Angiotensin-Converting Enzyme, Bradykinin, Angiotensin, and Cerebral Vessel Reactivity


SUMMARY Bradykinin (BK) produced concentration-related relaxations of cat middle cerebral arteries and was ineffective in cat basilar arteries. On rabbit basilar arteries, BK initially produced concentration-related relaxations; however, when repeated at 2-hour intervals, BK eventually produced pure contractile responses. After preincubation of the tissues with cycloheximide, BK produced reproducible relaxation responses. The angiotensin-converting enzyme inhibitors, SQ 14,225, BPP, and BPP, had no effect on the concentration-effect curves of BK, All, or 5-HT with any of the preparations, but responses to AI were inhibited. These results suggest that, in these tissues, angiotensin-converting enzyme is important for conversion of AI to All, but apparently not for the degradation of BK. (Hypertension 5 (supp 5): V-34-V-37, 1983)

Key Words • converting enzyme • bradykinin • angiotensin • cerebral vessel

ANGIOTENSIN I converting enzyme (ACE) (kininase II, peptidyl dipeptidase, EC 3.4.15.1) plays an important role in both the renin-angiotensin and the kallikrein-kinin systems by releasing the hypertensive vasoconstrictive peptide angiotensin II (AI) from its precursor angiotensin I (AI) and by inactivating the hypotensive vasodilator peptide, bradykinin (BK). 1 ACE is widely distributed and appears to be localized to the luminal surface of vascular endothelial cells of all tissues studied.2,3

Kininase-like activity has been demonstrated in bovine cerebral microvessels,4 and recent studies have demonstrated that the relaxant response to BK of cat superficial pial arterioles in vivo and cat middle cerebral arteries in vitro are unaffected by kininase II inhibitors.5 The apparent insensitivity of the cat basilar artery to BK6,7 may reflect a high turnover of BK in this tissue.

This study investigates the effect of the angiotensin-converting enzyme inhibitors (ACEIs), SQ 14,225, BPP, and BPP, on the responses of cat middle cerebral and basilar arteries in vitro to AI, All, BK, and 5-hydroxytryptamine (5-HT). The rabbit basilar artery is also included for comparison, since this tissue is also known to relax to BK.8

Methods

Cats and rabbits of both sexes were anesthetized with pentobarbitone (30 mg/kg-1 i.v.). After exsanguination, the brains were removed and the basilar and middle cerebral (cat only) arteries were carefully dissected out. Under a binocular dissecting microscope, small segments (4-5 mm) of these arteries were mounted on L-shaped stainless steel holders (diameter, 0.2 mm) and then placed in 10 ml tissue baths containing Krebs-Henseleit solution maintained at 37°C and gassed with 95% O2 and 5% CO2. The Krebs-Henseleit solution had the following composition (mM): NaCl, 118.3; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25.0; glucose, 11.0, and contained the following substances: indomethacin (2.8 X 10-6 M), phentolamine (10-6 M), (±)-propranolol (10-6 M), and atropine (10-6 M). Changes in tension were recorded isometrically by means of a Bell and Howell transducer connected to one of the two metal holders (the other being firmly attached to a tissue holder) and displayed on a Rikadenki single channel recorder.

Tissues were allowed to equilibrate for 2 to 3 hours, and studies were performed on vessels under a resting tone of 500 mg (BK, AI, All, and 5-HT) and after contraction with 5-HT, 5 X 10-8 M (BK only). Cumulative concentration-effect (C-E) curves were produced to each agent in the presence and absence of the ACEIs, SQ 14,225 (5 X 10-6 M), BPP, (10-6 M), and BPP, (10-6 M). Contractile responses to AI, All, and 5-HT were expressed as a percentage of their maximum obtained response. Relaxant responses to BK on tissues under resting tension and contracted with 5-HT were expressed as a percentage of the maximum relaxation response obtained with papaverine (10-4 M) as described previously.5,7

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The drugs used were: 5-hydroxytryptamine creatinine sulphate, indomethacin, papaverine hydrochloride, angiotensin I, angiotensin II, cycloheximide (Sigma); phenotolamine mesylate (Ciba); atropine sulphate (BDL); bradykinin triacetate, BPP₅₄, BPP₆₄ (Bachem); SQ 14,225 (Squibb).

Results

Bradykinin (BK) produced concentration-related relaxations of the cat middle cerebral artery in vitro under resting tension and when contracted with either 5-HT (5 × 10⁻⁴ M) or KCl (18 mM). Responses obtained with 5-HT and KCl were qualitatively the same, and so only data obtained with 5-HT are presented.

Maximum relaxations of 34.5 ± 4.2% (SEM, n = 6) and 13.4% ± 3.2% (SEM, n = 6) were obtained with 10⁻⁵ M BK on tissues contracted with 5-HT or under resting tension respectively. The C-E curves with BK in the presence of SQ 14,225, 5 × 10⁻⁶ M, were virtually superimposable with the curves produced in its absence in tissue contracted with 5-HT (fig. 1, upper graph) and under resting tension (fig. 1, lower graph). Results obtained with BPP₅₄ (10⁻⁵ M) and BPP₆₄ (10⁻⁵ M) were similar to those obtained with SQ 14,225. The cat basilar artery was insensitive to the action of BK under resting tension and when contracted with 5-HT. None of the ACEIs increased the sensitivity of the tissues to BK.

In the rabbit basilar artery under resting tension and when contracted with 5-HT, BK produced concentration-related relaxations. Construction of C-E curves to BK at 2-hour intervals resulted in a reduced relaxant effect of BK until finally at 8–10 hours after the initial exposure to BK, pure contractile effects were observed (fig. 2). Preincubation of the tissues with cycloheximide (7.2 × 10⁻⁵ M) during the equilibration period resulted in superimposable C-E curves to BK at 2-hour intervals similar to that seen at time 0 in figure 2. No such change in sensitivity was seen with 5-HT or KCl applied at the same time intervals.

C-E curves of BK on tissues preincubated with cycloheximide were unaffected by SQ 14,225, BPP₅₄, or BPP₆₄ (fig. 3). Similarly, C-E curves of BK on tissues

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**Figure 1.** Concentration-effect curves of bradykinin in the cat middle cerebral artery (n = 6 to 8) contracted with 5-HT (5 × 10⁻⁴ M) (upper graph) under resting tension (lower graph) in the absence (□) and presence (○) of SQ 14,225 (5 × 10⁻⁶ M).

**Figure 2.** Concentration-effect curves to bradykinin constructed at 2-hour intervals on rabbit basilar artery under resting tension (n = 6 to 8).

**Figure 3.** Concentration-effect curves to bradykinin in the absence (circles) and presence (squares) of SQ 14,225 on rabbit basilar arteries that had been preincubated with cycloheximide (broken lines) or preparations in the absence of cycloheximide (solid lines). Results in the absence of cycloheximide were obtained in tissues that had been challenged with bradykinin at 2-hour intervals for 8 to 10 hours.
FIGURE 4. Concentration-effect curves of angiotensin I in the absence (circles) and presence of SQ 14,225, 5 × 10^{-6} M (downpointing triangles), BPP_{	ext{II}} 10^{-5} (squares), and BPP_{	ext{II}} 10^{-6} M (upward triangles) in cat middle cerebral artery (a), cat basilar artery (b), and rabbit basilar artery (c). Concentration-effect curves to angiotensin II are also shown (diamonds); n = 6 to 8.

that had been challenged for 8 to 10 hours at 2-hour intervals (in the absence of cycloheximide) were unaffected by SQ 14,225 (5 × 10^{-6} M), BPP_{	ext{II}} (10^{-5} M), or BPP_{	ext{II}} (10^{-6} M) compared to controls (fig. 3).

AI and All produced concentration-related contractions of cat and rabbit basilar and cat middle cerebral arteries (fig. 4). AI was approximately 10 times less potent than All on each tissue. On each of these tissues, SQ 14,225 (5 × 10^{-6} M), BPP_{	ext{II}} and BPP_{	ext{II}} produced significant rightward shifts of the C-E curves of AI (fig. 4) but were without effect against C-E curves of All or 5-HT compared to controls.

Discussion

Results from this study demonstrate that BK produced concentration-related relaxations of the cat middle cerebral and rabbit basilar arteries but was ineffective in cat basilar arteries. These effects were seen in tissues under resting tension and when contracted with 5-HT or KCl. They are consistent with previous observations. The receptor type mediating the relaxant response of the cat middle cerebral artery appears to be of the B_{2}-type whereas the cat basilar artery appears to be devoid of kinin receptors. The initial relaxant effect of BK on the rabbit basilar artery appears to be of the B_{2}-type, but on repeated application of BK, a contractile effect is seen, which can be blocked by pretreating the tissues with the protein synthesis inhibitor cycloheximide (this study) or by the selective B_{1}-receptor antagonist des-Arg^{9}-Leu^{10}-BK, suggesting that the contractile response to BK is mediated via generation de novo of a BK B_{1} receptor as described by Regoli and Barabé.

The relaxant effect of the kinins in the present study is believed not to involve the release of a relaxant prostaglandin, since indomethacin, which inhibits prostaglandin synthesis, was present in the bathing medium. From the present study, however, it is not possible to say whether the kinins are relaxing the tissue directly or indirectly via release of a non-prostaglandin endothelium-derived factor, which has been shown to occur in other vascular preparations with BK and other vasodilators.

The relaxant or contractile effects of BK on any of the tissues were unaffected by the ACEIs, SQ 14,225, BPP_{	ext{II}}, or BPP_{	ext{II}}. Thus, it may be concluded from these observations that ACE or kininase II is not present in these vascular preparations. In addition, the lack of sensitivity of the cat basilar artery is not due to a high turnover of BK but presumably to a lack of receptors for BK in the tissue as suggested previously. The lack of kininase II has also been demonstrated in other vascular preparations such as the rabbit aorta and dog carotid artery and cat pial arteries in vitro and in vivo.

Very few studies compare the effect of BK and AI in the same tissue at the same time in the absence and presence of ACEIs. Perhaps the most interesting observations made here were that all ACEIs produced significant antagonism of the contractile responses to AI without affecting responses to All or 5-HT. It is difficult to reconcile these responses since from the results with BK it is apparent that there is an absence of kininase II, which is the same enzyme as ACE. It may be possible that there are other enzymes present in these vascular preparations that degrade BK but do not convert AI to All. One such possibility is a carboxypeptidase-N type enzyme, which is present in plasma and cleaves off the C-terminal Arg, producing des-Arg^{9}-BK, which is a less potent agonist than BK at B_{2}-receptors and more potent than BK at B_{1}-receptors. This enzyme is thought to be present in such vascular preparations as the rabbit aorta and mesenteric vein.
Thus, in the presence of ACEIs, some of the BK may be broken down to the less potent contractile agent des-Arg⁹-BK by this second enzyme, and the overall effect is the algebraic sum of the effects of BK and des-Arg⁹-BK. This would be consistent with the ineffectiveness of the ACEIs against BK-induced relaxations of the cat middle cerebral and rabbit basilar artery. However, this hypothesis would not be consistent with the ineffectiveness of the ACEIs on the BK-induced contractions of the rabbit basilar artery since this appears to be mediated via B₁-receptors in which des-Arg⁹-BK is more potent than BK. These effects require further investigation.

In conclusion, the results from this study appear to suggest that in cat and rabbit cerebral vessels in vitro, ACE is important for the conversion of AI to AII but apparently not for the degradation of BK.

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