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 Martin⁴ 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 Takemori⁶ who concluded that pure agonists interact with opioid receptors in a different way than do mixed agonist-antagonist drugs. More recently, numerous reports have appeared⁷ 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



data which suggest that only 1 covalently binds to opioid

- (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).



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 IC₅₀¹⁷ was determined. The IC₅₀ ratio represents the IC₅₀ of morphine after treatment with β -FNA (2 × 10⁻⁸ M) divided by the IC₅₀ of morphine for the same preparation. Each point represents the mean of three experiments (±SD).

receptors even though each of the compounds contains an identical alkylating moiety and forms a receptor complex.

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 5¹¹ with the monomethyl ester of fumaroyl chloride¹² in aqueous THF containing Na₂CO₃. 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 Et₂O-hexane: yield 68%; mp 101-103 °C; EIMS m/e 454 (M⁺); TLC R_f (silica gel; EtOAc-NH₄OH, 100:1) 0.54; NMR (CD₃COCD₃) 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-Et₂O (1:1): mp >285 °C; $[\alpha]_D$ -164° (c 0.5, MeOH). Anal. (C₂₅H₃₁N₂O₆Cl) C, H, N.

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:

- (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).

CIMS m/e 415 (M + 1); TLC R_f (silica gel, EtOAc-MeOH-NH₄OH, 98:2:1) 0.50; NMR (CD₃COCD₃) 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-Et₂O (1:1): mp >250 °C; $[\alpha]_D$ -144° (c 0.36, MeOH). Anal. (C₂₂H₂₇N₂O₆Cl·0.5MeOH) C, H, N.

 β -FNA (1) behaved as a potent reversible agonist with an IC₅₀ of 4.8×10^{-9} M when tested on the electrically stimulated guinea pig ileal longitudinal muscle preparation¹⁴ (morphine IC₅₀ = 2.4×10^{-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



was incubated with 1 $(2 \times 10^{-8} \text{ M})$ for different time periods and then washed $(20\times)$, 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; $5 \times 10^{-8} \text{ M}$). 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 (IC₅₀ = 2.7 × 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, re-

(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 NaCNBH₃ to the



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 CHCl₃).
(14) H. P. Rang, Br. J. Pharmacol. Chemother., 22, 356 (1965).

- (14) H. P. Rang, Br. J. Pharmacol. Chemother., 22, 356 (1965).
 (15) E. J. Simon and J. M. Hiller, Ann. Rev. Pharmacol. Toxicol.,
- (15) E. J. Simon and J. W. Hiner, Ann. Rec. Furmacol. 10 18, 371 (1978).

spectively, 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

Acknowledgment. This research was supported by NIDA Grants DA01533 and DA00289. We thank the capable technical assistance of Miss Joan Naeseth and Mrs. Masako Ikeda.

Philip S. Portoghese,* Dennis L. Larson Lawrence M. Sayre, David S. Fries Department of Medicinal Chemistry College of Pharmacy

A. E. Takemori

Department of Pharmacology School of Medicine University of Minnesota Minneapolis, Minnesota 55455 Received December 17, 1979

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 collaborators¹ 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 grouping³ 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-

⁽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 IC₅₀ is the concentration of morphine required to inhibit the muscle twitch by 50%.

⁽¹⁾ Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. Nature (London) 1976, 263, 663.

⁽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.