Neuropeptides 7: 369-380, 1986

REASSESSMENT OF OPIOID BINDING SITES IN THE RAT BRAIN

E.Castanas,D.Blanc*,N.Bourhim,A.Cupo*,P.Cantau,P.Giraud
Laboratoire de Neuroendocrinologie Expérimentale,ERA 982 CNRS,UER Nord,Bd.P.Dramard,F 13326,Marseille,Cedex 15, (*) Centre d'Immunologie INSERM-CNRS,Luminy, Marseille,FRANCE. (reprint request to EC).

Abstract

Opioid binding sites have been characterized pharmacologically in membranes from different areas of the rat brain. Delta,mu and sites belonging to the kappa family (K1,K2,K3) have been detected.Delta sites were more abundant in cortex and striatum,mu sites in striatum and hypothalamus,while kappa binding site concentration was higher in deeper enkephalic structures (brainstem,cerebellum,hypothalamus) and the pituitary gland. A distinct distribution of each subtype of the kappa site was found: kappa 1 sites were higher in the spinal cord,kappa 2 sites in the brainstem and kappa 3 sites in cerebellum. The distribution of delta and kappa sites in the central nervous system was correlated with the distribution of proenkephalin-A derived peptides and precursors, suggesting that these peptides could be their endogenous ligands.

Introduction

Previous studies from our laboratory and by others (see 1 for a discussion) have shown a heterogenous distribution of the three families of opioid peptides (proenkephalin A,proenkephalin B and proopiomelanocorticotropin) in the rat brain.On the other hand,peptides derived from these three families present different affinities for opioid binding sites. Three major classes of opioid sites have been found: the delta (or enkephalin) sites; the mu site on which B-endorphin binds with high affinity; the kappa site on which C-terminal extended Met- and Leu-enkephalin peptides bind with high affinity (2-4). The kappa site has been found to be heterogenous:two major subtypes have been found in the guinea-pig spinal cord (5). We have recently characterized pharmacologically a new subtype of the kappa binding site in the bovine adrenal medulla which was named kappa 3 site. It presents a high affinity for Met-enkephalin-Arg^{6}-Phe^{7} (6-7).It seemed therefore interesting to investigate the distribution of this new site in different structures of the central nervous system in relation to the other opioid binding sites.

Material and Methods

Animals : Adult female rats of a local strain (Lou rats) were decapitated;different brain areas were dissected,frozen on dry ice and immediately treated for membrane preparation (7) or extracted for proenkephalin A peptide content.

Binding studies : Binding was performed in triplicate as described previously (8-9).Opioid site determination was performed by the use of specific ligands and

effectors as described elsewhere (7-9).

Determination of proenkephalin-A derived peptides (10-12).

Current data support the hypothesis that M-Enk, and M-Enk-Arg^6-Gly^7-Leu^8 are derived from the same precursor molecule, named proenkephalin-A. (See 13 for a review). This precursor molecule contains four copies of M-Enk, and one copy of L-Enk, M-Enk-Arg^6-Phe^7, M-Enk-Arg^6-Gly^7-Leu^8. Post-transcriptional maturation of the precursor gives rise to intermediary peptides containing the sequence of M-Enk which is followed by one or two basic aminoacids. In the present investigation the concentrations of M-Enk and M-Enk-Arg^6-Gly^7-Leu^8 were assayed directly in the acidic extracts of tissues, while the proenkephalin A content was estimated by TCA precipitation of the tissue extracts and tryptic treatment of the pellet.

1. Extraction : Brain areas were homogeneized by sonication in 0.5 ml 0.1 N HCl for 20 sec, boiled during 15 min, centrifuged and the supernatant was stored at -20°C until assay.

2. Enzymatic treatment : M-Enk containing fragments of proenkephalin A were assayed by the M-Enk-Arg^6 immunoreactivity generated after trypsin treatment as described elsewhere (10) . Briefly, one volume of the tissue acid extract was mixed with an equal volume of 10% (w/v) trichloroacetic acid in water. Samples were let to stand for 15 min on ice, centrifuged and the pellet after two washes with diethylether, was resuspended in the activation buffer (0.05 M Tris-HCl, 0.01 M NaCl, 0.05 M $CaCl_2$, pH 8.0). 0.2 mg of TPCK treated trypsin was then added and the mixture was incubated at 37°C for three hours. Reaction was stopped by heat-inactivation of the enzyme (95°C, 15 min) and the M-Enk-Arg^6 formed was assayed by radioimmunoassay.

3. Radioimmunoassays : Table I summarizes the characteristics of antisera used in the present study. Radioiodinations were performed using the chloramine-T method. For the M-Enk-Arg^6 determination samples and standards were oxidized by chloramine-T (2 mg chloramine-T per 100 µl of sample aliquot) prior to the assay.

Results

Table II presents the distribution of opioid binding sites in different areas of the rat brain. A distinct distribution pattern for each opioid site was found : delta sites were predominant in cortex and striatum; the highest number of mu sites was found in hypothalamus; the kappa sites were present in all areas examined with K_1 and K_3 sites predominant in the pituitary gland and K_2 sites predominant in the hypothalamus. The highest concentration of opioid sites (expressed as fmoles/mg of membrane protein) was found in the hypothalamus and the pituitary (313.0 and 333.7 fmoles/mg protein respectively), while the lowest concentration was found in the cerebellum (55.0 fmoles/mg protein), brainstem (75.1 fmoles/mg protein) and spinal cord (93.8 fmoles/mg protein). Taking into consideration the weight of tissues, the highest concentration of total opioid sites was found in hypothalamus (9.49 pmoles/g tissue) followed by the pituitary (6.62 pmoles/g), cortex (5.82 pmoles/g), striatum (5.37 pmoles/g), spinal cord (5.26 pmoles/g), braistem (3.42 pmoles/g) and cerebellum (1.45 pmoles/g).

Table I : Characteristics of the different RIA methods used in the present study.

Peptide (assay buffer)	Antiserum No Dilution	Radioactive ligand IC_{50}	Cross Reactivities
M-Enk (0.1M phosphate 0.15M NaCl, BSA 1g/l, NaN_3, 0.1g/l pH 7.2)	# 2158 From our laboratory 1/75000	^{125}I-M-Enk IC_{50}:4.5×10^{-11}M	M-Enk sulfoxide 1.6% L-Enk 0.5% M-Enk-Arg^{6}Phe^7 0.2% M-Enk-Arg^{6}Gly^7 Leu^8 0.1%
M-Enk-Arg^6 sulfoxide (Tris-HCl 0.05M, NaCl 0.15M, BSA 1g/l NaN_3 0.1g/l, pH 8.25)	# 1205 From our laboratory	^{125}I-M-Enk-Arg sulfoxide IC_{50}:3×10^{-9}M	L-Enk-Arg^6 0.002% M-Enk-Thr^6 0.01% M-Enk-Arg^6 1% M-Enk-Arg^6-Gly^7-Leu^8 sulfoxide 0.15% M-Enk sulfoxide <0.001% B-Endorphin 61-69 <0.001%
M-Enk-Arg^6-Gly^7-Leu^8 (0.01M phosphate, 0.15M NaCl, BSA 1g/l, NaN_3 0.1g/l, pH 7.2)	# 2227 From our laboratory 1/25000	^{125}I-M-Enk-Arg^6 Gly^7-Leu^8 IC_{50}:4×10^{-10}M	M-Enk <0.001% M-Enk-Arg^6-Phe^7 <0.001%

Table II : Opioid binding sites in different structures of the rat brain. Structures from a couple of animals (three animals for pituitary and hypothalamus) were pooled for each binding type determination. Mean ± SEM of three or four determination. The number of individual experiments is prescuted in parantheses. N.D. = Not Detectable.

Structure / Protein yield (% of wet tissue)	fmoles/mg protein				
	Delta	Mu	Kappa		
			K_1	K_2	K_3
Cortex (n=4) 3.05	60.9±10.0	12.3± 8.4	34.7±22.8	53.4±27.1	29.3±16.9
Striatum (n=4) 3.61	62.5±19.9	24.9±11.1	7.8± 5.7	53.2±20.4	N.D.
Brainstem (n=4) 4.55	N.D.	6.7± 4.9	9.9± 2.1	46.1±24.3	12.4± 7.9
Cerebellum (n=4) 2.63	6.0± 2.2	N.D.	15.8± 4.7	N.D.	33.2±10.4
Hypothalamus (n=3) 3.03	57.2± 9.0	58.0±33.0	61.4±46.3	79.4±31.1	57.0±33.1
Pituitary (n=3) 1.99	N.D.	N.D.	233.7±89.4	N.D.	100.0±20.2
Spinal cord (n=4) 5.62	10.2± 5.3	17.2± 9.1	47.4±18.3	11.5± 8.3	7.5± 4.3

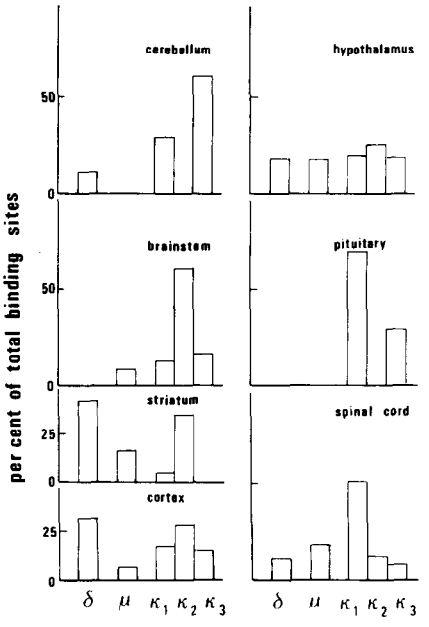

Fig. 1

Relative distribution of delta, mu and kappa opioid binding sites in different areas of the rat brain.

Fig. 1 presents the relative distribution of opioid binding sites in the different areas studied. A predominance of delta sites was found in striatum representing 42.1 % of total binding sites and in cortex (32% of total sites) while no delta sites were detectable in the brainstem and the pituitary. Mu sites were absent from pituitary and cerebellum. They represented 18.5%, 18.3%, 16.8%, 8.9%, and 6.5% in the hypothalamus, spinal cord, stiatum, brainstem and cortex respectively. Kappa binding sites were present in all structures studied. They were the predominant sites in the pituitary (100%) and cerebellum (90%) while the lowest relative concentrations was found in the striatum.

Kappa sites have been found to be heterogenous (see 2, 7-9 for discussion). The characterization of the three subtypes of the kappa site revealed specific tissue patterns : the K_2 subtype was absent from the pituitary and the cerebellum, while it represented 61% of the total binding sites in the brainstem. The K_1 subtype present in all tissues studied represented 70% of the total binding sites in the pituitary and only 5.3% in the striatum. The K_3 subtype was most abundant in the cerebellum (60% of total binding) and the pituitary (30% of total sites) while it was absent from striatum.
Proenkephalin A derived peptides have been found by others (16, 32) and by us (13) to be present in all areas of the rat brain while proenkephalin B and proopiomelanocrotin presents a much more limited distribution (1). Furtermore, these peptides can bind to all types of the opioid receptor. Notably, M-Enk binds to the delta and K_3 site (K_I 6.3 and 234 nM respectively), M-Enk-Arg^6 to the mu, K_1 and K_2 sites (K_I 29, 2.3 and 120 nM), M-Enk-Arg^6-Gly^7-Leu^8 to the K_2 and K_3 sites (K_I 39 and 19 nM respectively) and M-Enk-Arg^6-Phe^7

Table III : Radioimmunoassay of proenkephalin-A derived peptides in different structures of the rat brain.

The number of separate experiments are given in parenthesis. Mean ± SEM. (1) Assay performed after tryptic enzymatic treatment and oxydation (8).

	pmoles / g wet tissue		
Structures	M-Enk	M-Enk-Arg^6-Gly^7-Leu^8	M-Enk-Arg^6 (1)
Cortex (n=3)	99 ± 36	44 ± 9	not assayed
Striatum (n=3)	435 ± 173	123 ± 20	873 ± 164
Brainstem (n=3)	171 ± 63	77 ± 16	404 ± 35
Cerebellum (n=3)	70 ± 5	2 ± 0.03	not assayed
Hypothalamus (n=3)	358 ± 100	151 ± 15	409 ± 92
Pituitary (n=2)	242 ± 77	128 ± 6	424 ± 91
Spinal cord (n=2)	124 ± 25	127 ± 31	427 ± 6

all five binding types (K_I 145, 464, 47, 4.6 and 1.2 nM for the delta, mu, K_1, K_2 and K_3 site respectively) (8,9). It was therefore of interest to investigate the distribution of these peptides in different areas of the rat brain, in relation with the distribution of the opioid receptors. This distribution is presented in Table III. The high lipid content in cerebral cortex and cerebellum did not allow the determination of M-Enk-Arg^6 content after enzymatic treatment. The highest M-Enk content was found in the striatum followed by the hypothalamus and pituitary. M-Enk-Arg^6-Gly^7-Leu^8/ M-Enk ratio was equal to about four in most structures. After tryptic treatment, the M-Enk-Arg^6/M-Enk was about 6/4 and M-Enk-Arg^6/M-Enk-Arg^6-Gly^7-Leu^8 about 6/1. These results are in agreement with the molar rations of these peptides in the proenkephalin-A molecule. Significant correlations were found between : 1) the M-Enk levels and the delta site number (Spearman's rho 0.91, $p<0.01$); 2) the M-Enk levels and the sum of delta plus K_3 site number (rho 0.89, $p<0.05$); 3) the levels of M-Enk-Arg^6-Gly^7-Leu^8 and the total number of kappa sites (rho 0.88, $p<0.05$); 4) the proenkephalin-A precursor content of tissues, estimated by TCA precipitation and tryptic analysis of the samples, and the delta site number (rho 0.999, $p<0.05$).

Discussion

In the present study we have examined the pharmacological characterization of opioid studies in grossly dissected regions of the rat brain. Although opioid sites of the delta and mu type have been found during the first period of opioid site characterization, the identification of kappa type opioid binding was more difficult. Indeed, in early studies this type of sites were not detected (14-17) perhaps because of their low concentrations in the rat strain used (18,19) and/or the ability of "kappa" ligands (EKC, bremazocine) to bind to delta and mu sites (8,9). Finally Gillian and Kosterlitz (2) have identified kappa sites in brain homogenates of a local rat strain. They have used EKC to identify these sites after suppression of the drug interaction

with delta and mu sites by the use of saturating concentrations of delta and mu ligands. The same technique of specific masking of types of opioid sites has been previously used for the identification of opioid binding in the adrenal medulla (7-9) and in this study.

A problem encountered in this study was the availability of tissues. Although samples from a pair of animals have been mixed the protein concentration was relatively low. Recently Bardo et al (20) reported a modification of the filtration technique permiting to assay opioid binding sites in very diluted samples. It consisted in using GF/C filters pretreated with water saturated by isoamylalcohol. This technique diminishes dramatically non specific binding to the filters and has permited us to detect opioid sites in samples containing as little as 30 µg protein/ml (ex. hypothalamus, pituitary).

A specific distribution of each type of opioid binding sites was found in the rat brain : delta sites predominate in the cortex and in striatum. They became less abundant in other structures being even undetectable in the brainstem while they reappear at the spinal cord. Kappa sites showed an inverse distribution pattern : lower concentrations were found in neoenkephalic structures (cortex) and striatum while they represent more than 60% of the sites detected in the deeper areas of the brain.

Kappa sites were found to be heterogenous (4,5,9). Three subtypes (K_1, K_2 and K_3) can be detected. In the rat brain, the distribution of the K_3 site was different from K_1 and K_2 sites. The highest concentration has been found in the pituitary and the cerebellum. No K_3 site was found in the striatum. Conversely, K_2 site concentrations were highest in the striatum and brainstem, and undetectable in the pituitary and cerebellum. K_1 sites were present in all areas studied. The highest concentrations were found in the pituitary and spinal cord and the lowest ones in the striatum, the brainstem, and the cerebellum.

Table IV summarizes some of recent reports of other investigators on opioid binding sites in the rat brain. These results are difficult to compare mainly because different techniques have been used for their determination as well as different rat strains. Nevertheless, in studies using the whole brain (2, 21,22) a predominance of µ sites was found. However in our study and in another one examining specific brain areas (29) no such predominance was observed. No major differences were found between this study and the results of Bardo et al (20) in the hypothalamus (314 towards 460 fmoles/mg protein) and striatum (147 towards 127 fmoles/mg protein) and the results reported by Tsang et al (22) in the cerebellum (55 towards 30 fmoles/mg protein) and the brainstem (64 towards 40 fmoles/mg protein) while on the contrary three times more sites have been identified in the cortex (191 towards 60 fmoles/mg protein). In order to compare our results with these studies, and in accord with previous investigations from our laboratory (8,9) the assumption that [^{3}H]Naloxone used by these investigators identifies all binding sites was made. On the contrary about half as much sites were found in the present study than in the one of Pfeiffer and Herz (29). The ratio of delta to kappa sites was about the same in the two studies while much less mu sites have been identified in the present investigation. Furthermore, we do not find the predominance of delta and mu sites in hypothalamus reported by this group (27). Strain (and methodological) differences could explain these discrepancies : indeed, in unpublished observations from our laboratory marked differences were found in the

Table IV

Rat Type	Technique	Brain area	Binding sites detected	Ref
Aberdeen colony	Binding	total brain	δ=6.7, μ=7.3, K=2.0 *	2
Sprague-Dawley	Binding Computer itineration	total brain	δ=2.2, μ=10.7, K=2.6 *	21
Sprague-Dawley	Binding	total brain	δ=140 μ=175 (2) **	22
Sprague-Dawley	[^{3}H]Naltrexone Binding High affinity binding	total brain	δ + μ + K=293 **	23
? (3)	Autoradiography [^{3}H]Etorphine	posterior pituitary	Existence of [H]Etorphine stereo-selective binding on posterior pituitary cells	24
Winstar	Autoradiography [^{3}H]FK 33824	locus coeruleus	Existence of binding sites (1),(2).	25
Sprague-Dawley	In vivo assessmend DA metabolites	striatum	Existence of and sites(1)	26
Sprague-Dawley	Autoradiography [^{3}H] DADLE, [^{3}H] Etorphine.	hypothalamus thalamus.	predominance of sites predominance of sites	27

Notes (1)δ receptors not detected ,(2) receptors not detected ,

(3) rat strain not detected , * pmoles/g tissue ;
** fmoles/mg protein .

Table IV (continued)

Rat type	Technique	Brain area	Binding sites detected	Ref
Sprague-Dawley	[^{3}H]Naloxone Binding	hypothalamus	δ + μ + K=460**	20
		striatum	δ + μ + K=127	
Sprague-Dawley	[^{3}H]Naloxone Binding	forebrain	δ + μ + K= 60**	28
		cerebellum	δ + μ + K= 30**	
		brainstem	δ + μ + K= 40	
"	[^{3}H]Met-Enk Binding	forebrain	20**	29
		cerebellum	0**	
		brainstem	5	
? (3)	Binding+ computer itinination	cortex	δ=5.5 μ= 6.9, K=7.1*	30
		striatum	δ=8.5 μ= 6.9, K=6.1*	
		midbrain	δ=1.7 μ= 7.8, K=4.8	
Winstar	[^{3}H]Sufentanil Binding	striatum	μ=12.9* (1),(2)	
		hypothalamus	μ=11.2* (1),(2)	
		cerebellum	μ= 0.7* (1),(2)	
		cortex	μ= 8.7 (1),(2)	

Notes (1) δ receptors not detected , (2) K receptors not detected, (3) rat strain not detected, * pmoles/g tissue; ** fmoles/mg protein.

opioid binding sites identified in the cortex of Sprague-Dawley and Lou rats (local strain) (δ sites 61.9 towards 60.9, μ sites 55.2 towards 12.3, K_1 sites 20.3 towards, 34.7, K_2 sites 225.2 towards 53.4 and K_3 sites 115.2 towards 29.3; all results are expressed as fmoles/mg protein).

The distribution of proenkephalin-A derived peptides has been also studied. As it was found in a previous study from our laboratory (1) striatum contains the highest concentration of opioid peptides followed by hypothalamus, pituitary, brainstem and cortex. The radioimmunoassay of M-Enk-Arg^6 after enzymatic treatment and the compassion of molar ratios between M-Enk and M-Enk-Arg^6 on one hand and M-Enk-Arg^6-Gly^7-Leu^8 on the other indicated that all these peptides, derived from proenkephalin-A (31,32). Their regional distribution is correlated with the distribution of kappa and delta sites confirming pharmacological evidences that these peptides are the endogenous ligands of kappa and delta receptor (8,9,33,34).

We should like to discuss specifically three structures of the rat brain :

The hypothalamus. It was the only structure which contains almost equal concentrations of all five binding sites. Opioid peptides derived from proenkephalin-A (1 and this study), proenkephalin-B (1) and proopiomelanocortin family (35) have been equally found in high concentration in this structure. They could therefore account for a specific opioid regulation of pituitary secretion at the hypothalamic level.

The pituitary. Recent studies (see 36 for a Discussion) show the existence of proenkephalin A and proenkephalin-B derived peptides in the posterior pituitary, while their concentration in the anterior lobe is low (1). The whole pituitary gland has been assayed in the present study. This could account for the high M-Enk concentrations reported here. The spectrum of opioid binding sites was rather porr : only K_1 and K_3 sites were found. In a recent study (24) posterior pituitary cells were found to pocede stereoselective and saturable ^{3}H Etorphine binding sites. Furthermore, in our laboratory, we have found that ethylketocyclazocine (a K_1 ligand) and etorphine (a K_3 ligand) had different effects on prolactin secretion in primary cultures of hypophyseal cells (37). The fact that dynorphin (a potential K_1 ligand) and M-Enk-Arg^6-Phe^7 (a potential K_3 ligand) are present in hypothalamo-hypophyseal portal blood (38) could account for by opioid effects at the pituitary level.

The cerebellum. In earlier investigations this tissue was not included in binding studies. Opioid binding sites have been described recently in cerebellar membranes (28,30,39). In the rat, like in the guinea-pig (39) most the observed sites belong to the kappa family. The new K_3 site represents 60% of the total detected sites, making cerebellum a tissue of choice for the study of this subtype of the kappa binding site.

In the present study, only a rough determination of binding sites was made. On the contrary a complete pharmacological characterization of opioid binding was undertaken in the anterior pituitary gland. Our results (37) showed a pharmacological profile (saturation and displacement binding, cell culture studies) in accord with the data presented here. Furthermore, these results indicate that the opioid control at the pituitary level might be exerced via kappa receptors. Further studies in other brain structures are needed in order to determine possible physiological implications of opioid sites in physiological phenomena.

Acknowledgements

Supported by INSERM (CRL 83 60 12), CNRS (ERA 07 0982 and ATP 380/83) and Fondation pour la Recherche Médicale Française grants. We thank Hoffmann-LaRoche Co (Basel) for the gift of levorphanol, and Pr B. Roques (Paris) for the gift of DSLET. We are particularly grateful to Pr C. Oliver for his criticism and constructive discussion of the paper. We thank Mrs Garozzo for her secretarial assistance.

References

1. Giraud P.,Castanas E.,Patey G.,Oliver C. and Rossier J.(1983) Regional distribution of Methionine-enkephalin-Arg^{6}-Phe^{7} in the rat brain:Comparative study with the distribution of other opioid peptides. J.Neurochem.41:154-160
2. Gillian M.G.C. and Kosterlitz H.W.(1982) Spectrum of the μ,δ and k-binding sites in homogenates of rat brain. Br.J.Pharmacol. 77:461-469
3. Ho C.L.,Hammonds R.G.and Li C.H. (1983) B-endorphin:Characteristics of binding sites in the rabbit cerebellar membranes.Biochem.Biophys.Res.Commun. 111:1096-1104
4. Audigier Y.,Attali B.,Guarderès C.,Mazarguil H. and Cros J.(1983) Multiplicity of kappa opiate sites in the central nervous system. in Ruckenbusch Y., Tontain J. and Koritz H (eds) Veterinary Pharmacology and Toxicology MTP Press pp 451-463
5. Attali B.,Guarderès C.,Mazarguil H.,Audigier Y. and Cros J. (1982) Evidence for multiple "kappa" binding sites by use of opioid peptides in the guinea pig lumbosacral spinal cord. Neuropeptides 3: 53-64
6. Castanas E.,Giraud P.,Bourhim N.,Cantau P. and Oliver C. (1984) Kappa 3: A novel subtype of the kappa opioid site in the bovine adrenal medulla highly selective for Met-enkephalin-Arg^{6}-Phe^{7}. Neuropeptides 5 : 133-136
7. Castanas E.,Giraud P.,Audigier Y.,Drissi R.,Boudouresque F.,Conte-Devolx B., and Oliver C. (1984) Adrenal medullary opiate receptors: Pharmacological characterization in bovine adrenal medulla and a human pheochromocytoma. Mol. Pharmacol. 25:38-45
8. Castanas E., Bourhim N., Giraud P., Cantau P. and Oliver C. (1985) Interaction of opiates with opioid binding sites in the bovine adrenal medulla. I. Interaction with delta and mu sites. J. Neurochem. 45: 677-687.
9. Castanas E., Bourhim N., Giraud P., Cantau P. and Oliver C. (1985) Interaction of opiates with opioid binding sites in the bovine adrenal medulla. II. Interaction with kappa sites. J. Neurochem.45: 688-699.
10. Cupo A., Pontarotti P.A., Jarry T. and Delaage M. (1984) A new immunonological approach to the detection and the quantification of the Met^{5}-enkephalin precursors in rat brain. Neuropeptides 4 : 375-387
11. Cupo A. and Jarry T. (1985) Detection of Methionine-enkephalin at the 10^{-16} mole level. J. Neuroimmunol. (in press)
12. Cupo A., Eybalin M., Patey G., Rossier J. and Jarry T. (1984) Quantification and localization of Met-enkephalin-Arg-Gly-Leu in rat brain using higly sensitive antibodies. Neuropeptides 4 : 389-401

13. Hölt V. (1983) Multiple endogenous opioid peptides. Trends Neurosci. 6: 24-26

14. Hiller J.M. and Simon E.J. (1979) ^{3}H-ethylketocyclazocine binding : lock of evidence for a separate k receptor in rat CNS. Eur. J. Pharmacol. 60 : 389-390

15. Hiller J.M. and Simon E.J. (1980) Specific, high affinity ^{3}H-ethylkectocyclazocine binding in the rat central nervous systems : lock of evidence for k receptor. J. Pharmacol. Exp. Ther. 214 : 516-519

16. Snyder S.H. and Goodman R.R. (1980) Multiple neurotransmiter receptors. J. Neurochem. 35 : 5-15

17. Harris D.W. and Sethy V.H. (1980) High affinity binding of ^{3}H ethylketocyclazocine to rat brain homogenates. Eur. J. Pharmacol. 66 : 121-123

18. Wood P.L., Charleson S.E., Lone D. and Hudgin R.L. (1981) Multiple opiate receptors : differential binding of μ, k and δ agonists. Neuropharmacology 20 : 1215-1220

19. Chang K.J., Hazoum E. and Cuatrecasas P. (1981) Novel opiate binding site selective for benzomorphan drugs. Proc. Natl. Acad. Sci. USA 78 : 4141-4145

20. Bardo M.T., Bhatnagar R.K. and Gebhart G.F. (1982) An improved filtration procedure for measuring opiate receptors in small regions of rat brain. J. Neurochem. 39 : 1751-1754

21. Pfeiffer A., Sadée W. and Herz A. (1982) Differential regulation of the μ-, δ - and k- opiate receptor subtypes by guanyl nucleotides and metal ions. J. Neurosci. 2 : 912-917

22. Chang K.J., Blanchard S.G. and Cuatrecasas P. (1983) Unmasking of magnesium-dependant high affinity binding sites for DAla^{2}, DLeu^{5} enkephalin after pretreatment of brain membranes with guanyl nucleotides. Proc. Natl. Acad. Sci. (USA) 80 : 940-944

23. Fischel S.V. and Medzihradsky F. (1981) Scatchard analysis of opiate receptor binding. Mol. Pharmacol. 20 : 269-279

24. Lightman S.L., Ninkovic M., Hunt S.P. and Iversen L.L. (1983) Evidence for opiate receptors on pituicytes. Nature (London) 305 : 235-237

25. Beaudet A., Tremeau O., Menez A. and Droz B. (1979) Visualisation des récepteurs aux opiacés dans le locus coeruleus du rat : étude radioautographique à haute résolution après administration d'un analogue tritié de la Met-Enkephaline. C.E. Acad. Sci. (Paris) 289 : 591-593

26. Wood P.L., Sanschagrin D., Richard J.W. and Thakur M. (1983) Multiple opiate receptor affinities of kappa and agonist/antagonist analgesics : in vivo assessment. J. Pharmacol. Exp. Ther. 226 : 545-550

27. Duka T., Schubert P., Wüster M., Stoiber R. and Herz A. (1981) A selective distribution of different opiate receptors in certain areas of rat brain as revealed by in vitro autoradiography. Neurosci. Letters 21 : 119-124

28. Tsang D., Ng S.C., Ho K.P. and Ho W.K.K. (1982) Ontogenesis of opiate binding sites and radioimmunoassayable B-Endorphin and enkephalin in regions of rat brain. Devel. Br. Res. 5 : 257-261

29. Pfeiffer A. and Herz A. (1981) Demonstration and distribution of an opiate binding site in rat brain with high affinity for ethylketocyclazocine and SKF 10047. Biochem. Biophys. Res. Commun 101 : 38-44

30. Leysen J.E., Gommeren W. and Niemegeers C.J.E (1983) ^{3}H Sufentanil,a superior ligand for μ-opiate receptors : binding properties and regional distribution in rat brain and spinal cord. Europ. J. Pharmacol. 87 : 209-225

31. Hölt V. (1983) Multiple endogenous opioid peptides. Trends Neurosci. 6 : 24-26

32. Kojima K., Kilpatrick D., Stern A., Jones B. and Udenfriend S. (1982) Proenkephalin : a general pathway for enkephalin biosynthesis in animal tissues. Arch. Biochem. Biophys. 215 : 638-643

33. Attali B., Guarderès C., Mazarguil H., Audigier Y. and Cros J. (1982) Evidence for multiple "kappa" binding sites by use of opioid peptides, in the guinea-pig lumbosacral spinal cord. Neuropeptides 3 : 53-64

34. Audigier Y., Attali B., Guarderes C., Mazarguil H. and Cros J. (1983) Multiplicity of kappa opiate sites in the central nervous system. in Ruchebusch Y., Tontain J. and Koritz H. (eds) Veterinary Pharmacology and Toxicology MTP Press pp 451-463

35. Bloom F., Bayon A., Battenberg E., French E., Koda E., Koob G., Le Moal M., Rossier J. and Schoemaker W. (1980) Endorphins : Developmental, cellular and behavioral aspects. In Costa E. and Trabucchi M. Advances in Biochemical Psychopharmacology vol 22 Raven Press, N.Y. pp 619-632

36. Vanderhaeghen J.J., Lotstra F., Liston D.R. and Rossier J. (1983) Proenkephalin, Met-enkephalin and oxytocin immunoreactivities are colocalized in bovine hypothalamic magnocellular neurous. Proc. Natl. Acad. Sci. (USA) 60 : 5139-5143

37. Castanas E., Jaquet P., Gunz G., Cantau P. and Giraud P. (1984) Are opioid binding sites implicated in prolactin release by lactotrophs ? Symposium PRL Paris p 66

38. Castanas E., Giraud P., Drissi R., Chabrier P.R., Conte-Devolx B., Boudouresque F., Cantau P., Cesselin F., Cupo A., Eiden L.E. and Oliver C. (1984) Characterization of enkephalins and related peptides in rat hypophyseal portal blood. Brain Research 310 : 1-6

39. Maurer R., Foote R.W., Robson L.E. and Kosterlitz H.W. (1983) K-opioid binding sites in the guinea-pig cerebellum. In International Narcotic Research Conference, Garmisch-Partenkirchen L26

Received 24/2/86 Accepted 7/4/86