Differential Contribution of Angiotensinergic and Cholinergic Receptors in the Hypothalamic Paraventricular Nucleus to Osmotically Induced AVP Release

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ABSTRACT

We studied the involvement of periventricular and hypothalamic angiotensinergic and cholinergic pathways in osmotically induced arginine vasopressin (AVP) release into the blood. In conscious Wistar rats, i.c.v. injections of 0.2, 0.3 and 0.6 M hyperosmolar saline (5 µl) resulted in concentration-dependent increases in AVP release (5.2 ± 1.5, 10.6 ± 2.2 and 18.0 ± 2.2 pg/ml, respectively, vs. 2.0 ± 0.1 in controls). The two lower saline concentrations did not affect arterial blood pressure (non-pressure-associated AVP release), whereas 0.6 M saline induced increase in blood pressure (pressure-associated AVP release).

In the first set of experiments, periventricular angiotensin AT1, muscarinic or nicotinic receptors were blocked by i.c.v. administration of losartan (10 nmol), atropine (100 nmol) or hexamethonium (100 nmol), respectively, before i.c.v. hyperosmolar saline injections. Losartan significantly reduced the 0.2 M and 0.3 M, but not the 0.6 M, saline-induced increase in AVP release. The 0.3 M saline-induced AVP release was blocked by atropine and hexamethonium, whereas the 0.6 M saline-induced AVP release was blocked by atropine only. In the second set of experiments, losartan (4 nmol), atropine (200 nmol) or hexamethonium (200 nmol) was injected bilaterally into the paraventricular nucleus before i.c.v. hyperosmolar saline injections. Losartan reduced 0.3 M and potentiated 0.6 M saline-induced AVP release. On the other hand, atropine and hexamethonium significantly reduced both 0.3 and 0.6 M saline-induced AVP release. We conclude that afferents arising from periventricular osmosensitive neurons to the hypothalamic paraventricular nucleus, which are involved in non-pressure-associated osmotically induced AVP release, are both angiotensinergic and cholinergic, whereas those mediating pressure-associated AVP release are cholinergic in nature.

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ABBREVIATIONS: AVP, arginine vasopressin; PVN, paraventricular nucleus; SON, supraoptic nucleus; CVO, circumventricular organs. BP, blood pressure; ANG II, angiotensin II; SFO, subfornical organ; OVLT, organum vasculosum of the lamina terminalis; NA, nonadrenaline.
adrenoceptors located on the magnocellular neurons, mediates the release of AVP.

Besides ANG II and catecholamines, central ACh is believed to be an important neurotransmitter mediating AVP release (Bhargava et al., 1972; Gregg, 1985; Yamaguchi and Hama, 1989). It has been demonstrated that hyperosmolar saline, when applied peripherally, affected the electrical activity of neurosecretory cells in the PVN and that the effect of hyperosmolar saline was blocked by pretreatment with (Sar^{1}, Ala^{8})-ANG II, atropine and hexamethonium, the respective angiotensinergic, muscarinic and nicotinic cholinergic receptor antagonists (Akaishi and Negoro, 1983). These data suggest the involvement, in osmotically induced AVP release, of angiotensinergic as well as cholinergic receptor mechanisms in the PVN. In addition, water deprivation and local changes in osmolality in the PVN resulted in an increased release of ANG II and ANG III within this nucleus (Harding et al., 1992; Qadri et al., 1994). Taken together, these data imply the participation of central angiotensinergic and cholinergic systems in the control of body fluid homeostasis via the release of AVP.

The present experiments were designed to examine specifically the contribution of periventricular and hypothalamic angiotensinergic and cholinergic mechanisms to AVP release in response to central osmotic stimulation by i.c.v. injections of hyperosmolar saline. We chose the hypothalamic PVN to study the involvement of the angiotensinergic and cholinergic systems in osmotically induced AVP release because neurons from the PVN respond to small increases in extracellular osmolality with a marked increase in their firing rate (Akaishi and Negoro, 1983; Gutman et al., 1988). Further, a single i.p. injection of hyperosmolar saline caused not only an increased firing rate but also changes in neuronal morphology in the PVN (Beagley and Hatton, 1994). Furthermore, the PVN contains angiotensinergic and cholinergic innervations and receptors (Hatton and Mason, 1985; Imboden et al., 1989, 1992; Lind et al., 1985; Oldfield et al., 1989; Obermüller et al., 1991; Pow and Morris, 1989).

In the first part of the study, angiotensinergic AT1 or cholinergic receptor antagonists were administered i.c.v. before the i.c.v. injections of hyperosmolar saline. In the second part of the study, angiotensinergic or cholinergic receptor antagonists were administered bilaterally into the PVN before i.c.v. saline injections.

Our data demonstrate that both angiotensinergic and cholinergic pathways are involved in PVN-mediated AVP release and, further, that the individual recruitment of these pathways depends on the strength of the osmotic signal.

Materials and Methods

Animals

Male Wistar rats weighing 300 to 350 g were obtained from Dr. Karl Thomae GmbH (Biberach/Riss, Germany). The animals were kept under controlled temperature, humidity and light/dark period (0600 h on, 1800 h off) and were allowed free access to food (Altromin standard rat diet, 0.2% sodium) and water.

Implantation of Chronic Intracerebral Guide Cannulas for the Microinjection of Drugs into the PVN

Rats were anesthetized with chloralhydrate (400 mg/kg b.w., i.p.). Intracerebral guide cannulas were implanted bilaterally 2 mm above the PVN with a Kopf-stereotaxic apparatus. The guide cannulas were fashioned from 21-gauge stainless steel tubing and fitted with indwelling stylets. They were secured to the skull with two stainless steel screws and dental cement as described earlier (Veltmar et al., 1992). According to the rat brain atlas by Paxinos and Watson (1986), the coordinates for PVN were 1.5 mm caudal to the bregma, 0.7 mm lateral to the midline and 5.5 mm ventral to the dural surface. Injections into the PVN were performed using a 31-gauge stainless steel tubing that extended 2.0 mm beyond the tip of the intracerebral guide cannula—7.5 mm below the dural surface. Besides the implantation of the guide cannula into the PVN, a polypropylene catheter was inserted into the lateral brain ventricle as described earlier (Unger et al., 1981).

One week after implantation of the i.c.v. cannula and intracerebral guide cannulas, a polypropylene catheter for blood sampling was placed in the right femoral artery under chloralhydrate anesthesia. The catheter was filled with heparinized physiological saline, sealed, exteriorized and secured at the nape of the neck. Experiments were started 24 hr after implantation of the femoral catheter.

Experimental Protocols

Involvement of periventricular angiotensinergic and cholinergic receptors in AVP release in response to i.c.v. hyperosmolar saline. Protocol 1: Animals received i.c.v. hyperosmolar saline injections of 0.2, 0.3 or 0.6 M. Ninety seconds later, 1 ml of blood was drawn from the left femoral artery to measure plasma AVP levels. Blood volume was substituted i.a. with 1 ml of isotonic saline. In all experiments, 0.15 M isotonic saline was used for control i.c.v. injections.

Protocol 2: The specific angiotensin AT1 receptor antagonist losartan (10 nmol) was injected i.c.v. 5 min before i.c.v. 0.2, 0.3 or 0.6 M saline injection. Ninety seconds later, 1 ml of blood was drawn from the left femoral artery to measure plasma AVP levels as described in protocol 1. The dose of losartan was sufficient to block periventricular AT1 receptors completely (Veltmar et al., 1992; Qadri et al., 1993).

Protocol 3: The specific muscarinic receptor antagonist atropine (100 nmol) or the nicotinic receptor antagonist hexamethonium (100 nmol) was injected i.c.v. 5 min before i.c.v. 0.2, 0.3 or 0.6 M saline injection. Ninety seconds later, 1 ml of blood was drawn from the left femoral artery to measure plasma AVP levels. The doses of the cholinergic receptor antagonists were chosen on the basis of previously published data (Akaishi and Negoro 1983; Iitake et al., 1986).

Contribution of angiotensinergic and cholinergic receptors in the PVN to AVP release in response to i.c.v. hyperosmolar saline. Protocol 4: Losartan (4 nmol) was injected bilaterally into the PVN 10 min before i.c.v. 0.3 or 0.6 M saline injections. Ninety seconds later, 1 ml of blood was drawn from the left femoral artery to measure plasma AVP levels. The doses of atropine, hexamethonium and losartan were chosen on the basis of previously published data (Akaishi and Negoro, 1983; Iitake et al., 1986; Veltmar et al., 1992; Qadri et al., 1993).

Verification of Microinjection Location

After completion of the experiments, animals were sacrificed. Poi-tamine sky blue solution (200 nl) (Gurr BDH, U.K.) was injected bilaterally into the PVN. Then brains were removed, kept in 10% formaldehyde for at least 5 days and sectioned to verify the correct localization of the microinjections. Only data from animals in which the microinjection needle was correctly placed in the PVN were processed (table 1).
Drugs and Chemicals

Losartan was a gift from Dr. R. Smith, DuPont-Merck, Wilmington, DE; atropine and hexamethonium were purchased from Sigma (München, Germany). All drugs were dissolved in isotonic saline and kept in 20-ml aliquots at 2°C until used. Different concentrations of hyperosmolar saline were prepared freshly in distilled water for each experiment.

Drug Administration

Different concentrations of hyperosmolar saline solutions (0.2, 0.3 or 0.6 M) were injected i.c.v. in a total volume of 5 µl/60 sec and flushed with 3 µl of isotonic saline. Angiotensinergic or cholinergic receptor antagonists were microinjected into the PVN in a volume of 200 nl/60 sec.

Different concentrations of hyperosmolar saline were injected into the lateral brain ventricle in random order. Separate groups of animals were used in each individual set of experiments. Each animal received at most two different concentrations of saline injected on separate days (experimental protocols 2, 3, 4 and 5). Only the animals in experimental protocol 1 were treated with different concentrations of saline on separate days for 4 days. The time interval between individual injections into the lateral brain ventricle or PVN was at least 24 to 48 hr (table 1).

AVP Assay

Plasma AVP levels were measured by radioimmunoassay after acetone extraction as described previously (Rascher et al., 1981).

Results

Effect of i.c.v. hyperosmolar saline injections on mean arterial BP and on AVP release into the circulation. I.c.v. injections of 0.2 and 0.3 M saline had no effect on systemic blood pressure, whereas 0.6 M saline induced an increase in blood pressure (n = 4) (fig. 1). Basal plasma AVP levels in unrestrained Wistar rats were 1.82 ± 0.08 pg/ml (n = 9). The i.c.v. injection of isotonic saline had no effect on plasma AVP levels (2.39 ± 0.36 pg/ml, n = 9). Hyperosmolar saline injected i.c.v. at concentrations of 0.2, 0.3 (non-pressure-associated) and 0.6 M (pressure-associated) increased AVP release in a concentration-dependent fashion (fig. 2).

Effect of periventricular angiotensin AT1 receptor blockade on i.c.v. hyperosmolar saline-induced AVP release

<table>
<thead>
<tr>
<th>Experimental Protocol</th>
<th>Site of Drug Application</th>
<th>Drug Treatment (day)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>i.c.v.</td>
<td>0.15 M/0.2 M</td>
<td>0.2 M/0.15 M</td>
</tr>
<tr>
<td>2</td>
<td>a) i.c.v.</td>
<td>Losartan</td>
<td>0.2 M/0.3 M</td>
</tr>
<tr>
<td></td>
<td>b) i.c.v.</td>
<td>Losartan</td>
<td>0.6 M/0.15 M</td>
</tr>
<tr>
<td>3</td>
<td>a) i.c.v.</td>
<td>Atropine</td>
<td>0.3 M/0.15 M</td>
</tr>
<tr>
<td></td>
<td>b) i.c.v.</td>
<td>Atropine</td>
<td>0.6 M</td>
</tr>
<tr>
<td></td>
<td>c) i.c.v.</td>
<td>Hexamethonium</td>
<td>0.3 M/0.15 M</td>
</tr>
<tr>
<td></td>
<td>d) i.c.v.</td>
<td>Hexamethonium</td>
<td>0.6 M</td>
</tr>
<tr>
<td>4</td>
<td>a) PVN</td>
<td>Losartan</td>
<td>0.3 M/0.15 M</td>
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<tr>
<td></td>
<td>b) PVN</td>
<td>Atropine</td>
<td>0.6 M</td>
</tr>
<tr>
<td>5</td>
<td>a) PVN</td>
<td>Atropine</td>
<td>0.3 M/0.15 M</td>
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<td></td>
<td>b) PVN</td>
<td>Losartan</td>
<td>0.6 M</td>
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<td></td>
<td>c) PVN</td>
<td>Hexamethonium</td>
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<td></td>
<td>d) PVN</td>
<td>Hexamethonium</td>
<td>0.6 M</td>
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</table>

Hyperosmolar saline, angiotensin or cholinergic receptor antagonists were applied into the PVN randomly in each group, whenever the animals were used for more than 1 day. Separate groups of animals were used in the individual experimental protocols designated as a), b), c) and d) in the table. Abbreviations: 0.3 M and 0.6 M, different concentrations of hyperosmolar saline solution; 0.15 M, isotonic saline solution. Doses used in i.c.v. experiments: losartan: 10 nmol in 1 µl; atropine or hexamethonium: 100 nmol in 1 µl. Doses used for direct microinjections into the PVN: losartan: 4 nmol in 200 nl; atropine or hexamethonium: 200 nmol in 200 nl.

Fig. 1. Effects of i.c.v. injections of 0.2, 0.3 and 0.6 M saline on mean arterial BP in conscious Wistar rats. Saline injections (5 µl) were given over 1 min. Values are means ± S.E.M.; n = 4; * P < .05, vs. vehicle (0.15 M saline).
release. Blockade of periventricular AT1 receptors by i.c.v. administration of losartan (10 nmol) significantly inhibited the 0.2 M and 0.3 M saline-induced AVP release, whereas the 0.6 M saline-induced AVP release showed a tendency to increase upon losartan pretreatment (fig. 3). The i.c.v. administration of losartan followed by i.c.v. isotonic saline (control group) had no effect on basal AVP release (table 2).

Effect of periventricular muscarinic or nicotinic receptor blockade on i.c.v. hyperosmolar saline-induced AVP release. Blockade of periventricular muscarinic receptors by i.c.v. administration of atropine (100 nmol) inhibited both 0.3 M and 0.6 M saline-induced AVP release, whereas blockade of periventricular nicotinic receptors with hexamethonium (100 nmol) inhibited only 0.3 M but not 0.6 M saline-induced AVP release (fig. 4). In control experiments, i.c.v. injections of atropine or hexamethonium followed by i.c.v. isotonic saline did not affect basal plasma AVP levels (table 2).

Effect of angiotensin AT1 receptor blockade in the PVN on i.c.v. saline-induced AVP release. Losartan (4 nmol) injected bilaterally into the PVN reduced 0.3 M saline-induced, but increased 0.6 M saline-induced, AVP release (fig. 5). In control experiments, bilateral administration of losartan into the PVN followed by i.c.v. isotonic saline did not affect plasma AVP levels (table 2).

Effect of blockade of cholinergic (muscarinic and nicotinic) receptors in the PVN on i.c.v. hyperosmolar saline-induced AVP release. Atropine (200 nmol) and hexamethonium (200 nmol) injected bilaterally into the PVN inhibited both 0.3 and 0.6 M saline-induced AVP release (fig. 6). In control experiments, bilateral microinjections of atropine or hexamethonium into the PVN followed by isotonic saline did not affect basal plasma AVP levels (table 2).

**Discussion**

Involvement of periventricular angiotensinergic and cholinergic receptors in osmotically induced AVP release. In the present study, we were able to differentiate between 1) central mechanisms of AVP release involved in acute, moderate increases in osmolality (produced by i.c.v. injections of 0.2 and 0.3 M saline) without changes in BP and 2) mechanisms involved in acute, drastic increases in osmolality (produced by 0.6 M saline) accompanied by changes in BP. Administration of hyperosmolar saline into the lateral ventricle (produced by 0.6 M saline) accompanied by changes in BP.
injections of saline. Values are means ± S.E.M.; n = 6 to 8; * P < .05 (F = 108) vs. 0.3 M, *** P < .001 (F = 48) vs. 0.6 M saline.

brain ventricle elicited a concentration-dependent increase in plasma AVP levels. Pretreatment with atropine significantly reduced the effect of both non-pressure-associated (0.3 M saline) and pressure-associated (0.6 M saline) release of AVP. On the other hand, pretreatment with hexamethonium or losartan reduced only the effect of non-pressure-associated AVP release in response to hyperosmolar saline. These data suggest that cholinergic (muscarinic and nicotinergic) and angiotensinergic pathways are activated in non-pressure-associated osmotically induced AVP release, whereas only muscarinic cholinergic pathways are activated in pressure-associated osmotically induced AVP release.

Our findings are compatible with data published recently by Rohmeiss et al. (1995a, b) on the effect of subpressor and pressor concentrations of hyperosmolar saline on natriuresis in conscious normotensive rats. Intracerebroventricular injections of a low concentration of hyperosmolar saline (0.23 M) induced an increase in natriuresis without influencing mean arterial BP. This effect, as in the case of osmotically induced AVP release in the present study, was mediated via periventricular AT1 receptors (Rohmeiss et al., 1995a) as well as AT1 receptors found in the osmosensitive subfornical organ (Rohmeiss et al., 1995b). Further, the natriuresis associated with the increase in mean arterial BP induced by i.c.v. injections of 0.6 M saline was unaffected by i.c.v. pretreatment with losartan. These data, together with the present findings, suggest a common central pathway between osmotically induced AVP release and natriuresis in response to low or high concentrations of hyperosmolar saline.

We have previously calculated that CSF osmolality is increased by about 0.5% and 1.6% after the i.c.v. injection of 5 μl of 0.2 and 0.3 M saline solution, respectively (Höhle et al., 1996). Regardless of cholinergic receptor specificity, our findings imply that small changes in extracellular osmolarity affect i.c.v. hyperosmolar saline administration induce the release of ACh as a neurotransmitter. These findings further suggest that in the chain of events, an interneuron situated between the periventricular osmoreceptor and the AVP-secreting cell in hypothalamic magnocellular neurons releases ACh across the cholinergic synapses, resulting in AVP secretion into the blood. In the present experiments, stimulation of periventricular osmoreceptors by hyperosmolar saline may have induced ACh release in the hypothalamic nuclei in both non-pressure-associated and pressure-associated AVP release. In addition to ACh, angiotensin peptides are also involved in the non-pressure-associated AVP release, because 0.2 and 0.3 M saline-induced AVP release was inhibited by i.c.v. pretreatment with the AT1 receptor antagonist losartan. Angiotensin may function directly as a neurotransmitter or indirectly as a neuromodulator influencing ACh in the hypothalamic nuclei. It has been shown that ANG II can induce ACh release in the peripheral nervous system (McCubbin 1974) and the CNS (Elie and Panniset, 1970). Thus, in the present experiments, angiotensinergic afferents may have affected the cholinergic system in the hypothalamic nuclei.

In contrast to the data presented here, Yamaguchi and Hama (1989) reported that neither i.c.v. ANG II- nor hyperosmolar saline-induced AVP release was affected by i.c.v. pretreatment with atropine or hexamethonium, whereas carbachol-induced AVP release was inhibited by atropine but not by hexamethonium, which suggesting that ACh is not involved in ANG II- or hyperosmolar saline-induced AVP release. The discrepancies between our and their data could be explained in two ways: 1) Yamaguchi and Hama used only pressure-associated hyperosmolar saline concentrations, whereas we differentiated between non-pressure-associated and pressure-associated hyperosmolar saline concentrations. 2) The dose of atropine used in their study may not have been high enough to inhibit cholinergic pathways involved in angiotensin- and osmotically induced AVP release, although it was obviously sufficient to inhibit the effect of exogenously applied carbachol (28 nmol vs. 100 nmol in our study). Further, in our study we did observe an inhibitory effect of i.c.v. hexamethonium in non-pressure-associated but not in pressure-associated osmotically induced AVP release.

On the other hand, our findings are in agreement with those of previous investigators who reported that cholinergic agents, such as carbachol and ACh, act centrally to stimulate AVP release via muscarinic rather than nicotinic receptors (Bhargava et al., 1972; Hoffman, 1979; Hatzikostas et al.,...
1980; Iitake et al., 1986), results that support our present in vivo observations that stimulation of periventricular osmoreceptors with hyperosmolar saline may trigger ACh release, which acts on postsynaptic muscarinic receptors to induce an increase in AVP release.

It is obvious that the exact site(s) of action of cholinergic and/or angiotensinergicafferent mechanisms in osmotically induced AVP release cannot be determined when the antagonists are applied i.c.v., because afferents from periventricular organs reach both the PVN and SON and can stimulate them simultaneously. Therefore, in the second part of our study we investigated whether angiotensinergic and/or cholinergic mechanisms within the hypothalamic PVN are involved in the osmotically induced release of AVP.

**Contribution of angiotensinergic and cholinergic receptors in the PVN to AVP release in response to i.c.v. saline.** The data in the present study provide in vivo evidence that AVP release after stimulation of periventricular osmoreceptors is mediated via angiotensinergic and cholinergic (muscarinic and nicotinic) receptors in the PVN. We were able to differentiate possible mechanisms involved in non-pressure-associated and pressure-associated hyperosmolar saline-induced AVP release induced by low (0.3 M) and high (0.6 M) concentrations of hyperosmolar saline, respectively. We found that stimulation of periventricular osmoreceptors with low concentrations of hyperosmolar saline involves angiotensinergic receptors and muscarinic and nicotinic cholinergic receptors in the hypothalamic PVN, whereas stimulation of osmoreceptors with high concentrations of hyperosmolar saline involves only muscarinic and nicotinic cholinergic receptors, but not angiotensinergic receptors, in the PVN.

In a parallel study, we observed that blockade of muscarinic receptors in the hypothalamic SON inhibited, whereas nicotinic receptors potentiated, the non-pressure-associated and pressure-associated osmotically stimulated release of AVP (Waldmann et al., 1994). On the other hand, in the present study, blockade of muscarinic and nicotinic receptors in the PVN inhibited both the non-pressure-associated and the pressure-associated osmotically stimulated AVP release. The apparent discrepancy between the role of muscarinic and nicotinic receptors in the PVN and in the SON may be due to the heterogeneity of neurosecretory cells within the PVN. A plausible explanation for the discrepancy may be that in the PVN, both muscarinic and nicotinic receptors are facilitatory, whereas in the SON, muscarinic receptors are facilitatory and nicotinic receptors inhibitory in nature. The inhibitory and stimulatory receptors can be located on the same neuron. The concept of a balance between nicotinic and muscarinic receptors in not new (Gregg, 1985). Furthermore, several authors have reported that there are both nicotinic and muscarinic receptors in the PVN facilitating AVP release (Moss et al., 1972; Michels et al., 1986; Okuda et al., 1993).

Our findings are consistent with those of previous investigators who reported that an intracarotid injection of 0.6 M NaCl increased the firing rate of neurosecretory cells in the PVN. The firing rate of 60% of cells stimulated by hyperosmolar saline was blocked by local application of hexamethonium into the PVN, 80% after atropine pretreatment and 40% after the angiotensin antagonist saralasin (Akaishi and Negoro 1983). Thus our present data and the findings of Akaishi and Negoro both suggest the involvement of cholinergic and angiotensinergic mechanisms in osmotically induced AVP release.

One discrepancy between our present data and those reported by Akaishi and Negoro is that we observed an involvement of angiotensin AT1 receptors on non-pressure-associated (0.3 M saline), but not on pressure-associated (0.6 M saline), osmotically stimulated AVP release. It is possible that in their study, 0.6 M saline introduced into the carotid artery was already diluted before it reached the periventricular osmoreceptors. Therefore, the effect of 0.6 M saline given peripherally may be comparable to the low concentrations of saline (0.2 or 0.3 M) in the present study where the hyperosmolar saline was applied directly into the brain ventricle.

At this point, we are unable to explain the potentiating effect of losartan pretreatment on 0.6 M saline-induced AVP release. It is possible that 0.6 M saline given i.c.v. stimulated angiotensinergic pathways toward PVN as well as SON and that there is a cross-talk between PVN and SON. Blockade of AT1 receptors in the PVN may have stimulated the angiotensinergic system in the SON and hence the potentiated increase in AVP levels in the blood. Another possibility is that blockade of AT1 receptors within the PVN unmasked the facilitatory effect of AT2 receptors. In a previous study, we found that blockade of periventricular AT2 receptors with PD 123177 via the i.c.v. route reduced the 0.6 M, but enhanced the 0.2 M, saline-induced release of AVP (Höhle et al., 1996). This suggests that periventricular AT2 receptors, unlike the AT1 receptors, may suppress non-pressure-associated, but facilitate pressure-associated, osmotically induced AVP release. The role of AT2 receptors within the hypothalamic PVN in osmotically stimulated AVP release needs to be further investigated in view of discrepant reports demonstrating the presence (Obermüller et al., 1991) and the absence of AT2 receptors in the PVN (Jöhren et al., 1996; Lenkei et al., 1996; Reagan et al., 1996).

In summary, a moderate increase in osmolality in the CFS (non-pressure-associated) stimulates periventricular osmoreceptors found on osmosensitive circumventricular neurons. This may lead to an increased release of ACh and angiotensin across synapses in the hypothalamus. ACh, in turn, by acting on postsynaptic muscarinic and nicotinic receptors, and angiotensin, by acting on postsynaptic AT1 receptors in the PVN, both stimulate receptor-mediated release of AVP. A large increase in CSF osmolality (pressure-associated) also stimulates periventricular osmoreceptors found in the CVO leading to what may be a predominant ACh release across synapses in the PVN. ACh acting on muscarinic and nicotinic receptors found on magnocellular neurons in the PVN stimulates AVP release from the neurohypophysis. The contribution of angiotensinergic pathways to pressure-associated hyperosmolar saline-induced AVP release remains to be investigated.

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