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INVOLVEMENT OF ENDOPEPTIDASE 24.15 IN THE INACTIVATION OF BRADYKININ BY RAT BRAIN SLICES

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Summary. The effect of peptidase inhibitors on the degradation of [^{3}H]-bradykinin by rat hypothalamic slices was studied using HPLC to separate and identify the products. The degradation appears to be mainly mediated by an enzyme which cleaves the peptide at the Phe^5-Ser^6 bond and is inhibited by 1,10-phenanthroline, dynorphin(1-13) and carboxyphenylethyl-Ala-Ala-Phe-p-aminobenzoate. This suggests the involvement of a membrane bound variant of the soluble metalloendopeptidase (EC3.4.24.15) isolated from rat brain which degrades neurotensin, angiotensin and other neuropeptides as well as bradykinin.

As well as being present in peripheral tissues and blood, the nonapeptide, bradykinin (BK; Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) has also been identified in brain (18). It is localized in neuronal pathways (9) and has behavioural effects when injected centrally (16) suggesting that it may function as a neurotransmitter (20). Both the enzyme responsible for its synthesis (kallikrein) and its precursor (kininogen) have been demonstrated in brain (4,19). An important component of all neurotransmitter systems is the mechanism for the removal and inactivation of the transmitter after its release. In vitro studies with tissue homogenates and purified enzymes have shown that the brain contains several peptidases capable of degrading BK (2,11,8,21). However it is not clear which, if any, of these enzymes control extracellular levels of BK in the brain.

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We have therefore examined the effects of various peptidase inhibitors on the degradation of ^{3}H-BK superfused over rat hypothalamic slices, the brain region with the highest levels of BK [18].

MATERIALS AND METHODS

Male Wistar rats (~200g) were stunned, decapitated and the brain rapidly removed onto ice. The hypothalamus was excised and frontal sections (0.25mm) prepared using a Vibratome. The slices were divided into two down the midline, washed in oxygenated (95% O_2/5% CO_2) Krebs bicarbonate buffer (2x5ml) and placed into the superfusion chambers (maintained at 37°C) in 1ml of Krebs buffer containing [Pro^{2,3}-H^{3}]-bradykinin (^{3}H-BK; 0.1μCi; Amersham) and inhibitor at the appropriate concentration. Solutions were pumped over the slices at 50μl/min and collected into 10% trifluoroacetic acid (TFA; 100μl). The degradation products were separated by reversed phase HPLC and determined by liquid scintillation counting as before [15]. Each inhibitor and control superfusion was made in triplicate. Inhibitors were obtained from the following sources: N-[1(RS)-carboxy-2-phenylethyl]-Ala-Ala-Phe-p-aminobenzoate (CPE-Ala-Ala-Phe-pAB) - gift from Dr M Orlowski, Mount Sinai School of Medicine, New York; phosphoramidon, bestatin, 1,10-phenanthroline - Sigma; dynorphin(1-13) - Peninsula Labs Europe; captopril - Squibb. The products of BK degradation by a soluble metalloendopeptidase purified from rat brain [14] were separated by reversed phase HPLC and their amino acid compositions determined after acid hydrolysis. The two major products were BK(1-5) and BK(6-9), the same as obtained with a peptidase purified from human brain [13].

RESULTS

In the absence of inhibitors, ^{3}H-BK was degraded by 40-60% to give one major radioactive product on HPLC which co-eluted with BK(1-5) and some unidentified minor products (Fig 1A). The most effective inhibitors of ^{3}H-BK degradation were dynorphin(1-13) (Fig 1C) and 1,10-phenanthroline (Table). Dynorphin(1-13) is an effective inhibitor of ^{3}H-neurotensin degradation by rat hypothalamic slices and the soluble metalloendopeptidase [15]. However, dynorphin(1-13) may inhibit other CNS peptidases capable of degrading BK (eg endopeptidase 24.11) and therefore its inhibition may not be specific. CPE-Ala-Ala-Phe-pAB, a specific transition state inhibitor of the soluble metalloendopeptidase [6,17] also causes significant inhibition of ^{3}H-BK degradation by the slices (Fig 1B & Table). Phosphoramidon, (an inhibitor of endopeptidase 24.11; Fig 1D), captopril (an inhibitor of

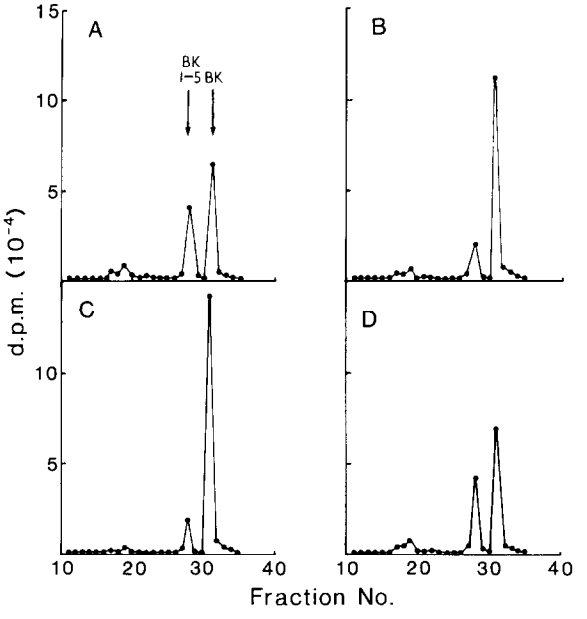

Figure 1. Reversed-phase HPLC separation of the products of ^{3}H-bradykinin degradation by rat hypothalamic slices in the presence of inhibitors. A - no inhibitor; B - CPE-Ala-Ala-Phe-pAB; C - dynorphin(1-13); D-phosphoramidon. Novapak C18 radial compression cartridge; 2.1-37% acetonitrile (over 15min at 2ml/min) containing 11mM-TFA; trifluoroacetic acid. Fractions (0.3min) were collected and radioactivity determined by scintillation counting. BK = bradykinin.

angiotensin converting enzyme) and bestatin (a broad specificity aminopeptidase inhibitor) are all virtually without effect (Table).

DISCUSSION

The inhibition of BK degradation by CPE-Ala-Ala-Phe-pAB, 1,10-phenanthroline and dynorphin(1-13) points to the involvement of the metalloendopeptidase which hydrolyses BK at Phe^{5}-Ser^{6} being involved in its catabolism in hypothalamic slices. This enzyme, which also hydrolyses neurotensin (at Arg^{8}-Arg^{9}) and angiotensin-I (at Pro^{7}-Phe^{8}) as well as other neuropeptides, has unusual specificity, being directed mainly by hydrophobic residues at positions P_1, P_1' and P_3' around the hydrolysis site [7,17], and there is evidence for its involvement in the catabolism of neurotensin by rat synaptic membranes [5] and rat hypothalamic slices [15]. It seems likely that the rabbit enzyme termed endopeptidase-A [1,3] is

TABLE 1

EFFECT OF INHIBITORS ON THE DEGRADATION OF ^{3}H-BRADYKININ BY RAT HYPOTHALAMIC SLICES

Inhibitor	Concentration	BK-degrading activity remaining (%)
None	-	100
1,10-Phenanthroline	1mM	24.4 ± 2.3
CPE-Ala-Ala-Phe-pAB	50μM	47.0 ± 4.1
Dynorphin(1-13)	7μM	25.5 ± 5.3
Phosphoramidon	50μM	96.6 ± 9.6
Captopril	50μM	89.6 ± 8.4
Bestatin	10μM	89.6 ± 7.7

In the absence of inhibitor, degradation of ^{3}H-BK was 40-60%. The results (n=3, ± SD) are expressed as a percentage of the degradation occuring in the absence of inhibitor.

the equivalent peptidase in this species despite some differences in specificity and inhibitor sensitivity. Degradation of BK by homogenates of cultured neuroblastoma and glioma cells also appears to be mainly mediated by a endopeptidase-A like enzyme [10]. Although initially isolated from the soluble fraction of brain, the metalloendopeptidase is also present on brain membranes [5; McDermott et al, unpublished] and the activity in neuroblastoma/glioma cells is partly associated with the particulate fraction. Very little peptidase activity is released into the medium during the superfusion of hypothalamic slices [15]. From this it can be proposed that the degradation of BK is partly mediated in brain tissue by a membrane bound metalloendopeptidase related to an enzyme (EC 3.4.24.15) isolated from brain soluble fractions [3,14,17] which hydrolyses the peptide at Phe^{5}-Ser^{6} and which is sensitive to CPE-Ala-Ala-Phe-pAB. The physiological importance of this process has yet to be established.

The finding that dynorphin(1-13) inhibits the degradation of exogenous BK, neurotensin (15) and probably angiotensin-I in brain slices may have important implications for the central effect of this peptide. It has recently been pointed out that inhibition of degradation pathways for endogenous

peptides rather than receptor-mediated actions may explain some of the pharmacological effects of centrally injected neuropeptides (12).

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