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Rapid communication

'ENKEPHALINASE' IS DISTINCT FROM BRAIN 'ANGIOTENSIN-CONVERTING ENZYME'

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We have recently shown the presence in cerebral membranes of 'enkephalinase', a dipeptidyl-carboxypeptidase, releasing ^{3}H-Tyr-Gly-Gly from ^{3}H-enkephalins (ENKs) which could be associated with the control of enkephalinergic transmission. This possibility is mainly suggested by the enzyme's distribution paralleling that of opiate receptors and its increase following morphine treatment (Malfroy et al., 1978).

The possible identity of 'enkephalinase' with 'angiotensin-converting enzyme' (ACE) i.e. peptidyl-dipeptide-hydrolase (EC 3.4.15.1) was raised because: (i) ACE is present in brain (Yang and Neff, 1972), (ii) this enzyme, of broad substrate specificity, when purified to homogeneity also cleaves the ENKs at the Gly-Phe bond (Erdös et al., 1978), (iii) like ACE, 'enkephalinase', appears to be a metallo-enzyme (Swerts et al., 1979).

On the other hand, in contrast with ACE, 'enkephalinase' is not activated by Cl^- and is inhibited only in the presence of comparatively high concentrations of SQ 14,225 or SQ 20,881, suggesting that the two enzymes are different.

Because the activity of these potent inhibitors had generally been assessed on solubilized ACE, we could not entirely exclude that 'enkephalinase' was different from an ACE activity when in membrane-bound form (Swerts et al., 1979).

We have now compared, on the same membrane preparations from mouse or rat brain, the activities of ACE defined as the enzyme activity releasing His-Leu from Hip-His-Leu and of 'enkephalinase', i.e. the enzyme activity releasing ^{3}H-Tyr-Gly-Gly from ^{3}H-Leu ENK, under a variety of physiological and pharmacological conditions.

The data in table 1 show that the regional distribution and ontogenic development of and the effects of morphine on the two enzyme activities differ markedly. Thus the marked differences in ACE activity between regions of rat brain, in agreement with those reported by Yang and Neff (1972), are not correlated with those of 'enkephalinase' activity. The regional distribution of the enkephalinase appears similar in rats (table 1A) and mice in which it is correlated with that of opiate receptors (Malfroy et al., 1979).

In addition, whereas both enzyme activities are low in the striatum of newborn rats (G. Patey, S. de la Baume, C. Gros and J.C. Schwartz, in preparation), 'enkephalinase' activity develops earlier: at 15 days its level, expressed per mg protein, is much higher than in the adult, and, if calculated per total striatum, has reached the adult level. The pattern observed for ACE is again different (table 1B).

Furthermore ACE activity is not modified in the striatum of mice chronically treated with morphine whereas a substantial increase in 'enkephalinase' activity occurs, (table 1C) as previously found using a different assay (Malfroy et al., 1978).

Finally the two enzyme activities from the same membrane preparation have clearly distinct substrate (or inhibitor) specificity. Thus the K_i values of SQ 14,225 (1.7 nM) and SQ

TABLE 1

Comparison of the properties of enkephalin-dipeptidyl carboxypeptidase ('enkephalinase') and peptidyldipeptide hydrolase (ACE), from brain preparations.

Particulate fractions from rat or mouse cerebral structures obtained as described by Malfroy et al. (1978). 'Enkephalinase' activity estimated by 15 min incubations at 25°C in 0.05 M Tris-HCl buffer pH 7.4 with 20 nM ^{3}H-Leu-ENK (Amersham, 39 Ci/mmol) and 0.1 mM puromycin. ^{3}H-Tyr-Gly-Gly formation evaluated by either Porapak Q column chromatography (A, C, D) or TLC (B) as previously described (Malfroy et al., 1979), both methods giving very similar results. ACE activity measured fluorimetrically using 1 mM Hip-His-Leu as a substrate according to Yang and Neff (1972), except that p-chloromercuribenzoate was replaced by 0.1 mM puromycin. In experiment C, 14 groups of 2 mice were sham-operated or implanted for 3 days with a 75 mg morphine base pellet and killed 1 day after extraction of the pellet. Basal 'enkephalinase' activity in striatum (fmoles min^{-1} mg $protein^{-1}$) was 191 ± 19 in rat and 237 ± 10 in mouse. Striatal ACE activity (nmoles min^{-1} mg $protein^{-1}$): 2.3 ± 0.2 in rat and 2.0 ± 0.1 in mouse. Means ± S.E.M. of 3-14 experiments on pooled structures from 2-3 animals with triplicate assays. Activities are expressed as % of the activity in the striatum (A) and of adults (B) and controls (C).

	'Enkephalinase'	ACE
(A) Distribution in rat brain regions		
Striatum	100 ± 6	100 ± 4
Cortex	55 ± 5	6 ± 0.3
Cerebellum	25 ± 2	46 ± 2
(B) Ontogenetic development in rat striatum		
Adults	100 ± 8	100 ± 7
15-day old	179 ± 8	90 ± 2
(C) Effects of morphine in mouse striatum		
Controls	100 ± 4	100 ± 3
Morphine pellets	122 ± 5	100 ± 4
(D) Effects of inhibitors (IC_{50}) in mouse striatum		
Leu-ENK	1.1 ± 0.1 10^{-5} M	1.2 ± 0.2 10^{-3} M
Met-ENK	1.4 ± 0.5 10^{-6} M	2.1 ± 0.9 10^{-3} M
Gly-Gly-Phe-Met	1.7 ± 0.8 10^{-6} M	>3.0 10^{-3} M
SQ 14,225	1.0 10^{-5} M	1.6 ± 0.2 10^{-8} M
SQ 20,881	>1.0 10^{-4} M	2.4 ± 1.7 10^{-7} M

20,881 (100 nM) for ACE, which are in the same range as those reported by various authors for this enzyme in pure form, are approximately 1,000 fold lower than the K_is for 'enkephalinase'. In contrast, the K_i values of ENKs and Gly-Gly-Phe-Met for ACE are 100-1,000 fold higher than for 'enkephalinase'. The IC_{50} of Leu-ENK for 'enkephalinase' is substantially lower than previously reported by Malfroy et al. (1978) a difference due to the poor reliability of the previous method which involved the analysis of biphasic saturation curves, the high affinity component of which represented less than 20%.

Our data show that the two enzymes are distinct species co-existing in cerebral membranes but indicate that the ENKs might also be cleaved by ACE, although with a low affinity. ACE seems not to be involved in the cleavage of ENKs in moderate concentrations: Tyr-Gly-Gly formation by rat striatal membranes in the presence of 10^{-6} M SQ 14,225 is not modified as long as the concentration of the substrate, Leu-ENK, is below 10^{-5} M and is decreased by only 9 ± 2% for 10^{-4} M Leu-ENK (not shown). This result is in agreement with the IC_{50} of Leu-ENK, for ACE, in the mM range (table 1D).

Hence it is likely that, in contrast with

'enkephalinase', cerebral ACE does not play a significant physiological role in the inactivation of endogenous or exogenous ENKs.

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