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# Proposal for the biologically active conformation of opiates and enkephalin

(opiate receptor/neuroblastoma × glioma/morphine/x-ray crystallography)

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**ABSTRACT** **A model for the opiate receptor has been defined by using a computer-based molecular display and x-ray crystallographic input data. The model can explain the stereochemical fashion in which the morphine, morphinan, and oripavine classes of compounds interact with the receptor. The minimal structural unit of the enkephalins demonstrated to be pharmacologically active, Tyr-Gly-Gly-Phe, was also fitted to this model by using a systematic search of conformational space.**

The discovery, in the central nervous system, of endogenous peptides possessing opioid activity has prompted proposals comparing the structural similarity of the enkephalins with the opiate compounds of the morphine and oripavine family (1–4). There is, unfortunately, much dispute regarding the topography of the chemical groups responsible for the biological activity (5–8) of the opiates. Because of the diversity of chemical structures having narcotic activity, it has been suggested that different sites exist on the narcotic receptors capable of binding chemically distinct groups corresponding to the different classes of narcotics (6, 8). Alternatively, it is possible that there is more than one type of opioid receptor mediating the opiates' analgesic effect. This latter possibility seems to be discounted in such *in vitro* preparations as rat neostriatum (9) and neuroblastoma-glioma hybrid cell line NG108-15 (10). In both systems, opiates and enkephalins have been shown to depress intracellular cyclic AMP levels by inhibiting a prostaglandin-stimulated adenylate cyclase (11). This physiological response is completely antagonized by naloxone. In the neuroblastoma-glioma cell line, displacement of radioactive naloxone by opiates appears to be competitive, and analysis of opiate binding suggests a single class of opioid receptors (11, 12). Similar binding behavior has been observed for bioassays such as guinea pig ileum (13) and mouse vas deferens (14). In these preparations, electrically stimulated contractions are inhibited by both opiates and enkephalins and this inhibition is reversed by naloxone. For guinea pig ileum and mouse vas deferens, good correlation has been demonstrated between *in vitro* potency and *in vivo* analgesic efficacy with most opiates (15). Analogous evaluation of the enkephalins is hampered by their rapid *in situ* degradation (16). However, their pharmacologic action is identical to that of the opiates in these *in vitro* systems and is antagonized by naloxone (9, 10). Because of their complex pharmacology (7, 17), it is likely that the opiates and, perhaps, the enkephalins act *in vivo* at several species of receptors. However, the *in vitro* systems cited above provide a means of examining structure-activity relationships for opiates and enkephalins on a presumably homogeneous class of narcotic receptors.

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## PROPOSED OPIATE PHARMACOPHORE

X-ray crystallographic data of morphine hydrochloride (18, 19), naloxone hydrochloride (20), 7-α-[1-(*R*)-hydroxyl-1-methylbutyl]-6,14-*endo*ethenotetrahydrothebaine hydrobromide (THT) (21), and methadone hydrobromide (22) were compared by using a PDP 11/40 computer-based molecular graphics system with GT-40 display similar to that described in ref. 23 (Fig. 1). THT is the 3-methoxy analog of the 6,14-C-bridged oripavine, etorphine (24). As was the case with morphine and methadone, etorphine was shown by Klee and Nirenberg (25) to displace [$^3$H]naloxone in the neuroblastoma × glioma hybrid line NG108-15 (25). The five fused rings of the morphinans and the six fused rings of the bridged thebaines considerably restrict their conformational freedom. However, x-ray data reveal that the C ring† atomic positions are different among the different compounds with the exception of C5 and C14 (Table 1). THT's bicyclo (2:2:2) octene cage formed by the 6,14-*endo*etheno bridge considerably distorts what would be equivalent to morphine's C ring into a pseudo-chair. However, the C18 and C17 atoms of the *endo*etheno bridge assume positions that correspond to morphine's C7 and C8, respectively, and reestablish the spatial arrangement of the C ring. Because of their obvious similarities to the diaromatic enkephalins, the 5-phenylbenzomorphans and methadone series were explored. The 5-phenylbenzomorphan derivative, GPA 1657, was computer-simulated by using the skeleton of morphine with the C ring replaced by a 5-substituted phenyl ring. The phenyl ring was positioned to correspond to published nuclear magnetic resonance data (26). Good correlation was found between the 1″ and 2″ positions of the phenyl ring corresponding to the C5 and C6 atoms of morphine (Table 1; Fig. 2). Similar results with space-filling models have been obtained for methadone (data not shown).

Earlier proposals suggested that opiate activity was conferred by the particular spatial arrangement of the phenolic ring of morphine's phenanthracene nucleus, separated from a tertiary amine by two or three methylenes (27, 28). These two chemical substituents are necessary but not sufficient conditions to define such an opiate pharmacophore. Tyramine possesses these attributes and sufficient conformational freedom to adopt the appropriate spatial configuration but is inactive as an opiate. The model we propose differs from a simple tyramine moiety in that C5 and C6 of the C ring of morphine are considered to play an important role in the opiate pharmacophore. This additional site of interaction of the pharmacophore can account

Abbreviation: THT, 7-α-[1-(*R*)-hydroxyl-1-methylbutyl]-6,14-*endo*ethenotetrahydrothebaine hydrobromide.

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† C ring corresponds to morphine's ring III of Mackay and Hodgkin (19).

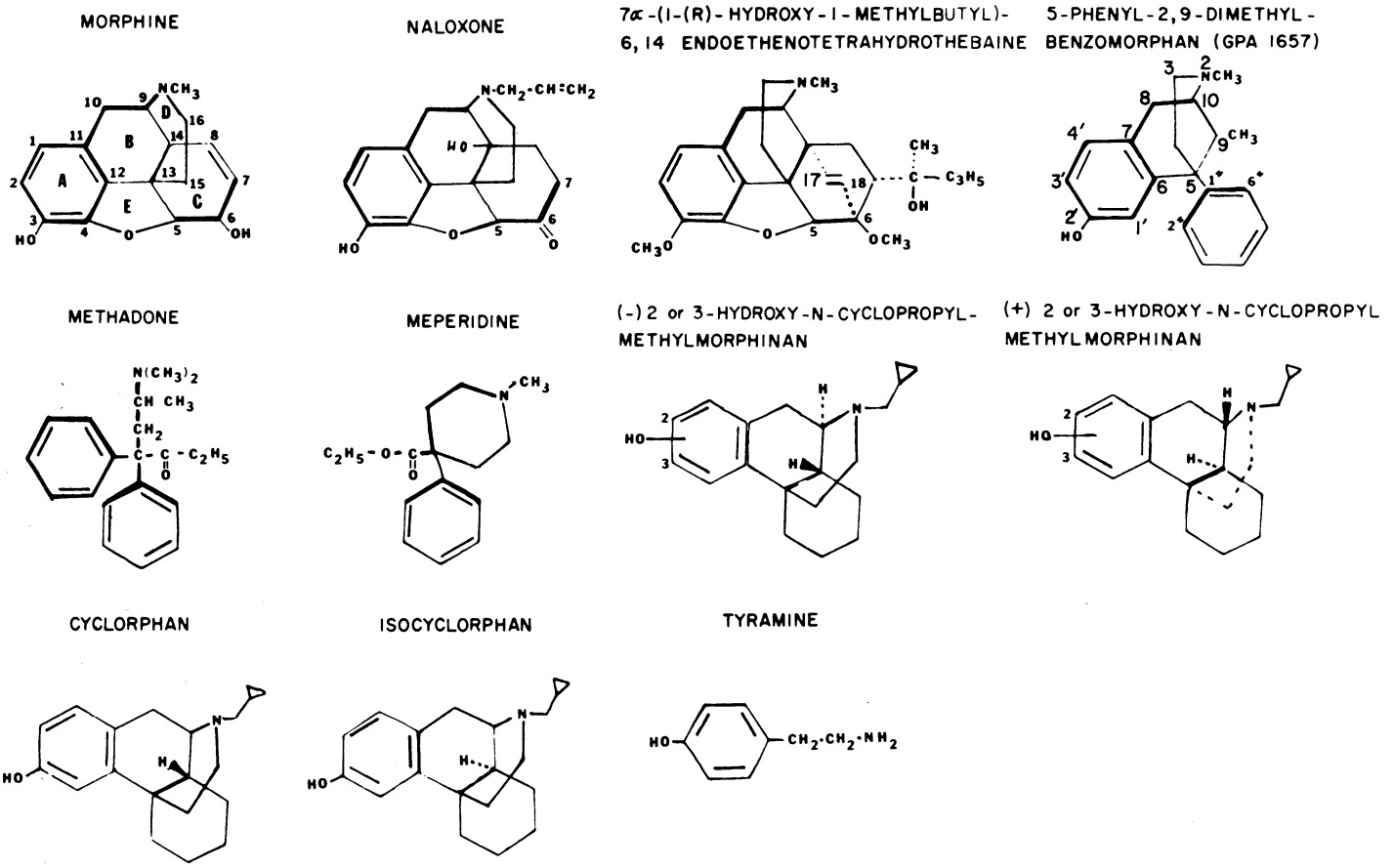



FIG. 1. Structures of compounds that are either opiate agonists or antagonists. Tyramine has no opioid activity.

for the stereospecificity of opiate activity. The importance of C-ring orientation is demonstrated by cyclorphan (Fig. 1), a potent agonist possessing a *trans* A/C ring configuration analogous to that of the the morphines and morphinans. Its epimer, isocyclorphan, which has a *cis* A/C configuration, has little or no agonistic activity, although both compounds possess considerable antagonistic activity (30).

Even when existing in the proper *trans* A/C ring configuration, it is likely that subtle spatial reorientation of C6, and perhaps C7, affect opiate potency. Examination of the distance between the aromatic ring center to C7 demonstrates the considerable disparity (0.6 Å) between the antagonist naloxone and the agonists morphine and THT (Table 1). In THT, it is C18 of the *endo*etheno bridge which corresponds to C7 of the C ring

Table 1. Interatomic distances in several opiate compounds

| | Distance, Å | | | | Mean (±SD) interatomic distance, Å |
|---|---|---|---|---|---|
| | Morphine | Naloxone | THT* | GPA 1657† | |
| Nitrogen atom to: | | | | | |
| C5 | 4.37 | 4.36 | 4.31 | 4.35 (1′) | 4.35 ± 0.03 |
| C6 | 5.10 | 5.22 | 4.84 | 5.18 (2′) | 5.09 ± 0.17 |
| C7 | 4.90 | 4.93 | 4.87 (C18) | | 4.90 ± 0.03 |
| C8 | 3.82 | 3.81 | 3.86 (C17) | | |
| C14 | 2.45 | 2.43 | 2.46 | 2.47 | 2.45 ± 0.02 |
| Aromatic ring center to: | | | | | |
| C5 | 3.37 | 3.30 | 3.36 | 3.32 (1′) | 3.34 ± 0.03 |
| C6 | 4.16 | 3.91 | 4.28 | 4.05 (2′) | 4.10 ± 0.16 |
| C7 | 3.97 | 4.57 | 3.91 (C18) | | 4.15 ± 0.26)‡ |
| C8 | 3.77 | 3.96 | 3.49 (C17) | | |
| C14 | 3.66 | 3.70 | 3.49 | 3.66 | 3.63 ± 0.09 |

* 7-α-[1-(*R*)-Hydroxymethyl butyl]-6,14-*endo*ethenotetrahydrothebaine.

† 1-β-2′-Hydroxy-2,9-dimethyl-5-phenyl-6,7-benzomorphan.

‡ Mean interatomic distance excluding naloxone = 3.94 ± .03. (See *text* for details.)

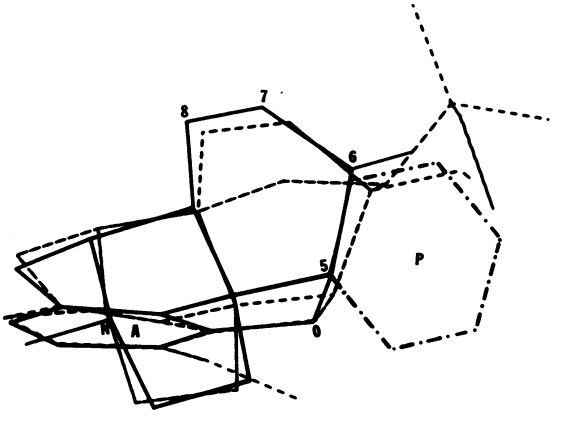



FIG. 2. Computer display of x-ray structures of morphine (—), THT (- - - -), and GPA 1657 (— · — · —) demonstrating correspondence of phenolic A rings (A), nitrogen atoms (N), and the 5-substituted phenyl ring of GPA 1657 (P) with C5 (5) and C6 (6) of morphine and THT.

of morphine and naloxone. This disparity between agonists and antagonists is due to the conversion of the hydroxyl at C6 to a ketone and C14-*cis*-hydroxyl substitution in naloxone (Fig. 1). The C7-C8 double bond of morphine is saturated in both THT and naloxone. Using guinea pig ileum, Kosterlitz and Waterfield (5) have shown with the *N*-cyclopropylmethylmorphine series that C6 ketone substitution alone increases both agonal properties and binding affinity. C14-Hydroxyl substitution in conjunction with C6 keto modification abolishes agonal potency while maintaining comparable binding affinity. An analogous study with *N*-cyclopropylmethylmorphinans demonstrated that C14-hydroxyl substitution alone converts cyclorphan (*N*-cyclopropylmethylmorphinan) to a potent antagonist with binding affinity about comparable to that of the parent compound. Thus it appears that C14-hydroxyl substitution confers antagonistic properties by yet some unknown mechanism, whereas C6 keto modification reorients C6 and, more dramatically, C7 to enhance receptor binding affinity. In this paper we intend to define only the agonist pharmacophore. There is evidence to suggest that two forms of the receptor exist and show different specificities, one agonal and the other antagonal (31).

Recently reported studies of analogs of enkephalins support the requirement of an opiate pharmacophore consisting of a phenolic ring separated from a tertiary amine by two or three methylenes. [desamino-Tyr$^1$, Met$^5$]-Enkephalin is biologically inactive and incapable of binding to rat brain tissue (32). Chemical modification of the hydroxyl of the phenolic ring reduces potency and the Phe$^1$ derivative of [Leu$^5$]-enkephalin shows only slight biological activity on mouse vas deferens and guinea pig ileum (33). Furthermore, it has been shown that the tripeptide Tyr-Gly-Gly is pharmacologically inactive (34) and incapable of displacing the specifically bound component of naloxone from brain tissue (32). The tetrapeptide Tyr-Gly-Gly-Phe appears to be the minimal structural unit capable of binding to brain tissue and eliciting a biological response (33–35). Despite its considerable proteolytic lability (16), the tetrapeptide has been reported to have 1% of the biological activity of [Met$^5$]-enkephalin when tested on guinea pig ileum and mouse vas deferens (33, 34). Its binding affinity for synaptic plasma membranes is said to be 3% of that of [Met$^5$]-enkephalin (35). In contrast, the tripeptide Tyr-Gly-Gly shows no binding affinity or biological activity whatsoever in the same preparations. It is our contention that an essential feature for agonist activity has been lost by the elimination of residue 4, similar to the lack of activity seen with tyramine. The low potency (1–3%) seen with the tetrapeptide is not relevant to the definition of the essential requirements for activity but may reflect differences in affinity, distribution, and proteolytic lability compared to the parent pentapeptides.

## MODELING ENKEPHALIN TO THE OPIOID PHARMACOPHORE

A minimum of 10 rotatable bonds is required to fit the smallest biologically active fragment of enkephalin (Tyr-Gly-Gly-Phe) to the proposed opiate pharmacophore. To decrease the conformational freedom of residue 2, D-alanine was used because [D-Ala$^2$, Met$^5$]-enkephalinamide has been shown to be a potent enkephalin analog (36). The side chain torsional angles of Tyr$^1$ were set at $\chi_1 = 197°$ and $\chi_2 = -106°$ so that the amino terminus and phenolic ring of Tyr$^1$ spatially corresponded to the phenolic A ring and nitrogen atom of morphine. This gave an aromatic ring center-to-nitrogen distance of 4.54 Å for the peptide compared to morphine's 4.54 Å, naloxone's 4.48 Å, and THT's 4.50 Å as measured from x-ray crystallographic data.

Table 2. Description of conformer of Tyr-D-Ala-Gly-Phe consistent with proposed opiate pharmacophore

| | |
|---|---|
| $\chi_2$ | (Tyr$^1$) = − 163° |
| $\chi_1$ | (Tyr$^1$) = − 106° |
| $\Psi_1$ | (Tyr$^1$) = 129° |
| $\Phi_2$ | (D-Ala$^2$) = 160° |
| $\Psi_2$ | (D-Ala$^2$) = −87° |
| $\Phi_3$ | (Gly$^3$) = − 118° |
| $\Psi_3$ | (Gly$^3$) = 98° |
| $\Phi_4$ | (Phe$^4$) = − 87° |
| $\chi_1$ | (Phe$^4$) = − 87° |
| $\chi_2$ | (Phe$^4$) = − 56° |

The major additional assumption of this model, as opposed to previous models (2–4), is a correspondence between the *para* and *meta* positions of Phe$^4$ and the C5 and C6 atoms of the C ring of morphine suggested by the orientation of 1″ and 2″ of the phenyl ring in 5-phenyl-2,9-dimethylbenzomorphan. Two target points were positioned relative to the phenolic ring and amino terminus of Tyr$^1$ so as to correspond spatially to C5 and C6. A conformational search program, BURLESK (37), systematically explored the torsional angle space of the eight rotatable bonds of the backbone of the tetrapeptide and the sidechains of Phe$^4$ with 31° increments. By using a hard-sphere approximation, each sterically allowed conformation was examined to see if it was within 0.4 Å of the positions of the target atoms. After 5.5 days of computations, a single conformer was found in which the *meta* position of Phe$^4$ was within 0.22 Å of the position of the C6 target. A summary of angles is shown in Table 2.

**Other Opiate Models.** Several investigators have proposed that an F ring corresponding to the 19-phenethyl substituent on 7-(1-phenyl-3-hydroxybutyl-3)-*endo*ethenotetrahydrothebaine (PET) enhances the potency of certain opiate agonists (Fig. 3) but is not essential for conferring opioid activity (2, 4, 38). These investigators postulated that the aromatic ring of Phe$^4$ of the enkephalins corresponds to this F ring. It has been proposed by Bradbury *et al.* (2) that the two glycyl residues in enkephalin assume the position of a $\beta$-bend with a hydrogen bond between the carbonyl group of [Tyr$^1$] and the amide nitrogen group of Phe$^4$. However, if one extends the potency argument of the F ring to enkephalin, one expects that, although

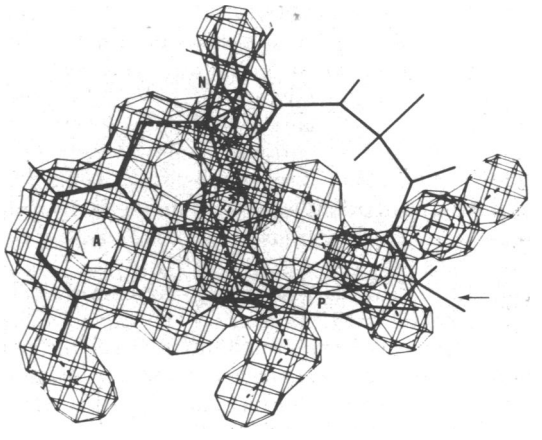
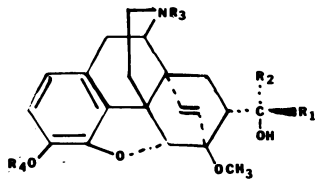



FIG. 3. Superposition of the computer-generated conformer of Tyr-D-Ala-Gly-Phe with a space-filling representation of THT. There is correspondence between (*i*) the phenolic rings of THT and [Tyr$^1$] (A), (*ii*) the nitrogen atoms of the D ring of THT and nitrogen terminus of the peptide (N), and (*iii*) the C6 of THT and the *meta* position of the phenyl ring of [Phe$^4$] (P). The arrow indicates the carboxyl terminus of the tetrapeptide fragment of enkephalin.




| | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| ETORPHINE | $-C_3H_7$ | $-CH_3$ | $-CH_3$ | $-OH$ |
| "PET" | $-C_2H_4$  | $-CH_3$ | $-CH_3$ | $-OCH_3$ |
| ALLETORPHINE | $-C_3H_7$ | $-CH_3$ | $-CH_2CH{=}CH_2$ | $-OH$ |
| CYCLOPROPYLMETHYLETORPHINE | $-C_3H_7$ | $-CH_3$ | $-CH_2$  | $-OH$ |

FIG. 4. Members of the C-bridged oripavine ($R_4$ = OH) and thebaine ($R_4$ = $OCH_3$) families. These compounds are two to three orders of magnitude more potent agonists than morphine despite their nitrogen substituents. The phenethyl substituent of "PET," $R_1$, corresponds to the F-ring model of Feinberg *et al.* (4). "PET," 7-(1-phenyl-3-hydroxybutyl-3-)-*endo*ethenotetrahydrothebaine.

Tyr-Gly-Gly-Phe should be more potent than Tyr-Gly-Gly, the latter compound should exhibit at least weak binding to the opioid receptor in neuroblastoma × glioma and brain tissue homogenates, but several studies have found this fragment to be totally inactive (12, 32). Furthermore, emphasis of this F-ring model tends to obscure the significant opioid activity of members of the morphine, morphinan, and etorphine families which do not possess an F ring. Several compounds cited by Feinberg *et al.* (4), such as phenazocine, fentanyl, etonitazene, methopholine, and *N*-methylphenylmorphan, can fit the model proposed in this paper as well as the F-ring model.

The conformation of enkephalin obtained by the systematic search suggests, in fact, that the $R_1$ sidechain of the C-bridged oripavines are better modeled to the side chains of $Met^5$ or $Leu^5$ than to $Phe^4$ as in the F-ring model (Fig. 3). With Dreiding models, the $\beta$ and $\gamma$ carbons of these side chains approximately correspond to the $R_1$ = butyl in etorphine. Heuristically, this observation does correspond with the important potency role played by $R_1$ and $R_2$ substitutions on the 19-substituted ethyl carbinols of the thebaine and oripavine families (Fig. 4). In the *N*-methyl, *N*-allyl, and *N*-cyclopropylmethyl derivatives of thebaine and oripavine, if $R_2$ = methyl, agonist potency increases with $R_1$ alkyl chain length, maximizing activity in the *n*-propyl to *n*-butyl range (38, 39). Alletorphine, *N*-cyclopropylmethylnoretophine, and etorphine are powerful analgesics, two to three orders of magnitude more potent than morphine. Both [$Leu^5$]- and [$Met^5$]-enkephalin have $\beta$ and $\gamma$ carbons in their side chains that can correspond to the side chain of these powerful analgesics. Our model of the opiate pharmacophore argues for a potency role for the fifth residue of enkephalin and is based on the observation that Tyr-Gly-Gly-Phe is the minimal structural unit possessing biological activity. The model further suggests that increasing the aliphatic side chain of the fifth residue should be accompanied by a progressive increase in activity as one goes from $Gly^5$ or $Ala^5$ to $Val^5$, $Ile^5$, and $Leu^5$, assuming the proteolytic lability of the pentapeptide remains unchanged for the different substituents. More precise modeling of the fifth residue of enkephalin is now being done with the cyclohexano derivative of 6,14-*endo*ethenotetrahydrooripavine which has been found to be 1000 times more potent than morphine (38).

***N*-Alkyl Substitution Conferring Antagonism.** This proposed model of the opiate receptor does not explain antagonistic properties that result from *N*-substitution among the morphine, morphinan, and benzomorphan families. Oxymorphone (7,8-dihydro-14-hydroxymorphinone), a potent agonist possessing a C-ring configuration analogous to the "pure" antagonist naloxone, is converted to the latter by replacement of an *N*-methyl with an *N*-allylic group. Such *N*-substitution is ineffective for the methadones and meperidines and is anomalous for the C-bridged *endo*ethenothebaines. In the latter instance, *N*-cyclopropylmethylnoretorphine is an analgesic 1000 times more potent than morphine, and alletorphine is 50 to 100 times more potent than morphine (Fig. 4). The importance of C14-hydroxyl substitution in the introduction of antagonistic properties in morphinans and morphines has been discussed previously. Such a substitution could conceivably affect the conformation of the C ring, the conformation of the six-membered nitrogen D ring, and the spatial orientation of the *N*-alkyl substituent. One might argue that the agonal potency of the bridged *endo*ethenothebaines is immune to *N*-substitution because the *endo*etheno bridge locks the C ring configuration. However, this argument still leaves in question the mechanism by which the antagonistic transformation of the morphine, morphinan, and benzomorphan families occurs with *N*-alkylic substituents.

Feinberg *et al.* (4) suggested that the antagonistic nature is conferred to morphinan compounds by confining the *N*-substituent side chain to an equatorial position relative to morphine's D ring. According to the diagrams of their model, a "pure" agonist has its *N*-alkyl substituent confined to the axial position, and C14-hydroxyl substitution favors the equatorial position. A stereochemical question is then posed because most evidence suggests that the opiates, with $pK_a$ values of about 9.0, are protonated at the receptor site. This is consistent with enkephalin's amino terminus having a $pK_a$ of approximately 9. It is well documented that noncyclic quaternary amines can undergo rapid stereochemical inversion (40). There is evidence, however, that quaternization of morphine's nitrogen atom occurs in a stereospecific fashion (41, 42). In fact, it has been our conclusion from x-ray data that crystals of all the acidic salts of morphine, naloxone, THT, and codeine are protonated in a stereospecific fashion and localize the *N*-alkyl substituent solely in the equatorial configuration. Energy calculations (43) that assume a rigid D ring, as does the model of Feinberg *et al.* (4), calculate the "best" axial configuration of morphine's methyl group to be 5.7 kcal/mol less stable than that of the optimized equatorial configuration. For oxymorphone (a potent agonist) and naloxone (a "pure" antagonist), the equatorial conformations are favored by 12 and 20 kcal, respectively, making it unlikely that differences in biological activity of these latter two compounds could be due to different equilibria of the *N*-alkyl axial-equatorial configurations. Belleau *et al.* (44) demonstrated, by the synthesis of five-membered nitrogen D ring analogs of morphine (which were totally inactive), that orientation of the nitrogen lone pair is critical for biological activity. Conversely, the stereochemistry of the *N*-alkyl group must be an important consideration. Nuclear magnetic resonance spectral analysis of protonated opiates would indicate whether specific quaternization of a ring-constrained nitrogen is possible in the morphine alkaloids as has been observed, for instance, with 3-phenyltropidine hydrobromide (45).

**Reservations.** This model for the analgesic pharmacophore utilizes the previously recognized requirement of the phenolic ring and tertiary amine of morphine. To be consistent with the stereospecific activity of the morphine, morphinan, and ori-

pavine classes of compounds, it is also proposed that atoms C5 and C6 of the C ring of morphine are an additional requirement. It is assumed that there is correspondence between the tyramine moiety and the aromatic ring and nitrogen of $Tyr^1$ and also between C5, C6, and the aromatic ring of $Phe^4$. With these constraints, a single conformation was found for the smallest active fragment of enkephalin when conformational space was searched on a 31° grid. Two kinds of objections can be raised against this model. The first is the designation of the pharmacophore and assignment of corresponding groups in enkephalin. The justification in these choices is presented above but may bear modification as additional structure-activity data are collected. A second objection is the coarseness of the grid scanned for acceptable conformations. The actual orientation of the ring observed only showed correspondence with C6. Further refinement of the ring orientation to show alignment with C5, C7, or C8 is under consideration. Due to the combinatorial nature of the systematic search, however, the sheer number of computations places severe constraints on the number of increments to be scanned for each rotatable bond. For this reason, the conformation of Tyr-Gly-Gly-Phe proposed as the one bound to the analgesic receptor must be viewed as a working hypothesis against which active analogs can be checked for consistency. All active analogs that we have examined (approximately 40) can adopt the conformation proposed.

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