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Effect of angiotensin AT_2 and muscarinic receptor blockade on osmotically induced vasopressin release

Susanne Höhle *, Juraj Culman, Mathias Boser, Fatimunnisa Qadri, Thomas Unger

Department of Pharmacology, University of Kiel, Hospitalstr. 4, 24105 Kiel, Germany

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Abstract

Recently, we have shown that angiotensin II-induced AT_1 receptor-mediated vasopressin release can be potentiated by blockade of periventricular AT_2 receptors. In the present study, we investigated whether the AT_2 receptor also exerts an inhibitory effect on osmotically induced vasopressin release. In addition, we tested the effect of the muscarinic receptor antagonist, atropine, on hyperosmolar saline-induced vasopressin release. Plasma vasopressin levels were determined 90 s after intracerebroventricularly applied hyperosmolar saline (0.2, 0.3 and 0.6 M, 5 μl) with or without intracerebroventricular pretreatment with 1 nmol of the selective AT_2 receptor antagonist, PD 123177 (1-(4-amino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid-2HCl), or with 15 nmol of the muscarinic receptor antagonist, atropine. PD 123177 potentiated 0.2 M saline-induced vasopressin release (4.7 ± 0.8 pg/ml vs. 2.2 ± 0.3 in vehicle-pretreated controls, $P < 0.05$), did not affect 0.3 M saline-induced vasopressin release (4.3 ± 0.7 pg/ml vs. 5.4 ± 0.6 pg/ml in vehicle-pretreated controls) and reduced 0.6 M saline-induced vasopressin release (10.0 ± 2.3 pg/ml vs. 17.9 ± 1.8 pg/ml in vehicle-pretreated controls, $P < 0.05$). Pretreatment with atropine reduced 0.3 M (2.3 ± 0.6 pg/ml vs. 5.4 ± 0.9 pg/ml in vehicle-pretreated controls, $P < 0.05$) and 0.6 M saline-induced AVP release (4.0 ± 1.5 pg/ml vs. 18.4 ± 2.4 pg/ml in vehicle-pretreated controls, $P < 0.05$) but did not affect 0.2 M saline-induced vasopressin release (2.1 ± 0.4 pg/ml vs. 3.2 ± 0.8 pg/ml in vehicle-pretreated controls). Our results suggest that the low saline concentration-induced, AT_1 receptor-mediated, vasopressin release is under inhibitory control by periventricular AT_2 receptors. Following high saline concentrations, a muscarinic mechanism seems to be predominant on which AT_2 receptor stimulation acts in a facilitating manner.

Keywords: Angiotensin AT_1 receptor; Angiotensin AT_2 receptor; Atropine; Osmoregulation, central; PD 123177; Vasopressin

1. Introduction

Centrally applied angiotensin II induces a well known neuroendocrine and behavioral response pattern including drinking, vasopressin release, natriuresis and pressor response. All these effects are mediated by the AT_1 receptor (for review see Steckelings et al., 1992). The effects of intracerebroventricularly (i.c.v.) applied hyperosmolar saline are strikingly similar to those of angiotensin II suggesting that an angiotensinergic pathway is involved. Along with this hypothesis, it has recently been demonstrated that hyperosmolar saline-induced effects such as natriuresis (Rohmeiss et al., 1995) and drinking (Blair-West et al., 1994) are mediated in part by the AT_1 receptor. In addition, vasopressin release induced by i.c.v. injected hyperosmolar 0.2 M saline and 0.3 M saline was shown to be abolished by i.c.v. pretreatment with the AT_1 receptor antagonist, losartan, whereas vasopressin release in response to 0.6 M saline was not affected (Waldmann et al., 1994). We have recently demonstrated that blockade of periventricular AT_2 receptors potentiated the angiotensin II-induced, AT_1 receptor-mediated vasopressin release and drinking response (Höhle et al., 1995). We now tested the hypothesis that AT_2 receptors also inhibit hyperosmolar saline-induced vasopressin release, by investigating the effect of i.c.v. pretreatment with the AT_2 receptor antagonist, PD123177, on hyperosmolar saline-induced vasopressin release. In addition, we addressed the question as to whether a cholinergic mechanism is also involved in this response since periventricular cholinergic receptor stimulation is known to be a potent stimulus for vasopressin release (Pickford, 1947; Iitake et al., 1986) and since cholinergic mechanisms are known to be involved in

* Corresponding author. Tel.: +49-0431-597 3527; fax: +49-0431-597-3522.

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osmotically induced vasopressin release (Kühn, 1974; Gregg, 1986). Therefore, we also studied the effect of the muscarinic receptor antagonist, atropine, on hyperosmolar saline-induced vasopressin release.

2. Materials and methods

Male Wistar rats weighing 300–350 g obtained from Møllegaard (Berlin, Germany) were used.

2.1. Surgical methods

Rats were anesthetized with chloralhydrate (400 mg/kg i.p.). Using a Kopf stereotaxic apparatus a cannula was implanted i.c.v. and fixed to the skull with dental cement. The coordinates for the i.c.v. cannula were 0.6 mm caudal to bregma, 1.3 mm lateral to midline and 5.0 mm from dural surface. Five days later, the animals were anesthetized again, and catheters were inserted into the right femoral artery. The catheters were filled with heparinized saline (0.5%), exteriorized, sealed, and secured at the back of the neck. Following surgery, rats were housed individually in plastic cages under controlled temperature (24°C) and humidity on a 12 h light/dark cycle (lights on 06:00–18:00 h) and were allowed free access to food and water.

2.2. General procedures

Prior to arterial catheter implantation, 10 pmol angiotensin II was injected i.c.v. to test the correct position of the i.c.v. cannula. Only those animals which drank immediately after angiotensin II i.c.v. were used in the experiments. All experiments were performed in conscious and unrestrained rats 48 h after femoral surgery when the animals had regained their regular eating and drinking habits. Experiments were started when the animals were in a resting state, quietly lying on the sawdust. The AT_2 receptor antagonist and the muscarinic receptor antagonist were administered i.c.v. in a total volume of 1 μl and flushed with 4 μl of physiological saline. Control animals received 5 μl of physiological saline. Hyperosmolar saline solutions were injected in a volume of 5 μl flushed with 3 μl physiological saline. We used physiological saline as vehicle solution instead of artificial cerebrospinal fluid since, in previous experiments on vasopressin release, both treatments had yielded identical results (Veltmar et al., 1992). One group of rats received only one single dose of one antagonist. The experiments were performed as follows:

On the first day, half of the animals were injected with a single dose of antagonist followed by hyperosmolar saline. The other half was pretreated with vehicle followed by hyperosmolar saline. On the second day, treatment was reversed with respect to groups. Antagonists or vehicle were always injected 5 min prior to hyperosmolar saline.

Ninety seconds after the hyperosmolar saline injection, 1 ml blood was drawn from the femoral catheter for vasopressin determination, and the volume was replaced with 1 ml heparinized saline. The time interval of 90 s was chosen because it is known from previous studies that vasopressin release in response to i.c.v. angiotensin II injections is highest during the first 3 min (Hogarty et al., 1992). From each animal, plasma samples were drawn both after hypertonic saline with vehicle pretreatment and with antagonist pretreatment. The following doses or concentrations, respectively, were used: PD 123177, 1 nmol ($n = 7–9$); atropine, 15 nmol ($n = 6–8$). The doses were chosen on the basis of previous experiments in which 1 nmol PD 123177 potentiated the angiotensin II-induced vasopressin release (Höhle et al., 1995) and 15 nmol atropine significantly reduced the water deprivation-induced drinking response (Stauss and Unger, 1990). A similar dose of atropine inhibited carbachol-induced vasopressin release (Yamaguchi and Hama, 1989); hyperosmolar saline: 0.2, 0.3 and 0.6 M ($n = 6–9$).

The effects on vasopressin release of the antagonists injected i.c.v. alone were tested in separate experiments. Basal vasopressin levels in plasma were determined 90 s after a single i.c.v. injection of vehicle.

2.3. Vasopressin assay

Plasma vasopressin was measured by radioimmunoassay after acetone extraction as described elsewhere (Rascher et al., 1981). The used antibody cross-reacted with lysine-vasopressin (25%) but hardly with oxytocin ($< 0.1\%$) or with vasotocin ($< 0.7\%$) (Rascher et al., 1981). The intra-assay coefficient of variance is 7.6%, the inter-assay coefficient of variance is 12.2% (Rascher et al., 1981). The detection limit is 1.5 pg/ml (personal communication).

2.4. Drugs

The following substances were used: atropine sulfate and sodium chloride, Sigma, München, Germany; PD 123177 (1-(4-amino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid-2HCl) was a gift from Dr. David Taylor, Parke Davis Pharmaceutical Research, Ann Arbor, MI, USA.

2.5. Statistics

Results are expressed as means ± S.E.M. Statistics were performed using Student's *t*-test for paired samples since vasopressin plasma values in response to hypertonic saline i.c.v. after pretreatment with the vehicle and after pretreatment with PD 123177 or atropine were always obtained from the same animal. Significance was accepted at $P < 0.05$.

3. Results

3.1. Effect of pretreatment with the AT_2 receptor antagonist, PD 123177, on hyperosmolar saline-induced vasopressin release

PD 123177 at 1 nmol i.c.v. significantly enhanced the vasopressin release in response to 0.2 M saline i.c.v. (4.7 ± 0.8 vs. 2.2 ± 0.3 pg/ml in vehicle-pretreated controls, $P < 0.05$; Fig. 1). Vasopressin release in response to 0.3 M saline i.c.v. was not affected by PD 123177 (4.3 ± 0.7 pg/ml vs. 5.4 ± 0.6 pg/ml in vehicle-pretreated controls) while vasopressin release in response to 0.6 M saline i.c.v. was clearly reduced by PD 123177 (10.0 ± 2.3 pg/ml vs. 17.9 ± 1.8 pg/ml in vehicle-pretreated controls, $P < 0.05$; Fig. 1).

3.2. Effect of pretreatment with the muscarinic receptor antagonist, atropine, on hyperosmolar saline-induced vasopressin release

Atropine at a dose of 15 nmol i.c.v. did not affect 0.2 M saline-induced vasopressin release (2.1 ± 0.4 pg/ml vs. 3.2 ± 0.8 pg/ml in vehicle-pretreated controls). However, the 0.3 M and 0.6 M saline-induced vasopressin release was significantly reduced by atropine (2.3 ± 0.6 and 4.0 ± 1.5 pg/ml, respectively, vs. 5.4 ± 0.9 and 18.4 ± 2.4 pg/ml in vehicle-pretreated controls, $P < 0.05$; Fig. 2).

Neither PD 123177 nor atropine affected vasopressin release when injected i.c.v. alone (data not shown).

4. Discussion

Centrally applied hyperosmolar saline has been shown to engender a neuroendocrine, cardiovascular and behavioral response pattern, which closely resembles the one induced by angiotensin II, including drinking, vasopressin release, blood pressure increase and natriuresis (Thrasher et al., 1980; Waldmann et al., 1994; Rohmeiss et al., 1995). These observations, among others, led to the hypothesis that the hyperosmolar saline-induced effects were mediated at least partially through a central angiotensinergic pathway. Microdialysis and push pull technique experiments have demonstrated that angiotensin peptides can be released in the central nervous system upon hyperosmotic stimuli (Harding et al., 1992; Qadri et al., 1994).

This is an important prerequisite for angiotensin pep-

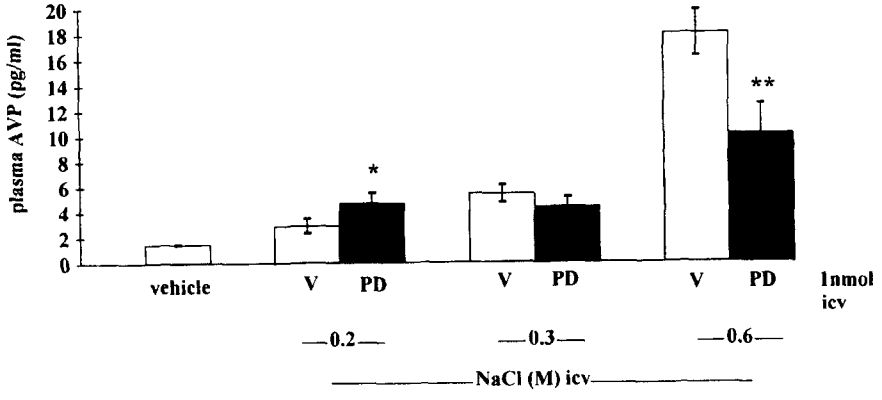

Fig. 1. Effect of i.c.v. pretreatment with PD 123177 (PD) on hyperosmolar saline-induced vasopressin release. PD 123177 was administered 5 min prior to hyperosmolar saline (0.2, 0.3 and 0.6 M). Values are means ± S.E.M., $n = 7$–9. * $P < 0.05$, ** $P < 0.01$ statistical comparison to the respective vehicle-pretreated group, calculated with Student's t-test for paired samples.

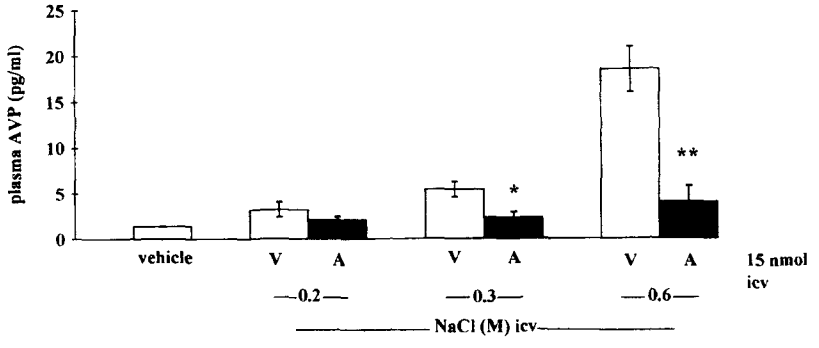

Fig. 2. Effect of i.c.v. pretreatment with atropine (A) on hyperosmolar saline-induced vasopressin release. Atropine was administered 5 min prior to hyperosmolar saline (0.2, 0.3 and 0.6 M). Values are means ± S.E.M., $n = 6$–8, * $P < 0.05$, ** $P < 0.01$ statistical comparison to the respective vehicle-pretreated group, calculated with Student's t-test for paired samples.

tides to act as neurotransmitters in osmoregulation. In agreement with the above hypothesis, hyperosmolar saline-induced vasopressin release, drinking and natriuresis were demonstrated to be reduced by i.c.v. pretreatment with the AT_1 receptor antagonist, losartan (Waldmann et al., 1994; Blair-West et al., 1994; Rohmeiss et al., 1995). Furthermore, vasopressin release induced by 0.2 M and 0.3 M saline was inhibited by i.c.v. pretreatment with the AT_1 receptor antagonist, losartan, while 0.6 M saline-induced vasopressin release was not affected (Waldmann et al., 1994).

We have recently demonstrated that the angiotensin II-induced, AT_1 receptor-mediated, vasopressin release and drinking response were potentiated by periventricular AT_2 receptor blockade (Höhle et al., 1995). We now tested the hypothesis whether the AT_2 receptor also exerts an inhibitory effect on AT_1 receptor-mediated vasopressin release induced by i.c.v. injected hyperosmolar saline.

Hyperosmolar saline, injected i.c.v. at concentrations of 0.2, 0.3 and 0.6 M stimulated vasopressin release dose-dependently. The vasopressin release in response to 0.2 M saline was potentiated by the AT_2 receptor antagonist, PD 123177. Together with our previous observation that the response to 0.2 M saline can be prevented by i.c.v. pretreatment with losartan (Waldmann et al., 1994), this finding suggests that after this low saline concentration (raising cerebrospinal fluid osmolality by about 0.5%) vasopressin release is mediated by the AT_1 receptor and that, under these conditions, the AT_2 receptor exerts a tonic inhibitory effect as we had demonstrated for angiotensin II-induced vasopressin release (Höhle et al., 1995). In contrast to the low saline concentration, the 0.6 M saline-induced vasopressin release was attenuated by i.c.v. pretreatment with PD 123177. This rather unexpected experimental result could be interpreted to mean either that after this strong osmotic stimulus vasopressin release is in fact mediated in part by the AT_2 receptor or that other non-angiotensinergic mechanisms are involved which are facilitated by the AT_2 receptor. Since 0.6 M saline-induced vasopressin release was reduced by atropine the latter possibility appears more likely.

It would also explain the fact that PD 123177 did not affect vasopressin release in response to 0.3 M saline (rise of cerebrospinal fluid osmolality by about 1.6%). In this case one could assume that both neurotransmitters, angiotensin and acetylcholine, are involved in vasopressin release and that the stimulatory and the inhibitory effect of the AT_2 receptor on these systems counterbalance each other.

The question as to why the AT_2 receptor acts in an inhibitory fashion on AT_1 receptor-mediated vasopressin release, on one hand, but is facilitatory concerning the same effect mediated by the muscarinic receptor, on the other hand, is at present difficult to answer. One could hypothesize that AT_2 receptor stimulation, regardless of the stimulus, facilitates the muscarinic receptor-mediated vasopressin release, but that this effect only becomes apparent after a strong osmotic stimulus when muscarinic mechanisms predominate. A weak osmotic stimulus such as 0.2 M saline i.c.v. activates only angiotensinergic pathways involving the AT_1 receptor. The effects of this AT_1 receptor stimulation, including vasopressin release, are under tonic inhibitory control by the AT_2 receptor as has been shown before (Höhle et al., 1995). After 0.6 M saline i.c.v., a concentration which induces a threatening increase in cerebrospinal fluid osmolality (rise of about 4.8%), vasopressin release is mainly mediated by cholinergic mechanisms so that the facilitatory effect of the AT_2 receptor on cholinergic transmission becomes apparent. Under these severe circumstances the potentiating effect exerted by the AT_2 receptor could help to prevent further dehydration by enforced release of antidiuretic hormone into the blood.

The intracellular mechanisms which are involved in the central AT_2 receptor-mediated effects are unknown. A phosphotyrosine phosphatase was reported to be either stimulated (Brechler et al., 1994) or inhibited (Kambayashi et al., 1993) by AT_2 receptor stimulation. Since muscarinic receptor stimulation was shown to result in phosphorylation of K^+ channels probably via a tyrosine kinase-dependent pathway, which resulted in a suppression of channel activity (Huang et al., 1994), it is possible that the AT_2 receptor interferes with the signalling pathway of the muscarinic receptor at the level of enzyme phosphorylation or dephosphorylation. Supposing that phosphotyrosine phosphatase activity is decreased by AT_2 receptor stimulation, this could result in reduced dephosphorylation and, therefore, in stronger suppression of K^+ channels. If this takes place in a vasopressinergic cell, a higher excitability could ensue with an increased release of vesicular vasopressin upon an osmotic stimulus.

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