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Angiotensin AT_1 receptor-mediated vasopressin release and drinking are potentiated by an AT_2 receptor antagonist

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Abstract

Stimulation of angiotensin II AT_2 receptors has been shown to inhibit AT_1 receptor-mediated actions in peripheral tissues. The role of AT_2 receptors in the central actions of angiotensin is not well understood. In the present study, plasma vasopressin levels and water intake in response to intracerebroventricular angiotensin II (10 pmol) were determined after intracerebroventricular pretreatment with 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), a selective AT_2 receptor antagonist (10, 100 and 1000 pmol), or with losartan (2-*n*-butyl-4-chloro-5-hydroxy-methyl-1-2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl)methylimidazole, potassium salt), a specific AT_1 receptor antagonist (0.2, 2 and 10 nmol). Blood samples for vasopressin determination were drawn 90 s after angiotensin II injection and the drinking response was determined in a time interval of 10 min after intracerebroventricular angiotensin II. Losartan at a dose of 2 nmol or higher completely prevented vasopressin release and drinking response to angiotensin II. The drinking response was already attenuated after pretreatment with the lowest dose of losartan. In contrast, PD 123177 potentiated the angiotensin II-induced vasopressin release (39.7 ± 2.7 pg/ml after 1000 pmol PD 123177 vs. 21.3 ± 2.9 pg/ml in vehicle-pretreated controls, $P < 0.05$). The dipsogenic response to angiotensin II was also potentiated by PD 123177 (9.5 ± 0.7 ml after 1000 pmol PD 123177 vs. 5.1 ± 1.3 ml in vehicle-pretreated controls, $P < 0.05$). Our results suggest that the angiotensin II-induced vasopressin release and drinking, mediated by central AT_1 receptors, are under inhibitory control by central AT_2 receptors.

Keywords: Angiotensin AT_1 receptor; Angiotensin AT_2 receptor; Losartan; PD 123177; Vasopressin; Drinking response

1. Introduction

The biological actions of angiotensin II are mediated at least by two types of receptors referred to as AT_1 and AT_2. These receptors differ in their binding properties as well as in their signal transduction mechanisms. AT_1 receptors can be subdivided into two populations of receptors termed AT_{1A} and AT_{1B} (for review see Steckelings et al., 1992; Bottari et al., 1993; Timmermans et al., 1993). Both the AT_1 and AT_2 receptor have been cloned and their amino acid sequence has been identified (Murphy et al., 1991; Sasaki et al., 1991; Mukoyama et al., 1993; Kambayashi et al., 1993).

AT_1 and AT_2 receptors are both expressed in adult rat brain; however, their distribution pattern is heterogenous. AT_1 receptors are concentrated in areas involved in central control of fluid homeostasis such as circumventricular organs and in brain areas related to blood pressure control such as the paraventricular nucleus and nucleus of the solitary tract. AT_2 receptors are expressed in areas involved in sensory and motor control such as the ventral thalamic nucleus, the locus coeruleus and the inferior olive (for review see Saavedra, 1992). However, Obermüller et al. (1991), using a more sensitive method, could also detect small quantities of AT_2 receptors in circumventricular organs and in the median preoptic nucleus, structures belonging to the lamina terminalis which play an important role in central mechanisms of fluid and electrolyte control (for review see Thrasher, 1985).

Evidence has accumulated that the known central

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actions of angiotensin II such as pressor response, dipsogenic effects and the release of vasopressin into the circulation are mediated by AT_1 receptors since they could be antagonized by selective AT_1 receptor antagonists (Wong et al., 1990; Stauss and Unger, 1990; Fregly and Rowland, 1991; Beresford and Fitzsimons, 1992; Veltmar et al., 1992; Qadri et al., 1993). On the other hand, the in vivo use of the specific non-peptide antagonists of AT_2 receptors, PD 123177 and PD 123319, has yielded conflicting results, and no direct evidence for an involvement of central AT_2 receptors in any known action of angiotensin II has been presented so far. For instance, a blockade of periventricular AT_2 receptors was reported to either not affect the angiotensin II-induced vasopressin release into the circulation (Qadri et al., 1993) or to abolish this response (Hogarty et al., 1992). The AT_2 receptor antagonist, PD 123319, did not attenuate the pressor response to central angiotensin II, whereas treatment of rats with the the AT_1 receptor antagonist, losartan, combined with PD 123319 was reported to be more effective in inhibiting the pressor response to angiotensin II than losartan alone (Toney and Porter, 1993). However, in most of the studies relatively high doses of the AT_2 receptors antagonist were used, and since these antagonists at higher concentrations were demonstrated to bind to the AT_1 receptor (Whitebread et al., 1991), non-specific interactions of these antagonists with periventricular AT_1 receptors cannot be excluded.

In the present study we investigated the effects of pretreatment with multiple doses of the AT_1 receptor antagonist, losartan, and the AT_2 receptor antagonist, PD 123177, on the angiotensin II-induced release of vasopressin and drinking reponse in conscious rats. We found that losartan effectively inhibited both responses already at low doses. On the other hand, intracerebroventricular (i.c.v.) pretreatment with 1000 pmol PD 123177 potentiated the angiotensin II-induced vasopressin release and dipsogenic response suggesting that periventricular AT_2 receptors can exert an inhibitory action on both of these central responses to angiotensin II.

2. Materials and methods

Male Wistar rats weighing 300–350 g obtained from Dr. Karl Thomae (Biberach/Riss, Germany) were used.

2.1. Surgical methods

Rats were anesthetized with chloralhydrate (400 mg/kg i.p.). Using a Kopf stereotaxic apparatus the i.c.v. cannula was implanted 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

Before rats were catheterized, 10 pmol angiotensin II was injected i.c.v. to test the position of the i.c.v. cannula. Only those animals which drank immediately after angiotensin II i.c.v. were chosen for the experiments. All experiments were performed in conscious and unrestrained rats 48 h after femoral surgery when the animals had regained their regular drinking habits. Experiments were started when the animals were in a resting state, quietly lying on the sawdust. Angiotensin II and the AT_1 and AT_2 receptor antagonists 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. We used physiological saline as vehicle solution instead of artificial cerebrospinal fluid since, in previous experiments, 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 received a single dose of antagonist followed by angiotensin II (10 pmol). The other half of the animals was pretreated with vehicle followed by angiotensin II (10 pmol). On the second day, treatment was reversed with respect to groups. Antagonists or vehicle were always injected 5 min prior to angiotensin II. Ninety seconds after the angiotensin II injection, 1 ml blood was drawn from the femoral catheter for vasopressin determination, and 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 is highest in the first 3 min after treatment (Hogarty et al., 1992). Water intake was determined in a period of 10 min after blood samples were drawn. The following doses of antagonist were used: losartan: 0.2 ($n = 7$), 2 ($n = 6$) and 10 ($n = 5$) nmol; PD 123177: 10 ($n = 6$), 100 ($n = 7$) and 1000 ($n = 11$) pmol. The effects of the antagonists injected i.c.v. alone on vasopressin release and drinking response were tested in separate experiments. Basal vasopressin levels in plasma were determined 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).

2.4. Drugs

The following substances were used: angiotensin II from Bachem, Heidelberg, Germany; losartan (2-*n*-butyl-4-chloro-5-hydroxy-methyl-1-2′-(1*H*-tetrazole-5-yl)biphenyl-4-yl)methylimidazole, potassium salt) was a gift from Dr. R.D. Smith, DuPont-Merck, Wilmington, DE, USA; 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 obtained from Parke Davis Pharmaceutical Research, Ann Arbor, MI, USA.

2.5. Statistics

Results are expressed as means ± S.E.M. Statistics were performed using one-way analysis of variance (ANOVA) followed by comparison of individual means with the Newman-Keuls post-hoc test. Significance was accepted at $P < 0.05$.

3. Results

3.1. Effect of pretreatment with the AT_1 receptor antagonist, losartan, on angiotensin II-induced vasopressin release and water intake

The lowest dose of losartan (0.2 nmol) attenuated the drinking response to 10 pmol angiotensin II (2.7 ± 1.1 ml vs. 5.0 ± 0.6 ml in vehicle-pretreated controls, $P < 0.05$) without affecting the vasopressin release

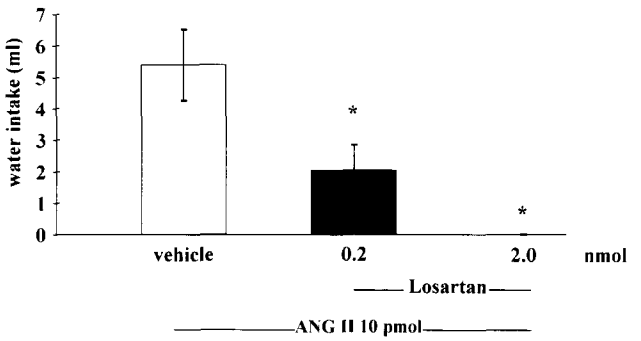

Fig. 2. Effect of i.c.v. pretreatment with losartan (0.2 and 2.0 nmol) on i.c.v. angiotensin II-induced water intake. The observation period was 10 min. Significant effects were observed at 0.2 and 2.0 nmol ($F = 15.93$, $P < 0.001$). Values are means ± S.E.M., $n = 5–7$. * $P < 0.05$ vs. 10 pmol angiotensin II with vehicle pretreatment.

(18.3 ± 3.5 pg/ml vs. 17.2 ± 3.7 pg/ml in vehicle-pretreated controls). Higher doses of losartan (2 and 10 nmol) completely abolished both responses (Figs. 1 and 2).

3.2. Effect of pretreatment with the AT_2 receptor antagonist, PD 123177, on angiotensin II-induced vasopressin release and water intake

PD 123177 at 1000 pmol significantly increased the vasopressin release in response to angiotensin II (39.7 ± 2.7 vs. 21.2 ± 2.9 pg/ml in vehicle-pretreated controls, $P < 0.05$; Fig. 3). The lower doses of PD 123177 did not significantly change this response although a tendency to increase was observed after 100 pmol (Fig. 3). Water intake in response to 10 pmol angiotensin II was also significantly potentiated by pretreatment with 1000 pmol PD 123177 (9.5 ± 0.7 ml vs. 5.3 ± 1.3 ml in vehicle-pretreated controls, $P < 0.05$; Fig. 4). As with vasopressin release, a tendency to increase was noted after 100 pmol (Fig. 4).

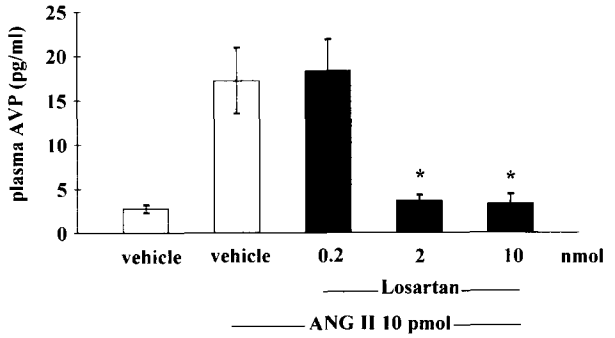

Fig. 1. Effect of i.c.v. pretreatment with losartan (0.2, 2.0 and 10.0 nmol) on i.c.v. angiotensin II-induced vasopressin release. Losartan was administered 5 min prior to angiotensin II. Significant effects were observed at 2.0 and 10.0 nmol losartan ($F = 12.03$, $P < 0.001$). Values are means ± S.E.M., $n = 5–7$. * $P < 0.05$ vs. 10 pmol angiotensin II with vehicle pretreatment.

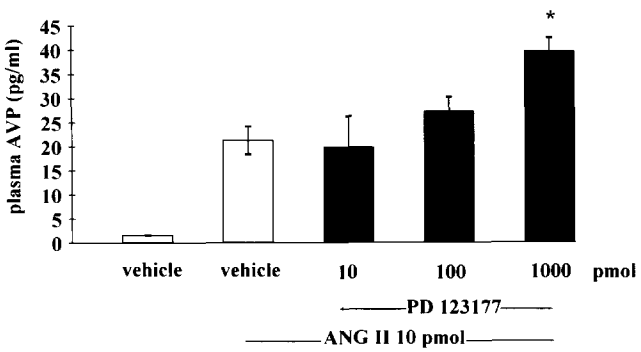

Fig. 3. Effect of i.c.v. pretreatment with PD 123177 (10, 100 and 1000 pmol) on i.c.v. angiotensin II-induced vasopressin release. A significant effect was observed at 1000 pmol PD 123177 ($F = 14.73$, $P < 0.001$). Values are means ± S.E.M., $n = 6–10$. * $P < 0.05$ vs. 10 pmol angiotensin II with vehicle pretreatment.

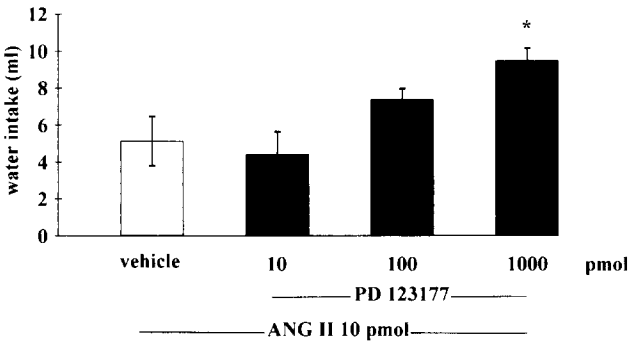

Fig. 4. Effect of i.c.v. pretreatment with PD 123177 (10, 100 and 1000 pmol) on i.c.v. angiotensin II-induced water intake. The observation period was 10 min. A significant effect was observed at 1000 pmol PD 123177 ($F = 6.04$, $P < 0.0025$). Values are means ± S.E.M., $n = 7–11$. * $P < 0.05$ vs. 10 pmol angiotensin II with vehicle pretreatment.

Neither losartan nor PD 123177 affected the basal vasopressin release or drinking behavior when injected i.c.v. alone (data not shown).

4. Discussion

Over the past few years, it has become apparent that both types of angiotensin receptors, AT_1 and AT_2, are present in the adult rat brain, and participation of individual angiotensin receptors in central angiotensin II-mediated effects has been intensively studied.

It is generally accepted that the central angiotensin II-induced cardiovascular and endocrine responses, such as induction of drinking, stimulation of natriuresis and release of vasopressin are mediated by AT_1 receptors (for review see Steckelings et al., 1992; Saavedra, 1992).

Several groups, including ours, have attempted to characterize the role of AT_2 receptors in the central actions of angiotensin II. These studies have yielded contradictory results, partly due to the fact that different doses of angiotensin II and of specific AT_2 receptor antagonists or ligands were used in these studies (Hogarty et al., 1992; Qadri et al., 1993; Toney and Porter, 1993).

In the present study, we investigated the effect of selective inhibition of periventricular AT_1 and AT_2 receptors on the angiotensin II-induced vasopressin release and dipsogenic response. We employed a low dose of angiotensin II (10 pmol) to approximate more closely the physiological effects of the peptide on the circumventricular organs and multiple doses of each antagonist, starting at low doses that had not been used before, to increase the probability of a selective interaction of individual agonists with the respective type of receptor.

Intracerebroventricular injection of angiotensin II (10 pmol) resulted in a 10-fold increase in the plasma vasopressin concentration and in an immediate drinking response. As expected, i.c.v. pretreatment with 2 nmol of the specific AT_1 receptor antagonist, losartan, effectively attenuated both effects, the drinking response to angiotensin II being more sensitive to the AT_1 receptor blockade than the vasopressin release. A similar dose of losartan (0.7 μg = 1.4 nmol) had previously failed to completely prevent the angiotensin II-induced vasopressin release when a higher dose of peptide (50 ng i.c.v.) was used (Hogarty et al., 1992). Accordingly, 5–10 times higher doses of losartan were required to inhibit the angiotensin II-induced vasopressin release or the increase in blood pressure when a dose of 100 ng angiotensin II was injected i.c.v. (Qadri et al., 1993; Toney and Porter, 1993).

The lowest dose of losartan used in this study (0.2 nmol) significantly abolished the dipsogenic response to angiotensin II while leaving the vasopressin response unaffected. The most probable explanation for this observation is that periventricular AT_1 receptors mediating the drinking response to angiotensin II are more readily accessible to the antagonist than those mediating the vasopressin release. This would imply that both responses are mediated by different populations of AT_1 receptors.

Pretreatment with the specific AT_2 receptor antagonist, PD 123177, injected i.c.v. at the dose of 1000 pmol potentiated both the angiotensin II-induced vasopressin release from the posterior pituitary and the dipsogenic response to angiotensin II. Concerning the dipsogenic response our findings are in agreement with a report by Dourish et al. (1992). These authors observed that the AT_2 receptor antagonist, WL-19, given subcutaneously (s.c.), enhanced the s.c. angiotensin II-induced dipsogenic response.

Studies employing quantitative autoradiography did not reveal any AT_2 receptors in circumventricular organs (Tsutsumi and Saavedra, 1991). In contrast, we have previously found small quantities of AT_2 receptors in the subfornical organ, organum vasculosum laminae terminalis and in the periventricular nucleus (Obermüller et al., 1991) using radioligand binding to membranes isolated from these areas, a method more sensitive than quantitative autoradiography (Bottari et al., 1993). Our present data provide pharmacological evidence for the presence of functionally active AT_2 receptors in periventricular areas of the adult rat brain.

AT_2 receptors have been defined as binding sites having a high affinity for PD 123177 ($K_i = 10–100$ nM) and CGP 42112A ($K_i = 1$ nM) and a low affinity for losartan ($K_i = 1$ μM) (Bottari et al., 1993). Since appropriate doses of PD 123177 were used in the present study it can be assumed that the observed stimulatory effect of PD 123177 on angiotensin II-induced vaso-

pressin release and drinking response resulted exclusively from a specific interaction of the antagonist with the AT_2 receptor.

In our previous experiments, PD 123177 had failed to affect the vasopressin response to angiotensin II (Qadri et al., 1993). Hogarty et al. (1992) even reported that this AT_2 receptor antagonist had an inhibitory effect on vasopressin release and no effect on the dipsogenic response to i.c.v. angiotensin II. However, significantly higher doses of the AT_2 receptor antagonist were used in both above-mentioned studies than in the present experiments. As PD 123177 at higher doses or concentrations can also interact with AT_1 binding sites (Whitebread et al., 1991; Timmermans et al., 1993) unspecific inhibition of periventricular AT_1 receptors with PD 123177 at a dose of 7 μg (14.58 nmol) (Hogarty et al., 1992) may account for the observed inhibition of angiotensin II-induced vasopressin release. Pretreatment with a lower dose of PD 123177 (5 μg = 10.4 nmol) did not affect the central responses to angiotensin II (Qadri et al., 1993). This dose of PD 123177 may, in addition to the inhibition of AT_2 receptors, also antagonize AT_{1B} receptors to which at least PD 123319 was shown to possess a higher affinity than to AT_{1A} receptors (Ernsberger et al., 1992). The simultanous inhibition of AT_2 and a part of AT_1 receptors could thus explain the lack of PD 123177 at a dose of 5 μg to affect the central responses to angiotensin II.

Multiple doses of losartan and PD 123319 were used to investigate the central pressor responses induced by angiotensin II (Toney and Porter, 1993). Losartan was reported to inhibit the vasopressin component of the angiotensin II-induced pressor response in a dose-dependent manner while PD 123319 was without effect. To determine the vasopressin component of the angiotensin II-induced pressor response, the authors measured mean arterial pressure after peripheral administration of the ganglionic blocker, chlorisondamine. This approach may not be sensitive enough to discern small changes in vasopressin levels in the circulation. The direct measurement of vasopressin levels in plasma used in the present study seems to be the more adequate method for these purposes.

In the same study, combined treatment with losartan and 3.5 μg PD 123319 (6.03 nmol) was demonstrated to be more effective in reducing the pressor response to angiotensin II than the treatment with high doses of losartan alone (Toney and Porter, 1993). An inhibition of periventricular AT_{1B} receptors with PD 123319 may account for the observed effects since AT_{1B} receptors are expressed in the circumventricular organs and since these receptors have been reported to display a higher affinity for PD 123319 than for losartan (Kakar et al., 1992; Bottari et al., 1993; Ernsberger et al., 1992).

We have demonstrated that an inhibition of periventricular AT_1 and AT_2 receptors affects AVP release and dipsogenic response to angiotensin II in an opposite manner. This is not surprising in view of the fact that the effects of a selective stimulation of either AT_1 or AT_2 receptors have been shown to oppose each other in various biological systems. Thus, angiotensin II induced a heterogenous response in neurons co-cultured from the hypothalamus and brain stem. A major population of neurons showed an increase in potassium outward current which was blocked by PD 123177 and PD 123319 but not by losartan, indicating that this effect was mediated by the AT_2 receptor, whereas the decrease in potassium outward current in a small population of neurons showed the opposite specificity with regard to angiotensin antagonists (Kang et al., 1993). Further, it has been demonstrated that angiotensin II and angiotensin III can produce a biphasic arterial blood pressure response. The pressor response was mediated by AT_1 while the depressor response was reported to be related to AT_2 receptors (Scheuer and Perrone, 1993). Recently, we have been able to demonstrate in endothelial cells that angiotensin II exerts different growth-modulating actions depending on the type of angiotensin receptor present on the respective cells: growth promoting effects mediated by the AT_1 receptor are counteracted by inhibitory actions on growth mediated by the AT_2 receptor (Stoll et al., 1994).

In summary, selective antagonists of angiotensin receptors have been used to characterize the role of central AT_1 and AT_2 receptors in mediating the angiotensin II-induced vasopressin release and water intake. Our results support previous findings that both responses to angiotensin II are mediated by AT_1 receptors. In addition, the present study reveals an inhibitory effect of central AT_2 receptors on both angiotensin II-induced release of vasopressin and drinking. This finding suggests that central AT_1-mediated actions of angiotensin II are under inhibitory control by the AT_2 receptor.

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References

Beresford, M.J. and J.T. Fitzsimons, 1992, Intracerebroventricular angiotensin II-induced thirst and sodium appetite in rat are blocked by the AT_1 receptor antagonist, losartan (DuP 753), but not by the AT_2 receptor antagonist, CGP 42112B, Exp. Physiol. 77, 761.

Bottari, S.P., M. De Gasparo, U.M. Steckelings and N.R. Levens, 1993, Angiotensin II receptor subtypes: characterization, sig-

nalling mechanisms, and possible physiological implications, Front. Neuroendocrinol. 14(2), 123.

Dourish, C.T., J.A. Duggan and R.J.A. Banks, 1992, Drinking induced by subcutaneous injection of angiotensin II in the rat is blocked by the selective AT_1 receptor antagonist DuP 753 but not by the selective AT_2 receptor antagonist WL 19, Eur. J. Pharmacol. 211, 113.

Ernsberger, P., J. Zhou, T.H. Damon and J.G. Douglas, 1992, Angiotensin II receptor subtypes in cultured rat renal mesangial cell, Am. J. Physiol 263 (Renal Fluid Elektrolyte Physiol. 32), F411.

Fregly, M.J. and N.E. Rowland, 1991, Effect of a nonpeptide angiotensin II receptor antagonist, DuP 753, on angiotensin-related water intake in rats, Brain Res. Bull. 27, 97.

Hogarty, D.C., E.A. Speakman, V. Puig and M.I. Phillips, 1992, The role of angiotensin, AT_1 and AT_2 recptors in the pressor, drinking and vasopressin responses to central angiotensin, Brain Res. 586, 289.

Kakar, S.S., K.K. Riel and J.D. Neill, 1992, Differential expression of angiotensin II receptor subtype mRNAs (AT-1A and AT-1B) in the brain, Biochem. Biophys. Res. Commun. 185(2), 688.

Kambayashi, Y., S. Bardhan, K. Takahashi, S. Tsuzuki, H. Inui, T. Hamakubo and T. Inagami, 1993, Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition, J. Biol. Chem. 268(33), 24543.

Kang, J., C. Sumners and P. Posner, 1993, Angiotensin II type 2 receptor-modulated changes in potassium currents in cultured neurons, Am. J. Physiol. 265 (Cell Physiol. 34), C607.

Mukoyama, M., M. Nakajiama, M. Horiushi, H. Sasamura, R.E. Pratt and V. Dzau, 1993, Expression cloning of type 2 angiotensin II receptor reveals a unique class of seven-transmembrane receptors, J. Biol. Chem. 268(33), 24539.

Murphy, T.J., R.W. Alexander, K.K. Griendling, M.S. Runge and K.E. Bernstein, 1991, Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor, Nature 351, 233.

Obermüller, N., Th. Unger, J. Culman, P. Gohlke and S.P. Bottari, 1991, Distribution of angiotensin II receptor subtypes in rat brain nuclei, Neurosci. Lett. 132, 11.

Qadri, F., J. Culman, A. Veltmar, K. Maas, W. Rascher and Th. Unger, 1993, Angiotensin II-induced vasopressin release is mediated through alpha-1 adrenoceptors and angiotensin II AT1 receptors in the supraoptic nucleus, J. Pharmacol. Exp. Ther. 267(2), 567.

Rascher, W., E. Weidmann and F. Gross, 1981, Vasopressin in the plasma of stroke-prone spontaneously hypertensive rats, Clin. Sci. 61, 295.

Saavedra, J.M., 1992, Brain and pituitary angiotensin, Endocr. Rev. 13(2), 329.

Sasaki, K., Y. Yamano, S. Bardhan, N. Iwai, J.J. Murray, M. Hasegawa, Y. Matsuda and T. Inagami, 1991, Cloning and expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor, Nature 351, 230.

Scheuer, D.A. and M.H. Perrone, 1993, Angiotensin type 2 receptors mediate depressor phase of biphasic pressure response to angiotensin, Am. J. Physiol. 264 (Regul. Integr. Comp. Physiol. 33), R917.

Stauss, H. and Th. Unger, 1990, Role of angiotensin in the drinking response after water deprivation, Naunyn-Schmied. Arch. Pharmacol. 343 (Suppl.), R106.

Steckelings, U.M., S.P. Bottari and Th. Unger, 1992, Angiotensin receptor subtypes in the brain, Trends Pharmacol. Sci. 13, 365.

Stoll, M., U.M. Steckelings, S.P. Bottari, M. Paul, R. Metzger and Th. Unger, 1994, The angiotensin AT_2-receptor mediates inhibition of cell proliferation coronary endothelial cells, J. Clin. Invest. (in press).

Thrasher, T.N., 1985, Circumventricular organs, thirst, and vasopressin secretion, in: Vasopressin, ed. R.W. Schrier (Raven Press, New York) p. 311.

Timmermans, P.B.M.W.M., P.C. Wong, A.T. Chiu, W.F. Herblin, P. Benfield, D.J. Carini, R.J. Lee, R.R. Wexler, J.A.M. Saye and R.D. Smith, 1993, Angiotensin II receptors and angiotensin II receptor antagonists, Pharmacol. Rev. 45(2), 205.

Toney, G.M. and J.P. Porter, 1993, Functional role of brain AT_1 and AT_2 receptors in the central angiotensin II pressor response, Brain Res. 603, 57.

Tsutsumi, K. and J.M. Saavedra, 1991, Quantitative autoradiography reveals different angiotensin II receptor subtypes in selected rat brain nuclei, J. Neurochem. 56, 348.

Veltmar, A., J. Culman, F. Qadri, W. Rascher and Th. Unger, 1992, Involvement of adrenergic and angiotensinergic receptors in the paraventricular nucleus in the angiotensin II-induced vasopressin release, J. Pharmacol. Exp. Ther. 267, 1253.

Whitebread, S.E., V. Taylor, S.P. Bottari, B. Kamber and M. De Gasparo, 1991, Radioiodinated CGP 42112A: a novel high affinity and highly selective ligand for the characterization of angiotensin AT_2 receptors, Biochem. Biophys. Res. Commun. 181(3), 1365.

Wong, P.C., W.A. Price, A.T. Chiu, J.V. Duncia, D.J. Carini, R.R. Wexler, A.L. Johnson and P.B.M.W.M. Timmermans, 1990, Nonpeptide angiotensin II receptor antagonists. VIII. Charaterization of functional antagonism displayed by DUP 753, an orally active antihypertensive agent, J. Pharmacol. Exp. Ther. 252, 719.