Basic Res Cardiol 93: Suppl. 2, 63 – 68 (1998)
© Steinkopff Verlag 1998

Y. Nishimura
T. Xu
O. Jöhren
W. Häuser
J. M. Saavedra

The angiotensin AT_1 receptor antagonist CV-11974 regulates cerebral blood flow and brain angiotensin AT_1 receptor expression

Abstract We studied cerebral blood flow autoregulation by laser Doppler flowmetry, and expression of brain angiotensin II AT_1 receptors by quantitative autoradiography, after administration of an angiotensin AT_1 receptor antagonist, CV-11974 (Candesartan, 0.5 or 1.0 mg/kg·day) for two weeks via subcutaneously implanted osmotic pumps in adult normotensive Wistar Kyoto and spontaneously hypertensive male rats (SHR). In SHR, the autoregulation curve was shifted towards higher blood pressures, when compared with that of normotensive Wistar Kyoto rats. Administration of CV-11974 shifted the autoregulation curve toward lower blood pressures in both Wistar Kyoto and SHR, partially normalizing the autoregulation curve in SHR. CV-11974 treatment markedly decreased the expression of AT_1 receptors in Wistar Kyoto rats, both in areas outside the blood brain barrier (subfornical organ, 95 % decrease) and inside the blood brain barrier (nucleus of the tractus solitarius, 87 % decrease, and paraventricular nucleus, 96 % decrease). Our results demonstrate that blockade of AT_1 receptors tends to normalize the shift to higher pressures in the autoregulation curve of genetically hypertensive rats, and has a profound modulatory role in brain angiotensin II AT_1 receptors.

Key words Cerebral blood flow – autoregulation – Angiotensin II receptors – Wistar-Kyoto rats – Spontaneously hypertensive rats (SHR) – AT_1 receptor expression

Y. Nishimura · T. Xu · O. Jöhren · W. Häuser
J. M. Saavedra (✉)
Section on Pharmacology
National Institute of Mental Health
10 Center Drive, Bldg 10/2D57
Bethesda, MD 20892-1264, USA
e-mail: Saavedrj@irp.nimh.nih.gov

Introduction

Angiotensin II (ANG II) modulates blood pressure in part by vasoconstriction of peripheral arteries, which results from stimulation of ANG II AT_1 receptors in smooth muscle cells (11). All known actions of ANG II seem to be mediated through stimulation of AT_1 receptors, and blockade of AT_1 receptors with selective inhibitors is an effective treatment of hypertension in humans (11). In addition to its actions in the regulation of the peripheral circulation, there is evidence to suggest the possibility of a direct modulation of cerebrovascular flow (CBF) by ANG II. ANG II receptors have been described in cerebral microvessels (8). The presence of large amounts of ACE in cerebral microvessels suggests that ANG II could be locally produced in cerebral arteries (1). Thus, both circulating and locally formed ANG II could modulate CBF.

We have recently reported (3) that ANG II contracts perfused rat cerebral arteries by an AT_1 related mechanism. However, in young, immature rats (two weeks old), cerebral arteries of the Willis polygon express substantial amounts of AT_2, not AT_1, ANG II receptors, as determined by autoradiography (12). Acute administration of both AT_1 antagonists and AT_2-selective receptor ligands was reported to modulate the upper and lower limits of the CBF autoregulation in rats (2, 9).

Since the goal of anti-hypertensive treatments is the prevention of end organ damage, the question was raised whether the anti-hypertensive treatment with AT_1 receptor antagonists could prevent or reverse the cerebral complications arising from chronic hypertension. We have initiated a series of studies to answer this question. As the first step in that direction, we investigated the effects of repeated administration of AT_1 antagonists on the regulation of CBF in normotensive and

© Steinkopff Verlag 1998

genetically hypertensive rats. We chose to administer the AT_1 antagonist CV-11974, because of its potency and previously demonstrated effects in cerebrovascular control (13).

Materials and methods

Animals

Adult male SHR and age-matched WKY rats, weighing 250 to 300 g, were purchased from Taconic Farms, Germantown, NY, and kept under standard conditions, with lights on from 07:00 AM to 07:00 PM, water and rat chow ad libitum. All procedures were approved by the NIMH Animal Care and Use Committee.

Treatment with CV-11974

Animals were anesthetized with ketamine, 100 mg/kg i.p., and xylazine, 10 mg/kg i.p., and Alzet osmotic pumps (Alza Scientific Products, Palo Alto, CA) containing CV-11974, (a gift from Astra, Wedel, Germany) dissolved in 1 mol/l sodium carbonate and further diluted in isotonic saline, to be delivered at a rate of 0.5 mg/kg·day or 1.0 mg/kg·day for cerebral blood flow determinations, or at a rate of 1.0 mg/kg·day, for AT_1 receptor binding experiments, were implanted subcutaneously. Control animals received osmotic pumps filled with vehicle, pH 7.5 to 8.0.

Surgery for cerebral blood flow measurements

Rats were anesthetized with ketamine, 100 mg/kg i.p., and xylazine, 10 mg/kg i.p. After intratracheal intubation and spontaneous respiration with supplemental oxygen, the femoral artery and vein were cannulated. Baseline arterial blood pressure was measured through the femoral artery cannula with a Spectramed P23XL transducer (Spectramed, Oxnard, CA), under normal pH and concentrations of blood gasses (pH 7.35 to 7.45, pCO_2 40–45 and pO_2 80 to 100 mmHg), measured with a Stat Profile 3 analyzer (NOVA Biochemical, Walthan, MA, USA). The rats were then ventilated with 0.35 % halothane in 70 % nitrous oxide and 30 % oxygen with a Ugo Basile 7025 Rodent Respirator (Stroelting, Wood Date, IL, USA). After the animals were placed in a stereotaxic frame, their skull was exposed and a small, 2 x 2 mm, hole was drilled in the parietal bone under a microscope, leaving the dura mater intact. During the drilling, the exposed skull was flushed with saline to prevent thermal injury, and the skull window was enlarged to a 4 x 7 mm size by use of a bone ronger.

Cerebral blood flow measurement

To measure the CBF, a laser-Duppler flowmetry probe (BPM2, Vasamedics, Inc., St. Paul, MN, USA) was fixed under microscopic control in a position close (less than 1 mm) to the exposed dural surface, taking care to avoid the proximity of the probe to any larger or medium size cerebral vessels. Blood pressure and laser-Doppler flowmetry signals were recorded simultaneously using a Grass model 79 polygraph (Grass Instrument Co., Quicy, MA, USA). Arterial blood for gas analysis was drawn from the arterial cannula, and ventilation was adjusted to keep the pH between 7.35 and 7.45, $PaCO_2$ between 32 and 40 mmHg, and the PaO_2 between 130 and 180 mmHg. Rectal temperature was maintained between 36.5 and 37.5 °C with a heating pad, and monitored with a rectal thermometer.

To increase blood pressure, phenylephrine was infused through the femoral vein in increasing amounts (0.1 to 10 µg/min) until a sudden elevation of the CBF indicated the loss of autoregulation. Results were expressed plotting the percent CBF relative to the baseline (100 %), against the mean arterial blood pressure (MABP) to generate CBF autoregulation curves. The upper limit of CBF autoregulation was defined as the mean arterial blood pressure at which CBF increased to a value double that of the baseline value. Results were expressed as means ± S.E.M. The upper limits of CBF autoregulation were compared with unpaired t-tests.

Quantitative autoradiography

Animals were treated with CV-11974 infused with osmotic pumps implanted subcutaneously for fourteen days, at a dose of 1.0 mg/kg·day. After analysis of the CBF, the brains were removed immediately, frozen at –30 °C by immersion in isopentane on dry ice, and kept frozen at –80 °C until used. Consecutive coronal brain sections, 16 µm thick, were cut in a cryostat and incubated to determine the levels of ANG II receptors. ANG II AT_1 receptors were determined by incubation of the sections with 0.5×10^{-9} M [^{125}I] Sar^1-ANG II (2200 Ci/mmol, iodinated at New England Nuclear, Boston, MA) for total binding, and a consecutive section was incubated with the ligand in the presence of 10^{-5} M of the AT_1 antagonist losartan (Dupont Merck, Wilmington, DE). The number of AT_1 receptors was determined as the difference between total binding and binding remaining after the displacement with the AT_1 antagonist (12).

After binding sections were dried and exposed to Hyperfilm-[^{3}H]. Optical densities of autoradiograms were measured by computerized microdensitometry using the Image 1.61 program (NIMH, Bethesda, MD) quantified by comparison with [^{125}I]Micro-scales (Amersham Corporation, Arlington Heights, IL) and transformed to corresponding values of

fmol/mg protein (4). Data were analyzed with the GraphPad Prism 2.0 software (San Diego, CA). Brain regions were identified by comparison with toluidine blue staining of consecutive brain sections.

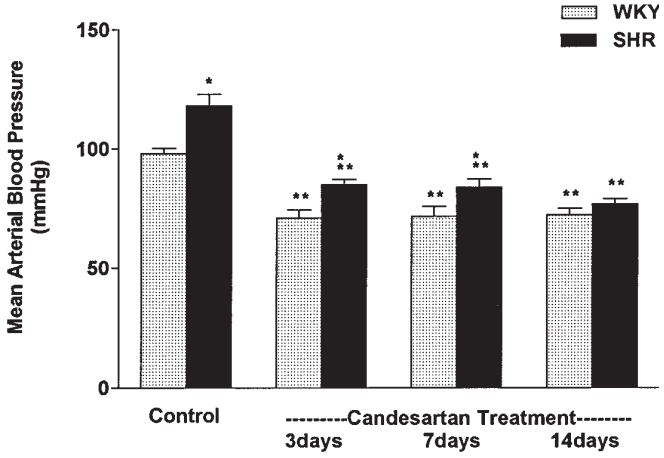


Fig. 1 Mean arterial blood pressures after repeated treatment with CV-11974. WKY: Wistar Kyoto rats. SHR: spontaneously hypertensive rats. CV-11974 was administered at 0.5 mg/kg·day with minipumps implanted subcutaneously. * Statistically significant ($p < 0.05$) SHR vs WKY. ** Statistically significant ($p < 0.05$) treated vs nontreated groups. N: six to eight animals per group.

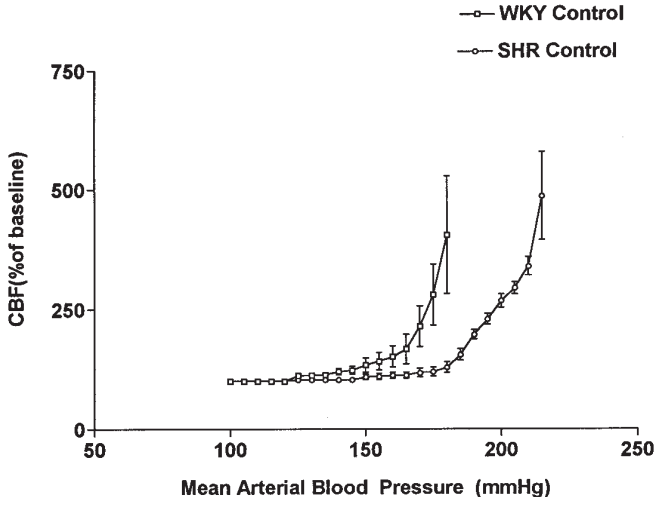


Fig. 2 Cerebrovascular autoregulation in genetically hypertensive and normotensive control rats. Spontaneously hypertensive rats (SHR) (open circles) show a shift towards higher blood pressures when compared to normotensive controls (WKY rats) (open squares). N: six to eight animals per group ($P < 0.05$).

Results

Effects of CV-11974 on blood pressure

Treatment with CV-11974, 0.5 mg/kg·day, administered through minipumps implanted subcutaneously, reduced the mean blood pressure in both WKY and SHR, as early as 3 days after treatment, and the reduction was maintained for the 14 days of the experiment. The blood pressure differences between untreated WKY and SHR disappeared after treatment with CV-11947 throughout the entire treatment (Fig. 1).

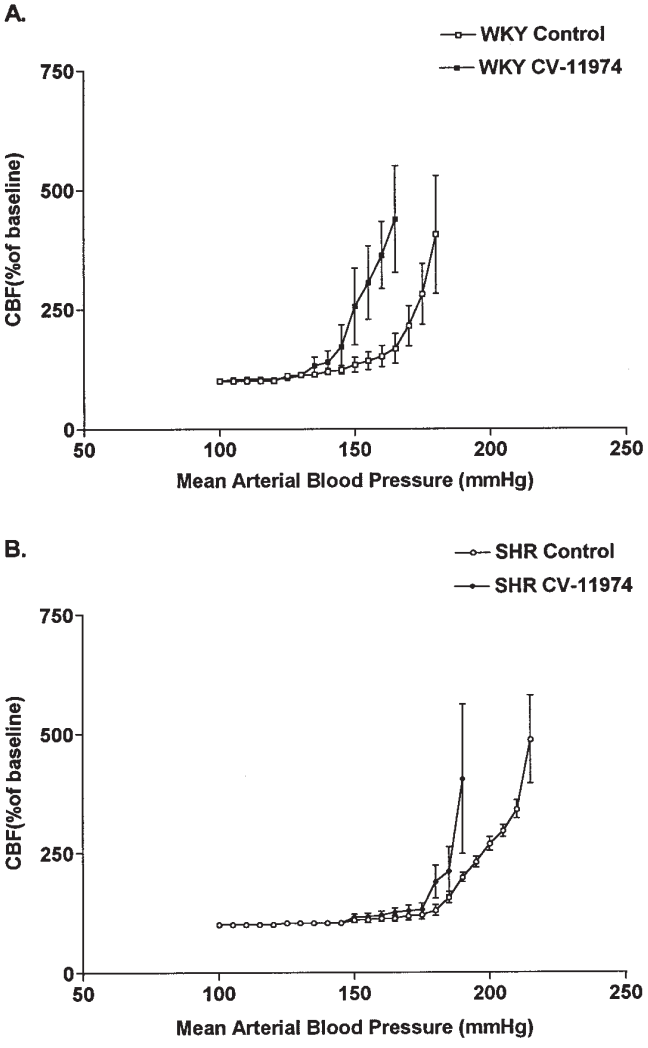


Fig. 3 Effect of repeated treatment with CV-11974 on cerebrovascular autoregulation in genetically hypertensive and normotensive control rats. Repeated treatment with CV-11974 significantly shifted the cerebrovascular autoregulation towards lower blood pressures in normotensive (WKY) rats (Fig. 3A, closed squares) and in hypertensive rats (SHR) (Fig. 3B, closed circles). N: six to eight animals per group ($P < 0.05$).

© Steinkopff Verlag 1998

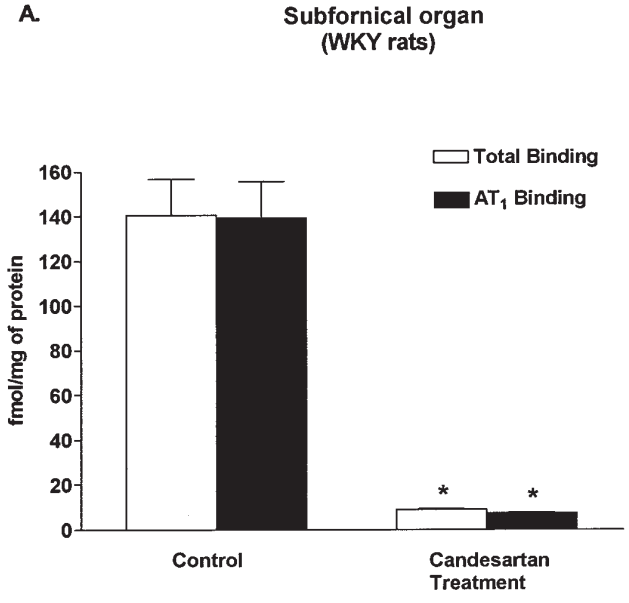
Effects of CV-11974 on the cerebrovascular autoregulation in WKY and SHR

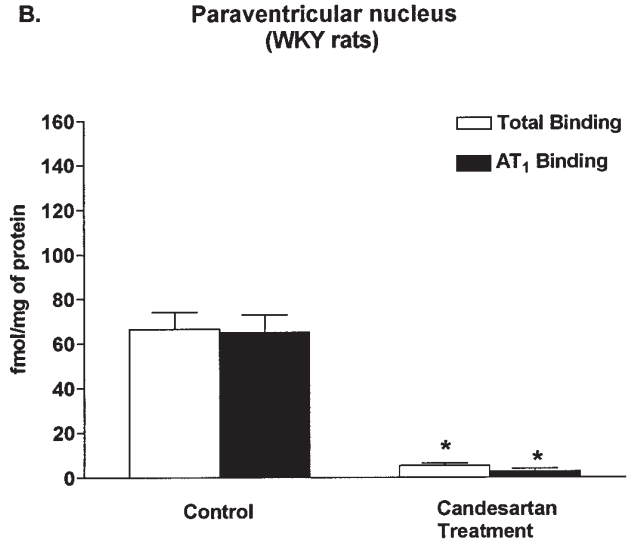
In untreated adult SHR, the autoregulation curve is significantly shifted to the right, towards higher blood pressures, when compared to that of WKY (Fig. 2). Treatment with CV-11974, 0.5 mg/kg·day, significantly shifted the autoregulation towards the left in both WKY and SHR (Fig. 3). After 14 days of treatment, the differences between the autoregulation curves in SHR and WKY were partially diminished (Fig. 3). A similar change in CBF in both WKY and SHR was observed after treatment with CV-11974 for fourteen days, at a dose of 1.0 mg/kg·day (results not shown).

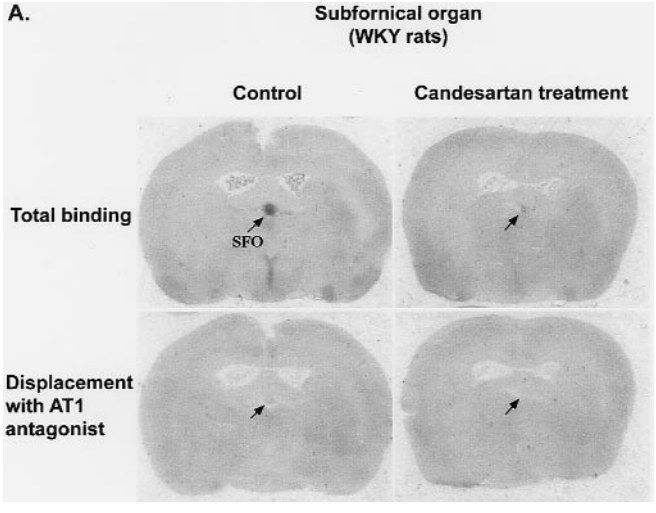
Effects of CV-11974 on brain ANG II AT_1 receptor expression

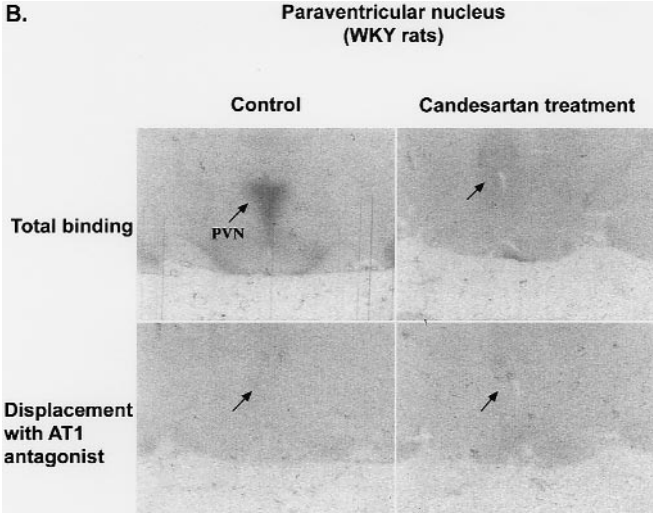
Treatment with CV-11974, 1 mg/kg·day, with subcutaneously implanted minipumps, profoundly modified the expression of

Fig. 4 Quantitative autoradiography of brain AT_1 receptors in normotensive rats after repeated treatment with CV-11974. **Fig. 4A:** Subfornical organ; **Fig. 4B:** Paraventricular nucleus; **Fig. 4C:** nucleus tractus solitarius. CV-11974 was administered for fourteen days at a dose of 1.0 mg/kg·day. Total binding was binding in the presence of [^{125}I]Sar^1-ANG II. In consecutive sections, binding was displaced by the AT_1 antagonist losartan (see text). N: four to six animals per group; * $P > 0.05$.





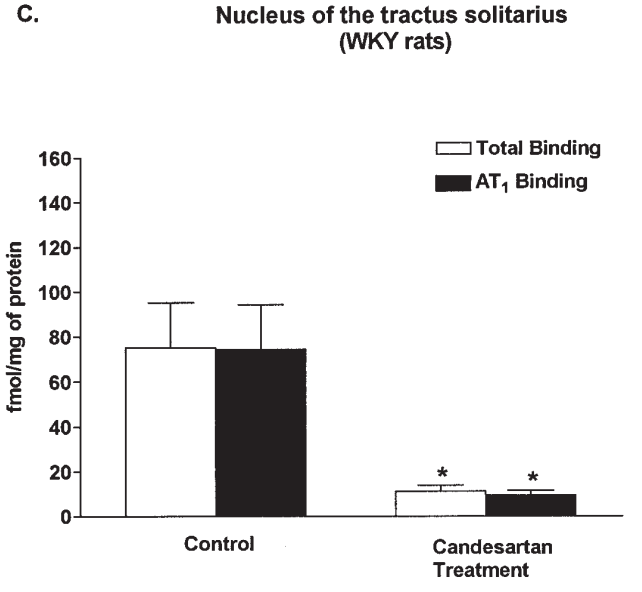


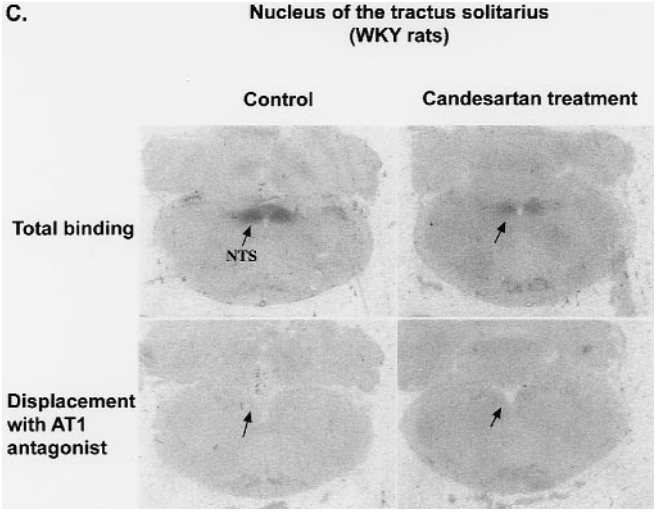


AT_1 receptors in WKY rats. The number of AT_1 receptors decreased by about 85 % to 95 % in the subfornical organ, paraventricular nucleus and nucleus of the solitary tract (Fig. 4).

Discussion

The main observations of our study were that repeated peripheral administration of CV-11974 affected cerebrovascular autoregulation and dramatically changed ANG II receptor AT_1 binding in the brain.





The dose of CV-11974 used in this study (0.5 mg/kg·day, for fourteen days) lowered the mean arterial blood pressure in both WKY and SHR. In the SHR, the decrease in blood pressure was progressive, and after fourteen days of treatment the blood pressure in hypertensive animals was not different from that of normotensive controls.

Determination of cerebral blood flow required total anesthesia, a procedure which might have influenced cerebral blood flow and vascular reactivity. However, since the anesthesia was similar for both hypertensive and control animals, the changes observed are not likely to be the result of the anesthesia procedure.

While baseline cerebral blood flow was not changed by CV-11974 (results not shown) this AT_1 antagonist shifted the upper limit of autoregulation towards lower blood pressures. This effect was similar to that described earlier (13) after acute, intravenous administration of CV-11974. The results obtained with the repeated administration of CV-11974 in hypertensive rats are similar to those previously reported after administration of angiotensin converting enzyme (ACE) inhibitors in both animals and humans, that is, a normalization of the autoregulation curve shift to higher blood pressures in genetic hypertension (7). It has been hypothesized that both the inhibition of ANG II synthesis or the blockade of ANG II AT_1 receptors inhibit local affects of ANG II at the level of the cerebral arteries and that, as a result, the vascular tone maintained by ANG II is inhibited (13). In large cerebral arteries of the rat, the predominant ANG II receptor subtype is the AT_2 subtype (12). However, in the isolated basilar artery of the rat, ANG II produces a vasoconstrictive effect which is antagonized by AT_1 blockers (3). ANG II has been reported to produce vasoconstriction or vasodilatation in the cerebral vessels, and the effects depend on the conditions of the experiment, the species used, and the vascular bed studied (13). In addition, the nature of the ANG II receptors present in the small cerebral arteries and precapillary vessels is not known. Further experiments will be necessary to clarify the precise role of each ANG II receptor subtype in cerebrovascular flow. Nevertheless, the present and previous (13) experiments indicate that blockade of ANG II AT_1 receptors, and specially repeated treatment as presented here, allows altered cerebral blood flow during hypertension to re-adapt towards normal. Treatment with ACE inhibitors or AT_1 antagonists may represent a clear advantage (6), because other antihypertensive drugs, such as the calcium antagonists, are cerebral vasodilators, and have the potential for paralyzing the autoregulation and raising intra cranial pressure (10).

We have reported earlier (2, 9) that acute administration of the AT_1 blocker losartan shifts the autoregulation curve towards higher blood pressures, an effect opposite to the one described here and by others (13) for the AT_1 blocker CV-11974. It is not likely that the differences are related to differential actions of AT_1 antagonists, but rather to differences in

© Steinkopff Verlag 1998

the anesthesia protocol and the Doppler measurement procedures. Because the studies utilizing CV-11974 revealed similar results after acute and chronic administration, in two different laboratories, and because the effects of blockade of AT_1 receptors on the cerebral circulation is similar to that of ANG II synthesis inhibition with ACE inhibitors, we believe that the results presented here are an accurate demonstration of the effects of suppression of ANG II effects on the cerebrovascular flow.

Our results do not clarify whether treatment with AT_1 blockers can protect from the effects of cerebral ischemia. It is tempting to speculate, however, that blockade of ANG II effects may have effects similar to that of blockade of ANG II synthesis. For example, administration of ACE inhibitors attenuates the metabolic effects observed after cerebral ischemia, such as increased lactate and decreased ATP levels (6) and improves the neurologic outcome of rats after cerebral ischemia (14).

Preliminary experiments presented here reveal that large (1 mg/kg·day for fourteen days) doses of CV-11974 result in a dramatic decrease of ANG II AT_1 binding in brain areas both outside and inside the blood brain barrier. These results demonstrate that, at least in high doses, CV-11974 may exert central effects by blocking brain AT_1 receptors. Since brain ANG II receptors have been shown to be up-regulated in genetic hypertension, in areas related to cardiovascular and autonomic control (5) it is possible that central effects may play a role in the anti-hypertensive effect and the modulation of the cerebral blood flow in genetic hypertension.

The mechanism of the decrease in brain AT_1 binding by CV-11974 has not been clarified. Perhaps the decrease in binding is a result of receptor occupancy by the AT_1 antagonist. Alternatively, decreased AT_1 binding may be the consequence of alterations in transcription or translation of brain AT_1 receptors. Nevertheless, decreased binding occurs not only in brain areas outside the blood brain barrier, such as the subfornical organ, but in brain areas inside the blood brain barrier such as the nucleus tractus solitarius. Whatever the mechanism of action, administration of CV-11974 produces an apparent decrease AT_1 binding throughout the brain.

Our results raise the possibility of a regulatory effect of AT_1 receptor antagonists both in cerebrovascular flow and in the expression and/or function of central AT_1 receptors. Future experiments will perhaps clarify whether AT_1 antagonists could prevent or protect from alterations of cerebral blood flow such as those occurring during stroke, and whether AT_1 receptor blockade could result in behavioral effects of interest.

Acknowledgment W. Häuser and T. Xu were supported by grants from Astra GmbH, Germany.

References

1. Brecher P, Tercyak A, Chobanian AV (1981) Properties of angiotensin-converting enzyme in intact cerebral microvessels. Hypertension 3: 198–204
2. Näveri L, Strömberg C, Saavedra JM (1994) Angiotensin II AT_2 receptor stimulation extends the upper limit of cerebral blood flow autoregulation: Agonist effects of CGP 42112 and PD 123319. J Cereb Blood Flow Metab 14: 38–44
3. Näveri L, Strömberg C, Saavedra JM (1994) Angiotensin II AT_1 receptor mediated contraction of the perfused rat cerebral artery. NeuroReport 5: 2278–2280
4. Nazarali AJ, Gutkind JS, Saavedra JM (1989) Calibration of [^{125}I]-polymer standards with [^{125}I]-brain paste standards for use in quantitative receptor autoradiography. J Neurosci Meth 30: 247–253
5. Saavedra JM, Correa FMA, Plunkett LM, Israel A, Kurihara M, Shigematsu K (1986) Binding of angiotensin and atrial natriuretic peptide in brain of hypertensive rats. Nature 320: 758–760
6. Sadoshima S, Kenichiro F, Ooboshi H, Ibayashi S, Fujishima M (1993) Angiotensin converting enzyme inhibitors attenuate ischemic brain metabolism in hypertensive rats. Stroke 24: 1561–1567
7. Sadoshima S, Nagao T, Ibayashi S, Fujishima M (1994) Inhibition of angiotensin-converting enzyme modulates the autoregulation of regional cerebral blood flow in hypertensive rats. Hypertension 23: 781–785
8. Speth RC, Harik SI (1985) Angiotensin II receptor binding sites in brain microvessels. Prov Natl Acad Sci USA 82: 6340–6343
9. Strömberg C, Näveri L, Saavedra JM (1992) Angiotensin AT_2 receptors regulate cerebral blood flow in rats. Neuroreport 3: 703–704
10. Strandgaard S, Paulson OB (1995) Cerebral blood flow in untreated and treated hypertension. Neth J Med 47: 180–184
11. Timmermans PBMWM, Inagami T, Saavedra JM, Ardaillou R, Rosenfeld CR, Mendelsohn FAO (1995) Angiotensin receptor subtypes and their parmacology. In: Cuello AC, Collier B (eds) Pharmacological Sciences: Perspectives for Research and Therapy in the Late 1990's. Birkhäuser Verlag, Basel, Switzerland, pp 37–58
12. Tsutsumi K, Saavedra JM (1991) Characterization of AT_2 angiotensin II receptors in rat anterior cerebral arteries. Am J Physiol 261: H667–H670
13. Vraamak T, Waldemar G, Strandgaard S, Paulson S (1995) Angiotensin II receptor antagonist CV-11974 and cerebral blood flow autoregulation. J Hypertens 13: 755–761
14. Werner C, Hoffman WE, Kochs E, Rabito SF, Miletich DJ (1991) Captopril improves neurologic outcome from incomplete cerebral ischemia in rats. Stroke 22: 910–914