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Effect of arachidonic acid on specific binding of [^{3}H]naloxone to opioid receptors

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Abstract. The effects of arachidonic acid (AA) on binding of [^{3}H]naloxone to the agonist and antagonist configurations of opioid receptors were investigated in rat brain. Equilibrium binding parameters of the agonist and antagonist configurations of the receptors were evaluated from homologue displacement data in the presence or absence of AA. Addition of AA at a concentration of 0.6 mM (1.5 μ mole/mg of protein) reduced by 22% and 53% the maximal number of binding sites (B_{max}) respectively in the absence or presence of 100 mM NaCl. Binding affinity (K_D) was not altered significantly (P<0.05) either in the presence or absence of 100 mM NaCl and AA. We conclude that AA mediated reduction in [^{3}H]naloxone specific binding was chiefly due to a decrease in the number of binding sites.

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The properties and function of many membrane-bound receptors are effected by their lipid environment and the lipid bilayer as a whole. The sensitivity of opioid receptors to lipids has been also well documented. In an earlier study, McGee and Kenimer, (1982) studied the effect of alterations in membrane fatty acid composition on opioid receptor binding in clonal NG108-15 cells where addition of unsaturated fatty acids to cells caused a decrease in [^{3}H]etorphine binding. In 1987 Hasegawa et al. (1987) showed that binding affinity of isolated opioid receptors can be restored by addition of lipids. Remmers et al. (1990) have shown that cis and trans isomers of fatty acids influence both agonist and antagonist opioid ligand-receptor interactions differentially.

It is a well documented phenomena that, being a potent intracellular messenger, AA and some of its metabolites are capable of modulating the activity of a variety of molecules, including protein kinases (McPhail et al. 1984, Murakami and Routtenberg 1985, Piomelli et al. 1989), ion channels (Ordway et al. 1989, Urushidani et al. 1996), and membrane bound receptors (Hasegawa et al. 1987, Nielsen et al. 1988, Koenig and Martin 1992). Previous research conducted in our laboratory revealed that AA inhibited [^{3}H]naloxone specific binding to rat brain opioid receptors in a receptor subtype dependent manner and with an IC_{50} of 0.6 mM (Öktem and Apaydin 1998). We have also demonstrated that, in addition to AA, another PL-A_2 derived group of cellular messengers, lyso- phospholipids, were also capable of modulating opioid receptor binding (Apaydin and Öktem 1998).

In this study we aimed to investigate the effect of AA on the equilibrium binding parameters (K_D and B_{max}) of the agonist and antagonist configurations of rat brain opioid receptors.

Experiments were performed on male Sprague--Dawley rats weighing 200-250 g, from Başkent University Animal House, Ankara. Brain membranes were prepared as described previously (Öktem and Apaydin 1998). Protein concentration of membranes were determined by the Bradford method (Bradford 1976). Membranes (0.4 mg protein) were incubated with 3 nM final concentration of [^{3}H]naloxone in the absence (total binding) and presence (non-specific binding) of different concentrations of non-radioactive naloxone in 1 ml assay medium for 60 min at 0 C. When necessary, NaCl (100 mM) and AA (0.6 mM final concentration) were included in the assay media. Results were compared by means of two way ANOVA ($P<0.05$). Experimental data were analysed by the LIGAND program of Munson (Munson and Rodbard 1980).

Effect of AA on equilibrium binding parameters of opioid receptors was studied by homologue displacement experiments for both the agonist and antagonist (100 mM NaCl in assay media) configurations of opioid receptors. Throughout the experiments 0.6 mM final concentration of AA was employed. This is known to abolish 50% of [^{3}H]naloxone specific binding to rat brain membranes (Öktem and Apaydin 1998).

Computer analysis of homologue displacement data revealed a one site model for the interaction of [^{3}H]naloxone with the membrane bound total receptor sites in the presence and absence of 0.6 mM AA at both the agonist and antagonist configurations of the receptors. Results of the experiments in which binding parameters of [^{3}H]naloxone was determined under different conditions are given in Table I. K_D of [^{3}H]naloxone interaction to unmodified membrane was calculated to be 13.1 nM and B_{max} was 1030 fmol/mg of protein (Table I). In the presence of 100 mM Na^+ binding was characterised with K_D and B_{max} values of 11.7 nM and 2811 fmol/mg of protein (Table I). As expected, Na^+ ions increased the maximal binding capacities of [^{3}H]naloxone where as K_D values were not significantly effected. Following treatment with 0.6 mM AA, B_{max} values were reduced while the K_D values were not affected significantly ($P>0.05$). In the agonist configuration of the receptors, AA abolished 22% of the binding sites

TABLE I

Binding parameters of [^{3}H]naloxone binding to rat brain opioid receptors

AA[a]	NaCl[b]	K_D (nM)	B_{max} (fmol/mg of protein)
-	-	13.1 ± 2.48	1030 ± 343
+	-	5.7 ± 1.06	800[c] ± 152
-	+	11.7 ± 0.28	2811 ± 465
+	+	5.4 ± 0.49	1256[c] ± 214

[a] AA was added to the assay medium at a final concentration of 0.6 mM. This concentration is equivalent to 1.5mole AA/mg protein in the assay medium; [b] Final concentration in the assays were 100 mM; [c] Value is different from control at 5% significance level. All experiments were carried out in duplicate and repeated three times on separate batches of membrane preparations. Values given are means and standard error of means of these experiments.

whereas 53% of the sites were affected in the antagonist configuration.

The present results show that AA did not significantly influence the affinity of the agonist and antagonist configuration of opioid receptors towards nalaxone. In both configurations, the maximum number of binding sites was significantly reduced by AA and the antagonist configuration of the opioid receptors were observed to be more susceptible to AA inhibition.

The inhibitory effect of AA that was observed in our studies can not be explained simply by a negative feedback mechanism between the second messenger and the receptor site, since the concentration of AA that was used in our experimental system is much above the free physiological concentration of AA. Therefore, the effect of AA must be due to an indirect action at the lipid-protein boundary layer, most probably causing a decrease in membrane fluidity. Such a mechanism was also postulated by others (Remmers et al. 1990, Koenig and Martin 1992).

In summary our results suggested a reduction in interaction of opioid receptors with at least antagonist ligands, in the presence of AA.

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Apaydin S., Öktem H.A.1998. Modulatory role of lysophosphatidic acid on opioid receptor binding. Neurobiology 6:421-427.

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Hasegawa J.-I., Loh H.H., Lee N.M. 1987. Lipid requirement for X opioid receptor binding. J. Neurochem. 49: 1007-1012.

Koenig J.A., Martin I.L. 1992. Effect of free fatty acids on $GABA_A$ receptor ligand binding. Biochem. Pharmacol. 44: 11-15.

McGee R. Jr., Kenimer J. G. 1982. The effects of exposure to unsaturated fatty acids on opiate receptors, prostaglandin E1 receptors, and adenylate cyclase activity of neuroblostoma x glioma hybrid cells. Mol. Pharmacol. 22: 360-368.

McPhail L.C., Clayton C.C., Snyderman R. 1984. A potential second messenger role for unsaturated fatty acids: activation of Ca^{2+}-dependent protein kinase. Science 224: 622-625.

Munson J.P., Rodbard D. 1980 Ligand: a versatile computerised approach for characterisation of ligand binding systems. Anal. Biochem. 107: 220-239.

Murakami K., Routtenberg A. 1985. Direct activation of purified protein kinase C by unsaturated fatty acids (oleate and arachidonate) in the absence of phospholipids and Ca^{2+}. FEBS 192: 189-193.

Nielsen M., Witt M-R., Thøgersen H. 1988. [^{3}H]Diazepam specific binding to rat cortex in vitro is enhanced by oleic, arachidonic and docosahexenoic acid isolated from pig brain. Eur. J. Pharmacol. 146: 349-353.

Ordway R.W., Walsh J.W.Jr., Singer J.J. 1989. Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. Science 244: 1176-1179.

Öktem H.A., Apaydin S. 1998. Arachidonic acid modulation of [^{3}H]naloxone specific binding to rat brain opioid receptors. Neurobiology 6: 323-332.

Piomelli D., Wang J.K., Sihra T.S., Nairn A.C., Czernik A.J., Greengard P. 1989. Inhibition of Ca^{2+}/calmodulin-dependent protein kinase II by arachidonic acid and its metabolites. Proc. Natl. Acad. Sci. USA 86: 8550-8554.

Remmers A.E., Nordby G.L., Medzihradsky F. 1990. Modulation of opioid receptor binding by cis and trans fatty acids. J. Neurochem. 55: 1993-2000.

Urushidani T., Shimasue K., Hagiwara M., Nagao T. 1996. Effects of anadamide and arachidonic acid on specific binding of (+)-PN200-110, diltiazem and (-)-desmetho--xyverapamil to L-type Ca^{2+} channel. Eur. J. Pharmacol. 296: 347-350.

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