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KAPPA 3 : A NOVEL SUBTYPE OF THE KAPPA OPIOID SITE IN BOVINE ADRENAL MEDULLA, HIGHLY SELECTIVE FOR MET-ENKEPHALIN-ARG^6-PHE^7

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^{3}H etorphine is supposed to identify with high affinity delta,mu,and kappa 2 opioid binding sites.In the bovine adrenal medulla and in the presence of high (5 µM)concentrations of DADLE,a residual ^{3}H etorphine site was found. An attempt for its characterization was made in the present investigation. This residual site (16% of total ^{3}H etorphine identified sites) was stereoselective,saturable and sensitive to proteolytic enzymes and N-ethylmaleimide.Displacement studies revealed a high affinity interaction with bremazocine,pentazocine,etorphine and diprenorphine and a moderate interaction with ethylketocyclazocine and levorphanol. We propose that this residual etorphine-identified site might represent a novel subtype of the kappa opioid site named kappa 3. Met-enkephalin-Arg^{6}Phe^7 might be its endogenous ligand.

Introdution

In previous works from our laboratory we have investigated opioid binding site spectrum in the bovine adrenal medulla (1, 2). Delta, mu and kappa binding sites have been detected. Further investigation at kappa sites have led to the characterization of kappa subtypes, merely kappa 1 (EKC labelled, DADLE insensitive) and kappa 2 or benzomorphan (EKC and Etorphine labelled,DADLE sensitive), according to the classification of Attali et al (3). A component of ^{3}H Etorphine high affinity binding is equally detected in this tissue, which is not sensitive to DADLE masking. In this study we have tried to analyse this high affinity interaction in terms of pharmacological labelling and competition studies.

Material and Methods

Bovine adrenals were obtained from a local slaughterhouse . Membrane preparation and opioid binding were carried as described previously (1). Enzymatic treatments were made by the use of appropriate concentration of enzymes at 37°C for 30 min. Reactions were stopped by washing membranes (-chymotrypsin and pepsin), or by adding soybeen trypsin inhibitor (trypsin) or reduced glutathion (N-ethylmaleimide).

Results

Table I presents the interaction of ^{3}H Etorphine with bovine adrenal medullary opioid sites. As previously reported, etorphine identifies with high affinity

Effectors	Saturation binding		Hill coefficient
	Bmax (fmoles/mg prot)	K_D (nM)	
0	125 ± 3	0.16 ± 0.01	0.6 ± 0.03
DADLE (5µM)	20 ± 2	0.19 ± 0.01	1.0 ± 0.02
Morphiceptin (1µM)	99 ± 3	0.24 ± 0.02	0.65 ± 0.1
DSLET (0.1µM)	94 ± 5	0.25 ± 0.01	0.69 ± 0.2
DSLET + Morphiceptin	89 ± 3	0.20 ± 0.02	0.80 ± 0.05

Table I : Analysis of saturation binding of ^{3}H Etorphine in bovine adrenomedullary membranes.

delta, mu and kappa 2 or benzomorphan sites in the bovine adrenal medulla. All three sites are masked by micromolar concentrations of DADLE (1).Interestingly a high affinity compament of ^{3}H Etorphine binding persists after DADLE addition which represents 16% of total ^{3}H Etorphine identified sites. This component is stereoselective (Table II) and sensitive to proteolytic enzymes and N-ethylmaleimide treatment (Table III). Displacement experiments with opiates show a particular profile of this component. From all opioid peptides tested Met-Enkephalin-Arg^6-Phe^7 was the most affine on this site, followed by Met-Enkephalin-Arg^6-Gly^7-Leu^8 and Met-Enkephalin (Table IV).

	Effectors		
	0	DADLE (5µM) cpm bound	DSLET + Morphiceptin (0.1 + 1µM)
Bo	4,468 ± 205	2731 ± 103	3865 ± 32
Dextrorphan (10µM)	4,212 ± 107	2701 ± 85	3850 ± 24
Levorphanol (10µM)	2,391 ± 26	2357 ± 35	2403 ± 18

Table II : Stereoselectivity of ^{3}H Etorphine binding (300000 cpm) in the bovine adrenal medulla. Total binding is presented. (mean ± standard error of three separate experiments).

Discussion

^{3}H Etorphine presents particular binding characteristics in bovine adrenal medullary membranes. This ligand identifies with high affinity delta, mu and subpopulations of the kappa binding sites (Table I). Delta and mu sites are blocked by DSLET and morphiceptin while kappa 2 or benzomorphan sites are blocked by micromolar concentrations of DADLE (3). In such a case, a residual DADLE insensitive high affinity component is detectable, corresponding to 16%

Treatment	Effectors	
	DADLE (5µM)	DSLET (0.1µM) + Morphiceptin (1µM)
	% of total binding	
0	100	100
Levorphanol (10µM) (NSB)	37	23
Trypsin (20µg/mg) 30 min	41	44
Pepsin (100µg/mg) 30 min	63	63
α-Chymotryspin (50µg/mg) 30 min	40	38
NEM (05mM) 30 min	7	33
Levorphanol (10µM) (NSB)	37	23

Table III : Effect of proteolytic enzymes and N-Ethylmaleimide on ^{3}H Etorphine binding in bovine adrenomedullary membranes. Results are expressed as a percentage of ^{3}H Etorphine bound in the absence of any treatment. In these conditions, binding was 6.8% of total in the presence of DADLE and 11.8% of total in the presence of DSLET and morphiceptin. For comparison non specific binding (NSB) in the presence of levorphanol is also presented.

of total high affinity etorphine labelled sites. Binding was analysed in various graphical systems (4) and found to correspond to one site. Interestingly, etorphine binding in the presence of DSLET and morphiceptin (conditions in which kappa 2 and the new etorphine binding component are labelled) shows a Hill coefficient of 0.8 , which could correspond to two sites identified with an equal affinity but present in different concentrations. This new binding component is stereoselective and peptide sensitive (Tables II and III) rulling out the possibility of a non specific binding to membrane component or during the separation. No major differences were found using different opiates. Conversely displacement experiments using opioid peptides (which were carried out at 0°C to minimize proteolysis in the presence of inhibitors (5)) showed a quite different affinity pattern (Table IV) while DSLET and morphiceptin were used as effectors (in which case the ratio of kappa 2 to the residual site was about 3/1 - cf Table I -) or DADLE was present in the incubation mixture (in which case only the residual site can be detected). We therefore propose that etorphine can identify a new binding site in the bovine adrenal medulla. Its high affinity for etorphine, diprenorphine, bremazocine and pentazocine suggests that it might be a subtype of the kappa binding site. We therefore propose the name of kappa 3 site. The naturally occuring heptapeptide Met-Enkephalin-Arg^6-Phe^7 could be its endogenous ligand (6).

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Displacer	DADLE	DSLET + Morphiceptin	Displacer	DADLE	DSLET + Morphiceptin
	IC 50 nM			IC 50 nM	
Pentazocine	3.7	7.1	Met-Enkephalin	1000	>5000
Cyclazocine	40	8.6	Leu-Enkephalin	>5000	>5000
Ketocyclazocine	220	100	Met-Enkephalin-Arg^6	>5000	629
Ethylketocyclazocine	30	80	Met-Enkephalin-Arg^6-Phe^7	5	19
Fentanyl	100	309	Met-Enkephalin-Arg^6-Gly^7-Leu^8	100	160
Levorphanol	24	45	Dynorphin 1-13	>5000	63
Morphine	1410	890	DADLE	>5000	323
Etorphine	1.2	0.9			
Bremazocine	4.2	1.1			
Naloxone	47	40			
Diprenorphine	2.4	0.8			

Table IV : Competition for ^{3}H Etorphine binding by opiates and opioid peptides. Binding studies were performed at 37°C during 30 min for opiates and at 0°C during 150 min for opioid peptides. Bestatin (10µM) thiorphan (0.2µM), aprotinin (200 Trypsin inhibitor units/ml) and Leu-Leu (1mM) were added in this latter case, to inhibit peptide degradation. Binding at 0°C does not affect the K_D of ^{3}H Etorphine, while an 25% reduction of Bmax is observed.

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