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Effect of methadone on plasma arginine vasopressin level and urine production in conscious dogs

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The aim of this study was to examine the effect of i.v. methadone on the plasma arginine-vasopressin (AVP) levels and urine production in 9 conscious dogs. A highly significant increase from the baseline plasma AVP values of below 3 pg/ml occurred within 5 min following methadone administration. Maximum levels were reached within 30–50 min post-injection and varied from 18.5 to 100 pg/ml. A significant decrease in urine production was not seen under these experimental conditions. Mean arterial blood pressure did not change significantly during the experiment. Apart from the partial influence of the methadone-induced respiratory acidosis, we postulate a direct relationship between i.v. administration of methadone and the increased plasma AVP levels in dogs.

Methadone; Arginine-vasopressin; (Dog)

1. Introduction

The purpose of this study was to examine the possible effect of i.v. opiate administration on plasma arginine-vasopressin (AVP) level and urine production in the dog. To this date, only sparse and conflicting information about opiate-induced changes in AVP release in the dog has been presented since De Bodo's report in 1944 (De Bodo, 1944). Furthermore, due to variations in experimental species, states of hydration or mental awareness (conscious vs. anesthetized) and experimental protocol, extrapolation from a study on one species to one on another species is hazardous.

Montastruc et al. (1980) found a morphine-induced, naloxone-sensitive, antidiuresis in normal dogs but not in dogs with diabetes insipidus. However, experiments performed by Huidobro-Toro (1980) showed that the antidiuretic response following morphine administration was similar in Brattleboro and in normal rats. This would imply that AVP does not play a major role in the morphine-induced antidiuresis. Rockhold et al. (1983) reported a rise in plasma AVP after morphine administration in dogs. These authors postulated a causative relationship between the decreased blood pressure after opiate administration and the concurrent rise in plasma AVP levels.

We recorded plasma AVP levels and urine production in conscious euhydrated dogs after i.v. administration of methadone, like morphine, a μ -type opiate agonist (Iwamoto and Martin, 1981) frequently used in canine anesthesia.

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2. Materials and methods

2.1. Experimental animals

Nine adult mixed breed dogs (8 males and 1 female) were used for the experiment. Body weight varied between 14.3 and 38.0 kg with a median value of 26.5 kg. Food, but not water, was withheld for 18 h before the start of the experiment.

2.2. Experimental protocol

The dogs were trained to lie on their side during the experiment. Percutaneous placement of cephalic vein (Braunule, 18 gauge, B. Braun, Melsungen, West-Germany) and femoral artery (Secalon, 16 gauge, British Viggo, Swindon, Great Britain) catheters was performed. Insertion of the femoral catheter was facilitated by s.c. infiltration with 5 ml of a lidocaine HCl solution. The bladder was catheterised (Portex Ltd., Hythe, Kent, Great Britain) and emptied less than 2 min before the start of the experiment. Methadone (methadoni HCl, 10 mg/ml, Gist-Brocades, Delft, the Netherlands), in a dose of 1 mg/kg body weight, was injected into the cephalic vein. Blood samples were drawn in heparinized syringes via the arterial line at -1, 5, 10, 15, 20, 30 and 60 min. At this time (60 min) methadone was injected and the sampling sequence was repeated (65, 70, 75, 80, 90, 120 min) and extended by 2 additional samples at 150 and 180 min.

2.3. AVP extraction and radioimmunoassay

Plasma was frozen immediately after centrifugation and was stored at -80°C for serial assaying of AVP.

AVP extraction was based on a method using small columns packed with octadecasilyl silica (C18 Sep-Paks, Waters Assoc., Milford, MA, USA) as described previously (La Rochelle et al., 1980; Glanzer et al., 1984; Galard et al., 1985). Extraction recovery was determined by the addition of various concentrations of AVP to a plasma pool from a dog which had received a water load. The extraction efficiency amounted to $82 \pm 3\%$ (mean \pm S.D.).

The antiserum, 'WIE', raised against synthetic AVP showed the following cross-reactivities at 50% displacement of ^{125}I -AVP, AVP-(4-9): 100%; vasotocin: 100%; lysine-8-vasopressin: 10% and oxytocin: $< 0.01\%$, respectively. The intra-assay coefficient of variation averaged 5.9%. Synthetic AVP (Sigma, St. Louis, MO, USA) was used for preparing standards ranging from 0.25 to 250 pg/ml, in RIA buffer consisting of 0.1 M phosphate buffer (pH 7.5), 1 mM EDTA and 0.1% (w/v) HSA (Serva, Heidelberg, West-Germany). The RIA tubes, containing 0.2 ml standard or plasma extract and 0.1 ml antiserum (1 : 200 000 in assay buffer) were incubated for 20 h at 4°C . Subsequently, 100 μl of (3- ^{125}I -iodotyrosyl)-AVP (6250 d.p.m.; specific activity 2100 Ci/mmol, Amersham International, Great Britain) was added and incubated for another 20 h. For separation of bound and free tracer, 0.1 ml second antibody-coated cellulose (Saccel, Wellcome Laboratories) was added, mixed thoroughly and incubated for 30 min. After addition of 1 ml distilled water (4°C), the tubes were centrifuged at $3000 \times g$ for 15 min at 4°C . The supernatants were aspirated, and bound ^{125}I -AVP was estimated in a gamma counter. The detection limit of the assay was determined at 0.5 pg/ml AVP. The results were calculated from a linear regression curve obtained by logit-log transformation of the standard curve (Rodbard and Lawald, 1970).

2.4. Urine production and osmolality determination

Urine production was measured by a dilution method. For that purpose, a 20 ml volume of distilled water was instilled in the bladder which had been emptied at the beginning of the experiment. The water was labeled by means of 1 mCi of $^{99\text{m}}\text{Tc}$ -DTPA (diethylene triamine penta acetic acid). During refilling, the water was thoroughly mixed with the remainder of the bladder content by repeated removal and administration. Uptake of the radioactive isotope by the bladder wall was checked by repeated blood sampling; no specific radioactivity was detectable in these samples. A 1.04 ml sample was withdrawn at 15 min intervals from the bladder and its specific radioactivity was measured in a gamma counter (Gamma 4000,

Beckman, Fullerton, CA, USA).

The volume of the bladder content could be determined by comparison of the specific activity of the samples with a standard which was a known fraction of the amount of activity administered. The bladder content that remained after emptying was determined from a sample taken immediately after the start of the experiment. Taking this value and the predrawn volumes into account, the accumulated volume of the urine produced was then calculated as a function of time. The time derivative of this volume curve yields the urine production rate as a function of time.

A constant production rate would imply a linear increase in the accumulated volume with respect to time. Since this may not be assumed a priori, a polynomial regression of the third degree was applied to the accumulated volume data instead of a linear regression. The production rate was calculated as the time derivative of this polynomial.

The osmolality of the samples was measured by a vapor pressure method (Wescor Inc., Logon, Utah, USA). The osmolality of the urine produced was calculated as a function of time from these values and the results of the volume measurements.

2.5. Biochemical monitoring

All blood samples were processed immediately for blood gas and acid-base analysis (Acid Base Analyzer, Instrumentation Laboratory Type 1302, Allied, London, Great Britain) and Packed Cell Volume (PCV, microhematocrit method). After centrifugation at $1000 \times g$, serial determinations of plasma sodium, potassium and chloride concentrations were performed using flame photometry (Klinafame, Beckman, Fullerton, CA, USA) and colorimetry (Chlorocounter, Marius, Utrecht, the Netherlands), respectively. Plasma osmolality was measured with a vapor pressure method (Wescor Inc., Logon, UT, USA).

2.6. Hemodynamic monitoring

Pulsatile and mean arterial pressure were recorded on a Gould Brush 440 recorder (Gould,

Oxnard, CA, USA) by means of a Statham P23 ID (Gould, Oxnard, CA, USA) strain gauge transducer and a HP 78205 C amplifier (Hewlett Packard, Waltham, MA, USA). Heart rate was recorded from an ECG tracing, lead II.

2.7. Statistical analysis

The results were analyzed using the two-way analysis of variance and the Student-Newman-Keuls multiple comparison test. Differences were considered to be significant at the $P < 0.05$ level.

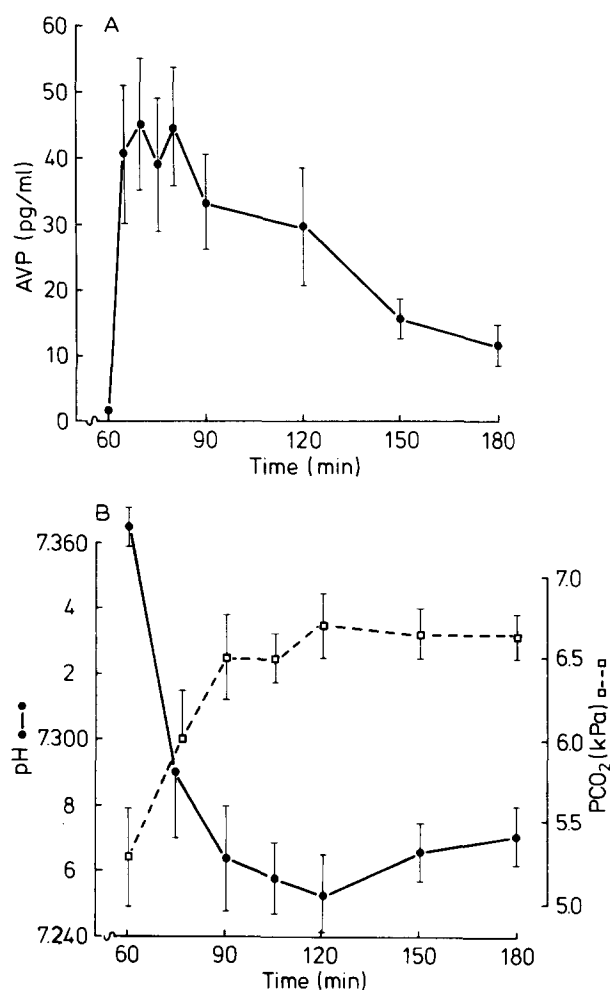


Fig. 1. (A) Effect of i.v. methadone administration (time 60) on plasma AVP levels. Data presented as means \pm S.E.M. (B) Effect of i.v. methadone administration (time 60) on arterial pH and pCO₂ levels. Data presented as means \pm S.E.M.

3. Results

3.1. Plasma AVP levels

In samples collected before methadone administration, plasma AVP levels varied from 0.9 to 2.5 pg/ml with an average of 1.7 ± 0.2 pg/ml. Within 5 min after methadone administration, the plasma AVP levels increased to or above 10 pg/ml AVP level (fig. 1A). Maximum levels were reached within 30-50 min post-injection and varied from 18.5 to 100 pg/ml. The mean maximum increase to 45.1 ± 10.4 pg/ml was reached 10 min post-injection. In 5 of the 9 dogs, the plasma AVP levels had not returned to below 5 pg/ml 2 h after injection of methadone. All values obtained after methadone administration were calculated to be significantly different from the baseline values.

3.2. Urine production and osmolality

The polynomial regression of the urine volume on time showed that the urine production rate increased slightly but significantly from a baseline value of 6.52 ± 1.35 ml/10 kg body weight/h (table 1). The urine osmolality data presented (table 1) are the osmolalities of the urine produced in the particular time interval. There were no significant differences from the baseline value of 1440 ± 87 mOsm/l.

TABLE 1

Values for urine production and osmolality before and after methadone administration at $t = 60$. Urine production, corrected for duration of time interval, is expressed as ml/10 kg body weight/h. Data presented as means \pm S.E.M.

Time interval (min)	Urine production (ml/10 kg body weight per h)	Osmolality (mOsm/l)
0- 60	6.52 ± 1.35	1440 ± 87
60- 75	7.24 ± 1.32	1311 ± 178
75- 90	7.56 ± 1.36	1454 ± 164
90-105	7.92 ± 1.40	1387 ± 111
105-120	8.24 ± 1.44	1897 ± 307
120-150	8.84 ± 1.66	1462 ± 163
150-180	9.62 ± 2.30	1401 ± 120

3.3. Biochemical monitoring

The basal PCV values for individual animals varied from 38 to 57% with an average of $44.8 \pm 1.0\%$. The PCV was significantly elevated following methadone administration. The group average rose to $48.1 \pm 0.7\%$. The baseline values (time 0) for plasma electrolytes (Na^+ -147.4 ± 0.7 meq/l, K^+ -3.6 ± 0.1 meq/l, Cl^- -107.7 ± 0.9 meq/l) and plasma osmolality (291 ± 2 mOsm/l) did not show any consistent significant change during the experimental procedure.

A primarily respiratory acidosis (fig. 1B) occurred in all experimental animals. The values for pH and arterial pCO_2 were significantly different at 15 min post-injection in 6 animals and at 30 min post-injection in 8 of the 9 animals. One hour after methadone injection, the pH had decreased to a mean of 7.253 ± 0.012 which was significantly different from the baseline value of 7.373 ± 0.004 . Arterial pCO_2 values were significantly elevated from a pre-injection mean value of 5.22 ± 0.03 kPa to 6.7 ± 0.2 kPa at 60 min post-injection. Arterial pO_2 and bicarbonate levels did not show any consistent significant change. Base excess values decreased slightly but significantly from -1.5 ± 0.1 to -4.4 ± 0.2 mmol/l prior to and following methadone administration respectively.

3.4. Hemodynamic monitoring

Mean arterial blood pressure calculated for the whole group showed no significant change from the baseline value of 117 ± 2.8 mm Hg during the experiment. The heart rate showed a significant and pronounced decrease in all animals. This occurred directly after methadone administration. Group averages for the heart rate were 101.5 ± 2.8 and 68.5 ± 2.5 beats per min before and after methadone administration, respectively.

4. Discussion

In order to study the relationship between opioid administration, plasma AVP levels and urine production in the dog, methadone was selected on account of its frequent use in canine

anesthesia and because it is, like morphine, a true representative of the opiate agonist group.

Plasma AVP levels were all within the physiological range of 0-5 pg/ml (Cowley, 1982) before methadone administration. Since plasma AVP levels remained above the basal value during the entire post-injection period and in view of the fact that the plasma half-life of AVP is relatively short (Lauson, 1974), the continuous release of AVP over the 2 h test period should be considered. Furthermore various reports on metabolic clearance of AVP (Harvey et al., 1967; Matsui et al., 1983; Share et al., 1985) rule out the possibility of a decreased metabolic clearance rate with high plasma AVP levels.

Despite the absence of a time control group, we conclude that the rise in plasma AVP level cannot be attributed to a physiological variation during the time of the experiment. This statement is based on the magnitude of the increase as well as on the lack of increased plasma AVP during the first (control) hour of the experiment.

Thus, the question remains as to which factor is the principal stimulus for the supra-normal elevation of the plasma AVP levels. A recent report by Rockhold et al. (1983) has designated the morphine-induced fall in arterial blood pressure as the main factor elevating plasma AVP levels. This is contradicted by results from other experiments performed in rats (Aziz et al., 1981; Haldar, 1982; Grell et al., 1985) and rabbits (Firemark and Weitzman, 1979). No substantial fall in arterial blood pressure could be shown in the present experiments. Therefore, a cause and effect relationship between arterial blood pressure and plasma AVP levels is very unlikely in our experiments.

A factor which itself could cause a rise in plasma AVP level is the increased arterial $p\text{CO}_2$. The AVP elevating properties of a hypercapnic and acidotic state have been well substantiated (Raff et al., 1983; Rose et al., 1984; 1985; Wang et al., 1984), although the 4.75-fold increase in AVP levels recorded in similar states of hypercapnic acidosis (Rose et al., 1985) is less than the rise we now saw. Considering the difference in magnitude of response and the difference in time course between plasma AVP levels (fig. 1A) and changes

in blood gas and acid-base variables (fig. 1B) in this experiment we postulate that other factors besides the contribution of the respiratory acidosis must be jointly responsible for the elevated AVP levels.

It has been postulated in several reports that opioids play a regulatory role in the release of endogenous AVP (Aziz et al., 1981; Rockhold et al., 1983; Firemark and Weitzman, 1979; Van Wimersma Greidanus et al., 1979), although disagreement exists as to whether it is an inhibitory or stimulatory role. Van Wimersma Greidanus and Ten Haaf (1984) concluded in a recent review that the literature favors a suppressive influence of opiates and opioid peptides on vasopressin release although there is great species variability. It would seem that under the conditions of the present experiment the influence of methadone in the dog is a stimulatory one which further augments the response due to respiratory acidosis. The question whether this opiate influence takes effect through binding to specific opiate receptors remains to be elucidated by a study of the effect of naloxone-mediated inhibition of opiate binding to opiate receptors.

AVP is considered to be one of the major determinants of urine production and urine osmolality. While the AVP levels in this experiment were 10 pg/ml or higher, a level considered sufficient for maximal antidiuresis, there was no significant decrease in urine production. The explanation for this phenomenon might be that the urine produced before methadone administration seemed maximally concentrated (1440 ± 87 mOsm/l). This implies that AVP, at least under these experimental circumstances, induces neither antidiuretic effect nor concentration of the urine produced. The fact that these experimental animals produced concentrated urine under basal conditions is a peculiarity which, in our opinion, was not due to the methodology but to the behavioral pattern of the dogs. Despite continuous access to drinking water, these animals drank only sufficient to maintain their fluid balance, with normal plasma AVP and osmolality levels as the result while they excreted a concentrated urine. The increase in urine production rate found during the experiment cannot be ascribed to changes in

plasma AVP level or to hemodynamic changes. Since this phenomenon could be reproduced under similar circumstances during a different experiment (manuscript in preparation) it cannot be considered an accidental finding. Further investigations are in progress to clarify the underlying mechanisms.

Fieldman et al. (1985) ascribed an important role to the tonicity of the renal medulla and suggested that urine production and composition was influenced more by electrolyte load than by plasma AVP concentration. The lack of a change in urine solute concentration in our experiments could not substantiate this possibility. Furthermore, altered renal perfusion, or even distribution of perfusion within the kidney, could cause alterations in urine production and composition. Several studies (Liard et al., 1982; Moursi et al., 1985) have shown that total renal perfusion was unaltered after an AVP infusion which raised plasma AVP levels well above the physiological limits. Investigations on the distribution of intrarenal bloodflow however revealed bloodflow shifts from outer cortex to inner cortex and outer medulla (Akatsuka et al., 1977). The role of angiotensin II and the newer atrial peptides in regulating urine production and electrolyte excretion will also have to be studied more closely.

The experimental procedure included the handling of fully to semiconscious animals. Emotional stress is stated to cause a rise in the plasma AVP level (Rydin and Verney, 1939) and PCV (Berns et al., 1979). However, the stability of PCV in the pre-methadone time period excludes stress as a significant factor in increasing PCV or plasma AVP values. The rise in PCV after methadone administration could have been induced by the respiratory acidosis, as reported by Raff et al. (1983). The moderately severe respiratory acidosis can be ascribed to the sedative and respiratory depressive effect of methadone (Short et al., 1970). The decrease in base excess, which was within the physiological limits for dogs at all times (Pickrell et al., 1971), could have been caused by a reduction in peripheral perfusion due to a changed perfusion pattern following methadone administration (Heyndrickx et al., 1976; Liard et al., 1982).

In agreement with investigations which incorporated extensive circulatory monitoring, the relative bradycardia can be at least partially accounted for by the increased AVP level (Malayan et al., 1980; Montani et al., 1980; Liard et al., 1981; Undesser et al., 1985). These reports show that when plasma AVP levels are increased to an extent comparable with the levels reached in this experiment, there is an increase in total peripheral resistance without concurrent increase in mean arterial pressure. This is caused by a fall in cardiac output which is postulated to be centrally mediated through direct interaction of AVP with the central nervous system structures, possibly involving baroreceptor reflex pathways (Montani et al., 1980; Liard et al., 1981). Furthermore, the parasympathomimetic effect of methadone causes a decrease in heart rate (Hall and Clark, 1983).

The absence of changes in the high pressure system however does not exclude the possibility of changes in the low pressure system (Henry et al., 1967; Bie and Warberg, 1983). This is of special interest in view of the regulation of AVP under daily circumstances, which is thought to be mediated primarily through the low pressure system (Henry et al., 1967). Future studies in which circulatory variables, such as central venous pressure, left atrial or pulmonary artery wedge pressure, cardiac output and total peripheral resistance, are monitored may shed some light on this matter.

In conclusion, we report here a supra-physiological elevation of plasma AVP levels after the i.v. administration of methadone to conscious dogs. We propose that this elevation is independent of any major blood pressure change and only partially dependent on the concomitant rise of arterial $p\text{CO}_2$. Plasma AVP does not exhibit an antidiuretic effect under these experimental circumstances, in which the animals receive no water load and are already producing maximally concentrated urine during the control period.

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