Dopaminergic Modulation of the Renal Effect of Arginine-Vasopressin in Water-Loaded Rats

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Abstract—In order to confirm whether dopamine inhibits the antidiuretic action of vasopressin in mammalian kidney, we examined interactions among arginine vasopressin (AVP), dopamine and haloperidol in water-loaded ethanol anesthetized rats. The submaximal dose of AVP causing antidiuresis was 80 μU in this preparation. Dopamine at the doses of 0.11, 1.1 and 11 μg/100 g body weight (i.v.) inhibited the antidiuretic effect of 80 μU AVP by 18±7, 27±6 and 36±14%, respectively. The effect of 1.1 μg/100 g body weight dopamine in inhibiting the action of AVP was completely reversed by haloperidol at 2.3 μg/100 g body weight. Single administration of dopamine or haloperidol was without effect on urine flow. These observations support the view that dopamine inhibits the antidiuretic action of vasopressin by dopaminergic receptors also in the mammalian kidney.

It is well-known that administration of dopamine increases urinary excretion of water and sodium chloride (1, 2). It has been suggested that this effect is caused by renal vasodilatation via stimulation of dopamine receptors in the kidney (1-5). Since administration of dopamine increases fractional excretion of sodium, dopamine may also exert direct renal tubular action (6, 7).

In 1970, Deis and Alonso (8) reported for the first time that dopamine caused diuresis in antidiuretic rats treated with vasopressin, suggesting an antagonistic action of dopamine against vasopressin. However, they did not show any evidence that this effect is caused by the stimulation of dopaminergic receptors. Dopamine, at a large dose, also stimulates α-adrenergic receptors (3). Since α-adrenergic stimulation also inhibits the renal tubular action of vasopressin (9, 10), it is important to segregate the action on the dopamine receptors from that on the α-adrenergic receptors.

It has been also reported that dopamine inhibits hydro-osmotic response of the toad urinary bladder to vasopressin (11, 12). Arruda and Sabatini (12) provided convincing evidence that the inhibitory action of dopamine on the hydro-osmotic response to vasopressin in the toad bladder was caused by the stimulation of dopamine receptors. The purpose of this report is to provide evidence that dopaminergic stimulation inhibits the antidiuretic effect of vasopressin also in the mammalian kidney.

Materials and Methods

Animal preparation
Male Wistar rats weighing 200–400 g fasted overnight were anesthetized by oral administration of ethanol to inhibit secretion of endogenous vasopressin. One ml/100 g body weight of 24% ethanol was given orally 2 or 3 times every thirty minutes through a gastric tube. After a polyethylene cannula was inserted into the right femoral vein, constant i.v. infusion of a hypotonic solution consisting...
of 1.2% ethanol, 1.7% glucose and 0.3% NaCl was started at a rate of 0.1–0.2 ml/min. After a small midsection of the lower abdominal wall, the urinary bladder was exposed and a polyethylene tube (PE 190) was inserted into the bladder through the incised wall to make a bladder fistule.

Experimental protocols

Experimental protocols were usually started at 3–4 hr after the operation when urine flow became constant at a rate of more than 0.15 ml/min. Urine was collected into small measuring syringes every 10 min. Drugs were injected bolusly into the femoral vein in a volume of 0.1 ml. Drugs were injected repeatedly in the same animal after urine volume returned to the control level. The following 4 experimental protocols were conducted:

Protocol 1: A dose-response relationship between changes in urine volume and doses of bolusly injected arginine vasopressin (AVP) was examined to determine the submaximal dose of AVP.

Protocol 2: This protocol was designed to examine whether dopamine modulates the antidiuretic action of AVP. After observing the antidiuretic effect of 80 μU AVP, various doses of dopamine in combination with 80 μU of AVP were injected. The doses of dopamine were 0.11, 1.1 and 11 μg/100 g body weight. These doses were expected to correspond to 10⁻⁷, 10⁻⁶ and 10⁻⁵ moles/liter plasma concentration, respectively, assuming that plasma volume is 7% of body weight.

Protocol 3: This protocol was designed to examine whether the action of dopamine can be blocked by a dopaminergic antagonist, haloperidol. Bolus injections of AVP, AVP plus dopamine, and AVP plus dopamine plus haloperidol were performed, consecutively. Doses of AVP, dopamine and haloperidol were 80 μU, 1.1 μg/100 g body weight, and 2.3 μg/100 g body weight, respectively.

Protocol 4: In order to confirm that dopamine or haloperidol has no effect on basal urine flow, effect of single administration of 0.1, 1.1 and 11 μg/100 g body weight of dopamine or 2.3 μg/100 g body weight of haloperidol was observed.

Statistical analysis

The urine flow rate in 10 min after administration of agents (A) was divided by that in the 10 min before administration of agents (B). The ratios of (A-B)/B were used as indices of the effect of the agent(s) in question. The data were expressed as means ± S.E., and differences between the data of two groups were evaluated by Student's t-test for paired samples.

Chemicals

Drugs used were AVP (Sigma), dopamine hydrochloride (Sigma) and haloperidol hydrochloride (Dainippon Pharmaceutical Co.). Injected AVP solution contained 0.03% acetic acid in 0.9% saline. Dopamine solution contained 0.1% ascorbic acid in 0.9% saline.

Results

Dose-response relationship of AVP (Protocol 1): After i.v. injection of various doses of AVP, urine flow reduced immediately. It took 10 to 50 min before urine flow returned to the control level, depending on the doses of vasopressin. The reduction rate of urine flow during the first 10 min were taken as an index of the response to vasopressin. Doses of vasopressin used were 20, 40, 80 and 160 μU/rat. As summarized in Table 1, the index was dose-dependent from 20 to 80 μU/rat, and the maximum effect of vasopressin was observed between 80 and 160 μU/rat. Based on this observation, we assumed that 80 μU/rat was the submaximal dose of vasopressin in this preparation.

Modulation by dopamine of the action of AVP (Protocol 2): After confirming that 80 μU/rat AVP caused a marked reduction in urine flow, we examined whether dopamine modulates the effect of AVP according to protocol 2. The results are summarized in Table 2. It is clear that dopamine administration inhibited the action of AVP. This inhibitory effect was much more at 1.1 μg/100 g body weight than 0.1 μg/100 g body weight of dopamine, but no further increase in inhibitory effect was observed at 11 μg/100 g body weight of dopamine.

Effect of haloperidol (Protocol 3): In order to confirm whether the inhibitory effect of dopamine on AVP action is virtually mediated by a dopaminergic mechanism, we examined
the effect of haloperidol on this inhibitory effect of dopamine according to protocol 3. After confirming that in the same animals the action of AVP was inhibited by 1.1 μg/100 g body weight of dopamine, we tested whether this effect of dopamine can be prevented by 2.3 μg/100 g body weight of haloperidol. The results summarized in Table 3 clearly indicate that haloperidol could in fact prevent the action of dopamine in inhibiting the effect of AVP.

Direct effects of dopamine or haloperidol on urine flow rate (Protocol 4): In order to evaluate the observations described above, it is necessary to confirm that dopamine or haloperidol does not cause significant change of urine flow rate in this preparation. For this purpose, we undertook a series of studies in which effects of 0.1, 1.1 and 11 μg/100 g body weight dopamine or 2.3 μg/100 g body weight of haloperidol were observed. The results are summarized in Table 4. Although dopamine at a dose of 11 μg/100g body weight tended to cause an increase in urine flow, this was not statistically significant. Haloperidol by itself also did not influence urine flow rate in this preparation.
Table 3. Antagonistic action of haloperidol (HP) against dopaminergic (DA) inhibition of the antidiuretic action of arginine vasopressin (AVP)

<table>
<thead>
<tr>
<th></th>
<th>AVP (μU/rat)</th>
<th>DA (μg/100 gBW)</th>
<th>HP (μg/100 gBW)</th>
<th>Urine flow (ml/10 min)</th>
<th>Percent inhibition of urine flow (%)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>(a) 5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.12±0.22</td>
<td>63±6</td>
<td>&lt;0.05 (b vs. a)</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0</td>
<td>0</td>
<td>0.74±0.07</td>
<td>63±6</td>
<td>&lt;0.05 (b vs. a)</td>
</tr>
<tr>
<td>(b) 5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.19±0.24</td>
<td>51±5</td>
<td>&lt;0.05 (b vs. a)</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.1</td>
<td>0</td>
<td>1.05±0.09</td>
<td>51±5</td>
<td>&lt;0.05 (b vs. a)</td>
</tr>
<tr>
<td>(c) 5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.27±0.21</td>
<td>66±5</td>
<td>&lt;0.05 (c vs. b)</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.1</td>
<td>2.3</td>
<td>0.78±0.15</td>
<td>66±5</td>
<td>&lt;0.05 (c vs. b)</td>
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</table>

Values are means±S.E.

Table 4. The single effect of dopamine (DA) and haloperidol (HP)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (μg/100 gBW)</th>
<th>N</th>
<th>Urine flow (ml/10 min)</th>
<th>P</th>
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<tbody>
<tr>
<td>DA</td>
<td>0.11</td>
<td>9</td>
<td>C 2.29±0.17</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E 2.29±0.24</td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>1.1</td>
<td>9</td>
<td>C 2.21±0.14</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E 2.10±0.25</td>
<td></td>
</tr>
<tr>
<td>DA</td>
<td>11</td>
<td>9</td>
<td>C 2.25±0.13</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E 2.57±0.43</td>
<td></td>
</tr>
<tr>
<td>HP</td>
<td>2.3</td>
<td>4</td>
<td>C 2.10±0.22</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E 2.17±0.28</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: C=control period, E=experimental period, N=number of experiments, NS=not significant (E vs. C). Values are means±S.E.

Discussion

Dopamine is known to regulate renal water and sodium excretion. Since dopamine caused vasodilatation relatively selectively in the renal vessels, diuresis or natriuresis by dopamine has been mainly attributed to its renal vascular effect (1-5). However, evidence has been accumulated in support of the view that dopamine acts directly on the renal tubules (6, 7, 13). Bello-Reuss et al. (13) reported that dopamine inhibited sodium transport across the rabbit proximal straight tubule. In addition to direct renal tubular action, dopamine might have inhibitory action on the hydro-osmotic effect of vasopressin, thereby increasing urinary excretion of water (6, 11, 12).

In the present study, we demonstrated that dopamine inhibited the antidiuretic effect of AVP in the rat receiving i.v. hypotonic saline infusion, confirming the observation of Deis and Alonso (6), although the protocols were somewhat different. However, this is contradictory to the observation of Cadnapaphornchai et al. (14) that in hypophysectomized dogs, i.v.-infused dopamine did not modulate the effect of 100 mU vasopressin. The reason for this discrepancy is unknown at present. However, it should be noted that the dose of vasopressin in their study ranged from 3 to 5 mU/kg, whereas in our study, it ranged from 0.2 to 0.4 mU/kg, the latter being carefully selected as a submaximal dose. It is possible that the dose of vasopressin in their study was too high to detect an inhibitory effect of dopamine. In the present study, we further confirmed that this inhibitory effect of dopamine on vasopressin action was in fact mediated by dopaminergic receptors, since haloperidol clearly antagonized the action of dopamine. Therefore, the hydro-osmotic effect of vasopressin is inhibited by a dopaminergic mechanism also in the mam-
malian kidney as it is in toad bladder (11, 12).

It has been reported that α-adrenergic stimulation inhibits the action of vasopressin (9, 10). In toad bladder, Handler et al. (15) demonstrated that norepinephrine inhibited the hydro-osmotic effect of vasopressin via stimulation of α-adrenergic receptors. This was recently confirmed in the mammalian collecting tubule by Krothapalli and Suki (10), who provided evidence that the inhibitory effect of catecholamines is mediated by α2-receptors. This inhibitory effect of catecholamine by α2-receptors was shown to be caused by inhibition of vasopressin stimulated cyclic AMP generation (9). Since a large dose of dopamine is known to stimulate α-adrenergic receptors (3), it is possible that the inhibitory effect of dopamine on AVP in our study is simply caused by the stimulation of α-receptors. The observation that the effect of dopamine was inhibited by haloperidol favors the view that the dopaminergic receptors are participating. Although it is impossible to exclude the possibility that haloperidol also acts as an α2-antagonist, the fact that dopamine exerted the inhibitory action at a low dose suggests that the effect of dopamine in this condition is not mediated by α2-receptors. Thus it is possible that the action of vasopressin is dually modulated by dopamine receptors as well as by α2-receptors. Such dual modulation is observed in toad bladder (11, 12). Bentley (11) reported that the effect of dopamine inhibiting the hydro-osmotic action of vasopressin in toad urinary bladder was antagonized by phentolamine, an α-adrenergic antagonist. On the other hand, Arruda and Sabatini (12) demonstrated that the inhibitory action of dopamine on the hydro-osmotic effect of vasopressin in toad urinary bladder was antagonized by metoclopramide, a dopaminergic antagonist.

The mechanisms by which dopamine inhibits the hydro-osmotic effect of vasopressin is unknown at present time. It has been reported that dopamine increased urinary excretion of cAMP in man (16), increased cAMP content in the venous effluent of perfused rat kidney (17), and stimulated adenylate cyclase activity of the rat kidney particulate preparation (18). Therefore, it is clear that D1 receptors exist in the kidney. However, it is uncertain whether renal tubular epithelia also have D1 receptors. It may be worth while to examine whether the vasopressin stimulated adenylate cyclase is modulated by dopamine in isolated nephron fragments in order to elucidate the mechanism of dopaminergic modulation.

In summary, we demonstrated in water loaded rats that dopamine inhibits the antidiuretic effect of vasopressin by the stimulation of dopaminergic receptors. This effect may play, at least in part, a significant role in the mechanism of diuresis caused by dopamine.

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