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KAPPA-OPIATE RECEPTOR IN BLOOD VESSELS

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ABSTRACT [^{3}H]etorphine was used in Scatchard analysis and displacement studies on membrane preparations from both rat brain and rabbit mesentery and aorta (adventitia stripped off). Scatchard plots revealed an affinity constant, K_D, for rat brain of 0.30 nM and a maximum binding, B_{max}, of 12 fmol/mg tissue. Rabbit blood vessel had a higher K_D of 0.61 nM and a much lower B_{max} of 0.17 fmol/mg tissue. The subtypes of opiate receptors were analysed with Tyr-Pro-NMePhe-D-Pro-NH_2 (a specific mu agonist), D-Ala^2-D-Leu^5-enkephalin (delta agonist), Dynorphin$_{1-13}$ (kappa agonist), and Met^5-enkephalin-Arg^6-Phe^7 (an opioid peptide distributes in some peripheral tissues with concentrations much higher than that in the brain). Their IC_{50} for displacing [^{3}H]etorphine binding in the brain were 820, 82, 12, and 34 nM respectively. But in the blood vessel, only dynorphin could displace [^{3}H]etorphine binding with an IC_{50} of 20 ± 5.5 nM (SD) while the other 3 opioid peptides showed very weak inhibition ($IC_{50}>1000$ nM). These results suggest an existence of opiate receptor in blood vessels which is different from that in the brain, and is mainly kappa in nature.

KEY WORDS opiate receptors; blood vessels; dynorphin; enkephalins

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Table 1. Concentrations (nM) for inhibiting 50% of [^{3}H]etorphine (0.4 nM) binding in membrane preparations from rat brains and rabbit vessels. $\bar{x} \pm SD$.

Inhibitor	IC_{50} (nM)			
	Brain	Blood vessel	Expts	Rabbits
Dynorphin$_{1-13}$ (D_{1-13})	12	20±5.5	4	9
D-Ala^2-D-Leu^5-enkephalin (DADL)	82	>1000	5	9
Tyr-Pro-NMePhe-D-Pro-NH_2(PLO17)	820	>1000	5	9
Met^5-enkephalin-Arg^6-Phe^7(MEAP)	34	>1000	2	5
Naloxone	25	830±17.0	2	5
Etorphine	6	19	1	3

The cardiovascular effect of opioids is well known, but it is usually considered as a central regulation. Recently we have demonstrated opiate receptors in rabbit mesentery and aorta, suggesting that opioids might act directly on blood vessels.

MATERIALS AND METHODS

[^{3}H]etorphine ([^{3}H]E), 44 Ci/mmol, chemical purity 95%, an dunlabeled opiates were produced by Lab of Isotopes and Dept of Pharmaceutical Chemistry respectively in this College. Tyr-Pro-NMePhe-D-Pro-NH_2 (PLO17) was a generous gift of Dr J K Chang, Peninsula Labs Inc, San Carlos CA, USA. Dynorphin$_{1-13}$ (D_{1-13}), D-Ala^2-D-Leu^5-enkephalin (DADL) and Met^5-enkephalin-Arg^6-Phe^7 (MEAP) were purchased from the same Company. Captopril (D-3-mercapto-methylpropanoyl-L-proline) was kindly provided by Squibb & Sons In, Princeton NJ, USA.

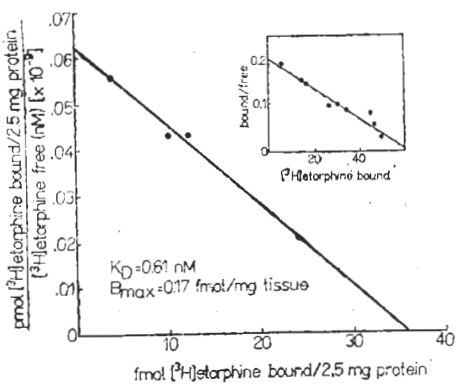

Fig 1. Scatchard plots of [^{3}H]etorphine (0.06-4.0 nM) binding to membrane preparations from rat brain (8 mg tisssue, upper right) and rabbit mesentery and aorta (50 mg tissue)

Membrane preparation Albino rabbits weighing 2-3 kg were killed by a blow on the head. The mesentery of small intestine was excised 1 cm apart from the retroperitonium, so that most of the nervous plexus were avoided. The aorta was taken out with its adventitia stripped off. The weighed tissue was chopped, homogenized in 30 volumes of ice-cold 50 mM Tris-HCl (pH 7.7), and centrifuged at 483 × g, 4℃ for 10 min. After the fat layer on the top was removed the supernatant was centrifuged at 16,500 × g, 4℃ for 20 min. The pellet was resuspended with the original amount of buffer and filtered with 6 layers of gauze. The filtrate was incubated at 36℃ for 30 min, then centrifuged at 16,500 × g, 4℃ for 20 min. The pellet was resuspended with Tris-HCl (pH 7.7) to a concentration of 2.5 mg protein/0.8 ml for assay. Protein was determined with biuret method[1]. The rat brain homogenate was prepared as previously described[2].

Receptor binding assay Scatchard plots and displacement assay were performed [3]. When MEAP was used as displacer, 0.2 μM of captopril was added to protect MEAP from degradation by dipeptidyl carboxypeptidase.

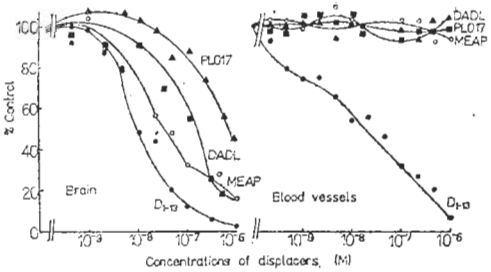

Fig 2. IC_{50} of 4 opioid peptides for inhibiting [^{3}H]etorphine (0.4 nM) binding in rat brain preparation (0.8 mg protein/ml/assay tube) and blood vessel preparation (2.5 mg protein/ml/assay tube). Each point = $\bar{x}$ of 5 (MEAP) and 9 (D_{1-13}, DADL & PLO17) animals.

RESULTS AND DISCUSSION

Scatchard plots (Fig 1) revealed a K_D for rat brain of 0.3 nM and B_{max} of 12 fmol/mg tissue. Blood vessel preparation had a slightly higher K_D of 0.61 nM and a 75 times lower B_{max} of 0.17 fmol/mg tissue, suggesting a very low density of opiate receptor in blood vessels. [^{3}H]E was chosen because it binds to mu, delta and kappa binding sites with quite similar affinity. We have already obsered that [^{3}H]naloxone showed poor specific binding in blood vessel preparation (data not shown). Subtypes of receptor were analysed with ligands relatively specific for each subtype. Dynorphin is a kappa agonist[4] and DADL is usually used as adelta ligand[5]. PLO17 is an analog of a mu agonist, morphiceptin[6]. Its specificity to mu binding site is 10-fold higher than that of morphiceptin in bioassay (data from Dr J K Chang), but in our radio-receptor assay PLO17 was not as potent as it was in bioassay. MEAP distributes in some peripheral organs (such as lund intestine) with extremely high contents[7]. We observed a high affinity with opiate receptors in the lung by MEAP[8] and expected that it might show high affinity with opiate receptor in blood vessels as well. Surprisingly, all opioid peptides studied except D_{1-13}, had very low affinity with opiate receptors in blood vessels though all of them displaced [^{3}H]E binding very well in the brain (Fig 2). Their concentrations for inhibiting 50% of [^{3}H]E binding, IC_{50}, were summarized in Table 1. The IC_{50} of D_{1-13} was very close to that of etorphine itself in blood vessel preparation. suggesting that the opiate receptors in blood vessels are mainly kappa subtype in nature.

The location of opiate receptors in blood vessels deserves further studies. In the mesentery, opiate receptors might exist as neuronal receptors on nerves accompanying the mesenteric arteries. Their existence suggests a peripheral mediation on blood pressure by opioid peptidergic system, a useful clue for cardiovascular studies.

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