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New Opiate–Receptor Model

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Abstract □ A new opiate–receptor model is proposed in which only one conformation of the receptor is needed for binding of both agonists and antagonists. There are two different spacially fixed amine-binding sites in this model: one agonist and one antagonist. The opiates undergo binding to their amine-binding sites *via* the lone electron pair on nitrogen. The role of the *N*-allyl or other such group in imparting antagonist properties is explained in terms of the steric requirements of this group. For this group to be accommodated without imposing severe steric interactions in the rest of the opiate molecule, the piperidine ring must assume a flexible (skew boat) conformation; in this conformation, the *N*-lone-pair electron lobe assumes the characteristic directionality of an antagonist toward its amine-binding site. If the *N*-lone-pair lobe is not rigorously maintained in this direction, the opiate molecule assumes both antagonist and agonist conformations and mixed antagonist–agonist activity is observed. The observed differences in the effect of sodium on the degree of binding of an agonist *versus* an antagonist can be explained in this model by the different effects of sodium on the two amine-binding sites. The antagonist activity of an *N*-methyl antagonist can be rationalized on the basis of the proposed model.

Keyphrases □ Opiate–receptor model—proposed, only one receptor conformation needed for binding both agonists and antagonists □ Model, opiate–receptor—proposed, only one receptor conformation needed for binding both agonists and antagonists □ Binding—opiate agonists and antagonists to receptor, new model proposed

Recently, Snyder and coworkers (1, 2) proposed a model of the opiate receptor to explain structure–activity relationships of opiate agonists and antagonists. They postulated that the receptor can exist in two different conformations: the antagonist conformation (a sodium-binding form) and the agonist conformation (no sodium form). On the basis of the qualitative conformational analysis of a series of opiate agonist and antagonist molecules, the following spacially fixed binding sites on the receptor were proposed (2): lipophilic, amine binding, agonist binding, and specific antagonist binding (Fig. 1).

To account for the observation that substitution of the *N*-methyl of an opiate agonist by an *N*-allyl or *N*-cyclopropylmethyl usually confers antagonist activity, Snyder and coworkers (2) suggested that: "Presumably, the *N*-allyl or other such group interacts with a portion of the opiate receptor regulating antagonist activity." This portion of the receptor in Fig. 1 is the specific antagonist binding site. Their view, therefore, is one interpretation of the data.

BACKGROUND

In the Snyder model, the amine-binding site is defined as the ionic site that interacts with the amine nitrogen. The protonated amine has been considered (3, 4) the active form at the receptor site. From the pKa of morphine (7.88), Beckett and Casy (3) estimated that it would be 80% protonated at physiological pH. However, it is not unreasonable to believe that the unprotonated amine is, in fact, the active species because of the nature of the amine-binding site. In this respect, a relatively high pKa value (a thermodynamic, not kinetic, value) may have an adverse effect on binding.

Figure 1 indicates that, in the Snyder model, the *N*-lone-pair electron lobe of naloxone is pointed away from the amine-binding site, while the *N*-lone-pair lobe of morphine is directed toward this binding site. It seems improbable that interaction between naloxone and the amine-binding site would occur in the juxtaposition shown in this figure. Only if the interaction between the amino nitrogen and the amine-binding site were chemical could a binding interaction in the conformation shown in Fig. 1 be reconcilable, and then only as a minor collision process in a cross-

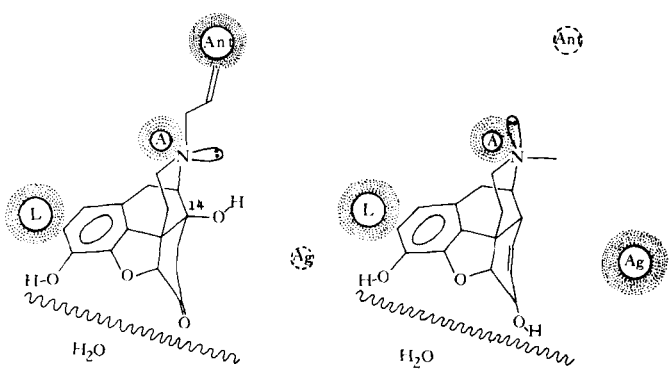

Figure 1—*Snyder's model for binding of naloxone (antagonist) and morphine (agonist) to the receptor. Key: left, naloxone bound to the antagonist conformation of the receptor; right, morphine bound to the agonist conformation of the receptor; L, lipophilic site; A, amine-binding site; Ag, agonist-binding site; and Ant, specific antagonist-binding site.*

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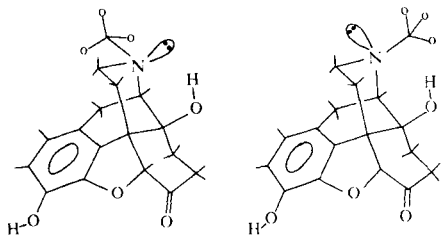


Figure 2—*Some conformations of oxymorphone. Key: left,* N-*methyl equatorial conformation; right,* N-*methyl axial conformation; and small circles, hydrogens of the* N-*methyl group. Other hydrogens are not shown.*

reaction (5). Therefore, if it can be demonstrated that the amine-binding site interacts with the N-lone-pair electron lobe of opiate bases, the Snyder model for the binding of naloxone to the receptor is not tenable.

Figure 1 also shows that, in the Snyder model, specific conformations of morphine and naloxone bind to the receptor and exhibit activity. The reason for the choice of these particular conformations was not made clear. Moreover, these conformations are not the most stable ones (*vide infra*).

While morphine (Fig. 1), oxymorphone (Fig. 2), and levorphanol were depicted by Snyder (1) in conformations in which the N-methyl is axial–chair, conformations in which the N-methyl of these compounds is equatorial–chair should be more stable. Thus, simple Van der Waals calculations indicate that more than 90% of N-methylpiperidine will be in the conformation in which N-methyl is equatorial–chair (6). Also, on the basis of dipole moment measurements on different systems, the axial–equatorial equilibrium for the N-methyl of N-methylpiperidine (in the chair conformation) was determined (ΔG = 1.5 kcal/mole) and showed that the equatorial placement of N-methyl was much preferred (6). Also, X-ray analysis of morphine hydroiodide dihydrate showed the equatorial–chair orientation of the N-methyl (7); however, extrapolation of this result to the solution state would be tenuous.

Loew and Berkowitz (8) showed, by PCILO calculations, that the conformation of N-methylpiperidine in which the N-methyl is axial–chair is less stable by 5.7 kcal/mole than the corresponding equatorial conformer. They also calculated that the axial–chair N-methyl conformer of oxymorphone (Fig. 2) is 12 kcal/mole less stable than the equatorial–chair conformer (8). Finally, there is now very strong experimental evidence that the N-methyl group in N-methylpiperidine prefers the equatorial position by about 3.0 kcal/mole (9).

Loew and Berkowitz (8) concluded that "the energy difference of 5.7 kcal/mole between the best axial and equatorial conformers would make the axial conformer only barely accessible to the drug by interaction in the biophase of the receptor." On this basis, they proposed that the more stable, equatorial–chair conformers of morphine and oxymorphone are most likely to bind to the receptor site. In contrast, the Snyder model indicates that the less stable, N-methyl axial–chair conformations of morphine and oxymorphone bind to the receptor.

On the other hand, with respect to naloxone, Snyder and coworkers (1, 2) considered the equatorial–chair conformation as the most stable (Fig. 1) and the one responsible for binding to the receptor. However, in view of the fact that the N-allyl group of naloxone might sterically influence the relative stabilities of its conformers, a qualitative conformational analysis was performed. As suspected, this steric effect is quite impressive and, surprisingly, it is very apparent in the equatorial–chair conformation while least apparent in the pseudoequatorial–skew boat conformation. The latter, therefore, appears to be the most stable conformation of naloxone. This observation, together with those already noted, led to the proposed new opiate–receptor model.

CONFORMATIONAL ANALYSIS[1]

A molecular model of naloxone was built according to that depicted by the Snyder model (1, 2). In this conformation (Fig. 3, I), the piperidine ring is in the chair conformation and the N-allyl group is equatorial.

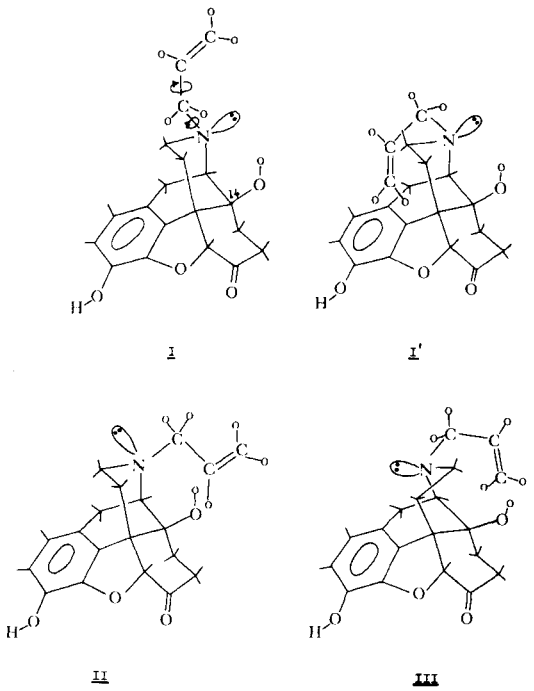


Figure 3—*Some conformations of naloxone. Small circles represent hydrogens of the* N-*allyl and 14-OH. Other hydrogens are not shown. Conformations (except I) are shown to point out the most unfavorable steric interactions.*

Snyder and coworkers (1, 2) referred to this conformation as the one allowing free N-allyl rotation and being the most stable compared to the N-allyl axial–chair conformer (Fig. 3, II) in which N-allyl rotation is reduced by interaction with the 14-OH.

However, careful study of the molecular model of the equatorial–chair rotamers (Fig. 3, I and I') reveals that the stated free rotations around the single bonds, N–C–C=C, are, in fact, subjected to very severe steric interactions. On the contrary, if the piperidine ring assumes the skew boat (flexible) form in which N-allyl is pseudoequatorial (Fig. 3, III), the N-allyl group exhibits virtually complete rotational freedom without introducing any apparently unfavorable interactions (the molecular models illustrate that eclipsing of ring hydrogens is absent in this skew boat form as well as in the chair form).

On these grounds, it is reasonable to conclude that the most stable form of naloxone is the skew boat conformation in which the N-allyl is pseudoequatorial.

Qualitative conformational analysis of other opiate molecules in this study (*vide infra*) was performed by the following method. The equatorial–chair, axial–chair, pseudoequatorial–boat, and pseudoaxial–boat conformations of the drug molecule were built. The N-substituent in each of these four conformations was rotated, and the least stable rotamer was found. The least stable rotamers must be considered because they reduce the overall stability of the system, even though there is an infinite number of more stable rotamers. The order of stability of these conformations was established. (Any conformational analysis book can be used for classifying unfavorable interactions—1,3-interactions, eclipsing, *etc.*—and for evaluating the energies of these interactions as well as the energies of chair–boat interconversion, *etc.*) Numerous examples (5, 10) illustrate the order of magnitude of unfavorable interactions necessary to force the piperidine ring into a boat conformation.

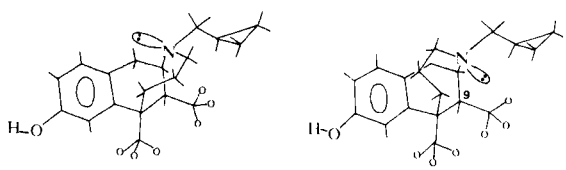
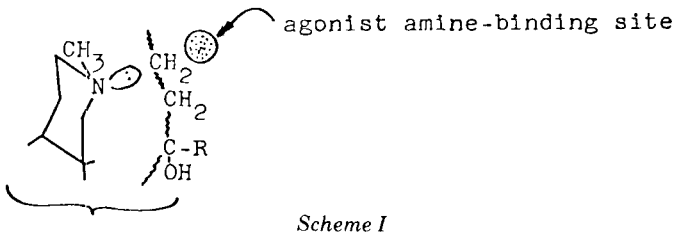


Figure 4—*Some conformations of* trans-*homobenzomorphan cyclazocine homolog. Key: left, conformation with a nonhindered* N-*lone pair; right, conformation with hindered* N-*lone pair; and small circles, methyl hydrogens. Other hydrogens are now shown.*

[1] Qualitative conformational analysis was performed on Japanese HGS Maruzen B models. A quantitative conformational analysis of morphine-type agonists and antagonists by ^{13}C-NMR spectrometry is planned.



Scheme I

RESULTS AND DISCUSSION

It is reasonable to believe that the most stable form of naloxone is the skew boat conformation in which the *N*-allyl is pseudoequatorial (*vide supra*) and that this form interacts with the receptor. On this basis, the antagonist activity of naloxone may be explained differently from that of the Snyder model. Thus, it is seen from conformation III that the *N*-lone-pair electron lobe is not hindered and is pointed approximately to the left, in a line parallel to one passing through the 3-OH and 6-keto groups. It is proposed that an antagonist amine-binding site is situated in this direction and that the primary action on a receptor by an antagonist may be through its *N*-lone pair that is pointing toward the antagonist amine-binding site. The antagonist effect of naloxone, therefore, would be derived indirectly through the *N*-allyl group whose steric effect forces the *N*-lone pair in the direction of the antagonist amine-binding site[2].

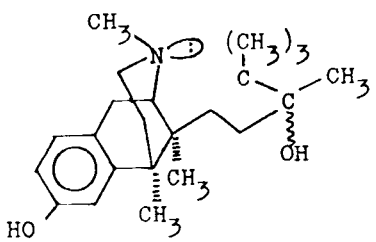
If the *N*-allyl group of naloxone is replaced by an *N*-methyl group, oxymorphone, an agonist, is obtained (Fig. 2). The most stable conformation (*N*-methyl equatorial–chair) of oxymorphone will probably bind to the receptor. The *N*-lone-pair lobe in this conformation is pointed to the right at about 45° from the plane in which the model sits. It is proposed that, in this direction, an agonist amine-binding site is placed and that the *N*-lone-pair lobe of an agonist interacts with this amine-binding site.

Similar conformational analysis of *cis*- and *trans*-homobenzomorphan cyclazocine homologs (*N*-cyclopropylmethylazacycloheptane opiates) is performed by moving the *N*-cyclopropylmethyl group between the axial and equatorial positions (10) and by the easy flipping of the azacycloheptane ring through its conformations, which are known to be flexible (11, 12). Thus, the *trans*-compound (antagonist) exists in two stable conformations (Fig. 4). The *N*-lone-pair electron lobe of one conformer is hindered by the 9-methyl, while the *N*-lone-pair lobe of the other conformer is pointed in the antagonist direction and is not hindered.

The *cis*-isomer assumes the analogous antagonist conformation but can also exist in another stable agonist conformation (Fig. 5). Thus, the *cis*-compound should show mixed activity, as was found to be the case (2). The *trans*-isomer cannot assume this agonist conformation without imposing severe steric restrictions on the 9-methyl group.

The same type of conformational analysis would account for the agonist, antagonist, and mixed activity of levorphanol, etorphine, nalorphine, levallorphan, benzomorphans, pentazocine, cyclazocine, and related compounds.

The introduction of the F-ring (2) or a moiety simulating it into the opiate molecule makes the analysis of the structure–activity relationship more complicated because of the new variables simultaneously introduced. Thus, the observed strong agonist effect imparted by the introduction of an F-ring (2) may compensate or overcome the antagonist effect of the molecule. The strong binding of the F-ring to the agonist



IV

[2] Concrete evidence for the importance of the *N*-lone electron pair orientation in stereospecific opiates for productive interaction with the opiate receptor was reported [B. Belleau, T. Conway, F. R. Ahmed, and A. D. Hardy, *J. Med. Chem.*, 17, 907 (1974)].

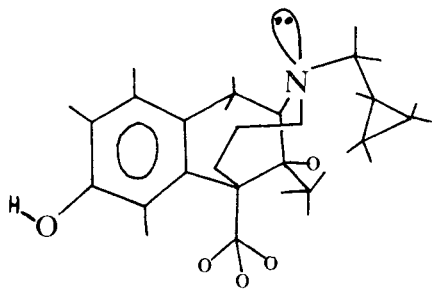


Figure 5—*Agonist conformation of* cis-*homobenzomorphan cyclazocine homolog. Small circles are hydrogens of the* trans-*methyl. Other hydrogens are not shown.*

binding site (Fig. 1) may slightly change the juxtaposition of the molecule with respect to the receptor and shift the *N*-lone-pair lobe away from the antagonist amine-binding site.

Furthermore, it is possible that a long alkyl side chain (*e.g.*, at a carbinol carbon of an *N*-methyloripavine), instead of folding into the F-ring conformation and causing agonist activity, will assume a different conformation that will block the *N*-lone pair of the agonist conformation, thereby obstructing interaction with its amine-binding site (Scheme I). The piperidine ring may flip into a skew boat conformation, which would permit hydrophobic interactions of the *N*-methyl with the alkyl side chain as well as polar interactions of the *N*-lone pair with the antagonist amine-binding site, the overall effect being antagonist activity.

The fact that an *N*-methyl compound has now been found to possess antagonist properties strongly supports the proposed concept. Michne *et al.* (13) reported two isomers, differing only in the chirality of the carbinol carbon, of Compound IV. One isomer (mp 242–247°) showed strong antagonist activity (of at least 60% of the potency of nalorphine). The reversed chirality at the carbinol carbon of the other isomer (mp 219–223°) destroyed the antagonist activity. The large difference in antagonist activity effected merely by a change in chirality at the carbinol carbon can be easily rationalized by Scheme I.

The proposed model was applied to numerous compounds with known activities. In no instance did the model predict antagonist activity for a compound that was an agonist, or *vice versa*, or in any other way fail to explain classic features of opiate pharmacology.

In summary, the salient features of this model are as follows. Only one conformation of the receptor is needed for binding of both agonists and antagonists. While lipophilic and agonist binding sites are the same as in the Snyder model, the amine-binding site and specific antagonist binding site are changed. There are two distinct, spacially fixed, amine-binding sites in this proposed model: one agonist amine-binding site and one antagonist amine-binding site. Agonists and antagonists interact with their respective amine-binding sites *via* the lone-pair electrons on their nitrogen atoms.

In this model, it is suggested that the primary action of an antagonist on the receptor may be through the interaction of the antagonist *N*-lone pair and its amine-binding site and not through lipophilic interaction of the *N*-allyl chain with the specific antagonist binding site. The main role of the *N*-allyl (or *N*-cyclopropylmethyl) chain is to force the *N*-lone-pair lobe of an antagonist molecule in the direction of its amine-binding site. This situation is achieved when the antagonist assumes a geometry in which its piperidine ring is in a flexible (skew boat) conformation and the *N*-allyl chain is pseudoequatorial. The specific antagonist binding site of the Snyder model thus becomes less important, or even not important, for antagonist activity.

Finally, in the proposed model, a change in sodium concentration affects the two amine-binding sites differently, resulting in the observed (1, 2) differences in the effect of sodium on the degree of binding by an agonist *versus* an antagonist.

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Modified Technique Using Perfused Isolated Guinea Pig Lung to Determine Effect of an Aerosol Constituent on Pulmonary Dynamics

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Received August 5, 1977, from the *Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John's University, Jamaica, NY 11439.* Accepted for publication November 10, 1977.

Abstract □ Polyethylene glycol 400, a commonly used plasticizer in many cosmetic aerosol hair sprays, was tested to determine its effect on perfusion pressure, perfusion vascular flow rate, and tidal volume in the perfused isolated guinea pig lung. Negative pressure was maintained within the chamber housing the intact lungs, and initial perfusion of the pulmonary vasculature was accomplished *via* the right side of the heart *in situ* after guinea pigs were sacrificed by decapitation. Polyethylene glycol 400 was injected into the pulmonary arterial system in doses of 0.01–30 μg/ml after pretreatment with isoproterenol (1 μg/ml). Isoproterenol was then readministered, followed by nebulized doses of the cosmetic chemical into the trachea at 0.3, 3, and 30% concentrations. Nebulized polyethylene glycol 400 was also administered in 50, 70, and 90% concentrations. Polyethylene glycol 400 significantly increased perfusion pressure and flow rate after injection into the pulmonary arterial system of the isolated lung. In addition, nebulized administration in concentrations of 0.3–30% significantly increased the perfusion flow rate; following the 50–90% nebulized doses, a significant increase in both perfusion pressure and flow rate was observed. Tidal volume decreased, regardless of the route of administration, as increasing doses of the cosmetic constituent were delivered to the isolated lung.

Keyphrases □ Polyethylene glycol 400—effect on pulmonary dynamics in perfused isolated guinea pig lung □ Pulmonary dynamics—effect of polyethylene glycol 400 in perfused isolated guinea pig lung □ Aerosol constituents—polyethylene glycol 400, effect on pulmonary dynamics in perfused isolated guinea pig lung

The relation between inhalation of airborne particles and pulmonary disease has been known for some time (1). Numerous cases of pulmonary thesaurosis were attributed to the inhalation of cosmetic hair sprays, although the question of whether cosmetic aerosols actually cause pulmonary disease is still controversial (2–5). Zuskin and Bouhoys (6) observed, under controlled experimental conditions, that short lasting and significant decreases in the air expiratory flow rate from the lung occurred following acute exposures to various commercial hair sprays. The purposes of this investigation were to develop a simplified, improved version of the perfused isolated guinea pig lung and to employ it as a model by which responses to the administration of a cosmetic constituent (polyethylene glycol 400) could be monitored to determine whether or not it alters pulmonary dynamics.

EXPERIMENTAL

Lung Chamber and Perfusion Apparatus—A modified method of Bhattacharya and Delaunois (7) was used for perfusion of the isolated guinea pig lung. The apparatus that housed the lungs consisted of a cylindrical Plexiglas chamber, 16 cm high and 11 cm wide. Resting within the chamber on three pegs, cemented to and situated in a triangular pattern around the inner wall, was a Plexiglas funnel with its bottom located directly above a glass mason jar. The top of the jar was screwed into the bottom of the chamber with a single hollow nylon nut and bolt.

A Plexiglas elbow and connecting tube, cemented to the exterior bottom of the chamber, led from the inside to the outside through a small hole in the chamber floor. The base of a three-way tube was connected to the outside Plexiglas tube of the elbow. One side of the three-way tube led into a calibrated mercury manometer[1]; the other side was conected to a second three-way tube, which had its base ttached to the expiration outlet of a small animal respirator[2]. The other side of the second three-way tube was connected to the air intake valve of a vacuum pump[3] (Fig. 1).

The chamber's upper end was closed with a Plexiglas plate. A piece of polyethylene tubing[4] that served as the perfusion cannula was passed through a small opening in the top of the chamber. The other end of the tubing was attached to a metal needle (20 gauge), which had the male end of a three-way valve inserted into its base. This valve served as an injection port through which test materials were administered into the pulmonary arterial vasculature of the isolated guinea pig lung.

Perfusion of the pulmonary vasculature was accomplished *via* a solid-state veristaltic pump[5]. It pumped aerated Tyrode solution from

[1] Fisher Scientific Co., Springfield, N.J.
[2] Model V5KG, E&M Instrument Co., Houston, Tex.
[3] Model 0211V45F, Gast Manufacturing Corp., Benton Harbor, Mich.
[4] PE-60 (0.08 cm i.d., 0.12 cm o.d.), Clay Adams, Becton, Dickinson and Co., Parsippany, N.J.
[5] Portable combination pressure/vacuum pump (model 0211-V45F. G8CX), Manostat Corp., New York, N.Y.

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