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PROPERTIES OF "ENKEPHALINASE" FROM RAT KIDNEY : COMPARISON OF DIPEPTIDYL-CARBOXYPEPTIDASE AND ENDOPEPTIDASE ACTIVITIES

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The "enkephalinase" i.e. the metallopeptidase cleaving the Gly^3-Phe^4 amide bond of enkephalins from rat kidney was studied in its membrane-bound form as well as in a highly purified preparation. It seems identical or very close to three other enzyme activities : "enkephalinase" from cerebral membranes, an endopeptidase from bovine pituitary and the "neutral endopeptidase" from rabbit kidney. Specificity constants of substrates were higher for peptides with a free terminal carboxylate as compared to amidified or typical endopeptidase substrates which were also cleaved. The dipeptidyl carboxypeptidase specificity of "enkephalinase" is attributable to the presence of a critical arginine residue in its active site.

INTRODUCTION

The weak and short-lasting biological activity of enkephalins can be ascribed to their rapid hydrolysis by various peptidases (for a review see 1). Among them an enzyme primarily associated with synaptic membranes (2)(3) and termed "enkephalinase" hydrolyses the opioid pentapeptides at the level of their Gly^3-Phe^4 amide bond (4). It displays many features suggesting that it physiologically participates in the inactivation of endogenous enkephalins released from their neuronal stores. Particularly a potent and selective inhibitor of this enzyme, thiorphan, has been recently shown to protect endogenous (Met^5)enkephalin released from depolarised brain slices (5) and to display antinociceptive activity mediated by opiate receptors (6).

Initial studies on the substrate specificity of "enkephalinase" were performed on cerebral membranes and suggested a high selectivity of this peptidase towards the enkephalins (4). However it was thereafter shown that "enkephalinase" was able to recognise a variety of non-opioid peptides (1). In addition, using ^{3}H-enkephalin as substrate, an apparently similar enzyme activity was detected (but not characterised) in a variety of peripheral organs, among which the kidney displayed the highest specific activity (7).More recently, an endopeptidase purified from bovine pituitary was shown to cleave the Gly^3-Phe^4 bond from enkephalins and to be present in brain, raising the possibility that it might be identical to "enkephalinase" (8,9).

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We now report that the chemical specificity of the rat kidney enzyme in its membrane-bound form or in a highly purified preparation is closely similar to that of cerebral "enkephalinase" as well as to those of the bovine pituitary endopeptidase (8) and of a thermolysin-like "neutral endopeptidase" previously isolated from rabbit kidney (10,11). Nevertheless the comparison of specificity constants of various substrates indicates that "enkephalinase" optimally functions as a dipeptidylcarboxypeptidase.

MATERIALS AND METHODS

^{3}H-(Leu^5) or ^{3}H-(Met^5)enkephalins were from Amersham, ^{3}H-(D-Ala^2,Leu^5)enkephalin was prepared in the laboratories of Drs B.P. Roques and J.L. Morgat as described (27). The non-labelled enkephalins were purchased from Bachem. Insulin B chain was obtained from Boehringer Mannheim, aminopeptidase M (EC.3.4.11.2) from Pierce Chemicals. The substrates Hip-Arg-Arg-Leu-β NA, Hip-Arg-Arg-Ala-β NA and Hip-Arg-Arg-Gly-β NA were kindly donated by Dr Sherwin Wilk. Phosphoramidon was a kind gift from Dr Umezawa. We are also indebted to Pr B.P. Roques and his colleagues for providing us with thiorphan, thiorphan-NH_2, and several peptides used in this study.

Rat kidney membrane fraction : Rats were killed by cervical dislocation, their kidneys removed and stored on ice. Homogeneisation was performed in HEPES 0.05 M, pH 7.5 containing 10 mM $MgCl_2$ (10 ml for 2 kidneys) using a glass-teflon Potter homogeneiser (Braun), and the homogenate left at 4°C for 15 min. The supernatant obtained after a 12 min centrifugation at 1,500 g, was recentrifuged at 15000 g for 20 min. After superficial washing, the final pellet was resuspended in ice cold HEPES buffer (0.05 M, pH 7.5) using a Dounce pestle. Prior to use the membrane preparation was diluted to give a protein concentration close to 20 µg/ml.

Purified rat kidney "enkephalinase" : A membrane fraction from the kidneys of 60 rats was solubilised using TRITON X 100. The solubilised enzyme was then purified by DEAE A50, concanavalin A, and hydroxylapatite chromatography. Sodium dodecylsulfate polyacrylamide electrophoresis revealed one major protein band (MW 96000) and three other faint bands. The purified "enkephalinase" releases 3 nmole/mg protein/min of ^{3}H-Tyr-D-Ala-Gly from 20 nM ^{3}H-Tyr-D-Ala-Gly-Phe-Leu at 25°C in 0.05 M pH 7.5 HEPES buffer. This value corresponds to a 1,000 fold enrichment as compared to the rat kidney homogenate and a specific activity about 50,000 times higher than that of rat brain. As electrophoresis of the purified enzyme preparation revealed one major band with an apparent M.W. of 96,000, this value was used for kcat calculations. Details of the purification procedure will be published elsewhere (Malfroy et al., manuscript in preparation)

Determination of enzyme activity : Incubations were performed in 0.05 M HEPES buffer pH 7.5. The hydrolysis of the ^{3}H-enkephalins (20 nM in 100 µl) was stopped by addition of 50 µl 0.2 N HCl, the tritiated metabolite, either Tyr-Gly-Gly or Tyr-D-Ala-Gly being isolated by polystyrene bead column chromatography (26,27). The identity of products was checked by H.P.L.C. analysis. Iodination of the B chain of insulin as well as the quantification of its hydrolysis were performed as described by Kenny (19). The hydrolysis of naphtylamides (NA) of the type Hip-Arg-Arg-X-β NA (X : Leu, Ala or Gly) was followed using the two step procedure described by Almenoff et al. (9). Briefly, the incubations were stopped by addition of dithiothreitol (1 mM final concentration) and further incubated for 1 h at 37°C with 10 µg of aminopeptidase M, to release naphthylamine from X-β NA. Blanks (dithiothreitol added before the substrate) were performed in all experiments.

Table I : Comparison of membrane-bound "enkephalinase" activity from mouse striatum and rat kidney

SUBSTRATE	PARAMETERS	ENZYME SOURCE	
		STRIATUM	KIDNEY
^{3}H-Tyr-D-Ala-Gly-Phe-Met-NH_2	K_M (μM)	72 ± 17	61 ± 22
	Vmax ⁂	262 ± 35	9350 ± 620
^{3}H-Tyr-D-Ala-Gly-Phe-Leu	K_M (μM)	19 ± 3	18 ± 1
	Vmax ⁂	188 ± 20	8100 ± 450
	Ki of :		
	. Thiorphan (nM)	1.6 ± 0.1	1.9 ± 0.2
	. Thiorphan-NH_2 (nM)	16.8 ± 1.9	17.0 ± 2.5
	. Phosphoramidon (nM)	4.3 ± 2.1	3.8 ± 1.7
	. EDTA (mM)	2.7 ± 1.0	1.8 ± 0.4

⁂ in pmole/mg protein/min

Striatal membranes obtained as previously described (4) and kidney membranes (see Materials and Methods) were incubated for 30 min at 25°C in 0.05 M HEPES NaOH buffer pH 7.5. The formation of ^{3}H-Tyr-D-Ala-Gly was evaluated by polystyrene bead column chromatography. Each value represents the mean (± S.E.M.) from at least three independent experiments (triplicate determinations).

RESULTS

The properties of the "enkephalinase" activities i.e. the enzyme activities cleaving the Gly^3-Phe^4 amide bond of ^{3}H-enkephalins in crude particulate fractions from mouse striatum and rat kidney, respectively, have been first compared (Table 1). Thus radioactive substrates containing a D-Ala residue in position 2 were used in order to avoid interferences by aminopeptidases present in these preparations and the identity of the characteristic tripeptide product of the reaction has been checked by H.P.L.C. chromatography. The specific activity of the enzyme is much higher in the particulate fraction from rat kidney (enriched in microsomes) than in the striatal pellet, as shown by the 35-40 fold difference in Vmax of the two preparations. However both preparations display closely similar properties. In particular Km values of the two ^{3}H-substrates do not differ significantly and the same remark holds true for the Ki values (calculated by assuming a competitive inhibition) of thiorphan (6), its amidified analogue and phosphoramidon (12). In addition both enzyme activities are inhibited at similar concentrations of the chelator EDTA (Table 1). The purified "enkephalinase" preparation from rat kidney hydrolyses ^{3}H-(D-Ala^2,Leu^5)enkephalin with a Km value similar to that of the enzyme in its membrane-bound state (not shown) and this activity is inhibited by thiorphan, its amidified analogue and phosphoramidon with apparent affinities (Fig. 1) corresponding closely to those regarding the original particula-

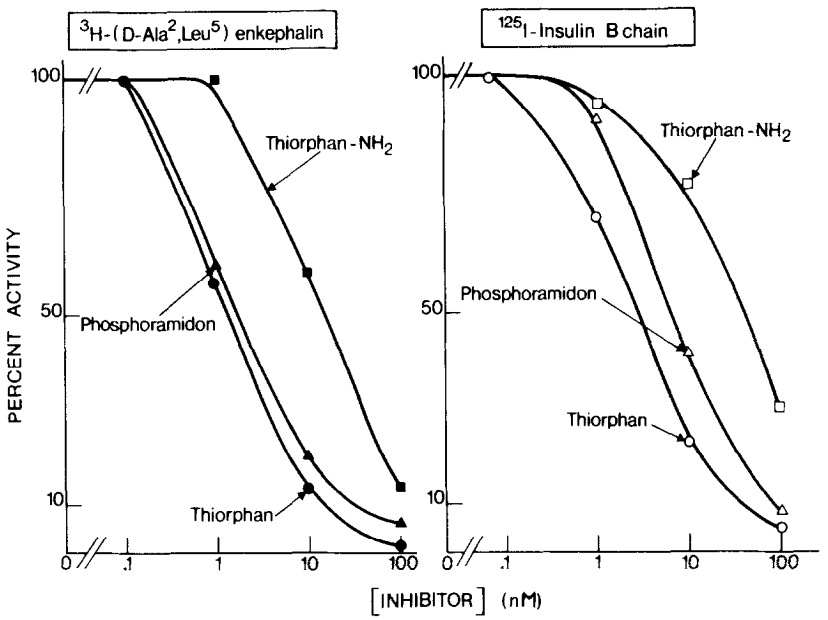

Figure 1 : Enhibition of the hydrolysis of ^{3}H-(D-Ala^{2},Leu^{5})enkephalin or ^{125}I-insulin B chain by purified rat kidney "enkephalinase"

The enzyme (3 ng/0.1 ml) was incubated for 30 min at 25°C in the presence of either 20 nM ^{3}H-(D-Ala^{2},Leu^{5})enkephalin or ^{125}I-insulin B chain (10.000 cpm/0.1 ml). Hydrolysis products separated from substrate by polystyrene bead column chromatography (enkephalin) or trichloroacetic acid preparation (insulin) according to Kenny (19). Values represent the means of triplicates.

te preparation (Table 1). In addition, the purified enzyme preparation hydrolyses ^{125}I-insulin B chain and this activity is also inhibited by the three compounds with IC_{50} values similar to those required to inhibit the enkephalin hydrolysing activity (Fig. 1). The substrate specificity of the purified rat kidney "enkephalinase" has been assessed by determining Km and kcat values (assuming homogeneity of the preparation) towards the free enkephalin derivatives and calculating the various specificity constants. Table II reports these values which are, in some cases, compared with the corresponding values found by Almenoff et al. for the endopeptidase activity purified from bovine pituitary gland by these authors (9).

Whereas the two fluorogenic substrates IV and V were hydrolysed in an easily quantifiable manner, the hydrolysis of compound VI was not detectable. The specificity constant was the highest for (Met^{5})enkephalin while (D-Ala^{2}, Met^{5})enkephalinamide and compounds IV and V displayed about 10-200 times lower specificity constants.

Finally the changes in specificity constants of various substrates were determined after pretreatment of the purified renal enzyme by butanedione, a reagent blocking selectively and irreversibly guanidium groups when used in

Table II : Kinetic parameters for hydrolysis of various substrates by purified rat kidney "enkephalinase"

	SUBSTRATE	Km (μM)	Kcat (min^{-1})	SPECIFICITY CONSTANT (Kcat/Km)
	P'_3 P'_2 P'_1↓ P_1 P_2			
I	Tyr-Gly-Gly-Phe-Met	8 (40)	1268 (21.2)	160 (0.5)
II	Tyr-D-Ala-Gly-Phe-Met	13	1044	80
III	Tyr-D-Ala-Gly-Phe-Met-NH_2	320	2521	8
IV	Hip-Arg-Arg-Leu-βNA	350 (180)	4320 (240)	12 (1.3)
V	Hip-Arg-Arg-Ala-βNA	380 (300)	410 (8.8)	1 (0.03)
VI	Hip-Arg-Arg-Gly-βNA	negligible hydrolysis		

Values in parenthesis taken from Almenoff et al. (1981)
kcat and Km values for all substrates (except II) were determined as described under methods, after 30 min incubations at 37°C with 0.33 (I), 1.67 (III, IV) or 16.7 (V) ng/0.1 ml enzyme.
The kcat value for substrate II was determined in 1 h incubation with 2.5 mM substrate and 25 ng/0.1 ml enzyme, Tyr-D-Ala-Gly release being quantified by spectrophotometry at 254 nm after HPLC separation (Bondapak C18 ; mobile phase : acetate buffer 1 mM, pH 4, 2,5 ml/min ; retention time : 13 min). The Km value for substrate II in fact represents the Ki value towards the hydrolysis of 20 nM ^{3}H-(D-Ala^2,Leu^5)enkephalin (2.5 ng/0.1 ml enzyme). Each value is the mean of three determinations.

borate buffer (13). Using this reagent a critical arginine residue has been previously evidenced in the active site of "enkephalinase" from striatal membranes, whose function seems to bind the free terminal carboxyl group of peptide substrates (14,15, Malfroy et al., manuscript in preparation). While the initial velocity of the enzyme pretreated with butanedione towards enkephalins with a free terminal carboxylate group decreases by about 80 %, the change is much lower towards an amidified enkephalin or a fluorogenic endopeptidase substrate in which this group is also amidified (Table III). It was checked that the butanedione effect was not due to modification of substrates, since the effect was still found on an enzyme preparation from which the excess of reagent was eliminated by Sephadex G 25 column chromatography.

DISCUSSION

Because most previous studies on "enkephalinase" have been performed on the cerebral enzyme (for a review see 1), it was important to assess whether the apparently high enzyme activity recently detected in rat kidney but not characterised therein (7) corresponds to the former.

This seems to be the case inasmuch as the renal enzyme, either in its membrane-bound state (Table I), or in its highly purified form (Fig. 1), displays properties closely similar to those of "enkephalinase" from mouse stria-

Table III : Hydrolysis of various substrates by purified rat kidney "enkephalinase" modified by butanedione

SUBSTRATE	ACTIVITY (nmole/mg/min)		
	CONTROL	BUTANEDIONE TREATED	PERCENT DECREASE
Tyr-Gly-Gly-Phe-Met (3)	4.9 ± 1.1	0.83 ± .20	-83 ± 6%
Tyr-Gly-Gly-Phe-Leu (3)	2.2 ± .4	0.46 ± .11	-79 ± 4%
Tyr-DAla-Gly-Phe-Leu (5)	1.6 ± .1	0.27 ± .04	-83 ± 3%
Tyr-DAla-Gly-Phe-Met-NH_2 (5)	.64 ± .07	0.30 ± .06	-53 ± 6%:::
Hip-Arg-Arg-Leu- βNA (4)	1720 ± 144	1324 ± 210	-23 ± 16%:

: $p < .005$, ::: $p < .002$ as compared to the decrease for Tyr-DAla-Gly-Phe-Leu

Butanedione treatment (10 mM, 1 h at 25°C) was performed on 0.5 µg of enzyme in 0.025 M borate buffer at pH 8, and stopped by addition of 20 volumes of buffer. Initial velocity of the hydrolysis of the four enkephalins was measured by incubating 20 nM of each ^{3}H-compound with 0.0025 µg enzyme for 30 min at 25°C, ^{3}H-Tyr-Gly-Gly or ^{3}H-Tyr-D-Ala-Gly being isolated by polystyrene bead chromatography. Hydrolysis of Hip-Arg-Arg-Leu-β-NA was measured at 10^{-4} M substrate concentration with 0.025 µg enzyme, for 45 min at 25°C. The number of experiments is in parenthesis.

tum. Thus both the Km values of (D-Ala^2,Leu^5)enkephalin or (D-Ala^2,Met^5)enkephalinamide and the Ki values of three potent inhibitors i.e. thiorphan, thiorphan-NH_2 (6) and phosphoramidon (12) do not significantly differ regarding the renal and the striatal enzymes, in spite of a variation by more than 10,000 fold in the apparent affinity constants of the various compounds tested. In addition the inhibitory action of the chelator EDTA (observed for the same concentrations of this compound) indicates that the enzyme in the two tissues (Table I) is a metallopeptidase, most probably a Zn-containing enzyme since the activity can be optimally restored upon addition of Zn salts (16,14,15). Finally the action of butanedione in borate buffer demonstrates the presence of an arginine residue, critical for the activity of the renal (Table III) as well as the striatal enzyme (14,15, Malfroy et al., manuscript in preparation) Taken together these observations strongly suggest that the renal enzyme is identical or, at least, closely similar to cerebral "enkephalinase".

In the course of isolation of the enkephalin-hydrolysing activity from rat kidney microsomes it was noticed that this "enkephalinase" activity was co-purified, until the final steps, with an enzyme activity hydrolysing the ^{125}I-insulin B chain (Malfroy et al., manuscript in preparation). The latter activity has already been characterised (10) and purified (11) from rabbit kidney microsomes under the name of "neutral endopeptidase". This observation raises the possibility that "enkephalinase" is identical to this "neutral endopeptidase". The substrate specificity of the latter resembles that of thermolysin, a metallo-endopeptidase of bacterial origin (18) in the sense that it also hy-

drolyses the peptide bond comprising the amino group of hydrophobic aminoacids. It is interesting to note that P1 subsite in "enkephalinase" is the most adequately satisfied by an aromatic or hydrophobic aminoacid residue (18). Although the substrate specificity of the "neutral endopeptidase" was not described in sufficient details to allow a clearcut comparison of kcat, Km or Ki values of large series of compounds to those found in the case of "enkephalinase", available data suggest the probable identity of the two enzymes. Thus the highly purified (by more than 1,000 fold) "enkephalinase" from rat kidney still hydrolyses ^{125}I-insulin B chain and this activity is potently inhibited by several compounds with IC_{50} values in the same range as those found using ^{3}H- enkephalins as substrates (Fig. 1). Moreover, among these compounds, phosphoramidon, originally described as a thermolysin inhibitor (12), potently inhibits the rabbit kidney "neutral endopeptidase" (19). Other common feature of "enkephalinase" and "neutral endopeptidase" are to be found in the apparently similar molecular weights of the two enzymes (95,000 - 100,000 daltons) and the inhibition by chelating agents, suggesting the presence of a metal (probably Zn) in their active sites.

More recently, a metallo-endopeptidase with an apparent molecular weight of 90,000 daltons has been purified from bovine pituitary using synthetic substrates of the type Hip-Arg-Arg-X-naphtylamide to follow the purification, and its possible identity with both "enkephalinase" and the "neutral endopeptidase" has been hypothetised (9). In order to check this hypothesis we have determined on the purified rat kidney "enkephalinase" the Km and kcat values of various compounds including three fluorogenic substrates used by these authors and kindly provided by Dr S. Wilk.

These values, as well as the specificity constants i.e. the ratios of kcat/Km, are reported in Table II in which they are compared to be corresponding values reported for the bovine pituitary endopeptidase. The Km values of the various substrates for the two preparations seem in rather good agreement, the larger difference being of 5-fold, for (Met^{5})enkephalin, and the fluorogenic substrate VI was not detectably hydrolysed by either preparation. In contrast kcat values were notably different being 20-60 fold higher in the rat kidney preparation than in the bovine pituitary preparation. However, it must be stressed that this does not rule out that the two enzymes are identical since the rank order of kcat values of the various substrates was the same. In fact this discrepancy might only reflect a higher degree of purity reached with the rat kidney "enkephalinase" preparation.

Hence, from all available data, it seems safe to conclude that the "enkephalinase" from rat kidney (and rodent brain) is identical with or, at least, closely similar to the bovine pituitary endopeptidase as well as the "neutral endopeptidase" from rabbit kidney.

In this case should the enzyme be considered as an endopeptidase or a dipeptidyl-carboxypeptidase ? Previous studies on substrate specificity of "enkephalinase" from brain were conducted on enkephalins or short peptides with an aromatic aminoacid residue (P1) in vicinity of an aminoacid (P2) with a free carboxylate, suggested that the enzyme is a dipeptidyl-carboxypeptidase. The fact that the enzyme is also able to cleave peptides in which the carboxylate of the P2 residue is amidified (as in compounds III, IV or V of Table II) or even engaged in peptide bonds as seems, for instance, the case for insulin B chain (19) or other biological peptides (9) leaves no doubt about "enkephalinase" being able to function as an endopeptidase. However it is very important to notice that, among the compounds tested, the enkephalins with a free terminal carboxylate were the best substrates, displaying specificity constants approximately 10-100 times higher than peptides with an amidified C-terminal residue. This feature is best examplified by comparing (D-Ala^2,Met^5)enkephalin to (D-Ala^2,Met^5)enkephalinamide, for which amidification results in a 90 % decrease in specificity constant (Table II). The importance of the free terminal carboxylate for recognition by "enkephalinase" has also been previously stressed when observing the decrease in inhibitory potency of peptides in which this group is amidified, esterified or replaced by an hydroxyl group (20,21,1,22). This suggests that the free terminal carboxylate enhances recognition of substrates (or inhibitors) by binding to some component of the active site of "enkephalinase".

In the active site of pancreatic carboxypeptidase A the free carboxylate of substrates initially forms a salt-link with a positively charged Arg residue (23). A critical Arg residue has also been demonstrated in angiotensin-converting enzyme, a dipeptidylcarboxypeptidase (24). The presence of a critical Arg residue in "enkephalinase" purified from rat kidney is also shown by the strong decrease in activity after treatment with butanedione, a reagent binding irreversibly with guanidinium residues when the reaction takes place in the presence of borate ions (13) (Table III). In the same way an Arg residue has been evidenced in striatal "enkephalinase" in its membrane-bound state, which seems located in the active site since the butanedione-induced inactivation is prevented in the presence of substrates or inhibitors (14,15, Malfroy et al., manuscript in preparation). Most interestingly, while the activity of the butanedione-treated enzyme is decreased by about 80 % towards substrates with a free terminal carboxylate, it is significantly less reduced towards an amidified enkephalin and only marginally affected towards an endopeptidase substrate (Table III). In the same way the butanedione pretreatment of striatal "enkephalinase" affects much more the potency of peptide inhibitors with a free terminal carboxylate than that of amidified compounds (14). Hence the role of the critical arginine residue in "enkephalinase" is most probably to

bind the negatively charged carboxylate of substrates of the enkephalin type as is well established in the case of carboxypeptidase A (23) and likely to be also the case in angiotensin-converting-enzyme (24). This hypothetical carboxylate-guanidinium salt linking would account for the improved catalysis when "enkephalinase" is functioning as a dipeptidyl-carboxypeptidase as compared to its functioning as an endopeptidase. In pancreatic carboxypeptidase A this linking seems imperative to initiate the catalytic mechanism, so that amidified substrates are not hydrolysed at all (23) but in the case of lysosomal carboxypeptidase A amidification of substrates only reduces their specificity constant by 4-fold (25). In comparison the ten-fold reduction in the case of "enkephalinase" illustrates the importance of the free carboxylate group (adjacent to a suitable P1 hydrophobic residue) for hydrolysis of substrates by this enzyme.

REFERENCES

1 . Schwartz, J C., Malfroy, B., and De la Baume, S. (1981) Life Sci. 29, 1715-1740
2 . Altstein, M., and Vogel, Z. (1980) in Neurotransmitters and their receptors U.Z. Littaver, Y. Dudai, I. Silman, V.I. Teichberg and Z. Vogel, Eds, J.W. Ley and Sons, Chichester, 497-507
3 . De la Baume, S., Patey, G., and Schwartz, J C. (1981) Neurosci. 6, 315-321
4 . Malfroy, B., Swerts, J.P., Guyon, A., Roques, B.P., and Schwartz, J.C. (1978) Nature, 276, 523-526
5 . Patey, G., De la Baume, S., Schwartz, J C., Gros, C., Roques, B.P., Fournié-Zaluski, M.C., and Soroca-Lucas, E. (1981) Science, 212, 1153-1155
6 . Roques, B.P., Fournié-Zaluski, M.C., Soroca, E., Lecomte, J.M., Malfroy, B. Llorens, C., and Schwartz, J C. (1980) Nature, 288, 286-288
7 . Llorens, C., and Schwartz, J C. (1981) Eur. J. Pharmacol., 69, 113-116
8 . Orlowski, M., and Wilk, S. (1981) Biochemistry 20, 4942-4950
9 . Almenoff, J., Wilk, S., and Orlowski, M. (1981) Biochem. Biophys. Res. Commun. 102, 206-214
10. George, S.G., and Kenny, A.J. (1973) Biochem. J. 134, 43-57
11. Kerr, M.A., and Kenny, A.J. (1974) Biochem. J. 137, 477-488
12. Suda, H., Aoyagi, T., Tacheuchi, T., and Umezawa, H. (1973) J. Antibiot. 26, 621-623
13. Glazer, A.N., Delange, R.J., and Sigman, D.S. (1975) Chemical modification of proteins, North-Holland/American Elsevier, 1-205
14. Malfroy, B., Llorens, C., Schwartz, J C., Soroca, E., Roques, B.P., Morgat J.L., Javoy-Agid, F., and Agid, Y. (1981) Abstract for the INRC Meeting, Kyoto, Japan
15. Schwartz, J C., De la Baume, S., Llorens, C., Malfroy, B., Soroca, E., Fournié-Zaluski, M.C., Roques, B.P., Morgat, J.L., Roy, J., Javoy-Agid, F. and Agid, Y. (1982) Proc. of the VIIIth Int. Congr. Pharmacol. (in press)
16. Swerts, J.P., Perdrisot, R., Malfroy, B., and Schwartz, J C. (1979) Eur. J. Pharmacol., 53, 209-210
17. Matsubara, H., Singer, A., Sasaki, R., and Jukest, H. (1965) Biochem. Biophys. Res. Commun. 21, 242
18. Llorens, C., Gacel, G., Swerts, J.P., Perdrisot, R., Fournié-Zaluski, M.C. Schwartz, J C., and Roques, B.P. (1980) Biochem. Biophys. Res. Commun. 96 1710-1717

19. Kenny, A.J. (1977) in Proteinases in mammalian cells and tissues., A.J. Barrett Ed., North Holland, 393-444
20. Fournié-Zaluski, M.C., Perdrisot, R., Gacel, G., Swerts, J.P., Roques, B.P. and Schwartz, J C. (1979) Biochem. Biophys. Res. Commun. 91, 130-135
21. Schwartz, J C., De la Baume, S., Malfroy, B., Patey, G., Perdrisot, R., Swerts, J.P., Fournié-Zaluski, M.C., Gacel, G., and Roques, B.P. (1980) Adv. Biochem. Psychopharmac. 22, 219-235
22. Huguenin, N.R., and Maurer, R. (1980) Brain Res. Bull. 5, 47-50
23. Quiocho, F.A., and Lipscomb, W.N. (1971) Adv. Prot. Chem. 15, 1-48
24. Bunning, P., Holmquist, B., and Riordan, J.F. (1978) Biochem. Biophys. Res. Commun. 83, 1442-1449
25. McDonald, J.K., Schwabe, C. (1977) in Proteinases in mammalian cells and tissues. A.J. Barrett Ed., North Holland, 311-391
26. Vogel, Z., and Altstein, N.M. (1977) FEBS Lett. 80, 332-335
27. Llorens C., Malfroy B., Schwartz J.C., Gacel, G., Roques, B.P., Roy, J., Morgat, Javoy-Agid, F., and Agid, Y. (1982), J. Neurochem. (In press).