Constrictor and Dilator Effects of Angiotensin II on Cerebral Arterioles

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Background and Purpose—In light of the equivocal data on the cerebral vasoconstrictor and vasodilator actions of angiotensin II (Ang II) and the potential clinical importance of this, we investigated the effects of Ang II on rat pial arterioles.

Methods—We determined the effect of Ang I (3.10^{-6} mol/L) in the absence and presence of the converting enzyme inhibitor, captopril (10^{-5} mol/L) in cerebral arterioles of male Wistar rats (open-skull preparation), and those of Ang II (3.10^{-12} to 3.10^{-6} mol/L) in the absence and presence of the Ang II receptor (AT_{1}) antagonist, telmisartan (10^{-5} mol/L) or the AT_{2} antagonist, PD123319 (10^{-5} mol/L). We examined the effect of PD123319 (10^{-5} mol/L) and the Ca^{2+}-activated K^{+} (BK_{Ca}) channel blocker, tetraethylammonium (10^{-4} mol/L) on the Ang II responses in the presence of telmisartan (10^{-5} mol/L).

Results—Ang II-induced dose-dependent constriction with a maximum decrease of −20.1±1.0% at 10^{-6} mol/L. Captopril significantly decreased Ang I-induced vasoconstriction (−4.0±0.9 versus −21.3±2.5%; n=4). Telmisartan reversed Ang II-induced vasoconstriction (9.5±2.5 versus −20.1±1.0% at 10^{-6} mol/L; n=5). PD123319 significantly increased Ang II-induced vasoconstriction (−12.9±0.8 versus −10.2±0.4% at 10^{-6} mol/L; n=5). PD123319 abolished (−2.6±0.7 versus 9.3±1.1% at 10^{-6} mol/L; n=5) whereas tetraethylammonium reversed (−12.1±1.6 versus 9.9±1.0% at 10^{-6} mol/L; n=4) Ang II-induced vasodilatation in the presence of telmisartan.

Conclusion—Angiotensin is converted locally into Ang II; the overall effect of Ang II is vasoconstrictor following stimulation of the AT_{1} receptor, but a vasodilator response can be evoked following stimulation of the AT_{2} receptor and activation of BK_{Ca}. (Stroke. 2005;36:2691-2695.)

Key Words: angiotensin AT_{1} receptor ■ angiotensin AT_{2} receptor ■ potassium channels, calcium-activated ■ rat

Blood pressure can be lowered in hypertensives—and even in elderly hypertensives1—without impairing cerebral blood flow (CBF) or CBF autoregulation.2 Such treatment-induced reversal of the cerebrovascular dysfunction associated with hypertension may have a beneficial effect on brain function.3 Recently, a case has been made for a specific effect of blockade of the renin-angiotensin system (RAS).4 An improvement in cerebrovascular autoregulatory dilatation could involve structural effects of blockade of the RAS5-6 and functional effects on vasomotion mediated by RAS activation.

The evidence of a cerebrovascular RAS was shown by applying renin on the surface of rabbit pial arterioles. This produced dilatation which was blocked by the angiotensin (Ang) I–converting enzyme (ACE) inhibitor, captopril.7 However, the effects of RAS on cerebral vasomotion are unclear. Using specific AT_{1} and AT_{2} antagonists, it was reported that Ang II induces contraction of the rat anterior cerebral artery via AT_{1} receptors.8 Other reports suggested that AT_{1} receptors were involved in dilatation. Several studies show that Ang II dilates small diameter microvessels such as the mesenteric9 and cerebral.10 Such Ang II-induced dilatation may involve endothelial factors such as prostaglandins10 or nitric oxide.11 Other reports, however, suggest that Ang II-induced vasodilatation occurs independently of any action on the endothelium and involves a direct smooth muscle cell dilatory action via calcium-activated potassium channels (BK_{Ca}).9 Furthermore, the receptor involved is open to question. Noting that the number of cerebral AT_{2} receptors and the degree of angiotensin-induced dilatation decreased with age, it was inferred that stimulation of the AT_{1} receptor is linked to dilatation.12 However, Haberl et al suggested that both AT_{1} and AT_{2} receptors are involved in Ang II-induced dila-
tation, whereas Feterik et al suggested that neither receptor subtype is involved.11

With the above in mind, we undertook a series of experiments on the effects of the RAS on pial arteriolar vasomotion in the rat. Using Ang I and the ACE inhibitor captopril, we showed that Ang I is globally vasoconstrictor via its conversion into Ang II, and that Ang II vasoconstricts via an AT1 receptor. When the latter is blocked vasodilatation occurs via an AT2 receptor. This vasodilator response may be direct and endothelium-independent because it is blocked by the BKCa channel blocker tetraethylammonium (TEA).14

**Methods**

**Animals and Operative Procedures**
Experiments were performed on 3 to 4 month-old male Wistar rats (Ifa-Credo, l’Arbresle, France; body weight: 415 ± 8; n = 32), housed at 24°C, exposed to 12 hours of light, and allowed free access to food and fluid. Experiments were performed in accordance with the guidelines of the French Ministry of Agriculture (Paris, France; permits 54 to 4 and 03575).

Animals were fitted with arterial and venous cannula under pentobarbital anesthesia as previously described.15

**Measurement of Arteriolar Diameter**
We measured the internal diameter of first-order cerebral arterioles through an open-skull preparation as previously described.15

Arteriolar diameter was monitored through a microscope (Stemi 200-C; Carl Zeiss Jena GMBH) connected to a video system with a final channel blocker tetraethylammonium (TEA).14

**Experimental Protocols**
A cumulative concentration-response was obtained for Ang II (10^{-12} to 3.10^{-5} mol/L). We examined if Ang I (3.10^{-6} mol/L) induced vasoconstriction following conversion to Ang II by using the converting enzyme inhibitor, captopril (N-(S)-3-mercaptop-2-methylpropionyl-L-proline),10^{-7} mol/L; n = 4).

We then explored the mechanisms by which Ang II induced vasoconstriction in cerebral arterioles. We examined the effects of the angiotensin AT1 receptor antagonist, telmisartan (4'-[1,4'-dimethyl-2'-propyl][2,6'-bi-1H-benzimidazol-1'-yl]methyl)-[1,1'-biphenyl]-2-carboxylic acid dimethyl ester, 10^{-5} mol/L), on arteriolar vasoconstriction induced by Ang II (n = 5).

As Ang II induced vasodilatation in the presence of telmisartan (see Results), we determined the mechanisms of such Ang II-induced vasodilatation using the angiotensin AT1 receptor antagonist, PD123319 (S(+)-1-[(4-dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid diirifluorooacetate, 10^{-3} mol/L), in the presence of telmisartan (n = 5). We also examined the effect of PD123319 on the vasoconstriction induced by Ang II (n = 5).

Because vasodilatation induced by stimulation of angiotensin AT1 receptors may be mediated by activation of BKCa channels,9 we examined effects of the BKCa channel blocker TEA (10^{-4} mol/L)15 on arterial vasodilatation induced by Ang II in the presence of telmisartan (n = 5). As Ang II induced vasoconstriction in the presence of telmisartan and TEA, we repeated the experiments in presence of telmisartan and TEA plus PD123319 (10^{-3} mol/L; n = 4).

In order to determine whether the effects of the various antagonists were specific to Ang II, experiments were also performed with an equipotent dose of another vasoconstrictor, 5HT (10^{-6} and 10^{-5} mol/L).

Given the complexity of the experimental designs, issues of reversibility and tachyphylaxis were dealt with in preliminary experiments. Repeated stimulation (every 10 minutes) with Ang II (10^{-6} mol/L; n = 4) showed no decrease in the response (1st stimulation = 10.8 ± 1.1%, 5th stimulation = 13.0 ± 1.9%; P > 0.05); similar results were obtained with serotonin (10^{-6} mol/L; n = 4; 1st stimulation = 17.2 ± 1.8%, 5th stimulation = 15.2 ± 1.7%; P > 0.05). Likewise, seroton (10^{-4} mol/L; n = 4) produced similar vasodilatation before (−15.2 ± 0.7%) and after (−14.3 ± 3.1%) Ang II (10^{-5} mol/L).

Because it was previously reported that Ang II can produce endothelium-dependent dilatation of cerebral arterioles17,18 and that pentobarbital may attenuate endothelium-dependent relaxation,19,20 we repeated the experiments in control experiments we showed that cerebral arterioles were capable of endothelium-dependent relaxation. Adenosine diphosphate, an endothelium-dependent vasodilator,21 induced dose-dependent relaxation of cerebral arterioles (11.4 ± 2.7 and 16.8 ± 2.1% at 10^{-3} and 10^{-4} respectively; P < 0.05). Furthermore, l-nitro arginine methyl ester induced a small nonsignificant dose-dependent contraction of cerebral arterioles (−0.5 ± 2.3 and −3.2 ± 1.6% at 10^{-4} and 10^{-5} respectively).

**Substances Used**
Ang II, serotonin, captopril, PD123319, l-nitro arginine methyl ester, and adenosine diphosphate were purchased from Sigma Chemical Company. Telmisartan was provided by Boehringer Ingelheim Pharma GmbH & Co KG. Sodium pentobarbital was purchased from Sanofi Santé Animale. KCl, MgCl2, CaCl2, NaCl, NaHCO3, urea, and glucose were purchased from Merck KGaA.

**Statistical Analysis**
Results are expressed as means ± SE. The probability level chosen was P ≤ 0.05.

**Results**
Ang II induced dose-dependent constriction of cerebral arterioles with a maximum of −9.8 ± 0.3 µm at 3.10^{-5} mol/L (Figure 1). Ang I-induced vasoconstriction was significantly attenuated by captopril, which had no significant effect on

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**Figure 1.** Cumulative concentration-response curve for arteriolar vasoconstriction evoked by Ang II (left; n = 7), and effects of the ACE, captopril (10^{-5} mol/L, solid bar), on vasoconstriction induced by Ang I, Ang II and SHT (right; n = 4). Results are expressed as % fall in diameter. Values are means ± SE. *P ≤ 0.05 vs baseline; †P ≤ 0.05 vs responses induced by Ang I.
baseline diameter (45±3 before versus 46±3 μm after) or on Ang II- or 5HT-induced vasoconstriction (Figure 1).

Telmisartan (10⁻⁵ mol/L) had no effect on baseline diameter (41±2 μm before and after; n=13) but reversed the effects of Ang II with an increase in internal diameter to a maximum of 4.7±0.6 μm at 10⁻⁴ mol/L (Figure 2). PD123319 (10⁻⁵ mol/L) had no significant effect on baseline diameter (58±3 before versus 61±3 μm after; n=5) but abolished vasodilatation after Ang II in the presence of telmisartan (Figure 3). PD123319 (10⁻⁵ mol/L) significantly increased constriction induced by Ang II but had no effect on vasoconstriction induced by 5HT when perfused alone or in presence of telmisartan (Figure 3).

The BKCa channel blocker TEA (10⁻⁴ mol/L) reversed the vasodilatation observed with Ang II in the presence of telmisartan (Figure 4). TEA had no effect on 5HT-induced vasoconstriction (Figure 4). PD123319 significantly decreased vasoconstriction produced by Ang II in the presence of telmisartan plus TEA (Figure 4).

Ang I, Ang II, 5HT, captopril, telmisartan, PD123319 and TEA, when perfused in the cranial window, had no significant effect on systemic hemodynamics or blood gas parameters (results not shown), which were stable over the duration of the experiment (results not shown).

**Discussion**

The present study shows that Ang I in vivo is transformed into Ang II, which binds to AT₁ receptors, producing arteriolar constriction. When AT₁ receptors are blocked with telmisartan, Ang II dilates arterioles by binding to AT₂ receptors and the subsequent opening of BKCa channels.

Our findings indicate that Ang II is globally vasoconstrictor principally via an AT₁ receptor. This is in agreement with other studies using rat brain vessels (e.g., the middle cerebral artery) but not with authors cited in the introduction and others who reported a globally vasodilator response or no effect of Ang II on baseline arteriolar diameter. Ang II diluted rat arterioles via an AT₂ receptor when AT₁ receptors were blocked, thus AT₂ receptors linked to vasodilatation exist in rat pial arterioles. This is further suggested by the fact that the AT₂ antagonist, PD123319, potentiates Ang II-induced vasoconstriction. Thus, the two receptors exist in rat pial arterioles and AT₁-associated vasoconstriction prevails. In other segments of the cerebrovascular network, in other species, the two receptors may exist but AT₂-mediated vasodilatation could predominate. The latter appears to involve smooth muscle cell BKCa channels and not endothelium because TEA reverses Ang II-induced dilation (in the presence of telmisartan). We cannot exclude the possibility that TEA is acting on other potassium channels or other types of neurohumoral transmission. However, we have shown that TEA inhibits vasodilatation induced by NS1619 (1-(2-hydroxy-5'-trifluoromethylphenyl)-5-trifluoro-methyl-2(3H)benzimidazolone), a selective activator of BKCa chan-
It should also be noted that TEA had no effect on responses to 5HT. We also observed that the AT2 receptor blocker, PD123319, reduced Ang II-induced vasoconstriction observed in presence of telmisartan and TEA, suggesting that Ang II induces vasoconstriction, at least partially, by stimulating receptor blocked by PD123319. Such Ang II type 2 receptor–mediated vasoconstriction has already been described in isolated mesenteric resistance arteries of spontaneously hypertensive rats (SHR).24

Captopril blocked the vasoconstrictor action of Ang I suggesting the existence of a physiological cerebrovascular RAS. This action of the ACE inhibitor is paradoxical at first sight because ACE is situated on the endothelial cell surface and Ang I, a large peptide, was applied to the outside of the arteriole. We suppose that conversion of Ang I to Ang II can occur elsewhere than in endothelium as has been recently reported in femoral arteries.25 Many authors have reported vasomotor effects of the angiotensins applied to the outside of arterioles (see Introduction). Although we and others7 have provided evidence for the existence of a cerebrovascular RAS, captopril alone did not modify pial arteriolar diameter in spite of the presumably activated state of the RAS after pentobarbital anesthesia.26 The explanation for this may lie in the relative lack of sensitivity of rat microvasculature to ACE inhibitors (unless associated with salidiuretic treatment).27 It has previously been reported that degradation products of angiotensin such as the hexapeptide Ang II-(3-8)18 rather than Ang II itself produce arteriolar vasomotor effects. Our experiments neither confirm nor refute this possibility.

Finally, the existence of both AT1 and AT2 receptors in the cerebrovascular network may have important clinical implications. It has been previously reported by Nishimura28 and Ito29 that the AT1 antagonists normalize CBF autoregulation in SHR. Several explanations have been given for this effect. In the light of our data, we propose a new explanation: AT1 blockade will enhance compensatory AT2-mediated vasodilatation. This has been developed further by Fournier and others30 who suggested that drugs which “activate” AT2 receptors (Ang II receptors (AT1) antagonists, calcium channels blockers, diuretics) have a more beneficial effect on stroke than drugs which “inhibit” AT2 receptors (ACE inhibitors, β blockers), for a given fall in blood pressure. However, this is still questionable because Ang II AT2 blockers and ACE inhibitors are equally effective in reversing cerebral arteriolar remodeling, suggesting that this effect is directly mediated by blockade of AT1 receptors.5 Furthermore, hypertension is a disease of the elderly and expression of AT2 receptors may decrease with age.12

We have recently reported that in an animal model of human essential hypertension (the SHR), there is a parallel increase in systemic pressure and the lower limit of CBF autoregulation, thus maintaining the cerebrovascular security margin.5 When blood pressure is lowered with an AT1 antagonist (telmisartan), the security margin is maintained. However for a similar blood pressure fall with an ACE inhibitor (ramipril) the security margin is reduced.5,31 This may be linked to compensatory activation of AT2 receptor–mediated vasodilatation after specific AT1 receptor blockade.

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References


