Efferent role of ADH in CNS-induced natriuresis

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WE HAVE PREVIOUSLY SHOWN that ventriculocisternal perfusion (VCP) or artificial cerebrospinal fluid (CSF) was performed in pentobarbital-anesthetized dogs. Renal function was studied in protocols consisting of a 1-h experimental period in which the animals received either CSF with an elevated sodium concentration (300 mM, high Na) via VCP or antidiuretic hormone (ADH) intravenously, bracketed by 1-h control and recovery periods. High Na VCP caused an increase in plasma ADH measured by radioimmunoassay (to 176% of control) that coincided with a natriuresis (to 180% of control). In a second set of experiments, these changes in endogenous ADH were mimicked experimentally with intravenous infusions of synthetic ADH in animals receiving continuous VCP with normal sodium artificial CSF. The dose response relationship between plasma ADH and urinary sodium excretion for the intravenous ADH experiments was not different from the relationship for those experiments in which ADH was elevated as a consequence of high Na VCP. These results suggest that ADH causes part, if not all, the natriuresis induced by high Na VCP.

Antidiuretic hormone (ADH) seems likely to be the hormonal mediator of CNS-induced natriuresis. There is strong evidence that the concentration of plasma ADH increases along with sodium excretion when CSF sodium is increased (1). Moreover, intravenous infusion or injection of ADH has been shown to produce natriuresis in sheep, cats, and dogs (11, 17, 25). However, it is not known whether the ADH increments in response to increased CSF sodium are capable of producing any or all of the observed natriuresis.

To investigate the role of ADH in CNS-induced natriuresis, changes in ADH that occur during high Na VCP experiments were mimicked in the same series of animals by intravenous infusion of ADH. We show that the dose-response relationship between plasma ADH and urinary sodium excretion during intravenous infusion of ADH was not different from the relationship during experiments in which ADH was elevated as a consequence of high Na VCP. These results suggest that ADH mediates at least a portion of the natriuresis induced by high Na VCP in anesthetized dogs.

METHODS

Animals and surgery. Mongrel dogs weighing 15–22 kg were studied. The animals had free access to water and standard laboratory chow prior to the experiment. They were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and given supplemental doses as needed during the experiment. An endotracheal tube was inserted to maintain an airway. The methods for VCP have been previously described in detail (21, 22). Briefly, sterile artificial CSF (excluding sterile glucose, which was added at the time of the experiment) was prepared by the Pharmacy Laboratory of the University of Michigan Hospital. High Na CSF (300 mM Na+) was made by adding desiccated NaCl to artificial CSF with a normal sodium concentration (150 mM). Needles were stereotaxically placed and held in the lateral ventricle (21-gauge) and cisterna magna (18-gauge). The tip of the outflow tubing from the cisterna magna was placed at the level of the external auditory meatus, which maintained CSF pressure slightly above atmospheric pressure (0-5 cmH2O) as determined by the pressure at the tip of the infusion needle. The artificial CSF was perfused at a rate of 100 μl/min (Harvard Apparatus pump).

Electrocautery was used to make all incisions. The right ureter (PE-160; Clay Adams Intramedic) was catheterized via a right flank incision. Both cephalic veins (PE-160) and the left femoral artery (PE-240) were cath...
eterized. All blood samples were taken from the femoral artery via a three-way valve in the arterial line. Both mean blood pressure (MAP) and CSF pressure were measured and recorded with pressure transducers (Statham) and a Grass polygraph. A rectal thermometer was inserted to monitor body temperature.

At the end of the protocol, methylene blue dye (saturated solution) was perfused (VCP) in order to mark the path of the perfusates. The carotid arteries were then cannulated to flush the vasculature with saline and preserve and harden the brain with 10% Formalin. The brains were later removed, sliced, photographed, and examined for proper needle placement (ventricular staining) and any abnormalities such as hemorrhage.

Analytical techniques. A radioimmunoassay for arginine vasopressin (ADH) was developed in our laboratory using a rabbit antiserum (courtesy of Dr. Gary Robert-son) that was raised as described by Oyama et al. (23). The assay buffer was the same as that described by Skowski et al. (27). 125I-labeled AVP (New England Nuclear) was used as tracer. Bound ADH was separated by the addition of rabbit gamma globulin (Miles Laboratories, fraction II) and polyethylene glycol 6,000 (J. T. Baker). Synthetic arginine vasopressin (Sigma, 350 IU/mg) was used for the standard curve. There was no detectable cross-reactivity with oxytocin, vasotocin, or ACTH and no interference from unknown plasma factors as determined by assaying serial dilutions of plasma and by assaying plasma from water-loaded animals.

ADH was extracted from plasma samples (2 ml) with octadecylsilane cartridges (Waters Associates, Sep-pak C18) using the modified method of LaRochelle et al. (14) (90% ethanol was substituted for 75% acetonitrile in the elution step). The dried extract was reconstituted to 1 ml in assay buffer and assayed in triplicate. Two milli liter plasma samples were used, and in that volume the assay could detect 1.25 pg/ml.

Arterial plasma renin activity (PRA) was measured using a New England Nuclear angiotensin I kit; the kit buffer was replaced by 2 M maleic acid buffer (pH 5.5). Creatinine and p-aminohippuric acid (PAH) were measured in plasma and urine using standard photometric methods (4, 28). Potassium and sodium were measured in plasma, urine, and CSF by flame photometry (Instrumentation Laboratory). Hematocrit was measured in microcapillary tubes.

Experimental protocols. After surgical preparation, VCP was performed continuously, and the animals received a continuous intravenous infusion of two saline solutions, each at a rate of 0.1 ml/min (Harvard pump). One solution contained 20 mg/ml PAH and 6 mg/ml creatinine. A prime of 10 ml was given just before the infusion was started. The other solution was either saline (0.9%) or saline with synthetic ADII (Sigma). There was a 1-h equilibration period before the protocol was started.

A 1-h experimental period was bracketed by a 1-h control period and 1.5-h recovery period. In all experiments, normal sodium artificial CSF was perfused (VCP) and saline was infused intravenously during the control and recovery periods. During the experimental period, in the high Na VCP experiments, the intravenous saline infusion was not changed but the normal CSF was switched to CSF containing 300 mM Na+. In the intravenous ADH experiments, the normal artificial CSF was not changed but the intravenous infusate was switched to a saline solution with ADH (3-50 mU·kg⁻¹·h⁻¹).

Continuous 15-min urine and CSF collections were made throughout the protocol. Blood samples (9 ml in sodium heparin for ADH and 7 ml in EDTA for all other assays) were taken at the midpoint of the last two 15-min clearance periods of the control, experimental, and recovery periods and were immediately replaced by an equal volume of 6% dextran (72,000 mol wt) in saline (0.9%). The blood samples were then chilled and centrifuged; the plasma was frozen immediately and stored at −20°C.

Results for grouped values are presented as means ± SE. Student's t test was used to compare group means, and paired sample analysis was used when appropriate. Bonferroni corrections were performed on the P values because three tests were performed for each variable. A probability level greater than 0.05 was considered not significant.

RESULTS

The measured variables for 10 high Na VCP experiments in 10 dogs (18.8 ± 0.56 kg) are summarized in Table 1; the variables for eight intravenous ADH experiments in six dogs (17.9 ± 0.83 kg) are summarized in Table 2. Sodium excretion, MAP, heart rate (HR), PRA, and GFR for both experimental groups are plotted as a function of time in Fig. 1.

Sodium, potassium, and water excretion. Both high Na VCP and intravenous ADH produced large increases in urinary sodium excretion (UNaV) (72 and 43%, respectively), urinary potassium excretion (UKV) (23 and 39%, respectively) and urine flow (41 and 21%, respectively) (Fig. 1; see Tables 1 and 2 for means and P values). These variables returned toward control values during the recovery period with the exception of urine flow and UKV in the intravenous ADH group. There were no significant differences in control values between groups for any of these variables.

ADH. In the first eight high Na VCP experiments and two of the intravenous ADII experiments, plasma ADII was determined at the middle of all four 15-min clearance periods of the experimental period (Fig. 2). In the remaining experiments ADH was measured only in the last two clearances of the experimental period. For all experiments in both experimental groups, ADH increased during the experimental period and fell during the recovery period (Tables 1 and 2). Therefore, during the experimental period the ADH values were significantly greater than the average of control and recovery values in both groups. The mean values of ADH during the control period for the two groups were not different. During the experimental period the mean values for the two groups were also not different.

The dose-response relationships between ADH and UNaV when ADH was elevated by either high Na VCP or intravenous ADH are shown in Fig. 3. The dose-response relationship in each experiment is indicated by the slope of the line connecting the average control
TABLE 1. Effects of VCP of high Na artificial CSF

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>High Na (II)</th>
<th>Recovery (R)</th>
<th>Paired-Sample Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow, ml.min⁻¹.kg⁻¹</td>
<td>1.98 ± 0.451</td>
<td>3.31 ± 0.360</td>
<td>2.53 ± 0.390</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Urine flow, μeq.min⁻¹.kg⁻¹</td>
<td>1.58 ± 0.313</td>
<td>1.94 ± 0.287</td>
<td>1.38 ± 0.186</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Pₚₚ, meq/liter</td>
<td>9.9 ± 1.41</td>
<td>14.0 ± 1.53</td>
<td>10.3 ± 0.95</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>MAE, mmHg</td>
<td>118.1 ± 4.40</td>
<td>124.0 ± 3.39</td>
<td>115.0 ± 3.94</td>
<td>NS</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>120.0 ± 9.78</td>
<td>122.1 ± 8.57</td>
<td>123.3 ± 11.13</td>
<td>NS</td>
</tr>
<tr>
<td>CSF pressure, cmH₂O</td>
<td>4.2 ± 1.27</td>
<td>4.7 ± 1.27</td>
<td>4.9 ± 1.23</td>
<td>NS</td>
</tr>
<tr>
<td>Pₚₚ, meq/liter</td>
<td>146.3 ± 1.22</td>
<td>144.6 ± 1.14</td>
<td>143.7 ± 1.07</td>
<td>NS</td>
</tr>
<tr>
<td>Pₚₚ, meq/liter</td>
<td>4.15 ± 0.076</td>
<td>4.00 ± 0.053</td>
<td>4.05 ± 0.097</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>37.8 ± 0.54</td>
<td>38.0 ± 0.61</td>
<td>38.2 ± 0.66</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>39.0 ± 1.38</td>
<td>39.0 ± 1.50</td>
<td>38.3 ± 1.23</td>
<td>NS</td>
</tr>
<tr>
<td>ADH, μg/ml</td>
<td>13.3 ± 2.04</td>
<td>36.7 ± 4.70</td>
<td>20.7 ± 4.60</td>
<td>NS</td>
</tr>
<tr>
<td>PRA, ng ANG I.ml⁻¹.h⁻¹</td>
<td>3.2 ± 0.44</td>
<td>2.4 ± 0.35</td>
<td>3.5 ± 0.31</td>
<td>NS</td>
</tr>
<tr>
<td>GFR, ml.min⁻¹.kg⁻¹</td>
<td>2.27 ± 0.153</td>
<td>2.43 ± 0.165</td>
<td>2.24 ± 0.131</td>
<td>*</td>
</tr>
<tr>
<td>RPF, ml.min⁻¹.kg⁻¹</td>
<td>6.29 ± 0.566</td>
<td>6.59 ± 0.596</td>
<td>6.06 ± 0.582</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE for the last half hour of each experimental period. n = 10 dogs. Renal data are for the right kidney only. *P < 0.05. **P < 0.005. $P < 0.001.

TABLE 2. Effects of eight intravenous infusions of ADH

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>ADH (A)</th>
<th>Recovery (R)</th>
<th>Paired-Sample Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow, μeq.min⁻¹.kg⁻¹</td>
<td>1.85 ± 0.508</td>
<td>2.73 ± 0.476</td>
<td>1.94 ± 0.504</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Urine flow, ml.min⁻¹.kg⁻¹</td>
<td>1.47 ± 0.203</td>
<td>2.04 ± 0.229</td>
<td>1.53 ± 0.213</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Pₚₚₚ, meq/liter</td>
<td>8.6 ± 1.48</td>
<td>10.4 ± 1.51</td>
<td>9.3 ± 1.12</td>
<td>NS</td>
</tr>
<tr>
<td>MAE, mmHg</td>
<td>122.8 ± 6.40</td>
<td>138.8 ± 6.82</td>
<td>121.8 ± 5.42</td>
<td>NS</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>147.5 ± 15.15</td>
<td>138.4 ± 10.95</td>
<td>148.4 ± 13.99</td>
<td>NS</td>
</tr>
<tr>
<td>CSF pressure, cmH₂O</td>
<td>9.0 ± 0.53</td>
<td>1.1 ± 0.59</td>
<td>2.1 ± 0.99</td>
<td>NS</td>
</tr>
<tr>
<td>Pₚₚₚ, meq/liter</td>
<td>142.7 ± 1.30</td>
<td>142.2 ± 1.78</td>
<td>141.9 ± 1.07</td>
<td>NS</td>
</tr>
<tr>
<td>Pₚₚₚ, meq/liter</td>
<td>3.87 ± 0.117</td>
<td>4.03 ± 0.107</td>
<td>3.99 ± 0.058</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>38.7 ± 0.74</td>
<td>38.8 ± 0.73</td>
<td>39.1 ± 0.75</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42.3 ± 0.72</td>
<td>41.0 ± 0.70</td>
<td>40.7 ± 0.58</td>
<td>NS</td>
</tr>
<tr>
<td>ADH, μg/ml</td>
<td>18.6 ± 2.26</td>
<td>55.0 ± 6.09</td>
<td>26.2 ± 5.50</td>
<td>*</td>
</tr>
<tr>
<td>PRA, ng ANG I.ml⁻¹.h⁻¹</td>
<td>2.8 ± 3.55</td>
<td>4.8 ± 1.63</td>
<td>7.3 ± 1.55</td>
<td>NS</td>
</tr>
<tr>
<td>GFR, ml.min⁻¹.kg⁻¹</td>
<td>2.76 ± 0.310</td>
<td>2.79 ± 0.285</td>
<td>2.66 ± 0.376</td>
<td>NS</td>
</tr>
<tr>
<td>RPF, ml.min⁻¹.kg⁻¹</td>
<td>5.81 ± 0.747</td>
<td>6.62 ± 0.739</td>
<td>6.13 ± 0.813</td>
<td>*</td>
</tr>
</tbody>
</table>

Values are means ± SE for the last half hour of each experimental period. n = 6 dogs. Renal data are for the right kidney only. *P < 0.05. **P < 0.005. $P < 0.001.

PRA. In both groups the PRA did not decrease significantly during the experimental period but increased during the recovery period.

The results of seven experiments were not included because of brain hemorrhage.

DISCUSSION

The principal finding of this report is illustrated in Fig. 3. The dose-response relationship between increments in ADH and increments in Uₙₙₙ in animals receiving intravenous ADH were remarkably parallel to the relationship in animals receiving high Na VCP. In every case, for both groups of animals, ADH and Uₙₙₙ both increased. The dose-response relationships of the two experimental groups can be compared in several ways. On inspection there were several cases in which both the control and experimental ADH values in a high Na VCP experiment were closely matched by those of an
FIG. 1. Effects of high Na VCP or intravenous ADH infusion on \( U_{Na}V \), MAP, HR, PRA, and GFR. Open circles represent changes induced by high Na VCP; closed circles represent changes induced by intravenous ADH. Data are means ± SE of changes from average control value for each animal.

FIG. 2. Effects of high Na VCP or intravenous ADH infusions on plasma ADH. Data for high Na VCP are shown with open circles; data for intravenous ADH are shown with closed circles.

FIG. 3. Relationship between log plasma ADH and \( U_{Na}V \) when ADH was elevated either by high Na VCP or intravenous infusion of ADH. Each pair of points represents values for a single high Na VCP or ADH infusion experiment. Lower point of each pair represents average of values for last half hour of control and recovery values. Upper point represents average of values for last half hour of high Na VCP or ADH infusion. For the 2 cases (A and B) in which 2 intravenous ADH experiments were performed on 1 dog, the order of the experiments is indicated. Absolute average control values for ADH in high Na VCP and intravenous ADH groups were 17.0 ± 4.06 and 22.4 ± 3.57 pg/ml, respectively; those for \( U_{Na}V \) were 2.23 ± 0.399 and 1.89 ± 0.480 μEq·min⁻¹·kg⁻¹, respectively.

Intravenous ADH experiment. In those cases, the natriuretic responses were very similar. In order to compare the dose-response relationships of all the experiments of the two experimental groups it would be helpful if we could assume that the dose-response relationships were linear throughout the full range of ADH encountered in each group. The fact that the dose-response lines were reasonably parallel even though the experiments spanned a large range in ADH values indicates that this assumption is valid; there was no tendency for the natriuretic response to "plateau" as ADH increased (log scale). Given the assumption of linearity, the average slope \( \Delta U_{Na}V/\Delta \log ADH \) for each experimental group can be compared. The similarity of the average slopes indicates...
that the changes in $U_{NaV}$ that occurred during 1-h of high Na VCP were caused, at least in part, by changes in ADH. We cannot exclude the possibility that over longer periods of testing, the response to high Na VCP could be demonstrated to involve additional mechanisms; this possibility is suggested by the divergence of the natriuretic responses in the latter part of the high Na/ADH period.

It was important that both the pattern and magnitude of changes in ADH during high Na VCP were mimicked in the ADH experiments. ADH rapidly increased and then tended to plateau during the high Na VCP period (Fig. 2). This pattern would be expected if the high Na stimulus caused a step increment in ADH secretion that was then sustained throughout the stimulus period. Fortunately, both the pattern and magnitude of these changes in ADH could be mimicked by a simple continuous infusion of ADH throughout the experimental period (Fig. 2). The increments in ADH resulting from high Na VCP ranged from 6.5 to 51.5 pg/ml. To achieve a similar range of changes, ADH was infused at rates between 3 and 50 mU·kg$^{-1}$·h$^{-1}$. The relationship between infusion rate and plasma ADH in this study is compared in Fig. 4 with that observed by Montani et al. (19). The close similarity of these two sets of data is a further verification of our ADH assay.

Because the approach taken in this study was to mimic changes that occur during high Na VCP, time-control experiments (no experimental interventions during the protocol) were not performed. We have previously reported the results of time-control experiments that were performed under conditions identical to the conditions in the present study (22). In those time controls, $U_{NaV}$, $U_{K}$, and PRA gradually increased and MAP gradually fell. Those changes, which were probably due to unknown effects of VCP and/or anesthesia, account for the failure of these variables in the present experiments to return completely to control levels after experimental treatment. To compensate for the changes in ADH that were not due to experimental interventions, the values during the experimental periods have also been compared statistically with the average of control and recovery values (Tables 1 and 2).

Intraventricular infusion of 0.5 M NaCl was first shown by Andersson (1) to cause natriuresis. This natriuresis was, however, accompanied by large changes in mean arterial pressure that could have accounted for the natriuresis (2, 8, 13, 26). We later demonstrated that VCP of high Na CSF (300 mM Na$^+$) could induce in anesthetized dogs a natriuresis without concomitant changes in mean arterial pressure or renal hemodynamics (22). We hypothesized that the hemodynamic changes observed by Andersson and other investigators were caused by a nonspecific effect of their infusate on neurons involved in the central control of blood pressure. This effect could have been caused by the extremely hypertonic NaCl solutions used or the dilution of other significant ions (e.g., Ca, K, Mg) because artificial CSF was not used. Because natriuresis induced in high Na CSF can occur without hemodynamic changes and, as discussed above, renal nerves do not appear to be involved, it seemed likely that the efferent pathway between the brain and the kidneys was endocrine.

Several known hormones including ADH have previously been examined as possible mediators of CNS-induced natriuresis. Zucker et al. (30) attempted to block CNS-induced natriuresis in anesthetized dogs by pretreating with high doses of deoxycorticosterone acetate and ADH. Theoretically this pretreatment would prevent any significant decrease in plasma mineralocorticoid or increase in ADH secondary to changes in endogenous release of aldosterone or ADH. The persistence of a natriuretic response led them to conclude that neither aldosterone nor ADH mediated the response. However, because the effective concentration of these hormones was not measured, it is conceivable that large increases in endogenous release of ADH could have produced the natriuresis. More likely, because the natriuresis was accompanied by increases in MAP and/or glomerular filtration rate, hemodynamic changes may have mediated the effect despite the lack of change in hormone levels. Aldosterone probably does not mediate the response because its effects last for more than an hour after the hormone itself is no longer present (24); yet the CNS-induced natriuresis occurs within a half hour.

Mouw et al. (22) investigated the possibility that the natriuretic factor described by Bourgoignie et al. (5) mediates CNS-induced natriuresis. They found that changes in the natriuretic factor that correlated with the natriuresis, although because of a lack of sensitivity in the assay method they could not totally exclude the role of natriuretic hormone.

Given the present findings, it is not inconceivable that ADH may have a physiologic role in the control of $U_{NaV}$ during severe dehydration. Although the magnitude of the high Na stimulus would not seem to be physiologic [it may be at a receptor level (22)], other work has shown that changes in ADH caused by simple water restriction (dehydration) (15, 18) or by hemorrhage (10) are associated with changes in $U_{NaV}$. The increments in ADH during 4 of the 10 high Na VCP experiments were actually less than those we measured in dogs that were severely water depleted (water deprived for 72 h, avg 18.8 pg/ml, $n = 2$). Some natriuresis, under conditions of severe dehydration and hypernatremia, may be physiologically appropriate in that it would provide an addi
ADH IN CNS-INDUCED NATRIURESIS

11. JOHNSON, M. D., L. B. KINTER, AND R. BEEUWKES III. Effects of ADH in CNS-induced natriuresis

REFERENCES

20. MOUW, D. K., M. MARTINEZ, B. WEINGARTEN, AND R. MALVIN. Antidiuretic hormone (AVP) does not cause the natriuretic response of rats to elevated cerebrospinal fluid (CSF) Na. Federation


