Role of Angiotensin Receptor Subtypes in the Response of Rabbit Brain Arterioles to Angiotensin

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Background and Purpose  Angiotensin II has been reported to induce either constriction or dilation in the cerebral microcirculation. The goal of this study was to determine whether binding to different angiotensin II receptor subtypes may account for the divergent responses.

Methods  Pial arterioles ranging in diameter from 28 to 136 μm were observed through a microscope in a closed cranial window preparation in anesthetized rabbits. Arteriolar responses to topical application of 10⁻⁷ mol/L angiotensin II or the vasoactive angiotensin II degradation products L-arginine/angiotensin-(3-8) were measured by videometry. The effect of the subtype 1 receptor antagonist losartan and the subtype 2 antagonist PD 123319 on these responses was examined in separate groups of animals.

Results  Topical coapplication of 10⁻⁳ mol/L losartan or 10⁻¹ mol/L PD 123319 produced 55% and 62% inhibition of the dilator response to 10⁻⁵ mol/L angiotensin II, respectively. Combined application of the antagonists caused 79% inhibition. Each of the antagonists almost completely blocked the response to L-arginine/angiotensin-(3-8). Acetylcholine-induced dilation of rabbit brain arterioles was unaffected by the antagonists.

Conclusions  Both of the known angiotensin II receptor subtypes appear to be involved in angiotensin II-induced dilation of rabbit cerebral arterioles. These results argue against the assumption that vasodilation is a specific function of one of these receptor subtypes, which might have explained the equivocal effects of angiotensin II by predominance of a certain receptor subtype in a given vascular bed. (Stroke. 1994;25:1476-1486.)

Key Words  • angiotensins • microcirculation • rabbits

See Editorial Comment, page 1480
Pial arterioles ranging in diameter from 28 to 136 μm (mean±SEM, 68±3 μm) were observed with a trinocular microscope (Leitz GmbH). The microscopic field in the window was illuminated with a 100-W halogen lamp and a fiberoptic ring probe fixed around the objective of the microscope. The field visualized through the microscope was recorded using a low-light-level video camera (Panasonic WV 1550/G) mounted on the phototube of the microscope, a video recorder (Panasonic AG 6200-EG), and a video monitor (Barco CD 233). Total magnification on the video monitor was ×80. Pial arteriolar diameters were measured with a personal computer image analysis system (Stemmer Elektronik GmbH). All solutions or combinations of solutions were topically applied to the brain surface by filling the space under the cranial window. Topical application of test solutions was started when baseline diameters were stable and did not change during repeated application of 1 mL mock CSF. Pial arteriolar diameters were measured at 1-minute intervals from 2 to 5 minutes after each topical application. Diameter readings were not made within 2 minutes after topical application because the moderate intracranial pressure increase during the addition of CSF may produce a transient pial arteriolar dilation. Previous experiments have shown that this dilation is reversible within 2 minutes after application. Five minutes after topical application, the window was flushed with 1 mL mock CSF, and vessels were allowed to return to baseline. The nonpeptide AT2 receptor antagonist losartan (E-7753) was generously provided by Dr. W. MacIntosh Merck. Losartan has previously been reported to specifically block the AT1 receptor in vascular in vivo and in vitro preparations. The AT2 receptor antagonist PD 123319 was a gift from Parke-Davis. This inhibitor was initially characterized by Dudley et al to be a specific AT2 antagonist. Ang II (acetate salt, human), acetylcholine, and L-arginine were purchased from Sigma Chemical Company. The Ang II fragment angiotensin-(3-8) was obtained from Peninsula.

The preparation was pretreated for 5 to 8 minutes with one of the antagonists to detect an effect on baseline diameters. When repeated diameter measurements indicated a new steady state, the substance to be blocked was topically applied together with the antagonist. The effect of Ang II itself and Ang II in the presence of losartan, PD 123319, or both receptor antagonists was studied in separate groups of animals. Angiotensin II was used at a concentration of 10^{-5} mol/L, which induced maximal dilation in a previous study. After three washouts with mock CSF, the response to 10^{-5} mol/L acetylcholine in the presence of the respective receptor antagonist was determined in each group.

In another group of animals we studied the hypothesis that the receptor antagonists may interfere with enzymatic breakdown of Ang II and the generation of vasoactive degradation products. Breakdown of Ang II, with production of the fragments angiotensin-(3-8) and L-arginine, has been found to beconditional for vasodilatation to occur. Dilution to the fragments themselves should be unaffected if the antagonists act through inhibition of fragmentation. To test this, 10^{-5} mol/L angiotensin-(3-8) was administered subsequent to topical application of 10^{-4} to 10^{-5} mol/L L-arginine, either in the absence or presence of one of the receptor antagonists.

Data are expressed as mean±SEM. Groups were compared by the Kruskal-Wallis test and Mann-Whitney test. Values of P<0.05 were considered statistically significant.

Results

Mean arterial blood pressure, blood gases, and blood pH were within the physiological range for rabbits, as previously reported. Topical application of Ang II, acetylcholine, and Ang II receptor antagonists had no effect on systemic blood pressure and blood gases.

The AT1 antagonist losartan (10^{-5} mol/L) produced 55% inhibition of the dilator response to 10^{-5} mol/L Ang II (Fig 1). Losartan itself induced vasodilation of 5.8±0.7% 2 minutes after topical application. Diameters were back to baseline after 5 minutes (0.6±1.3%). Repeated application of losartan without CSF washout had no further effect. Dilation in response to 10^{-5} mol/L acetylcholine was not affected by the presence of 10^{-5} mol/L losartan (Fig 1).

The AT2 antagonist PD 123319 (10^{-5} mol/L) produced 62% reduction of the response to 10^{-5} mol/L Ang II (Fig 1). PD 123319 itself produced dilatation of 8.0±2.1%, 2.4±1.9%, and 3.2±1.4% at 2, 5, and 8 minutes after topical application, respectively. Percent changes of vessel diameters at the presence of PD 123319 and Ang II were related to the steady-state diameter at 8 minutes after PD 123319 administration. PD 123319 had no effect on the dilation in response to acetylcholine (Fig 1).

Combined application of 10^{-5} mol/L losartan and 10^{-5} mol/L PD 123319 caused 79% inhibition of the dilation to 10^{-5} mol/L Ang II (4.4±2.9%, n=4, 12 vessels).

In separate groups the hypothesis was tested that the Ang II antagonists might inhibit dilation by affecting the generation of the vasoactive degradation products of Ang II rather than by receptor antagonism. Both inhibitors, however, eliminated the vasodilation that was seen when the peptide fragments L-arginine and angiotensin-(3-8) were topically applied in a cumulative manner (Fig 2). This argues against the assumption that the antagonists may work by preventing Ang II fragmentation into vasoactive components.

Discussion

This study demonstrates that vasodilation of rabbit brain arterioles in response to Ang II is inhibited by...
specific antagonists of both the AT₁ and the AT₂ receptor subtypes. The result is in agreement with a recent study in a rat closed window preparation from our laboratory. These investigations in different species argue against the possibility that the response of brain arterioles to Ang II may depend only on the subtype of receptor activated.

The specificity and selectivity of the receptor antagonists used were previously demonstrated in various in vitro preparations. Specificity for Ang II receptors in brain arterioles was confirmed by the lack of an inhibitory effect on acetylcholine-induced arteriolar dilation in this study. Concentrations of the antagonists higher than 10⁻⁸ mol/L were not applied because selective binding of the AT₁ or AT₂ receptor subtype may be affected. The finding of a moderate dilating effect 2 minutes after topical application of one of the antagonists may indicate inhibition of an Ang II–induced constriction tone. This is unlikely, however, because all interventions in previous studies, such as inhibition of Ang II breakdown by an aminopeptidase inhibitor or simultaneous elimination of endothelium-dependent dilation by methylene blue, failed to reveal constriction to Ang II. We speculate that losartan and PD 123319 may have partially agonistic effects. Alternatively, the antagonists may produce dilation by indirect effects such as the release of prostanoids, since losartan has previously been shown to stimulate the synthesis of prostacyclin in cultured smooth muscle cells.

Several possibilities exist that might explain the inhibition of Ang II–induced dilation by both the AT₁-selective losartan and the AT₂-selective PD 123319. The drugs may cross-react with the other receptor subtype. However, this seems unlikely with respect to previous studies showing high selectivity for the receptor subtypes in other smooth muscle preparations at the doses given here. Ang II may induce vasodilation by indirect mechanisms that are equally affected by losartan and PD 123319. One potential mechanism would be the release of a dilating prostaglandin, since AT₁ and AT₂ receptor inhibition have been reported to suppress prostaglandin E₂ release from cultured astrocytes in response to Ang II. Our failure to block Ang II–induced dilation with the cyclooxygenase inhibitor indomethacin in the rabbit closed window preparation argues against this possibility. Finally, our results may indicate that both receptor subtypes need to be activated for the response or that the antagonists act on an as yet unidentified Ang II receptor. The finding that combined application of both antagonists causes greater inhibition than each of the antagonists alone supports the conclusion of the involvement of two receptor subtypes.

The question of whether both antagonists may inhibit the dilation by an effect on Ang II degradation was directly addressed by our experiments. Previously we found that the aminopeptidase inhibitor amastatin blocks arteriolar dilation to Ang II, probably by preventing the generation of the peptide fragments L-arginine and angiotensin-(3-8). Cumulative application of L-arginine and angiotensin-(3-8) induced pial dilation similar to that induced by Ang II. This synergistic response was not affected by amastatin and was specific for the Ang II fragments. It was concluded that fragmentation of Ang II, with release of L-arginine and angiotensin-(3-8), is conditional for Ang II–induced dilation to occur in the rabbit pial preparation. A process that blocks or alters Ang II fragmentation may inhibit Ang II–induced dilation without affecting the response to the fragments. The receptor subtype antagonists, however, inhibited dilation in response to L-arginine/angiotensin-(3-8). This argues against an impairment of the production of the fragments. Another possibility is that the antagonists affect the metabolism of L-arginine to a vasoactive substance such as nitric oxide. Dilation to acetylcholine, however, which depends on conversion of L-arginine to nitric oxide in the endothelium, was not inhibited by the compounds. Alternatively, the antagonists may interfere with the effect of the hexapeptide angiotensin-(3-8). It has been suggested that angiotensin-(3-8) is a unique signaling peptide within the renin-angiotensin system, with a receptor different from the other Ang II receptor subtypes, a special pattern of immunoreactivity in the brain, and distinct physiological effects, such as an increase of renal blood flow. These authors proposed the term angiotensin IV to signify the independent role of angiotensin-(3-8) as a vascular and potentially neuronal transmitter. Stimulation of an angiotensin IV receptor, which mediates arteriolar dilation and is bound by losartan and PD 123319, may explain our results. Differences in affinity of the antagonists to the AT₁, AT₂, and angiotensin IV receptors may also account for the finding that each antagonist inhibited Ang II–induced vasodilation by 55% to 62% but almost abolished the response to L-arginine/angiotensin-(3-8). Further studies on the presence of an angiotensin IV receptor in the cerebral microcirculation, its pharmacology, and its function in regulation of cerebral blood flow are needed to support this speculation.

The present results do not provide a clue to previous results seen with Ang II in regulation of cerebral blood flow. Although it has only minor effects on resting cerebral blood flow, Ang II is known to change the autoregulatory capacity in the cerebral circulation. Inhibition of Ang II generation by the converting-enzyme blocker captopril shifts the limit of autoregulation toward lower pressures, whereas the receptor
antagonists, which were used in our study, both extended the upper limit of autoregulation toward higher pressures.\textsuperscript{32} Strömberg et al\textsuperscript{32} postulate constriction of cerebral vessels by stimulation of AT\textsubscript{2} receptors and dilation or no response through AT\textsubscript{1} receptor stimulation. This is not supported in the pial arterioles studied in the present investigation, which appear to dilate in response to activation of either receptor subtype. The possibility exists, however, that larger cerebral vessels upstream of those observed in the cranial window differ in their response to Ang II and their sensitivity to receptor subtype stimulation.

Taken together, our results do not support the hypothesis that constriction or dilation in response to Ang II may be determined by the receptor subtype. Stroke prevention by Ang II has been reported in some experimental stroke models and has been attributed to microcirculatory vasodilation.\textsuperscript{7,8} The question of whether Ang II--induced vasodilation is specific for only some species or may be produced by mechanisms that are as yet unidentified remains unresolved.

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References


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