Studies on the role of vasopressin in canine polyuria

Ilse Geerars - van Vonderen

Voor Ron en Jesse,
voor mijn ouders, Marit en Oma,
voor Miep en Arnold

Studies on the role of vasopressin in canine polyuria

Onderzoek naar de rol van vasopressine bij honden met polyurie

(met een samenvatting in het Nederlands)

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Aims and scope of the thesis

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Homeostasis, derived from the Greek word 'homoios', meaning of the same kind, and 'stasis', meaning condition, is a very important feature of all biological organisms. Equilibrium is required for many biophysical functions, amongst which the regulation of body temperature, cardiac frequency, blood pressure, and water- and electrolyte balance. Water constitutes the major component of the body, and thus its regulation is of vital importance for all living organisms.

In the general introduction of this thesis (**Chapter 2**), an overview is given of water homeostasis and its regulation, including the integration of water intake and water excretion, the mechanism of urine concentration, and the regulation and action of vasopressin (VP) and aquaporin-2 (AQP2). Polyuria and polydipsia (PUPD) represent an important disorder of water homeostasis, and are frequently encountered in the dog. Current knowledge of the pathogenesis of the different forms of canine polyuria is reviewed, in addition to the diagnostic approaches to PUPD.

Urine specific gravity and urine osmolality are used to assess the ability of the renal tubules to concentrate or dilute the glomerular filtrate and are therefore important variables for the documentation of PUPD. It has been stated that there is a high degree of individual biological variability in urine concentration. Although reference ranges can be found in many textbooks, these are only scarcely substantiated by investigatory data. To gain further insight into this topic, the intra- and interindividual variation in urine osmolality and urine specific gravity was studied in healthy dogs, together with the influence of age and gender on these variables (**Chapter 3**).

In dogs, secondary polycythaemia is associated with PUPD, but the underlying mechanism is not yet known. It was hypothesised that the hyperviscosity and increased blood volume occurring in secondary polycythaemia may affect VP release, resulting in PUPD. To test this hypothesis, the osmoregulation of VP release was studied in dogs with secondary polycythaemia due to renal neoplasm by investigating the VP response to hypertonic saline infusion (**Chapter 4**).

In all but one of the causes for PUPD, polyuria is the primary problem, and is compensated for by secondary polydipsia. In contrast, in primary polydipsia the polyuria is secondary to the increase in water intake. In dogs, primary polydipsia has been described as a behavioural problem. Results of indirect approaches not involving VP measurements suggest the presence of a normal hypothalamic-neurohypophyseal system, although an impaired osmoregulation of VP release has also been reported in dogs with primary polydipsia. **Chapter 5** describes VP

release during both water deprivation and hypertonic saline infusion in dogs with primary polydipsia identified by criteria not involving VP measurements.

Young dogs with polyuria form a separate category among dogs presented with PUPD. After exclusion of other causes of PUPD, the main diagnostic dilemma is to differentiate between central diabetes insipidus, primary polydipsia, and nephrogenic abnormalities. In **Chapter 6** eighteen young dogs presented with polyuria, in most cases since puppyhood, are described. The dogs were categorised according to their VP response to hypertonic saline infusion, which is regarded as the most powerful diagnostic tool for differentiation of polyuric conditions.

Unexpectedly high plasma VP concentrations have been found during hypertonic saline infusion in some dogs with polyuria. In these dogs it was not clear whether these high VP values should be regarded as pathological or as the result of a physiological pulsatile secretion pattern of VP. To determine whether VP is secreted in a pulsatile fashion in the dog, and whether stimulation of VP release changes the secretion pattern of VP, plasma VP concentrations were measured by frequent sampling under basal conditions, after water deprivation, and during osmotic stimulation with hypertonic saline in healthy dogs (**Chapter 7**).

Aquaporin-2 has been characterised as the VP-regulated water channel, localised in the apical membrane and the intracellular vesicles of the kidney collecting duct cells. Urinary AQP2 (U-AQP2) excretion closely parallels changes in VP action, and has been proposed as a marker for collecting duct responsiveness to VP. The immunohistochemical localisation of AQP2 in the canine kidney is described in **Chapter 8**. In addition, a radioimmunoassay was developed for the measurement of U-AQP2 excretion in dogs. To validate the assay, U-AQP2 excretion was measured in healthy dogs under basal conditions, after water loading, during hypertonic saline infusion, and after desmopressin administration.

In **Chapter 9** the results of the studies are summarised and discussed.

Chapter 2

General introduction on water homeostasis and polyuria/polydipsia

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1. Water homeostasis

1.1 Integration of water intake and water excretion

In healthy individuals, water homeostasis is accurately controlled, so that plasma osmolality (Posm) and its principal determinant, plasma sodium (Na), are maintained within a narrow range. This control is achieved by the close integration of water excretion and water intake (Robertson 1984, McKenna and Thompson 1998). Water intake is governed by the sensation of thirst, which is mainly regulated by osmosensitive neurons located in the anterior hypothalamus (Thrasher 1985, Robertson 1991). Studies in humans indicate that the thirst osmoreceptors possess an osmotic threshold, above which the sensation of thirst increases rapidly in direct proportion with Posm (Robertson 1984). In addition, hypovolaemia and hypotension detected by atrial and arterial baroreceptors are strong stimuli for water ingestion (Thrasher *et al*. 1982, Berl and Robertson 2000) (Figure 1). Oropharyngeal receptors play an important role in thirst regulation, as stimulation of these receptors by the ingestion of water leads to an early satiation of thirst before Posm or plasma volume change (Thrasher *et al*. 1981, Geelen *et al*. 1984, Salata *et al*. 1987, Appelgren *et al*. 1991).

Water excretion depends on the ability of the hypothalamic osmoreceptors to respond to changes in Posm, the ability of the atrial and carotid bifurcation baroreceptors to respond to changes in blood pressure or blood volume, and the subsequent release of the antidiuretic hormone, vasopressin (VP), via the hypothalamic-neurohypophyseal axis. In addition, renal medullary hypertonicity must be generated and maintained, and there must be an adequate number of functional nephrons with an appropriate response to VP (Chew and DiBartola 1989, Reeves and Andreoli 1992). The control of VP secretion and thirst sensation are partially interwoven, in that drinking not only leads to satiation of thirst but also to cessation of VP secretion (Thrasher *et al*. 1981, Geelen *et al*. 1984, Salata *et al*. 1987, Appelgren *et al*. 1991). Both thirst and VP release are influenced by similar stimuli, i.e. changes in Posm and pressure/volume status (Johnson and Cunningham 1987, Zimmerman *et al*. 1987) (Figure 1). In addition, osmoreceptors regulating VP secretion appear to be located in identical areas in the anterior hypothalamus as the thirst osmoreceptors (Robertson 1984, Thrasher 1985), although other studies indicate partial anatomic dissociation of thirst and VP regulatory areas (Andersson *et al*. 1967, Johnson and Cunningham 1987). The osmotic threshold for VP secretion is slightly lower than that for thirst perception, which permits maximum use of the antidiuretic mechanism to preserve water

balance, and a lower dependence on the thirst mechanism and a constant supply of water (Baylis and Robertson 1980, Robertson 1984). Despite the obvious vital importance of thirst during pathological situations of hyperosmolality and hypovolaemia, under normal circumstances water balance is accomplished more by free water excretion, regulated by VP, than by water intake, regulated by thirst (Robinson and Verbalis 2003).

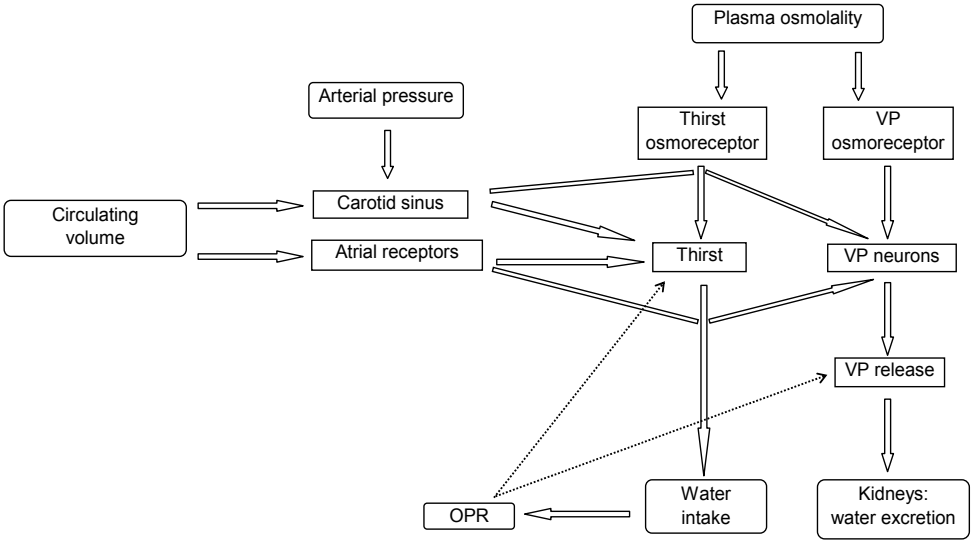

Figure 1. Schematic representation of the pathways influencing water intake and water excretion (VP = vasopressin, OPR = oropharyngeal reflex). In addition to these pathways, an increase in circulating volume will lead to central suppression of both thirst and VP secretion through the release of atrial natriuretic peptide (Gutkowska *et al.* 1997, McCann *et al*. 2003). On the other hand, stimulation of the juxtaglomerular apparatus and subsequent formation of angiotensin II in response to a decrease in circulating volume will stimulate both thirst and VP release, probably by a direct effect of angiotensin II on receptors in the circumventricular organs (Johnson and Cunningham 1987, Zimmerman *et al.* 1987, Giebisch and Windhager 2003a).

1.2 Mechanism of urine concentration

The kidney is an organ of both conservation and excretion: it conserves solutes and water needed by the body and simultaneously excretes excess solutes and metabolic wastes (Unwin and Capasso 2000). The functional unit of the kidney is the nephron, which is composed of a glomerulus, proximal tubule, loop of Henle, distal tubule, and collecting duct (Evans and Christensen 1993). In each nephron,

urine is formed by three basic processes: glomerular filtration, tubular reabsorption, and tubular secretion. The kidneys of the dog receive 10% to 20% of cardiac output, and about one fourth to one third of the blood plasma flowing through the glomerular capillaries becomes filtrate (approximately 3.7 l/kg body weight/day primary urine) (Finco 1995). The walls of the glomerular capillaries are porous to water and small-molecular-weight molecules, such as electrolytes, glucose, and amino acids. Blood cells and most plasma proteins are retained in the glomerular capillary lumen and leave the glomerular capillaries via the efferent arteriole. The tubular reabsorption of many substances, such as sodium, glucose, and amino acids, and the tubular secretion of organic ions, potassium, and protons, eventually determine the composition of urine (Rose 1994, Unwin and Capasso 2000).

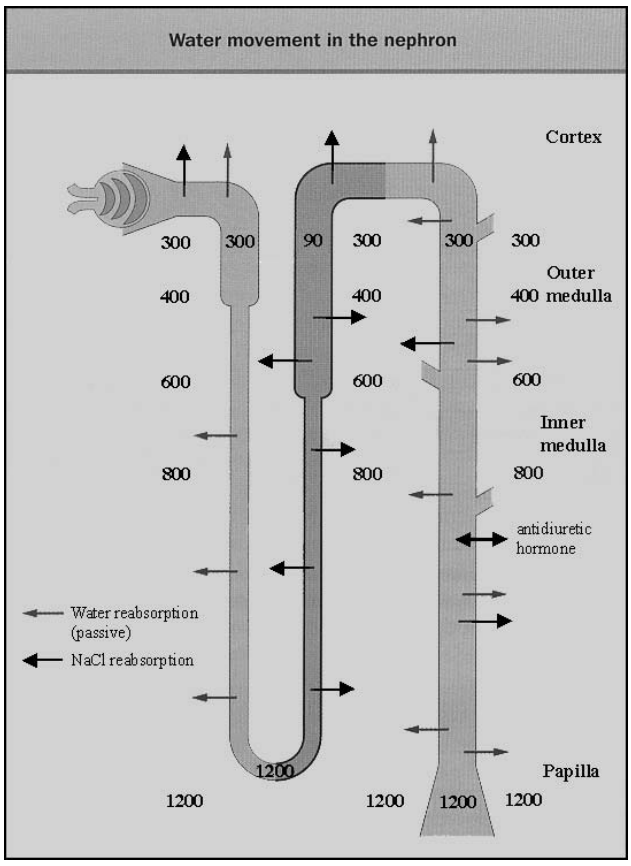

Figure 2. Schematic overview of water and sodium chloride (NaCl) movement in the nephron. The numbers indicate osmolalities and the cortico-medullary osmotic gradient. (Modified after Unwin RJ, Capasso G. Essential renal anatomy and physiology. Renal physiology. In: Johnson RJ, Fehally J, eds. Comprehensive Clinical Nephrology. Mosby: London; 2000: 2.1-2.11.)

The absorption of approximately 75% of filtered water occurs passively in the proximal tubule along osmotic gradients established by the active transport of

solutes such as sodium, potassium, bicarbonate, amino acids, and glucose (Ganong 1989) (Figure 2). Obligatory water reabsorption occurs in the proximal tubule regardless of the body's actual need for water. Isotonic fluid leaves the proximal tubule to enter the descending loop of Henle. The cells of the thin descending limb are highly permeable to water, but have a limited permeability to other small molecules, such as sodium and urea. Thus, tubular fluid becomes increasingly concentrated in the descending limb, as water is extracted from the tubule lumen by the high interstitial osmolality (Unwin and Capasso 2000). The entire ascending limb of the loop of Henle is impermeable to water. Passive diffusion of sodium into the interstitium takes place in the thin portion of the ascending limb, while active transport of chloride and sodium occurs in the thick ascending limb. The result of the outward movement of solutes and the restricted movement of water is a decrease in the osmolality of the fluid entering the distal tubule (Verlander 1997, Giebisch and Windhager 2003b).

The primary role of the loop of Henle is to provide a hyperosmotic medullary interstitium with which urine equilibrates during its passage through the collecting duct (Unwin and Capasso 2000) (Figure 2). Medullary hypertonicity is generated and maintained by the above-described sodium chloride pump in the thick ascending limb, passive sodium chloride transport out of the thin ascending limb, and urea transport from the inner medullary collecting duct. In the proximal part of the distal tubule sodium, chloride and water are reabsorbed independently of the VP concentration. The adjustment of water reabsorption needed to maintain the body water balance occurs in the latter parts of the distal tubule and in the collecting duct, and depends on the release and action of the hormones aldosterone and VP. Aldosterone, as part of the renin-angiotensin system, stimulates sodium and water reabsorption, and potassium secretion (Unwin and Capasso 2000, Rose 1994). Vasopressin facilitates the diffusion of water into the interstitium, as well as the diffusion of urea in the inner medullary collecting duct. Thus, the final concentration of urine is determined by the permeability of the collecting duct to water (Koeppen and Stanton 2000, Giebisch and Windhager 2003b).

1.3 Vasopressin regulation and secretion

The antidiuretic hormone VP is a nonapeptide produced in the neurosecretory neurons of the supraoptic, paraventricular, and suprachiasmatic nuclei of the hypothalamus, from which axons extend through the pituitary stalk to the posterior pituitary (Berl and Robertson 2000, Robinson and Verbalis 2003) (Figure 3). Vasopressin is synthesised as part of a large precursor molecule that is composed of a signal peptide, the hormone, a carrier protein termed neurophysin, and a glycopeptide known as copeptin (Robinson and Verbalis 2003). Like other peptide hormones destined for secretion, the protein precursor of VP is translocated into the endoplasmic reticulum, where the signal peptide is removed by a peptidase

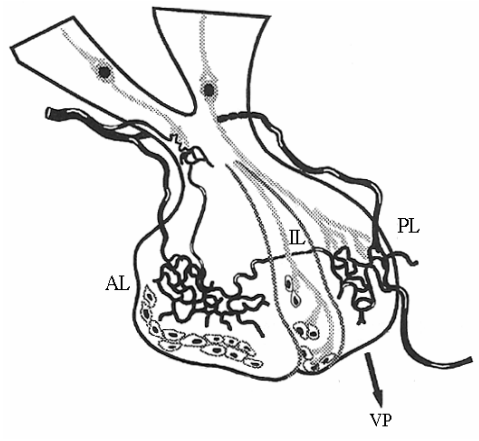

Figure 3. Schematic view of the hypothalamus and the pituitary gland. Vasopressin (VP) is produced in the hypothalamic magnocellular neurons and is released into the bloodstream in the posterior lobe (PL) of the pituitary gland (AL = anterior lobe; IL = intermediate lobe). (Modified after Rijnberk A. Hypothalamus-pituitary system. In: Rijnberk A, ed. Clinical Endocrinology of Dogs and Cats. Kluwer Academic Publishers: Dordrecht; 1996a: 11-34.)

and the prohormone folds and oligomerises, and moves from the Golgi apparatus into the neurosecretory granules (Robertson 2001). Binding of the N-terminus of the VP moiety into a pocket formed by the N-terminal domain of the neurophysin moiety facilitates proper folding of the prohormone, and is thought to play an important role in the production and trafficking of the hormone (Robertson 2001, Acher *et al*. 2002). As the granules move down the axons towards the axon terminal, the prohormone is further cleaved by endo- and exopeptidases, releasing VP, its neurophysin, and copeptin. In response to appropriate stimuli, the secretory granules fuse with the axon membranes and VP, neurophysin, and copeptin are released into the bloodstream, where they circulate independently of each other (Robertson 2001).

The secretion of VP is primarily controlled by osmoreceptors located in the organum vasculosum of the lamina terminalis and in areas of the adjacent anterior hypothalamus near the anterior wall of the third cerebral ventricle (Ramsay 1985, Robertson 1985, Wang and Goetz 1985, Wells 1998). In addition, baroreceptors situated in the atria, aortic arch, and carotid arteries may mediate haemodynamic influences to the neurohypophyseal system (Thrasher 1994). The afferent signals from these receptors are carried from the chest to the nucleus of the tractus solitarius and the magnocellular neurons through cranial nerves IX and X (Robinson and Verbalis 2003). Functionally, the two control systems are integrated in such a way that osmoregulation is altered but not disrupted by haemodynamic influences (Goetz and Wang 1985, Robertson and Ganguly 1986) (Figure 4). As little as a 1% increase in Posm may increase plasma VP, whereas blood volume and pressure need to decrease by 10% to 15% to stimulate VP release (Robinson and Verbalis 2003) (Figure 5). Although the action of VP in the kidney to retain water helps to restore the circulatory volume, the major hormonal regulation to

control volume actually involves the renin-angiotensin system (Robinson and Verbalis 2003).

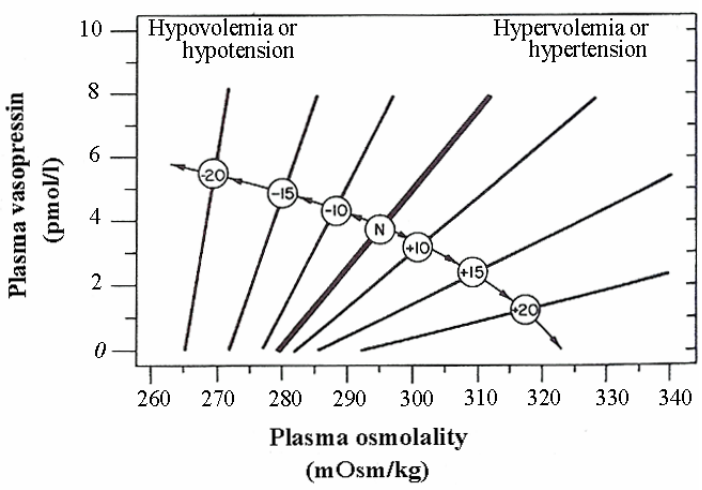

Figure 4. The effect of changes in blood volume or arterial pressure on the relation between plasma osmolality and plasma vasopressin activity. (Modified after Robertson GL, Athar S. The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. J Clin Endocrinol Metab 1976; 42: 613-620.)

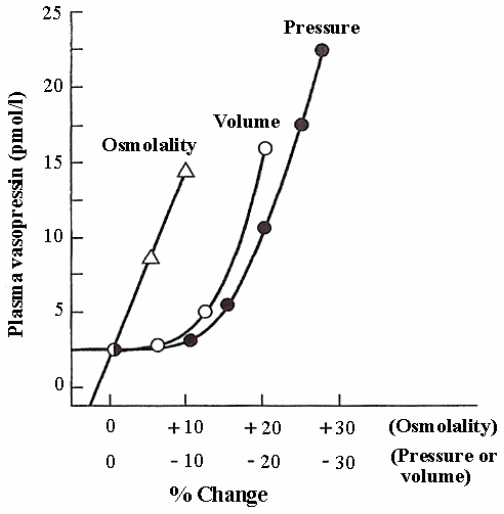

Figure 5. Comparison in humans of the sensitivity of vasopressin release to changes in plasma osmolality (increase), blood pressure, and blood volume (decrease). (Modified after Berl T, Robertson GL. Pathophysiology of water metabolism. In: Brenner BM, ed. Brenner and Rector's The Kidney, 6th ed. Saunders: Philadelphia; 2000: 866-925.)

Other important stimuli of VP release are nausea (Rowe *et al*. 1979), hypoglycaemia (Baylis and Heath 1977, Ellis *et al*. 1990), hypoxia/hypercapnia (Raff *et al*. 1983), pain (Kendler *et al*. 1978), and stress (Jørgensen *et al*. 2002). Apart from impairment of VP release, atrial natriuretic peptide also inhibits the renal responsiveness to VP (Dillingham and Anderson 1986, Iitake *et al*. 1986). In addition, a large number of drugs and hormones, amongst which glucocorticoids (inhibitory effect), angiotensin II, and corticotrophin-releasing factor (stimulatory effect), have been shown to influence VP secretion (Berl and Robertson 2000).

1.4 Vasopressin action

The biological effects of VP are mediated by three receptor subtypes: V1 receptors on blood vessels, V2 receptors on renal collecting duct epithelia, and V3 receptors (also termed V1b) responsible for the stimulation of adrenocorticotrophic hormone (ACTH) secretion from the anterior pituitary (Birnbaumer 2000, Robinson and Verbalis 2003). Although the term "vasopressin" was originally chosen because of the increase in blood pressure observed after administration of the hormone – a V1 receptor response – higher concentrations of the peptide are required to elicit these effects than for the antidiuretic action (Birnbaumer 2000). Apart from the antidiuretic effect, V2 receptors also regulate the stimulation of factor VIII production by VP (Robinson and Verbalis 2003).

Binding of VP to its V2-receptor in the basolateral membrane of the collecting duct cells initiates a signal transduction cascade consisting of activation of adenylate cyclase by the receptor-coupled Gs-GTP binding protein, acceleration of the production of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP), and activation of the catalytic subunit of protein kinase A (PKA) (Knepper 1997, Deen *et al*. 2000) (Figure 6). Subsequent phosphorylation of the water channel protein aquaporin-2 (AQP2) by PKA initiates the docking and fusion of AQP2-containing vesicles with the otherwise watertight apical membrane of collecting duct cells (Christensen *et al*. 2000, Deen *et al*. 2000). In the presence of these water-selective channels, water can move passively along an osmotic gradient, i.e., from the distal and collecting duct tubules to the hypertonic renal medulla. After VP withdrawal, AQP2 is redistributed into the cell by endocytosis, and water permeability decreases (Nielsen *et al*. 1995).

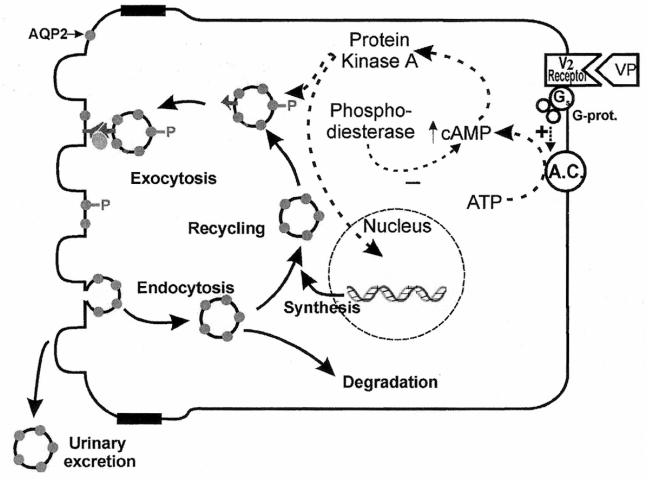

Figure 6. Regulation of aquaporin-2 (AQP2) trafficking and expression in kidney collecting duct cells. AC = adenylate cyclase, ATP = adenosine triphosphate, cAMP = cyclic adenosine monophosphate, VP = vasopressin (Modified after Nielsen S, Kwon T-H, Christensen BM, Promeneur D, Frøkiær J, Marples D. Physiology and pathophysiology of renal aquaporins. J Am Soc Nephrol 1999; 10: 647-663.)

1.5 Aquaporin-2 regulation and action

Diffusion-mediated water transport occurs through all biological membranes at a relatively low velocity. In the plasma membranes of highly water-permeable cells, aquaporins (AQPs), a family of integral membrane proteins, function as water-selective channels (Nielsen *et al*. 1995, Deen *et al*. 2000, Agre *et al*. 2002). At least 11 mammalian AQPs have been identified, and more than 200 members of this family have been found in plants, microbes, invertebrates, and other vertebrates (Agre *et al*. 2002, Ishibashi et al. 2002). The expression of AQPs in secretory and resorptive epithelia indicates that these proteins are involved in processes such as renal osmoregulation and water conservation, fluid secretion and absorption by epithelia of the gastrointestinal, respiratory, and reproductive tracts, and the formation of cerebrospinal fluid, amniotic fluid, and sweat (Deen *et al*. 2000, King and Yasui 2002). Members of the AQP family have six transmembrane domains which are connected by five loops (A-E), with the N- and C-termini located intracellularly (Fushimi *et al*. 1993, Jung *et al*. 1994, Sasaki *et al*. 1994) (Figure 7). Jung *et al*. (1994) proposed an hourglass model with the highly conserved asparagine-proline-alanine (NPA) sequence in loops B and E forming the aperture of the very tight structural water channel. Aquaporins are believed to be continuously open, and transmembrane water permeability is thought to be

regulated by the presence or absence of AQPs in the membrane (Deen *et al*. 2002). The single-channel water permeability of different AQPs varies greatly. Aquaporin-1, AQP2 and AQP4 have a high permeability, whereas AQP0 and AQP3 have a much lower permeability. Furthermore, some AQPs, including AQP3 and AQP9, also allow the passage of other molecules, including urea and glycerol (aquaglyceroporins) (Brown and Nielsen 2000).

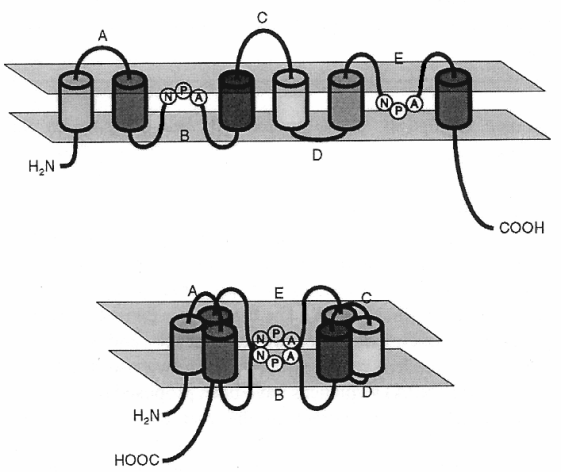

Figure 7. Schematic representation of the structural organisation of members of the aquaporin family. Aquaporins have six membrane-spanning domains connected by five loops, and both the amino- and carboxy-termini are cytoplasmic. The polypeptide is folded within the membrane bilayer, resulting in an hourglass-like structure, with the central necks of loops B and E including the highly conserved asparagine-proline-alanine (NPA) motifs forming the aperture of the water channel. (Modified after Jung JS, Preston GM, Smith BL, Guggino WB, Agre P. Molecular structure of the water channel through aquaporin CHIP. The hourglass model. J Biol Chem 1994; 269: 14648-14654.)

Seven different renal AQPs (AQP1-4, 6-8) have been defined thus far, correlating with well-defined segmental permeabilities in the nephron (King and Yasui 2002). Aquaporin-3 and AQP4 are localised in the basolateral membrane of the kidney collecting duct, and facilitate water transport from the cell to the interstitium (King and Yasui 2002, Schrier and Cadnapaphornchai 2003). Aquaporin-2 has been characterised as the major VP-regulated water channel and is predominantly localised in the apical membrane and the intracellular vesicles of collecting duct principal cells (Nielsen *et al*. 1993). Aquaporin-2 was first cloned

from rat kidney collecting duct and characterised as a functional water channel by Fushimi *et al*. (1993). In 1994, Sasaki *et al*. cloned human AQP2, a 271-amino acid protein with 91% homology with rat AQP2. Chromosomal mapping of the AQP2 gene assigned its location to chromosome 12q13 (Sasaki *et al*. 1994, Uchida *et al*. 1994).

Vasopressin-mediated regulation of collecting duct water permeability involves both short- and long-term regulation. Within a time frame of a few minutes, VP increases the water permeability of the kidney collecting duct by stimulating the translocation of AQP2 from an intracellular reservoir to the apical plasma membrane (Nielsen *et al*. 1993, 1995, Marples *et al*. 1995). Chronic VP exposure (>24 h) is associated with an increased maximal water permeability of the collecting duct epithelium and an increased expression of AQP2 (Nielsen *et al*. 1993, DiGiovanni *et al*. 1994, Kishore *et al*. 1996) as a result of increased transcription of the AQP2 gene (Hozawa *et al*. 1996) (Figure 6). During VP stimulation, 3% of total kidney AQP2, in part associated with small vesicles and larger membrane fragments, is excreted into the urine by a selective apical pathway (Rai *et al*. 1997, Wen *et al*. 1999) (Figure 6). Urinary excretion of AQP2 closely parallels changes in VP exposure, and has been proposed as a reliable marker for collecting duct responsiveness to VP in various physiological states of water homeostasis as well as disorders of water homeostasis in humans (Pedersen *et al*. 2001, Saito *et al.* 2001).

2. Polyuria and polydipsia (PUPD)

In companion animal medicine, polyuria is generally defined as a daily urine output of >50 ml/kg body weight per day, and polydipsia as a fluid intake exceeding 100 ml/kg body weight per day (Dunn 1990, Feldman and Nelson 1996, Taylor 2000). In general, there are two main causes of polyuria: osmotic diuresis and water diuresis. When the concentration of a solute present in the glomerular filtrate exceeds the proximal tubular capacity for reabsorption, passive water reabsorption is impaired. Even in the presence of VP, urine cannot be concentrated, resulting in osmotic diuresis. The permeability of the distal tubule and collecting duct depends on the presence of VP. Both a decreased release of VP and a decreased response to the hormone lead to a decrease in water permeability and water diuresis (Giebisch and Windhager 2003b).

The differential diagnosis of polyuria represents one of the classic problems of clinical medicine (Zerbe and Robertson 1981). Much more so than in other species, polyuria occurs frequently in dogs. This is illustrated by a relatively long list of conditions in which polyuria is a clinical feature. The conditions, well-known to be associated with polyuria, will be described below in brief sections.

2.1 Primary polydipsia

Primary polydipsia is the only disorder of water homeostasis in which the polydipsia is primary, and the polyuria is a compensatory response. In humans, primary polydipsia may result from a defect in the thirst center (dipsogenic polydipsia) or may be associated with mental illness (psychogenic polydipsia) (Robertson 1987, Robertson 1988). In dogs, primary polydipsia has been described as a behavioural problem and has also been called a psychological disorder occurring in young hyperactive dogs (Nichols 1992, Feldman and Nelson 1996). Nevertheless, VP release is impaired in some dogs with primary polydipsia (Biewenga *et al*. 1987).

2.2 Glucosuria

Glucose is filtered into the primary urine in the same concentration as in plasma. Normally, the resorption of all glucose takes place in the proximal tubule by active transport. In diabetes mellitus, the elevated glucose concentrations in plasma exceed the renal threshold of about 10 mmol/l, leading to glucosuria. The resulting osmotic diuresis causes polyuria and electrolyte loss. Both exogenous progestagens (used in estrus prevention) and endogenous progestagens (metestrus) may cause diabetes mellitus through growth hormone hypersecretion of mammary origin and peripheral insulin resistance (Selman *et al*. 1994). The classic picture is that of the middle-aged bitch presented in late metestrus with PUPD and weight loss despite a good appetite (Rijnberk 1996b).

Although glucosuria reflects hyperglycaemia in more than 95% of cases, primary renal glucosuria must also be taken into consideration. Dysfunction of the proximal tubule, such as in the Fanconi syndrome, may result in glucosuria and polyuria despite normoglycaemia (Meric *et al*. 1995).

2.3 Renal failure

Regardless of the primary cause of nephron loss, in chronic renal insufficiency some nephrons usually survive and subsequently adapt by enlarging and increasing the clearance per nephron. Initially, this adaptation process has a beneficial effect, maintaining whole kidney glomerular filtration rate and solute excretion. Ultimately, however, the glomerular hypertrophy and the increase in single-nephron glomerular filtration rate lead to focal glomerulosclerosis and further nephron loss. The osmotic diuresis in the remaining adapted nephrons impairs sodium and water conservation, resulting in a decreased renal concentrating ability. Besides PUPD, clinical manifestations may include anorexia, vomiting, weight loss, and anaemia (Luke 2000).

Acute pyelonephritis may produce a transiently decreased ability to concentrate urine but rarely leads to permanent renal damage. Chronic pyelonephritis refers to chronic interstitial nephritis resulting from infection of the

upper urinary tract. Many non-infectious diseases also cause an interstitial nephritis that is pathologically indistinguishable from chronic pyelonephritis. With persistent infection, chronic pyelonephritis may result in end-stage renal failure. Early signs of pyelonephritis may include fever, renal pain, and leukocytosis, but the renal problem is often occult (Stamm and Turck 1991, Kunin 2000).

2.4 Hyperadrenocorticism

The dominant clinical manifestations of canine hyperadrenocorticism are centripetal obesity, atrophy of muscles and skin, polyphagia, and PUPD. Many of these symptoms can be related to the increased gluconeogenesis and lipogenesis at the expense of protein caused by the glucocorticoids (Rijnberk 1996c). The polyuria associated with hypercortisolism is caused by an impaired osmoregulation of VP release affecting both the threshold and the sensitivity of the secretory system. A direct effect of glucocorticoids acting on the glucocorticoid receptors in the VP-producing magnocellular neurons seems likely (Biewenga *et al*. 1991). In addition, glucocorticoids interfere with the action of VP at the level of the kidney (Biewenga *et al*. 1991).

2.5 Pyometra

In the dog, pyometra may develop during the luteal phase of the estrus cycle, when cystic endometrial hyperplasia caused by repeated exposure of the endometrium to progesterone concurs with secondary bacterial infection. Clinical features may include anorexia, weight loss, vomiting, vaginal discharge, and PUPD (Schaefers-Okkens 1996, Johnston *et al*. 2001). The mechanism underlying the polyuria associated with pyometra is not well understood. From early studies by Åsheim (1963a, 1963b) it was concluded that an insufficiency of the neurosecretory system could not adequately explain the occurrence of polyuria in pyometra. According to Watts *et al*. (2001), endotoxinaemia in pyometra may cause increased plasma concentrations of prostaglandin F2α, which lead to polyuria, perhaps mediated by the in vivo antagonism between VP and prostaglandins (Anderson *et al*. 1975). Nevertheless, polyuria may also occur in dogs with pyometra which are negative for E. Coli endotoxin (Okano *et al*. 1998, Stone *et al*. 1998). A recent study by Heiene *et al*. (2003) provides evidence for the downregulation of kidney VP receptors in dogs with pyometra.

2.6 Hepatic failure

Polyuria in combination with failure to grow, cachexia, episodic central nervous system disturbances, and gastrointestinal problems may point to hepatoencephalopathy (HE). Rothuizen *et al*. (1987, 1995) have found that HE may be associated with hyperadrenocorticism. Hence, interference with the release and action of VP may play an important role in the polyuria associated with HE. It has

been postulated that an abnormal metabolism of amino acids in HE may give rise to 'false' dopaminergic neurotransmitters, which may increase ACTH secretion, and consequently cause hyperadrenocorticism. In addition to the hypercortisolism, an increased tonus of the gamma-amino butyric acid (GABA) neurotransmitter system may also result in a disturbed osmoregulation of VP release in HE (Rothuizen *et al*. 1995).

2.7 Hyperthyroidism

Most canine thyroid tumours are discovered as a painless mass in the midcervical or ventrocervical region, that with increasing size may give rise to dysphagia, hoarseness, and tracheal obstruction. In approximately 10% of cases the thyroid tumour may give rise to hypersecretion of thyroid hormone. Clinical manifestations of hyperthyroidism include weight loss despite good appetite, weakness, panting, and PUPD (Rijnberk 1996d). High levels of atrial natriuretic peptide may be involved in the polyuria of hyperthyroidism (Weissel *et al*. 1986).

2.8 Hypercalcaemia

Hypercalcaemia may be due to primary hyperparathyroidism (parathyroid adenoma, carcinoma, or hyperplasia) or to hypercalcaemia of malignancy (malignant lymphoma, mammary carcinoma, adenocarcinoma of the apocrine glands of the anal sac) (Rijnberk and Hazewinkel 1996). Clinical features include PUPD, anorexia, weakness, and weight loss. Hypercalcaemia has been postulated to impair the renal response to VP (Taylor 2000), which may be explained by downregulation of AQP2 (Wang *et al*. 2002). In humans, a raised serum calcium concentration prompts reciprocal changes in the secretion and action of VP. On the one hand, high calcium concentrations diminish the sensitivity of the antidiuretic response to VP, and on the other they increase the sensitivity of the VP secretory response to osmotic stimulation (Weiss and Robertson 1985).

2.9 Hyperaldosteronism

Primary hyperaldosteronism may lead to hypokalaemia, hypophosphataemia, alkalosis, and PUPD (Rijnberk *et al*. 2001). An elevated plasma aldosterone concentration and a low plasma renin activity are suggestive of primary hyperaldosteronism, which may be caused by an aldosterone-producing adrenal adenoma or idiopathic adrenocortical hyperplasia. In the dog, both VP resistance and an increased osmotic threshold of VP release may play a role in the development of polyuria, similar to the situation in glucocorticoid excess (Rijnberk *et al*. 2001). In experimental animals, hypokalaemia leads to a form of acquired nephrogenic diabetes insipidus due to downregulation of renal AQP2 (Marples *et al*. 1996, Amlal *et al*. 2000). Aquaporin-2 downregulation may play a role in the VP resistance seen in primary hyperaldosteronism in the dog.

2.10 Polycythaemia

Polycythaemia is characterised by an increase in the packed cell volume, and can be classified into secondary polycythaemia and polycythaemia vera. Polycythaemia vera is a myeloproliferative disorder which results from the proliferation of pluripotential stem cells in the bone marrow, leading to an increased red cell mass and variable increases in granulocytes and platelets (De Wolf *et al*. 1992). Secondary polycythaemia is the consequence of excessive production of erythropoietin, which in companion animals is most often associated with renal lesions (Campbell 1990). The clinical signs may include erythema of mucous membranes, bleeding diatheses, disturbances of the central nervous system, and PUPD (Peterson and Randolph 1983). Many of these clinical manifestations may be related to the increased red blood cell mass, which causes hyperviscosity of the blood and an increased blood volume, but the exact mechanism leading to polyuria is as yet unclear (Peterson and Randolph 1983, Campbell 1990).

2.11 Syndrome of inappropriate VP release (SIADH)

In this rare syndrome, the high VP secretion is considered inappropriate because it occurs in the presence of plasma hypo-osmolality (Zerbe *et al*. 1980). The hypotonicity leads to generalised cellular edema, and potentially hypotonic encephalopathy. In humans, water excretion is impaired in SIADH, and urine osmolality (Uosm) is inappropriately high for the concomitant Posm. Similar findings have been reported in dogs with SIADH (Fleeman *et al*. 2000, Brofman *et al*. 2003), although others have reported a somewhat paradoxical PUPD as an important clinical feature of SIADH (Rijnberk *et al*. 1988). This polyuria can be explained by downregulation of renal receptors due to chronic exposure of the kidney to increased VP levels (Rijnberk *et al*. 1988, Rijnberk 1996a).

2.12 Central diabetes insipidus

Diabetes insipidus refers to the passage of large quantities of dilute urine, and is synonymous with polyuria. In central diabetes insipidus, polyuria results from a complete or partial deficiency in VP synthesis or secretion. Besides an idiopathic form, possible causes include primary or metastatic neoplasia involving the hypothalamus or the posterior pituitary gland, trauma to the skull, and pituitary surgery (Taylor 2000). In humans at least 30 different mutations in the gene encoding for the hypothalamic precursor protein (prepro-VP-neurophysin) have been described to cause a familial form of central diabetes insipidus (Calvo *et al*. 1999, Siggaard *et al*. 1999). The major manifestations of central diabetes insipidus are insatiable polydipsia and voluminous polyuria, besides symptoms due to the underlying cause (Rijnberk 1996a).

2.13 Nephrogenic diabetes insipidus

Nephrogenic diabetes insipidus is a disorder in which the kidney tubules are insensitive to VP. Many of the above-mentioned causes of polyuria, such as hyperadrenocorticism and hypercalcaemia, seem to be characterised by secondary nephrogenic diabetes insipidus, also termed acquired nephrogenic diabetes insipidus. Primary or congenital nephrogenic diabetes insipidus is a rare condition in dogs (Takemura 1998), and may be due to a mutation affecting the affinity of the renal V2-receptor (Luzius *et al*. 1992). In humans, besides mutations in the VP receptor gene (Pasel *et al*. 2000), primary nephrogenic diabetes insipidus may also be caused by mutations in the AQP2 gene, leading to impaired routing of the water channel and its subsequent degradation (Deen *et al*. 1994, Marr *et al*. 2002).

3. Diagnostic approaches

Several causes of PUPD, such as glucosuria, hypercalcaemia, and renal failure, may be detected by a thorough medical history and physical examination, followed by blood and urine examination. Depending on these results, more specific examinations, such as diagnostic imaging (uterus, adrenal glands, liver, kidneys), thyroid scintigraphy, and/or measurements of urinary corticoid/creatinine ratios, may be necessary to establish the final diagnosis.

When physical and laboratory examinations do not identify a definite cause for polyuria, the next diagnostic step is the modified water deprivation test. In this indirect test for VP secretory capacity, Posm is increased by water deprivation to stimulate VP release. The effect is measured indirectly, by measurements of Uosm during the test. At the end of the water deprivation test, exogenous VP is administered and its effect on Uosm is determined (Mulnix *et al*. 1976). Although it may be possible to distinguish complete central diabetes insipidus, complete nephrogenic diabetes insipidus, and primary polydipsia, the result of the water deprivation test is not always conclusive (Biewenga *et al*. 1987).

Measurement of plasma VP concentration during osmotic stimulation with hypertonic saline constitutes a direct method to assess VP release (Biewenga *et al*. 1987). In humans, this test is considered the "gold standard" for the diagnostic interpretation of polyuria (Diederich *et al*. 2001). Especially differentiation between partial forms of central diabetes insipidus, nephrogenic diabetes insipidus, and SIADH may be possible during hypertonic saline infusion. However, chronic dehydration and overhydration may alter the VP response to hypertonicity (Moses and Clayton 1993, Moses and Scheinman 1993), and this effect must be taken into account when interpreting the results of the hypertonic saline infusion test.

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Chapter 3

Intra- and interindividual variation in urine osmolality and urine specific gravity in healthy pet dogs of various ages

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Abstract

Urine specific gravity (Usg) and urine osmolality (Uosm) are used routinely to assess renal concentrating ability, but limited data on these variables are available for healthy dogs. Consequently, we studied the intra- and interindividual variations in Usg and Uosm in healthy dogs as well as the influence of age and gender on these variables.

Dogs were selected for health and anestrus in females through the use of a detailed questionnaire. Eighty-nine owners collected morning and evening urine samples from their dogs on 2 consecutive days. In 8 dogs in which the Uosm of different samples varied more than 50%, owners collected urine for 24 hours at 2-hour intervals during the day and at 4-hour intervals at night. The possible effect of changes in adrenocortical function with age was assessed by measurements of urinary corticoid/creatinine ratios.

Among all samples, Uosm ranged from 161 to 2830 mOsm/kg and Usg from 1.006 to >1.050. In the morning, Uosm (1541 ± 527 mOsm/kg, range 273 – 2620 mOsm/kg) and Usg (1.035 ± 0.010, range 1.009 - >1.050) were higher than in the evening (Uosm: 1400 ± 586 mOsm/kg, range 161 – 2830 mOsm/kg; Usg: 1.031 ± 0.012, range 1.006 - >1.050). The interindividual coefficient of variation in Uosm was 34.2% for morning urine samples and 41.9% for evening samples. In 8 dogs with large differences in urine concentration, there were 2- to 3-fold increases or decreases in Uosm during the day, and the intraindividual coefficient of variation was 33.0%. There was no relation between gender and urine concentration. Urine concentration in both the morning and evening samples decreased with age. Urinary corticoid/creatinine ratios did not change with age.

It can be concluded that Uosm and Usg vary widely among healthy dogs. Urine concentration is generally lower in the evening than in the morning and is not related to gender. Urine concentration decreases with age, and this cannot be ascribed to an associated increase in endogenous corticoids.

In some dogs, Uosm varies widely during the day, with an intraindividual coefficient of variation approaching the interindividual coefficient of variation. This may be regarded as biologic variation but also could represent an early undiagnosed clinical abnormality.

Introduction

Urinary concentrating capacity depends on the ability of the hypothalamic osmoreceptors to respond to changes in plasma osmolality, the ability of the atrial and carotid bifurcation baroreceptors to respond to changes in blood pressure or blood volume, and the release of the antidiuretic hormone vasopressin (VP) from the neurohypophysis. In addition, medullary hypertonicity must be generated and maintained, and there must be an adequate number of functional nephrons with an appropriate response to VP (Chew and DiBartola 1989, Reeves and Andreoli 1992).

In the healthy dog, urine osmolality (Uosm) values as low as 50 mOsm/kg are reached after infusion of large quantities of water, i.e., in the absence of VP (Schrier and Berl 1972, Schrier *et al.* 1972, Anderson *et al*. 1974, Cadnapaphornchai *et al*. 1974). In states of dehydration, urine specific gravity (Usg) and Uosm are increased to as much as 1.076 and 2738 mOsm/kg, respectively (Hardy and Osborne 1979).

Urine specific gravity and Uosm are used to assess the ability of the renal tubules to concentrate or dilute glomerular filtrate (Stevens and Osborne 1974). Randomly collected urine samples from normally hydrated dogs are reported to have Usg values ranging from 1.015 to 1.045, whereas Uosm values usually vary between 500 and 1200 mOsm/kg (Bloom 1960, Doxey 1971, Osborne *et al*. 1972, Coles 1974, Stevens and Osborne 1974, Bush 1975, Bovee 1984).

There appears to be a high degree of individual biologic variability in Usg or Uosm values of urine samples obtained at different times of the day from the same dog (Bovee 1969, Chew and Dibartola 1989). Therefore, a single urine sample may be misleading, and a second sample or a series of samples should be obtained to confirm any abnormality (Doxey 1971, Bush 1975, Bovee 1984). A Usg value of 1.025 or more has been considered evidence of adequate renal concentrating ability (Hardy and Osborne 1979, Finco 1989).

Over the years, these statements, which have appeared in textbooks and which are based on clinical experience and not substantiated by investigatory data, have been used as guides for the assessment of urinary concentrating ability. Except for one report of 20 dogs (Hardy and Osborne 1979), there has been no systematic study of Uosm and Usg in healthy pet dogs. Also, the effects of gender and age on Usg and Uosm values have not been investigated in normal pet dogs.

This study was designed to investigate the influence of age and gender on Uosm and Usg and the fluctuation of these variables during the day in healthy pet dogs. Aged dogs have an elevated basal hypothalamic-pituitary-adrenocortical activity characterised by increased concentrations of adrenocorticotrophic hormone (ACTH) and cortisol in plasma and increased urinary corticoid excretion

(Rothuizen *et al*. 1993). Consequently, we also measured urinary corticoid/creatinine (C/C) ratios in the dogs in this study to evaluate the possible effect of increased corticoid production on renal concentrating ability (Joles *et al*. 1980, Biewenga *et al*. 1991).

Materials and Methods

Animals

Thirty-four male (5 castrated) and 55 female (27 spayed) dogs, ranging in age from 6 months to 15.4 years, were investigated. The sample population consisted of 21 mongrel dogs and 68 pure-bred dogs comprising 31 different breeds. Among the breeds represented were 8 Labrador Retrievers, 5 Golden Retrievers, 5 Bouviers des Flandres, and 5 Border Collies. Other breeds were represented by 4 or fewer dogs.

The dogs were judged to be healthy according to the information provided by their owners in a detailed questionnaire. Dogs were included when there had been no signs of polyuria, polydipsia, or urinary incontinence during the past 3 months and when there had been no signs of illness or any treatment that could influence urinary concentrating ability. Furthermore, only anestrous female dogs were included. The questionnaire also provided information on the availability of food during the day and the composition of the food.

Urine collection

On 2 consecutive days, owners collected 4 urine samples by free catch: 2 in the morning during the first walk and 2 in the evening during the last walk, resulting in 4 urine samples per dog. In 8 dogs with variations in Uosm values of >50%, the owners collected urine at 2-hour intervals during the day and at 4-hour intervals at night for a period of 24 hours.

Methods

In all urine samples, Usg was measured in duplicate by refractometry (Clinical table refractometer, Atago, Tokyo, Japan). Urine osmolality was determined in duplicate by freezing point depression (Cryostatic osmometer 030, Gonotec GmbH, Berlin, Germany). One urine sample from each dog was examined for the presence of glucose by a dipstick method (TES-TAPE, Eli Lilly and Co, Indianapolis, USA). In 176 morning urine samples and in the urine samples collected during 24 hours in 4 dogs, C/C ratios were determined as described previously (Stolp *et al*. 1983, Rijnberk *et al.* 1988).

Statistical analysis

Values are expressed as mean ± SD. The significance of changes in Uosm, Usg and C/C ratio with age was examined by linear regression analysis. The interindividual variation in Uosm of morning and evening urine samples and the intraindividual variation in Uosm of urine samples collected during 24 hours were expressed by coefficients of variation. Inter- and intraindividual differences were tested with unpaired and paired, two-tailed Student's t-tests, respectively. A P value <0.05 was considered significant. In 27 dogs, Usg of one or more samples exceeded the upper scale limit of the refractometer (1.050), and Usg values of these samples were not used for further analysis.

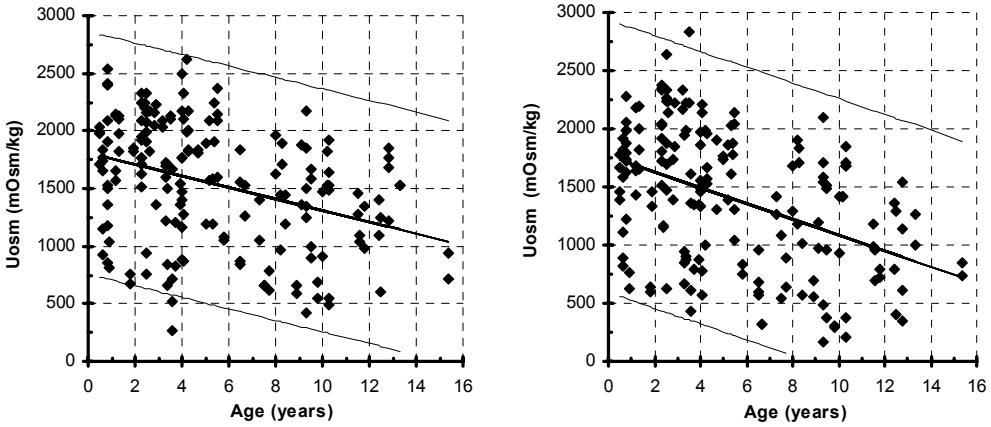

Figure 1. Relation between age and urine osmolality (Uosm) in 89 healthy pet dogs, in which Uosm was measured on 2 consecutive days in morning samples (left panel) and evening samples (right panel). Regression lines ± 2SD are shown:
Y = 1809 - 50.0X, r=0.36 (P<0.001), n=178 (left panel)
Y = 1764 - 67.8X, r=0.44 (P<0.001), n=178 (right panel)

Results

The Uosm of morning samples ranged from 273 to 2620 mOsm/kg, whereas the Uosm values of evening samples ranged from 161 to 2830 mOsm/kg. The Uosm of morning samples (1541 ± 527 mOsm/kg) was significantly higher than that of evening samples (1400 ± 586 mOsm/kg) ($P<0.001$). Neither the Uosm of morning samples of 2 consecutive days nor the Uosm of 2 evening samples differed significantly. The interindividual coefficients of variation for Uosm of morning and evening samples were 34.2% and 41.9%, respectively. The Uosm of both morning and evening samples decreased significantly with age ($P<0.001$, Figure 1).

The morning Uosm of male (1522 ± 427 mOsm/kg) and female (1553 ± 539 mOsm/kg) dogs did not differ significantly. Also, the Uosm values of evening samples of male (1309 ± 531 mOsm/kg) and female (1456 ± 562 mOsm/kg) dogs were not significantly different.

The Usg of morning samples of all dogs ranged between 1.009 and >1.050 and that of the evening samples between 1.006 and >1.050. The 27 dogs in which Usg of one or more samples was >1.050 were significantly younger than the remaining 62 dogs ($P<0.001$). After excluding the samples of these 27 dogs, analysis of data from the remaining 62 dogs showed a significant decrease in Usg of the evening samples with age ($P<0.001$, Figure 2), but no relationship was found between age and Usg of morning samples. In these 62 dogs, the Usg of the morning samples (1.035 ± 0.010) was significantly higher than the Usg of the evening samples (1.031 ± 0.012) ($P<0.001$). Neither the Usg of the 2 morning samples nor the Usg of the 2 evening samples differed significantly.

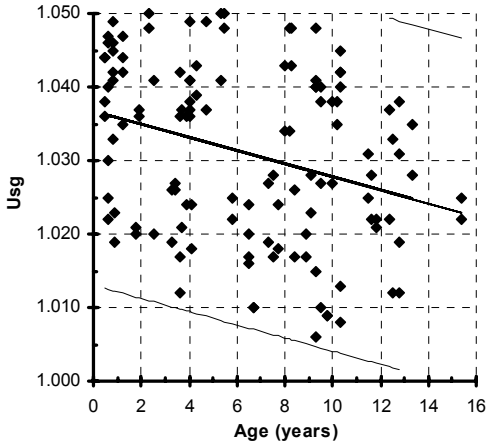

Figure 2. Relation between age and urine specific gravity (Usg) in 62 healthy pet dogs, in which Usg was measured in evening samples on 2 consecutive days. The regression line ± 2SD is shown:
Y = 1.037-0.0009X, r=0.31
($P<0.001$), n=124

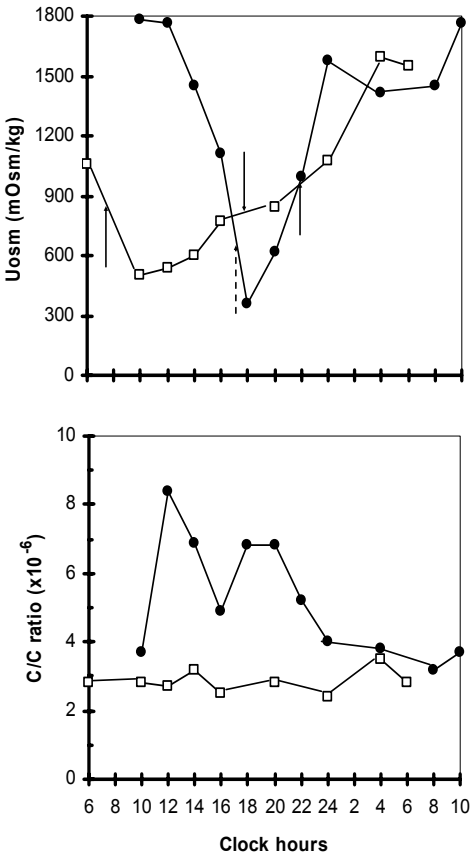

Figure 3. Fluctuation of urine osmolality (Uosm, upper panel) and the urinary corticoid/creatinine ratio (C/C ratio, lower panel) during the day in a 9.5-year-old castrated male schnauzer (closed circle, dotted arrow) and a 7.5-year-old female Belgian shepherd (open square, uninterrupted arrow). Arrows indicate feeding times.

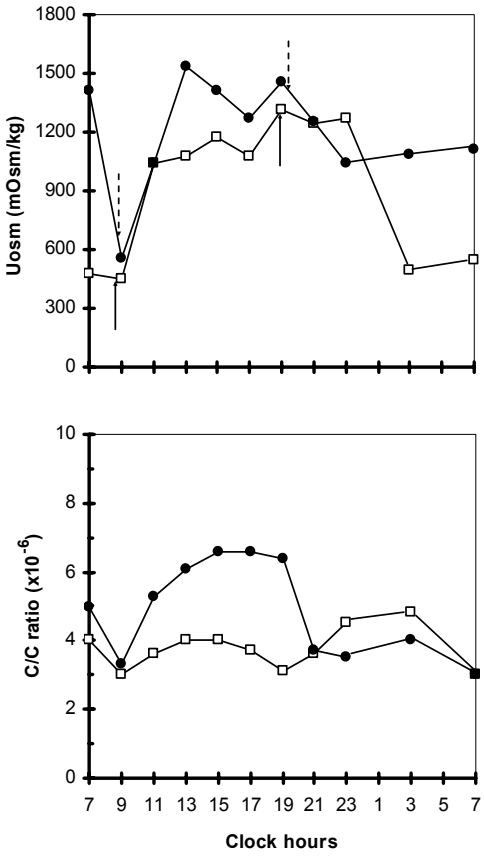

Figure 4. Fluctuation of urine osmolality (Uosm, upper panel) and the urinary corticoid/creatinine ratio (C/C ratio, lower panel) during the day in a 2.5-year-old male Border collie (closed circle, dotted arrow) and a 12.5-year-old castrated female longhaired German pointer (open square, uninterrupted arrow). Arrows indicate feeding times.

In the 8 dogs that were sampled frequently because of the large variation in Uosm of different samples, 2- to 3-fold increases or decreases in Uosm often occurred within 2 hours (Figures 3 and 4). After a meal, Uosm decreased as often

as it increased. The mean intraindividual coefficient of variation for Uosm was 33.0%.

The C/C ratios measured in the morning samples of 88 dogs ranged between 0.3 x 10^{-6} and 8.3 x 10^{-6}, with a mean of 2.9 x 10^{-6} (± 1.4 x 10^{-6}). The mean of the C/C ratios measured in the 2 morning samples did not change significantly with age (Figure 5). In the dogs with large intraindividual variations in Uosm in which measurements were made frequently during 24 hours, the C/C ratios varied with Uosm in some, whereas in others the ratios changed very little (Figures 3 and 4). Glucose was not found in any of the urine samples examined.

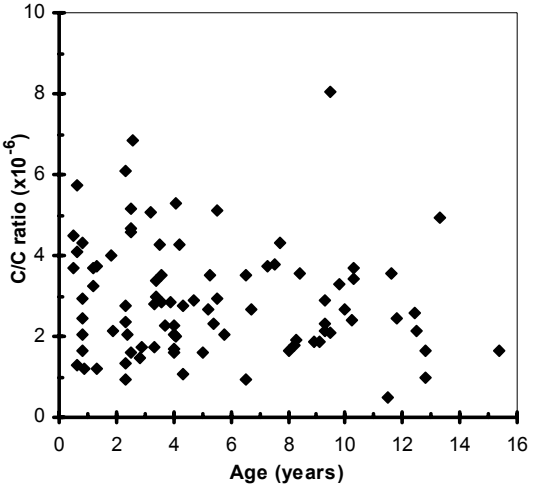

Figure 5. Relation between age and the mean urinary corticoid/creatinine (C/C) ratio in 2 consecutive morning samples from 88 healthy pet dogs.

Discussion

In 1775, De Beaumarchais (French author, 1732-1799) stated, "Boire sans soif ... c'est ce qui distingue l'homme des autres animaux." (Drinking without thirst ... is what distinguishes man from other animals.) Similar statements have been made by Adolph (1939, 1947, 1950) and Guyton (1991). Adolph (1939, 1947, 1950) demonstrated that after water deprivation, dogs do not drink an amount greater than the measured deficit: "In no instance is water ingested voluntarily in an excess sufficient to give rise to water diuresis, and in no instance is water excreted in a diuresis that gives rise to water drinking" (Adolph 1950).

In contrast to these statements and reports, we found large intra- and interindividual variations in Uosm and Usg in healthy pet dogs. The ranges of Usg (1.006 - >1.050) and Uosm (161 - 2830 mOsm/kg) found among the 89 dogs

examined are also considerably larger than those reported in current veterinary textbooks for normally hydrated dogs (Bloom 1960, Doxey 1971, Osborne *et al*. 1972, Coles 1974, Stevens and Osborne 1974, Bush 1975, Bovee 1984).

There are very few publications addressing Uosm as a measure of renal concentrating ability (Bovee 1969). Hardy and Osborne (1979) found Uosm values ranging from 976 to 2546 mOsm/kg and Usg values ranging from 1.023 to 1.064 in 20 healthy experimental dogs with free access to water. Our results exceed these values at both ends of the range.

The differences in Uosm and Usg between morning and evening samples were significant, whereas those between the morning or evening samples of 2 consecutive days were not. The lower values in the evening urine samples could reflect differences in water intake between day and night.

A significant decrease in Uosm and in evening Usg occurred with aging. It has been reported that Usg and Uosm have excellent correlation (Hendriks *et al*. 1978, Van Vonderen *et al*. 1995), so a significant decrease in morning Usg with age also would have been expected. The absence of such a decrease may have resulted from the fact that Usg values in 27 younger dogs could not be used in the analysis because they exceeded the upper scale limit of the refractometer (1.050). Because Usg was higher in the morning than in the evening, omission of these high Usg values presumably had a great effect on the mean morning Usg, causing the decrease of morning Usg with age to be insignificant.

Rothuizen *et al*. (1993) described an elevated basal hypothalamic-pituitary-adrenocortical activity in aged dogs, characterised by increased concentrations of ACTH and cortisol in plasma and increased urinary corticoid excretion. In the present study, no effect of age on the C/C ratio was observed. A possible explanation for this discrepancy could be the fact that in the study by Rothuizen *et al*. (1993), samples were not collected at home but in the clinic (i.e., under stressful circumstances). In addition, the dogs used in the study by Rothuizen *et al*. (1993) were all relatively old (11 - 15 years). Our results agree with the range of C/C ratios in healthy dogs reported by Stolp *et al.* (1983). Thus, in the age range studied there was no evidence of increased adrenocortical function and, as a consequence, the decrease in urinary concentrating ability cannot be ascribed to increased corticoid production.

Urinary concentrating ability decreases in senescent humans (Lindeman *et al*. 1966) and rats (Bengele *et al*. 1981, Beck *et al*. 1982). Apart from basal renal concentrating ability, maximum Uosm after water deprivation and that obtained after VP administration have been shown to decrease with advancing age in the rat (Miller 1985, Corman and Michel 1987, Geelen and Corman 1992). Furthermore, there is an increase in VP secretion with age in the rat (Frolkis *et al*. 1982, Fliers and Swaab 1983). These findings provide evidence that impaired renal

concentrating ability during aging in the rat is not a consequence of decreased VP release from the neurohypophysis, but rather a result of an age-associated reduction in renal responsiveness to VP (Bengele *et al*. 1981, Miller 1985, Geelen and Corman 1992). Increased secretion of VP in the aging rat could also be a consequence of impaired renal concentrating ability, with water loss and resultant hypovolaemia leading to stimulation of VP release (Miller 1985). Conversely, it is possible that increased VP secretion is a primary event, resulting from aging effects on the central nervous system and hypothalamus, leading to downregulation of renal membrane receptors for VP (Miller 1985).

Although VP secretion also increases with aging in humans (Kirkland *et al*. 1984, Lucassen *et al*. 1994), impaired renal concentrating ability has not been reported to be caused by a decrease in the renal response to VP, but rather by a decrease in glomerular filtration rate (GFR) and renal blood flow (RBF) during aging (Lewis and Alving 1938, Lindeman *et al*. 1960). It is indeed well established that GFR and RBF decrease with age in humans (Hollenberg *et al*. 1974). Furthermore, it has been demonstrated in dogs (Levinsky *et al*. 1959) as well as in humans (Levitt *et al.* 1958, Gullick and Raisz 1960) that a decline in GFR leads to a decrease in urine concentration.

In some dogs, a large intraindividual variation in urine concentration was found. Two- to 3-fold increases or decreases in Uosm often occurred within 2 hours. In these dogs, there was no relationship between urinary corticoid excretion and Uosm. A possible explanation for fluctuation in Uosm during the day could be the effect of activity on drinking behaviour. During the day drinking is associated with the activity of dogs, and water intake is larger in active dogs than in quiet dogs (O'Connor 1975, Golob *et al.* 1977), although drinking stops before the ingestion of enough water to cause a water diuresis (O'Connor 1975).

Another factor responsible for the fluctuation in Uosm during the day could be the effect of feeding on drinking behaviour. Some researchers have found a very close temporal relationship between eating and drinking (Adolph 1939, Ardisson *et al*. 1975, Fitzsimons 1979). At least 70% of the total intake of water is consumed just before, during, and immediately after meals (Fitzsimons 1979). Golob *et al*. (1977) reported that the amount of protein and carbohydrate in the meal is the major factor determining the postprandial water intake. Other reports indicate, however, that feeding and drinking evolve according to independent circadian rhythms (Ardisson *et al*. 1975, Fitzsimons 1979). In our dogs, Uosm decreased as often as it increased after a meal, so that it is unlikely that the fluctuation in Uosm during the day can be explained solely by the effect of feeding on drinking behaviour.

In dogs, an early satiation of thirst occurs during drinking before any changes are detected in plasma osmolality, plasma volume, or blood pressure as a

result of absorption of water (Adolph 1939, 1950, Thrasher *et al*. 1981). This anticipatory response protects the dog from drinking an amount of water that would exceed its physiological need (Salata *et al*. 1987). Furthermore, plasma VP concentration decreases significantly in dogs within 6 minutes after drinking, in the absence of changes in plasma composition (Thrasher *et al*. 1981, 1987). Similar findings have been reported in humans (Salata *et al*. 1987, Geelen *et al.* 1984). Stimulation of oropharyngeal receptors by the ingestion of water probably leads both to the initial inhibition of VP secretion and the temporary satiety after drinking (Thrasher *et al*. 1981, Geelen *et al.* 1984, Salata *et al*. 1987, Thrasher *et al.* 1987). Nevertheless, our observations indicate that these mechanisms do not prevent all dogs from drinking excessive amounts of water. In some dogs, drinking behaviour led to large fluctuations in Uosm, although apparently not to such a degree that the owners perceived the dogs to be polydipsic or polyuric. It may very well be that in more pronounced cases the iatrotropic threshold is surpassed, and the animals are presented to the veterinarian because of polyuria and polydipsia.

It does not seem appropriate to present reference values for Uosm and Usg in dogs, because the range found in healthy dogs is extremely wide. A low Usg in one urine sample does not exclude the possibility of finding a high Usg in another sample. Therefore, a low Usg does not automatically indicate polyuria and polydipsia of pathological origin. Whenever the suspicion of polyuria and polydipsia arises, urine samples should be collected every 2 hours to measure Uosm. Also in humans it is now recognised that Uosm should be established by repeated measurements, and that this approach may prevent further clinical evaluation (Mevorach *et al*. 1995).

It can be concluded that Uosm and Usg vary widely among dogs. In general, Uosm is lower at night than in the morning. In some dogs, Uosm varies widely during the day, with an intraindividual coefficient of variation approaching the interindividual coefficient of variation. There are no differences in Uosm values related to gender. The decrease in Uosm with age cannot be ascribed to an associated increase in corticoid production.

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Chapter 4

Polyuria and polydipsia and disturbed vasopressin release in two dogs with secondary polycythaemia

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Abstract

In dogs, secondary polycythaemia (SP) may be associated with polyuria and polydipsia (PUPD). The pathogenesis of this PUPD has not yet been explained. We hypothesised that hyperviscosity and increased blood volume in SP might affect vasopressin (VP) release, resulting in PUPD. This hypothesis was tested in two dogs with SP caused by renal neoplasia, PUPD being the main presenting problem. Osmoregulation of VP release was studied by a water deprivation test and by investigating the VP response to hypertonic saline infusion.

Water deprivation test results were consistent with an inability to produce concentrated urine despite increasing plasma osmolality. During hypertonic saline infusion, the osmotic threshold of VP release was markedly increased in both dogs, resulting in a delayed VP response to increasing plasma osmolality. The sensitivity of VP release was low normal in both dogs. We conclude that blood hyperviscosity and increased blood volume led to impaired VP release and polyuria.

Introduction

Polycythaemia is characterised by an increase in packed cell volume (PCV), red blood cell (RBC) count, and hemoglobin concentration (Brodsky 1980, Peterson and Randolph 1983). Based on its pathogenesis, polycythaemia can be classified as relative polycythaemia (RP), secondary polycythaemia (SP), or polycythaemia vera (PV).

Relative polycythaemia is the result of severe dehydration and disappears after correction of the fluid imbalance (Jain 1986, Drazner 1989, Giger 1992). Polycythaemia vera is a myeloproliferative disorder that results from proliferation of pluripotential stem cells in the bone marrow, leading to an increased red cell mass and variable increases in granulocytes and platelets (De Wolf *et al*. 1992). Secondary polycythaemia is the consequence of excessive production of erythropoietin (Epo) or other erythroid-stimulatory substances, such as androgens and adrenal steroids (Campbell 1990). Erythropoietin is produced primarily in the kidney, probably in interstitial or capillary endothelial cells. Increased production of Epo can be physiologically appropriate (i.e., the result of poor tissue oxygenation), or it can be physiologically inappropriate (Brodsky 1980, Peterson and Randolph 1983). Poor systemic tissue oxygenation may be caused by circulatory or respiratory diseases or hemoglobinopathies (Jain 1986, Drazner 1989). The most common cause of inappropriate SP in companion animal practice is the presence of space-occupying renal lesions such as cysts (Peterson and Randolph 1983, Drazner 1989), hydronephrotic lesions (Peterson and Randolph 1983, Drazner 1989), chronic pyelonephritis (Waters and Prueter 1988), or renal neoplasia (Scott and Patnaik 1972, Peterson and Zanjani 1981, Nelson *et al*. 1983, Gorse 1988, Waters and Prueter 1988, Crow *et al*. 1995). Renal tumours associated with SP in the dog include adenocarcinoma (Scott and Patnaik 1972, Peterson and Zanjani 1981, Waters and Prueter 1988, Crow *et al*. 1995), lymphosarcoma (Nelson *et al*. 1983), and fibrosarcoma (Gorse 1988). The space-occupying lesion causes local changes in renal blood flow, leading to a decrease in renal tissue oxygenation (Berk 1992, Cowgill 1992, Swinney *et al*. 1992). In addition, unregulated secretion of Epo may occur from a neoplasm (Berk 1992, Cowgill 1992, Swinney *et al*. 1992). In humans, polycythaemia also has been found to be associated with many non-renal tumours, including adrenal carcinoma, hepatoma, cerebellar hemangioblastoma, pheochromocytoma, and uterine leiomyoma (Golde *et al*. 1981, Conley 1987). In the dog, a nasal fibrosarcoma has been associated with SP (Couto *et al*. 1989).

In the veterinary literature, Epo assays have been reported to differentiate PV from SP (Jain 1986, Drazner 1989, Campbell 1990). The increased PCV and plasma Epo concentrations in dogs with SP due to renal neoplasia have decreased after removal of the neoplasm (Peterson and Zanjani 1981, Waters and Prueter

1988). In humans (Erslev and Caro 1984, Conley 1987, De Wolf *et al*. 1992) and dogs (Cook and Lothrop 1994), there is considerable overlap of Epo concentrations among patients with SP and PV, and thus Epo assays have limited diagnostic value (De Wolf *et al*. 1992, Cook and Lothrop 1994).

The clinical signs of PV and SP include erythema of mucous membranes, bleeding diatheses, and disturbances of the central nervous system (seizures, paraparesis, lethargy) (Gorse 1988, Crow *et al.* 1995). Many of these clinical manifestations are related to the increased RBC mass, which increases blood viscosity and expands blood volume (Peterson and Randolph 1983, Conley 1987). Hyperviscosity slows blood flow and expanded blood volume distends capillaries and small vessels, resulting in an increased risk of hypoxia, thrombosis, and rupture of these vessels (Peterson and Randolph 1983, Conley 1987). Another clinical sign reported in association with SP is polyuria/polydipsia (PUPD) (Scott and Patnaik 1972, Peterson and Zanjani 1981, Waters and Prueter 1988). It has been suggested that SP interferes with the ability of the kidneys to concentrate urine (Waters and Prueter 1988).

As hyperviscosity and expansion of blood volume may have consequences for the release of vasopressin (VP), we investigated the osmoregulation of VP release in two dogs with SP due to renal neoplasia by measuring the VP response to hypertonic saline infusion.

Materials and methods

The VP response to hypertonic saline was investigated by intravenous infusion of 20% NaCl for 2 hours at a rate of 0.03 ml/kg body weight/min. Samples for measurement of plasma VP concentration, collected in EDTA-coated tubes placed in ice, and for plasma osmolality (Posm) were obtained from the jugular vein at 20-min intervals. Plasma osmolality was measured by freezing point depression immediately after collection of the samples. Plasma for measurement of VP was separated by centrifugation at 4°C and stored at -20°C until assayed for VP by radioimmunoassay (Biewenga *et al*. 1991). Nomograms for the relation between Posm and plasma VP have been described (Biewenga *et al*. 1987). The slope of the regression line was used to describe the sensitivity of the osmoregulatory system and the intercept with the 5 pmol/l line provided a measure of its threshold value (Biewenga *et al*. 1987).

A water deprivation test was performed as described by Mulnix *et al.* (1976). Glomerular filtration rate (GFR) was determined from the plasma clearance of ^{99m}technetium-diethylenetriaminepenta-acetate (^{99m}Tc-DTPA) (Van den Brom

and Biewenga 1981). Renography was performed for 20 minutes after intravenous injection of ^{99m}Tc-DTPA (Biewenga and Van den Brom 1985). Red cell mass (ml/kg body weight) was computed from the dilution of radioactivity, measured in a sample taken 1 hour after intravenous injection of ^{51}Cr-labelled autologous erythrocytes (Sisson 1978).

Case reports

Dog 1

A 9½-year-old, castrated male Old English sheepdog weighing 43 kg was referred to the Department of Clinical Sciences of Companion Animals of Utrecht University because of PUPD which began suddenly 2½ months before and exercise intolerance for 6 months. According to the owner, the dog was less active and had signs of caudal weakness.

On physical examination the mucous membranes were noted to be brick red. No other abnormalities were observed. Laboratory findings included increased PCV, and slightly decreased plasma albumin concentration (Table 1). Urine specific gravity was 1.009. The diagnosis of absolute polycythaemia was confirmed by the finding of an increased red cell mass of 57 ml/kg (reference range 25-50 ml/kg). The glomerular filtration rate was normal. On ultrasonography of the abdomen, there was a hypoechoic mass approximately 15 mm in diameter in the caudal pole of the right kidney and a much larger hyperechoic mass in the cranial pole. Cytologic examination of the ultrasonography-guided biopsies of the mass in the cranial pole of the right kidney revealed poorly differentiated malignant cells, possibly of neuro-epithelial origin. There was no evidence of pulmonary metastasis on thoracic radiography.

During the water deprivation test, urine osmolality increased, but did not reach the level of normal concentrating capacity despite a marked loss of body weight (5%) and increase in Posm (from 310 to 320 mOsm/kg) (Figure 1). The VP response to hypertonic saline infusion was abnormal (Figure 2): The threshold value was 352 mOsm/kg (reference values 276-309 mOsm/kg), and the sensitivity of the VP response was 0.28 pmol/l per mOsm/kg (reference values 0.24-2.47 pmol/l per mOsm/kg). Euthanasia was performed at the owners request, but necropsy was not permitted.

Dog 2

A 9-year-old castrated male Labrador retriever weighing 37.5 kg was presented because of PUPD, lethargy, and weight loss for four weeks. The mucous

Table 1. Laboratory values of a 9.5-year-old Old English Sheepdog (dog 1), and a 9-year-old Labrador retriever (dog 2) on admission.

	Unit	Reference ranges	Dog 1	Dog 2
Packed cell volume	l/l	0.42 - 0.57	0.77	0.79
Leucocytes	10^9/l	5.9 - 13.8	7.4	7.8
Platelets	10^9/l	150 - 400	256	228
Urea	mmol/l	3.0 - 12.5	6.5	10.4
Creatinine	µmol/l	<60 + 1.2xBW	94	127
Glucose	mmol/l	3.9 - 5.0	ND	3.9
Sodium	mmol/l	141 - 149	145	147
Potassium	mmol/l	3.6 - 5.0	4.5	4.3
Calcium	mmol/l	2.2 - 3.0	2.70	2.47
Alkaline phosphatase	U/l	25 - 117	21	29
Bile acids	µmol/l	<8	ND	<8
Total protein	g/l	54 - 70	69	49
Albumin	g/l	25 - 37	23	16
Plasma osmolality	mOsm/kg	295 - 320	316	ND
Urinary total protein	g/l	0 - 0.56	0.12	2.01
Urinary corticoid/ creatinine ratio	x 10^{-6}	<10	3.7	3.1
λo		0.0162 - 0.0238	0.0213	0.0214

ND=not done; BW=body weight; λo=rate constant of fractional turnover of ^{99m}Tc-DTPA (^{99m}technetium-diethylenetriaminepenta-acetate)

membranes and skin were hyperemic and the abdomen was tense. Laboratory findings included increased PCV, decreased plasma albumin concentration, and slightly increased plasma creatinine concentration (Table 1). Urine specific gravity was 1.008 and proteinuria was present. Ultrasonography of the abdomen disclosed a mass in the caudal pole of the right kidney. There was no evidence of pulmonary lesions on thoracic radiography.

The animal was hospitalised for additional studies. Initial treatment consisted of reduction of the PCV to 51 % by three phlebotomies and replacement of 1365 ml of blood by lactated Ringer's solution. During the following days, the PCV increased again to 64 %. There were no signs of dehydration and Posm and plasma concentrations of creatinine, sodium, and potassium were within the reference range. Urine specific gravity was 1.005 and urine osmolality was 142 mOsm/kg. The VP response to a hypertonic saline infusion was abnormal (Figure 2): The threshold value was 336 mOsm/kg and the sensitivity of the response was 0.28 pmol/l per mOsm/kg. Plasma clearance of ^{99m}Tc-DTPA was slightly decreased, but the fractional turnover of ^{99m}Tc-DTPA was normal indicating

normal GFR. The renogram revealed equal excretion of ^{99m}Tc-DTPA by both kidneys.

After removal of the right kidney the dog recovered without complications. The tumour mass consisted of white lobulated, solid tissue and was well defined. A renal cell carcinoma with tubular, solid, and papillary growth patterns was observed on histological examination. By the day after surgery, the PCV had decreased to 53 % and remained at this level until the dog was discharged from the clinic one week later. Eight months postoperatively, the dog continued to do well without evidence of recurrence of the renal tumour or associated polycythaemia and PUPD.

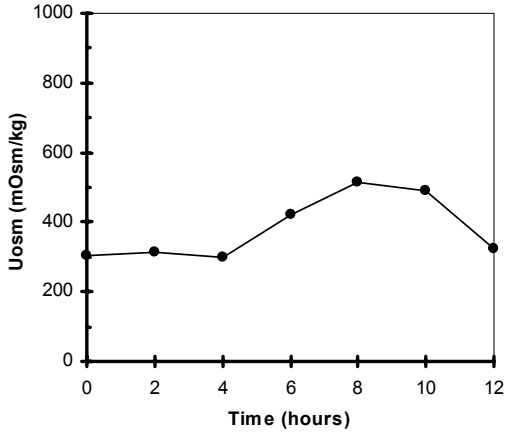

Figure 1. Urine osmolality (Uosm) during a 12-hour water deprivation test in a 9.5-year-old castrated male Old English sheepdog with polyuria and polydipsia and secondary polycythaemia due to a renal neoplasm.

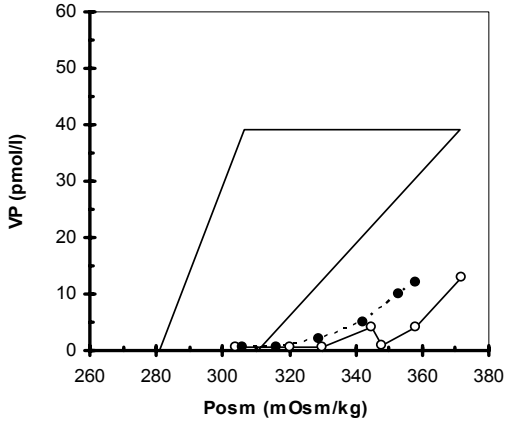

Figure 2. Relation between plasma vasopressin (VP) concentration and osmolality (Posm) in a 9-year-old castrated male Labrador retriever (dotted line) and a 9.5-year-old castrated male Old English sheepdog (uninterrupted line) with polyuria and polydipsia and secondary polycythaemia due to renal neoplasia. The outlined area represents the range of responses to infusion of hypertonic saline in 11 healthy dogs (Biewenga *et al.* 1987).

Discussion

This paper describes two dogs with SP due to renal neoplasia. In both dogs, PUPD was the main reason for the owners to seek veterinary help. In addition, there was loss of endurance. Only the condition of dog 2 required phlebotomy. Nevertheless, three days later when the additional studies were done the PCV was again abnormally high. In dog 2 red cell mass was not measured, but RP was considered unlikely because of the absence of clinical signs and laboratory features of dehydration. The remission of polycythaemia after nephrectomy provided evidence that the polycythaemia was caused by the neoplasm.

In several dogs with SP due to renal cell carcinoma or chronic pyelonephritis, the disease was associated with PUPD (Scott and Patnaik 1972, Peterson and Zanjani 1981, Waters and Prueter 1988). Although PUPD may be a clinical feature in renal adenocarcinoma not associated with polycythaemia (Goldschmidt 1984), it also has been reported as a consequence of PV in the dog (Meyer *et al*. 1993) and the cat (Swinney *et al*. 1992). Therefore, it is likely that polycythaemia per se causes PUPD.

In the water deprivation test performed in dog 1, the inability to produce concentrated urine provided indirect evidence for impaired osmoregulation of VP release and/or resistance at the level of the kidney. During hypertonic saline infusion, the osmotic threshold of VP release was markedly increased in both dogs, resulting in a delayed VP response to increasing Posm. The sensitivity of the VP response was just above the lower limit of the reference range in both dogs.

The hallmarks of polycythaemia are blood volume expansion and hyperviscosity (Conley 1987). Increased blood volume may lead to atrial stretch and increased release of atrial natriuretic peptide (ANP) (Stokhof *et al*. 1994), which has been observed in two dogs with PV in our Department (unpublished data). This may result in PUPD, because ANP inhibits the water permeability response to VP in the renal collecting ducts (Dillingham and Anderson 1986). Furthermore, ANP inhibits basal as well as KCl-stimulated VP release (Obana *et al*. 1985, Iitake *et al*. 1986, Lee *et al*. 1987), which may explain the impaired VP release during hypertonic saline infusion in our dogs.

Vasopressin release is controlled mainly by hypothalamic osmoreceptors and atrial and carotid bifurcation baroreceptors in order to maintain osmotic and fluid balance (Reeves and Andreoli 1992). The PUPD in the two dogs described here was very likely a consequence of delayed VP release, resulting in a decrease in permeability of the renal collecting ducts to water. Hyperviscosity and increased blood volume may have stimulated baroreceptors which consequently caused the delay in VP release. In humans (Robertson and Athar 1976) as well as in dogs (Quillen and Cowley 1983), hypervolaemia impairs the VP response to hypertonic saline infusion. In dogs with experimentally induced hypervolaemia the threshold

of VP release is increased and the sensitivity of the response is decreased (Quillen and Cowley 1983), similar to what was observed in the two dogs with SP described here. In conclusion, our observations in two dogs with SP indicate that the associated polyuria was at least partially the result of impaired VP release.

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Chapter 5

Disturbed vasopressin release in four dogs with so-called primary polydipsia

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Abstract

Primary polydipsia is characterised by a marked increase in water intake and secondary polyuria, and in dogs often is described as a behavioural problem or a psychological disorder. We describe 4 dogs with primary polydipsia, diagnosed on the basis of a water deprivation test, in which further examination included serial measurements of urine osmolality (Uosm) and plasma vasopressin (VP) measurements during water deprivation and hypertonic saline infusion.

The dogs, ranging in age from 4 months to 4 years, all were presented for evaluation of polyuria and polydipsia. Physical examination, routine blood chemistry and urinalysis disclosed no specific cause for the polyuria. During serial measurements Uosm spontaneously reached high concentrations in 2 dogs, whereas in the other 2 dogs Uosm also fluctuated but on no occasion exceeded 1000 mOsm/kg. Primary polydipsia was diagnosed when (1) Uosm exceeded 1000 mOsm/kg at the end of the water deprivation test and (2) plasma osmolality did not exceed the upper limit of the reference range during testing. During water deprivation, plasma VP concentrations remained relatively low. The VP response to hypertonic saline infusion was abnormal, with an increased threshold value in 3 dogs, an increased sensitivity in 2 dogs, and an exaggerated response in 1 dog.

It is concluded that some dogs fulfilling current criteria for primary polydipsia produce concentrated urine spontaneously throughout the day in a pattern similar to what has been observed in healthy pet dogs. This finding can be regarded as diagnostic and precludes the need for a water deprivation test. During water deprivation, all 4 dogs produced highly concentrated urine in the face of low basal plasma VP concentrations. The observed abnormal VP release in response to hypertonic stimulation may be interpreted as a primary disturbance in the regulation of VP secretion, although it might also be the result of overhydration caused by a primary abnormality in drinking behaviour.

Introduction

Polyuria and polydipsia (PUPD) are associated with a wide variety of endocrine and metabolic disturbances (Nichols 1992). In companion animal medicine, polyuria generally is defined as daily urine output of >50 ml/kg body weight per day, and polydipsia as fluid intake exceeding 100 ml/kg body weight per day (Dunn 1990, Meric 1995, Feldman and Nelson 1996).

Water diuresis is expected when there is a disturbance in urine concentration. Urinary concentrating capacity depends on the ability of hypothalamic osmoreceptors to respond to changes in plasma osmolality (Posm), the ability of the atrial and carotid bifurcation baroreceptors to respond to changes in blood pressure or blood volume, and the release of the antidiuretic hormone vasopressin (VP) via the hypothalamic-neurohypophyseal axis. In addition, medullary hypertonicity must be generated and maintained, and there must be an adequate number of functional nephrons with an appropriate response to VP (Chew and DiBartola 1989, Reeves and Andreoli 1992). When one or more of these conditions is not fulfilled, polyuria ensues and is compensated by secondary polydipsia.

On the other hand, it is possible that the polyuria is secondary to compulsive water drinking. Primary polydipsia has been defined as a marked increase in water intake which cannot be explained as a compensatory mechanism for excessive fluid loss (Dunn 1990). The resulting decrease in Posm causes suppression of endogenous VP release, and consequently secondary polyuria (Finco *et al*. 1979). The results of physical examination and routine blood and urine examinations are usually unremarkable, except for a marginally low Posm and hypostenuria (Bovee *et al*. 1987, Feldman and Nelson 1996). According to descriptions of primary polydipsia in textbooks, definitive diagnosis is based on a water deprivation test in which the patient is shown to have urinary concentrating ability comparable to that of healthy subjects (Hardy and Osborne 1980, Bush 1988, Dunn 1990, Nichols 1992, Meric 1995, Feldman and Nelson 1996).

In humans, primary polydipsia may result from a defect in the thirst center (dipsogenic polydipsia) or may be associated with mental illness (psychogenic polydipsia) (Robertson 1987, 1988). In dogs, primary polydipsia has been described both as a psychological disorder and a behavioural problem (Feldman and Nelson 1989, Nichols 1992, Meric 1995). Despite numerous descriptions in textbooks of primary polydipsia in dogs, there are only 2 reports of documented cases (Mulnix *et al*. 1976, Biewenga *et al*. 1987).

In 1959, Barlow and De Wardener suggested that humans with primary polydipsia may have a defect in neurohypophyseal function causing impaired VP release. This suggestion was supported by Díes *et al.* (1961) who reported that some cases of primary polydipsia may represent a reversible type of diabetes

insipidus. Zerbe and Robertson (1981) found subnormal VP release during both water deprivation and hypertonic stimulation in several human patients with primary polydipsia. They concluded that these patients suffered from partial diabetes insipidus. Downward resetting of the osmoreceptors (Goldman *et al*. 1988), downregulation of VP release in response to hypertonicity (Moses and Clayton 1993), impaired VP secretion to non-osmotic stimulation (Baylis *et al*. 1981), and abnormal osmotic and non-osmotic thirst regulation (Thompson *et al*. 1991) also have been claimed to be present in humans with primary polydipsia.

Reports on VP secretion in dogs with primary polydipsia are somewhat contradictory. Feldman and Nelson (1996) state that VP secretion and renal responsiveness to VP are normal in dogs with primary polydipsia, but they presented no data to substantiate this statement. Results of indirect approaches not involving VP measurements by Mulnix *et al*. (1976) also suggested the presence of a normal hypothalamic-neurohypophyseal system in dogs with primary polydipsia. However, Biewenga *et al*. (1987) described 6 dogs in which primary polydipsia was found to be associated with impaired VP release during hypertonic saline infusion.

In an attempt to determine whether or not disturbed VP release in response to osmotic and non-osmotic stimulation occurs in dogs with primary polydipsia, we measured plasma VP concentrations during both water deprivation and hypertonic saline infusion in 4 dogs with primary polydipsia selected according to criteria not involving VP measurements.

Materials and methods

Routine laboratory examination

Routine blood examination included measurement of packed cell volume, total and differential leukocyte counts, and the following measurements in plasma: Posm and urea, creatinine, glucose, sodium (Na), potassium, calcium, phosphate, alkaline phosphatase, bile acids and thyroxine concentrations. In 2 morning urine samples collected at home by the owners, the corticoid/creatinine (C/C) ratio was determined as described previously (Stolp *et al.* 1983, Rijnberk *et al.* 1988a). Urinalysis included determination of specific gravity (Usg) by refractometry, pH, concentrations of total protein, glucose, and hemoglobin, and sediment examination.

Analytical methods

Plasma osmolality (reference range 295 - 320 mOsm/kg) and Uosm were determined in duplicate by freezing point depression (Cryostatic Osmometer 030, Gonotec GmbH, Berlin, Germany). Plasma VP concentration (reference range 1 - 6 pmol/l) was measured with an antibody against arginine-vasopressin and the method of the kit as purchased from Nichols Institute (Wijchen, The Netherlands), and validated for the dog (Hellebrekers *et al*. 1987, Biewenga *et al.* 1991). The detection limit was 1 pmol/l and the intra- and interassay coefficients of variation were 6 and 10%, respectively.

Serial measurements of Uosm

Owners collected urine at 2-hour intervals during the day and at 4-hour intervals during the night for a period of 24 hours. In dog 3, urine samples were not collected at night. Urine osmolality was determined in all urine samples.

Water deprivation test

A water deprivation test was performed after a period of 12 hours in which food was withheld but water remained available ad libitum (Mulnix *et al.* 1976). At the start of the test, water was removed and the dog was weighed. A blood sample was drawn from the jugular vein for determination of Posm and plasma VP and Na concentrations. The bladder was emptied by catheterisation and Uosm was determined. Every 2 hours, the dog was weighed, the bladder was emptied by catheterisation, and blood was collected. The water deprivation test was concluded when (1) body weight loss was >5% or (2) Uosm increased to at least 1000 mOsm/kg. The diagnosis of primary polydipsia was made when Uosm exceeded 1000 mOsm/kg during water deprivation while Posm did not exceed the upper limit of the reference range.

Hypertonic saline infusion

Hypertonic saline infusion was performed at least 2 days after the water deprivation test. Food was withheld for 12 hours but water was available ad libitum until the start of the test. The VP response to hypertonic saline was investigated by intravenous infusion of 20% NaCl for 2 hours at a rate of 0.03 ml per kg body weight per minute. Samples for measurement of plasma VP concentration, collected in EDTA-coated tubes placed in ice, and for Posm were obtained from the jugular vein at 20-min intervals. Plasma osmolality was measured immediately after collection of samples. Plasma for measurement of VP was separated by centrifugation at 4°C and was stored at -20°C until assayed for VP. Nomograms for the relation between Posm and plasma VP concentration have been described previously (Biewenga *et al*. 1987). Briefly, reference values for basal Posm and plasma VP were obtained from the calculation of the inner 90% confidence

intervals of the 2.5 and 97.5 percentiles of values of 25 healthy dogs, aged 2 - 12 years (8 Beagles, 4 Bouviers, 1 Boxer and 12 mongrels). In 11 of these dogs, VP measurements were performed during osmotic stimulation with hypertonic saline. The slope of the regression line was used to describe the sensitivity of the osmoregulatory system (reference range 0.24 - 2.47 pmol/l per mOsm/kg) and the intercept with the 5 pmol/l line provided a measure of its threshold value (reference range 276 - 309 mOsm/kg) (Biewenga *et al.* 1987). The ranges for the values observed in the 11 healthy dogs are presented in the figure depicting the relation of VP to Posm (Figure 3).

Results

Dog 1

A 4-month-old male Jack Russell terrier weighing 5.5 kg was admitted to the Department of Clinical Sciences of Companion Animals of Utrecht University for evaluation of PUPD and urinating in the house for a few days. Physical examination and routine blood and urine examination disclosed no abnormalities except for a Usg of 1.010.

During serial measurements of Uosm, the urine was well concentrated early in the morning, in the evening and at night, but not during the day (Figure 1). During the water deprivation test, Uosm was >1000 mOsm/kg within 4 hours (Figure 2). The initial values of both Posm (299 mOsm/kg) and plasma Na (143 mmol/l, reference range 141 - 149 mmol/l) were at the lower limits of their respective reference ranges. A small weight loss (2.2%) and slight increases in Posm and plasma Na concentrations occurred, but plasma VP concentration remained low during water deprivation. The VP response to hypertonic saline infusion was abnormal, with extremely high plasma VP concentrations. The osmotic threshold for VP secretion was normal (306 mOsm/kg), whereas the sensitivity of the VP response was increased (6.4 pmol/l per mOsm/kg) (Figure 3, upper panel).

Eight months later, the dog's drinking behaviour had returned to normal according to the owners. The polyuria and urinating in the house had ceased. However, in 2 more urine samples collected by the owners, Uosm was 1804 mOsm/kg in the morning and 345 mOsm/kg in the evening. At the time of writing, the dog was 3 years of age and its drinking behaviour and micturition pattern were virtually unchanged, and the owners had learned to live with this peculiarity.

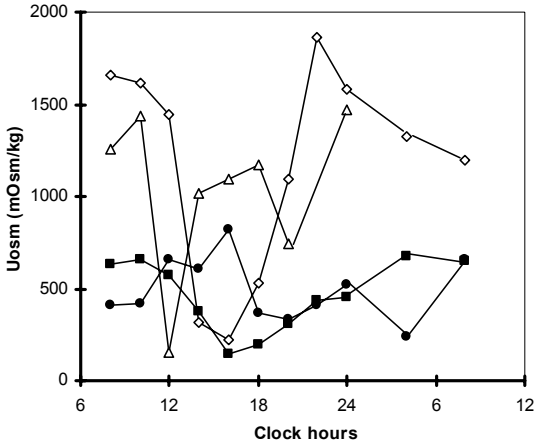


Figure 1. Serial measurements of urine osmolality (Uosm) in 4 dogs with polyuria and polydipsia: dog 1 (open diamond), dog 2 (closed circle), dog 3 (open triangle) and dog 4 (closed square).

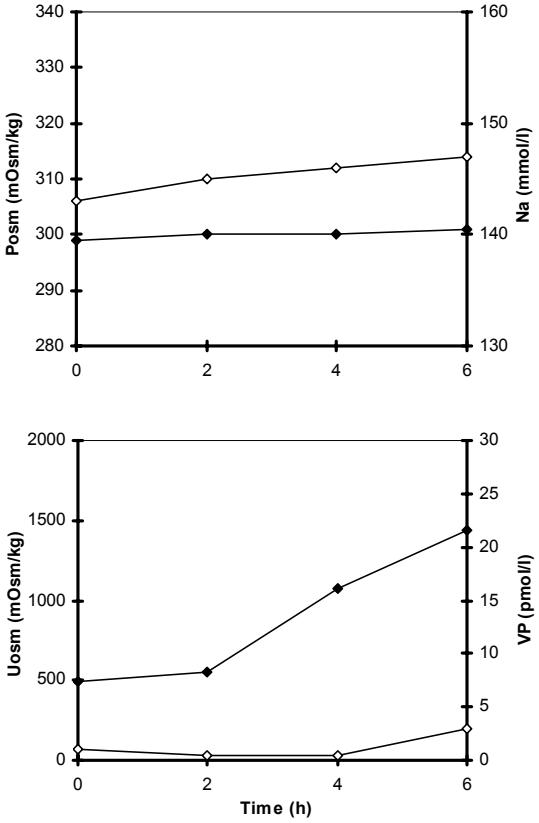
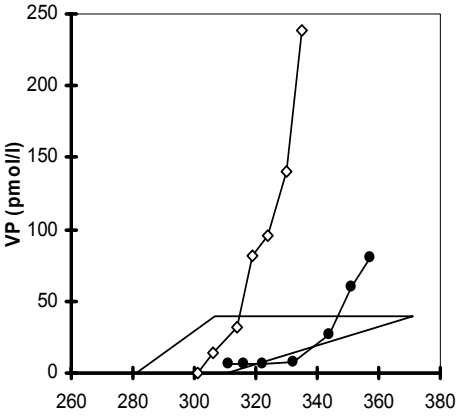
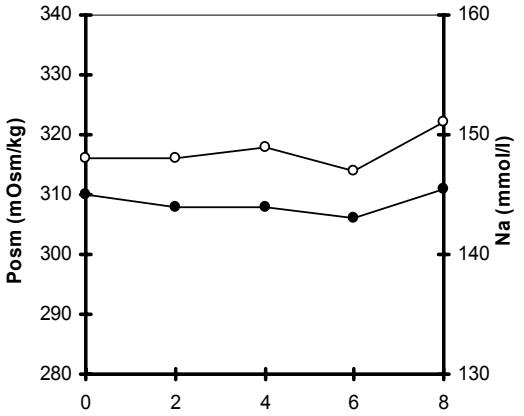
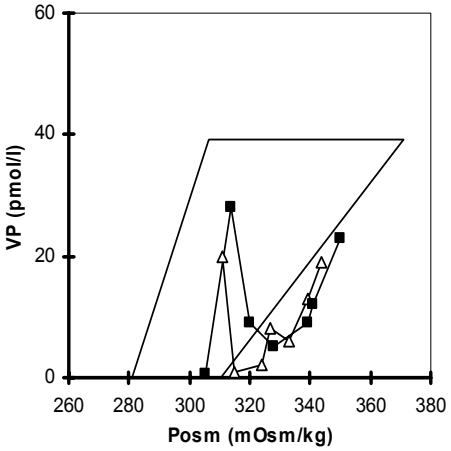


Figure 2. Results of a water deprivation test in a 4-month-old Jack Russell terrier with polyuria and polydipsia (dog 1). Upper panel: plasma osmolality (Posm, closed diamond) and plasma sodium concentration (Na, open diamond). Lower panel: urine osmolality (Uosm, closed diamond) and plasma vasopressin concentration (VP, open diamond).







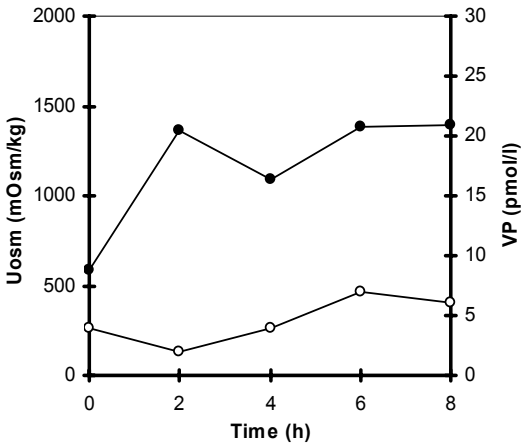


Figure 3. Relation between plasma vasopressin (VP) concentration and osmolality (Posm) in 4 dogs with polyuria and polydipsia. Upper panel: dog 1 (open diamond) and dog 2 (closed circle). Lower panel: dog 3 (open triangle) and dog 4 (closed square). The outlined area represents the range of responses to infusion of hypertonic saline in 11 healthy dogs (Biewenga *et al*. 1987).

Figure 4. Results of a water deprivation test in a 15-month-old Bernese mountain dog with polyuria and polydipsia (dog 2). Upper panel: plasma osmolality (Posm, closed circle) and plasma sodium concentration (Na, open circle). Lower panel: urine osmolality (Uosm, closed circle) and plasma vasopressin concentration (VP, open circle).

Dog 2

A 15-month-old female Bernese mountain dog weighing 34.0 kg was presented for evaluation of PUPD and urinating in the house for 4 weeks. Physical examination disclosed no abnormalities. Results of routine laboratory examinations were within reference ranges except for a low Usg (1.007). During serial measurements of Uosm, urine concentration fluctuated at low values of osmolality (Figure 1). After only 2 hours of water deprivation, Uosm was >1000 mOsm/kg (Figure 4). A small loss of weight (2.2%) occurred and Posm and plasma Na and VP concentrations remained relatively stable at the upper limit of their reference ranges. The VP response during hypertonic saline infusion was abnormal (Figure 3, upper panel). The threshold value was abnormally high at 333 mOsm/kg, and the sensitivity was abnormally high at 2.93 pmol/l per mOsm/kg.

Six months later, the owners reported that all problems had disappeared. Their repeated attempts to collect urine samples were unsuccessful.

Dog 3

A 1.5-year-old male beagle dog weighing 9.0 kg was presented for evaluation of PUPD. The dog was a laboratory dog used for stress and behavioural research, but during the examinations for PUPD no other tests were performed. Physical examination disclosed no abnormalities. Routine laboratory examinations disclosed a low plasma Na concentration (139 mmol/l), a low Posm (294 mOsm/kg), and a low Usg (1.008). During the day large fluctuations in Uosm occurred (Figure 1). During the water deprivation test, the dog produced well-concentrated urine after 6 hours (Figure 5). A slight loss of weight (2.8%) occurred, but very small changes in Posm or plasma Na concentrations were detected, both of which were at the lower limit of their reference ranges. After a high initial value (16 pmol/l), plasma VP concentration remained below the limit of detection during the water deprivation test. The VP response to hypertonic saline infusion was abnormal (Figure 3, lower panel). The starting value (20 pmol/l) was abnormally high and an increased threshold value (325 mOsm/kg) was detected, whereas sensitivity was normal (0.60 pmol/l per mOsm/kg).

Dog 4

A 4-year-old female Rottweiler dog weighing 42.5 kg was referred for evaluation of PUPD. Physical examination and routine laboratory examination disclosed no abnormalities except for a low Usg (1.015). During the day, Uosm was very low (Figure 1). Urine was more concentrated at night and early in the morning, but high Uosm values were not detected. During the water deprivation test, Uosm increased steadily, and after 6 hours time a value >1000 mOsm/kg was obtained (Figure 6). The loss of weight was 3% and there was a small increase in Posm, but plasma Na concentration changed very little. Both Posm and plasma Na

concentration were at the upper limit of their reference ranges. Plasma VP concentration remained below the level of detection. The VP response to hypertonic saline infusion was abnormal (Figure 3, lower panel). The plasma VP concentration (28 pmol/l) was high before the gradual rise in response to the increasing hypertonicity. The threshold value was abnormally high (331 mOsm/kg), whereas sensitivity was normal (0.81 pmol/l per mOsm/kg).

Three months later, the owners reported that the situation was unchanged. In 2 evening urine samples, Uosm was 1014 and 1365 mOsm/kg, and in 2 morning urine samples Uosm was 552 and 648 mOsm/kg.

Discussion

In dogs 1 and 3, Uosm reached high values spontaneously during serial measurements. In dogs 2 and 4, Uosm also fluctuated, but on no occasion exceeded 1000 mOsm/kg. The large fluctuations in Uosm observed during serial determinations in dogs 1 and 3 were very similar to those found in some healthy dogs without PUPD (Van Vonderen *et al.* 1997). Apparently, water intake in dogs 1 and 3 exceeded the variation observed in healthy dogs to such an extent that the owners sought veterinary help. These observations also indicate that a water deprivation test is not needed in all polyuric dogs. When pronounced fluctuations are found in Uosm during serial measurents and some Uosm values are >1000 mOsm/kg, the diagnosis of primary polydipsia seems justified.

Basal Posm and plasma Na concentration were subnormal or at the lower limit of the reference ranges in dogs 1 and 3, whereas in dogs 2 and 4 Posm and plasma Na were at the upper limit of normal. This observation suggests that dogs diagnosed as having primary polydipsia may comprise two groups, as has been reported for humans with primary polydipsia: polydipsic patients with and without hyponatraemia (Goldman *et al.* 1996).

During water deprivation, both plasma VP concentration and Posm are known to increase markedly in healthy dogs (Szczepanska-Sadowska *et al.* 1983, Wade *et al.* 1983). According to Reeves and Andreoli (1992), a 2% increase in Posm or a 10% decrease in effective circulating volume can cause stimulation of osmo- and baroreceptors, respectively, leading to detectable increases in VP release. In dog 1, plasma VP concentration was low to undetectable until there was a slight increase at the end of the period of water deprivation. This response appeared to be the result of increases in Posm and plasma Na concentration, although the values remained within the reference range. In dog 2, plasma VP concentration remained around the upper limit of the reference range during water deprivation, which agrees with the relatively high Posm and plasma Na concentration in this dog. This

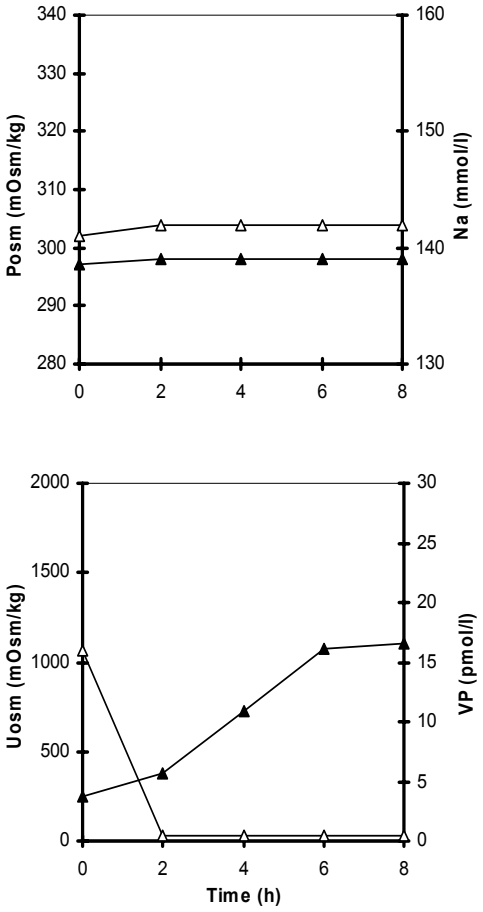


Figure 5. Results of a water deprivation test in a 1.5-year-old beagle dog with polyuria and polydipsia (dog 3). Upper panel: plasma osmolality (Posm, closed triangle) and plasma sodium concentration (Na, open triangle). Lower panel: urine osmolality (Uosm, closed triangle) and plasma vasopressin concentration (VP, open triangle).

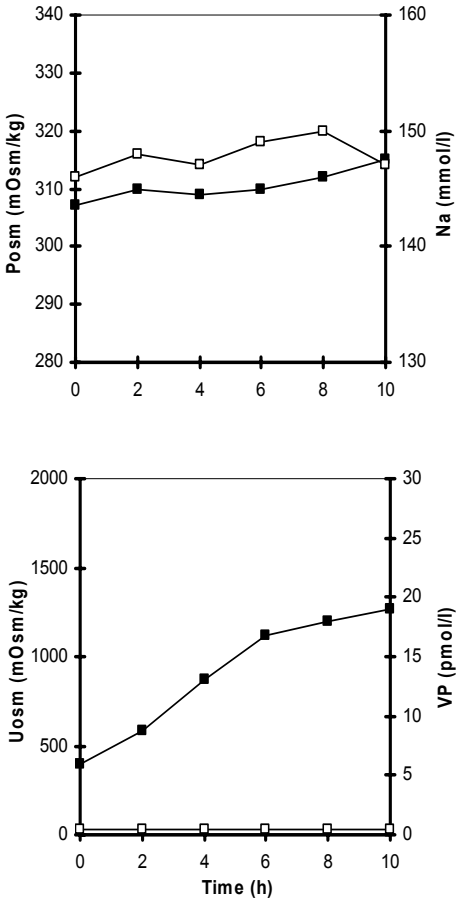


Figure 6. Results of a water deprivation test in a 4-year-old Rottweiler with polyuria and polydipsia (dog 4). Upper panel: plasma osmolality (Posm, closed square) and plasma sodium concentration (Na, open square). Lower panel: urine osmolality (Uosm, closed square) and plasma vasopressin concentration (VP, open square).

observation suggests some peripheral resistance to the effect of VP, although water deprivation rapidly led to an increase in Uosm. Apparently in dogs 3 and 4, the changes in body weight and Posm during water deprivation were not sufficient to

cause measurable VP concentrations. Despite the low plasma VP concentrations, Uosm increased to >1000 mOsm/kg in all dogs, which supports the findings of Hellebrekers *et al.* (1989) that production of highly concentrated urine can occur with relatively low plasma VP concentrations (<5 pmol/l).

Current criteria for the diagnosis of primary polydipsia do not seem to encompass a uniform syndrome. There is heterogeneity not only in basal Posm and plasma Na concentration, but also in the course of Uosm throughout the day and Posm and plasma Na concentration during water deprivation. Dogs 1 and 3 both reached Uosm values of 1500 mOsm/kg or more spontaneously during serial measurements, whereas during water deprivation Posm and plasma Na concentrations remained at the lower limit or in the middle of their reference ranges. In dogs 2 and 4, however, Uosm did not exceed 1000 mOsm/kg during serial measurements, whereas during water deprivation both Posm and plasma Na concentration were at the upper limit of their reference ranges. Also, the VP response to hypertonic saline infusion was not uniform, an increased threshold value was found in 3 dogs, an increased sensitivity in 2 dogs, and an exaggerated response in 1 dog.

As mentioned above, plasma VP concentrations were low or unmeasurable during water deprivation in all dogs, but were apparently still sufficient to result in an increase in Uosm to >1000 mOsm/kg. In contrast, the osmotic threshold for VP release calculated during hypertonic saline infusion was increased in dogs 2-4, which suggests that insufficient VP concentrations occurred at the Posm values reached during water deprivation. A delayed VP response to osmotic stimulation therefore was combined with an adequate VP concentration for urinary concentration during water deprivation in these dogs, suggesting adequate response only to the combined action of the osmo- and baroreceptors.

The abnormal VP response to stimulation of the osmoreceptors may indicate a primary disturbance in osmoreceptor function and regulation of VP secretion. Polyuria and polydipsia thus could be a result of impaired VP release to osmotic stimulation, as demonstrated in the dogs with an increased threshold value during hypertonic saline infusion. Conversely, chronic overhydration might downregulate VP release in response to hypertonicity (Moses and Clayton 1993). In this situation polydipsia is primary and leads to decreases in Posm and consequently to low or absent VP release. Moses and Clayton (1993) have reported decreased sensitivity and an increased threshold value for the VP response to hypertonic saline infusion in humans with primary polydipsia. These observations in humans are supported by other studies in humans (Robertson and Athar 1976) and by studies in dogs (Quillen and Cowley 1983), in which it was found that hypervolaemia or hypertension lead to a shift in the relationship of plasma VP concentration to Posm. This phenomenon may be an explanation for the increased threshold value found in 3 of the dogs described here.

Another explanation for the observations in dogs 1 and 3 could be a mild form of the syndrome of inappropriate VP secretion (SIADH). In both dogs, Posm and plasma Na concentrations were relatively low, which in SIADH is caused by persistent VP release in the absence of hypovolaemia or hypotension (Rijnberk *et al.* 1988b). In dog 1, the VP response to osmotic stimulation was extremely high, whereas in dogs 3 and 4 the initial VP concentrations during the hypertonic saline test were high, as was the initial result in the water deprivation test of dog 3. The latter finding suggests bursts of VP release unrelated to an osmotic stimulus, as has been observed in SIADH (Rijnberk *et al.* 1988b).

As already mentioned above, in dogs 2 and 4 the situation was different. Their basal Posm and plasma Na concentrations were not low. During water deprivation, their Posm and plasma Na concentrations tended to exceed the reference ranges. The responsiveness of VP secretion to hypertonic saline stimulation was delayed, although in dog 4 early in the test, VP concentration was high, apparently out of proportion to the rise in Posm. The latter may indicate occasional erratic VP release, as has been observed in humans with primary polydipsia (Zerbe and Robertson 1981). However, in healthy dogs the linear relationship of plasma VP to Posm can be irregular in some individuals (Meij *et al.* 1997). This observation might be a reflection of the pulsatile character of VP release (Redekopp *et al.* 1986, Livesey *et al.* 1988).

We have demonstrated that dogs fulfilling the commonly accepted criteria of primary polydipsia may exhibit abnormalities of VP release. These abnormalities may include episodes of hypersecretion as well as delayed responses to plasma hypertonicity. It is not clear whether these changes in VP release are the result or the cause of PUPD.

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Chapter 6

Vasopressin response to osmotic stimulation in 18 young dogs with polyuria and polydipsia: Vasopressin responsiveness to hypertonicity still the "gold standard"?

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Submitted

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Abstract

Common disorders of water homeostasis leading to polyuria include a variety of endocrine, metabolic and renal disturbances. After exclusion of most of these conditions, there may remain the diagnostic dilemma of differentiating between central diabetes insipidus, primary polydipsia, and nephrogenic abnormalities. Here, we report on 18 young dogs with polyuria, in most cases since puppyhood. The conditions were categorised according to the plasma vasopressin (VP) response to hypertonicity.

The VP response to osmotic stimulation was tested by intravenous infusion (0.03 ml per kg body weight) of 20% NaCl for 2 hours. The slope of the regression line was used to describe the sensitivity of the osmoregulatory system and the intercept with the 5 pmol/l line provided a measure of the threshold value. In all dogs the VP response was abnormal. Three categories could be distinguished: (a) a hyperresponse (n=3), (b) a hyporesponse (n=4), and (c) a non-linear response with high VP values unrelated to increases in plasma osmolality (n=11).

When these categories were considered together with the results of other diagnostic procedures (serial measurements of urine osmolality, water deprivation, and response to desmopressin administration) it appeared that the VP response to hypertonicity did not consistently distinguish between different clinical entities. In the 9 dogs with variations in urine osmolality compatible with primary polydipsia, hyper-, hypo-, and non-linear responses were observed. The VP peaks of the 11 dogs with non-linear responses might represent the erratic secretory bursts known to occur in the syndrome of inappropriate VP release. However, the early peaks in particular might also reflect the pulsatile release pattern of VP, which may be either physiological or induced by the hypertonicity.

The present data question the generally accepted notion that VP measurements during hypertonic saline infusion form the “gold standard” for the diagnostic interpretation of polyuria. There is a need for in-depth studies of the peripheral reflection in plasma of the pulsatile VP release in healthy and polyuric individuals, with and without provocation.

Introduction

In healthy individuals, water homeostasis is accurately controlled, so that plasma osmolality (Posm) and its principal determinant, plasma sodium (Na), are maintained within a narrow range. This control is achieved by close integration of the antidiuretic action of vasopressin (VP), which regulates water excretion, and the sensation of thirst, which governs water intake (Robertson 1984, McKenna and Thompson 1998). Disturbances of the secretion and/or action of VP, or of the regulation of thirst and drinking behaviour, can cause profound abnormalities in Na and water homeostasis (McKenna and Thompson 1998).

In humans, the capacity to concentrate urine develops progressively during infancy, and the adult capacity is reached at approximately 18 months of age (Poláček *et al.* 1965). In rats, urinary concentrating capacity increases in parallel with the expression of aquaporin-2, the VP-dependent renal water channel and reaches adult levels between 4 and 6 weeks of age (Yasui *et al.* 1996). It has been postulated that a low expression and/or deficiency of aquaporin-2 is a factor underlying the low urinary concentrating capacity of infants (Yasui *et al.* 1996, Robertson 2001). Also in dogs there is a gradual increase in concentrating ability during infancy (Horster and Valtin 1971).

Polyuria and polydipsia (PUPD) occur in a wide variety of endocrine and metabolic disorders, and may result from either water diuresis or solute diuresis (Nichols 1992). Polyuria in infancy and childhood usually indicates an underlying neurological, renal or metabolic disorder (Leung *et al.* 1991, Cheetham and Baylis 2002). Primary polydipsia is a less common disorder, but may be more prevalent in young children than previously recognised (Joshi *et al.* 1987, Horev and Cohen 1994, Matsumoto *et al.* 2000, Cheetham and Baylis 2002). It is not always easy to distinguish between central diabetes insipidus, primary polydipsia and polyuria of renal origin (nephrogenic diabetes insipidus) as causes of polyuria in children (Cheetham and Baylis 2002). The most powerful diagnostic tool for differentiation of these conditions is the hypertonic saline infusion test, with measurement of Posm and plasma VP concentration (Diederich *et al.* 2001), although the interpretation of the test results may pose problems (Moses and Clayton 1993).

In this context, the dog deserves attention because many polyuric syndromes are known to occur in this species. In puppies these may include conditions such as hepatoencephalopathy due to a congenital portosytemic shunt and congenital renal dysplasia (Meric 1995, Lees 1996, Sterczer *et al.* 1998, Greco 2001). Following exclusion of these conditions there may remain the differential diagnostic dilemma, similar to that described above for children, i.e., the distinction between central diabetes insipidus, primary polydipsia and nephrogenic abnormalities (Nichols 1992, Belshaw 1995, Greco 2001). There are no reports documenting these conditions with VP measurements in a series of polyuric young

dogs. In a preliminary report on four cases we suggested that what is called primary polydipsia may comprise several conditions, including hyperresponsiveness of VP release (Van Vonderen *et al.* 1999).

In this report, we describe our observations in 18 young dogs presented with polyuria, in most cases since puppyhood. The dogs were categorised according to their VP response to hypertonicity. Apart from this test, the protocol included serial measurements of urine osmolality (Uosm) during ad libitum water intake with and without desmopressin administration, and a water deprivation test.

Materials and methods

Dogs

Thirteen male and 5 female (2 spayed) dogs with PUPD, ranging in age from 3 to 32 months (median: 8 months), were studied. Three mongrel dogs and 15 pure-bred dogs comprising 12 different breeds were included. Dogs were included when the history, physical examination and routine laboratory examination did not adequately explain the PUPD.

In 13 dogs the owners had noticed the PUPD at the first arrival of their pet at 8-10 weeks of age, and in dog 4 at 8 months of age. In dogs 1, 3 and 7, the PUPD was first noticed at 14, 6, and 8.5 months, respectively. In dog 13, a laboratory dog, the onset of the PUPD was unknown. The owners of 8 dogs also reported in-house micturition.

In all dogs physical examination revealed no abnormalities. Results of routine blood and urinary examination were unremarkable in 15 dogs, except for Uosm, which was low in all cases. Plasma Na concentrations in dog 5 (135 mmol/l), dog 12 (136 mmol/l) and dog 13 (139 mmol/l) were below the reference range (141-149 mmol/l), as well as Posm values (dog 5: 281 mOsm/kg; dog 12: 293 mOsm/kg; dog 13: 294 mOsm/kg; reference range 295-320 mOsm/kg).

Serial measurements of Uosm

In 14 dogs serial measurements of Uosm, at 2-hour intervals during the day and at 4-hour intervals at night for 24 hours, were performed during ad libitum water intake (Van Vonderen *et al.* 1997). Subsequently, seven dogs were treated with desmopressin (Minrin^{R}, Ferring B.V., Hoofddorp, The Netherlands), 1 drop at 8-hour intervals in the conjunctival sac for 4 days, and serial measurements of Uosm were repeated on the fourth day. The variation in Uosm was judged by determination of the factor between the highest and the lowest Uosm. The response to desmopressin was expressed as the absolute increase and the percentage increase

in mean Uosm. In 4 dogs Uosm was measured in a single urine sample before and after desmopressin administration, and in 3 dogs the response to desmopressin was tested at the end of the water deprivation test. The response to desmopressin was classified into 3 categories, namely, small (<25% increase in Uosm), medium (25-75% increase), and large (>75% increase).

Water deprivation test

A water deprivation test was performed in all dogs, as described previously by Mulnix *et al.* (1976). Briefly, after an overnight fast the water bowl was removed, the dog was weighed, and basal urine and blood samples were collected for the determination of Uosm, Posm, and plasma VP and Na concentrations. This protocol was repeated every 2 hours until Uosm had increased to at least 1000 mOsm/kg or had reached a plateau of 3 similar values. The water deprivation test was also terminated when body weight loss was >5%. Maximum urinary concentrating ability was divided into 4 categories: low (<500 mOsm/kg), low-to-medium (500 to 750 mOsm/kg), medium-to-high (>750 to 1000 mOsm/kg), and high (>1000 mOsm/kg).

Hypertonic saline infusion

The VP response to osmotic stimulation was tested in all dogs by intravenous infusion of 20% NaCl for 2 hours at a rate of 0.03 ml per kg body weight per minute (Biewenga *et al.* 1987). Blood samples for the measurement of plasma VP concentration, collected in EDTA-coated tubes pre-chilled in ice, and for Posm were obtained from the jugular vein at 20-min intervals. Plasma osmolality was measured immediately after the collection of samples. Plasma for measurement of VP was separated by centrifugation at 4°C and was stored at -20°C until assayed. Nomograms for the relation between Posm and plasma VP have been described previously (Biewenga *et al.* 1987). The slope of the regression line was used to describe the sensitivity of the osmoregulatory system (reference range 0.24-2.47 pmol/l per mOsm/kg), and the intercept with the 5 pmol/l line provided a measure of its threshold value (reference range 276-309 mOsm/kg). If the correlation coefficient of the regression line was less than 0.8, VP peaks were excluded from the regression analysis, and the VP response was termed non-linear (dogs 8-18).

Vasopressin assay

Vasopressin was extracted from plasma by the addition of 5.2 ml 96% ethanol (4°C) to 0.8 ml plasma, and incubation by end-over-end rotation for 30 min at 4°C. After centrifugation for 30 min at 5000xg and 4°C, the supernatant was collected and dried overnight using a speedvac vacuum concentrator. Extracts were dissolved in 0.8 ml assay buffer. The recovery of VP amounted to a mean value of

75 ± 1%. Vasopressin concentrations were measured by radioimmunoassay (Nichols Institute, Wijchen, The Netherlands), validated for the dog by measuring a serial dilution of an extract of canine plasma with a high VP concentration that resulted in a curve parallel to the standard curve. The detection limit was 1 pmol/l. The intra-assay coefficient of variation was 12% at 8 pmol/l, and the inter-assay coefficient of variation was 20% at 1.5 and 4 pmol/l, and 10% at 8.5 pmol/l.

Table 1. Laboratory data collected during hypertonic saline infusion in 18 young polyuric dogs, categorised according to the plasma vasopressin (VP) response to hypertonicity.

VP response	Sex	Age	Osmotic threshold	Sensitivity	Plasma VP S-E values	VP peak values	Correlation coefficient	
							incl. VP peaks	excl. VP peaks
		months	mOsm/kg	pmol/l per mOsm/kg	pmol/l			
Hyper-response								
dog 1	f	15	333	2.93	7-80		0.97	nd
dog 2	m	5	309	2.73	22-114		0.94	nd
dog 3	m	6	306	6.36	1-239		0.94	nd
Hypo-response								
dog 4	m	10	ns	ns	1-0		ns	nd
dog 5	f	4	318	0.42	1-16		0.98	nd
dog 6	m	8	321	0.21	1-12		0.93	nd
dog 7	fc	9	317	0.25	2-12		0.89	nd
Non-linear response								
dog 8	m	26	309	1.90	1-77	96	0.16	0.94
dog 9	m	8	314	0.68	1-35	34	0.67	0.98
dog 10	fc	32	ns	ns	1-6	68/16	0.41	ns
dog 11	m	12	324	0.19	0.4-8	6.3	0.79	0.92
dog 12	m	4	318	0.13	2-8	14	0.22	0.95
dog 13	m	18	325	0.60	1-19	20	0.21	0.92
dog 14	m	8	324	0.37	1-15	10	0.73	0.95
dog 15	m	7	307	0.51	4-22	19	0.61	0.91
dog 16	m	25	299	0.22	5-15	20	0.25	0.91
dog 17	m	3	337	0.44	6-14	14	0.25	0.99
dog 18	f	3.5	314	0.58	27-24	115/32	0.47	0.99
Reference values			276-309	0.24-2.47 (1)				

(1) Biewenga *et al*. (1987)

f=female; fc=female castrate; m=male; S-E value=start-to-end value; ns= not significant; nd= not done

Results

Vasopressin hyperresponse

Dogs 1-3 had a very high VP response to hypertonic saline infusion compared to the reference range obtained in healthy adult dogs (Table 1, Figure 1, r = 0.94 - 0.97). The osmotic threshold for VP secretion was increased in dog 1, whereas the sensitivity of the VP response was increased in all three dogs (Table 1).

In dog 1 Uosm varied by a factor of 2.2 during the day (Table 2). In dog 3 Uosm varied considerably, with a factor of 7.4 between the highest and lowest Uosm value. In this dog Uosm reached values of >1000 mOsm/kg during basal serial measurements. In dog 1 the desmopressin response was not measured because high Uosm values were reached during water deprivation. The response to desmopressin in dog 2 was large. In dog 3 desmopressin was administered at the end of the water deprivation test and the response was small (Table 2).

In dogs 1 and 3, Uosm exceeded 1000 mOsm/kg during water deprivation, with hardly any weight loss or increase in Posm and plasma Na concentration (Table 3). During water deprivation, dog 2 had a low-to-medium concentrating ability, with a large weight loss (8.2%), but hardly any increase in Posm. Plasma VP concentrations during water deprivation in dogs 1 and 3 varied at a low level (≤7 pmol/l).

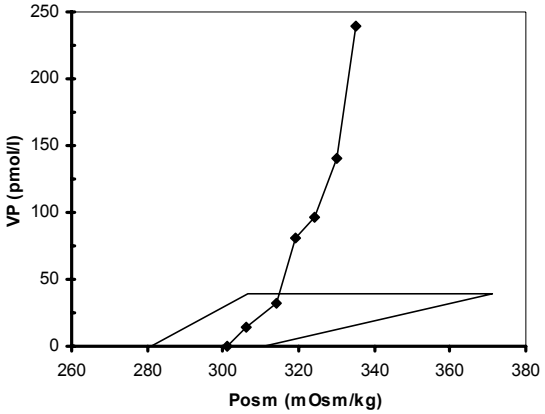

Figure 1. Plasma vasopressin concentration during hypertonic saline infusion in a 6-month-old Jack Russell terrier (dog 3) with polyuria and polydipsia, illustrating hyperresponsiveness. The outlined area represents the range of responses to infusion of hypertonic saline in 11 healthy dogs (Biewenga *et al.* 1987). VP = vasopressin; Posm = plasma osmolality.

Table 2. Laboratory data collected during serial measurements of urine osmolality (Uosm) in 18 young polyuric dogs, categorised according to the plasma vasopressin (VP) response to hypertonicity.

VP response	Minimum basal Uosm	Maximum basal Uosm	(Mean) basal Uosm (1)	Factor	Desmopressin response	
					Absolute increase	Percentage increase
	mOsm/kg				mOsm/kg	
Hyper-response						
dog 1	371	823	496	2.2	nd	nd
dog 2	nd	nd	510 (1)	nd	450	88 *
dog 3	223	1658	1168	7.4	249	17 **
Hypo-response						
dog 4	nd	nd	113 (1)	nd	639	565*
dog 5	467	836	682	1.8	0	0 *
dog 6	119	346	202	2.9	195	97
dog 7	390	1257	767	3.2	346	45
Non-linear response						
dog 8	88	1387	695	15.8	439	63
dog 9	87	308	141	3.5	188	133
dog 10	140	695	286	5.0	97	34
dog 11	266	1489	741	5.6	nd	nd
dog 12	68	679	217	10.0	131	60
dog 13	157	1468	1044	9.4	108	10 **
dog 14	159	1482	975	9.3	nd	nd
dog 15	229	503	379	2.2	nd	nd
dog 16	nd	nd	nd	nd	15	2 **
dog 17	nd	nd	338 (1)	nd	77	23 *
dog 18	169	308	237	1.8	93	39

(1) In dogs 2, 4, and 17 the starting point for measurement of desmopressin response in a single urine sample (dog 5: 445 mOsm/kg)
nd=not done; * measured in a single urine sample; ** measured after water deprivation

Vasopressin hyporesponse

In dogs 4-7 the VP response during hypertonic saline infusion was low, with no significant response in dog 4, and low values in dogs 5-7 (Table 1, Figure 2, r = 0.89 – 0.98). The osmotic threshold for VP secretion was increased in dogs 5-7, and the sensitivity of the VP response was decreased in dog 6 (Table 1).

In dogs 5-7 Uosm varied during the day by a factor of 1.8-3.2 (Table 2). In dog 7 Uosm reached values of >1000 mOsm/kg during basal serial measurements. The response to desmopressin was large in dogs 4 and 6, medium in dog 7, and absent in dog 5 (Table 2).

Urine concentration during water deprivation in dogs 5 and 6 was low-to-medium, with a moderate weight loss (2.7-4.1%), and a variable increase in Posm (+5 to +11 mOsm/kg) and plasma Na concentration (+1 to +5 mmol/l) (Table 3). Dog 4 had a poor concentrating ability during water deprivation, with a large weight loss (6.2%) and a large increase in Posm (+12 mOsm/kg). In dog 7, Uosm almost reached the level of 1000 mOsm/kg during water deprivation. This was associated with a large weight loss and a considerable increase in Posm. In dog 6 plasma VP concentrations during water deprivation varied at a low level (≤3 pmol/l).

Table 3. Laboratory data collected during water deprivation in 18 young polyuric dogs, categorised according to the plasma vasopressin (VP) response to hypertonicity.

VP response	Maximum Uosm	Weight loss	Posm S-E values	Plasma Na S-E values	Plasma VP S-E values	VP peak values
	mOsm/kg	%	mOsm/kg	mmol/l	pmol/l	
Hyper-response						
dog 1	1397	2.2	310-311	148-151	4-7	
dog 2	713	8.2	300-302	nd	nd	
dog 3	1436	2.2	299-301	143-147	1-3	
Hypo-response						
dog 4	463	6.2	296-308	nd	nd	
dog 5	557	2.7	291-296	141-142	nd	
dog 6	539	4.1	305-316	144-149	1-3	
dog 7	937	6.5	307-314	147-150	nd	
Non-linear response						
dog 8	1293	5.2	306-321	146-151	2-29	13
dog 9	834	2.1	308-309	146-145	3-1	
dog 10	623	2.0	303-306	142-144	1-2	
dog 11	nd	nd	nd	nd	nd	
dog 12	420	6.4	293-302	136-144	1-3	
dog 13	1107	3.4	297-298	141-142	<1	16
dog 14	nd	nd	nd	nd	nd	
dog 15	1620	2.0	306-312	143-149	nd	
dog 16	900	2.9	314-314	144-148	nd	
dog 17	1008	4.8	304-314	143-150	4-6	23
dog 18	333	7.2	315-324	146-150	7-16	44/42/26
Reference values			295-320	141-149		

Uosm=urine osmolality; Posm=plasma osmolality; Na=plasma sodium concentration
S-E value=start-to-end value; nd=not done

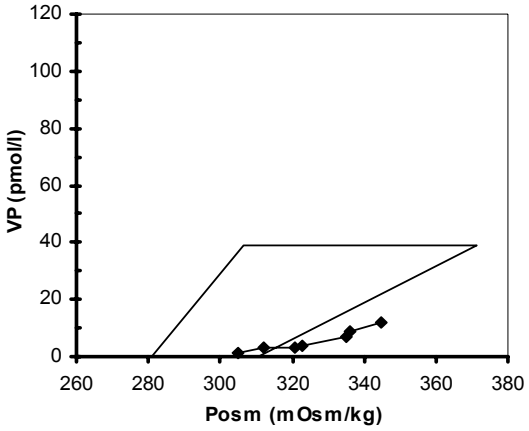

Figure 2. Plasma vasopressin concentration during hypertonic saline infusion in a 8-month-old Flatcoated Retriever (dog 6) with polyuria and polydipsia, illustrating hyporesponsiveness. See also legend to Figure 1.

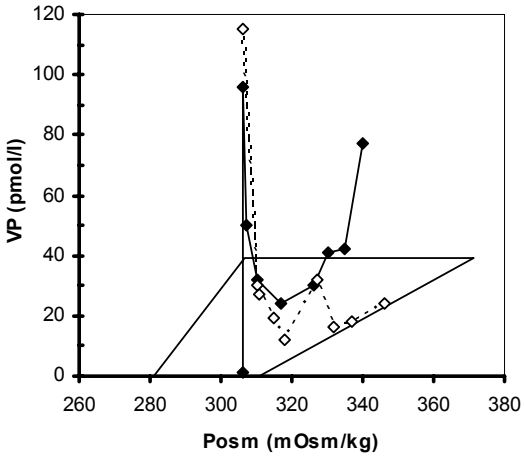

Figure 3. Plasma vasopressin concentrations during hypertonic saline infusion in a 26-month-old Maltese (dog 8, uninterrupted line and closed diamond), and a 3.5-month-old Scottish terrier (dog 18, dotted line and open diamond) with polyuria and polydipsia, illustrating peak responses unrelated to the gradual rise in plasma osmolality. See also legend to Figure 1.

Non-linear VP response

During hypertonic saline infusion in dogs 8-18 abrupt VP rises occurred unrelated to the gradual increase in Posm (Table 1, Figure 3, r = 0.16 – 0.79). Omission of these peak values from the regression analysis improved the correlation coefficients to 0.91 – 0.99, while the remaining VP response in dog 10 was not significant. The osmotic threshold was increased in dogs 9,11-14, and 17-18, and the sensitivity was decreased in dogs 11,12, and 16 (Table 1).

Serial measurements of Uosm revealed a large variation in Uosm during the day, with Uosm differing by a factor of 5 or more in 6 out of 11 dogs (Table 2). In 4 dogs (no 8,11,13,14) Uosm reached values of >1000 mOsm/kg. The response to desmopressin was small in 3 dogs (no 13, 16, 17), medium in 4 dogs (no 8, 10, 12, 18), and large in 1 dog (no 9). In the 3 remaining dogs high Uosm values occurred spontaneously during basal serial measurements and/or water deprivation, and therefore desmopressin was not administered (Table 2).

During the water deprivation test, Uosm exceeded 1000 mOsm/kg in dogs 8,13,15 and 17. These dogs showed a variable weight loss (2.0- 5.2%) and increase in Posm (+1 to +15 mOsm/kg) (Table 3). In dogs 11 and 14, initial Uosm was above 1400 mOsm/kg, and the water deprivation test was discontinued. Urinary concentrating ability during water deprivation was medium-to-high in dogs 9 and 16, low-to-medium in dog 10, and low in dogs 12 and 18. In the 2 dogs with a low concentrating ability, weight loss was large (6.4 and 7.2%) and there was a large increase in Posm (+9 mOsm/kg) and plasma Na concentration (+4 and +8 mmol/l). In the dog with low-to-medium concentrating ability, weight loss (2%) and the absolute increase in Posm (+3 mOsm/ kg) were small, as they were in the dogs with medium-to-high concentrating ability (weight loss 2.1 and 2.9% and increase in Posm 0 and +1 mOsm/kg). In 3 dogs plasma VP concentrations varied at a low level during water deprivation (≤3 pmol/l). In 4 dogs high VP peaks occurred, ranging from 13 to 44 pmol/l.

Discussion

In agreement with the general belief that the VP response to hypertonic saline infusion is the most powerful tool in the differential diagnosis of PUPD (Diederich *et al.* 2001), we categorised our group of 18 young dogs with PUPD on the basis of these results. All dogs had an abnormal VP response to osmotic stimulation, in that there was either a hyperresponse with increased sensitivity, a hyporesponse, or a non-linear response with VP peaks unrelated to increases in Posm. In addition, there were abnormalities in the threshold value of the VP response.

In the three hyperresponsive dogs, plasma VP concentrations reached high values with an increased threshold value in one dog and an increased sensitivity in all three dogs, when compared to the reference values (Biewenga *et al.* 1987). This increased VP response could be a form of the syndrome of inappropriate VP release (SIADH), in which plasma VP levels may be abnormally high in relation to Posm (Rijnberk *et al.* 1988), although the increased threshold value does not fit in

with this theory. The increased sensitivity may also be seen as a compensation mechanism for the slow start (i.e. increased threshold) of the VP response system. An alternative explanation for the VP results in the hyperresponsive dogs could be an alteration in the responsiveness of the hypothalamo-neurohypophyseal system due to chronic dehydration and overhydration (Moses and Scheinman 1993). During chronic dehydration upregulation of VP release occurs, which is associated with a higher sensitivity of the VP response (Moses and Scheinman 1993), or a lower osmotic threshold for VP secretion (Robertson and Athar 1976). In dogs, short-term hypovolaemia is also known to lead to a shift in the relationship between plasma VP and Posm, i.e., a decreased osmotic threshold and an increased sensitivity (Quillen and Cowley 1983). In another study Quillen *et al.* (1984) demonstrated that chronically altered volume states do not affect the VP response to increases in Posm. Nevertheless, we cannot rule out the possibility that hypovolaemia due to polyuria may have affected the VP response to osmotic stimulation in the three hyperresponsive dogs. The polyuria in these dogs could also have been due to VP resistance at the level of the kidneys, e.g. a disorder of the VP receptor or the renal water channel aquaporin-2, leading to an increased VP response (Moses and Scheinman 1993, Robertson 1995). However, the strong Uosm fluctuations with high values and the response to desmopressin administration argue against a causative abnormality at the kidney level.

In four dogs there was a hyporesponse in VP release during hypertonic saline infusion, which was characterised by an increased threshold value and a low sensitivity. At least in two of these dogs the diagnosis partial diabetes insipidus was supported by the good response to desmopressin administration. One dog concentrated its urine spontaneously to >1000 mOsm/kg during serial Uosm measurements. Downregulation of the VP response due to overhydration caused by primary polydipsia seems the most likely explanation in this dog (Moses and Clayton 1993). In the remaining dog the diminished VP response was accompanied by hyponatraemia and hypo-osmolality under basal conditions. A form of SIADH with low VP concentrations, due to production of a different antidiuretic substance or due to VP receptor upregulation (Kern *et al.* 1986, Sugama *et al.* 1992), cannot be ruled out in this dog.

In 11 dogs unexpected high VP peaks unrelated to increases in Posm occurred during hypertonic saline infusion. After elimination of the peaks, in all but two dogs the remaining VP response was abnormal with regard to the threshold value and/or sensitivity. In an earlier study, we described similar VP responses in dogs with PUPD (Van Vonderen *et al.* 1999). These high VP values might reflect the erratic bursts of VP release which are known to occur in SIADH (Robertson *et al.* 1976, Rijnberk *et al.* 1988). Nevertheless, initially dog 8 responded well to desmopressin treatment, which was started before the results of the VP measurements were available. During treatment, hyponatraemia and hypo-

osmolality developed, associated with episodes of restless behaviour and tremor. The desmopressin dose was reduced, Posm and plasma Na concentration returned to within their reference ranges, and the neurological signs disappeared. Apparently in this dog, the ongoing VP release due to the SIADH, in combination with the desmopressin therapy, caused hyponatraemia to the extent that neurological signs developed. In two other dogs the concurrence of hyponatraemia with high VP values make SIADH with desensitisation of renal VP receptors a likely cause of the PUPD.

In the dogs with a non-linear VP response, there was a wide range of VP responses to increases in Posm, with strong individual variations in threshold value and sensitivity of the response. This is very similar to what has been found in healthy humans (Robertson *et al.* 1976) and dogs (Meij *et al.* 1997), although in some publications the impression of a linear response is created because data are presented as idealised curves or as single dots not related to the individual studied. The present non-linear responses might reflect spontaneous (patho)physiological variations in plasma VP concentrations. Several studies have demonstrated episodic VP secretion (Weitzman *et al.* 1977, Hammer and Engell 1982, Livesey *et al.* 1988). The observed peaks might also be a reflection of the pulsatile character of VP release and not a pathological condition.

Considering all dogs, thus irrespective of categorisation according to VP measurements during hypertonic saline infusion, it appears that similar changes occurred in all these categories. For example, nine dogs (no 1,3,7-8,11,13-15,17) concentrated well spontaneously during basal serial measurements and/or during water deprivation, which complies with the diagnosis of primary polydipsia. In accordance with our previous report (Van Vonderen *et al.* 1999), we found different abnormalities in VP release in our dogs with so-called primary polydipsia. Whether or not these abnormalities are consequence or cause in the development of PUPD is not clear. Primary polydipsia is the only disorder of water metabolism in which drinking is the primary response, and the polyuria is a compensatory response. In humans, primary polydipsia can be divided into two categories: (1) psychogenic polydipsia (schizophrenia, neurosis), in which a generalised cognitive defect leads to excessive fluid intake, and (2) dipsogenic diabetes insipidus, in which there is an abnormality in the thirst mechanism (Robertson 1995). In psychogenic polydipsia, VP release may be suppressed by the low Posm caused by overhydration (Robertson 1995), but the occurrence of SIADH in schizophrenic patients with psychogenic polydipsia has also been described (Delva *et al.* 1990), as well as a defective thirst osmoregulation (Goldman *et al.* 1988). Intermittent hyponatraemia has been reported as a feature of psychogenic polydipsia (Vieweg *et al.* 1988), which fits in with the observations in dog 13. In dipsogenic diabetes insipidus, the osmotic thirst threshold is decreased, with or without associated abnormalities in VP osmoregulation (Robertson 1984, 1987, Thompson *et al.*

1991). Normally, drinking leads to an early satiation of thirst and to a complete cessation of VP secretion before Posm or plasma volume change (Thrasher *et al.* 1981, Geelen *et al.* 1984, Salata *et al.* 1987, Appelgren *et al.* 1991). In dipsogenic diabetes insipidus, patients drink more than healthy controls in response to an increase in Posm, and drinking fails to suppress thirst (Thompson *et al.* 1991). It is entirely possible that dogs with primary polydipsia have abnormalities in the thirst mechanism in addition to the described abnormalities in VP release.

What remains is the group of dogs with an inadequate concentrating ability during water deprivation. Similar to the situation in the dogs with primary polydipsia these dogs were present in all three categories of VP responses. In view of the response to desmopressin administration a (partial) VP deficiency seems likely in four of these dogs. In 2 of the latter dogs there were also sudden high VP values, but these peaks may have been too short to have sufficient effect on urine concentration. In one dog, insufficient urine concentration during dehydration in combination with an inadequate response to desmopressin administration may be explained by insensitivity to VP. Upregulation of VP release (as a result of dehydration) may have caused the sudden high VP values during water deprivation and hypertonic saline infusion in this dog. The other dogs with a diminished concentrating ability have been discussed above.

Thus in all three categories there were dogs with strong spontaneous fluctuations in Uosm and dogs with inadequate urinary concentrating capacity. It seems that the VP response to hypertonicity does not consistently distinguish between different clinical entities. This may in part be due to the relatively infrequent sampling. The observed non-linear (or erratic) responses compiled in the third category may in part represent spontaneous peaks in VP concentrations, whereby it is not clear whether this should be interpreted as a spontaneous (patho)physiological event or as due to the hypertonic stimulus. The present data question the generally accepted notion that VP measurements during hypertonic saline infusion are the "gold standard" for the diagnostic interpretation of polyuria. There is a need for in-depth studies of the peripheral reflection in plasma of the pulsatile VP release in healthy and polyuric individuals, with and without provocation. Although the pulsatile nature of VP release has been known for several years, this phenomenon is not commonly considered when the results of VP measurements in response to hypertonicity are interpreted.

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Chapter 7

Pulsatile secretion pattern of vasopressin under basal conditions, after water deprivation, and during osmotic stimulation in dogs

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Abstract

Measurement of plasma osmolality (Posm) and plasma vasopressin (VP) concentration in response to hypertonicity is regarded as the "gold standard" for the assessment of VP release in polyuric conditions. Yet the interpretation of the VP curve as a function of Posm may be hampered by the occurrence of VP pulses. To determine whether VP is secreted in a pulsatile fashion in the dog and whether stimulation of VP release changes the secretion pattern of VP, we investigated the secretion pattern of VP by blood sampling at 2-min intervals for 2 hours under basal conditions, after 12 hours of water deprivation, and during osmotic stimulation with hypertonic saline (20%) in 8 healthy dogs. Volume and electrolyte losses due to blood sampling were substituted by intravenous infusion of Ringer's lactate. The secretion pattern of VP was analysed by means of the Pulsar programme.

Vasopressin was secreted in a pulsatile fashion with a wide variation in number of VP pulses, VP pulse duration, and VP pulse amplitude and height. After water deprivation, total and basal VP secretion, the number of significant VP pulses, as well as the pulse characteristics did not differ from the basal situation. During osmotic stimulation, there was a large increase in both basal and pulsatile VP secretion. The number of VP pulses and the VP pulse height and amplitude were significantly increased compared to the basal and water-deprived state. The VP pulse amplitude correlated significantly with the basal plasma VP concentration during osmotic stimulation.

It is concluded that VP is secreted in a pulsatile manner in healthy dogs. The basal and pulsatile VP secretion increases during osmoreceptor-mediated stimulation. The VP pulses may occur to the magnitude that they may be interpreted as erratic bursts, when occurring in the hypertonic saline infusion test.

Chapter 7

Introduction

The release of vasopressin (VP), like that of other pituitary hormones, is the integrated result of intrinsically controlled episodic secretion and environmental factors. Vasopressin is secreted in a pulsatile pattern in humans (Hammer and Engell 1982, Wood *et al*. 1994), although there is some controversy about whether pulsatile VP secretion occurs under basal conditions, i.e., without stimulating factors such as activity, posture changes and osmotic stimulation. Some studies on the ultradian rhythm of VP reported only minimal changes in plasma VP concentrations in recumbent subjects (Luboshitzky *et al*. 1978, Lavie *et al*. 1980), whereas others demonstrated episodic VP secretion during bed rest and sleep, although considerably less than during daytime activity (Katz *et al*. 1979). The pattern of VP secretion varies considerably both inter-individually and intra-individually (Rubin *et al*. 1975, 1978). In well-hydrated humans the amplitude of plasma VP pulses may be 2-6 times higher than the basal plasma VP level (Uhlich *et al*. 1975, Lavie *et al*. 1977, Hammer and Engell 1982, Wood *et al*. 1994). The pulsatile secretion pattern of VP in humans is superimposed on a circadian rhythm that may vary with the sleep-wake cycle (Nadal 1996, Forsling *et al*. 1998).

The fluctuations in plasma VP concentrations have also been addressed in animal studies. A circadian VP rhythm has been found in several species, including the dog (Gordon and Lavie 1985). The pulsatile secretion pattern of VP has been most elegantly studied in the horse by measurements in the pituitary effluent, both under basal conditions and during osmotic stimulation (Redekopp *et al*. 1986, Irvine *et al*. 1989). In these studies it became clear that the detection of hormone pulses is highly dependent upon the intensity of the sampling regimen (Alexander *et al*. 1994). Episodic secretion of VP has also been documented in sheep (Engler *et al*. 1989), cats (Coleman and Reppert 1985), and rats (Ohno 1981).

Studies on episodic VP release in dogs are limited. Bie *et al*. (1982) found a few unexpectedly high plasma VP values in some euhydrated anaesthetized dogs, and significantly more during osmotic stimulation. Weitzman *et al*. (1977) studied VP release in dogs during intravenous water loading, dehydration and osmotic stimulation. During both water loading and minimal dehydration plasma VP levels remained low. Episodic VP release occurred in response to severe dehydration and osmotic stimulation, with a tendency for larger VP peaks in animals with higher baseline VP values. Unfortunately, in the latter study VP measurements were not performed under basal conditions and sampling took place for less than 1 hour.

In earlier studies, we reported the occurrence of unexpectedly high plasma VP concentrations during hypertonic saline infusion in dogs with polyuria (Van Vonderen *et al*. 1999, 2003). In these dogs it was not clear whether these high VP values had to be regarded as pathological or as the result of the physiological

pulsatile secretion pattern of VP. Hypertonic saline infusion with measurement of plasma osmolality (Posm) and plasma VP concentration is regarded as a very powerful diagnostic tool for the differentiation of polyuric conditions in both humans and dogs (Biewenga *et al*. 1987, Diederich *et al*. 2001). In this test, hypertonic saline is infused and blood is collected for VP measurements at 20-minute intervals for 2 hours (Zerbe and Robertson 1981). The plasma VP concentrations are plotted as a function of Posm. In principle, the interpretation of the curves thus obtained may be hampered by the infrequent sampling and the occurrence of VP pulses. However, so far there are no reports on the possible interference of VP pulses with the (linear) Posm-plasma VP relationship during hypertonic saline infusion.

To determine whether VP is secreted in a pulsatile fashion in the dog and whether stimulation of VP release changes the secretion pattern of VP, we investigated VP secretion in 8 healthy dogs by blood sampling at 2-min intervals for 2 hours under basal conditions, after water deprivation, and during osmotic stimulation with hypertonic saline.

Materials and methods

Dogs

Eight healthy castrated female dogs (2 cross-breds and 6 beagle dogs), with ages ranging from 1 to 8 years (median 5 years), were studied. All dogs were accustomed to the laboratory environment and experimental procedures. The dogs were housed singly or in pairs in indoor-outdoor runs, fed on a standard commercial dog food once daily, and given water ad libitum.

Studies

Studies were conducted in a random order and were at least 4 weeks apart. Food was withheld for 12 hours before all studies.

Study 1: Secretion pattern of VP under basal conditions. Water remained available until the start of the study. Blood samples for measurement of plasma VP concentration were collected from the jugular vein every 2 min for 2 hours. In addition, every hour blood was collected for the measurement of Posm. To compensate for the blood withdrawn, the dogs received a continuous infusion of Ringer's lactate (RL) in the cephalic vein, with a total volume equal to that withdrawn.

Study 2: Secretion pattern of VP after 12 hours of water deprivation. The protocol for this study was identical to that of study 1, with the exception that the blood sampling started after 12 hours of water deprivation.

Study 3: Secretion pattern of VP during hypertonic saline infusion. Saline solution (20%) was infused intravenously at a rate of 0.03 ml/kg body weight/min for 1 hour. In addition, the dogs received RL to compensate for the blood withdrawn. The infusion volumes in the first hour (20% NaCl and RL) and the second hour (RL) were the same. Blood samples were collected as above, except that samples for measurement of Posm were collected every 20 min.

Vasopressin determination

Blood samples for plasma VP measurement were collected in EDTA-coated tubes pre-chilled in ice. Plasma was obtained by centrifugation at 4°C and stored at –20°C until assayed. Vasopressin was extracted by the addition of 5.2 ml 96% ethanol (4°C) to 0.8 ml plasma, and incubation by end-over-end rotation for 30 min at 4°C. After centrifugation for 30 min at 5000xg and 4°C, the supernatant was collected and dried overnight using a speedvac vacuum concentrator. Extracts were dissolved in 0.8 ml assay buffer. The recovery of VP amounted to a mean value of 75 ±1%. Vasopressin concentrations were measured by radioimmunoassay (Nichols Institute, Wijchen, The Netherlands), validated for the dog by measuring a serial dilution of an extract of canine plasma with a high VP concentration that resulted in a curve parallel to the standard curve. The detection limit was 1 pmol/l. The intra-assay coefficient of variation was 12% at 8 pmol/l, and the inter-assay coefficient of variation was 20% at 1.5 and 4 pmol/l, and 10% at 8.5 pmol/l.

Data analysis

The secretion pattern of VP was analysed by means of the Pulsar programme developed by Merriam and Wachter (1982). The programme identifies secretory pulses by height and duration from a smoothed baseline, using the assay standard deviation (SD) as a scale factor. The cut-off parameters G1-G5 of the Pulsar programme were set at 3.98, 2.40, 1.68, 1.24 and 0.93 times the assay SD as criteria for accepting pulses 1, 2, 3, 4, and 5 points wide, respectively. The smoothing time, a window used to calculate a running mean value omitting pulses, was set at 1.5 hours. The splitting cut-off parameter was set at 0.5 and the weight assigned to pulses was 0.05. The A, B, and C values of the Pulsar programme, used to calculate the variance of the assay, were set at A=0.01, B=12.5 and C=37. Data below the detection limit of the VP assay were set at 1.0 pmol/l. The values extracted from the Pulsar analyses included the mean of the smoothed baseline (as a measure of the mean basal plasma VP concentration), the overall mean, the total number of pulses, the mean pulse duration, the mean pulse amplitude, the mean pulse height, and the area under the curve (AUC). The AUC was calculated above

the zero level as a measure of the total amount of VP secreted. The AUC above the baseline was used as a measure of the amount of VP secreted in pulses, while the AUC under the baseline was used as a measure of the basal secretion of VP.

The distribution of the data extracted from the Pulsar programme was tested using the Kolmogorov-Smirnov and the Shapiro-Wilk tests. The mean basal plasma VP concentration, the VP pulse duration, and Posm were normally distributed. Changes in these variables were further evaluated by analysis of variance (ANOVA) for repeated measurements. Subsequently, multiple comparisons were performed for data with significant differences ($P<0.05$) using the Student-Newman-Keuls test. These values are presented as mean ± SEM. The overall mean plasma VP concentration, the number of VP pulses, the VP pulse amplitude, the VP pulse height, and the AUC values were not normally distributed. For these variables differences between studies were analysed with the Friedman test and multiple comparisons were conducted using the Student-Newman-Keuls test. These values are expressed as median and range. Differences in AUC between the first and second hour of the studies were compared using the Wilcoxon signed ranks test. Differences in mean Posm between the first and second hour of study 3 were compared using a paired Students t-test. The significance of the relationship between the basal plasma VP concentration and the pulse amplitude was examined by linear regression analysis. $P<0.05$ was considered significant.

Ethics of experimentation

The studies were approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University.

Results

Study 1

Vasopressin was secreted in a pulsatile fashion (Figure 1). The mean basal plasma VP concentration was 3.2 ± 0.3 pmol/l. The AUC above the zero line varied between 3.0 and 20.4 pmol/l • 2 h, with a median value of 7.1 pmol/l • 2 h. The median AUC above the baseline was 1.2 pmol/l • 2 h (range 0.13 – 11.8 pmol/l • 2 h). The median AUC under the baseline was 6.1 pmol/l • 2 h (range 2.6 – 8.8 pmol/l • 2 h). The median of the overall mean plasma VP concentration was 3.6 pmol/l, and varied between 1.4 and 10.1 pmol/l. The number of VP pulses ranged from 0 to 5 during the period of blood sampling, with a median value of 2. The mean VP pulse duration was 5 ± 2 min. The median of the mean VP pulse amplitude was 7.3 pmol/l (range 4.4 – 81.3 pmol/l), and the median of the mean

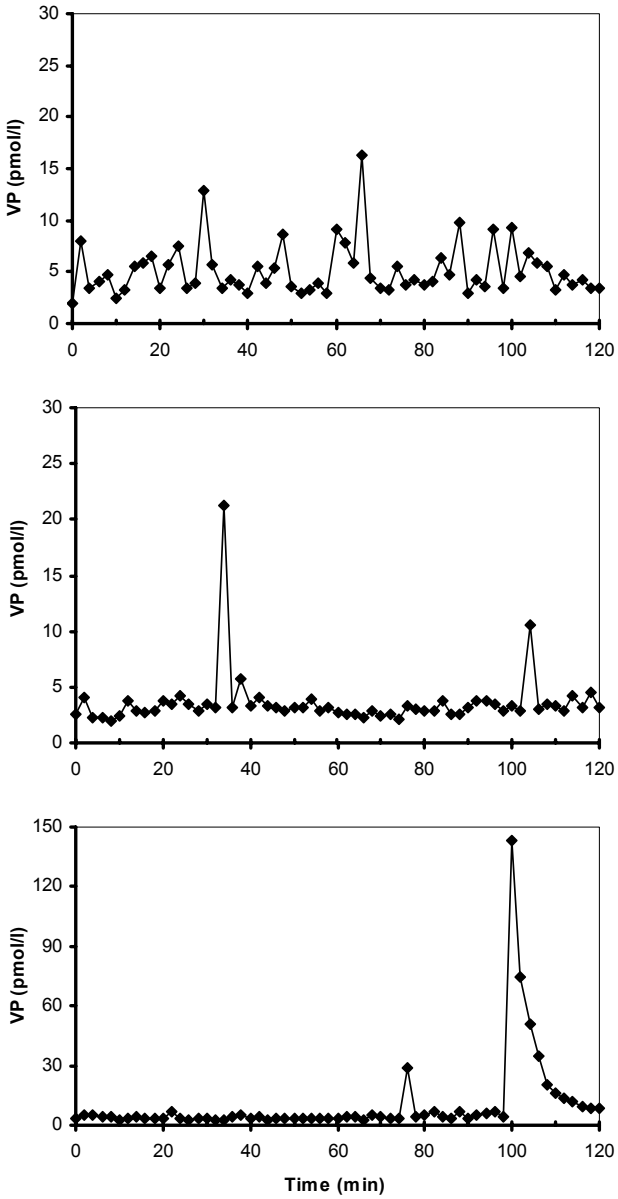

Figure 1. Basal plasma vasopressin (VP) concentrations in blood samples collected every 2 min for 2 hours. Volume and electrolyte losses due to blood sampling were substituted by intravenous infusion of Ringer's lactate. Upper panel: a 6-year-old beagle dog, middle panel: a 5-year-old beagle dog, lower panel: a 2-year-old beagle dog. (Note differences in scale of y-axis.)

VP pulse height was 10.9 pmol/l (range 7.0 – 86.1 pmol/l). Maximum VP pulse height was 143 pmol/l.

There was a small but significant decrease in Posm during the study (305 ± 1 ⇒ 303 ± 1 mOsm/kg). There were no significant differences in the AUC above the zero line, the AUC above the baseline, and the AUC under the baseline between the first and the second hour of sampling. After omission of one extreme value, there was no significant correlation between VP pulse amplitude and basal plasma VP concentration.

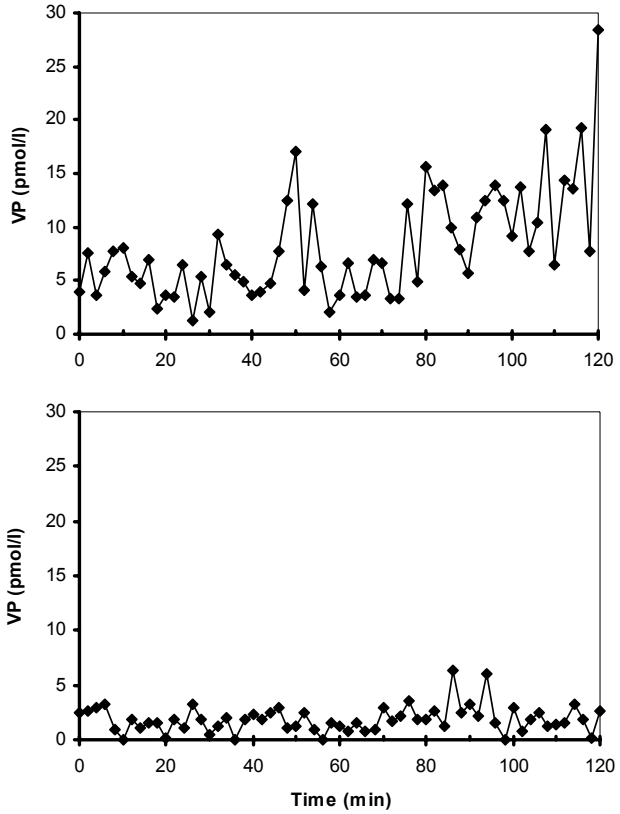

Figure 2. Plasma vasopressin (VP) concentrations after 12 hours of water deprivation in blood samples collected every 2 min for 2 hours. Volume and electrolyte losses due to blood sampling were substituted by intravenous infusion of Ringer's lactate. Upper panel: a 6-year-old beagle dog (same dog as in Figure 1), lower panel: a 5-year-old beagle dog (same dog as in Figure 1).

Study 2

After 12 hours of water deprivation VP was secreted in a pulsatile fashion similar to that in study 1 (Figure 2). The mean basal plasma VP concentration after water deprivation was 3.4 ± 0.7 pmol/l. The AUC above the zero line varied between 3.5 and 16.1 pmol/l • 2 h, with a median value of 6.6 pmol/l • 2 h. The median AUC above the baseline was 0.43 pmol/l • 2 h (range 0.12 – 2.8 pmol/l • 2 h). The median AUC under the baseline was 5.7 pmol/l • 2 h (range 3.0 – 15 pmol/l • 2 h). The median of the overall mean plasma VP concentration was 3.3 pmol/l, with a range of 1.7 – 8.2 pmol/l. The number of VP pulses in study 2 varied between 0 and 6, with a median of 1. The mean VP pulse duration was 6 ± 2 min. The median of the mean VP pulse amplitude was 8.2 pmol/l (range 2.3 - 10.6 pmol/l), and the median of the mean VP pulse height was 12.2 pmol/l (range of 4.6 – 16.3 pmol/l). Maximum VP pulse height was 42 pmol/l.

There was a small but significant decrease in Posm during the study (310 ± 3 ⇒ 307 ± 3 mOsm/kg). There were no significant differences in the AUC above the zero line, the AUC above the baseline, and the AUC under the baseline between the first and the second hour of sampling. The correlation between VP pulse amplitude and basal plasma VP concentration was not significant.

There was no significant difference in Posm between studies 1 and 2. There were also no significant differences in the basal plasma VP concentration, the AUC above the zero line, the AUC above the baseline, the AUC under the baseline, and the overall mean plasma VP concentration between studies 1 and 2. Also with regard to the number of significant VP pulses and the pulse characteristics there were no significant differences between studies 1 and 2.

Study 3

The pulsatile secretion pattern of VP during osmotic stimulation was more pronounced than in studies 1 and 2 (Figure 3). The mean basal plasma VP concentration in study 3 was 15.3 ± 3.4 pmol/l. The AUC above the zero line varied between 9.3 and 206.4 pmol/l • 2 h, with a median value of 55.1 pmol/l • 2 h. The median AUC above the baseline was 24.1 pmol/l • 2 h (range 2.0 – 136.1 pmol/l • 2 h). The median AUC under the baseline was 25.8 pmol/l • 2 h (range 6.9 – 70.3 pmol/l • 2 h). The median of the overall mean plasma VP concentration was 27.5 pmol/l (range 4.6 – 104.1 pmol/l). The number of VP pulses varied between 2 and 14, with a median of 10. The mean VP pulse duration was 5 ± 0.5 min. The median of the mean VP pulse amplitude was 66.2 pmol/l (range 10.8 – 159.9 pmol/l), and the median of the mean VP pulse height was 78.6 pmol/l (range 20.0 – 197.9 pmol/l). Maximum VP pulse height was 683 pmol/l.

Plasma osmolality increased significantly during the first hour of the study (309 ± 1 ⇒ 330 ± 1 mOsm/kg). During the second hour of sampling Posm decreased significantly to 325 ± 1 mOsm/kg, which was still significantly higher

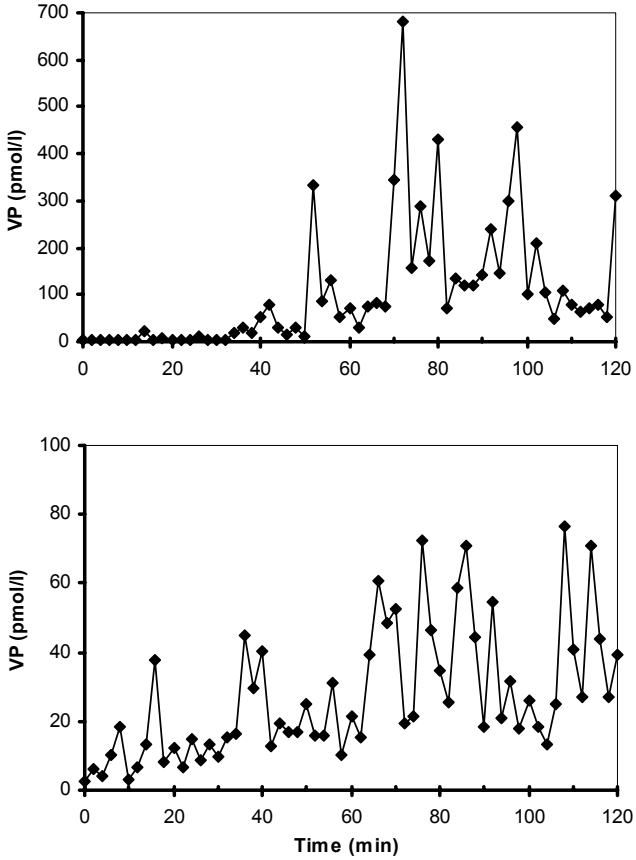

Figure 3. Plasma vasopressin (VP) concentrations in blood samples collected every 2 min for 2 hours during osmotic stimulation. Hypertonic saline (20% NaCl 0.03 ml/kg/min) was infused for 1 hour. Volume and electrolyte losses due to blood sampling were substituted by intravenous infusion of Ringer's lactate. Upper panel: a 6-year-old beagle dog (same dog as in Figures 1 and 2), lower panel: a 5-year-old beagle dog (same dog as in Figures 1 and 2). (Note differences in scale of y-axis, also as compared to Figures 1 and 2.)

than at the start of the measurements. The mean Posm during the second hour (326 ± 2 mOsm/kg) was significantly higher than the mean Posm during the first hour (320 ± 1 mOsm/kg). In the second hour the median AUC above the zero line (37.7 pmol/l • h, range 4.5 – 172.6 pmol/l • h) and the median AUC under the baseline (16.5 pmol/l • h, range 4.3 – 56.6 pmol/l • h) were significantly higher than in the first hour (17.5 pmol/l • h, range 4.3 – 42.5 pmol/l • h, and 9.4 pmol/l • h, range 2.6

– 13.7 pmol/l • h, respectively). The median AUC above the baseline in the second hour (17.7 pmol/l • h, range 0.2 – 116 pmol/l • h) was also higher than in the first hour of the study (6.4 pmol/l • h, range 0 – 33.5 pmol/l • h), but this difference did not reach statistical significance. There was a significant positive correlation between VP pulse amplitude and basal plasma VP concentration (r=0.65).

The basal plasma VP concentration, the AUC above the zero line, the AUC above the baseline, the AUC under the baseline, as well as the overall mean plasma VP concentration were significantly higher during osmotic stimulation than in studies 1 and 2. There was no significant difference in VP pulse duration between studies 1-3. The number of VP pulses, the VP pulse amplitude, and the VP pulse height were significantly higher in study 3 than in studies 1 and 2.

Discussion

The level of the mean basal plasma VP concentration measured under basal conditions was consistent with the reference range of VP (1-6 pmol/l) reported earlier in dogs (Biewenga *et al*. 1987, Hellebrekers *et al*. 1987). In both the hydrated state (study 1) and after 12 hours of water deprivation (study 2), basal and total plasma VP secretion did not change during the 2 hours of blood sampling. Thus, it seems justified to assume that the volume and electrolyte losses due to blood sampling were adequately substituted. Plasma osmolality, basal and total VP secretion, as well as VP pulse characteristics in study 2 were not significantly different from those in study 1. This indicates that water deprivation for 12 hours does not change the secretion pattern of VP in healthy dogs. Basal and total VP secretion in study 3 were significantly higher than those under basal conditions and after water deprivation. Thus, in agreement with other studies (Wang and Goetz 1985, Robertson and Ganguly 1986), osmotic stimulation proved to be a very strong stimulus for VP secretion.

In study 1 significant VP pulses were recognised by the Pulsar programme, indicating that, even under basal conditions, VP is secreted in a pulsatile fashion in the dog. The pulsatile secretion pattern of VP was characterised by large individual variations in number of VP pulses, VP pulse amplitude, VP pulse height and VP pulse duration. Studies on the pulsatile secretion pattern of VP under basal conditions have not been reported before in dogs. In an earlier study, plasma VP levels remained relatively constant in dogs with minimal dehydration, while significant VP pulses with variable amplitude and timing were found in dogs with severe dehydration (Weitzman *et al*. 1977). We did not find that water deprivation altered the number of VP pulses and the VP pulse characteristics. Apparently, VP

pulsatile secretory activity is not influenced by mild dehydration to the extent that it leads to detectable changes when sampled at 2-min intervals. During osmotic stimulation, the amount of VP secreted in pulses increased considerably, with an increase in number, amplitude and height of the VP pulses. Weitzman *et al*. (1977) have reported higher VP pulses in dogs with higher baseline VP levels during severe dehydration and osmotic stimulation. Although we could not confirm a difference in total VP secreted in pulses between the first and second hour of blood sampling in study 3, there was indeed a significant increase in VP pulse amplitude with increasing basal plasma VP concentration.

The half-life of endogenous VP has been reported to vary between 2 and 5 min in dogs, and between 5 and 15 min in humans (Wilson *et al*. 1981, Vilhardt 1990, Robinson and Verbalis 2003). Weitzman and Fisher (1978) determined the existence of two exponential components in the disappearance curve of synthetic VP after pulse injection. The first component resembled distribution over the total plasma volume (half-life 1.4 min), while the second component corresponded to VP clearance (half-life 4.1 min). Occasionally, we observed a much faster decline in plasma VP concentration following large pulses. Katz *et al*. (1979) reported a similar discrepancy between VP pulse decline and hormone half-life, and suggested that VP half-life in the basal state may differ from that during stimulation.

The secretion of VP is primarily controlled by osmoreceptors located in the anterior hypothalamus near the cell bodies of the VP-synthesising magnocellular neurons (Robertson 1985, Wang and Goetz 1985, Robertson and Ganguly 1986). In addition, baroreceptors situated in the heart, aorta, and carotid arteries may mediate haemodynamic influences to the neurohypophyseal system. Functionally, the two control systems are integrated in such a way that osmoregulation is altered but not disrupted by haemodynamic influences (Robertson and Ganguly 1986). An increase in Posm by as little as 1% may increase plasma VP, whereas blood volume and pressure must decrease by more than 10% to 15% to stimulate VP release (Robinson and Verbalis 2003). In the present study, volume loss was substituted and Posm decreased only minimally. Therefore, osmo- and/or baroreceptor-mediated VP stimulation will not have contributed to the occurrence of significant VP pulses in the basal state. As dehydration was minimal after 12 hours, the same probably accounts for study 2. The increase in VP pulse height and amplitude and the increase in total number of VP pulses upon infusion of hypertonic saline can solely be attributed to the stimulation of osmoreceptors.

Other important stimuli of VP release are nausea (Rowe *et al*. 1979), hypoglycaemia (Baylis and Heath 1977, Ellis *et al*. 1990), hypoxia/hypercapnia (Raff *et al*. 1983), pain (Kendler *et al*. 1978), and stress (Jørgensen *et al*. 2002). The presence of hypoglycaemia, hypoxia or hypercapnia does not seem to have played a role in our studies. All dogs were accustomed to the laboratory

environment and handling, and catheters had been inserted prior to the studies. Nausea, however, cannot be completely excluded during hypertonic stimulation. In previous studies, we observed nausea in some dogs during the last 15 min of a 2-hour-infusion of hypertonic saline. As in our study 3 no signs of nausea were observed and hypertonic saline was only infused for one hour, it is unlikely that nausea played a role in stimulating VP release.

Our studies in healthy dogs were initiated because of uncertainty about the possible confounding role of spontaneous VP pulses in the test that is regarded as the "gold standard" for the assessment of VP release in polyuric conditions, i.e. hypertonic saline infusion with measurements of Posm and plasma VP concentrations (Biewenga *et al*. 1987, Diederich *et al*. 2001). The results of our study demonstrate that in healthy dogs VP pulses may occur of a height that would lead to high points outside the nomogram for the (linear) relation between Posm and plasma VP concentration (Zerbe *et al*. 1980). Such VP pulses may erroneously be interpreted as erratic, and consequently, a false-positive diagnosis of the syndrome of inappropriate antidiuresis could be made. The present observations may explain, in part, the non-linear VP response to osmotic stimulation observed previously in polyuric dogs (Van Vonderen *et al*. 1999, 2003). In some of these dogs, VP peaks occurred that did not seem to relate to the gradual rise in Posm following hypertonic saline infusion. However, a pathological origin of the VP peaks in these polyuric dogs cannot be excluded.

In conclusion, in healthy dogs VP is secreted in a pulsatile fashion. Basal and pulsatile VP secretion increase during osmoreceptor-mediated stimulation. The magnitude of the VP pulses may be sufficient for the pulses to be considered as erratic bursts when occurring during the hypertonic saline infusion test, a test used to assess VP release in polyuric conditions.

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Chapter 8

Urinary aquaporin-2 excretion in dogs: a potential marker in the differentiation of polyuric conditions

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Abstract

Water conservation by the kidney is mediated by both short- and long-term regulation of aquaporin-2 (AQP2), the vasopressin (VP)-dependent water channel in the apical membrane and the intracellular vesicles of the collecting duct cells. In humans, the urinary AQP2 (U-AQP2) excretion closely parallels changes in VP action and has been proposed as a marker for collecting duct responsiveness to VP. This report describes the development of a radioimmunoassay for the measurement of U-AQP2 excretion in dogs. Urinary AQP2 measurements were performed in states of high and low VP exposure. In addition, the localisation of AQP2 in the canine kidney was investigated by immunohistochemistry.

Basal U-AQP2 excretion was highly variable among healthy dogs. Two hours after oral water loading, the mean U-AQP2/creatinine ratio decreased significantly from 231 ± 30 x 10^{-9} to 60 ± 15 x 10^{-9}, while the median plasma VP concentration decreased from 4.2 pmol/l (range 2.2 – 4.8 pmol/l) to 1.2 pmol/l (range 1.0 – 1.9 pmol/l). Subsequent intravenous administration of desmopressin led to a significantly increased mean U-AQP2/creatinine ratio of 258 ± 56 x 10^{-9}. Desmopressin administration via the conjunctival sac did not result in a significant change in the U-AQP2/creatinine ratio. Two hours of intravenous hypertonic saline infusion (20% NaCl, 0.03 ml/kg body weight/min) significantly increased the mean U-AQP2/creatinine ratio from 86 ± 6 x 10^{-9} to 145 ± 23 x 10^{-9}, while the median plasma VP concentration increased significantly from 2.2 pmol/l (range 1.1 – 6.3 pmol/l) to 17.1 pmol/l (range 8.4 – 67 pmol/l). Immunohistochemistry revealed extensive labeling for AQP2 exclusively in the kidney collecting duct cells. The AQP2 labeling was distributed throughout the cytoplasm, but predominantly localised in the apical and subapical region.

As in humans, U-AQP2 excretion in dogs closely reflects changes in VP exposure, elicited by water loading, hypertonic saline infusion, and intravenous desmopressin administration. Urinary AQP2 excretion may become a diagnostic tool in dogs for the differentiation of polyuric conditions such as (partial) central or nephrogenic diabetes insipidus, primary polydipsia, and inappropriate VP release.

Chapter 8

Introduction

Aquaporin-2 (AQP2) has been characterised as the major vasopressin (VP)-regulated water channel and is predominantly localised in the apical membrane and intracellular vesicles of the kidney collecting duct principal cells (Nielsen *et al*. 1993, 1995). Water conservation by the kidney is mediated by both short- and long-term regulation of AQP2 (Wen *et al*. 1999). Upon binding of VP to its V2-receptor in the basolateral membrane of collecting duct cells, a chain of signalling events is initiated, resulting in the translocation of AQP2 from an intracellular reservoir to the apical plasma membrane and an increased passage of water within a few minutes (Nielsen *et al*. 1993, 1995, Marples *et al*. 1995a). After VP withdrawal, AQP2 is redistributed into the cell by endocytosis and water permeability decreases (Nielsen *et al*. 1995). Chronic VP exposure (>24 h) leads to increased expression of AQP2 and, consequently, maximal water permeability of the collecting duct epithelium (DiGiovanni *et al*. 1994, Hozawa *et al*. 1996, Kishore *et al*. 1996).

Studies of urinary AQP2 (U-AQP2) excretion in rats indicate that 3% of total kidney AQP2 is excreted into the urine by a selective apical pathway (Rai *et al*. 1997, Wen *et al*. 1999). Of the AQP2 excreted into the urine, 35% - 45% is glycosylated (Baumgarten *et al*. 1998) and 40% is associated with small vesicles and larger membrane fragments (Kanno *et al*. 1995, Deen *et al*. 1996, Wen *et al*. 1999). In humans and rats, U-AQP2 excretion closely parallels changes in VP action, and thus has been proposed as a marker for collecting duct responsiveness to VP (Elliot *et al*. 1996, Mitsuma *et al*. 1998, Wen *et al*. 1999, Pedersen *et al*. 2001). Indeed, U-AQP2 excretion decreases during water loading and increases during water deprivation, osmotic stimulation with hypertonic saline, and administration of the VP-analogue desmopressin (Elliot *et al*. 1996, Saito *et al*. 1997, Wen *et al*. 1999, Pedersen *et al*. 2001).

A defective function and/or regulation of AQP2 plays a key role in several disorders of water homeostasis (Wen *et al*. 1999, King and Yasui 2002). In hereditary nephrogenic diabetes insipidus, mutations in the AQP2 gene may lead to impaired routing of AQP2 to the apical membrane or to misfolding of the protein. The molecule is retained in the endoplasmic reticulum and is subsequently degraded (Deen *et al*. 1994, Marr *et al*. 2002). Animal models of acquired nephrogenic diabetes insipidus are based on downregulation of AQP2 as a result of long-term lithium treatment, hypokalaemia, and hypercalcaemia (Marples *et al*. 1995b, 1996, Wang *et al*. 2002). Saito *et al*. (1998, 2001) demonstrated an exaggerated U-AQP2 excretion in humans with the syndrome of inappropriate VP release (SIADH), and concluded that U-AQP2 is a potent marker for VP excess. In addition, U-AQP2 excretion may contribute in the differentiation of polyuric

conditions such as central diabetes insipidus, nephrogenic diabetes insipidus, and primary polydipsia in humans (Kanno *et al*. 1995, Saito *et al*. 1997, Mitsuma *et al*. 1998, Saito *et al*. 1999, Matsumoto *et al*. 2000).

Much more so than in other species, polyuria is a feature of disease in the dog. In several endocrine diseases such as hyperadrenocorticism, hyperthyroidism, and hyperparathyroidism, polyuria is the dominating symptom (Biewenga *et al*. 1991, Rijnberk 1996, Van Vonderen *et al*. 2003a). After exclusion of these conditions, there remains a group of largely unresolved polyurias. Particularly the differentiation between primary polydipsia, SIADH, partial central diabetes insipidus, and partial nephrogenic diabetes insipidus may pose problems (Van Vonderen *et al*. 1999, 2003b). As in humans, U-AQP2 excretion may be of value in the differentiation of polyuric conditions.

Studies on the presence and function of AQPs in dogs are scarce. Two studies have demonstrated a large similarity between the cDNA and amino acid sequences of AQP1 and the N-terminal part of AQP2 of dogs, humans, and rats (Madsen *et al*. 1997, Higa *et al*. 2000). Urinary AQP2 excretion has not been measured before in dogs, and there is no commercially available test for U-AQP2 in animals (Cohen and Post 2002). In this report, we describe the development of a radioimmunoassay for the measurement of U-AQP2 excretion in dogs. To validate the assay, U-AQP2 excretion was measured in healthy dogs under basal conditions, after water loading, during hypertonic saline infusion, and after desmopressin administration. In addition, the immunohistochemical localisation of AQP2 in the canine kidney is described.

Materials and methods

Dogs

All dogs were accustomed to the laboratory environment, and handling such as collection of blood samples. The dogs were housed singly or in pairs in indoor-outdoor runs, fed on a standard commercial dog food once daily, and given water ad libitum. Food was withheld for 12 hours before all studies, while water remained available until the start of the measurements.

Studies

Study 1: Desmopressin administration in the conjunctival sac. Six healthy beagle dogs (2 male dogs and 4 neutered female dogs), aged 4 to 11 years (median: 7 years), were studied. Four urine samples for the measurement of urine osmolality (Uosm), urinary creatinine concentration (Ucreat), and U-AQP2 concentration

were collected via catheterisation at hourly intervals. After the first 2 basal urine samples (h-1/h0), 2 drops of desmopressin (Minrin^{R}, Ferring B.V., Hoofddorp, The Netherlands) were administered in the conjunctival sac. Urine sampling was continued for 2 hours after desmopressin administration (h1/h2).

Study 2: Hypertonic saline infusion. The group of dogs studied was the same as in study 1. Four urine samples for the measurement of Uosm, Ucreat, and U-AQP2 were collected via catheterisation at hourly intervals. After the first 2 basal urine samples (h-1/h0), 20% NaCl was infused intravenously at a rate of 0.03 ml/kg body weight/min for 2 hours (Biewenga *et al*. 1987). Blood samples for the measurement of plasma VP concentration and plasma osmolality (Posm) were collected every 20 min, starting 40 min before the hypertonic saline infusion (9 samples). Urine sampling was continued every hour during the hypertonic saline infusion (h1/h2).

Study 3: Water loading and intravenous desmopressin administration. Four healthy male beagle dogs, all of them 4 years of age, were studied. A total of 8 urine samples for the measurement of Uosm, Ucreat, and U-AQP2 were collected via catheterisation. The first 4 urine samples were collected at hourly intervals. After 2 basal urine samples (h-1/h0), the dogs were given an oral water load (42 ml/kg), and 2 additional urine samples were collected (h1/h2). Next, 0.3 µg desmopressin per kg body weight was administered intravenously in 100 ml 0.9% NaCl over a period of 20 min. After the start of the desmopressin infusion, urine samples were collected at 30-min intervals (h2.5/h3/h3.5/h4). Samples for measurement of Posm, and plasma concentrations of VP, sodium (Na), and total protein (TP) were collected at the same times as the urine samples during basal sampling and water loading.

Plasma VP determination

Blood samples for plasma VP measurement were collected in EDTA-coated tubes pre-chilled in ice, separated by centrifugation at 4°C, and stored at -20°C until assayed. Vasopressin was extracted from plasma by the addition of 5.2 ml 96% ethanol (4°C) to 0.8 ml plasma, and incubation by end-over-end rotation for 30 min at 4°C. Next, the tubes were centrifuged for 30 min at 5000xg and 4°C. Supernatants were dried overnight using a speedvac vacuum concentrator. Extracts were dissolved in 0.8 ml assay buffer. The recovery of VP amounted to a mean value of 75 ± 1%. Vasopressin concentrations were measured by radioimmunoassay (Nichols Institute, Wijchen, The Netherlands), validated for the dog by measuring a serial dilution of an extract of canine plasma with a high VP concentration that resulted in a curve parallel to the standard curve. The detection limit was 1 pmol/l. Data below the detection limit of the VP assay were set at 1.0 pmol/l. The intra-assay coefficient of variation was 15% at 7 pmol/l, and the inter-assay coefficient of variation was 20% at 1.5 and 4 pmol/l, and 10% at 8.5 pmol/l.

Urinary AQP-2 determination

Samples for measurement of U-AQP2 were placed on ice immediately after collection, separated from debris by centrifugation at 4°C (10 min at 3000xg), and stored at –20°C. All samples were analysed within 10 days. The AQP2 antibody was a generous gift from Dr. M.A. Knepper, National Heart, Lung, and Blood Institute, National Institutes of Health (Bethesda, Maryland, USA). The AQP2 antibody had been raised in rabbits against a BSA-linked synthetic peptide corresponding to the 15 carboxy-terminal amino acids of rat AQP2 (DiGiovanni *et al*. 1994). The synthetic rat AQP2 peptide was purchased from Alpha Diagnostic International (San Antonio, TX, USA) and provided with an N-terminal tyrosin for labeling. Iodination of tyrosin-linked AQP2 was performed by the chloramine T method. To 1 µg of AQP2 (dissolved in destilled water at 1 µg/5 µl), 3 µl $^{125}I^-$ (11.1 MBq) (Amersham Pharmacia Biotech, Buckinghamshire, UK), 5 µl 10 mM HCl, 10 µl 0.5 M sodium phosphate (pH 7.4), and 10 µl choramine T (1 mg/ml) were added. After 1 min the reaction was stopped by addition of 10 µl sodium metabisulphite (2 mg/ml). ^{125}I-labeled AQP2 was separated from free $^{125}I^-$ on a sephadex G25 column (Amersham Pharmacia Biotech, Uppsala, Sweden). The assay buffer consisted of 63 mM sodium phosphate (pH 7.4), 13 mM sodium EDTA, 0.05% (v/v) Tween-20, 2% (v/v) Trasylol, and 0.25% (w/v) BSA.

The mixture of 100 µl of standard or urine sample, 100 µl of antibody (final dilution 1:6000), and 100 µl of tracer was incubated for 24 h at 4°C. After incubation with the second antibody (100 µl of Sac-cel Anti-rabbit^R, IDS Boldon, Tyne & Wear, UK) for 30 min at 4°C, and subsequent centrifugation (10 min, 4°C, 5000xg), the precipitate (bound fraction) was counted in a gamma counter for 1 min. All samples were analysed in duplicate. A standard curve was made with the concentrations 0.1 - 100 ng/ml of the synthetic AQP2 peptide. Serial dilution curves of canine urine samples paralleled that of the standard. The detection limit was 0.05 ng/ml. The intra-assay coefficient of variation was 7.2% at 7.3 ng/ml, and the inter-assay coefficient of variation was 8.3% at 1.7 ng/ml, and 5.3% at 6.8 ng/ml. The U-AQP2 concentrations were converted to pmol/ml using the molecular weight of the synthetic AQP2 peptide (M=1858). In addition to the U-AQP2 concentration, the U-AQP2/creatinine ratio is reported.

Immunohistochemistry

The immunohistochemical localisation of AQP2 in the canine kidney was studied using both the avidin-biotin-peroxidase complex (ABC) technique and immunofluorescence. Control sections were stained according to the same protocol with omission of the primary antibody. For the ABC technique, tissue samples fixed in 10% buffered formalin were processed by conventional methods, embedded in paraffin wax, and sectioned at 5 µm. After deparaffination and blockage of endogenous peroxidase activity, sections were incubated with the

AQP2 antibody (1:800) at room temperature for 60 min. Next, they were incubated with goat biotinylated anti-rabbit IgG (1:1600, 30 min) and treated with the ABC standard kit for 45 min (Vector, Amsterdam, The Netherlands). The peroxidase activity was "visualised" with 0.5% 3.3'-diaminobenzidine tetrahydrochloride (DAB, Sigma-Aldrich Chemie BV, St. Louis, USA) and H_2O_2 0.3% in 0.05 M Tris-HCl. All sections were counterstained with Mayer's haematoxylin for 1 min.

Immunofluorescense microscopy was performed on 5-µm cryosections of the canine kidney. Sections were dried (room air) for 10 min, and then fixed in cold aceton for an additional 10 min. The sections were then washed in PBS (3 x 5 min) and incubated for 30 min at 37ºC with the AQP2 antibody described above at a 1:100 dilution. After washing, the sections were incubated with a fluorescein-conjugated secondary antibody (swine anti-rabbit 1:100). Final washing was done in PBS (2 min) and destilled water.

Data analysis

In all studies, changes in U-AQP2 concentration and U-AQP2/creatinine ratio were assessed by analysis of variance (ANOVA) for repeated measurements. Subsequently, multiple comparisons were performed for data with significant differences ($P<0.05$) using the Student-Newman-Keuls test. Values are presented as mean ± SEM and range. The mean of the first two urine samples (h-1/h0) was used to calculate basal Ucreat (study 2), basal U-AQP2 concentration, basal U-AQP2/creatinine ratio, and basal Uosm (all studies). In study 3, urine data after desmopressin administration were pooled for analysis, because one dog produced insufficient urine in the second hour. In study 2, the mean of the first three blood samples was used to calculate the basal plasma VP concentration. In study 3, the mean of the first two blood samples was used to calculate basal Posm and basal plasma concentrations of Na, VP, and TP. Differences in Uosm (studies 1-3), Ucreat (study 2), Posm (studies 2-3), and plasma concentrations of Na and TP (study 3) were assessed by ANOVA for repeated measurements and the Student-Newman-Keuls test for multiple comparisons. Values are presented as mean ± SEM. Because of the pulsatile nature of VP secretion (Van Vonderen *et al*. 2003c), differences in plasma VP concentrations in studies 2 and 3 were analysed with the non-parametric Friedman test and multiple comparisons were conducted using the Student-Newman-Keuls test. These data are presented as median and range.

Results

Study 1

The mean basal U-AQP2 concentration was 1.28 ± 0.33 pmol/ml (range 0.04 – 2.82 pmol/ml) and the mean basal U-AQP2/creatinine ratio was 125 ± 20 x 10^{-9} (range 18 – 219 x 10^{-9}). After desmopressin administration in the conjunctival sac, mean Uosm increased from 723 ± 138 mOsm/kg to 1021 ± 188 mOsm/kg (h1) and 1292 ± 108 mOsm/kg (h2). The mean Uosm 2 hours after desmopressin administration (h2) was significantly higher than the basal value. The mean U-AQP2 concentration increased significantly to 2.05 ± 0.47 pmol/ml (range 0.06 – 3.64 pmol/ml, h1) and 2.22 ± 0.47 pmol/ml (range 1.21 – 4.37 pmol/ml, h2). The mean U-AQP2/creatinine ratio did not change significantly, with values of 117 ± 29 x 10^{-9} (range 21 – 224 x 10^{-9}, h1) and 109 ± 20 x 10^{-9} (range 65 – 201 x 10^{-9}, h2).

Study 2

The mean basal U-AQP2 concentration was 1.45 ± 0.32 pmol/ml (range 0.53 – 4.34 pmol/ml) and the mean basal U-AQP2/creatinine ratio was 86 ± 6 x 10^{-9} (range 59 – 136 x 10^{-9}). During hypertonic saline infusion, the mean Uosm did not change significantly, while the mean Ucreat decreased significantly. Parallel with Ucreat, the mean U-AQP2 concentration decreased significantly to 0.54 ± 0.11 pmol/ml (range 0.13 – 0.83 pmol/ml, h1) and to 0.24 ± 0.05 pmol/ml (range 0.10 – 0.45 pmol/ml, h2). The mean Posm increased significantly from 303 ± 1 mOsm/kg (basal) to 336 ± 2 mOsm/kg (h2), while the median plasma VP concentration increased significantly from 2.2 pmol/l (basal, range 1.1 – 6.3 pmol/l) to 17.1 pmol/l (h2, range 8.4 – 67 pmol/l) (Figure 1). The mean U-AQP2/creatinine ratio increased to 108 ± 18 x 10^{-9} (range 55 – 181 x 10^{-9}, h1) and 145 ± 23 x 10^{-9} (range 87 – 233 x 10^{-9}, h2) (Figure 1). The mean U-AQP2/creatinine ratio after 2 hours of osmotic stimulation (h2) was significantly higher than the basal value.

Study 3

The mean basal U-AQP2 concentration was 3.62 ± 1.03 pmol/ml (range 0.76 – 8.06 pmol/ml), and the mean basal U-AQP2/creatinine ratio was 231 ± 30 x 10^{-9} (range 125 – 482 x 10^{-9}). After water loading, the mean basal Uosm (909 ± 157 mOsm/kg) decreased significantly to 416 ± 252 mOsm/kg (h1) and 59 ± 6 mOsm/kg (h2). In addition there were significant decreases in the mean basal Posm (309 ± 1 mOsm/kg) to 293 ± 1 mOsm/kg (h1) and 296 ± 2 mOsm/kg (h2), the mean basal plasma Na concentration (147 ± 1 mmol/l) to 140 ± 1 mmol/l (h1) and 141 ± 1 mmol/l (h2), and the mean basal plasma TP concentration (60 ± 1 g/l) to 55

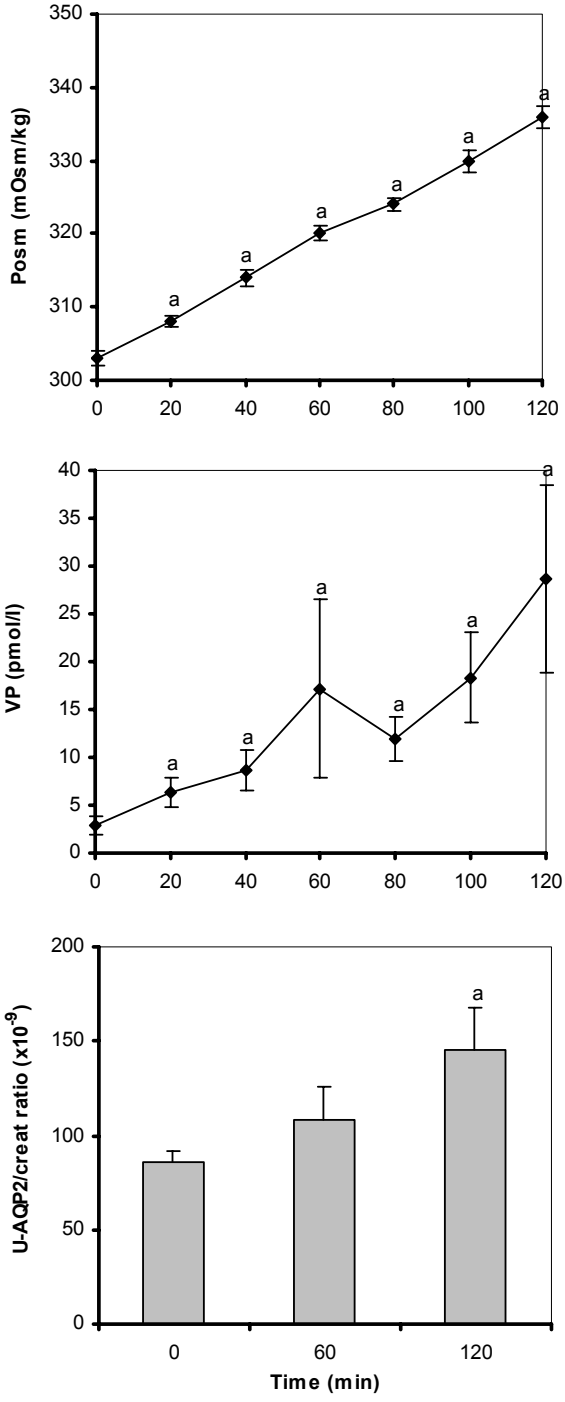

Figure 1. Plasma osmolality (Posm), plasma vasopressin (VP) concentration, and urinary aquaporin-2/creatinine (U-AQP2/ creat) ratio in 6 healthy beagle dogs under basal conditions and during 2 hours of intravenous osmotic stimulation with 20% NaCl infusion. Means ± SEM are shown. (a = significant difference from the basal value)

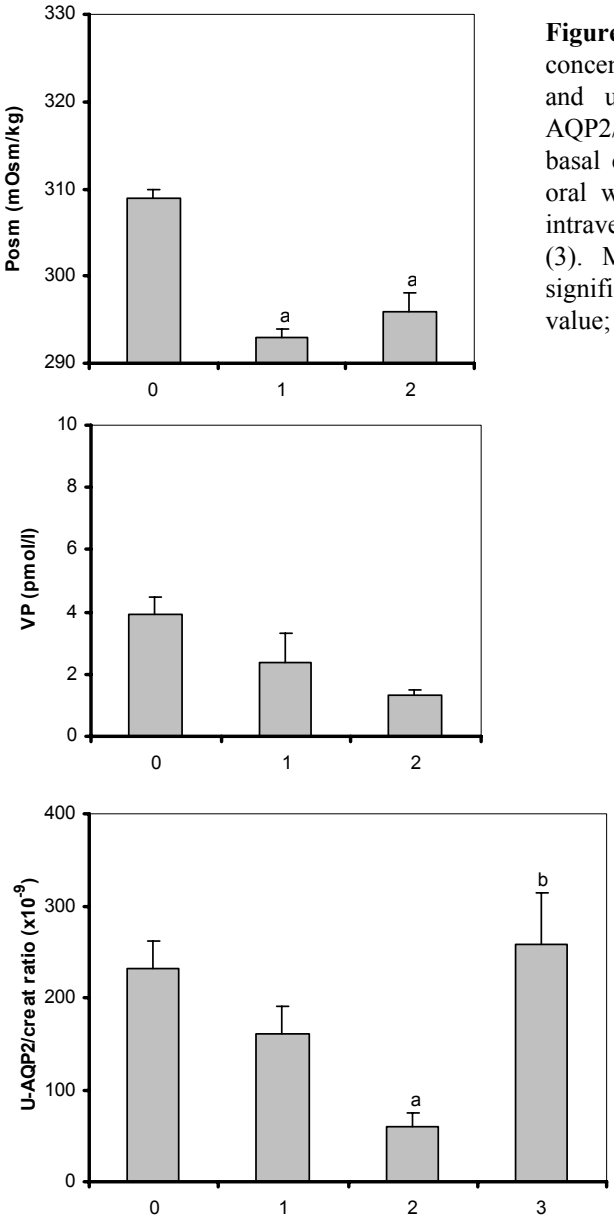

Figure 2. Plasma vasopressin (VP) concentration, plasma osmolality (Posm), and urinary aquaporin-2/creatinine (U-AQP2/creat) ratio in 4 healthy dogs under basal conditions (0), 1 and 2 hours after oral water loading (1 and 2), and after intravenous desmopressin administration (3). Means ± SEM are shown. (a = significant difference from the basal value; b = significant difference from h2)

± 1 g/l (h1) and 57 ± 1 g/l (h2). The median plasma VP concentration (4.2 pmol/l, range 2.2 – 4.8 pmol/l) decreased to 1.8 pmol/l (range 1.0 – 5.1 pmol/l, h1) and 1.2 pmol/l (range 1.0 – 1.9 pmol/l, h2) (Figure 2). The mean U-AQP2 concentration decreased significantly to 1.0 ± 0.55 pmol/ml (range 0.09 – 2.6 pmol/ml, h1) and

0.05 ± 0.01 pmol/ml (range 0.03 – 0.08 pmol/ml, h2). The mean U-AQP2/creatinine ratio decreased to 160 ± 30 x 10^{-9} (range 86 – 228 x 10^{-9}, h1) and 60 ± 15 x 10^{-9} (range 31 – 94 x 10^{-9}, h2) (Figure 2). The mean U-AQP2/creatinine ratio 2 hours after water loading (h2) was significantly lower than the basal value.

After desmopressin administration, the mean Uosm significantly increased to 836 ± 135 mOsm/kg compared to values 1 and 2 hours after water loading. The mean U-AQP2 concentration increased significantly to 3.12 ± 0.6 pmol/ml (range 1.54 – 4.48 pmol/ml) after desmopressin compared to its concentration 1 and 2 hours after water loading. The mean U-AQP2/creatinine ratio after desmopressin administration (258 ± 56 x 10^{-9}, range 158 – 417 x 10^{-9}) was significantly higher than that 2 hours after water loading (Figure 2).

Immunohistochemistry

Immunohistochemistry revealed extensive labeling for AQP2 exclusively in the kidney collecting duct (Figure 3). The AQP2 labeling was distributed throughout the cytoplasm, but predominantly localised in the apical and subapical region. Control sections revealed no labeling. Results obtained with the ABC and immunofluorescence techniques were similar.

Discussion

Immunohistochemistry revealed exclusive AQP2 labeling of collecting duct cells in the canine kidney. This pattern of labeling is identical to that described earlier in the human and rat kidney (Nielsen *et al*. 1993, Sasaki *et al*. 1994). The subcellular distribution of AQP2 depends on the presence or absence of VP. Prior to VP exposure, AQP2 labeling is predominantly localised in intracellular vesicles in apical, central, and basal parts of the cells, but also in the apical plasma membrane (Nielsen *et al*. 1993, 1995). After VP exposure, the AQP2 labeling of the apical membrane increases markedly, and the vesicles are located mainly in the subapical region. Following VP washout, there is a decrease in labeling of the apical plasma membrane relative to the intracellular vesicles, and the labeled vesicles are distributed throughout the cells. We found AQP2 labeling to be distributed predominantly in the apical and subapical region of the canine collecting duct cells, which is in accordance with some VP exposure. The results also indicate that the rat AQP2 antibody specifically recognises canine AQP2, so that what is measured as U-AQP2 can be regarded as derived from renal AQP2.

There was a large individual variation in basal U-AQP2 excretion, expressed as the U-AQP2/creatinine ratio, as has also been found in humans (Elliot

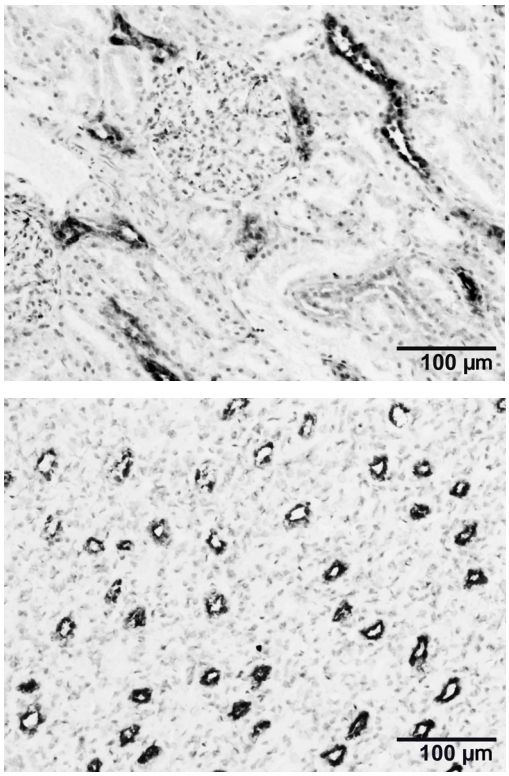

Figure 3. Immunohistochemical localisation of aquaporin-2 in 5-µm sections of a canine kidney. Upper panel: cortex, lower panel: papilla. Labeling was exclusively seen in the collecting duct cells, mainly in the apical and subapical region.

et al. 1996, Rai *et al*. 1997, Saito *et al*. 1997). This variation may in part be due to differences in basal hydration state, plasma VP concentration, or kidney collecting duct sensitivity (Elliot *et al*. 1996, Rai *et al*. 1997). The levels of U-AQP2 excretion in dogs seem to be of a similar magnitude compared to those described in humans (Saito *et al*. 1998, 1999, 2001), although the studies are not fully comparable due to differences in radioimmunoassay and antibody.

Urinary AQP2 excretion in dogs closely reflected changes in exposure of the collecting duct to VP, elicited by water loading, hypertonic saline infusion, and intravenous desmopressin administration. Water loading leads to retrieval of AQP2 from the apical membrane of kidney collecting duct cells to the intracellular vesicles, and thus to decreased water permeability (Marples *et al*. 1995a). In humans and rats, U-AQP2 excretion decreases up to threefold depending on water load volume and preceding water deprivation (Kanno *et al*. 1995, Elliot *et al*. 1996, Saito *et al*. 1997, Wen *et al*. 1999). In agreement with these findings, we found a decrease in U-AQP2 excretion in our dogs, to 26% of the basal level, 2 hours after water loading.

Stimulation by endogenous and exogenous VP results in the translocation of AQP2 from the intracellular vesicles to the apical membrane of kidney collecting duct cells and to an increase in water permeability (Nielsen *et al*. 1993, DiGiovanni *et al*. 1994, Marples *et al*. 1995a, Nielsen *et al*. 1995). It has been established that thirst (in rats and humans) and osmotic stimulation by hypertonic saline infusion (in humans) increase U-AQP2 excretion (Kanno *et al*. 1995, Elliot *et al*. 1996, Saito *et al*. 1997, Wen *et al*. 1999, Pedersen *et al*. 2001). Our findings of an increased U-AQP2 excretion during hypertonic saline infusion are in accordance with these observations. Also our observations in dogs after intravenous desmopressin administration agree with the desmopressin-induced increase in U-AQP2 excretion found in humans and rats (Kanno *et al*. 1995, Saito *et al*. 1997, Wen *et al*. 1999). However, in study 1 U-AQP2 excretion did not increase significantly after conjunctival administration of desmopressin. These dogs had a relatively high Uosm indicative of mild antidiuresis, at the start of the study, which limits the potential response to desmopressin (Marples *et al*. 1995a). Prior water loading, as in study 3, might have led to a significant response in U-AQP2 excretion after desmopressin administration via the conjuctival sac.

Urinary AQP2 excretion has been proposed as a reliable marker for collecting duct responsiveness to VP in various physiological states of water homeostasis as well as disorders of water homeostasis in humans (Kanno *et al*. 1995, Elliot *et al*. 1996, Saito *et al*. 1997, 1998, Mitsuma *et al*. 1998, Saito *et al*. 1999, Matsumoto *et al*. 2000, Pedersen *et al*. 2001, Saito et al. 2001). Also in dogs U-AQP2 excretion parallels changes in VP exposure, as demonstrated by the responses to oral water loading, osmotic stimulation, and intravenous desmopressin administration. As in humans, U-AQP2 excretion may be of use as a diagnostic tool in canine polyuria, for the differentiation of (partial) central diabetes insipidus, (partial) nephrogenic diabetes insipidus, primary polydipsia, and SIADH. In earlier studies we found that interpretation of VP measurements during osmotic stimulation still leaves unresolved issues in the differentiation of these polyuric conditions (Van Vonderen *et al*. 1999, 2003b). In these situations, measurement of AQP2 excretion may be of value. Moreover, compared to VP measurements, the easier sample collection and the less time-consuming radioimmunoassay without extraction might make U-AQP2 measurement a more convenient and practical tool for diagnostic purposes.

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Chapter 9

Summarising discussion and conclusions

Summarising discussion and conclusions

It is generally accepted that there is a high degree of individual biologic variability in urine specific gravity (Usg) and urine osmolality (Uosm) of urine samples obtained at different times of the day from the same dog (Chew and DiBartola 1989, Finco 1989). Therefore, basing the assessment of urinary concentrating ability on a single urine sample may be misleading, and a second sample or a series of samples should be obtained to confirm any abnormality (Chew and DiBartola 1989). However, few studies have investigated the normal range of these variables in healthy dogs. The observations in 89 healthy dogs described in **Chapter 3** indicate that the ranges of Usg (1.006 - >1.050) and Uosm (161 – 2830 mOsm/kg) values are much larger than previously thought. Urine concentration was significantly higher in the morning than in the evening, and was not related to gender. Urinary concentrating ability decreased significantly with age, which could not be ascribed to an associated increase in glucocorticoid production. In parallel to the situation in other species, the impaired urine concentration during aging may be caused by an age-associated reduction in renal responsiveness to vasopressin (VP) (Geelen and Corman 1992), or to a decline in glomerular filtration rate (Lindeman *et al*. 1960).

In addition to the large interindividual variation in Uosm, in some dogs Uosm varied widely during the day with the intraindividual coefficient of variation approaching the interindividual coefficient of variation. Two- to three-fold increases or decreases in Uosm often occurred within two hours. Possible factors underlying the fluctuation in Uosm during the day may be the effect of activity and feeding on drinking behaviour (O'Connor 1975, Meyer *et al*. 1994). Stimulation of oropharyngeal receptors leads to an early satiation of thirst (Thrasher *et al*. 1981), but apparently this does not prevent some dogs from drinking excessive amounts of water, although not to an extent that the owners perceived the dogs to have polyuria and polydipsia (PUPD). The results of the study reported in **Chapter 3** lead to the conclusion that whenever the suspicion of PUPD arises, urine samples should be collected every two hours to measure Uosm, and this approach may prevent the need for further studies.

The results of the study reported in **Chapter 4** demonstrate that VP release is impaired in dogs with secondary polycythaemia due to renal neoplasm. During hypertonic saline infusion, the osmotic threshold of VP release was markedly increased and the sensitivity of the response was low. Polyuria and polydipsia are an important clinical feature of secondary polycythaemia in dogs (Peterson and Randolph 1983, Campbell 1990). The increase in red blood cell mass leads to hyperviscosity of the blood and to an increased blood volume (Peterson and Randolph 1983, Campbell 1990), and these factors may stimulate the atrial and

carotid bifurcation baroreceptors controlling VP release. It is indeed well known that hypervolaemia impairs the VP response to osmotic stimulation in humans and dogs, resulting in an increased threshold and a decreased sensitivity of the osmoregulatory VP response (Robertson and Athar 1976, Quillen and Cowley 1983). In addition, the increased blood volume in secondary polycythaemia may lead to an increased release of atrial natriuretic peptide due to the atrial stretch. Atrial natriuretic peptide not only inhibits the renal responsiveness to VP, but may also impair VP release (Dillingham and Anderson 1986, Iitake *et al*. 1986). Therefore, both the hypervolaemia per se and the resulting increase in atrial natriuretic peptide may underlie the impaired osmoregulation of VP release in dogs with secondary polycythaemia. The results of the study indicate that, in secondary polycythaemia, the associated polyuria is at least in part the result of impaired VP release.

Primary polydipsia is characterised by a marked increase in water intake that cannot be explained as a compensatory mechanism for excessive fluid loss (Dunn 1990). Therefore, there is no urinary concentrating defect, and the diagnosis can be made when Uosm exceeds 1000 mOsm/kg during water deprivation, while plasma osmolality (Posm) remains below the upper limit of its reference range. The results of the study described in **Chapter 5** indicate that some of the dogs fulfilling the criteria of primary polydipsia produced concentrated urine spontaneously during the day. Serial measurements of Uosm (collected every 2 hours for 24 hours, with an interval of 4 hours during the night) demonstrated large fluctuations in Uosm in these dogs with some Uosm values exceeding 1000 mOsm/kg. This pattern of Uosm was very similar to that of some healthy pet dogs without noticed PUPD described in **Chapter 3**. Apparently, the water intake in the dogs with primary polydipsia exceeded the variation observed in healthy dogs to such an extent that the owners sought veterinary help. Based on the results of the studies described in **Chapters 3 and 5**, it can be concluded that a water deprivation test need not be performed in all polyuric dogs. When pronounced fluctuations are found in Uosm during serial measurements and some Uosm values exceed 1000 mOsm/kg, the diagnosis of primary polydipsia seems justified.

Dogs fulfilling the criteria of primary polydipsia do not seem to encompass a uniform syndrome. In the dogs that produced concentrated urine spontaneously during the day, Posm and plasma sodium (Na) concentration remained at the lower limit or the middle of their reference ranges. Other dogs only reached the limit of 1000 mOsm/kg during water deprivation, while both Posm and plasma Na concentration were at the upper level of their reference ranges. These observations suggest that dogs diagnosed as having primary polydipsia may comprise two groups, as has been reported for humans with primary polydipsia: polydipsic patients with and without hyponatraemia (Goldman *et al*. 1996). Apart from this

heterogeneity, also the VP response to osmotic stimulation was not uniform, albeit abnormal in all dogs. These abnormalities included episodes of hypersecretion, as well as delayed responses to plasma hypertonicity. In several dogs initial high plasma VP levels were found during water deprivation and/or osmotic stimulation. These findings may represent bursts of VP release, similar to those that occur in the syndrome of inappropriate VP secretion (SIADH) (Rijnberk *et al*. 1988), or they may reflect the (normal) pulsatile character of VP release. The disturbed VP response to osmotic stimulation in dogs with primary polydipsia may be interpreted as a primary disturbance in the regulation of VP secretion, although it might also be the result of overhydration caused by a primary abnormality in drinking behaviour.

Common disorders of water homeostasis leading to polyuria include a variety of endocrine, metabolic, and renal disturbances. After exclusion of most of these conditions, there may remain the diagnostic dilemma of differentiating between central diabetes insipidus, primary polydipsia, and nephrogenic abnormalities (Nichols 1992, Greco 2001). The most powerful diagnostic tool for differentiation of these conditions is the hypertonic saline infusion test, with measurement of Posm and plasma VP concentration (Diederich *et al*. 2001). The study described in **Chapter 6** reports on eighteen young polyuric dogs categorised according to the plasma VP response to hypertonic saline infusion. In all dogs the VP response was abnormal. Three categories could be distinguished: (a) a hyperresponse, (b) a hyporesponse, and (c) a non-linear response with high VP values unrelated to increases in Posm. Similar to the situation in the polyuric dogs described in **Chapter 5**, the VP peaks (up to 150 pmol/l) of the dogs with a non-linear response might represent the erratic secretory bursts known to occur in SIADH. However, the early peaks, in particular, could also reflect a pulsatile release pattern of VP, which may be either physiological or induced by the beginning increase in tonicity. In accordance with the results of the study described in **Chapter 5**, (seemingly) abnormal VP responses to hypertonicity were found in the dogs with variations in Uosm compatible with primary polydipsia. When the three categories of VP response were considered together with the results of other diagnostic procedures (serial measurements of Uosm, water deprivation, and response to desmopressin administration), it appeared that the VP response to hypertonicity did not consistently distinguish between the different clinical conditions. In all three categories, there were dogs with an inadequate concentrating ability and dogs diagnosed as having primary polydipsia. Thus, the data presented in **Chapter 6** question the generally accepted notion that VP measurements during hypertonic saline infusion are the “gold standard” for the diagnosis of polyuric conditions.

The results of the study described in **Chapter 7** indicate that in dogs VP is secreted in a pulsatile fashion with a wide variation in the number of VP pulses, VP pulse duration, and VP pulse amplitude and height. Episodic VP secretion during severe dehydration and osmotic stimulation has been reported earlier in dogs (Weitzman *et al*. 1977), but **Chapter 7** describes the first report documenting the pulsatile secretion of VP under basal conditions. By means of the Pulsar programme developed by Merriam and Wachter (1982), secretory pulses were identified by their height and duration from a smoothed baseline, using the assay standard deviation as a scale factor. Thus, apart from pulse characteristics and total VP secretion, basal VP secretion and pulsatile VP secretion could be determined. After twelve hours of water deprivation, total and basal VP secretion, the number of significant VP pulses, as well as the pulse characteristics did not differ from those under basal conditions. Apparently, VP secretory activity is not influenced by mild dehydration to the extent that changes in VP secretion could be detected in samples collected at 2-min intervals. During osmotic stimulation with hypertonic saline, there was a large increase in both basal and pulsatile VP secretion. The number of VP pulses and the VP pulse height and amplitude were significantly increased compared to the basal and water-deprived state. As volume loss due to blood sampling was substituted, and the presence of other stimuli on VP release, such as nausea, was unlikely, the increase in VP pulsatile secretion upon infusion of hypertonic saline can solely be attributed to the stimulation of osmoreceptors.

The pulsatile secretion pattern of VP in dogs may explain part of the non-linear VP responses to osmotic stimulation, as observed in the polyuric dogs described in **Chapters 5 and 6**. In some of these dogs VP peaks occurred that did not seem to relate to the gradual rise in Posm in response to the hypertonic saline infusion. In addition, in some cases the plasma VP concentration at the start of osmotic stimulation or water deprivation was high. A pathological origin of the VP peaks in these polyuric dogs cannot be excluded. The results reported in **Chapter 7** demonstrate that in healthy dogs VP pulses can occur of a height that would lead to high points outside the nomogram for the (linear) relation between Posm and plasma VP concentrations (Zerbe *et al*. 1980). Such VP pulses may erroneously be interpreted as erratic, and consequently, a false positive diagnosis of SIADH could be made. Thus, apart from the finding that the VP response to hypertonicity did not consistently distinguish between different clinical entities, as reported in **Chapter 6**, the results of the study reported in **Chapter 7** indicate that the occurrence of spontaneous VP pulses may severely hamper the interpretation of the curve describing the relationship between Posm and plasma VP concentration during osmotic stimulation.

Chapter 8 is the first report to describe the development of a radioimmunoassay for the measurement of urinary aquaporin-2 (U-AQP2)

excretion in dogs. Aquaporin-2, the VP-regulated water channel, is localised in the apical membrane and the intracellular vesicles of the kidney collecting duct cells (Nielsen *et al*. 1993). Studies in rats revealed that 3% of total kidney AQP2 is excreted into the urine by a selective apical pathway (Rai *et al*. 1997). Basal U-AQP2 excretion in healthy dogs, expressed as the U-AQP2/creatinine ratio, varied similarly to the large individual variation in U-AQP2 excretion found in humans (Saito *et al*. 1997). This variation may in part be due to differences in basal hydration state, plasma VP concentration, or kidney collecting duct sensitivity (Rai *et al*. 1997). The levels of U-AQP2 excretion in dogs seem to be of a similar magnitude compared to those described in humans (Saito *et al*. 2001). Urinary AQP2 excretion in dogs closely reflected changes in the collecting duct exposure to VP elicited by water loading, hypertonic saline infusion, and intravenous desmopressin administration. After oral water loading, U-AQP2 excretion decreased significantly, whereas stimulation by endogenous and exogenous VP resulted in a significant increase in U-AQP2 excretion, which is in accordance with earlier studies in humans (Saito *et al*. 1997, Pedersen *et al*. 2001). Immunohistochemistry of canine kidney tissue demonstrated extensive labeling for AQP2 predominantly in the apical and subapical region of the collecting duct cells, consistent with the localisation of the VP-regulated water channel in the intracellular vesicles (Nielsen *et al*. 1993).

Urinary AQP2 excretion has been proposed as a reliable marker for collecting duct responsiveness to VP in various physiological states of water homeostasis as well as disorders of water homeostasis in humans. In water retention disorders, U-AQP2 excretion is exaggerated (Saito *et al*. 2001), whereas downregulation of AQP2 is found in hereditary and acquired nephrogenic diabetes insipidus (Deen *et al*. 1994, Wang *et al*. 2002). Urinary AQP2 excretion may contribute to the differentiation of polyuric conditions such as central diabetes insipidus, nephrogenic diabetes insipidus, and primary polydipsia (Kanno *et al*. 1995). Also in dogs, U-AQP2 excretion seems to parallel changes in VP action, as demonstrated by the responses to oral water loading, osmotic stimulation, and intravenous desmopressin administration. As in humans, U-AQP2 excretion may be of use as a diagnostic tool in canine polyuria, for the differentiation of (partial) central diabetes insipidus, (partial) nephrogenic diabetes insipidus, primary polydipsia, and SIADH. As described in **Chapters 5-7**, the interpretation of VP measurements during osmotic stimulation still leaves unresolved issues regarding the differentiation of these polyuric conditions. Particularly in this dilemma, measurements of U-AQP2 excretion may be helpful.

The following conclusions can be drawn:

- Because of the wide spontaneous fluctuations in Uosm occurring in healthy dogs, documentation of polyuria cannot be based on the measurement of Uosm in a single urine sample.
- Measurement of Uosm at 2-hour intervals may allow the diagnosis of primary polydipsia, thereby avoiding further clinical studies.
- Polyuria in dogs with secondary polycythaemia due to renal neoplasm is associated with an impaired osmoregulation of VP release.
- In dogs supposedly having primary polydipsia a wide variation in VP responses to hypertonic stimulation can be found.
- As the VP response to hypertonic saline infusion does not consistently distinguish between some forms of polyuria, it is questionable whether VP measurements during osmotic stimulation are the "gold standard" for differentiation of canine polyuria.
- In the dog, VP is secreted in a pulsatile fashion with a wide variation in the number of VP pulses, VP pulse duration, and VP pulse amplitude and height.
- Mild dehydration does not influence VP pulsatile secretion, whereas osmotic stimulation significantly increases basal and pulsatile VP release in the dog.
- The occurrence of spontaneous VP pulses may severely hamper the interpretation of the curve describing the relationship between Posm and plasma VP concentration during osmotic stimulation.
- In healthy dogs, U-AQP2 excretion closely reflects changes in collecting duct exposure to VP.
- Measurement of U-AQP2 excretion in polyuric dogs may be helpful in the differentiation of (partial) central diabetes insipidus, (partial) nephrogenic diabetes insipidus, primary polydipsia, and SIADH.

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Chapter 10

Samenvatting en conclusies

Samenvatting en conclusies

In de laatste decennia is de indruk ontstaan dat er een grote intraindividuele variatie bestaat van het soortelijk gewicht (Usg) en de osmolaliteit (Uosm) van urinemonsters verzameld op verschillende momenten van de dag bij dezelfde hond (Chew en DiBartola 1989, Finco 1989). Het gebruik van één urinemonster om het concentrerend vermogen van de nieren te beoordelen kan dan ook misleidend zijn. Onderzoek van enkele urinemonsters kan nodig zijn om een afwijking vast te stellen (Chew en DiBartola 1989). Er zijn echter maar weinig rapportages over deze variabelen bij gezonde honden. De waarnemingen bij 89 gezonde honden, zoals beschreven in **Hoofdstuk 3** tonen aan dat de variaties van Usg (1.006 - >1.050) en van Uosm (161 – 2830 mOsm/kg) veel groter zijn dan tot voor kort werd aangenomen. De concentratie van de urine was 's ochtends significant hoger dan 's avonds, en was niet gerelateerd aan het geslacht. Er was een significante afname van de urineconcentratie met de leeftijd, hetgeen niet verklaard kon worden door een toename in glucocorticoidproductie. Evenals bij de mens en de rat kan de daling van de urineconcentratie met de leeftijd wellicht verklaard worden door een afname van de gevoeligheid van de nieren voor vasopressine (VP) (Geelen en Corman 1992), of een afname van de glomerulaire filtratiesnelheid (Lindeman *et al.* 1960).

Naast de ruime interindividuele variatie van de Uosm, was er bij sommige honden ook een grote fluctuatie van de Uosm tijdens de dag, waarbij de intraindividuele variatiecoëfficiënt overeenkwam met de interindividuele variatiecoëfficiënt. Twee- tot drievoudige toe- of afnamen van de Uosm traden vaak binnen een tijdsbestek van twee uur op. De fluctuatie in Uosm kan wellicht verklaard worden door het effect van lichaamsbeweging en voeding op het drinkgedrag (O'Connor 1975, Meyer *et al.* 1994). Stimulatie van orofaryngeale receptoren geeft normaliter in een vroeg stadium verzadiging van de dorstbehoefte (Thrasher *et al.* 1981). Blijkbaar kan dit niet voorkomen dat sommige honden grote hoeveelheden water drinken, al leidde dit niet tot voor de eigenaar merkbare polyurie en polydipsie (PUPD). De bevindingen beschreven in **Hoofdstuk 3** hebben tot een andere diagnostische aanpak geleid van patiënten verdacht van PUPD. Verzameling van urinemonsters met een interval van twee uur voor de bepaling van Uosm kan bij sommige honden verder onderzoek voorkomen.

De resultaten van het onderzoek beschreven in **Hoofdstuk 4** laten een verminderde VP-afgifte zien bij honden met secundaire polycythemie als gevolg van een niertumor. Tijdens osmotische stimulatie met een hypertoon zoutinfuus was de drempelwaarde van de VP-afgifte verhoogd en de sensitiviteit van de respons laag. Polyurie en polydipsie zijn belangrijke klinische symptomen van secundaire polycythemie bij de hond (Peterson en Randolph 1983, Campbell

1990). De toegenomen massa van de rode bloedcellen leidt tot een stijging van de viscositeit van het bloed en een toegenomen bloedvolume (Peterson en Randolph 1983, Campbell 1990), hetgeen de baroreceptoren die de VP-afgifte beïnvloeden kan stimuleren. Het is inderdaad bekend dat een toegenomen bloedvolume de VP-respons op osmotische stimulatie vermindert, resulterend in een hogere drempel en een lagere sensitiviteit van de VP-afgifte (Robertson en Athar 1976, Quillen en Cowley 1983). Daarnaast kan het toegenomen bloedvolume bij secundaire polycythemie ook leiden tot een stijging van de afgifte van het atriale natriuretische peptide. Dit peptide remt niet alleen de gevoeligheid van de nieren voor VP, maar kan ook de VP-afgifte verzwakken (Dillingham en Anderson 1986, Iitake *et al.* 1986). Zowel het toegenomen bloedvolume, als de resulterende toename in het atriale natriuretische peptide kunnen dus de gestoorde osmoregulatie van de VP-afgifte bij honden met secundaire polycythemie verklaren.

Primaire polydipsie wordt gekenmerkt door een toename van de wateropname, die niet gerelateerd is aan een verhoogd vochtverlies (Dunn 1990). Derhalve is er geen defect in het concentrerend vermogen van de nieren, en kan de diagnose gesteld worden wanneer Uosm >1000 mOsm/kg bedraagt tijdens wateronthouding, terwijl de plasma osmolaliteit (Posm) binnen het referentiegebied blijft. De resultaten van het onderzoek beschreven in **Hoofdstuk 5** laten zien dat sommige honden met kenmerken van primaire polydipsie spontaan kunnen concentreren. Tijdens een dagcurve van Uosm (elke 2 uur gemeten gedurende de dag, en ’s nachts met een interval van 4 uur) treden er bij deze honden grote fluctuaties op van de Uosm, met op sommige momenten een Uosm >1000 mOsm/kg. Dit patroon van Uosm is vergelijkbaar met dat van enkele gezonde honden zonder merkbare PUPD (**Hoofdstuk 3**). Blijkbaar varieert de wateropname bij honden met primaire polydipsie sterker dan bij sommige gezonde honden. Op basis van de resultaten van de onderzoeken beschreven in **Hoofdstukken 3 en 5** kan geconcludeerd worden dat een dorstproef niet noodzakelijk is bij alle honden met polyurie. Wanneer er grote fluctuaties in Uosm optreden tijdens een dagcurve, en sommige waarden van Uosm >1000 mOsm/kg bedragen, lijkt de diagnose primaire polydipsie gerechtvaardigd.

Honden die voldoen aan de criteria van primaire polydipsie vertonen geen uniform beeld. Bij de honden die spontaan een goede urineconcentratie bereikten tijdens de dag, bleven Posm en de plasma natrium concentratie ($[Na^+]$) bij de ondergrens of in het midden van het referentiegebied. Andere honden daarentegen bereikten pas een Uosm van 1000 mOsm/kg tijdens wateronthouding, terwijl zowel Posm als $[Na^+]$ zich bij de bovengrens van de referentiegebied bevonden. Deze waarnemingen suggereren een tweedeling bij de groep honden met primaire polydipsie, zoals al eerder beschreven bij de mens: polydipsie met en zonder lage $[Na^+]$ (Goldman *et al.* 1996). Daarnaast was ook de VP-afgifte tijdens hypertoon

zoutinfuus niet uniform bij honden met primaire polydipsie, hoewel er een afwijking was bij alle honden. Naast een vertraagde VP-afgifte tijdens osmotische stimulatie, kwamen er ook episoden met hypersecretie voor. Bij sommige honden waren de initiële plasma-VP-concentraties tijdens wateronthouding en/of osmotische stimulatie hoog. Deze hoge waarden kunnen passen bij de plotselinge uitstoot van VP zoals bekend van het syndroom van onaangepaste VP-afgifte (SIADH) (Rijnberk *et al.* 1988), of ze kunnen passen bij een pulsatiele afgifte van VP. De afwijkende VP-afgifte tijdens osmotische stimulatie bij honden met een beeld passend bij primaire polydipsie kan geïnterpreteerd worden als een primaire afwijking van de regulatie van de VP-afgifte, maar kan ook het gevolg zijn van overhydratie passend bij een primaire afwijking in het drinkgedrag.

Verstoringen van de waterhomeostase leidend tot polyurie omvatten een groot scala aan endocriene, metabole en renale afwijkingen. Na het uitsluiten van het merendeel van deze oorzaken, resteert het diagnostische dilemma van het onderscheid tussen centrale diabetes insipidus, primaire polydipsie en nefrogene afwijkingen (Nichols 1992, Greco 2001). Het belangrijkste diagnostische hulpmiddel hierbij is de zoutbelasting, waarbij zowel Posm als plasma-VP-concentraties worden gemeten (Diederich *et al.* 2001). **Hoofdstuk 6** beschrijft 18 jonge honden met polyurie, ingedeeld in categorieën aan de hand van de plasma-VP-respons tijdens zoutbelasting. De VP-afgifte was bij alle honden afwijkend, waarbij er drie categorieën werden onderscheiden: (a) een hyperrespons, (b) een hyporespons, en (c) een non-lineaire respons met hoge VP-waarden los van stijgingen van de Posm. Vergelijkbaar met de situatie bij de honden met polyurie beschreven in **Hoofdstuk 5**, kunnen de hoge VP-waarden (tot 150 pmol/l) van de honden met een non-lineaire respons passen bij de grillige VP-afgifte zoals die bekend is van SIADH. In het bijzonder de vroege VP-pieken kunnen een afspiegeling zijn van een pulsatiel afgiftepatroon van VP, dat zowel fysiologisch kan zijn alsook geïnduceerd door de beginnende osmotische stimulatie. In overeenstemming met de resultaten van het onderzoek beschreven in **Hoofdstuk 5**, was de VP-afgifte tijdens osmotische stimulatie afwijkend bij de honden met variaties in Uosm passend bij primaire polydipsie. Bij het beoordelen van de samenhang tussen de VP-respons en de resultaten van de andere diagnostische testen (dagcurve, dorstproef, en de respons op desmopressine toediening), bleek dat de VP-respons tijdens osmotische stimulatie niet altijd onderscheid kon maken tussen verschillende klinische afwijkingen waarbij polyurie optreedt. In alle drie categorieën bevonden zich honden met onvoldoende concentrerend vermogen en honden met primaire polydipsie. Dit doet de vraag rijzen of VP-bepalingen tijdens hypertoon zoutinfuus wel de “gouden standaard” vormen voor de diagnostiek van polyurie bij de hond.

De resultaten van het onderzoek beschreven in **Hoofdstuk 7** laten zien dat VP bij de hond pulsatiel wordt afgegeven, met een grote variatie in het aantal pulsen, de pulsduur, de pulshoogte en de pulsamplitude. Het voorkomen van VP-pieken tijdens dehydratie en osmotische stimulatie was al eerder gerapporteerd bij de hond (Weitzman *et al.* 1977), maar de pulsatiele afgifte van VP onder basale omstandigheden was niet eerder beschreven. Met behulp van het Pulsar programma (Merriam en Wachter 1982) werden significante pulsen geïdentificeerd aan de hand van pulsduur en pulshoogte vanaf een berekende basaallijn. Naast de totale VP-afgifte en de pulskarakteristieken, konden ook de basale VP-afgifte en de pulsatiele VP-afgifte worden bepaald. Na twaalf uur wateronthouding waren de totale VP-afgifte, de basale VP-afgifte, het aantal significante VP-pulsen en de pulskarakteristieken identiek aan de basale situatie. Blijkbaar wordt het VP-afgiftepatroon niet zodanig beïnvloed door milde dehydratie dat er veranderingen worden gevonden wanneer met een interval van 2 minuten VP wordt gemeten. Tijdens osmotische stimulatie met hypertoon zout was er een duidelijke toename van de basale en de pulsatiele VP-afgifte. Het aantal VP-pulsen en de VP-pulshoogte en –amplitude waren significant hoger dan onder basale omstandigheden en tijdens wateronthouding. Aangezien het vochtverlies tijdens bloedafname gesubstitueerd werd, en de aanwezigheid van andere stimulerende factoren zoals misselijkheid onwaarschijnlijk lijkt, kan de toename van de pulsatiele afgifte van VP tijdens hypertoon zoutinfuus worden toegeschreven aan de stimulatie van osmoreceptoren.

Het pulsatiele afgiftepatroon van VP bij de hond verklaart (voor een deel) de non-lineaire VP-afgifte tijdens osmotische stimulatie bij de honden met polyurie beschreven in **Hoofdstukken 5 en 6**. Bij sommige van deze honden werden VP-pieken gemeten die niet proportioneel gerelateerd waren aan stijgingen van de Posm. Daarnaast was in sommige gevallen de initiële plasma-VP-concentratie voorafgaand aan wateronthouding of osmotische stimulatie al vrij hoog. Desalniettemin kan een pathologische oorzaak van deze VP-pieken niet geheel worden uitgesloten. De resultaten beschreven in **Hoofdstuk 7** laten zien dat bij gezonde honden VP-pulsen kunnen voorkomen die buiten het nomogram vallen dat de (lineaire) relatie tussen Posm en plasma-VP-concentratie beschrijft (Zerbe *et al.* 1980). Zulke VP-pulsen kunnen abusievelijk worden aangezien voor de grillige afgifte zoals bekend bij SIADH, waarmee een foutieve diagnose gesteld zou worden. Naast de bevinding dat de VP-respons tijdens osmotische stimulatie niet altijd onderscheid kan maken tussen verschillende klinische afwijkingen waarbij polyurie optreedt, zoals beschreven in **Hoofdstuk 6**, blijkt uit het onderzoek beschreven in **Hoofdstuk 7** ook dat het voorkomen van spontane VP-pulsen de interpretatie van de resultaten van de zoutbelasting ernstig kan bemoeilijken.

Hoofdstuk 8 beschrijft de ontwikkeling van een radioimmunoassay voor de bepaling van de uitscheiding van aquaporine-2 (AQP2) in hondenurine. Aquaporine-2, het VP-gereguleerde waterkanaal, bevindt zich in de apicale membraan en de intracellulaire vesikels van de verzamelbuiscellen in de nieren (Nielsen *et al.* 1993). Onderzoek bij ratten heeft aangetoond dat 3% van de totale hoeveelheid AQP2 in de nieren met de urine wordt uitgescheiden via een selectief apicaal pad (Rai *et al.* 1997). De basale uitscheiding van AQP2 in de urine van gezonde honden, uitgedrukt als de AQP2/kreatinine ratio, vertoonde dezelfde grote individuele variatie als de AQP2-uitscheiding bij mensen (Saito *et al.* 1997). Deze variatie berust waarschijnlijk op verschillen in basale hydratatiestatus, plasma-VP-concentraties en/of gevoeligheid van de nieren voor VP (Rai *et al.* 1997). De totale hoeveelheid AQP2 in de urine van de hond en de mens is van een vergelijkbaar niveau (Saito *et al.* 2001). De uitscheiding van AQP2 met de urine bij gezonde honden bleek een goede afspiegeling te zijn van de respons van de verzamelbuizen op VP, zowel bij waterbelasting als bij hypertoon zoutinfuus en intraveneuze desmopressine-toediening. Na orale waterbelasting nam de uitscheiding van AQP2 met de urine significant af, terwijl stimulatie door middel van endogeen en exogeen VP leidde tot een significante toename van de uitscheiding van AQP2. Deze bevindingen komen overeen met de resultaten van eerdere studies bij mensen (Saito *et al.* 1997, Pedersen *et al.* 2001). Bij immunohistochemisch onderzoek van nierweefsel van de hond werd uitgebreide AQP2-kleuring in het cytoplasma van de verzamelbuiscellen gevonden, passend bij de localisatie van AQP2 in de intracellulaire vesikels (Nielsen *et al.* 1993).

De uitscheiding van AQP2 met de urine lijkt een betrouwbare afspiegeling te zijn van de respons van de verzamelbuizen op VP tijdens fysiologische omstandigheden en bij stoornissen van de waterhomeostase bij mensen. Bij ziektebeelden waarbij vocht wordt vastgehouden is de uitscheiding van AQP2 met de urine verhoogd (Saito *et al.* 2001), terwijl verminderde uitscheiding van AQP2 met de urine wordt gevonden bij erfelijke en verkregen vormen van nefrogene diabetes insipidus waarbij de nieren minder gevoelig zijn voor VP (Deen *et al.* 1994, Wang *et al.* 2002). Daarnaast kan de uitscheiding van AQP2 met de urine ook bijdragen aan het onderscheid tussen diverse vormen van polyurie bij de mens, zoals bijvoorbeeld centrale diabetes insipidus, nefrogene diabetes insipidus en primaire polydipsie (Kanno *et al.* 1995). Ook bij de hond lijkt de uitscheiding van AQP2 met de urine een goede afspiegeling te zijn van de reactie van de verzamelbuizen op VP, zoals in dit onderzoek aangetoond aan de hand van de reacties op waterbelasting, osmotische stimulatie en intraveneuze desmopressinetoediening. Vergelijkbaar met de situatie bij mensen zou de bepaling van de uitscheiding van AQP2 met de urine wellicht waardevol kunnen zijn voor de diagnostiek van polyurie bij de hond, en dan vooral voor het onderscheid tussen (partiële) centrale diabetes insipidus, (partiële) nefrogene diabetes insipidus,

primaire polydipsie en SIADH. Zoals beschreven in **Hoofdstukken 5-7**, resteren er in diverse gevallen na interpretatie van de plasma-VP-concentraties tijdens osmotische stimulatie nog vragen. Voor de beantwoording van deze vragen zou de AQP2-uitscheiding met de urine van nut kunnen zijn.

De volgende conclusies kunnen worden getrokken:

- Aangezien er bij gezonde honden spontaan grote fluctuaties kunnen optreden in de Uosm, kan de documentatie van polyurie niet gebaseerd worden op de meting van Uosm in één enkel urinemonster.
- Wanneer de Uosm elke 2 uur wordt gemeten tijdens een dagcurve kan in sommige gevallen de diagnose primaire polydipsie al worden gesteld, en is verder onderzoek niet nodig.
- Polyurie bij honden met secundaire polycythemie als gevolg van een niertumor gaat gepaard met een afwijkende osmoregulatie van de VP-afgifte.
- Bij honden met kenmerken van primaire polydipsie kunnen verschillende afwijkingen van de VP-afgifte tijdens osmotische stimulatie worden gevonden.
- Aangezien de VP-respons tijdens osmotische stimulatie niet altijd onderscheid kan maken tussen verschillende klinische afwijkingen waarbij polyurie optreedt, is het twijfelachtig of VP-bepalingen tijdens hypertoon zoutinfuus als de “gouden standaard” beschouwd kunnen worden voor de diagnostiek van polyurie bij de hond.
- Bij de hond wordt VP in een pulsatiel patroon afgegeven met een grote variatie in het aantal pulsen, de pulsduur, de pulshoogte en de pulsamplitude.
- Milde dehydratie beïnvloedt het pulsatiele afgiftepatroon van VP bij de hond niet, terwijl osmotische stimulatie leidt tot een significante toename van de basale en pulsatiele VP-afgifte.
- Het voorkomen van spontane VP-pulsen kan de interpretatie van de curve, die het verband tussen de Posm en de plasma-VP-concentratie tijdens osmotische stimulatie beschrijft, bemoeilijken.
- Bij gezonde honden is de uitscheiding van AQP2 met de urine een goede afspiegeling van de reactie van de verzamelbuizen op VP.
- Bepaling van de AQP2-uitscheiding met de urine bij honden met polyurie kan waardevol zijn voor het onderscheid tussen (partiële) centrale diabetes insipidus, (partiële) nefrogene diabetes insipidus, primaire polydipsie en SIADH.

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Curriculum vitae

De schrijfster van dit proefschrift werd op 19 maart 1972 geboren in Amsterdam. De lagere school werd doorlopen aan de 'International Community School' in Zürich, Zwitserland. De middelbare schoolopleiding werd gestart aan het 'Freies Gymnasium' in dezelfde stad, en vervolgens voltooid aan het Christelijk Gymnasium te Utrecht (1984-1990). In 1990 startte de schrijfster met de studie diergeneeskunde aan de Faculteit der Diergeneeskunde van de Universiteit Utrecht. Tijdens deze studie nam ze in 1994 deel aan het 'Excellent tracé', waarbij ze gedurende 1 jaar onderzoek heeft verricht bij de Hoofdafdeling Geneeskunde van Gezelschapsdieren naar de achtergronden van polyurie bij de hond, afgerond met het diploma 'Master of Veterinary Research'. De studie diergeneeskunde werd in 1998 'cum laude' voltooid. Hierna werd schrijfster aangesteld bij de Hoofdafdeling Geneeskunde van Gezelschapsdieren voor een algemene klinische roulatie (1998-1999). Aansluitend volgde een klinische roulatie gericht op de interne geneeskunde van gezelschapsdieren bij dezelfde hoofdafdeling (1999-2000). Van 2001 tot 2003 heeft ze promotieonderzoek verricht naar de rol van vasopressine bij honden met polyurie. De schrijfster is in 2001 getrouwd met Ron Geerars en in 2002 moeder geworden van Jesse Michael.

The author of this thesis was born in Amsterdam, The Netherlands, on March 19th 1972. After primary school education at the International Community School in Zurich, Switzerland, secondary school education was received at the 'Freies Gymnasium' in Zurich and at the 'Christelijk Gymnasium' in Utrecht, The Netherlands (1984-1990). In 1990 the author started to study veterinary medicine at the Faculty of Veterinary Medicine of Utrecht University. In 1994 she was invited to participate in the Faculty's 'Excellent track' research programme. At the Department of Clinical Sciences of Companion Animals she investigated mechanisms underlying canine polyuria for 1 year, and received the MVSc degree. After graduating as a DVM with honours, the author completed a clinical internship in companion animal medicine (1998-1999), and thereafter she concentrated on internal medicine of companion animals at the same department (1999-2000). From 2001 to 2003 the author performed her PhD research on the role of vasopressin in canine polyuria. In 2001 the author married to Ron Geerars and in 2002 she became mother of Jesse Michael.

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