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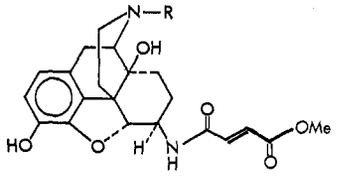
Communications to the Editor

Different Receptor Sites Mediate Opioid Agonism and Antagonism

Sir:

Many of the narcotic actions of morphine and other opioid ligands are believed to be mediated through interaction with μ opioid receptors.[1] These effects are promptly reversed by the opioid antagonists, naloxone and naltrexone, which are generally believed to compete with the same receptor that mediates the action of morphine. For this reason, naloxone and naltrexone have become important tools for investigating the pharmacological action of opioids. In this report we present evidence which suggests that antagonists reverse the effects of μ agonists by interaction with a separate recognition site that is coupled to the μ opioid receptor.

Recently, studies with β-funaltrexamine (β-FNA) and



β-FNA, R = $CH_2CH(CH_2)_2$

β-FOA, R = CH_3

its *N*-methyl analogue, β-fuoxymorphamine (β-FOA), have prompted us to question the belief that agonism and antagonism are mediated through identical receptor sites.[2,3] Though each of these ligands differ only in the nature of the N-substituent, only β-FNA irreversibly blocks the agonist action of μ agonists. Thus, β-FOA is a reversibly acting μ agonist with no detectable irreversible activity. These results can be explained either by (a) the presence of distinct receptor sites for μ agonism and antagonism or (b) by a difference in the nature of the interaction of agonists and antagonists with a single receptor type.

In an effort to distinguish between these possibilities, we have evaluated the ability of opioid agonists and antagonists to protect against the irreversible antagonism of morphine's effects by β-FNA. The protection studies were conducted in the following manner. After the guinea pig ileal longitudinal muscle (GPI)[4] was prepared and equilibrated, a control IC_{50} value of morphine was determined.

Table I. Protection of the Irreversible β-FNA Antagonism by Various Opioid Agonists and Antagonists

protector	concn	*N*	morphine IC_{50} ratio[a] ± SE	IC_{50}, nM	K_e[b]
none		7	6.1 ± 0.5		
morphine	1 μM	4	4.0 ± 0.7	24	88
β-FOA	500 nM	5	5.2 ± 0.7	22	
RX 783006[c]	1 μM	4	2.3 ± 0.6	28	
DAME[d]	1 μM	4	3.1 ± 0.1	21	
etorphine	1 nM	5	2.3 ± 0.3	0.3	
ethylketazocine	10 nM	4	4.6 ± 1.2	0.6	
nalorphine	200 nM	4	1.9 ± 0.2	28	4.5
	1 μM	4	1.4 ± 0.3		
naloxone	2 nM	4	3.4 ± 0.5		1.2
	20 nM	4	1.9 ± 0.3		
	200 nM	4	1.1 ± 0.2		
naltrexone	2 nM	4	2.4 ± 0.6		0.38
	20 nM	4	1.0 ± 0.1		
	200 nM	4	1.0 ± 0.1		
diprenorphine	3 nM	4	1.8 ± 0.2		0.13

[a] IC_{50} after treatment/control IC_{50}; statistical analyses were determined by the method of Finney.[20] [b] K_e (equilibrium constant) values were taken from Kosterlitz and Watt.[7] [c] [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin.[21] [d] [D-Ala^2,Met^5]enkephalinamide.

Then the protecting compound was incubated for 10 min with the GPI, followed by the addition of 20 nM β-FNA into the incubating medium for 30 min. This treated GPI was thoroughly washed with 20–30 changes of incubating medium, and the IC_{50} of morphine was redetermined.[5] The effect of the protector was expressed as morphine IC_{50} ratio (IC_{50} after treatment/control IC_{50}).

When very high concentrations of very potent agonists were used for protection, the treatment caused a residual interference on the ability of the GPI to contract. Thus, the concentrations of the agonist protectors were chosen so that they were the highest concentrations that could be used without residual effects on the contractions of the GPI after washing.

Most of the agonists afforded relatively poorer protection of β-FNA antagonism than that exhibited by the antagonists (Table I). The opioid peptide RX 783006 and etorphine afforded the best protection among the agonists. However, even at the very high concentration of RX 783006 used, it did not provide complete protection. Etorphine at a low concentration afforded relatively good but not full protection. Etorphine could not be tested at

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(5) The longitudinal muscle was stimulated during all incubations. Under such conditions, acute tolerance of the GPI is an unlikely possibility in view of identical IC_{50} values of morphine after exposure of the preparation to 1 μM morphine for 1–4 h. (Vaught, J. L.; Takemori, A. E., unpublished observations).

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higher concentrations because of its very potent agonist activity. Other μ agonists (morphine, β-FOA, and DAME) and the κ agonist (Ethylketazocine) exhibited relatively less protection against β-FNA irreversible antagonism.

The capability of the compounds to protect against the irreversible antagonism of β-FNA did not correlate with their agonist activity (IC_{50} values in Table I) but appeared to correspond to their antagonist potency. Thus, as protectors, the antagonists had a rank potency of nalorphine < naloxone < naltrexone < diprenorphine. It is of interest that comparison of this protective ability of the ligands with their K_e values from the literature[6] reveals the same rank order (Table I). Naloxone and naltrexone afforded complete protection of β-FNA antagonism. Higher concentrations of nalorphine and diprenorphine could not be used because of the interference from their agonist activity.

The effectiveness of narcotic antagonists to protect μ receptors in the GPI preparation against irreversible blockage by β-FNA is in contrast to the relatively poor protection afforded by a variety of opioid agonists. The fact that a variety of agonists, including morphine, possess an antagonistic component[6,7] in the GPI preparation and the apparent parallel relationship between this antagonism (K_e values) and their ability to protect against β-FNA tend to suggest the presence of separate recognition sites for μ agonists and antagonists. Since both β-FOA and β-FNA contain an identical electrophilic moiety at the C-6 position, the unreactivity[2,3] of β-FOA at μ receptors is consistent with its interaction at a site that does not possess an accessible requisite nucleophile for covalent association. Moreover, the inability of β-FOA to protect against β-FNA-induced, irreversible antagonism strongly implicates separate sites for these ligands.

The presence of separate recognition sites for μ agonists and antagonists provides a reasonable basis for rationalizing the differential effects of various types of treatments on the binding of opioid agonists and antagonists. These include protein-modifying reagents,[8] enzymatic treatments,[9] heat,[10] and exposure to various cations[11,12] and GTP.[13] Also, separate agonist and antagonist sites have been postulated from in vivo data.[14]

Since β-FNA specifically and irreversibly antagonizes the effects of μ agonists without affecting κ or δ opioid activity,[3,15,16] it appears likely that β-FNA interacts covalently with a site that is uniquely coupled to the μ receptor. A plausible functional role for this site is the regulation of μ receptors in response to endogenous μ ligands. Thus, the data suggest that naloxone and naltrexone may exert their antagonistic effect by interacting with a neighboring regulatory site, ρ, which is allosterically coupled to the μ receptor.

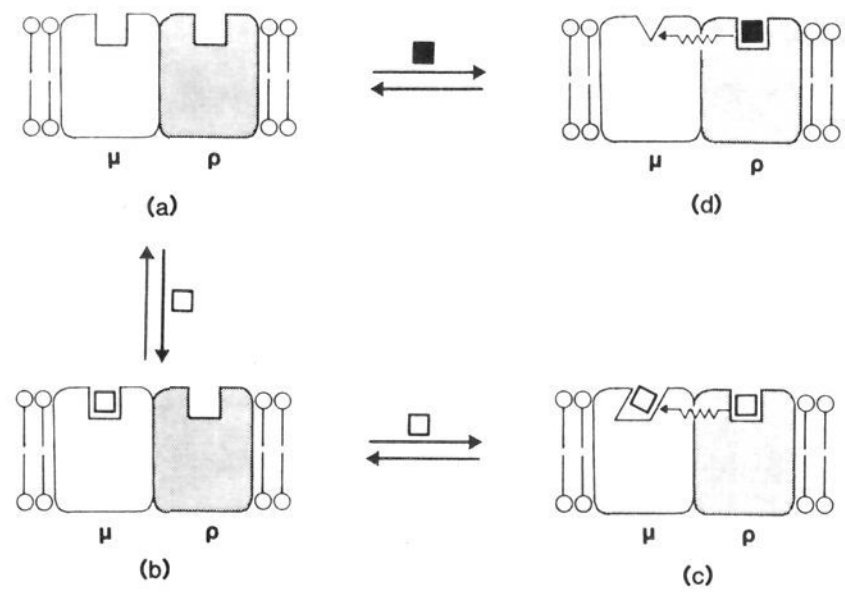

Figure 1. A model of the interaction of a μ agonist (□) and antagonist (■) with the μ opioid receptor and a neighboring regulatory site ρ. An agonist at low concentration binds selectively to the μ receptor (b) and at higher concentrations to the ρ regulatory site (c). The latter interaction triggers a vectorial decrease in the affinity of the μ receptor (symbolized by ←ww—). The selective interaction of an antagonist at the ρ site induces a vectorial loss of affinity of the μ receptor.

A model consistent with these data is illustrated schematically in Figure 1. We propose that a μ receptor subunit is associated with a regulatory subunit that contains a recognition site ρ that is topographically similar, but not identical, with its neighboring μ receptor. Agonists have higher affinity for the μ receptor, while antagonists possess higher affinity for the regulatory site ρ. We envisage that occupation of the ρ site induces a unidirectional (vectorial) decrease in the affinity of the agonist binding site μ. The ρ site becomes bound by an agonist only after its neighboring μ receptor is occupied, whereas narcotic antagonists interact selectively with the ρ site. The vectorial change induced by the antagonist is manifested by a considerably greater affinity loss at the μ receptor than the interaction of agonists with the ρ site.

The presence of separate recognition sites for opioid agonists and antagonists suggest a number of intriguing possibilities, which are outlined below. (1) The "purity" of the agonistic effect is determined by the relative affinity of a ligand for the μ and ρ sites. Accordingly, partial agonists are ligands whose affinity for the ρ site is of sufficient magnitude to afford a submaximal concentration–response relationship via the μ receptor. In this connection, it is conceivable that modulation of μ agonist binding by Na^+ and GTP resides in the vectorial coupling component of the complex. (2) In the context of this model, tolerance can be viewed as an increase in the number of μ receptor subunits that are functionally coupled with the regulatory subunit ρ. This could be effected by an agonist-induced increase of ρ subunits or by a mobile receptor[17,18] mechanism involving the association between monomeric μ and ρ subunits. This would explain the increased sensitivity to a narcotic antagonist by mice soon after they have been exposed to a μ agonist.[19]

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Finally, the possible presence of separate recognition sites provides a physiological mechanism for the fine tuning of responses to endogenous opioid ligands. It also has implications regarding the structure–activity relationship of μ opioid agonists because such agonism should reflect the relative affinity at two sites rather than one.

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Registry No. β-FNA, 72782-05-9; β-FOA, 72782-06-0; Rx 783006, 78123-71-4; DAME, 61090-95-7; morphine, 57-27-2; etorphine, 14521-96-1; ethylketazocine, 36292-66-7; nalorphine, 62-67-9; naloxone, 465-65-6; naltrexone, 16590-41-3; diprenorphine, 14357-78-9.

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Articles

Role of Substrate Lipophilicity on the N-Demethylation and Type I Binding of 3-*O*-Alkylmorphine Analogues

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A series of 3-*O*-alkylmorphine analogues was synthesized to determine if there was a good correlation between the rate of metabolism, type I binding affinity, and lipid solubility. The data indicate that the K_m for the N-demethylation declines with increasing chain length from C_1 to C_9, while for increasing chain length the V_{max} for the N-demethylation increases to a maximum of 15.20 nmol min^{-1} (mg of protein)$^{-1}$ for the butyl analogue (C_4) and then slowly declines with analogues with chain lengths greater than butyl (C_4). The decyl (C_{10}) and dodecyl (C_{12}) analogues showed no activity. There was a good correlation between the lipophilicity and K_m values, except for codeine and the C_{10} and C_{12} analogues. The type I binding dissociation constants (K_s) also decreased with increasing alkyl chain length with an excellent correlation between the K_s and log P. The OD_{max} did not change with increasing the chain length of the analogues. Our data suggest that in male rat hepatic microsomes the catalytic site for N-demethylation and the site for type I binding in this series of compounds are similar but distinct.

Imai and Sato[1] and Remmer et al.[2] simultaneously reported that in the presence of various drugs, hepatic microsomes exhibited characteristic absorbance changes in the Soret spectrum of cytochrome P-450. Schenkman and co-workers[3-7] classified these interactions into three types termed type I, type II, and reverse type I. A number of studies suggest that type I, but not the other two types, could be related to the metabolism of drugs.

On the basis of the concentration-dependent kinetics of the binding spectrum and metabolism, Schenkman et al.[5] proposed that the type I binding of some substrates is related to the binding of the drug to the catalytic site of the cytochrome P-450. They observed that for many substrates there was a similarity between the type I spectral dissociation constant, K_s, and the Michaelis constant, K_m.[5] However, Davies et al.,[8] in investigating the effect of sex and species differences on drug metabolism, did not find a direct relationship between metabolism and type I binding. Similarly, Hedwick and Fouts[9] reported no correlation between the type I binding and metabolism of a series of sympathominetics. Further, Al-Gailany et al.[10] reported that for a series of *p*-nitrophenyl alkyl ethers, the K_m's for the dealkylation were generally one order of magnitude lower than the corresponding K_s. In line with these observations, Anders et al.[11] found no correlation between the type I binding parameters and the rates of N-demethylation for several enantiomeric drugs. Finally, studies from our laboratory have suggested that the type I binding site and the catalytic site are probably separate sites.[12]

The effect of substrate lipophilicity on type I binding and metabolism has also been examined. Al-Gailany et al.[13] found a very good correlation between the binding

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