Osmoregulation in rats with long-term enhanced cerebrospinal fluid levels of vasopressin or vasoactive intestinal polypeptide

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ABSTRACT

The cerebrospinal fluid level of arginine vasopressin (AVP) or vasoactive intestinal polypeptide (VIP) was enhanced chronically by implantation of a device for controlled drug delivery in the lateral ventricle of the rat. Urine production, water consumption, urine osmolality as well as urinary AVP excretion were then measured for a period of 26 days. During this period the rats were studied under normal hydration and under conditions of osmotic stress induced by water deprivation (2 days) and the drinking of 2% (w/v) NaCl (6 days), in order to see whether the capacity of central systems to react adequately to osmotic stimuli was affected by high central peptide levels.

Immediately after the central AVP treatment was started, a temporary increase was found in urinary AVP levels which was not accompanied by a change in any of the other parameters but which decreased again to control levels within 10 days. After this burst of AVP excretion, AVP-treated rats showed a tendency during periods of normal hydration for a lower urine osmolality, combined with a higher water intake and urine production without changes in urinary AVP excretion. Since there was no clear-cut correlation between urinary AVP excretion and body water turnover, this could still indicate a slowly acquired and slight inhibition of pituitary AVP release by long-term centrally administered AVP. However, the capacity of these rats to respond to osmotic stimuli was not different from the controls.

In the VIP-treated rats a slight but significant reduction in urine production was found in all three periods of normal hydration. During the osmotic stress induced by the drinking of 2% NaCl the VIP-treated rats showed a lower increase in urinary AVP and fluid intake and a lower decrease in urine osmolality when compared with the response of the control rats. This has tentatively been interpreted as a potentiation by VIP of the activation of pituitary AVP release under these conditions.


INTRODUCTION

Vasopressin (AVP) is synthesized in the neuronal cell bodies of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus, and transported along axons to the posterior pituitary where it is stored and released in order to maintain body fluid homeostasis. In addition to the hypothalamo-neurohypophysial system, other sites in the central nervous system may also participate in the control of pituitary AVP secretion, for instance the septum and the periventricular area. Both these areas are, moreover, innervated by AVP fibres (De Vries, Buijs, Van Leeuwen et al. 1985). Their involvement is indicated by the observations that electrolytic lesioning of the medioventral septal area impaired basal and thirst-evoked AVP release (Iovino, Poenaru & Annunziato, 1983) and that after ablation of the periventricular tissue surrounding the anteroventral third ventricle, water balance and osmoregulation in rats was disturbed (Bealer, Crofton & Share, 1983). Therefore, in addition to the fact that central AVP is involved in brain hydration (Raichle & Grubb, 1978; Liszczak, Black & Foley, 1986; Rosenberg, Kyner, Fenstermacher & Patlak, 1986), it can be hypothesized that central AVP may be incorporated in body fluid regulation through an effect on pituitary AVP secretion. In several studies, therefore, the effect of central administration of AVP on body water metabolism has been examined. A central dipsogenic effect of centrally applied AVP has been reported (Szczepanska-Sadowska, Sobocinska & Sadowski,
1982) and release of pituitary AVP was shown to be inhibited following intracerebroventricular injection or infusion of AVP (Bhargava, Kulshrestha & Srivastava, 1977; Wang, Share & Crofton, 1982). However, in other studies no effects of such AVP treatments have been found on peripheral hydration and consummatory behaviour (Severs, Keil & Klase, 1978; Jerome, Barbella, Wurpel et al. 1983). Thus, a role of central AVP in osmoregulation is still a matter of dispute.

In the studies mentioned above, the effects were usually seen upon intracerebroventricular injection of AVP. Whenever AVP levels of cerebrospinal fluid (CSF) were continuously enhanced, this was always done under conditions of normal hydration and not during osmotic stimulation of the rat. The present study uses the latter approach, since in challenge situations the involvement of central AVP in the maintenance of body fluid homeostasis might be displayed more clearly. Levels of AVP in the CSF were raised permanently by implantation in the lateral ventricle of an AVP-loaded device for controlled drug delivery (Boer, Van der Woude, Kruisbrink & Van Heerikhuize, 1984). Previously it has been shown immunocytochemically that AVP released from such implants reaches a great number of brain areas (Ravid, Swaab, Van der Woude & Boer, 1986). Thereafter, body water turnover as well as urinary AVP excretion were followed in control situations and during osmotic stress. Additionally, under the same conditions, the central effect of vasoactive intestinal polypeptide (VIP) was studied to see whether the antidiuretic effect of VIP that has been reported previously (Itoh & Yoshikawa, 1985) could be confirmed and extended to other aspects of body water regulation.

MATERIALS AND METHODS

Animals and chemicals

Eighteen adult male Wistar rats (approximately 220 g body weight) were obtained from the Broekman Institute, Leuven, Belgium. Throughout the experiment the rats were housed individually in metabolism cages, under standard conditions (21 °C, lights on from 07.00 to 19.00 h, water and food available ad libitum) unless otherwise indicated. Synthetic AVP was obtained from Sigma (V9879; St Louis, MO, U.S.A.) and VIP from Biologicals Ltd (no. 1340; Cambridge, U.K.).

Implantation

After a simultaneous control period of 2 days, the 18 rats were randomly divided into three groups of six rats. Each group was given, under Hypnorm (Duphar, Weesp, The Netherlands) anaesthesia (0.1 ml/100 g body weight), a control, an AVP- or a VIP-loaded implant, as described previously (Boer et al. 1984). After the implantation, the rats were allowed a 5-day period of normal hydration (during this period one of the AVP-implanted rats proved to be ill and was killed). Thereafter the rats were dehydrated for 2 days by removing the water bottles. After a rehydration period of 4 days, the rats were given 2% (w/v) NaCl as drinking water from day 12 to day 17 after the operation and subsequently studied for another 9 control days.

Peptide levels in the CSF were raised by implantation in the lateral ventricle of an Accurel/collodion mini-device for controlled drug delivery. These devices were made of 5 mm pieces of Accurel polypropylene (P 9/80/1; 0.95 mm outer diameter, 0.6 mm inner diameter, kindly supplied by Mr D. Heitmann, ENKA Research Institute, Obnernburg, F.R.G.). Pieces were heat-sealed at one end and filled with 1 μl water (control), AVP or VIP in water (20 μg/μl), heat-sealed to close them and dip-coated with a membrane of collodion (Kruisbrink & Boer, 1984). The in-vivo release of these devices is, under the conditions used here, about 140 ng/day (Kruisbrink, Mirmiran, Van der Woude & Boer, 1987).

During the whole 4-week period, body weights were monitored every third day. Water intake and urine production were measured daily. Urine samples were stored at −20 °C until assay of osmolality and AVP content. Urine osmolality was determined by freezing point depression (Osmomat 030; Gonotec, Berlin, F.R.G.) and AVP content of the urine was determined by radioimmunoassay (see below).

After the entire experimental period, CSF samples of at least 100 μl were collected from the cisterna magna of the rats under Hypnorm anaesthesia by a direct puncture approach with a 25-gauge needle mounted on a standard syringe. These samples were completely free of contamination with blood and were again stored at −20 °C until assay for AVP (see below).

Extraction and assay of urinary AVP

Small columns packed with octadecasilyl-silica (C18 Sep-Pak; Waters Assoc., Inc., Milford, MA, U.S.A.) were used for extraction of AVP. Each Sep-Pak was attached to a 10 ml syringe and placed vertically in a specially designed mechanically driven extraction apparatus by which all fluids can be sucked up and pushed through 10 columns at the same time at the same speed (8 ml/min). This guarantees a reproducible extraction process for all samples and, moreover, a large number of samples can be processed within a
short period. The following steps (see LaRochelle, North & Stern, 1980) were applied: (a) the Sep-Pak columns were washed sequentially with 5 ml urea (8 mol/l), 20 ml water, 5 ml methanol and 20 ml water, (b) the urine samples (0.5 ml diluted with 4.5 ml 0.1 mol HCl/l) were loaded into the syringe and pushed more slowly than the other fluids (4 ml/min) through the column, (c) the columns were rinsed with 20 ml 4% acetic acid, (d) peptide fractions were obtained by elution with 4 ml methanol and collected in polystyrene or polypropylene tubes and (e) evaporated overnight in a Savant Speed Vac Concentrator.

The dried samples were redissolved in 250 μl assay buffer (0.14 mol NaCl/l, 1 mmol EDTA/l, 2 g bovine serum albumin/l and 0.05 mol Tris–HCl/l; pH 8.0). Except for the use of this buffer, the assay was performed as described previously (Dogterom, Van Wimersma Greidanus & De Wied, 1978). Recovery of AVP from rat urine averaged 75±8% (s.e.m., n=6).

RESULTS

On the 2 days before surgery no differences were measured between the three groups of rats in the four parameters investigated: urine production, water intake, urinary osmolality and AVP excretion. Both basal urinary osmolality and AVP content gradually decreased in periods of normal hydration in all rats over the 4-week period of the experiment. For control rats, the data from the first and last 2 days of the experiment are shown in Table 1. Body weights of the rats showed no differences between the groups during the experiment, and the average body weight (+ s.e.m.) of the control rats (n=6) increased from 227±3 g on day 2 before operation to 305±5 g on day 26 after the operation. In AVP-treated rats (n=5) these values were 221±2 and 300±3 g and in VIP-treated rats (n=6) 227±2 and 310±6 g respectively.

Effect of AVP

Rats implanted with a 20 μg AVP-loaded Accurcel device in the lateral ventricle had decreased urine production compared with control rats (−14% average decrease over 5 days; F(1,36)=10, P<0.005; Fig. 1), but no significant differences were found on individual days after operation. However, no concomitant effect was found on water intake (Fig. 2) or urine osmolality (Fig. 3) during the 5 days following the implantation of an AVP-loaded Accurcel device. Total 24-h AVP excretion in the urine was significantly increased (F(1,36)=51, P<0.001) in this period from 2.2±0.2 (5-day average ± s.e.m.) in control rats to 8.2±1.4 pmol in AVP-treated rats. However, this effect rapidly decreased in this first 5-day period of normal hydration (Fig. 4).

As a result of the 2-day period of water deprivation (days 6 and 7 after implantation), urine production decreased in the control rats while osmolality and

### TABLE 1. Water intake, urine production, urine osmolality and urinary vasopressin (AVP) excretion in control rats on the day before implantation and on the first and last 2 days of the period after implantation of a water-loaded control Accurcel/collodion device in the lateral ventricle. Values are means ± s.e.m.; n=6

<table>
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<tr>
<th>Day</th>
<th>Water intake (ml/100 g per 24 h)</th>
<th>Urine production (ml/100 g per 24 h)</th>
<th>Urine osmolality (mOsm/kg H₂O)</th>
<th>Urinary AVP (pmol/24 h)</th>
</tr>
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<td>−1</td>
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<td>5.8±0.4</td>
<td>1760±90</td>
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<tr>
<td>2</td>
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<td>7.6±0.4</td>
<td>1575±56</td>
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<tr>
<td>25</td>
<td>10.9±0.8</td>
<td>6.5±0.5</td>
<td>1190±50*</td>
<td>ND</td>
</tr>
<tr>
<td>26</td>
<td>10.4±0.7*</td>
<td>5.6±0.4</td>
<td>1290±75*</td>
<td>0.5±0.2*</td>
</tr>
</tbody>
</table>

*P<0.05 compared with day −1, 1 and day 2 (Student's t-test). ND, not determined.

Statistics

Two-way analysis of variance (ANOVA) for control compared with peptide treatment was used to test for significant changes over time in the different periods. These periods were: days 1–5, days 9–11 and days 19–26 for normal hydration, days 6–7 for dehydration and days 12–17 for when the rats were given 2% NaCl to drink. Student's t-test was used to test differences between groups on each day. P<0.05 was taken as the level of significance in all cases.
AVP content of the urine increased. The AVP-treated group behaved like the controls in this period as well as on the first day of rehydration. The increased AVP excretion found in the first 5 days after central AVP application was then no longer detectable. Also, on the next 3 days of rehydration (days 9-11 after operation) differences were absent for water intake and urine production but osmolality of the urine was decreased in the AVP-treated rats (from 1386 ± 61 to 1240 ± 55 mOsm/kg; F(1.27) = 7.4, P < 0.05), without
FIGURE 3. Urine osmolality responses in normally hydrated and osmotically stressed Wistar rats given an Accurel/collodion implant containing water, vasopressin (AVP) or vasoactive intestinal polypeptide (VIP). Implantation is shown by the arrow and the period of water deprivation by the solid bar. *P < 0.05 for AVP compared with controls; †P < 0.05 for VIP compared with controls (Student’s t-test).

FIGURE 4. Daily excretion of vasopressin (AVP) in the urine in normally hydrated and osmotically stressed Wistar rats given an Accurel/collodion implant containing water, AVP or vasoactive intestinal polypeptide (VIP). Implantation is shown by the arrow and the period of water deprivation by the solid bar. *P < 0.05 for AVP compared with controls (Student’s t-test).
significant differences on individual days. Urinary content of AVP was again increased compared with controls (2-4 ± 0-4 vs 1-4 ± 0-3 pmol/24 h; F(1,18) = 6-4, P < 0-05; Fig. 4). The osmotic challenge by drinking 2% NaCl for 6 days resulted, in control rats, in a steadily increasing fluid intake and urine production while urine osmolality slowly decreased. In AVP-treated rats, urine osmolality decreased slightly more in this period (average -8%; F(1,63) = 8-4, P < 0-01; Fig. 3) but the other parameters responded as seen in the control group. During the last control period (days 19–26), urine production remained increased compared with controls (average 18%; F(1,72) = 9-7, P < 0-005) and this was accompanied by an increase in water intake (average 13%; F(1,72) = 6-8, P < 0-05) and a lower urine osmolality (average -10%; F(1,72) = 7-9, P < 0-01). No differences were found in the total 24-h AVP excretion in the urine in this period.

Vasopressin content of CSF from control rats was below the detection limit (< 10 pmol/l) whereas, in the five AVP-treated rats, an AVP level in CSF of 355 ± 87 pmol/l was measured on the last day of the experiment.

**Effect of VIP**

After implantation of a 20 μg VIP-loaded Accurel device in the lateral ventricle urine production decreased when compared with controls (average -7%; F(1,40) = 6-5, P < 0-05; Fig. 1), but with no significant differences on individual days. There were no differences in either water intake (Fig. 2) or urine osmolality (Fig. 3). Total 24-h AVP content of the urine showed a small increase from 2-2 ± 0-2 to 2-9 ± 0-2 pmol over the first 5 days after operation (F(1,40) = 11-4, P < 0-005; Fig. 4). However, this difference was already present on the 2 days before the operation. During neither the 2 days of dehydration nor on the first day of rehydration were different responses found in any of the parameters investigated compared with the controls. During the following 3 control days urine production was decreased again (average -14%; F(1,30) = 11-1, P < 0-005) showing a significant difference on day 9. During the period when 2% NaCl was given as drinking water urine production and fluid intake remained lower than the control response for 25% (F(1,70) = 14-7, P < 0-001) and 18% (F(1,70) = 11-1, P < 0-005) respectively, although on individual days no significant differences were found. The same delayed response was found for urine osmolality (average 14%; F(1,70) = 19-1, P < 0-001) with significant differences on the first, second and third day of salt drinking. No significant differences were found in the 24-h urinary AVP content during this period. In the final control period urine production once again showed a decrease (average -15%; F(1,80) = 21-2, P < 0-001), but this time it was accompanied by a higher urine osmolality (average 10%; F(1,80) = 14-1, P < 0-001). Water intake and urinary AVP content were again not significantly affected.

**DISCUSSION**

Throughout the 29 days that the rats were housed in the metabolism cages, urine production and water intake did not change in the control rats during the three periods of normal hydration. The slow shift (up to -29%) of the urinary osmolality during the experiment was therefore a peculiar finding. Although it fits with the concomitant decrease (-79%) of the urinary AVP excretion, no clear explanation is available. It may point to a slow adaptation of the rat to the isolation and limited space of the metabolism cage. However, it does show that a change in urinary AVP excretion is not necessarily correlated with a change in body water turnover (see below). All animals were subjected to both dehydration and the salt stimulus and therefore the effect of the previous treatment and the passage of time may have complicated the results.

However, comparisons were always made with the control group receiving the same treatment.

**Control responses**

The rats with the control implants responded during water deprivation in a manner similar to that which has been described by others (Jones & Pickering, 1969; Miller & Moses, 1971). Under this condition, an increased synthesis of AVP has been found in the SON and PVN (Majzoub, 1985), together with an increased release of AVP from the pituitary, resulting in a several-fold enhanced plasma AVP level (Mens, Bouman, Bakker & Van Wimersma Greidanus, 1980). The increase in urinary AVP excretion after water deprivation reported by Miller & Moses (1971) was also found in this study. However, upon saline stress, which is reported to increase AVP synthesis (Burkett, De Hoop, Schmale et al. 1984) and plasma AVP levels (Zerbe & Palkovits, 1984), we did not find an enhanced urinary AVP excretion. However, in the present study, the rats drank a steadily increasing amount of liquid. Under these conditions, the renal excretory capacity of the rat might be such that sufficient free water could be retained from 2% saline and excess sodium excreted to prevent either dehydration or significant volume expansion. This is supported by the fact that in this study the rats did not reach the state of negative fluid balance (urine osmolality was always greater than that of the 2% saline imbibed) and consequently no increased AVP
release was then evoked. Further investigations should be carried out to ascertain whether this effect is found in rats that are put directly onto the salt stimulus and have no implant in their brains.

**Effect of AVP**

It was previously shown (Kruisbrink et al. 1987) that within 24 h of the intracerebroventricular placement of the AVP/Accurel/collodion mini-implant, a constant level of AVP in the CSF, which lasted for several weeks, was obtained. The presently observed enhanced AVP levels of approximately 300 pmol/l detected 26 days after implantation are fully comparable with the previous findings obtained for periods of up to 3 weeks (Kruisbrink et al. 1986, 1987). Thus, long-term and constant delivery of AVP (approx. 140 ng/24 h; see Kruisbrink et al. 1987), as well as constant levels of AVP in the ventricular compartment, can be assumed throughout the 26-day test period.

No dramatic changes were seen in body water metabolism during the first 5 days of enhanced levels of AVP in CSF. This confirmed the data of Jerome et al. (1983) who infused AVP into the third ventricle of conscious normally hydrated rats at rates of 0.5 or 50 ng/h for 5 h or 5 days (present 'infusion' rate was approx. 5 ng/h). The present observations were made in spite of the average threefold increase in daily urinary excretion of AVP in this period. The enhanced excretion, however, decreased within 10 days to control levels (Fig. 4) and might therefore be in agreement with the study mentioned above: at the end of a 5-h infusion period of 50 ng/h, plasma AVP had risen from 3-5 to 21 pmol/l, whereas after a 5-day infusion period no changes were detectable compared with control saline infusion (Jerome et al. 1983). It has been reported previously (Rolls, 1971) that enhanced plasma AVP levels obtained by intravenous infusion rates of up to 6 ng/h failed to alter drinking behaviour and urine production in the normally hydrated rat. Thus, the supposedly enhanced plasma levels of AVP reported by Rolls (1971) and in the present study at the onset of the intracerebroventricular treatment with AVP failed to alter body water metabolism. Apparently unknown mechanisms are involved that offset the expected decline of urine output when plasma AVP levels are increased (see Chan, 1971; Jerome et al. 1983). The temporarily enhanced peripheral AVP levels, as presently measured by urinary AVP excretion, are not likely to be derived directly from the AVP implant in the brain. First of all, the concentration of AVP in CSF remained constant throughout the test period (see above). Moreover, it can be calculated, using the fractional excretion of 5% as reported previously (Kruisbrink & Boer, 1986) that on day 1 after implantation, roughly 140 ng AVP more than control levels is cleared by the kidney. All centrally released AVP would, by then, have passed intact from the brain into the peripheral circulation, which is not very likely. However, some temporarily enhanced passage of AVP from the brain to the periphery due to the implantation procedure cannot be excluded (see Ravid et al. 1986).

The rats with centrally enhanced AVP can clearly cope with the osmotic stress of water deprivation and 2% salt drinking, giving responses not different from control rats. Therefore, under these conditions, a role for CSF AVP in body osmoregulation must be discarded.

**Effect of VIP**

An effect of VIP on osmoregulation was suggested since after intracarotid infusion of mg amounts of VIP, AVP was released from the pituitary (Ottesen, Hansen, Fahrenkrug & Fuchs, 1984). VIP is, moreover, found in the posterior pituitary (van Noorden, Polak, Bloom & Bryant, 1979) and VIP receptors are widely distributed in the central nervous system including the SON and PVN (Loren, Emson, Fahrenkrug et al. 1976; Shaffer & Moody, 1986). Itoh & Yoshikawa (1985) recently showed that intracerebroventricular injection of 2-10 μg VIP decreased water intake in dehydrated rats, an effect that was not seen after subcutaneous injection of 25 μg. In the present study centrally enhanced VIP was found to decrease urine production in all the periods of normal hydration. Although a much lower dose of VIP was used, this result is in agreement with the reported antidiipsogenic effect of central VIP. Although no assay was available to detect the level of VIP in the CSF, it could be derived from in-vitro studies with 125I-labeled VIP, that the release rate of VIP is comparable to that of AVP (see Kruisbrink et al. 1987).

During 2% salt drinking (not during water deprivation) important differences were observed in the responses of the VIP-infused rats. Urine production lagged behind and urine osmolality decreased much less compared with the control rats and this was still visible during the first 3 days of rehydration. Thus the VIP-treated rats responded less to the salt loading, which could argue for potentiation by VIP of the activation of the pituitary AVP release. To prove this hypothesis, assay of AVP in blood will be necessary after central administration of VIP. VIP may, however, also play a role as an inhibitor of thirst in the control of fluid intake during the salt-loading via an unknown mechanism. This should be comparable to its antagonistic effect of the dipsogenic action of angiotensin II (Itoh & Yoshikawa, 1985). To our knowledge, this is the first finding of osmoregulatory effects of central VIP when applied continuously.
Future studies will have to elucidate further the functional significance of endogenous VIP in central osmoregulatory processes.

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