Microinjections of Angiotensin II into the Supraoptic and Paraventricular Nuclei Produce Potent Antidiureses by Vasopressin Release Mediated through Adrenergic and Angiotensin Receptors

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ABSTRACT—We investigated the effects of angiotensin II (Ang II), microinjected into the supraoptic (SON) and paraventricular (PVN) nuclei of rats, on the urine outflow rate and underlying mechanisms. Ang II produced antidiuretic effects in a dose-dependent manner with ED50 values of 0.1 and 0.05 nmol in the SON and PVN, respectively. [Sar', Ile8]Ang II at 0.1 nmol diminished the Ang II (0.5 nmol)-induced antidiureses in the SON more markedly than in the PVN. A high dose of [Sar', Ile8]Ang II, 1 nmol, completely inhibited the effects in both the nuclei. In addition, the Ang II (1 nmol)-induced antidiuretic effects were partially inhibited by phenoxybenzamine (80 nmol) in the SON and by phenoxybenzamine, timolol (100 nmol) and propranolol (100 nmol) in the PVN. The microinjection of Ang II (1 nmol) into both the nuclei, after pretreatment with a vasopressin V1V2-antagonist, d(CH2)5-u-Tyr(Et)VAVP (i.v.), significantly increased the urine outflow rate. These findings suggest that 1) Two mechanisms account for the Ang II receptor-mediated antidiureses resulting from an increase in vasopressin release: direct stimulation on vasopressin-containing neurons and indirect stimulation on them through α-adrenoceptors in the SON and α- and β-adrenoceptors in the PVN; 2) The Ang II-induced antidiuretic effect in the SON is slightly less potent than that in the PVN; and 3) Ang II receptors in the nuclei may possibly produce the diureses through mechanisms that are not presently understood.

Keywords: Angiotensin II, Vasopressin, Adrenoceptor, Supraoptic nucleus, Paraventricular nucleus

A number of reports have shown that angiotensin II (Ang II), one of the neuropeptides in the central nervous system, induces antidiuretic effects or increases the plasma vasopressin level, when administered into the cerebroventricle (1 – 5). The primary action site(s) of Ang II is suggested to be an organ(s) surrounding the anteroventral third ventricle (AV3V), from the results that lesion or ablation of AV3V inhibits the antidiuretic effects (1, 3). There are Ang II-sensitive neurons from the circumventricular organs to the hypothalamic paraventricular nucleus (PVN) (6, 7). The PVN, being adjacent to the wall of the third ventricle, includes cell bodies of vasopressin-containing neurons. Therefore, Ang II injected into the ventricle will probably increase vasopressin release by indirect stimulation on vasopressin-containing neurons in the PVN. However, the PVN contains Ang II receptors, Ang II-immunoreactive cells and fibers (8 – 10). In addition, vasopressin-containing neurons in the PVN are sensitive to Ang II (11). Recently, Veltmar et al. (5) have shown that the microinjection of Ang II into the PVN stimulates vasopressin release directly through Ang II receptors in the nuclei.

On the other hand, the hypothalamic supraoptic nucleus (SON) is another nucleus in which cell bodies of vasopressin-containing neurons are localized. Vasopressin-containing neurons are activated by Ang II (12 – 14). Also, Ang II receptors, neurons and terminals of Ang II-containing neurons are involved in the SON, as well as in the PVN (6, 8, 15).

In this study, we investigated the effects of Ang II, directly microinjected into the SON, on the urine outflow rate and involvement of vasopressin release and adrenoceptors in the effects; data in the SON were compared with those in the PVN.

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MATERIALS AND METHODS

Animals

Male Wistar rats (9- to 10-week-old), housed with a 12 hr light/dark cycle at room temperature: 22±1°C, were used. The animals were fasted for approximately 17 hr, but had access to water ad libitum.

Procedures

The rats were orally loaded with a volume of 5 ml/100 g body weight of tap water; and 45 min later, they were anesthetized with the same volume of 12% ethanol, using stomach intubation. Three polyethylene cannulae were inserted into the trachea, the jugular vein and the urinary bladder. During the experiments, Locke's solution containing 3% ethanol was continuously infused at a rate of 0.1 ml/min through the cannula in the jugular vein. The animals were unilaterally implanted with a stainless steel cannula (diameter: 200 μm) into the SON or the PVN using a stereotaxic apparatus (Takahashi Co., Tokyo). The coordinates were determined according to the atlas of König and Klippel (16) (SON: 6.3 mm anterior from the lambda, 1.3 mm from the midline, 9.0 mm from the dural surface; PVN: 5.6 mm, 0.3 mm, 8.0 mm).

Drops of urine flowing from the bladder cannula were counted by a photoelectric drop counter (Unique Medical Co., Tokyo) every 10 min. Ethanol-anesthesia and -infusion maintain a constant urine outflow rate of 0.096 ±0.004 ml/min (n=139) for 4–5 hr from 30–60 min after the rats were fixed in the stereotaxic apparatus. A drug solution was administered: 1 pl for microinjections into the nuclei at a rate of 0.35 μl/min and 0.2 ml for intravenous injections. Drugs were dissolved in saline. Changes in the urine outflow rate were expressed as a percentage of the control level (values for 10 min before the injection).

Identification of injection sites

At the end of the experiments, the tip of the cannula was verified by the following methods: 1) An electric current (2 mV, 1 min) was run through the stainless steel cannula in the nucleus (Lesion Generator, RFG-4A; Radionics, Inc., Burlington, MA, USA). The brains were removed after decapitation and sectioned in 30-μm slices with a cryostat (Tissue-Tek II; Miles, Inc., Elkhart, IN, USA). The slices were stained with Haematoxylin-Eosin. The location of the tip of the cannula was verified under a microscope or 2) KCl at 400 or 800 nmol, a depolarizing dose (17, 18), was microinjected into the nuclei. When the KCl-induced antidiuretic effects were approximately equal to the ones by KCl that was correctly microinjected into the nuclei, the results were included in the data.

Statistical analysis

Data are expressed as means±S.E.M. ANOVA was used for statistical analysis between two means. P values under 0.05 were considered as significant differences.

Drugs

The following drugs were used: Angiotensin II and an angiotensin II antagonist, [Sar₁, Ile₈]angiotensin II (Sigma Chemical Co., St. Louis, MO, USA); an α-adrenoceptor antagonist, phenoxybenzamine hydrochloride (Nacalai Tasque, Kyoto). β-Adrenoceptor antagonists, timolol maleate and propranolol hydrochloride, and a vasopressin V₁, V₂-antagonist, 1-(β-mercaptop-β, β-cyclopentamethylene propionic acid)2-(O-ethyl)-D-tyrosine, 4-valine, arginine vasopressin: d(CH₂)₅-D-Tyr(Et)VAVP, were generous gifts from Sankyo Co. (Tokyo), Sumitomo Chemical and Industrial Co. (Tokyo) and Dr. K.G. Hofbauer (Cardiovascular Research Department, Pharmaceutical Division, Ciba-Geigy, Basel, Switzerland), respectively.

RESULTS

Effects of Ang II microinjected into the SON and PVN on urine outflow rate

As shown in the left panel of Fig. 1, the microinjection of Ang II at 0.02–1 nmol into the SON decreased the urine outflow rate in a dose-dependent manner. The antidiureses induced by 1 nmol Ang II were relatively slow in onset, showed the maximum response at 20–30 min after the microinjection and were of approximately 60-min duration. The ED₅₀ value for this effect was estimated to be 0.1 nmol from the dose-response curve (Fig. 2). In the PVN, Ang II also produced antidiuretic effects, having a similar time-course to that in the SON (Fig. 1, right panel). The ED₅₀ value was calculated to be 0.05 nmol, and the effect was slightly more potent than that in the SON (Fig. 2).

The microinjection of vehicle (saline) alone into the nuclei did not significantly change the urine outflow rate in the rats that had not been microinjected with Ang II into the nuclei (Fig. 1). However, after the microinjection of Ang II (0.1 nmol, n=3; 0.2 nmol, n=2; 0.5 nmol, n=3) into the SON, vehicle, microinjected into the same SON, diminished the urine outflow rate (41 ± 8.7% * at 10 min, 27±8.4%* at 20 min, 51±12%* at 30 min, 72±11%* at 40 min, 81±8.6%* of the control at 50 min after the injection; n=8; *P<0.05 vs vehicle shown in Fig. 1). Therefore, Ang II was injected into the nuclei only once in one rat.

Effects of antagonists on Ang II-induced antidiureses

To investigate mechanisms underlying the Ang II-induced antidiureses, effects of an Ang II antagonist, [Sar₁,
Ile$^8$Ang II; an $\alpha$-adrenoceptor antagonist, phenoxybenzamine; $\beta$-adrenoceptor antagonists, timolol and propranolol; and a vasopressin antagonist, d(CH$_2$)$_5$D-Tyr(Et)-VAVP, on the effects were examined (Fig. 3).

Pretreatment of the SON or PVN with an injection of [Sar$^1$, Ile$^8$]Ang II at 1 nmol completely blocked the Ang II (1 nmol)-induced antidiureses in the same nucleus (SON: n=4, PVN: n=4). In the SON, a lower dose of [Sar$^1$, Ile$^8$]Ang II, 0.1 nmol, also completely inhibited the effects of Ang II at 0.5 nmol (urine outflow rate after microinjection of Ang II in the absence vs presence of the antagonist: 89±9.0% vs 100±3.8% at 10 min, 19±4.1% vs 104±10%* at 20 min, 57±9.4% vs 105±6.9%* at 30 min and 85±10% vs 100±3.8% of the control level at 40 min after the injection; n=5 vs n=5; *P<0.05). However, in the PVN, this dose of [Sar$^1$, Ile$^8$]Ang II merely accelerated to return to the control level in the Ang II (0.5 nmol)-induced effects (urine outflow rate after microinjection of Ang II in the absence vs presence of the antagonist: 80±6.8% vs 101±3.5% at 10 min, 16±1.4% vs 24±7.5% at 20 min, 14±2.7% vs 49±8.2%* at 30 min and 25±5.3% vs 110±18%* of the control level at 40 min after the injection; n=4 vs n=3; *P<0.05).

The microinjection of phenoxybenzamine at 80 nmol and timolol at 100 nmol, which completely blocked norepinephrine (NE)- and isoproterenol-induced antidiureses in the nuclei (17, 18), respectively, was used. Phenoxybenzamine inhibited the Ang II (1 nmol)-induced effects in both the nuclei. However, weak, but significant antidiureses remained (SON: n=6, PVN: n=6). On the other hand, timolol attenuated the effects in the PVN (n=5), but not in the SON (n=6). In the PVN, pretreatment with propranolol at 100 nmol also diminished the effects (urine outflow rate after microinjection of Ang II in the absence vs presence of the antagonist: 89±5.2% vs 68±17%* at 20 min and 21±3.4% vs 61±16%* of the control level at 30 min after the administration; n=5 vs n=6; *P<0.05). The inhibitory effects of the two $\beta$-antagonists in the PVN were observed only at 20 and 30 min after the injection.

After pretreatment with an intravenous injection of d(CH$_2$)$_5$D-Tyr(Et)VAVP (16.7 µg), Ang II (1 nmol) conversely elicited diuretic effects in both the nuclei (SON:
n = 5, PVN: n = 5). These diuretic effects reached maximum at 20 min after the injection: 141 ± 10% and 146 ± 10% in the SON and PVN, respectively (P < 0.05 vs vehicle shown in Fig. 1), and they were of short duration (30–40 min).

The microinjections of [Sar', Ile8]Ang II, phenoxybenzamine or propranolol and the intravenous injections of d(CH2)5-D-Tyr(Et)VAVP, alone, did not significantly alter the urine outflow rate in both the nuclei. However, timolol increased the urine outflow rate up to 161 ± 23% in the SON (n = 5) and 173 ± 22% in the PVN (n = 5).

**DISCUSSION**

This study demonstrated that the microinjection of Ang II into the SON and PVN elicits the dose-dependent antidiureses which are inhibited by the Ang II antagonist [Sar', Ile8]Ang II and the vasopressin antagonist d(CH2)5-D-Tyr(Et)VAVP. Therefore, the antidiureses by Ang II were suggested to be produced by an increase in vasopressin secretion mediated through Ang II receptors in the nuclei. Ang II receptors and Ang II-sensitive vasopressin-containing neurons are present in the SON and PVN (8–15). It has been reported that the PVN includes regulation of vasopressin release by Ang II (5). The present result suggests that vasopressin release is also regulated by Ang II in the SON, as well as in the PVN. Therefore, under Ang II-containing neuron-activated conditions, vasopressin release is stimulated in the bilateral SON and PVN, and then the plasma vasopressin level accelerately elevates, because Ang II is the most potent vasopressin releasing agent (see below). Our previous studies (17–20) show that adrenergic and cholinergic mechanisms of urine production in the SON and PVN are similar to each other. However, as for opioid mechanisms, which are probably mediated through δ- and κ-subtypes, the PVN seems to involve more potent regulation than the SON (21, 22). Cooperation on urine production between the bilateral SON and PVN is interesting.

The ED50 values for the Ang II-induced antidiureses were considerably smaller, compared with those for antidiureses induced by adrenoceptor agonists (epinephrine, NE, isoproterenol) (17, 18); cholinooceptor agonists (ACh, oxotremorine, nicotine) (19); opioid agonists (Met-enkephalin, d-Ala2-Met5-enkephalinamide, morphine, fentanyl, d-Ala2-d-Leu5-enkephalin, dynorphin-(1–13), Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Arg-Leu-Arg-Gly aminopentylamide, U50488H) (21–25); and tachykinins (substance P, [D-Pro2, D-Trp7,9]substance P) (26) which we have already reported. In addition, no papers have reported more marked antidiureses or vasopressin release than the Ang II-induced ones. Probably Ang II in the SON and PVN plays an important role in the regulation of urine production. Sensitivity of Ang II to the regula-
tion in the SON seems to be different from that in the PVN. The dose-response curves in Fig. 2 show that the PVN is slightly more susceptible to Ang II than the SON. Therefore, the Ang II-induced effects are inhibited by a lower dose of [Sar\(^1\), Ile\(^8\)]Ang II in the SON than in the PVN. Also, this is consistent with evidence from autoradiographic binding studies that the Ang II receptor concentration is higher in the PVN than in the SON (8, 10).

It is suggested that Ang II receptors exist on cell bodies of vasopressin-containing neurons in the nuclei and directly promote vasopressin release (11–14). Also, Ang II receptors are reported to be present on terminals of adrenergic neurons in various tissues and regulate NE release (4). Adrenergic neurons are well-known to terminate in the nuclei from the caudal ventrolateral medulla and the locus coeruleus (27, 28). Therefore, we investigated the involvement of adrenoceptors in the Ang II-induced effects. The effects in the SON were inhibited by phenoxybenzamine, but not by timolol, whereas those in the PVN were inhibited by the \(\alpha\)- and \(\beta\)-adrenoceptor antagonists. All the inhibitory effects were partial. These findings seem to show a possibility that Ang II receptors in the nuclei exist on both cell bodies of vasopressin-containing neurons and terminals of adrenergic neurons. These Ang II-induced effects are caused via \(\alpha\)-adrenoceptors in the SON and \(\alpha\)- and \(\beta\)-adrenoceptors in the PVN. Adrenoceptor agonists microinjected into the nuclei produced antidiuretics that have a similar time-course to those of Ang II. NE decreased the urine outflow rate in the PVN more markedly than in the SON (17, 18). This may result in the difference in sensitivity to Ang II in the nuclei. Veltmar et al. (5) concluded that Ang II-induced vasopressin release involves the \(\alpha\)-subtype, not the \(\beta\)-subtype, in the PVN, and stimulation of \(\beta\)-adrenoceptors decreases vasopressin release. This is not consistent with the present result. The discrepancy is probably due to differences in experimental conditions. Conscious, nonhydrated animals may regulate urine production through stimulation of neurons different from the ones regulating urine production in anesthetized, hydrated animals. Isoproterenol, microinjected into the PVN of anesthetized, hydrated rats, caused antidiuresis (18); however, this effect did not involve increased vasopressin release (29). Morphine injected into the ventricle is reported to elicit antidiureses, increase in, decrease in or no change in vasopressin release under various conditions (30). Moreover, Veltmar and their colleagues bilaterally administered the drugs into the PVN, although we used a unilateral microinjection technique. This may be one of the reasons for the discrepancy. An electrophysiological study shows neural connections between the bilateral PVN (31).

After the pretreatment with a vasopressin \(V_1V_2\)-antagonist, \(d\)-(CH\(_2\))\(_5\)-D-Tyr(Et)VAVP (32), Ang II significantly increased the urine outflow rate. The microinjection of oxotremorine and \(d\)-Ala\(^2\)-d-Leu\(^3\)-enkephalin pretreated with the vasopressin antagonist did not elicit any effects on the urine outflow rate (20, 21). The vasopressin antagonist alone did not significantly change the rate. It is possible that Ang II stimulates two pathways for antidiureses by increased vasopressin release and diureses by unknown mechanisms. Because the antidiureses appear to be more potent than the diureses, Ang II after pretreatment with and without the vasopressin antagonist is probably able to cause the diureses and the antidiureses, respectively.

In conclusion, the SON and PVN include the powerful regulation of urine production by Ang II. This regulation in the PVN seems to be more sensitive than that in the SON. Two mechanisms are suggested to account for the Ang II receptor-mediated antidiuretic effects resulting from an increase in vasopressin release: 1) direct stimulation on vasopressin-containing neurons and 2) indirect stimulation on them through \(\alpha\)-adrenoceptors in the SON and \(\alpha\)- and \(\beta\)-adrenoceptors in the PVN. In addition, Ang II may also induce diureses through unknown mechanisms.

REFERENCES

1. Bealer SL, Phillips MI, Johnson AK and Schmid PG: Anterior ventral third ventricle lesions reduce antidiuretic responses to angiotensin II. Am J Physiol 236, E610–E615 (1979)
2. Hisada S, Fujimoto S, Kamiya T, Endo Y and Tsushima H: Antidiuresis of centrally administered amines and peptides and release of antidiuretic hormone from isolated rat neurohypophysis. Jpn J Pharmacol 27, 153–161 (1977)
3. Johnson AK, Hoffman WE and Buggy J: Attenuated pressor responses to intracranially injected stimuli and altered antidiuretic activity following preoptic-hypothalamic periventricular ablation. Brain Res 157, 161–166 (1978)
4. Timmermans PBWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JM and Smith RD: Angiotensin II receptor and angiotensin II receptor antagonists. Pharmacol Rev 45, 205–251 (1993)
5. Veltmar A, Cuiman J, Qadri F, Roscher W and Unger T: Involvement of adrenergic and angiotensinergic receptors in the paraventricular nucleus in the angiotensin II-induced vasopressin release. J Pharmacol Exp Ther 263, 1253–1260 (1992)
6. Ferguson AV and Wall KM: Central actions of angiotensin in cardiovascular control: Multiple roles for a single peptide. Can J Physiol Pharmacol 70, 779–785 (1992)
7. Tanaka J: Involvement of the median preoptic nucleus in the regulation of paraventricular vasopressin neurons by the subfornical organ in the rat. Exp Brain Res 76, 47–54 (1989)
8. Hwang BH, Wu J-Y, Wiczerek CM, Harding JW, Erickson JB and Wamsley JK: Different pharmacological anatomy in the paraventricular hypothalamic nucleus, supraoptic nucleus, and...
suprachiasmatic nucleus of rats: Quantitative autoradiography on angiotensin II receptor binding sites. Am J Anat 176, 243–247 (1986)

9 Lind RW, Swanson LW, Bruhn TO and Ganten D: The distribution of angiotensin II-immunoreactive cells and fibers in the paraventriculo-hypophysial system of the rat. Brain Res 338, 81–89 (1985)

10 Mendelsohn FAO, Quirion R, Saavedra JM, Aguilar G and Catt KJ: Autoradiographic localization of angiotensin II receptors in rat brain. Proc Natl Acad Sci USA 81, 1575–1579 (1984)

11 Akaishi T, Negoro H and Kobayasi S: Electrophysiological evidence for multiple sites of actions of angiotensin II for stimulating paraventricular neurosecretory cells in the rat. Brain Res 220, 386–390 (1981)

12 Okuya S, Inenaga K, Kaneko T and Yamashita H: Angiotensin II sensitive neurons in the supraoptic nucleus, subfornical organ and anteroventral third ventricle of rats in vitro. Brain Res 402, 58–67 (1987)

13 Jhamandas JH, Lind RW and Renaud LP: Angiotensin II may mediate excitatory neurotransmission from the subfornical organ to the hypothalamic supraoptic nucleus: an anatomical and electrophysiological study in the rat. Brain Res 487, 52–61 (1989)

14 Nicoll RA and Barker JL: Excitation of supraoptic neurosecretory cells by angiotensin II. Nature New Biol 233, 172–174 (1971)

15 Obermüller N, Unger T, Culman J, Gohlke P, Gaspar M and Bottari SP: Distribution of angiotensin II receptor subtypes in rat brain nuclei. Neurosci Lett 132, 11–15 (1991)

16 König JFR and Klippel RA: The Rat Brain, a Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore (1963)

17 Tsushima H, Mori M and Matsuda T: Antidiuretic effects of alpha- and beta-adrenocceptor agonists microinjected into the hypothalamic supraoptic nucleus in a water-loaded and ethanol-anesthetized rat. Jpn J Pharmacol 39, 365–374 (1985)

18 Tsushima H, Mori M and Matsuda T: Antidiuretic effects of alpha- and beta-adrenocceptor agonists microinjected into the hypothalamic paraventricular nucleus in a water-loaded and ethanol-anesthetized rat. Jpn J Pharmacol 40, 319–328 (1986)

19 Mori M, Tsushima H and Matsuda T: Antidiuretic effects of oxytocorine microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anesthetized rat. Jpn J Pharmacol 35, 27–36 (1984)

20 Mori M, Tsushima H and Matsuda T: Effect of vasopressin antagonist on antidiuresis by oxytocorine microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anesthetized rat. Jpn J Pharmacol 49, 357–364 (1989)

21 Tsushima H, Mori M and Matsuda T: Effects of D-Ala²-D-Leu⁵-enkephalin, microinjected into the supraoptic and paraventricular nuclei, on urine outflow rate. Jpn J Pharmacol 63, 181–186 (1993)

22 Tsushima H, Mori M and Matsuda T: Microinjection of dynorphin into the supraoptic and paraventricular nuclei produces antidiuretic effects through vasopressin release. Jpn J Pharmacol 63, 461–468 (1993)

23 Tsushima H, Mori M and Matsuda T: Antidiuretic effects of methionine-enkephalin and 2-d-alanine-5-methionine-enkephalinamide microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anesthetized rat. Jpn J Pharmacol 42, 507–515 (1986)

24 Tsushima H, Mori M and Matsuda T: Antidiuretic effects of morphine microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anesthetized rat. Jpn J Pharmacol 45, 449–457 (1987)

25 Tsushima H, Mori M and Matsuda T: Effects of fentanyl, injected into the hypothalamic supraoptic and paraventricular nuclei, in a water-loaded and ethanol-anesthetized rat. Neuropharmacology 29, 757–763 (1990)

26 Mori M, Tsushima H and Matsuda T: Substance P injected into the hypothalamic supraoptic nucleus causes antidiuresis through the release of arginine-vasopressin in water-loaded and ethanol-anesthetized rats. Jpn J Pharmacol 62, 49–56 (1993)

27 Day TA and Sibbald JR: Solitary nucleus excitation of supraoptic vasopressin cells via adrenergic afferents. Am J Physiol 254, R711–R716 (1988)

28 Renaud LP: Magnocellular neuroendocrine neurons: update on intrinsic properties, synaptic inputs and neuropharmacology. Trends Neurosci 10, 498–502 (1987)

29 Tsushima H, Fujimoto S and Matsuda T: Effects of β₁- and β₂-adrenocceptor agonists applied into the hypothalamic paraventricular nuclei of spontaneously hypertensive rats on urine production. Jpn J Pharmacol 64, 201–207 (1994)

30 van Wimersma Greidanus TB and ten Haaf JA: Opioids and the posterior pituitary. In Frontiers in Neuroscience, Opioid Modulation of Endocrine Function, Edited by Delitala G, Motta M and Serio M, pp 125–136, Ravan Press, New York (1984)

31 Moos F and Richard P: Paraventricular and supraoptic bursting oxytocin cells in rat are locally regulated by oxytocin and functionally related. J Pharmacol (Lond) 408, 1–18 (1989)

32 Hofbauer KG, Mah SC and Opperman JR: Chronic blockade of vasopressin receptors in rats. J Cardiovasc Pharmacol 8, Supp 7, S56–S60 (1986)