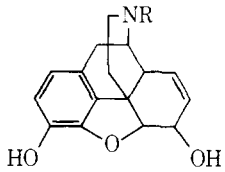
Quantum Chemical Studies of Morphine-Like Opiate Narcotics. Effect of N-Substituent Variations

Gilda H. Loew* and Donald S. Berkowitz

Department of Genetics, Stanford University Medical Center, Stanford, California 94305. *Received December 2, 1974*

Quantum chemical calculations including extensive conformational variations are performed on three morphine-like analgesics with varying N-substituents using the PCILO and INDO methods. The three compounds, morphine, nalorphine, and *N*-phenethylmorphine, have been shown experimentally to exemplify opiate narcotic agonism, antagonism, and increased agonism, respectively. In this study, these properties are correlated with the electronic and conformational results. The electronic properties of the fused ring skeleton including specifically the cationic region around the nitrogen are relatively unaffected by varying N-substituents. The properties studied include net charges, bond polarities, and the nature and energy of the highest filled and lowest empty molecular orbitals. The conformational behavior appears to be the main cause of differing receptor binding and interaction with the active site and is discussed in these terms.

Nalorphine (**1b**) and *N*-phenethylmorphine (**1c**) are two prototype N derivatives of morphine (**1a**). One is a potent



1a. R = CH_3
b. R = $CH_2CH{=}CH_2$
c. R = CH_2CH_2Ph

antagonist and the other a potent agonist of morphine, exemplifying the importance of the N-substituent in determining the nature of the pharmacological activity of this class of analgesics. Significant antagonism in morphine analgesics has been obtained only by replacement of the *N*-methyl group by a straight chain substituent of at least three carbons (e.g., nalorphine and *N*-methylcyclopropyl- and *N*-*n*-propylnormorphine).[1] Since the search for a nonadditive analgesic centers on obtaining a balance of agonist and antagonist properties, any insight that can be obtained relating specific molecular features of the opiates to enhanced agonism or antagonism should prove useful.

In this study, using semiempirical, quantum chemical methods, electronic and conformational properties of the three selected compounds (**1a–c**) are calculated and those properties which most likely affect the pharmacology are identified. Inherent in our study is the assumption that both enhanced potency and antagonism are at least partially due to effects at the receptor site, i.e., to drug–receptor interactions.

While not definitively established, the structural similarity of rigid opiate agonists and antagonists, together with recent progress in isolating and identifying a membrane-bound receptor,[2-4] points to their action at a specific receptor site. Other evidence for specific receptor sites in the central nervous system has recently been reviewed.[5]

Structural similarities of rigid opiates suggest that the region of the presumably quaternized nitrogen, a phenyl ring, and polar groups, particularly a 3-OH group, are vital parts of the opiate interaction with the receptor[6] (Jansen[5] and Casy, 1973). Based on the principle of complimentarity, a schematic model for the receptor has been proposed[7] (Beckett, 1954), consisting of an anionic site for the quaternized nitrogen, a flat portion to interact with the phenyl ring, and a cavity to accommodate the piperidine ridge atoms (C_{15}, C_{16} of Figure 2). No specific role of N-substituents or polar group variations was assigned in this early model of the receptor. In a previous study we have examined the role of polar group variation on the analgesic potency of morphine-like opiates.[8] In this study, attention is focused on the effect of N-substituents on observed pharmacological behavior.

Tables I and II summarize the experimental data available for the three compounds studied. Despite a lack of model system or intraventricular data for any *N*-phenethyl derivative, animal studies show this substituent to consistently enhance the potency of rigid opiates with a common 6,7-benzomorphan nucleus[9,10] (Table II). Lengthening or shortening of the ethyl chain as well as saturation of the phenyl ring detracts from its agonist potency. This behavior provides strong evidence for enhanced receptor interaction as an important factor on the agonist action of the *N*-phenethyl compounds.[9,10] Nalorphine is more strongly bound than morphine to both the guinea pig ileum[11] and the rat brain homogenate receptor.[2] The similarity of analgesic potency in model system studies,[11,12] in humans[13-15] and the many whole animal studies[10,15,16,17] (summarized in Table I), establishes nalorphine as a nearly equivalent agonist as morphine and leads to the conclusion that such similar analgesic activity is related to events at the receptor site despite known differences in the rate of penetration and nature of distribution in the brain tissue.[9]

Since first prepared in 1942,[18] nalorphine has been shown to exhibit strong antagonism to almost all the action of morphine in animals,[19,20] in man,[21,22] and in the guinea pig ileum model system.[11] As shown in Table I, antagonism occurs at much lower doses (3–5%) than required for its own agonist activity and appears to be totally competitive.[23,24] Antagonism then seems firmly established as a receptor-site event.

The electronic properties which we monitor as a function of changing N-substituent are net atomic charges, bond polarities, and the nature and energy of the highest filled (HOMO) and lowest empty (LEMO) molecular orbitals. Charges and bond polarities are relevant to how varying N-substituents can affect electrostatic, polarization, and van der Waals interactions with specific receptor subsites. Knowledge of the electron distribution and energy of HOMO and LEMO contributes to a further understanding of the electron-donating and -accepting ability of the analgesic relevant to possible charge-transfer interactions with the receptor.

Calculations of conformational characteristics of these compounds are also necessary to understand how the N-substituents might be accommodated at the receptor. They also contribute to an understanding of how the N substituent's orientation might alter the interaction of the opiate's cationic region with the receptor.

Experimental Section

Conformational calculations were performed using a semiempirical molecular orbital method called Perturbation Configuration

Table I. Pharmacological Behavior of Morphine and Nalorphine

	Relative potency,[a] ED_{50}, mg/kg				Guinea pig ileum,[f] rel potency	Relative receptor binding		Antagonism[k]	
	Human[b]	Tail flick[c] (rat)	Writhing[d] (mouse)	Electrica[e] mouse		Guinea pig ileum[f]	Rat brain[h] homogenate	Benzoquinone[i] writhing (mice)	Modified hot[j] plate (mice)
Morphine	0.2	3.8 sc	0.5 sc	0.8 sc	1	1	1	0.69[l] mg/kg	0.94[l] mg/100 g
Nalorphine	0.1–0.2	1.5 sc	1.0 sc	4.8 sc	1.5[g]–3[f]	22 ± 3	2.3	0.05[k] mg/kg	0.028[k] mg/100 g

[a] All data for in vivo results taken from ref 15. See references therein for original source. [b] Based on 50 kg of human body weight, same effective dose whether given orally (ref 13), subcutaneously (ref 15), or parenterally (ref 14). Effective oral dose of nalorphine varied from ½ to 1 of morphine. [c] Relative potency of nalorphine/morphine by this method varied significantly from ½ to 1/10. [d] "Typical" potency values from writhing techniques. However, in one study potencies were measured as a function of elapsed time; observed relative potencies varied from 3/1 to 1/10 for (nalorphine/morphine) (ref 17). [e] Reference 14. [f] Reference 11 (relative potency). [g] Reference 12 (relative potency). [h] Reference 2. [i] Reference 23. [j] Reference 24. [k] Amount of nalorphine required to double ED_{50}- (listed) of morphine. [l] ED_{50} (morphine) in absence of nalorphine.

Table II. Effect of N-Substituent (NR) on Analgesic Potency[a] in Three Different Rigid Opiate Series: Morphine, Morphinan, and Benzomorphan

Compound	NCH_3	$-CH_2-$ Ph	$-(CH_2)_2-$ Ph	$-(CH_2)_2-$ S[d]	$-(CH_2)_3-$ Ph	$-(CH_4)-$ Ph
Morphine	1[a]	<0.1[b]	6.1[b]	0.3[b]	<0.1[b,c]	
Morphinan	1[a]	None	6.3[e]	0.3[e]		0.18[e]
Benzomorphan	1[a]		23[e]	0[e]	0.22[e]	

[a] Analgesic potencies given relative to NCH_3 compound of each series. [b] Potency determined by rat tail flick method, ref 10. [c] The potency of the $-N(CH_2)_3Ph$ compound for morphine was not available. Instead the NCH_2COCH_2Ph and $NCH_2CHCHPh$ result is listed. [d] This compound is $N(CH_2)_2C_6H_{11}$, the saturated cyclic analog of $N(CH_2)_2Ph$. [e] Potency determined by mouse hot plate method by Eddy et al., ref 9.

Interaction using Localized Orbitals (PCILO) developed in the Laboratory of B. Pullman and described elsewhere in detail.[25] It has been used extensively for conformational studies of a variety of biological systems. Additional electronic properties were calculated using the Incomplete Neglect of Differential Overlap method (INDO) of Pople et al.[26] Our versions of the programs were essentially those of the authors with minor modifications to allow for calculations on these very large molecules.

Geometric parameters for the fused ring and polar group structures were held fixed at values obtained from a recent refinement[27] of the morphine crystal structure and from our previous calculations on the minimum energy rotational positions of the two OH groups.[8] Both polar groups were considered too far from the N-substituent to be significantly affected by its variation. The nonvarying geometric parameters for the N-substituents in nalorphine[28] and N-phenethylmorphine were taken from prototype crystal structures and are given in Figures 1a–c. Also given in these figures are the torsion angles which were varied in our conformational study of these three substituents. In all cases the convention used to define these torsion angles Ti (ABCD) was clockwise rotation of atom D into A while looking along the C-B axis from atom C to atom B.

The protonated form of the compound was used as it is believed to be the active form at the receptor.[5] Calculations on morphine base are included however to show the effect of protonation on electronic structure.

Staggered and eclipsed conformations of the morphine *N*-methyl hydrogens were studied as well as axial and equatorial conformations of the *N*-methyl carbon.

For nalorphine, 42 nested, systematic variations of the two-substituent torsion angles (τ_1, τ_2 of Figure 1b) were calculated with PCILO.

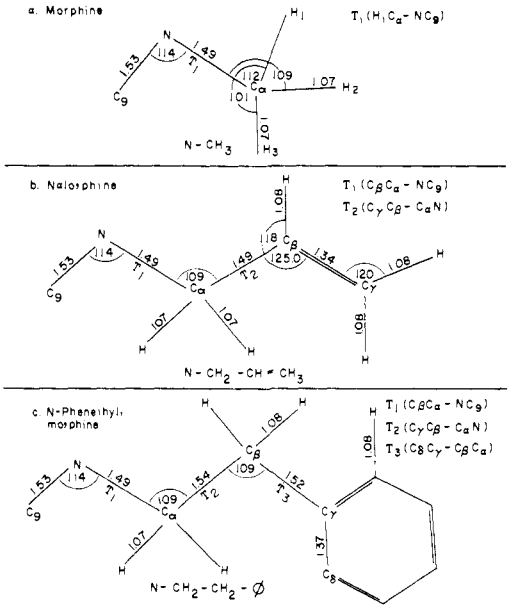


Figure 1. Input geometries and torsion angles for nitrogen substituents: (a) from ref 27; (b) ref 27 and 28; (c) ref 26.

Complete variations of the phenethyl substituent with three defining torsion angles were not feasible due to the large number of conformations required. By using the results of the nalorphine calculations based on τ_1 and τ_2 variations could be reduced to a manageable number. For nalorphine, values of τ_1 from 0 to 120° corresponding to the allyl group pointing toward the cyclohexane ring of morphine (Figure 2) were high-energy conformers. For the bulkier *N*-phenethyl group such interference with the fused ring atoms of morphine would be even more pronounced. This is easily seen by looking at a structural model of the *N*-phenethyl compound. Hence, the low-energy region of τ_1 from 180 to 300° was explored with nested rotations for τ_2 in this region. An optimum value of τ_3 for each run was chosen from simple steric considerations using an interactive coordinate program. Using this procedure, a local minimum at $\tau_2 = 180°$ was found for all values of τ_1 considered. Therefore, a full rotation about τ_1 (including the high-energy region previously ignored) was performed for this single value of τ_2, again optimizing the values of τ_3 for each point.

Results and Discussion

Electronic Properties Calculations. Table III shows the net atomic charges on the nitrogen atom and on all the atoms surrounding it (i.e., the cationic head) including its bonding atoms and the hydrogens bonding to them. Results are presented for the three compounds as calculated by both PCILO and INDO methods.

Table III. Net Atomic Charges[a] in Cationic Region of Morphine, Nalorphine, and N-Phenethylmorphine

	Morphine (base)		Morphine		Nalorphine		N-Phenethylmorphine	
Atom[b]	PCILO	INDO	PCILO	INDO	PCILO	INDO	PCILO	INDO
N_{13}	−0.13	−0.22	+0.07	+0.06	−0.053	−0.053	−0.040	+0.006
C_9	+0.11	+0.14	+0.10	−0.12	+0.100	+0.122	+0.100	−0.125
C_{16}	+0.09	+0.16	+0.09	+0.14	+0.090	+0.131	+0.090	+0.137
C_α	+0.06	+0.16	+0.05	+0.12	−0.074	−0.144	+0.072	−0.127
H_9[c]	−0.03	−0.04	+0.01	−0.00	+0.013	−0.006	+0.012	−0.007
H_{16}	−0.02	−0.03	+0.03	+0.01	−0.035	+0.010	−0.036	+0.003
H_α	−0.01	−0.03	+0.05	−0.02	+0.036	0.010	+0.038	+0.014
H_α	0.0	−0.03	+0.06	+0.04	+0.040	0.017	+0.036	−0.012
H_N			+0.16	+0.15	+0.158	+0.144	+0.160	+0.141
H_{16}	−0.02	−0.05	+0.05	+0.02	+0.043	+0.016	+0.043	+0.015
$\Sigma_a qa$[c]	+0.05	+0.06	+0.67	+0.68	+0.642	0.641	0.631	0.573
O_F			−0.11		−0.12		−0.12	
O_{Ph}			−0.15		−0.13		−0.13	

[a] In units of electron charge. [b] Atom numbering as in Figure 2. [c] Sum of net atomic charges (qa). [d] H_i = H on ith atom.

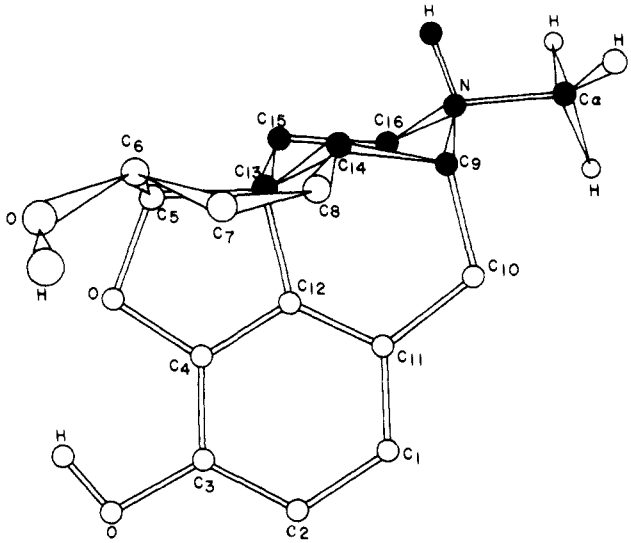


Figure 2. Minimum energy conformer of morphine in the plane of the phenyl ring.

Table IV. Nitrogen Bond Polarities[a] in Morphine, Nalorphine, and N-Phenethylmorphine

	Morphine			
Bond	Base	Protonated	Nalorphine, protonated	N-Phenethylmorphine, protonated
N–C_9	1.06–0.94	1.29–0.73	1.29–0.734	1.29–0.732
N–C_{16}	1.06–0.94	1.26–0.75	1.26–0.750	1.26 0.754
N–C_α	1.05–0.94	1.22–0.78	1.25–0.765	1.26–0.760
N–H		1.25–0.84	1.15–0.840	1.15–0.849

[a] Bond polarities expressed as the number of electrons in the bonding atomic orbital of each atom. E.g., in morphine base the N–C_9 bond has 1.06 electrons in the nitrogen orbital and 0.94 electrons in the C_9 atomic orbital, etc. Nonpolar bonds would be listed as 1–1, i.e., 1 electron on each atom. Results are from the PCILO method only since INDO does not readily yield such information.

Table IV gives the bond polarities of each nitrogen bond in terms of the number of electrons in the hybridized atomic orbital of each atom forming the bond. For example, the N–C_9 bond in morphine base has 1.09 electrons in the nitrogen atomic orbital and 0.94 electrons in the carbon orbital forming the bond. A value of one electron in each orbital corresponds to a totally nonpolar bond. Only PCILO results are given in Table IV since INDO calculations do not readily yield such information. The electron distributions given in Tables III and IV are insensitive to the conformation of the N-substituent in the three compounds. Therefore, similarities or differences among them in this regard are independent of the fact that only the most plausible part of conformational space was explored for the N-phenethyl compound. The most noteworthy feature of these results is the overall electronic similarity between the three protonated molecules in the region of the cationic head. Where small differences are seen, the order puts the strong antagonist and the strong agonist on the same side of the moderate agonist morphine. Thus, bond polarities and charges which might change the nature of weak, nonbonding interactions are essentially the same in the three protonated molecules. Note also the similarities between the results of PCILO and INDO with respect to net charges. This result was also obtained in our study of polar group variations for similar opiates.[8]

Tables III and IV also show the effect of protonation on the electronic structure of morphine. This effect is to change the cationic region from a neutral, relatively noninteracting structure to a fairly polar one more favorable to interactions with the postulated anionic site. Protonation imparts a net positive charge of approximately 0.7 electrons on the cationic region as a whole while diminishing the net N charge by 0.20 electrons. This confirms the predictions of Casy[5] on the delocalized nature of the positive charge on a protonated nitrogen based on the quantum chemical studies of the ammonium ion. It is also consistent with previous studies of the protonated, nonprotonated morphine and nalorphine.[29,30] Bond polarities in the cationic region change from nearly neutral (i.e., close to 1.00–1.00) in the base to strongly polar in the direction N^-–C^+ upon protonation. Thus the effect of protonation is to "activate" all the atoms in the region near the nitrogen (i.e., the cationic head) to electrostatic and polarizing forces rather than just the N atom itself. From these results it can be seen that variation of pharmacological properties cannot be attributed to the effects of N-substituent variation directly on the nature of the cationic head.

Table V. Nature and Energy of HOMO[a] and LEMO[b] in Compounds Studied

	Morphine$_{base}$	Morphine$_{H^+}$	Nalorphine$_{H^+}$	N-Phenethyl$_{H^+}$
E_{HOMO},[c] au	−0.3913	−0.5172	−0.5139	−0.5117
E_{LEMO},[d] au	+0.1512	−0.0540	−0.0499	−0.0378
Nature$_{HOMO}$[e]	$\pi_{\phi,O_F,O_{Ph}}$	$\pi_{\phi,O_F,O_{Ph}}$	$\pi_{\phi,O_F,O_{Ph}}$	$\pi_{\phi,O_F,O_{Ph}}$
Nature$_{LEMO}$[e]	π^*_{Ph}	$\sigma^*_{cationic}$	$\sigma^*_{cationic}$	$\sigma^*_{cationic}$

[a] HOMO = highest occupied molecular orbital. [b] LEMO = lowest empty molecular orbital. [c] E_{HOMO} = energy of HOMO ≅ ionization potential. A measure of ease of electron donation in atomic units. [d] E_{LEMO} = energy of lowest empty orbital ≃ electron affinity. A measure of ease of electron acceptance in atomic units. [e] $\pi_{\phi,O_F,O_{Ph}}$ = orbital with a node in the plane of the Ph ring, centered on ring, furan oxygen, and phenolic oxygen. $\sigma^*_{cationic}$ = nonbonding orbital with maximum electron density on nitrogen and its neighbors in the plane of the nitrogen bonds.

Table V shows the nature and energy of HOMO and LEMO for the compounds studied. As with the charge comparison, the variations for the three protonated molecules are small and put the potent agonist and antagonist on the same side of the parent compound morphine.

The electron distribution in the highest filled molecular orbital (HOMO) in all protonated molecules studied is a delocalized π orbital centered on the benzene ring, the phenolic oxygen, and the furan oxygen. It is the electrons in this most loosely bound orbital that would be most readily involved in electron transfer to the receptor.

The significant electron density in the two oxygen atoms in HOMO, together with their net negative charge, is indicative of their important electron-donating role in interactions at the receptor site, providing a basis for the observed requirement of at least the phenolic oxygen for analgesic activity.

LEMO in the morphine base is an antibonding π orbital centered on the phenyl ring but changes dramatically to a σ nonbonding orbital centered on the cationic head in all protonated molecules studied. Since LEMO can serve as an incipient acceptor of electrons from the receptor, its nature verifies the importance of the cationic head as an electron-accepting site. The dramatic change upon protonation is further evidence that protonation "activates" the cationic head. Evidence is also seen in the change in net charges and bond polarities upon protonation shown in Tables III and IV. In our previous study of the effect of polar group variation on morphine activity we have further shown that such protonation and interaction with an anionic site causes advantageous changes in the electrostatic features of the polar oxygen atoms.

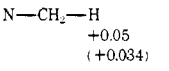
The net atomic charges on the substituent atoms themselves are given in Table VI. We see that there are no highly charged atoms in these substituents and that their interaction with a possible receptor site would come mainly through polarization and dispersion terms.

Based on our results, it is not possible to account for the variations in pharmacological behavior of the three compounds from the calculated charge distributions of the substituents themselves. Additionally, it seems that the various N-substituents cause no apparent change in the electronic properties of the cationic head itself. Thus it appears likely that spatial orientation is an important factor in understanding the differing pharmacological behavior of the compounds studied.

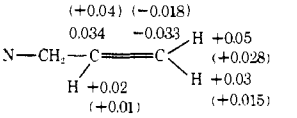
Conformational Results and Discussion. Although no crystal structure data on fused ring opiates exist showing an axial N-methyl or NR group, some investigators have presented hypotheses in which axial piperidine ring substituents were implicated in both enhanced agonism and antagonism.[31] An axial methyl group was, however, 5.7 kcal/mol higher energy than an equatorial one in a staggered arrangement and 7.0 kcal/mol higher in an eclipsed form. Further energy would probably be necessary to promote the methyl group over an energy barrier between the two structures. The energy difference of 5.7 kcal/mol between the best axial and equatorial conformers would make the axial conformer only barely accessible to the drug by interactions in the biophase of the receptor. The staggered, equatorial form shown in Figure 2, obtained as a minimum energy form (1.4 kcal/mol than the eclipsed form), agrees with crystal structure data and is assumed to be the most likely conformer at the receptor site. The larger dimensions and steric hinderance of other N-substituents would be expected to make an axial conformation even less likely. This result has been verified by our recent calculations of a series of NR derivatives of oxymorphone.[32] In these calculations, the best axial conformers of the N-(oxymorphone), N-allyl (naloxone), and N-dimethylcyclopropyl (naltrexone) groups were 12, 20, 18, and 17 kcal/mol above their equatorial counterparts. Thus we consider only equatorial conformations of the three compounds studied.

Table VI. Net Charge[a] on Substituent Atoms (R) of 3-Morphine NCH_2R Compounds

A. Morphine



B. Nalorphine



C. N-Phenethylmorphine

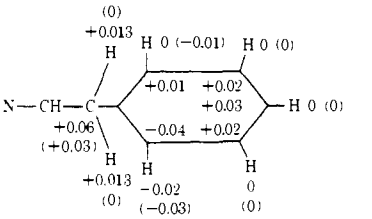


Figure 3 summarizes the conformational behavior of N-phenethylmorphine in the regions of relatively low-energy conformations. In this plot of ΔE vs. τ_1 for a family of τ_2 values, the optimized value of τ_3 used for each point is indicated in parentheses. Figure 3 shows a broad absolute minimum energy conformer at $\tau_1 = 300°$, $\tau_2 = 180°$, and $\tau_3 = 0°$ and additional low-energy local minima at $\tau_2 = 180°$ for all values of τ_1 studied; i.e., $\tau_1 = 180°$, 240°, 300°. These preferred conformers correspond to an extended chain arrangement of the substituent groups as shown in Figure 4. This figure, presented here for comparison with Figures 2 and 6, is drawn to the same scale and with respect to the common phenyl ring in the plane of the paper as in Figure 2. Superimposable atoms of Figures 2, 4, and 6 are shown darkened in both figures for reference. We see from Figure 4 that the entire N-phenethyl substituent lies to the side and somewhat tilted with respect to the reference phenyl

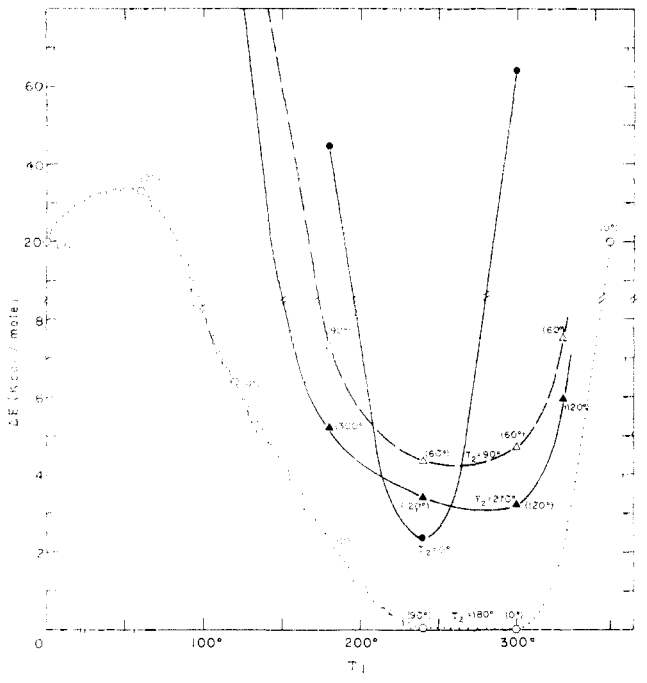


Figure 3. Energy conformation behavior of N-phenethylmorphine. Relative energy (ΔE) as a function of τ_1 for four values of τ_2: (○) τ_2 = 180°; (●) τ_2 = 0°; (△) τ_2 = 90°; (▲) τ_2 = 270°. Values of τ_3 in parentheses at each point. τ_1, τ_2, and τ_3 as defined in Figure 1.

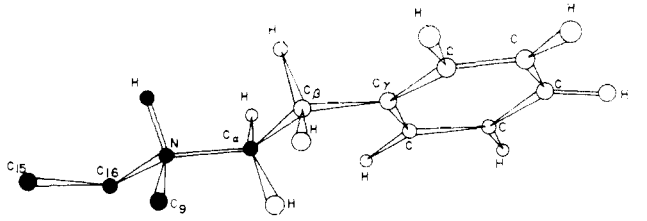


Figure 4. Minimum energy conformer of the N-phenethyl substituent of morphine in the plane of the skeleton phenyl ring.

ring. The distance from the N to the center of the substituent phenyl ring is 5.1 Å.

For nalorphine, the conformational results of systematic, nested rotations of τ_1 and τ_2 are summarized in Figure 5 (as a series of curves of ΔE vs. τ_1 for different values of τ_2). We see from this figure that for all values of τ_2, the low-energy values for τ_1 are between 180 and 300° with substantial energy barriers on either side of this region. The behavior of the N-allyl group differs from that of the N-phenethyl derivative in that two rather than one distinct low-energy local minima at τ_1 = 180 and 300° are obtained for almost all values of τ_2 with a low barrier between the two at τ_1 = 240°. The extended chain conformer τ_1 = 240°, τ_2 = 180° and in general all conformers with τ_2 = 180° are local maxima rather than local minima as in N-phenethylmorphine.

The absolute minimum (τ_1 = 180°, τ_2 = 240°) and its "mirror image" conformer (τ_1 = 300°, τ_2 = 120°), a local minima, are then predicted to be the most likely conformers at the active site. These two conformers are shown in Figure 6, drawn to be superimposable with the reference atoms of Figures 2 and 4.

While the methods we use are approximate and the conformations obtained are subject to possible change in the biological environment of the receptor, our results do seem to describe a biphasic conformational behavior of the allyl substituent. This behavior would be characterized by an extensive conformational region of high energy and a well-defined region of low-energy conformations accessible to

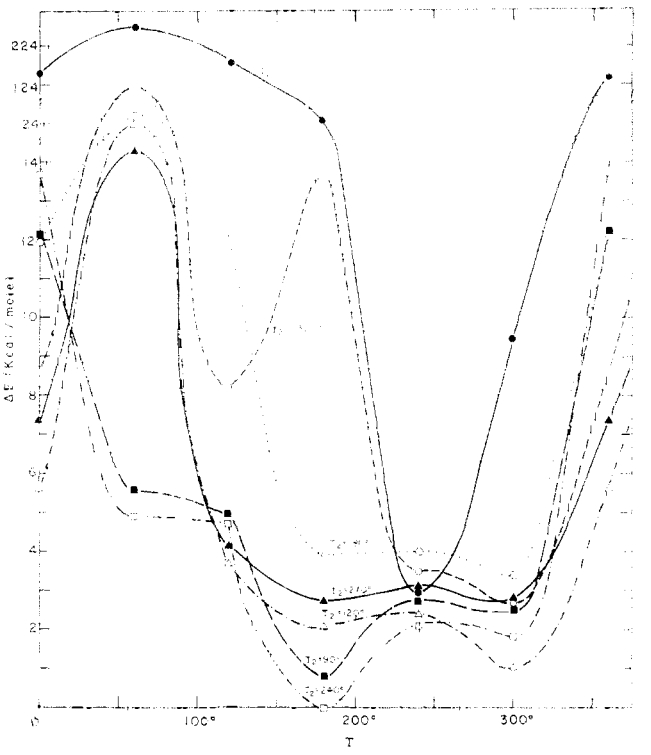
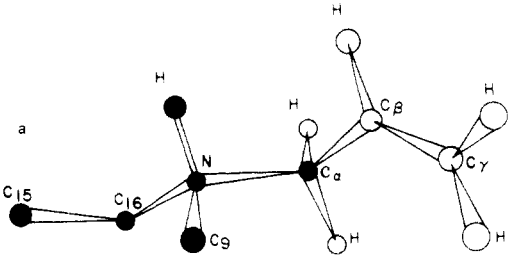


Figure 5. Energy conformation behavior of nalorphine relative energy (ΔE) as a function of τ_1 for six values of τ_2. τ_1 and τ_2 as defined in Figure 1.



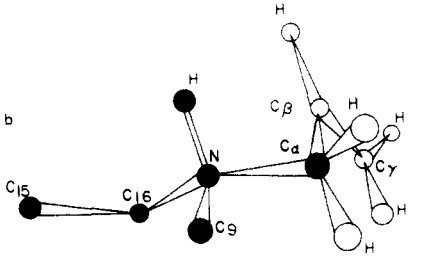


Figure 6. Two types of low-energy allyl group conformers in nalorphine: (a) low-energy, noninterfering (agonist) conformer; (b) minimum energy, protruding (antagonist) conformer.

the molecule through weak, nonbonding interactions with the receptor. In the region of low-energy states, the biphasic behavior would be characterized by an equilibrium mixture primarily between the two types of conformers for which distinct low-energy minima are found. Both types of conformers are candidates for the active site provided the requisite activation energy between them of approximately

3.4 kcal/mol is available from weak interactions in the biophase of the receptor. The type with $\tau_1 = 180°$, and the other with $\tau_1 = 300°$, both have several low-energy values of τ_2 combined with them to consistently project the allyl group either into or out of the plane of the reference phenyl group. This behavior, shown in Figure 6 for the two lowest local minima of each type, never favors an extended chain-like conformation ($\tau_2 = 180°$). We postulate then a thermal equilibrium between both types of conformers, so that their relative concentration is expressible in terms of a Boltzmann distribution

$$[N1]/[N2] = \exp(-(En1-En2)/kT)$$

where [N1]/[N2] is the relative concentration of the two isomers at temperature T and with an energy separation of En1–En2. For nalorphine at $T = 300$ K, with an energy difference of approximately 1.0 kcal/mol between the two lowest rotational isomers of each type, the proportion would be roughly 4:1 favoring the lower energy structure, but indicating significant amounts of both structures to be present. We associate the presence of these two qualitatively different conformers with the dual agonist–antagonist behavior of nalorphine. Such duality is absent in the behavior of the *N*-phenethyl substituent which is known to have only agonist activity.

By examining the calculated behavior for all three compounds, a consistent interpretation of our results for the variation in N-substituent behavior can be made.

For morphine, the classic analgesic agonist, the equatorial *N*-methyl group would seem to describe an area in the receptor where an accommodation if not attraction exists for the equatorial substituent.

For *N*-phenethylmorphine, both the experimental results and our results on the electronic and conformational behavior verify the hypothesis that the position of the phenyl substituent describes indirectly the location of an additional lipophilic binding site in the receptor. In potency studies, lengthening or shortening of the ethyl chain or saturation of the phenyl ring was shown to detract from its analgesic potency (Table II).[9,10] The electronic structure of the cationic head shows no appreciable deviation from that of morphine. The distinct minimum energy conformation of the phenyl substituent is oriented away from the cationic head and would thus not be expected to interfere with or alter its N-receptor interaction. For these reasons the behavior of this derivative seems directly attributable to increased binding and interaction of the substituent itself with the receptor.

For nalorphine, we postulate that at low concentrations it binds strongly in its minimum energy conformer to the same receptor site as morphine but with an additional binding site or "cleft" to accommodate the protruding allyl group. It is this accommodation which, we believe, leads to the observed enhanced binding and competitive antagonism seen in both in vivo and in vitro systems. According to current ideas of opiate contact with a receptor, qualitatively similar to the original Beckett scheme,[7] the allyl group in this position would make the initial contact with a receptor surface.[5] Its accommodation could lead to a small conformational change in the receptor which alters its contact with the crucial cationic head, thus preventing agonism while allowing nalorphine to effectively and competitively block almost all known effects of morphine.

At higher concentrations, significant amounts of the "mirror image", higher energy conformer (Figure 6a) would be present. Such a conformer would be less likely to interfere with normal cationic head contact with the receptor and could allow nalorphine to have its own agonism. The combined effect of the binding of both types of conformers might cause agonism to be accompanied by different side effects characteristic of nalorphine-type agonists. The accommodation of the allyl group in this position (Figure 6a), not unlike the *N*-phenethyl substituent, does not appear to enhance its potency. It may even detract from it, considering the wide range of observed agonist potencies of nalorphine (ranging from 2:1 to 1:6 using various techniques as shown in Tables I and II).

An alternate explanation of the apparently smaller enhancement (if any) of relative nalorphine agonism compared to *N*-phenethylmorphine is that the "agonist" conformer of nalorphine is only about 20% of the total concentration of the drug. Thus, for the same total concentration, the observed range of relative nalorphine potencies (about 1:1 using ED_{50}) corresponds to about the same intrinsic potency for the agonist conformer of nalorphine as for the *N*-phenethyl derivative.

In summary then, our hypothesis is that N-substituent conformations resembling *N*-phenethylmorphine in Figure 4 and *N*-allylmorphine in Figure 6a are associated with variable agonism depending on the nature and extent of accommodation of the substituent. N-Substituent conformations which resemble the lower energy structure of nalorphine (Figure 6b) are responsible for competitive antagonism, provided that the substituent can be accommodated by a "cleft" or open area in the receptor. In our model, such antagonism is due to enhanced binding and an altered drug–receptor contact particularly with respect to the cationic head. Partial support for our proposed molecular requirements for antagonism is the observation that three-carbon chain N-substituents are both necessary and optimum for morphine-type antagonists. Continuing work with N-substituent variations and particularly our analysis of conformational results recently obtained for a series of six N-substituted oxymorphones (oxymorphone, naloxone, nalmexone, naltrexone, nalbuphine, and *N*-phenethyl compounds) should shed further light on the requirements for agonist–antagonist properties.

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References and Notes

(1) F. M. Robinson, *Adv. Biochem. Psychopharmacol.*, 8, 21 (1974).
(2) C. B. Pert and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2243 (1973).
(3) H. H. Loh, T. M. Cho, Y. C. Wu, and E. L. Way, *Life Sci.*, **14**, 2231 (1974).
(4) A. Goldstein, *Life Sci.*, **14**, 614 (1974).
(5) A. F. Casy, *Mod. Pharmacol.*, 1 (1973).
(6) P. A. J. Janssen and C. A. M. van der Dyken, "Drugs Affecting the Central Nervous System", A. Burger, Ed., Vol. 2, Marcel Dekker, New York, N.Y., 1968, Chapter 2.
(7) A. H. Beckett and A. F. Casey, *J. Pharm. Pharmacol.*, **6**, 986 (1954).
(8) G. H. Loew, H. Weinstein, S. Srebrenik, and D. Berkowitz in "Molecular and Quantum Pharmacology", E. Bergmann and B. Pullman, Ed., D. Reidel Publishing Co., Holland, 1974.
(9) L. B. Mellett and L. A. Woods, *Fortschr. Arzneimittelforsch.*, **5**, 156 (1963).
(10) C. A. Winter, P. D. Orahovats, and E. G. Lehman, *Arch. Int.*

Pharmacodyn. Ther., 186 (1957).
(11) H. W. Kosterlitz and A. J. Watt, Br. J. Pharmacol. Chemother., 23, 266 (1963).
(12) B. M. Cox and M. Weinstock, Br. J. Pharmacol. Chemother., 27, 81 (1966).
(13) Dr. William Forrest, private communication, Department of Anesthesia, Stanford Medical Center.
(14) T. D. Pernine, L. Atwell, I. B. Tice, A. E. Jacobson, and E. L. May, J. Pharm. Sci., 61 (No. 1), 86 (1972).
(15) R. I. Taber, Adv. Biochem. Psychopharmacol., 8, 191 (1974).
(16) N. B. Eddy, H. Halbach, and O. J. Braenden, Bull. W. H. O., 14, 353 (1956).
(17) J. Pearl, H. Stander, and D. B. KcKean, J. Pharmacol. Exp. Ther., 167, 9 (1969).
(18) J. Weijland and A. E. Erickson, J. Am. Chem. Soc., 64, 868 (1942).
(19) K. Unna, J. Pharmacol. Exp. Ther., 79, 27 (1943).
(20) W. L. McCawley, E. R. Hart, and D. F. March, J. Am. Chem. Soc., 63, 314 (1941).
(21) A. R. Wikler, L. C. Fraser, and H. Isbell, Fed. Proc., Fed. Am. Soc. Exp. Biol., 11, 402 (1952).
(22) G. W. Gorodetzky, Adv. Biochem. Psychopharmacol., 8, 191 (1974).
(23) A. E. Takemori, H. J. Kupferberg, and J. W. Miller, J. Pharmacol. Exp. Ther., 169 (No. 1), 39 (1969).
(24) B. M. Cox and M. Weinstock, Br. J. Pharmacol., 22, 289 (1964).
(25) S. Diner, J. P. Malrieu, F. Jordam, and M. Gilliert, Theor. Chim. Acta, 15, 100 (1969), and references cited therein.
(26) J. Pople and D. Beveridge, "Approximate Molecular Orbital Theory", McGraw-Hill, New York, N.Y., 1970.
(27) L. Gylbert, Acta Crystallogr., Sect. B, 29, 1630 (1973).
(28) "Tables of Interatomic Distances and Configuration in Molecules and Ions", Chem. Soc., Spec. Publ., No. 11 (1958).
(29) J. J. Kaufman and E. Kerman, Int. J. Quantum Chem., 6, 319 (1972).
(30) J. J. Kaufman and E. Kerman, Int. J. Quantum Chem., Symp., 8, 289-313 (1974).
(31) Robert Willette, NIH, private communication.
(32) G. Loew and D. Berkowitz, unpublished results.

Conformation of Histamine Derivatives. 5. Molecular Orbital Calculation of the H_1-Receptor "Essential" Conformation of Histamine[1]

L. Farnell, W. G. Richards,*

Physical Chemistry Laboratory, Oxford University, Oxford OX1 3QZ, England

and C. R. Ganellin*

The Research Institute, Smith Kline & French Laboratories Ltd., Welwyn Garden City, Hertfordshire, England.
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Conformational energies of histamine and 4-methylhistamine monocations are calculated using the EHT molecular orbital procedure; the results are expressed as potential energy surfaces in which bond rotations (θ_1 for ring–C_β, θ_2 for C_β–C_α) are measured along the axes, and energy variation is indicated by contours. Using the classical Boltzmann partition function and Simpson's rule for normalization, corresponding probability surfaces are generated which take account of the potential surface entropy. Comparing the two surfaces provides regions which are within a given probability contour of histamine but outside this contour for 4-methylhistamine. Thus, at the 99% probability level, three conformational regions defined by the bond rotation angles are indicated as possible "H_1-essential" conformations of histamine: viz. trans (θ_1 = 290–330°, θ_2 = 150–210°) and gauche (θ_1 = 260–280°, θ_2 = 30–90° and θ_1 = 290–320°, θ_2 = 270–320°). This procedure provides a quantitative basis for comparison with other histamine derivatives and may have a general value for studying relationships between conformation and biological activity of closely related small molecules.

Two types of histamine receptor, H_1 and H_2, have recently been characterized by using selective histamine-like stimulants (agonists) and selective histamine-blocking agents (antagonists).[2] A selective agonist of considerable interest is 4-methylhistamine [4-methyl-5-(2-aminoethyl)-imidazole]; it has about half the activity of histamine at H_2 receptors but only $\frac{1}{500}$th of the activity at H_1 receptors. This marked effect of a 4-methyl substituent on H_1-receptor agonist activity poses an intriguing medicinal chemical problem and provides an opportunity to identify chemical properties of histamine likely to be involved in H_1-receptor stimulation.

We have previously shown[3] by EHT calculation that 4-methylhistamine may differ from histamine in its conformational properties. The calculations suggested that the methyl substituent influences the orientation of the imidazole ring with respect to the side chain and introduces a measure of rigidity through restricting ring rotation. We do not know whether these changes account for the observed biological difference but we can explore this as a possibility. If 4-methylhistamine is ineffective as an H_1-receptor stimulant because of restricted rotation or of its inability to assume a necessary conformation then we can define for histamine the "H_1-essential" conformations, i.e., conformations essential to drug activity which have to be adopted by drug molecules at some stage during productive interaction at the H_1-receptor site. To do this we must find those conformations which are *accessible* to histamine but *inaccessible* to 4-methylhistamine. We have previously argued this in a qualitative manner;[4] in the present paper we make it more quantitative.

Calculations were performed on histamine and 4-methylhistamine monocations in their N_3–H (N^τ–H)[6] tautomeric forms (Figure 1) using the nomenclature and geometry previously given.[5] As before, the conformation is described by the two torsion angles θ_1 and θ_2 which, respectively, represent rotation of the imidazole ring about the bond C_5–C_β, and rotation within the side chain about the bond C_β–C_α. The symmetrical ammonium group was held in a staggered position (θ_3 = 60°) with respect to C_α. In 4-methylhistamine the symmetrical methyl substituent was rotated to minimize the energy for given values of θ_1 and θ_2; for most of the surface the orientation θ_4 = 120° is most favorable, but as θ_1 approaches 0°, θ_4 tends toward 75°, and similarly as θ_1 approaches 360°, θ_4 tends toward 165°.

The total internal molecular energies were calculated