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 vasoconstrictor peptide angiotensin II (AII) from its precursor angiotensin I (AI) and by inactivating the hypotensive vasodilator peptide, bradykinin (BK). 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, 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 (ACEI), SQ 14,225, BPP₃, and BPP₆, on the responses of cat middle cerebral and basilar arteries in vitro to AI, AII, 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.6

### Methods

Cats and rabbits of both sexes were anesthetized with pentobarbitone (30 mg/kg 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, AII, and 5-HT) and after contraction with 5-HT, 5 X 10⁻⁶ 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), BPP₃ (10⁻⁴ M), and BPP₆ (10⁻³ M). Contractile responses to AI, AII, and 5-HT were expressed as a percentage of their own 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.6,7
The drugs used were: 5-hydroxytryptamine creatinine sulphate, indomethacin, papaverine hydrochloride, angiotensin I, angiotensin II, cycloheximide (Sigma); phenolamine mesylate (Ciba); atropine sulphate (BDM); bradykinin triacetate, BPP sub s, BPP sub s (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^{-6} 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^{-7} 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 BPP sub s (10^{-5} M) and BPP sub s (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, BPP sub s, or BPP sub s (fig. 3). Similarly, C-E curves of BK on tissues...
that had been challenged for 8 to 10 hours at 2-hour
intervals (in the absence of cycloheximide) were unaf-
fected by SQ 14,225 (5 × 10⁻⁶ M), BPP₅₅⁺ (10⁻⁵ M),
or BPP₆₀⁺ (10⁻³ M) compared to controls (fig. 3).
AI and All produced concentration-related contrac-
tions 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 tis-
sues, SQ 14,225 (5 × 10⁻⁶ M), BPP₅₅⁺, and BPP₆₀⁺
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 pro-
duced concentration-related relaxations of the cat mid-
dle cerebral and rabbit basilar arteries but was ineffect-
ive 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 obser-
vations. The receptor type mediating the relaxant re-
sponse of the cat middle cerebral artery appears to be
of the B₂-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
B₂-type, but on repeated application of BK, a contrac-
tile effect is seen, which can be blocked by pretreating
the tissues with the protein synthesis inhibitor cyclo-
heximide (this study) or by the selective B₁-receptor
antagonist des-Arg⁹-Leu⁸-BK⁹ suggesting that the con-
trastile response to BK is mediated via generation de
novo of a BK B₁ 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,¹⁰ 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-pros-
taglandin endothelium-derived factor, which has been
shown to occur in other vascular preparations with BK
and other vasodilators.¹¹,¹²

The relaxant or contractile effects of BK on any of
the tissues were unaffected by the ACEIs, SQ 14,225,
BPP₅₅⁺, or BPP₆₀⁺. 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.⁷,⁸ The lack
of kininase II has also been demonstrated in other
vascular preparations such as the rabbit aorta and dog
carotid artery⁶ and cat pial arteries in vitro and in vivo.⁵

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 ob-
servations 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.¹ 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 carboxy-
peptidase-N type enzyme, which is present in plasma
and cleaves off the C-terminal Arg,¹³ producing des-
Arg⁹-BK, which is a less potent agonist than BK at B₂-
receptors and more potent than BK at B₁-receptors.⁹
This enzyme is thought to be present in such vascular
preparations as the rabbit aorta and mesenteric vein.¹⁴
Thus, in the presence of ACEIs, some of the BK may be broken down to the less potent contractile agent des-Arg$^9$-BK by this second enzyme, and the overall effect is the algebraic sum of the effects of BK and des-Arg$^9$-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 B$_2$-receptors in which des-Arg$^9$-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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