Communications to the Editor

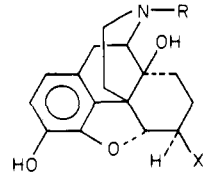
A Novel Opioid Receptor Site Directed Alkylating Agent with Irreversible Narcotic Antagonistic and Reversible Agonistic Activities

Sir:

In 1965 the concept of multiple opioid receptors and multiple modes of interaction with a single receptor was proposed based on a detailed analysis of a broad spectrum of structure–activity relationships among analgetic ligands.[1-3] The major lines of evidence for this concept were (1) the divergent stereochemical requirements for analgetic activity and (2) the different rank-order potencies among congeners, in two or more series, whose N substituent is modified in an identical manner.

Pharmacologic and biochemical studies have since provided additional support for this concept. Noteworthy among the early pharmacologic studies were those of Martin[4] who pointed to the competitive dualism of molecules with mixed agonist–antagonist activity, those of Takemori et al.[5] who indicated that opioid receptors in the gut are different from those in the CNS, and those of Smits and Takemori[6] who concluded that pure agonists interact with opioid receptors in a different way than do mixed agonist–antagonist drugs. More recently, numerous reports have appeared[7] which have suggested that multiple populations of opioid receptors are present in the CNS and in peripheral tissue.

We now report on another line of evidence which provides additional support for the multiple modality or multiple receptor concept.[1-3] Through use of the novel pair of receptor probes, 1 (β-FNA) and 2 (β-FOA), we present



	R	X
1 (β-FNA)	CH_2-c-C_3H_5	NHCOC(H)=C(H)COOMe
2 (β-FOA)	CH_3	NHCOC(H)=C(H)COOMe
3 (β-CNA)	CH_2-c-C_3H_5	$N(CH_2CH_2Cl)_2$
4 (β-COA)	CH_3	$N(CH_2CH_2Cl)_2$
5	CH_2-c-C_3H_5	NH_2
6	CH_3	NH_2

data which suggest that only 1 covalently binds to opioid receptors even though each of the compounds contains an identical alkylating moiety and forms a receptor complex.

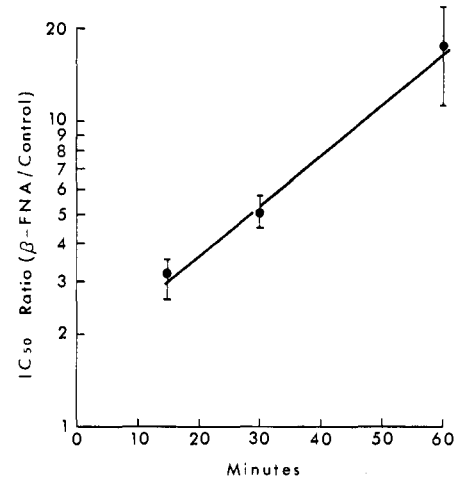


Figure 1. The time-dependent inhibition by 1 (β-FNA) of the effect of morphine on the electrically stimulated guinea pig ileal longitudinal muscle preparation. After the muscle was incubated with 1 at different time intervals and washed (20×), the morphine IC_{50}[17] was determined. The IC_{50} ratio represents the IC_{50} of morphine after treatment with β-FNA (2×10^{-8} M) divided by the IC_{50} of morphine for the same preparation. Each point represents the mean of three experiments (±SD).

The design rationale for 1 and 2 was based on the corresponding nitrogen mustard analogues, chlornaltrexamine 3 (β-CNA) and chloroxymorphamine 4 (β-COA), which possess potent, nonequilibrium narcotic antagonistic and agonistic properties, respectively.[8-10] As the aziridinium ion derived from the nitrogen mustard is highly reactive, we reasoned that a Michael acceptor, such as the fumaramate group, attached at an identical position would confer greater selectivity in covalent bond formation with opioid receptors due to its lower reactivity.

β-FNA (1) was synthesized by reaction of amine 5[11] with the monomethyl ester of fumaroyl chloride[12] in aqueous THF containing Na_2CO_3. The acid chloride was added over 15 min and stirring was continued for an additional 30 min. After extraction (EtOAc) and removal of solvent, the free base 1 was crystallized from Et_2O–hexane: yield 68%; mp 101–103 °C; EIMS *m/e* 454 (M^+); TLC R_f (silica gel; EtOAc–NH_4OH, 100:1) 0.54; NMR (CD_3COCD_3) δ 4.49 (d, $J_{5,6}$ = 7.6 Hz, C-5 H), 7.03 and 6.82 (J = 15.4 Hz, vinyls). The base 1 was converted to the HCl salt and crystallized from MeOH–Et_2O (1:1): mp >285 °C; $[\alpha]_D$ −164° (*c* 0.5, MeOH). Anal. ($C_{25}H_{31}N_2O_6Cl$) C, H, N.

β-FOA (2), mp 149–153 °C, was prepared in 71% yield from its amine precursor 6[13] using a similar procedure:

(1) P. S. Portoghese, *J. Med. Chem.*, 8, 609 (1965).
(2) P. S. Portoghese, *J. Pharm. Sci.*, **55**, 865 (1966).
(3) P. S. Portoghese, *Acc. Chem. Res.*, 11, 21 (1978).
(4) W. R. Martin, *Pharmacol. Rev.*, **19**, 463 (1967).
(5) A. E. Takemori, H. J. Kupferberg, and J. W. Miller, *J. Pharmacol. Exp. Ther.*, **169**, 39 (1969).
(6) S. E. Smits and A. E. Takemori, *Br. J. Pharmacol.*, **39**, 627 (1970).
(7) S. H. Snyder and S. R. Childers, *Ann. Rev. Neurosci.*, **2**, 35 (1979).
(8) P. S. Portoghese, D. L. Larson, J. B. Jiang, A. E. Takemori, and T. P. Caruso, *J. Med. Chem.*, 21, 598 (1978).
(9) P. S. Portoghese, D. L. Larson, J. B. Jiang, T. P. Caruso, and A. E. Takemori, *J. Med. Chem.*, **22**, 168 (1979).
(10) T. P. Caruso, A. E. Takemori, D. L. Larson, and P. S. Portoghese, *Science*, **204**, 316 (1979).
(11) J. B. Jiang, R. N. Hanson, P. S. Portoghese, and A. E. Takemori, *J. Med. Chem.*, **20**, 1100 (1977).
(12) R. M. Acheson, R. S. Feinberg, and A. R. Hands, *J. Chem. Soc.*, 526 (1964).

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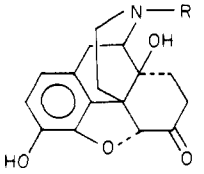
CIMS m/e 415 (M + 1); TLC R_f (silica gel, EtOAc–MeOH–NH_4OH, 98:2:1) 0.50; NMR (CD_3COCD_3) δ 4.45 (d, $J_{5,6}$ = 7.6 Hz, C-5 H), 7.03 and 6.82 (J = 15.4 Hz, vinyls). The base **2** was converted to its HCl salt and crystallized from MeOH–Et_2O (1:1): mp >250 °C; $[\alpha]_D$ −144° (c 0.36, MeOH). Anal. ($C_{22}H_{27}N_2O_6Cl$·0.5MeOH) C, H, N.

β-FNA (**1**) behaved as a potent reversible agonist with an IC_{50} of 4.8×10^{-9} M when tested on the electrically stimulated guinea pig ileal longitudinal muscle preparation[14] (morphine $IC_{50} = 2.4 \times 10^{-8}$ M). The agonist effect of **1** could be reversed at all incubation times by washing or addition of naltrexone (**7**). However, when the ileum



7, R = CH_2-c-C_3H_5
8, R = CH_3

was incubated with **1** (2×10^{-8} M) for different time periods and then washed (20×), a time-dependent, irreversible narcotic antagonistic effect against morphine was observed (Figure 1). The specificity of the blockage was suggested by the fact that the irreversible effects of **1** could be inhibited by prior addition of the reversible antagonist, naltrexone (**7**; 5×10^{-8} M). Norepinephrine receptors were unaffected by β-FNA treatment.

In view of the generally known chemical properties of Michael acceptors, the SH group is a good candidate for the nucleophile which forms a covalent bond with β-FNA (**1**). This nucleophile has been implicated in other studies on opioid receptors.[15]

Significantly, when the ileum was treated with **2** (β-FOA) under conditions identical with those for **1**, it produced a reversible agonistic effect ($IC_{50} = 2.7 \times 10^{-8}$ M) but no agonism or morphine antagonism was observed following washing (20×) after 80 min of incubation. The agonistic effect of **2** also was blocked by the irreversible action of **1**.

Since the data suggest that **1** but not **2** forms a covalent bond with opioid receptors, it is likely that the receptor environments which interact with the fumaramate ester moiety of **1** and **2** are different. *This indicates either that 1 and 2 interact differently with a single receptor or that they associate with different receptors, as proposed in the original concept.*[1]

It has been reported[9,10] that both the *N*-(cyclopropylmethyl) and *N*-methyl nitrogen mustards, **3** (β-CNA) and **4** (β-COA), form covalent bonds with receptor nucleophiles to afford sustained narcotic antagonism and agonism, respectively, in the guinea pig ileal longitudinal muscle preparation. This is in contrast to the action of the Michael acceptor analogues, where sustained action is observed with the *N*-(cyclopropylmethyl) compound **1** (β-FNA) but not with its *N*-methyl counterpart **2** (β-FOA). Since the results of the present study suggest that the receptor environments which interact with the fumaramate ester moieties of **1** and **2** differ, it is likely that a similar difference exists with the nitrogen mustards (**3** and **4**), but is not apparent due to the higher reactivity of the aziridinium ion. Specifically, in contrast to the receptor interaction for the *N*-(cyclopropylmethyl) compounds, wherein a receptor nucleophile is alkylated by both functionalities, for the *N*-methyl compounds a less reactive or less accessible nucleophile is readily alkylated by the nitrogen mustard group but not by the Michael acceptor moiety.[16]

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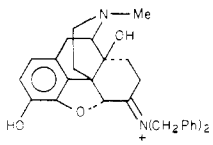
(13) The amine **6** was obtained stereospecifically in 66% overall yield from **8**. This involved azeotropic removal of H_2O from a mixture of **8** and dibenzylamine (both as benzoate salts) to afford the iminium salt **9**, reduction with $NaCNBH_3$ to the



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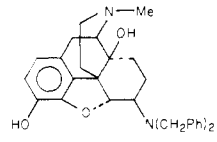

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dibenzylamino derivative **10**, followed by catalytic hydrogenolysis to **6**. The β configuration at C-6 was confirmed by NMR ($J_{5,6}$ = 6.8 Hz in $CHCl_3$).

(14) H. P. Rang, *Br. J. Pharmacol. Chemother.*, **22**, 356 (1965).

(15) E. J. Simon and J. M. Hiller, *Ann. Rev. Pharmacol. Toxicol.*, **18**, 371 (1978).

(16) This assumes that the *N*-methyl compounds (**2** and **4**) interact with opioid receptors in an identical fashion but differ in their mode of interaction from the corresponding *N*-(cyclopropylmethyl) analogues (**1** and **3**) whose binding modes are identical with one another.

(17) The IC_{50} is the concentration of morphine required to inhibit the muscle twitch by 50%.

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10,10-Difluoro-13-dehydroprostacyclin: A Chemically and Metabolically Stabilized Potent Prostacyclin

Sir:

The discovery of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation, by Vane, Moncada, and their collaborators[1] and its isolation and chemical characterization by Johnson et al.[2] represent a milestone in prostaglandin research. Immediately it became apparent that the presence of an unusually acid-labile enol ether grouping[3] seriously impeded the full realization of the therapeutic potential of this substance. As a result, a considerable number of more stable, biologically active analogues have been prepared, mainly by partial synthesis, involving replacement of the ether oxygen by sulfur,[4] ni-

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(2) Johnson, R. A.; Morton, D. R.; Kinner, J. H.; Gorman, R. R.; McGuire, J. C.; Sun, F. F.; Whittaker, N.; Bunting, S. ; Salmon, J.; Moncada, S.; Vane, J. R. *Prostaglandins* **1976**, *12*, 915.

(3) (a) Fried, J.; Barton, J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 2199; (b) Chiang, Y.; Kresge, A. J.; Cho, M. J. *J. Chem. Soc., Chem. Commun.* **1979**, 129.

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