Angiotensin Degradation Products Mediate Endothelium-Dependent Dilation of Rabbit Brain Arterioles

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This study demonstrates that the hexapeptide angiotensin II-(3–8) and L-arginine, generated through enzymatic degradation of angiotensin, mediate endothelium-dependent dilation in rabbit brain arterioles. Topical application of angiotensin II (10^-5 M) on the brain surface of anesthetized rabbits caused 21.6±4.5% (mean±SEM) cerebral arteriolar dilation. The cyclooxygenase inhibitor indomethacin did not change this dilation. The natural degradation product of angiotensin II in the brain, angiotensin III, also induced vasodilation at concentrations of 10^-7 to 10^-5 M. The dilation to angiotensin II and angiotensin III was eliminated in the presence of 10^-3 M methylene blue, a known inhibitor of endothelium-dependent vasodilation. Amastatin, an aminopeptidase inhibitor and blocker of enzymatic angiotensin degradation, also inhibited the response to angiotensin II and angiotensin III. The angiotensin fragment angiotensin II-(3–8), which lacks the amino-terminal L-arginine residue of angiotensin III, did not elicit an arteriolar response. When angiotensin II-(3–8) was topically applied subsequent to L-arginine, a 21.2±2.9% vasodilation was observed. L-Arginine itself induced only moderate vasodilation with a maximum of 4.0±0.9% at 10^-5 M L-arginine. The dilating response to angiotensin II-(3–8) after L-arginine was inhibited by methylene blue. It was not affected by amastatin. It is concluded that degradation products of angiotensin, rather than angiotensin II itself, induce endothelium-dependent dilation in rabbit brain arterioles without involvement of cyclooxygenase products. The finding that synergism of L-arginine and angiotensin II-(3–8) produced potent dilation indicates that, in addition to the availability of L-arginine, other factors such as angiotensin II-(3–8) are important in the regulation of endothelium-dependent response of brain arterioles. (Circulation Research 1991;68:1621–1627)

Angiotensin II (Ang II) is known as a constrictor substance in various vascular beds including the cerebral circulation in cats and hamsters. In a recent study, however, we have shown that Ang II may act as a vasodilator of cerebral arterioles in a rat closed cranial window preparation. The dilating response to Ang II was inhibited by methylene blue. Illumination of the arterioles with a mercury lamp in the presence of intravascular fluorescein dye (‘light plus dye’ injury) also blocked the dilating response. Since control experiments and studies by others have demonstrated that methylene blue and light plus dye injury of cerebral arterioles eliminate endothelium-dependent responses, without altering endothelium-independent reactions, we suggested that Ang II induces endothelium-dependent vasodilation of cerebral arterioles in rats. Ang II is rapidly degraded to angiotensin III (Ang III) and smaller fragments within the brain. The goals of the present study were to extend our previous findings in rats to another species and to examine the hypothesis that fragments of angiotensin rather than Ang II itself induce the endothelium-dependent response. With respect to reports that the amino acid L-arginine is the biochemical precursor of an endothelium-dependent relaxing factor (EDRF) in the peripheral circulation, the question was addressed whether the release of L-arginine from the amino-terminal position 2 of the peptide chain of Ang II may mediate the response seen with angiotensin.

Materials and Methods

Preparation of Animals

Experiments were carried out in 94 male New Zealand White rabbits (2.7–4.4 kg) anesthetized with sodium pentobarbital (25 mg/kg i.v.) and a mixture of...
urethane (560 mg/kg s.c.) and α-chloralose (38 mg/kg s.c.). Supplemental doses of pentobarbital were given as needed to maintain anesthesia. Under this regime of anesthetic drugs, surgical anesthesia is quickly induced with a minimum of respiratory depression, and the need for administration of additional doses of pentobarbital is reduced.

After tracheostomy, each animal was ventilated with a positive-pressure ventilator. The end-expiratory CO₂ of each animal was continuously monitored with an infrared CO₂ analyzer (Heyer GmbH, Bad Ems, FRG) and was maintained at a level of about 30 mm Hg throughout each experiment by adjusting the ventilator rate and volume. Arterial blood pressure was measured with a pressure transducer (Gould Inc., Cleveland, Ohio) connected to a cannula that was inserted into the right femoral artery. Arterial blood samples (0.1 ml) were periodically collected in a glass capillary and analyzed for arterial PCO₂, PO₂, and pH with an AVL 940 blood pH–blood gas analyzer (AVL GmbH, Bad Hamburg, FRG). The animals were kept at a constant temperature of 37.5±0.5°C by a rectal thermometer–controlled heating pad.

The pial microcirculation was visualized through a closed cranial window as previously described.⁸ The 10-mm-diameter cranial window was acutely implanted on the midline just caudal to the suture connecting the frontal and parietal bones. The dura over the sagittal sinus was left in place. Removal of the dura on each side of the sinus allowed observation of pial vessels and the brain surface on both cerebral hemispheres. Two or three pial arterioles were studied in each animal. The cranial window was equipped with three openings. Two openings on opposite sides of the window were used as the inlet and outlet for flushing the space under the window or filling the space with test solutions. The inlet and outlet port arrangement allowed the applied solutions to pass over the whole field of view under the window. The third window outlet was connected to a Gould pressure transducer for continuous measurement of intracranial pressure. The outflow of the window was set at a fixed height to maintain a 5 mm Hg intracranial pressure throughout the experiment. The plastic tubing connected to the three openings, as well as the space under the window, was filled with artificial cerebrospinal fluid (CSF).⁸ The artificial CSF had the following composition (mM): Na⁺ 153, K⁺ 3, Ca²⁺ 1.5, Mg²⁺ 0.6, Cl⁻ 140, glucose 3.7, urea 6, and HCO₃⁻ 25. Osmolarity was 315 mosm/l. This fluid was equilibrated with a mixture of 6.6% O₂, 5.9% CO₂, and 87.5% N₂, which gave a pH of 7.399±0.01 at 37°C.

Pial arterioles ranging in diameter between 19 and 148 μm (mean±SEM, 69±2 μm) were observed with a trinocular microscope (Leitz GmbH, Munich). The illumination came from a 100-W halogen lamp and a fiber-optic ring probe fixed around the objective of the microscope. A heat filter (to avoid warming the cranial window) and a green filter (for contrast enhancement, λ=546 nm) was placed between the halogen lamp and the illuminator. The field visualized through the microscope was recorded by 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, Kortrijk, Belgium). Total magnification on the video monitor was ×80. Pial arteriolar diameters were measured with a PC image analysis system (Stemmer Elektronik GmbH, Puchheim, FRG).

Preparation of Solutions

Ang II (acetate salt, human), Ang III (acetate salt, human), methylene blue, indomethacin, 2-chloroadenosine, bradykinin, acetylcholine, 1-tyrosyl-L-tyrosine (Tyr-Tyr), amastatin, L-arginine, D-arginine, and L-lysine were obtained from Sigma Chemical Co., Deisenhofen, FRG. The angiotensin fragments angiotensin II (3–8) (Ang-[3–8]; amino acid sequence, Val-Tyr-Leu-His-Pro-Phe) and angiotensin II (4–8) (Ang-[4–8]) were purchased from Peninsula Laboratories, Merseyside, England. An indomethacin stock solution (1 mg/ml) was prepared daily by dissolving indomethacin and sodium carbonate (3:1, wt/wt) in distilled water. All the other solutions were prepared directly in CSF before use and were equilibrated to 37°C in a heater immediately before application.

Experimental Procedure

All solutions or combinations of solutions were applied topically 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. Preliminary experiments have shown that this dilation is reversible within 2 minutes after application. At 5 minutes after topical application, the window was flushed with 1 ml mock CSF and vessels were allowed to return to baseline.

In experiments for which the effect of inhibitor substances was tested, the preparation was pretreated for 5 minutes with the inhibitor before the addition of the substance to be blocked. None of the inhibitor substances used had a significant effect on arteriolar diameter. Nevertheless, pial arteriolar diameters at 5 minutes after application of the inhibitor substances were used as the baseline to compute the effects of vasoactive substances in the presence of the inhibitor. Maximum arteriolar responses to all vasoactive substances used in this study were seen at 2 minutes after topical application. Diameter readings in the presence of inhibitors were therefore made at 2 minutes after coapplication with the vasoactive substances.
Effect of angiotensin II (diamonds) and angiotensin III (triangles) on cerebral arteriolar diameter. The angiotensin II data represent mean percent changes±SEM in 22 vessels of eight rabbits (baseline diameter, 67±4 μm). The effect of angiotensin III was tested in 14 arterioles of five rabbits with a baseline diameter of 60±7 μm. *Significantly different from baseline diameter (p<0.01).

Statistical Methods

The data are expressed as mean±SEM. Dose–response relations were compared with analysis of variance followed by t tests modified for multiple comparisons. Differences in responses induced by the presence of indomethacin, methylene blue, amastatin, and Tyr-Tyr were analyzed by t tests for unpaired observations.9 Values of p<0.05 were considered statistically significant.

Results

The mean arterial blood pressure in all rabbits was 81±2 mm Hg. Arterial blood gases and pH were PaO2 92±3 mm Hg, PaCO2 30.4±0.6 mm Hg, and pH 7.398±0.01. In all instances, topical application of the agents had no effect on systemic arterial blood pressure, arterial pH, or blood gases.

Effect of Angiotensin II on Pial Arterioles

Topical application of 10⁻⁵ M Ang II produced a 21.6±4.5% dilation of pial arterioles in rabbits (maximum effect at 2 minutes after topical application). Of 22 arterioles in eight animals studied, 16 dilated, five showed no response, and one was moderately constricted. The arteriolar responses to Ang II did not depend on the vessel size. Lower concentrations (10⁻⁷ and 10⁻⁶ M Ang II) did not induce a significant diameter response (Figure 1).

The dilation in response to 10⁻⁵ M Ang II was abolished in the presence of 10⁻⁵ M methylene blue, a known inhibitor of endothelium-dependent responses (Figure 2). Methylene blue itself had no effect (−0.9±2.1% in 25 vessels of nine rabbits). In a separate group, it was confirmed that methylene blue blocks the dilation to topical application of 10⁻⁵ M acetylcholine, the classical endothelium-dependent agonist. Acetylcholine (10⁻⁴ M) induced a 15.5±3.3% dilation of the arterioles that was eliminated in the presence of methylene blue (−0.9±2.5% in nine vessels of three rabbits; baseline diameter, 61±6 μm). The vasodilation in response to the endothelium-independent substance 2-chloroadenosine was not statistically changed by methylene blue (Figure 2).

Topical coapplication with the cyclooxygenase inhibitor indomethacin (3 μg/ml, 8.4 μM) did not affect the arteriolar dilation to 10⁻⁵ M Ang II in the rabbit (Figure 2). Indomethacin itself had no significant effect on the cerebral arterioles (3.3±1.6% in 15 vessels of five rabbits). The activity of the indomethacin solution used in this study was confirmed by its inhibitory effect on bradykinin-induced vasodilation (Figure 2).

Effect of Angiotensin III on Pial Arterioles

To study the hypothesis that breakdown products of Ang II, rather than Ang II itself, induce vasodilation, arteriolar responses to Ang III were examined. Ang III is reported to be the natural degradation product of Ang II in the brain. Ang III (10⁻² to 10⁻⁵ M) induced dose-dependent dilation of the cerebral arterioles (Figure 1). The maximum responses at 10⁻⁵ M Ang III and at 10⁻⁵ M Ang II were similar. The vasodilation to 10⁻² M Ang III was inhibited in the presence of 10⁻⁵ M methylene blue (−0.7±3% in 14 vessels of five rabbits; baseline diameter, 55±6 μm).

Inhibition of Angiotensin Degradation

Different aminopeptidases are involved in Ang II degradation in the brain.10–13 To examine whether...
Ang II degradation is required for the vascular effects to occur; we tested the effect of aminopeptidase inhibitors on the vascular responses to Ang II. Amastatin, a tetrapeptide, is a blocker of aminopeptidase A and aminopeptidase M and is reported to inhibit angiotensin metabolism by cerebral microvessels.\textsuperscript{10,12} In the presence of 10\textsuperscript{-5} M amastatin, the dilation to 10\textsuperscript{-5} M Ang II was abolished (Figure 3). Amastatin itself had no significant effect on arteriolar diameters (3.6±1.7% in 18 vessels of six animals). The endothelium-dependent responses to acetylcholine and bradykinin were not affected by amastatin (Figure 3). As a control, the effect of the structurally unrelated angiotensinase inhibitor Tyr-Tyr\textsuperscript{13,14} on Ang II–induced dilation was studied. Similar to amastatin, 10\textsuperscript{-5} M Tyr-Tyr inhibited the dilator response to Ang II (5.7±3.6% in 15 arterioles of five rabbits; baseline diameter, 57±4 μm; p<0.05) without altering arteriolar diameters by itself. These results support the concept that Ang II degradation products, rather than Ang II itself, induce dilation.

Inhibition of Ang II degradation by 10\textsuperscript{-5} M amastatin and simultaneous elimination of endothelium-dependent dilation by coapplication of 10\textsuperscript{-5} M methylene blue resulted in a 1.4±3.2% diameter change to 10\textsuperscript{-5} M Ang II (12 vessels in four rabbits; baseline diameter, 65±3 μm). This result argues against the possibility that the intact polypeptide Ang II has a vasoconstrictor effect that is counterbalanced by an endothelium-dependent dilation and is reduced by enzymatic destruction of the polypeptide.

\textbf{Figure 3.} Effect of amastatin (AMA) on the arteriolar responses to angiotensin II (Ang II) and angiotensin III (Ang III) (top panel) and to bradykinin (BK) and acetylcholine (Ach) (bottom panel). The responses to Ang II and Ang III in the presence of amastatin were tested in the same animals in random order. Eighteen vessels in six animals with a baseline diameter of 74±6 μm were studied. The effect of amastatin on bradykinin- and acetylcholine-induced dilation was studied in 15 vessels of five animals (baseline diameter, 79±7 μm) and nine vessels of three animals (baseline diameter, 73±4 μm), respectively. *Significantly different from response to Ang II or Ang III (p<0.01).

\textbf{Effect of Ang III Fragments on Rabbit Pial Arterioles}

Amastatin also eliminated the vasodilation to 10\textsuperscript{-5} M Ang III (Figure 3). Therefore, the possibility that a peptide fragment generated from Ang III through an amastatin-sensitive aminopeptidase may induce vasodilation was studied. Topical application (10\textsuperscript{-4} to 10\textsuperscript{-6} M) of the hexapeptide Ang-(3–8), which lacks the amino-terminal L-arginine residue of Ang III, did not cause an arteriolar response (nine arterioles of three rabbits; baseline diameter, 69±5 μm). When 10\textsuperscript{-4} M Ang-(3–8) was topically applied immediately after cumulative application of L-arginine (10\textsuperscript{-5} to 10\textsuperscript{-4} M), 21.2±2.9% dilation was observed (Figure 4). L-Arginine itself induced only moderate vasodilation with a maximum effect of 4.0±0.9% at 10\textsuperscript{-3} M (p<0.05). The dilation to Ang-(3–8), when given subsequent to cumulative L-arginine application, was inhibited by 10\textsuperscript{-5} M methylene blue. The aminopeptidase inhibitor amastatin did not change the response (Figure 5).

When the stereoisomer D-arginine or the basic amino acid L-lysine (10\textsuperscript{-4} to 10\textsuperscript{-3} M) were applied in a similar manner to that for L-arginine, no arteriolar dilation to 10\textsuperscript{-4} M Ang-(3–8) was seen. Topical application of a shorter angiotensin fragment, the heptapeptide Ang-(4–8), after L-arginine application did not have an arteriolar effect. Similarly, Ang III (10\textsuperscript{-5} M) in the presence of the aminopeptidase inhibitor amastatin, when applied after L-arginine, did not produce significant dilation (1.9±2.1% in nine vessels of three animals; baseline diameter, 75±2.5 μm). Therefore, L-arginine plus angiotensins other than Ang-(3–8) do not elicit dilation.

The possibility that the discrepancy between the moderate dilation to exogenous L-arginine and the dilation induced by Ang II or Ang III was due to a reduction of L-arginine breakdown by degradation products of angiotensin, such as Ang-(3–8), was considered. In a separate group of animals, the
response to 10−5 M l-arginine in the presence of 10−4 M Ang-(3–8) was studied. The dilation to l-arginine was similar to that seen in the absence of Ang-(3–8) (5.1±2% in six arterioles of two rabbits; baseline diameter, 78±6 μm).

Discussion

This study extends our previous finding of endothelium-dependent dilation to Ang II in rat cerebral arterioles to the rabbit brain microcirculation. Moreover, the study indicates that enzymatic degradation of angiotensin, with the release of the amino acid l-arginine and the angiotensin fragment Ang-(3–8), mediates the endothelium-dependent vasodilation in the rabbit.

In contrast to previously reported results in cat1,2,15 and hamster2 cerebral arterioles, angiotensin did not induce vasoconstriction in rabbits and rats. The reason for the conflicting results remains uncertain. Inhibition of angiotensin breakdown by amastatin and simultaneous elimination of endothelium-dependent dilation by methylene blue failed to reveal constriction to Ang II. Therefore, there is no evidence that a direct constrictor effect of Ang II on vascular smooth muscle was balanced by endothelium-dependent dilation or was hidden by rapid enzymatic destruction of the intact polypeptide.

The concentrations of angiotensins used in this study were rather high. The question needs to be addressed how they relate to normal physiology. Ang II determinations in CSF yielded undetectable16 or very low (in the fmol/ml range) concentrations in dogs, rats, sheep, and humans.17 However, there is uncertainty about local concentrations of angiotensins in the direct vicinity of cerebral vessels. Angiotensin metabolism in the brain is exceedingly fast,5,18 and ex vivo determinations in CSF may not reflect the angiotensin levels in the vascular tissue. The physiological relevance of the present data is supported by our recent finding that stimulation of endogenous angiotensin generation by topical application of 0.1 unit/ml renin induces similar cerebral arterial dilation as seen with 10−5 M Ang II and a cerebral blood flow increase through an l-arginine-utilizing mechanism.19

The suggestion that angiotensin-induced dilation in rabbit pial arterioles is endothelium dependent is based on the result that the response was inhibited in the presence of methylene blue. Methylene blue has been described to eliminate endothelium-dependent responses of cerebral arterioles in all species studied without altering the endothelium-independent effects of adenosine,4 8-bromo-cGMP,20,21 or nitroprusside.20 In control experiments, methylene blue blocked the vasodilation of rabbit pial arterioles to the classical endothelium-dependent substance acetylcholine, but it did not affect endothelium-independent dilation to 2-chloroadenosine. In our previous study of rats, the inhibition of Ang II–induced relaxation by methylene blue was reproduced using light plus dye injury of cerebral arterioles,4 a technique that functionally damages the endothelium.5 Although proof of endothelium dependence may be attained only by comparison of preparations before and after complete removal of the endothelial layer, several lines of evidence indicate that Ang II–induced dilation of cerebral arterioles is endothelium associated.

In contrast to our previous study of rats, the cyclooxygenase inhibitor indomethacin did not block Ang II–induced dilation in rabbit cerebral arterioles. Therefore, it is not likely that a vasodilator prostaglandin or the release of an oxygen radical via cyclooxygenase mediates the response in the rabbit. The difference to the result in the rat pial arteriolar preparation may be species related. Similar differences between species or vascular beds have been shown to exist for bradykinin, which induces cyclooxygenase-dependent, oxygen radical–mediated dilation of pial arterioles in rabbits and cats22,23 and endothelium-associated relaxation, not caused by free radical generation, in the canine basilar artery.24 While the response to Ang II in the rat is similar to the action of bradykinin in cat cerebral arterioles, our present data suggest that the vasodilation from angiotensin in rabbit cerebral arteries might be related to the release of an EDRF similar to that induced by acetylcholine.

The effect of Ang III on cerebral vessels has been previously examined in canine middle cerebral artery strips where it produced similar relaxation as did equimolar concentrations of Ang II.25 In the rabbit arteriolar preparation in vivo, Ang III was slightly more effective than Ang II at low concentrations and induced equal maximum dilation at 10−5 M. The inhibition by methylene blue also suggests an endothelium-dependent mechanism of Ang III–induced dilation.

Ang III and shorter angiotensin fragments, such as the hexapeptide Ang-(3–8), have been shown to be
produced within the brain by enzymatic degradation of Ang II.\(^{6,26}\) Aminopeptidases, which cleave single amino acids from the amino-terminal end of angiotensins,\(^{10,12}\) as well as dipeptidyl aminopeptidases\(^{13}\) have been identified in brain microvessels and brain homogenates. In a series of papers, it has been proposed that Ang II must be converted to Ang III to be biologically active in the brain.\(^{27-29}\) Conclusions are based on differential inhibitory effects of aminopeptidase blockers (e.g., amastatin and bestatin) on Ang II- or Ang III–induced central nervous responses. However, aminopeptidase blockers have been shown to be only marginally selective for inhibition of Ang II and Ang III degradation.\(^{30}\) In our preparation, the aminopeptidase inhibitor amastatin did not increase the vascular responses to Ang II or Ang III, as might be anticipated if one or the other substance was more vasoactive in the brain. Rather, all vascular responses to Ang II and Ang III were blocked in the presence of amastatin. Selectivity of the effect of amastatin was shown by the fact that it did not interfere with the response to acetylcholine or bradykinin, substances that putatively act by the release of different EDRFs in the brain circulation.\(^{23}\) Inhibition of Ang II–induced dilation could be reproduced by the aromatic dipeptide Tyr–Tyr, which has been described to block Ang II degradation by reducing dipeptidyl aminopeptidase III activity.\(^{13,14}\) These findings indicate that degradation of angiotensins and the release of an angiotensin peptide fragment shorter than Ang III are conditional for vasodilation.

There is evidence that the angiotensin fragment Ang-(3–8) and the basic amino acid L-arginine, when cleaved from the angiotensin peptide chain, synergistically mediate the endothelium-dependent dilation to angiotensin. Ang-(3–8), which lacks the amino-terminal L-arginine residue of Ang III, had no effect on cerebral arterioles. Ang-(3–8), when applied subsequently to topical L-arginine application, induced dilation similar to \(10^{-5}\) M Ang II or Ang III. This dilation was likely to be endothelium dependent because it could be abolished by methylene blue. Aminopeptidase inhibition by amastatin, which blocked the response to Ang II and Ang III, did not affect the dilation to Ang-(3–8) in the presence of exogenous L-arginine. The specificity of the synergistic effect of Ang-(3–8)/L-arginine was indicated by the lack of a response to L-arginine in the presence of the shorter fragment Ang-(4–8) and to L-arginine/ Ang III in the presence of amastatin. Coapplication of the enantiomer D-arginine or the basic amino acid L-lysine, instead of L-arginine, also was not effective. We therefore suggest that release of the angiotensin degradation products Ang-(3–8) and L-arginine specifically produce endothelium-dependent dilation of cerebral resistance vessels.

L-Arginine itself induced rather moderate vasodilation of the rabbit pial arterioles. This finding is in agreement with a study by Rosenblum et al.,\(^{31}\) who reported 7% vasodilation to L-arginine in the mouse open cranial window preparation. The release of L-arginine alone does not sufficiently explain the vasodilation to angiotensin. The failure to augment L-arginine–induced dilation by coapplication of Ang-(3–8) argues against the possibility that the angiotensin fragment might enhance the response to L-arginine by arginase inhibition. The reason for the stronger dilation seen when L-arginine application was followed by administration of Ang-(3–8) is unclear. In preliminary experiments, coapplication of L-arginine with amino acids contained in the Ang-(3–8) fragment did not produce vasodilation. We speculate that Ang-(3–8) or an as yet unidentified compound within Ang-(3–8) promotes L-arginine utilization by an endothelium-dependent pathway. Importantly, dilation was observed only when Ang-(3–8) was administered after cumulative application of \(10^{-8}\) to \(10^{-7}\) M L-arginine. It did not occur after application of a single dose of L-arginine. As suggested by Thomas and Ramwell,\(^{32}\) L-arginine may have to be incorporated into some compartment or biochemical moiety before EDRF biosynthesis.

Our findings have potential implications for the regulation of endothelium-dependent responses. In larger arteries, for which evidence has accumulated that L-arginine is the biochemical precursor of EDRF formation,\(^{7}\) L-arginine induces moderate vasodilation,\(^{33}\) dissimilar to the strong relaxation seen in response to acetylcholine. This discrepancy may be due to the fact that EDRF is generated from an as yet unidentified arginine derivative, rather than from L-arginine itself.\(^{32}\) Alternatively, we suspect that L-arginine utilization in the endothelium may be facilitated by other factors, such as Ang-(3–8).

Others have suggested that only peptides with L-arginine at the amino- or carboxy-terminal of the amino acid chain induce endothelium-dependent dilatation\(^{34}\) or stimulate guanylate cyclase activity.\(^{35}\) Our present observations indicate that Ang II, which contains the L-arginine inside the peptide chain, may be converted into an endothelium-dependent vasodilator through degradation by endogenous peptidases.

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**References**

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