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

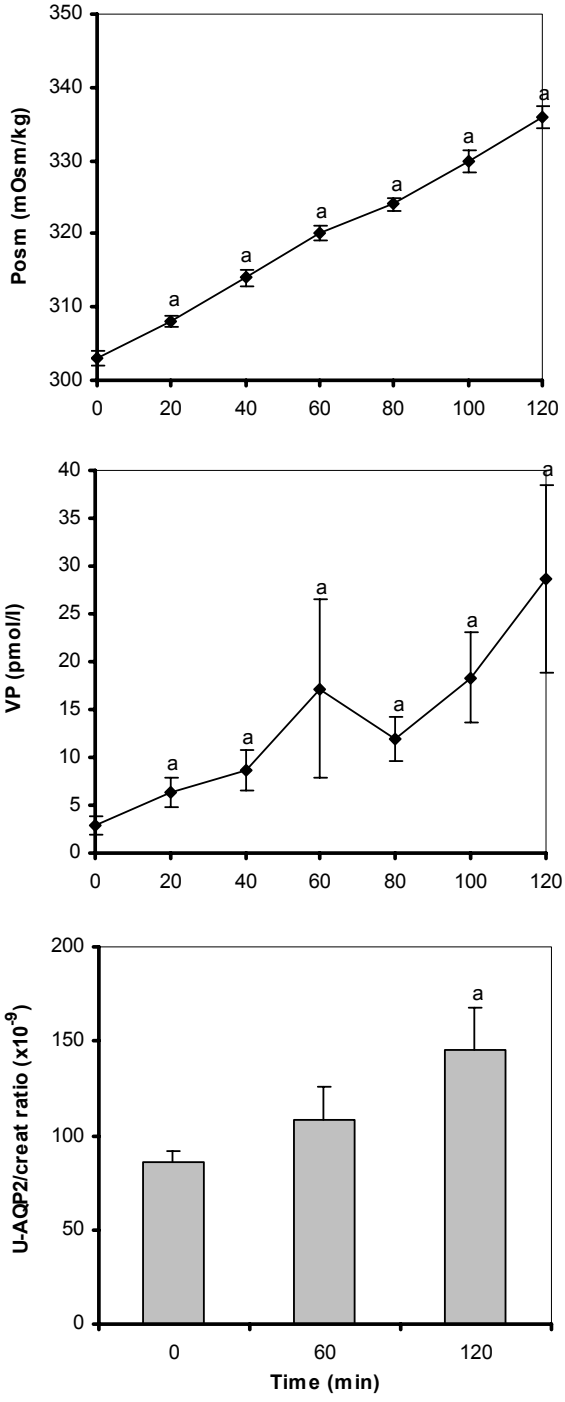

Figure 1. Plasma osmolality (Posm), plasma vasopressin (VP) concentration, and urinary aquaporin-2/creatinine (U-AQP2/ creat) ratio in 6 healthy beagle dogs under basal conditions and during 2 hours of intravenous osmotic stimulation with 20% NaCl infusion. Means ± SEM are shown. (a = significant difference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

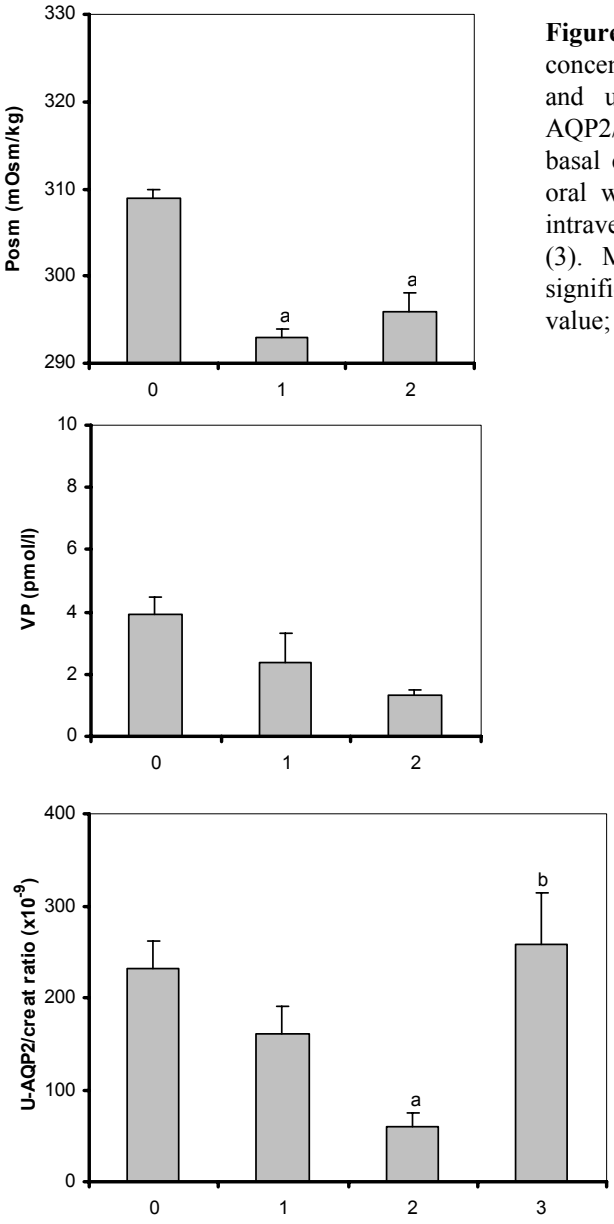

Figure 2. Plasma vasopressin (VP) concentration, plasma osmolality (Posm), and urinary aquaporin-2/creatinine (U-AQP2/creat) ratio in 4 healthy dogs under basal conditions (0), 1 and 2 hours after oral water loading (1 and 2), and after intravenous desmopressin administration (3). Means ± SEM are shown.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

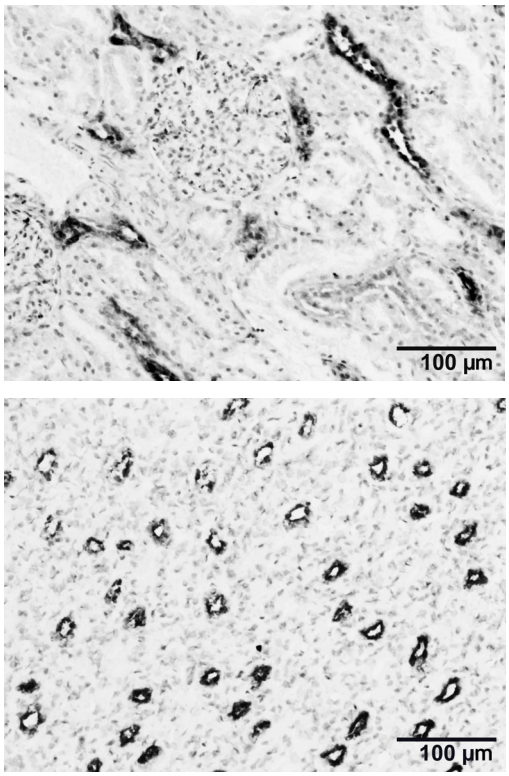

Figure 3. Immunohistochemical localisation of aquaporin-2 in 5-µm sections of a canine kidney. Upper panel: cortex, lower panel: papilla. Labeling was exclusively seen in the collecting duct cells, mainly in the apical and subapical region.

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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