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 (AT₁) antagonist, telmisartan (10⁻⁵ mol/L) or the AT₂ antagonist, PD123319 (10⁻⁵ mol/L). We examined the effect of PD123319 (10⁻² mol/L) and the Ca²⁺-activated K⁺ (BKCa) 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% 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 AT₁ receptor, but a vasodilator response can be evoked following stimulation of the AT₂ receptor and activation of BKCa. (Stroke. 2005;36:2691-2695.)

Key Words: angiotensin AT₁ receptor • angiotensin AT₂ receptor • potassium channels, calcium-activated • rat

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

However, the effects of RAS on cerebral vasmotion are unclear. Using specific AT₁ and AT₂ antagonists, it was reported that Ang II induces contraction of the rat anterior cerebral artery via AT₁ receptors. Other reports suggested that AT₂ receptors were involved in dilatation. Several studies show that Ang II dilates small diameter microvessels such as the mesenteric and cerebral. Such Ang II-induced dilatation may involve endothelial factors such as prostaglandins or nitric oxide. 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 (BKCa). Furthermore, the receptor involved is open to question. Noting that the number of cerebral AT₂ receptors and the degree of angiotensin-induced dilatation decreased with age, it was inferred that stimulation of the AT₁ receptor is linked to dilatation. However, Haberl et al suggested that both AT₁ and AT₂ receptors are involved in Ang II-induced dilatation.

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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 BK<sub>c</sub> channel blocker tetraethylammonium (TEA).<sup>14</sup>

### 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.<sup>15</sup>

#### Measurement of Arteriolar Diameter

We measured the internal diameter of first-order cerebral arterioles through an open-skull preparation as previously described.<sup>15</sup>

Arteriolar diameter was monitored through a microscope (Stemi 2000-C; Carl Zeiss Jena GMBH) connected to a video system with a final magnification of 400×. Images were digitized using a video frame grabber and diameter measured (Saisam; Microvision Instruments).

#### Experimental Protocols

A cumulative concentration-response curve was obtained (n=7) for Ang II (10⁻¹² to 3.10⁻⁴ 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),10⁻⁴ mol/L; n=4).

We then explored the mechanisms by which Ang II induced vasoconstriction in cerebral arterioles. We examined the effects of the ACE, captopril (10⁻⁶ 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 AT<sub>2</sub> 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 di trifluoracetate, 10⁻⁴ 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 AT<sub>2</sub> receptors may be mediated by activation of BK<sub>c</sub> channels,<sup>9</sup> we examined effects of the BK<sub>c</sub> channel blocker TEA (10⁻⁴ mol/L) on arteriolar 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 (10⁻⁵ 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 vasconstrictor, 5HT (10⁻⁸ and 10⁻⁶ mol/L).

### 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, mean. The experimental protocol was designed to use a paired t-test. 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 3.10⁻⁶ mol/L (Figure 1). Ang I-induced vasoconstriction was significantly attenuated by captopril, which had no significant effect on...
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 10⁻⁵ 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 vasodilation after Ang II in the presence of telmisartan (Figure 3). PD123319 (10⁻⁵ mol/L) significantly increased constriction produced 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 vasodilation 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 (eg, 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 dilated 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 predominates. 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 dilatation (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-
The effect of the BKCa channel blocker, TEA (10⁻⁵ mol/L, full bars) on responses induced by Ang II and 5HT in the presence of the angiotensin AT₁ receptor antagonist, telmisartan (10⁻⁵ mol/L; left; n=5) or in the presence of both the angiotensin AT₁ receptor antagonist, telmisartan (10⁻⁵ mol/L) and the angiotensin AT₂ receptor antagonist, PD123319 (10⁻⁵ mol/L; n=4). Open bars show responses obtained before and cross-hatched bars following wash-out of TEA for the left panel. Open bars show responses obtained before and cross-hatched bars responses obtained in presence of TEA plus PD123319 TEA for the left panel. Results are expressed as % fall in diameter. Values are means±SE. *P<0.05 vs baseline; †P<0.05 vs responses induced by Ang II or 5HT. $P<0.05 vs responses induced by Ang II in the presence of TEA.

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 blocker by PD123319. Such Ang II type 2 receptor–mediated vasoconstriction has already been described in isolated mesenteric resistance arteries of spontaneously hypertensive rats (SHR).

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. Many authors have reported vasomotor effects of the angiotensins applied to the outside of arterioles (see Introduction). Although we and others 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. The explanation for this may lie in the relative lack of sensitivity of rat microvasculature to ACE inhibitors (unless associated with salidramic treatment). It has been previously reported that degradation products of angiotensin such as the hexapeptide Ang II-(3-8) 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 Nishimura and Ito 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 others who suggested that drugs which “activate” AT₂ receptors (Ang II receptors (AT₁) antagonists, calcium channel 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. Furthermore, hypertension is a disease of the elderly and expression of AT₂ receptors may decrease with age.

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. 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. This may be linked to compensatory activation of AT₂ receptor–mediated vasodilatation after specific AT₁ receptor blockade.

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References


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