free bases of 22 and 23 with BBr_3 in $CHCl_3$ according to Rice.[10] Application of this procedure to 21 normally yielded 27. However, 24 could also be isolated after BBr_3 treatment of 21 by adding the theoretical amount of NH_3 to the ice-cooled reaction mixture and sufficient H_2O to dissolve the precipitating material. After the mixture was shaken for about 1 min, the $CHCl_3$ layer was separated and "dried" by filtration through several paper filters and evaporated under a stream of N_2. The target compound was isolated from the residue by PLC (10% MeOH in $CHCl_3$; R_f 0.35) and purified by crystallization from ethyl acetate.

Receptor Binding Assay. Binding experiments were performed, as previously described in more detail,[8] by incubating fixed amounts of a mitochondrial-synaptosomal fraction of rat brain homogenate in a medium of 50 mM Tris-HCl buffer of pH 7.4 at 25 °C, in the presence of 0.4 nM [^{3}H]fentanyl and either 40 nM levomoramide or 40 nM dextromoramide, to differentiate between opiate receptor binding and non-opiate-receptor-binding. Inhibitors were tested at three to five concentrations, and all incubations were carried out in duplicate. Incubations were terminated by rapid filtration through Whatman GF/B filters. Radioactivity on the filters was measured by liquid scintillation counting. Each experiment was repeated several times.

Statistical Evaluation of Receptor Binding Data. The combined variance of log IC_{50} determinations in our receptor binding assay was calculated from 137 independent observations on 47 test compounds by means of the SPSS program ONE WAY ANALYSIS OF VARIANCE (Version 70).[23] The combined variance was used in the Student's t test to evaluate the statistical significance of differences in ORA. In the case of two compounds both with $n = 3$, the difference is significant ($p < 0.05$) when the ratio of their mean IC_{50} values is larger than 1.563 or smaller than 0.640. Otherwise, it is denoted as nonsignificant (NS). In Tables I and II, most compounds are compared with fentanyl as the reference compound.

Acknowledgment. We are much indebted to Janssen Pharmaceutica for their help and their compounds ([^{3}H]fentanyl, levomoramide, and dextromoramide), to Endo Labs for their compounds (16 and 17), to H. J. A. Wijnne for the statistical analysis and discussions, to A. P. de Jong for his helpful comments, and to Mrs. B. Donkersloot and Mrs. H. Aveling for typing the manuscript.

(23) N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Brent, Eds., "SPSS, Statistical Package for the Social Sciences", 2nd ed., McGraw-Hill, New York, 1975, Chapter 22.

Allylprodine Analogues as Receptor Probes. Evidence That Phenolic and Nonphenolic Ligands Interact with Different Subsites on Identical Opioid Receptors

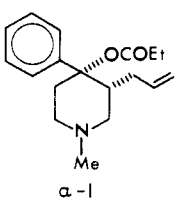
Philip S. Portoghese,* Bipin D. Alreja, and Dennis L. Larson

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
Received March 11, 1981

The *m*-hydroxy analogues of allylprodine and related structures have been synthesized and tested for narcotic agonist and antagonist activity on the electrically stimulated guinea pig ileum and by the hot-plate procedure in mice. It has been found that *m*-hydroxyallylprodine (α-2) is neither an agonist nor antagonist. Other phenolic congeners similarly have little or no activity. The fact that these results are in dramatic contrast with the structure–activity profile of morphine and closely related opiates has led to the proposal that the interaction of morphine and allylprodine (α-1) with the μ opioid receptor differs. This difference is postulated to arise from the recognition of the aromatic groups of morphine and α-1 by different aromatic-binding subsites of the receptor. These subsites are suggested to be identical with those which recognize the aromatic rings of the Tyr^1 and Phe^4 of the enkephalins and endorphins. A receptor model consistent with these results is proposed.

The role of the phenolic OH in enhancing the agonist potency of opiates and closely related compounds is well recognized. It has been proposed that the phenolic OH effects this enhancement by functioning as a hydrogen-bonding proton donor in the ligand–receptor association process.[1] The fact that the phenolic series often possess structure–activity profiles which differ substantially from the nonphenolic series has been attributed to divergent ligand–receptor binding modes.[1-3]

In order to investigate this phenomenon further we have synthesized and biologically evaluated phenolic analogues of allylprodine (α-1)[4-6] and its congeners. Allylprodine was selected for modification because it is considerably more potent than morphine.[5,6] Hence, it was of interest to determine the effect of a meta OH on agonist activity by analogy with the phenolic OH in morphine. If the phenolic analogue interacts with opioid receptors in a fashion similar to that of morphine, then such a modification should enhance potency. On the other hand, a substantial diminution of activity would be a manifestation of divergent modes of interaction with opioid receptors.



α-1

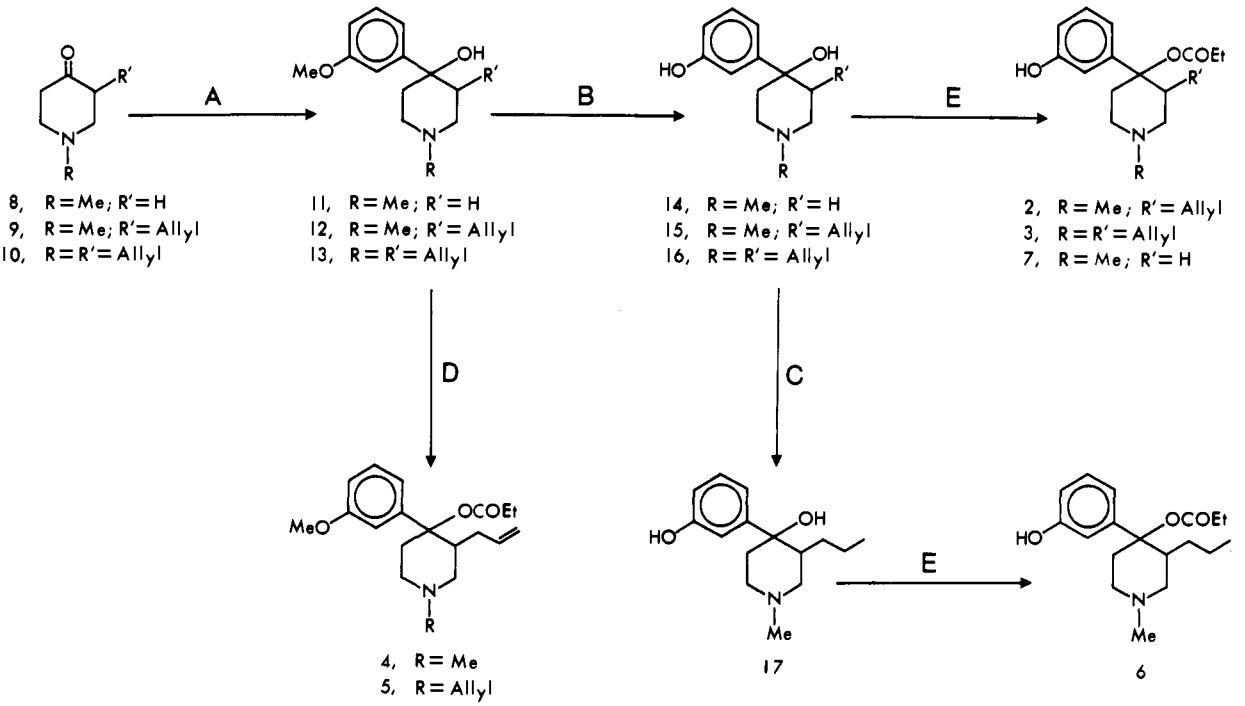
In this article we present evidence which suggests the latter possibility. A model consistent with the structure–activity relationship of the enkephalins[7] is proposed in order to account for the profoundly different structure–activity profiles between the allylprodine series and morphine-type compounds.

Chemistry. The first step leading to the piperidinol intermediates 11–13 in the synthesis of the target compounds (Table I) involved the condensation of *m*-anisyl-

(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) A. Ziering, A. Motchane, and J. Lee, *J. Org. Chem.*, 22, 1521 (1957).
(5) K. H. Bell and P. S. Portoghese, *J. Med. Chem.*, 16, 203 (1973).
(6) K. H. Bell and P. S. Portoghese, *J. Med. Chem.*, 16, 589 (1973).
(7) J. S. Morley, *Annu. Rev. Pharmacol. Toxicol.*, 20, 81 (1980).

0022-2623/81/1824-0782$01.25/0

Scheme I



lithium with the appropriately substituted piperidinones 8–10 (Scheme I). Piperidinone **10** was prepared by a procedure similar to that reported[5] for **9**; i.e., the Claisen rearrangement of 1-allyl-4-(allyoxy)-1,2,5,6-tetrahydropyridine. The piperidinols (**12** and **13**), which were derived from piperidinones **9** and **10** (method A), were formed as pairs of diastereomeric racemates in each case, with diastereomeric α/β ratios of approximately 16:1. The α racemates were purified by fractional crystallization of their HCl salts, while the β racemates were obtained in pure form by column chromatography.

The relative stereochemistry of the α racemate is *trans*-phenyl:allyl, while a cis disposition of the same substituents is the β form. The stereochemical identity of the racemates is based on numerous studies[8] which show a preponderance for the α racemate on the addition of phenyllithium to 1,3-disubstituted piperidinones. In this connection, an α/β ratio of 10:1 has been reported[5] in the synthesis of the piperidinol precursors of the closely related allylprodine (α-**1**).

Because of the susceptibility of the piperidinols **11–13** to dehydration under acidic conditions, the demethylation of the methoxyl function to afford the corresponding phenols **14–16** was carried out under conditions which assured the integrity of the C-4 hydroxyl group. This was accomplished with propanethiolate anion in refluxing DMF (method B).[9]

The saturated diastereomeric piperidinols **17** were prepared by catalytic hydrogenation of the 3-allyl group of **15** (method C). In order to prevent poisoning of the catalyst, it was necessary that the olefin not be contaminated with mercaptan from the prior demethylation step.

The acid lability of the piperidinols (**11–17**) also dictated the method selected for their esterification. Thus, the esterification method employed for the preparation of **4** and **5** (method D) involved the conversion to the lithium alkoxide by means of *n*-BuLi, followed by reaction with propionyl chloride.[10] An additional step was introduced into this procedure (method E) when the reaction was performed on the phenolic piperidinols (**14–17**). This involved the selective hydrolysis of the esterified phenolic function with $KHCO_3$ to afford the desired monoesters (**2**, **3**, **6**, and **7**).

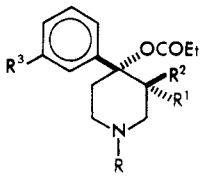
Pharmacology. The allylprodine congeners were evaluated by the subcutaneous route in mice using a modification[11] of the hot-plate procedure[12] and on the electrically stimulated guinea pig myenteric plexus longitudinal muscle preparation[13] (Table I).

With regard to agonist activity, (±)-allylprodine (α-**1**) was found to be 23 times more potent than morphine, which is close to the potency ratio of 15 reported[5] previously. In the ileal preparation, α-**1** possesses 4.5 times greater potency than morphine. By comparison, the phenolic analogue α-**2** was totally devoid of agonist activity in mice and on the ileal strip. The methylated derivative α-**4** was active but possessed reduced potency, having approximately one-sixtieth and one-fortieth the potency of allylprodine in vivo and in vitro, respectively. The 3-dealkyl congener **7** was $^1/_{200}$th and $^1/_{450}$th as potent as allylprodine (α-**1**) in the same tests. Analgesia due to compounds α-**1**, α-**4** and **7** is accompanied by the Straub tail phenomenon. All of the other congeners were inactive or considerably less potent as agonists.

The compounds were evaluated for narcotic antagonistic activity against an ED_{80} dose of morphine sulfate in mice. Only compound α-**3** had measurable antagonistic effects, with $^1/_{2500}$th the potency of naltrexone. Antagonism of the morphine response by α-**3** was accompanied by the disappearance of the morphine-induced Straub tail phenomenon. None of the synthesized compounds had any

(8) N. S. Prostakov and L. A. Gaivoronkaya, *Russ, Chem. Rev. (Engl. Transl.)*, **47**, 447 (1978).
(9) G. I. Feutrill and R. N. Mirrington, *Aust. J. Chem.*, **25**, 1719 (1972).
(10) E. M. Kaiser and R. A. Woodruff, *J. Org. Chem.*, **35**, 1198 (1970).
(11) P. S. Portoghese, Z. S. D. Gomaa, D. L. Larson, and E. Shefter, *J. Med. Chem.*, **16**, 199 (1973).
(12) N. B. Eddy and D. Leimbach, *J. Pharmacol. Exp. Ther.*, **107**, 385 (1953).
(13) H. P. Rang, *Br. J. Pharmacol.*, **22**, 356 (1965).

Table I. Narcotic Agonist and Antagonist Activities of Allylprodine Analogues



compd[a]	R	R^1	R^5	R^3	ED_{50},[b] μmol/kg (95% CL)	AD_{50},[b,c] μmol/kg (95% CL)	GPI rel agonist molar potency ± SEM[d]
α-1	CH_3	$CH_2CH{=}CH_2$	H	H	0.54 (0.35-0.84)		4.51 ± 1.08[e]
α-2	CH_3	$CH_2CH{=}CH_2$	H	OH	inact at 120	12% at 120	*f*
β-2	CH_3	H	$CH_2CH{=}CH_2$	OH	inact at 60	inact at 60	*f*
α-3	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	H	OH	inact at 110	74.13 (26.64-191.87)	*f*
β-3	$CH_2CH{=}CH_2$	H	$CH_2CH{=}CH_2$	OH	inact at 55	inact at 30	*f*
α-4	CH_3	$CH_2CH{=}CH_2$	H	OMe	34.67 (29.99-40.09)	inact at 15	0.12 ± 0.05[g]
β-4	CH_3	H	$CH_2CH{=}CH_2$	OMe	inact at 115	inact at 115	*f*
α-5	$CH_2CH{=}CH_2$	$CH_2CH{=}CH_2$	H	OMe	17% at 105	inact at 26	0.033[h]
α-6	CH_3	$CH_2CH_2CH_3$	H	OH	inact at 100	inact at 100	*f*
β-6	CH_3	H	$CH_2CH_2CH_3$	OH	inact at 100	inact at 100	*f*
7[j]	CH_3	H	H	OH	109.9 (86.6-139.0)	inact at 17	0.011[h]
morphine					12.42 (5.22-29.51)		1.0[i]
naltrexone						0.031 (0.02-0.04)	

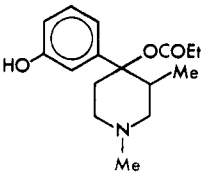
[a] All prodines (1-7) were tested in the form of racemic HCl salts; morphine and naltrexone were employed as the sulfate and HCl salts, respectively. [b] Molar potencies (ED_{50} and AD_{50}) derived from the mouse hot-plate procedure are based on the molecular weights of the free bases. [c] Compounds were evaluated for antagonist activity only at subagonistic dose levels. [d] Relative agonist molar potency (morphine = 1) on the electrically stimulated guinea pig myenteric plexus longitudinal muscle preparation. [e] Mean of 10 determinations. [f] Inactive at 10^{-6} M. [g] Mean of 3 determinations. [h] Mean of 2 determinations. [i] Mean of 18 determinations; morphine $IC_{50} = 6.97 \pm 4.75 \times 10^{-8}$ M. [j] Reported to be inactive as an agonist or antagonist.[19]

marked antagonistic effects against morphine on the ileal preparation. Thus, pA_2 values of ≤6 were observed in all cases (naloxone $pA_2 = 8.53 \pm 0.07$).

In order to determine whether allylprodine (α-1) and morphine are interacting with common receptors, the guinea pig ileal preparation was incubated with the irreversible receptor antagonist, β-FNA.[14-16] The IC_{50} values for morphine and α-1 before (control) and after incubation with β-FNA (treated) were determined and expressed as IC_{50} ratios [IC_{50} (treated)/IC_{50} (control)]. In three different ileal preparations the mean IC_{50} ratio was 5.9 for morphine and 5.7 for α-1. Although varying degrees of irreversible antagonism were produced by β-FNA, the difference between the IC_{50} ratios of morphine and α-1 obtained in the same preparation was small, suggesting that both agonists interact with identical receptors to equal extents.

Discussion

The dramatic loss of narcotic agonist activity upon introduction of a meta phenolic OH in allylprodine and propylprodine (Table I) is in marked contrast to the enhancement of agonist potency conferred by this group in morphine and conformationally related structures.[17,18] Since the phenolic analogues in the present series exhibit little or no narcotic antagonistic activity, it appears that the potency loss is related to a reduction of receptor affinity. Interestingly, the *N*-allyl substituent does not appear to confer substantial narcotic antagonist activity, as it does in the case of the morphine-related series. It is noteworthy that the phenolic analogues of α- and β-prodine (18) have been reported[19] to be devoid of agonist activity; however, the phenolic β-prodine 18 differs somewhat from the analogoues in the present series in that it behaves as a feeble narcotic antagonist.



18

Since the structure–activity profiles of allylprodine (α-1) and its analogues differ substantially from those of morphine and related structures, the possibility that α-1 mediates its effects through receptors that are distinct from those which bind morphine was investigated. This possibility was explored in experiments conducted with β-FNA, a specific irreversible blocker of μ opioid receptors.[14-16] These studies have revealed that the agonist effects of morphine and allylprodine (α-1) are blocked to nearly the same extent by β-FNA, thus suggesting that both of these ligands exert their effect at the same receptor.

The marked divergence in the structure–activity relationship between the allylprodine (α-1) series and the morphine series is, therefore, most likely due to differing modes of interaction at a common opioid receptor. Accordingly, the phenolic group contributes favorably to the

(14) β-FNA or β-funaltrexamine is the trivial name for 4,5α-epoxy-6β-[[3-(methoxycarbonyl)acryl]amido]-17-(cyclopropylmethyl)morphinan-3,14-diol.
(15) P. S. Portoghese, D. L. Larson, L. M. Sayre, D. S. Fries, and A. E. Takemori, *J. Med. Chem.*, **23**, 233 (1980).
(16) A. E. Takemori, D. L. Larson, and P. S. Portoghese, *Eur. J. Pharmacol.*, **70**, 445 (1981).
(17) J. Hellerbach, O. Schnider, H. Besendorf, B. Pellmont, N. B. Eddy, and E. L. May, "Synthetic Analgesics", Pergamon Press, Oxford, 1966.
(18) J. Reden, M. F. Reich, K. C. Rice, A. E. Jacobson, A. Brossi, R. A. Streaty, and W. A. Klee, *J. Med. Chem.*, **22**, 256 (1979).
(19) D. M. Zimmerman, R. Nickander, J. S. Horng, and D. T. Wong, *Nature* (London), **275**, 332 (1978).

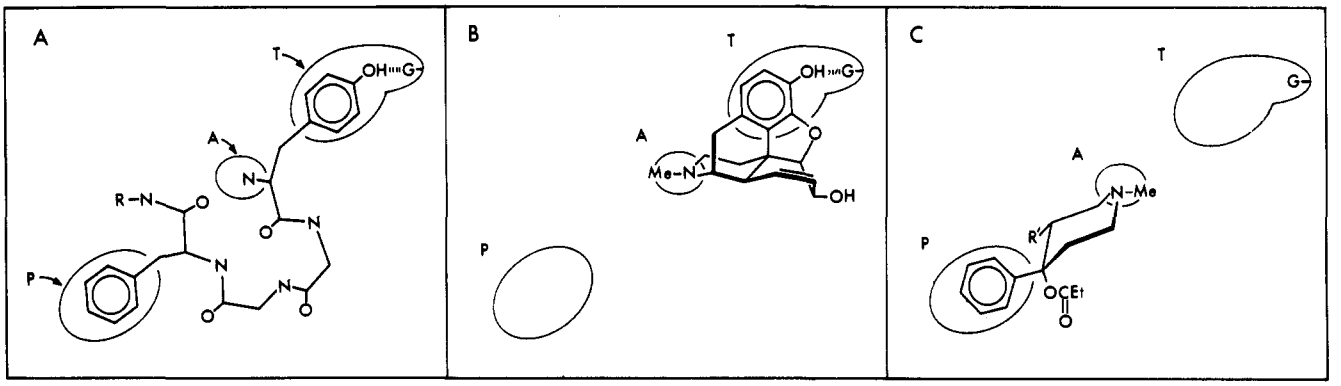
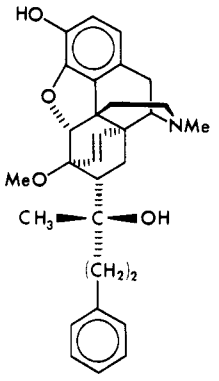


Figure 1. A schematic illustration of the interaction of enkephalins or endorphins (panel A), morphine (panel B), and allylprodine (panel C) with opioid receptor subsites (T and P) which recognize the aromatic residues of Tyr[1] and Phe[4] of the opioid peptides. The anionic site A is ion paired with the protonated nitrogen of the opioids in all three cases. Group G located on subsite T represents a hydrogen-bonding acceptor dipole.

binding of morphine but not to the binding of the allylprodine analogue α-**2**. Thus, the subsites responsible for the binding of the aromatic rings of morphine and of allylprodine may differ with respect to their recognition properties and location on the receptor.

The hypothesis of separate aromatic-binding subsites for morphine and allylprodine (α-**1**) is consistent with the fact that the endorphins and enkephalins contain two aromatic residues in their common recognition fragment, Tyr[1]-Gly[2]-Gly[3]-Phe[4].[7] This suggests the presence of complimentary subsites (designated in this discussion as T and P) on opioid receptors which recognize the aromatic residues of Tyr[1] and Phe[4], respectively. It has been proposed that analgesics such as morphine mimic the endogenous opioids by binding to the T subsite and that the high potency of oripavine derivative **19** is due to the simulta-



19

neous occupation of both the T and P subsites in a fashion analogous to the endorphins.[20] Numerous conformational studies on the enkephalins have been carried out in an effort to correlate the orientation of key pharmacophoric groups in such ligands with their biological activity.[21]

Structure–activity studies[7] of enkephalin analogues are consistent with the concept of separate subsites for the aromatic rings of morphine and allylprodine (α-**1**). For example, the requirement for the phenolic OH of Tyr[1] and the detrimental effect of a phenolic OH at the Phe[4] position reflect the different specificities of the T and P subsites. Furthermore, a number of structure–activity studies[7] suggest that the P subsite is more hydrophobic than the T subsite and that there is an additional hydrophobic area adjacent to the P subsite.

A model which is in harmony with this array of data is based on the multiple modality concept.[1] In order to illustrate the salient features of this model with respect to the difference between the modes of interaction of morphine and allylprodine, the solid-state conformation of [Leu[5]]enkephalin[22] is employed; however, this is not meant to imply that it is the same as the receptor-bound conformation. Accordingly, the aromatic ring of morphine and of allylprodine (α-**1**) are each envisaged to be capable of association with receptor subsites (T and P, respectively) which normally bind the aromatic residues of Tyr[1] and Phe[4] of enkephalin (Figure 1). Also, an anionic site which ion pairs with the NH_3^+ group of Tyr[1] is illustrated to be involved in the association with the cationic nitrogens of morphine and allylprodine. The phenolic group of morphine contributes to the binding by hydrogen bonding with a proton-acceptor group at the T subsite. This has been suggested[1] previously as a contributing factor in the enhancement of the analgesic potency of phenolic opiates (and closely related congeners), as compared to their methoxy and deoxy analogues. Binding to discrete subsites accounts for the remarkable difference in the structure–activity relationship of 3-allylprodine analogues from that of the morphine series, since the hydrophobic nature of the P subsite and its adjacent area might render unfavorable the presence of a hydrophilic phenolic OH. The fact that masking the phenolic OH of α-**2** with a methyl group (α-**4**) restores some of the analgesic activity (Table I) is consistent with this view.

The phenolic allylprodine analogue α-**2** is postulated not to bind to the T subsite because of its different conformation and constitution relative to that of morphine and structurally related phenols.[3] Thus, the preferred equatorial phenyl group and the 3-allyl substituent might in part play a role in the binding of allylprodine (α-**1**) at the P subsite, while these same features might not be as favorable for recognition at the T subsite. Moreover, as consequence of the equatorial aromatic group, the phenolic OH of α-**2** might not be in proper alignment with the receptor dipole which presumably is involved in the hydrogen bonding of the phenolic OH attached to the axially oriented aromatic ring of morphine.

It is conceivable that the expression of narcotic antagonism is associated with binding of the antagonist molecule to the T subsite of the opioid receptor. The fact that a phenolic OH in all cases enhances narcotic antagonist activity[23] is consistent with this view.

(20) A. F. Bradbury, D. G. Smith, and C. R. Snell, *Nature (London)*, **260**, 165 (1976).
(21) F. A. Gorin, T. M. Balasubramanian, C. G. Barry, and G. R. Marshall, *J. Supramol. Struct.*, **9**, 27 (1978).
(22) G. D. Smith and J. F. Griffin, *Science*, **199**, 1214 (1978).

Table II. 1,3-Dialkyl-4-aryl-4-piperidinols

compd	mp, °C	crystn solvent	yield,[a] %	formula[b]
11	112-113	hexane	72	$C_{13}H_{19}NO$
α-12	86-87	hexane	97[c]	$C_{16}H_{23}NO_2$
β-12	201-202 dec	acetone/ether		$C_{16}H_{23}NO_2 \cdot HCl$
α-13	116-117	*i*-PrOH/Et_2O	95[c]	$C_{18}H_{25}NO_2 \cdot HCl$
β-13	164-165	EtOAc		$C_{18}H_{25}NO_2 \cdot HCl$
14	237 dec	EtOH/Et_2O	57	$C_{12}H_{17}NO_2 \cdot HCl$
α-15	248 dec	EtOH/Et_2O	50	$C_{15}H_{21}NO_2 \cdot HCl$
β-15	213-214	EtOH/Et_2O	66	$C_{15}H_{21}NO_2 \cdot HCl$
α-16	182-183	EtOH/Et_2O	48	$C_{17}H_{23}NO_2 \cdot HCl$
β-16	211-212 dec	EtOH/Et_2O	65	$C_{17}H_{23}NO_2 \cdot HCl$
α-17	226-227	MeOH/Et_2O	87	$C_{15}H_{23}NO_2 \cdot HCl$
β-17	213-214	EtOH/Et_2O	61	$C_{15}H_{23}NO_2 \cdot HCl$

[a] Unless otherwise specified, this represents the percent yield after purification. [b] All compounds were within ±0.4% of theory for C, H, N analyses. [c] Combined yield of α and β racemates prior to separation by chromatography; ratio of $\alpha/\beta \approx 16$.

Table III. 1,3-Dialkyl-4-aryl-4-piperidinyl Propionates

compd	mp, °C	crystn solvent	yield,[a] %	formula[b]
α-2	179-180	acetone/EtOAc	61	$C_{18}H_{25}NO_3 \cdot HCl$
β-2	191-193	acetone/EtOAc	52	$C_{18}H_{25}NO_3 \cdot HCl$
α-3	188-189	2-butanone/EtOAc	78	$C_{20}H_{27}NO_3 \cdot HCl$
β-3	188-189	2-butanone/EtOAc	49	$C_{20}H_{27}NO_3 \cdot HCl \cdot 0.5H_2O$
α-4	147-148	2-butanone/ether	83	$C_{19}H_{27}NO_3 \cdot HCl$
β-4	179-180	2-butanone/ether	84	$C_{19}H_{27}NO_3 \cdot HCl$
α-5	137-138	2-butanone/ether	57	$C_{21}H_{29}NO_3 \cdot HCl$
α-6	131-134 dec	acetone	28	$C_{18}H_{27}NO_3 \cdot HCl \cdot 0.5H_2O$
β-6	202-204 dec	acetone	75	$C_{18}H_{27}NO_3 \cdot HCl \cdot 0.5H_2O$
7	196-198	acetone/EtOAc	63	$C_{15}H_{21}NO_3 \cdot HCl \cdot 0.5H_2O$

[a] Yield from the precursor alcohol after purification to analytical purity. [b] All compounds were within ±0.4% of theory for C, H, N analyses.

In summary, the receptor model proposed is a more detailed description of the version first published in 1965.[1] Structure–activity relationship studies of the endorphins and enkephalins suggest the possibility of at least two different binding modes for opioid ligands. The first, which is typified by morphine binding, involves occupation of the subsite (T) which normally interacts with the Tyr[1] aromatic ring of the enkephalins and endorphins. The second, which is exemplified by allylprodine (α-1) binding, involves the interaction of its phenyl group with the subsite (P) which is complimentary to the aromatic residue of Phe[4]. Each of the binding modes possesses a common receptor area which contains an anionic site as illustrated in Figure 1.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of the theoretical values. IR (Perkin-Elmer 281), NMR (Varian HFT-80, XL-100, and T-60D), and MS spectra (AEI MS-30) are consistent with the assigned structures. All chemicals and solvents were of reagent grade.

(±)-1,3-Bis(2-propenyl)-4-piperidinone (10). This was prepared by a procedure similar to that described for piperidinone 9.[5] *p*-Toluenesulfonic acid monohydrate (70.77 g, 0.37 mol) was refluxed in benzene (800 mL) during which water (8.4 mL) was collected in a Dean-Stark trap. After 225 mL of benzene had been distilled and the flask cooled, 1-allylpiperidin-4-one[24] (48.65 g, 0.35 mol), 2,2-dimethoxypropane (59.51 g, 0.56 mol), and allyl alcohol (69.81 g, 1.19 mol) were added. The mixture was heated under total reflux conditions for 3 h and then heated alternately on total reflux and collection modes over the next 12 h, collecting distillate at 56 °C. When IR analysis of an aliquot showed the disappearance of the C=O absorption (1720 cm^{-1}), toluene (300 mL) was added and distillates at 76 (benzene/allyl alcohol azeotrope) and 80 °C were collected. Refluxing for an additional 15.5 h gave total conversion to **10**. The layers were separated and the bottom layer was poured into water (40 mL). The upper toluene layer was washed with 40 mL of water, which was pooled with the lower aqueous layer. The combined aqueous solution was washed with hexane (2 × 20 mL), basified (pH 11) with NaOH (20% solution), and NaCl was added. Crystals of sodium *p*-toluenesulfonate, which separated upon standing and chilling, were filtered off and washed several times with ether. The product was extracted from the aqueous mixture by shaking with ether. The combined ethereal extract (1.8 L) was washed with brine (3 × 20 mL) and dried (Na_2SO_4). Removal of ether in vacuo gave an oil, which was twice distilled to afford 41.02 g (66%) of pure **10**: bp 54.5–55 °C (0.1 mmHg); TLC R_f 0.49 (silica gel GF, ether), MS (70 eV), *m/e* 179 (M^+). It is necessary to distil **10** immediately before use.

4-(3-Methoxyphenyl)-1,3-dialkyl-4-piperidinols (11–13). Method A. To a stirred THF (60 mL) solution of *m*-bromoanisole (0.1 mol) maintained under N_2 at −55 °C was added 0.1 mol of *n*-BuLi in hexane (54 mL) over 0.5 h. After the mixture was stirred at −50 °C for 2 h, the appropriate piperidinone (0.06 mol of **8–10**) in THF (15 mL) was added dropwise with stirring at −45 °C. The mixture was stirred at 25 °C for 0.5 h, cooled to −10 °C, and quenched with ice–water (350 mL). Following acidification to pH 3 with 6 N HCl, the mixture was extracted with Et_2O (3 × 125 mL). The aqueous phase was basified (pH 11) with aqueous NaOH (20%) and the product (**11–13**) was extracted with ether. After the solvent was dried (Na_2SO_4) and removed the piperidinols were obtained in yields ranging from 72 to 97%. The α-diastereomeric piperidinols (α-**12** and α-**13**) were purified by fractional crystallization of the HCl salt or free base. The α diastereomers (β-**12** and β-**13**) were obtained from the mother liquors of the crystallization of the α isomers by dry-column chromatography on silica gel. The α/β ratio of the isolated piperidinols was approximately 16:1.

4-(3-Hydroxyphenyl)-1,3-dialkyl-4-piperidinols (14–16). Method B. Piperidinols **11–13** (1 mmol) were stirred with NaH (6.6 mmol) in anhydrous DMF (6 mL) under N_2 at 25 °C. *n*-Propanethiol (4.75 mmol) was added dropwise, and the reaction mixture was refluxed (4 h), cooled (0 °C), quenched with ice–water

(23) S. Archer and W. F. Michne, *Prog. Drug Res.*, **20**, 45 (1976).
(24) N. Barbulescu, C. Greff, and C. Balaita, *Rev. Chim. (Bucharest)*, **20**, 193 (1969); *Chem. Abstr.*, **71**, 38844 (1969).

(15 mL), and acidified (pH 4) with 6 N HCl. After extraction with Et_2O, the aqueous phase was made basic with $NaHCO_3$ and extracted with EtOAc. The EtOAc extract was dried (Na_2SO_4), the solvent was removed in vacuo, and the products (14–16) were converted to the HCl salts with stoichiometric quantities of ethanolic HCl.

4-(3-Hydroxyphenyl)-1-methyl-3-propyl-4-piperidinol (17). Method C. A solution of the HCl salt of each diastereomeric piperidinol 15 (1 mmol) in 95% ethanol (4 mL) was added to prereduced PtO_2 (0.029 g) in the same solvent (20 mL) and was stirred in hydrogen gas (atmospheric pressure) at 20 °C until uptake was complete (2.5 h). The catalyst was removed by filtration and was washed with EtOH. The filtrate was taken to dryness in vacuo and residual water was removed as an azeotrope by successive additions and evaporation of absolute EtOH.

4-(3-Methoxyphenyl)-1,3-dialkyl-4-piperidinyl Propionates (4 and 5). Method D. The HCl salts of piperidinols 11–13 (1 mmol) in anhydrous THF (5 mL) were treated under N_2 at −45 °C with *n*-BuLi (4.8 mmol) in hexane (2.2 mL). After stirring for 1 h, the mixture was warmed to 0 °C, propionyl chloride (8 mmol) in THF (1 mL) was added in divided portions, and the mixture was refluxed for 4 h. The mixture was cooled, treated with ice–water (25 mL), adjusted to pH 3, and extracted with Et_2O. The aqueous solution was basified with saturated $NaHCO_3$ solution and extracted with ethyl acetate. The ethyl acetate extract was dried (Na_2SO_4), and the product was converted to the HCl salt with ethanolic HCl.

4-(3-Hydroxyphenyl)-1,3-dialkyl-4-piperidinyl Propionates (2, 3, 6, and 7). Method E. The HCl salts of piperidinols 14–17 (2 mmol) in anhydrous THF (10 mL) were treated dropwise with *n*-butyllithium (7.5 mmol) in hexane (4.0 mL). The mixture was stirred under N_2 at −40 °C for 20 min and at 25 °C for 10 min. Propionyl chloride (4.4 mmol) in THF (2 mL) was added dropwise, and the solution was stirred at 25 °C for 1 h and refluxed for 4 h. The mixture was adjusted to pH 4 and extracted with ether. The aqueous phase (50 mL) was treated with $KHCO_3$ (5 g) and methanol (40 mL) and stirred for 24 h at 23 °C. The product was extracted with ethyl acetate, dried (Na_2SO_4), and converted to the HCl salt by addition of ethanolic HCl.

Action of Compounds on the Guinea Pig Ileum Myenteric Plexus. The preparation used was a strip of longitudinal muscle[13] dissected from a segment of guinea pig ileum, taken from 10 to 15 cm above the ileocecal junction. The muscle strip was mounted under a tension of 1-g weight in a jacketed 10-mL organ bath containing bucarbonate-buffered Krebs solution at 37 °C an bubbled with 5% carbon dioxide in oxygen. The composition (in millimolar strengths) of Krebs solution was as follows: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.52; $MgSO_4$ 1.19; KH_2PO_4, 1.19; $NaHCO_3$, 25; dextrose, 11.48. Chlorpheniramine maleate (1.2 nM) was added as an antihistaminic. The preparation was stimulated by the application of coaxial supramaximal (90 V) pulses of 0.5-ms duration every 10 s from a Grass 44A square wave stimulator. The electrodes consisted of two vertical platinum wires mounted on either side of the muscle. The twitch-like contractions of the longitudinal muscle, amplified through a mechanoelectrical transducer (Statham 0019), were recorded isometrically on a Beckman Type R411 dynograph recorder. Compounds were applied by the addition of microliter quantities of aqueous solutions containing the HCl salt of the organ bath. Potencies of compounds that were active in concentrations of 10^{-7} M and below were evaluated in at least three independent preparations. Naloxone hydrochloride (2×10^{-6} M) was added to the organ bath to determine whether the inhibition of the twitch caused by the compound was reversible.

The antagonist potencies of the compounds were determined by the method of Kosterlitz and Watt.[25] Dose ratios of 2 and above were used for the determination of the pA_2 value. The dose ratio is the ratio of concentrations of morphine required to depress the twitch to the same extent in the presence or absence of a given antagonist concentration.

The interaction of allylprodine with opioid receptors in the guinea pig ileum was studied using the novel opioid receptor directed alkylating agent, β-FNA.[14-16] After IC_{50} values for morphine and allylprodine had been determined, the preparation was incubated with β-FNA (1×10^{-8} M) for 60 min. The preparation then was washed (20 times) and the IC_{50} values for allylprodine and morphine were evaluated on the FNA-treated ileum. The IC_{50} ratio, which represents the IC_{50} of the compound after treatment of the preparation with β-FNA divided by the control IC_{50} of the compound on the same preparation, was determined for allylprodine and morphine. For three preparations, the mean IC_{50} ratios for morphine and allylprodine, respectively, were (1) 3.73, 3.50; (2) 3.36, 2.72; and (3) 10.69, 10.80.

Evaluation of Narcotic Agonist and Antagonist Activity in Mice. The agonist potencies of the target compounds (Table I) were determined in mice using a modification[11] of the hot-plate procedure of Eddy.[12] The compounds were administered to the mice subcutaneously in physiological saline solution. A group of at least five 25–35 g male, white Swiss-Webster mice (Bio-Lab, St. Paul, MN) was used for each dose level examined. Reaction times were determined three times for every mouse at 15-min intervals before injecting the drug to establish a reaction time control value.

For the assessment of narcotic agonist activity, reaction times were recorded 15 min after subcutaneous administration of the phenolic diastereomers (2, 3, 6, and 7) and allylprodine (α-1). Reaction times were recorded 10 min after subcutaneous administration of methoxy compounds 4 and 5 and 30 min after the administration of morphine sulfate. Mice were judged to exhibit analgesia when their postinjection reaction time was greater than double their preinjection control reaction time. A cutoff time of 30 s was used for animals which did not leave the hot plate. The average of the preinjection reaction times for saline control (20 mice) was 7.26 (SD = 3.37) and the average for the postinjection interval was 6.83 (SD = 2.55). ED_{50} values were determined by probit analysis according to the methods of Stanley.[26]

Narcotic antagonist activity was assessed by reversal of morphine analgesia, using the quantal response of the hot-plate test. An approximate ED_{80} dose of morphine sulfate (8 mg/kg) was administered subcutaneously to groups of at least five mice per dose of compound, whose preinjection reaction times had been established. Compounds were administered 15 min after morphine pretreatment, and the hot-plate assay was conducted 30 min after the injection of morphine. Antagonist potency of naltrexone was determined by coadministration with morphine. The criterion for the assignment of analgesia was the same as was used for agonist potency. Injection of saline 15 min after morphine pretreatment, followed by hot-plate assay 15 min later, served as the control. The AD_{50} of the antagonist, which is the dose causing 50% reversal of the effect of an ED_{80} dose of morphine, was determined by probit analysis.

Acknowledgment. The authors thank Dr. Lawrence Sayre for valuable discussion during the preparation of this manuscript. This research was supported in part by NIDA Research Grant DA 02220.

(25) H. W. Kosterlitz and A. J. Watt, *Br. J. Pharmacol. Chemother.*, 33, 266 (1968).

(26) J. Stanley, "The Essence of Biometry", McGill University Press, Montreal, 1963, p 127.