caused substantial changes in excretion of chlorpheniramine and the monodesmethyl metabolite. In this study, the change in urine pH during two different urine collection intervals was expected and was presumably due to variations in diet.

The urinary pseudoephedrine excretion rate was enhanced under controlled acidic urine pH (3). However, the overall amount excreted unchanged was not dependent on urine pH. The excretion of negligible norpseudoephedrine, the principal metabolite, with and without pH control also supports this observation.

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Surface Properties of Membrane Systems: Interaction of Ketamine with Monomolecular Films of Gangliosides and Mitochondrial Lipids

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Abstract □ Ketamine solutions did not form a film ($\pi = 0$) but had an appreciable surface potential (ΔV = 500 mv), indicating a significant array of ± oriented charge dipoles at the air–water interface, as opposed to calcium chloride solutions whose ΔV was zero. The ΔV values of ganglioside films spread on the aqueous phase varied in the order water < sodium chloride < calcium chloride < ketamine hydrochloride. At equivalent concentrations, calcium chloride was 500 times as effective as sodium chloride, and ketamine at the clinical concentrations of 10–20 μg/ml (36–72 μ*M*) was 6000 times as effective as calcium chloride in raising the surface potential of gangliosides; the ΔV effect with mitochondrial lipid was in the reverse order: water < sodium chloride = ketamine hydrochloride < calcium chloride. This calcium–ketamine inversion indicates a unique specificity of ketamine for gangliosides. Since ketamine acts on the brain and did not affect mitochondrial respiration, the surface potential data suggest that part of the mechanism of action of ketamine could be its interaction with synaptic surfaces and, specifically, with the sialic acid of gangliosides and/or glycoproteins present on the synaptic membrane surface.

Keyphrases □ Membranes—surface properties, ketamine interaction with ganglioside and mitochondrial lipid monomolecular films □ Ketamine—effect on monomolecular films of gangliosides and mitochondrial lipids □ Gangliosides—monomolecular films, effect of ketamine □ Mitochondrial lipids—monomolecular films, effect of ketamine □ Analgesics—ketamine, effect on monomolecular films of gangliosides and mitochondrial lipids

Ketamine, a potent analgesic, acts on the cerebral cortex (1–5). Although the pharmacological effects of ketamine on the cardiovascular and respiratory systems have been defined clinically and experimentally (1–4), little is known about its mechanism of action at the molecular level. A relatively small molecule, which is prepared synthetically as the 2-(*o*-chlorophenyl)-2-(methylamine)cyclohexanone hydrochloride (mol. wt. 275), ketamine has been widely used and discussed as an analgesic, a dissociative anesthetic, and a preanesthetic in experimental and clinical surgery (1–4).

Although rapid induction of analgesia and cardiovascular stimulation with little effect on respiration and respiratory resistance are recognized effects of ketamine (1–4), it is believed that the major effects are a consequence of the direct action of ketamine on the central nervous system (1–4), specifically, the cerebral cortex (5). As a working hypothesis, it was assumed that the negatively charged surfaces of the synaptic membranes in the cerebral cortex are the target of this positively charged molecule.

The surface potential of the monomolecular film at the air–water interface is a suitable model in which to study the ionic properties of both the ketamine and the acidic synaptic components. Interaction of positively charged ketamine with negatively charged lipid films was expected to increase the surface potential (6, 7), which could then be related to film pressure and ion concentrations in the aqueous phase. Because of the role that Ca^{2+} plays in bioelectric phenomena and in the release of acetylcholine from synapses (5), the effects of Ca^{2+} and Na^{+} ions in the subphase were studied also.

The acidic lipid of choice was ganglioside; it is abundant in the synapses in the gray matter forming the cerebral cortex (8). For a comparison, a neutral phospholipid, dipalmitoyl phosphatidylcholine (dipalmitoyl lecithin), was used since the phosphatidylcholines (lecithins) are important constituents of cell membranes. The total lipid extract of mitochondria and isolated cardiolipin were studied for two reasons: (*a*) the mitochondrion is an important intracellular organelle, which controls vital biochemical processes; and (*b*) besides lecithin, the mitochondrial lipid contains about 10% cardiolipin (diphosphatidylglycerol), which is also acidic but is quite different from the ganglioside.

EXPERIMENTAL

Highly purified ketamine[1] and reagent grade electrolytes were used.

[1] Gift of Dr. A. M. Moore, Parke-Davis and Co., Ann Arbor, Mich.

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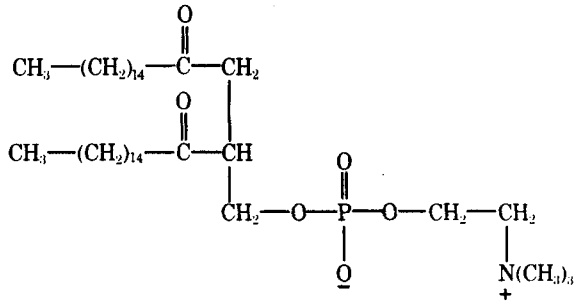
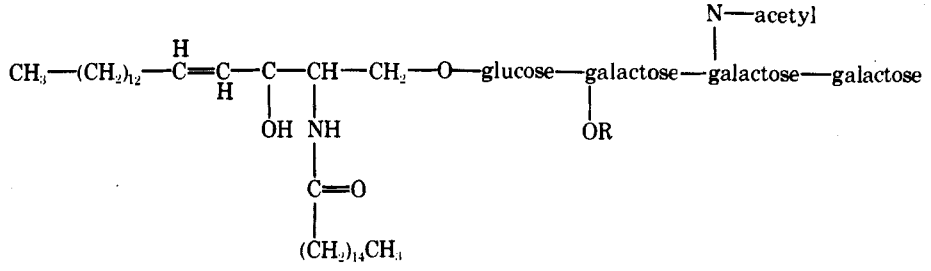
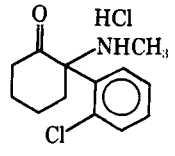


dipalmitoyl lecithin



ganglioside (monosialo) (R = *N*-acetyl neuraminic acid)



ketamine hydrochloride

Dipalmitoyl lecithin and beef heart cardiolipin (diphosphatidylglycerol) were chromatographically homogeneous[2]. Beef brain mixed ganglioside (9) and rat liver mitochondria (10) were prepared according to known methods[3]. Liver mitochondria were used, because they are more readily available than brain mitochondria and, for all practical purposes, the relative lipid composition is the same in various species and organs (11). The mitochondria exhibited a normal increase in respiratory rate upon addition of adenosine diphosphate[4].

The total lipid extract of the mitochondria was obtained by shaking the aqueous mitochondrial dispersion with four volumes of chloroform–methanol (2:1) and a second time with four volumes of chloroform. The mitochondrial lipids were identified by TLC on plates precoated with silica gel G, using chloroform–methanol–ammonia (65:35:5), the appropriate lipid standards, the work of Fleischer and Rouser (11), and various group reaction stains such as iodine vapors, the molybdenum trioxide spray for phosphorus, and the ninhydrin spray for the primary amino groups of phosphatidylethanolamine and phosphatidylserine (7).

The lipid solutions for the monolayer experiments were prepared in chloroform–methanol–hexane (50:40:20) and stored at −20°, but they were kept in ice during the experiments. Under these conditions, the lipids were stable for >2 weeks, as compared to 2 days when their solutions were stored at 0° (7). Since at −20° the ganglioside tended to precipitate out from the organic solvent, the solution was first warmed to complete clearness for 1 min at ~40° before it was put on ice for the duration of the experiment.

The procedures for the determination of surface pressure (π) and surface potential (ΔV) at the air–water interface have been described (12), except for minor modifications. Surface tension (γ) was measured by the Wilhelmy plate method, using a platinum blade suspended from a Cahn electrobalance; the film pressure (π) was calculated as the difference between γ_0 (without film) and γ (with film). The electrical potentials without film (V_0) and with film (V) were measured by a radioactive ^{226}Ra air electrode and a saturated calomel electrode connected with a high impedance electrometer; the surface potential (ΔV) was calculated as the difference $V - V_0$.

The lipid films were spread from organic solvent (1 mg/ml) on distilled water, ketamine hydrochloride, or electrolyte solutions in a circular glass trough whose useful area was 20 cm^2; ketamine films and other films were made by either spreading from organic solvent or adsorbing from aqueous solutions (13).

The electrolyte solutions were prepared with reagent grade salts and foamed to remove surface-active contaminants (12). The water was distilled twice, the second time from alkaline permanganate. A large long-neck flask and a tall fractionating Vigreux column were used upright in the distillation to avoid contamination of the distillate with permanganate sprays (14).

RESULTS AND DISCUSSION

Relation of Surface Activity to Molecular Structure—The structures of three molecules, dipalmitoyl phosphatidylcholine, a monosialoganglioside, and ketamine hydrochloride, are depicted. Although natural gangliosides are mixtures of molecules containing C_{18} and C_{20} sphingosine chains (8), only the C_{18} chain is specified for simplicity; similarly, details of the bonds between the sugar moieties were beyond the scope of the present article.

Unlike the molecules of dipalmitoyl phosphatidylcholine and monosialogangliosides, whose long hydrophobic chains confer the molecule asymmetry and amphiphilic or bimodal character and surface activity, the ketamine molecule is short and nearly spherical in its rotational volume and has a relatively symmetrical distribution of polar groups. Thus, ketamine hydrochloride is not expected to form stable films but, like Ca^{2+} and Na^+, should interact with water and anionic surfaces.

Spread Films—*Ketamine*—Upon application from organic solvent onto the surface of distilled water, ketamine did not spread as a film. The surface pressure was zero, but the surface potential oscillated erratically between 10 and 30 mv (Fig. 1). Similar results were obtained when ketamine was spread from aqueous solutions. The small fluctuating ΔV values may be ascribed to small local surface concentrations of ketamine,

[2] Applied Science Laboratories, State College, Pa.

[3] The ganglioside and mitochondria were kindly provided by Dr. R. Ledeen and Dr. D. Rezek, Departments of Neurology and Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461.

[4] D. Rezek, Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461, personal communication.

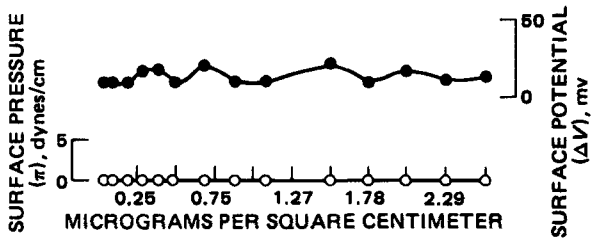


Figure 1—*Surface pressure and surface potential values on the distilled water surface after application of ketamine hydrochloride (micrograms per square centimeter).*

which diffused into the aqueous subphase until the surface potential became nearly zero.

Lipids—In marked contrast are the corresponding properties of spread films of dipalmitoyl lecithin and ganglioside. In Fig. 2, surface pressure (in dynes per centimeter) and surface potential (in millivolts) are related to the quantities of phospholipid and ganglioside (in micrograms per square centimeter) applied onto the distilled water surface. As with many known lipids, both the pressure and the surface potential curves of dipalmitoyl lecithin show the typical continuous rise to saturation. However, the ganglioside surface potential had an unusual maximum at ~25 dynes/cm, after which it decreased gradually from 220 to 200 mv, whereas the pressure rose regularly to a saturation value of 42 dynes/cm. Since the pressure continued to rise, the film was not collapsing.

Since the molecular correlates of the surface potential are not clearly established (7, 14–19), the cause for the unusual fall in the surface potential of the ganglioside is unclear. In accordance with the general meaning of the Helmoltz equation for the voltage of the parallel plate capacitor (17), possible reasons for lower potential are: (*a*) formation of hydrophilic interfacial structures having a high dielectric constant and (*b*) appearance of an excess of ∓ dipoles. The π–*A* curve of mitochondrial lipid on 0.15 *M* NaCl (not shown) resembled that of dipalmitoyl phosphatidylcholine, but the surface potential was lower (<500 mv), due probably to the anionic cardiolipin. Except for being more expanded because of the greater cross section of this lipid, the force–area curves of the latter and of mitochondrial lipid were very similar to those of dipalmitoyl lecithin and ganglioside. The ΔV–area curve of cardiolipin, however, differed markedly from those of ganglioside and mitochondrial lipid (20).

Adsorbed Ketamine Films—Since most of the experiments described later dealt with lipid films spread on either electrolyte or ketamine solutions, it was pertinent to determine the surface pressure and the surface potential of the aqueous hypophase. At concentrations of 10, 20, and 200 μg/ml, neither sodium chloride, calcium chloride, nor ketamine hydrochloride affected the surface tension; for all practical purposes, the film pressure was zero, as was that of distilled water. One concludes that the surface activity of ketamine was nil or <0.1 dyne/cm (the accuracy of the surface tension measurements). The surface potential, however, increased with the ketamine concentration (Fig. 3).

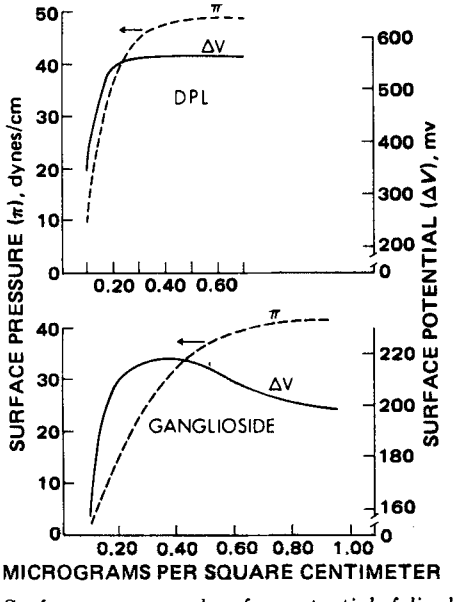


Figure 2—*Surface pressure and surface potential of dipalmitoyl lecithin (DPL) and beef brain mixed ganglioside films spread on distilled water. The lipid quantity applied on the aqueous phase surface is expressed in micrograms per square centimeter.*

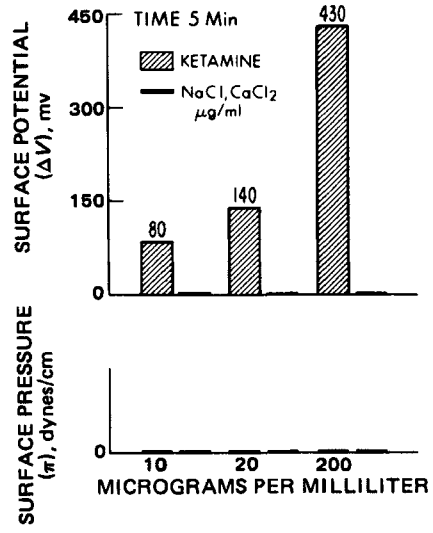


Figure 3—*Surface pressure and surface potential values of various sodium chloride, calcium chloride, and ketamine hydrochloride concentrations in distilled water 5 min after putting the solution in the trough.*

The zero pressure and large positive potential are consistent with a more marked effect of the ± ketamine hydrochloride dipole as compared to calcium chloride and sodium chloride, both of which had zero surface potential in the first 5 min. At 20 min, however, the ΔV values rose to 2, 5, and 530 mv with 200 μg of sodium chloride, calcium chloride, and ketamine hydrochloride/ml, respectively, in the subphase. With ketamine, a film pressure of 2 dynes/cm built up gradually between 10 and 20 min, whereas the pressure of the sodium chloride and calcium chloride solutions remained at zero. Similar results were observed with clinical preparations of ketamine hydrochloride[5].

Ketamine Interaction with Spread Lipid Films—The trough was filled with distilled water, the surface was cleaned (12), and measured volumes of concentrated aqueous ketamine solution were injected into the water with continuous magnetic stirring. After 2 min, the lipid was spread onto the aqueous subphase, and surface pressures and surface potentials were determined.

Gangliosides—In Fig. 4, the π and ΔV values are related to the quantity of ganglioside applied (in micrograms per square centimeter)

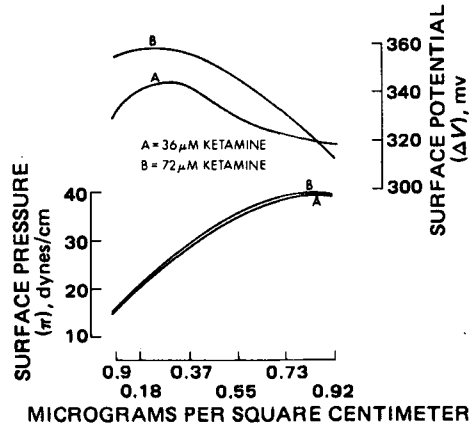


Figure 4—*Surface pressure and surface potential of ganglioside films spread on the aqueous subphase containing two different concentrations of ketamine hydrochloride The ganglioside quantity applied is expressed in micrograms per square centimeter.*

[5] G. Colacicco, A. Selis, and E. V. Cosmi, unpublished data.

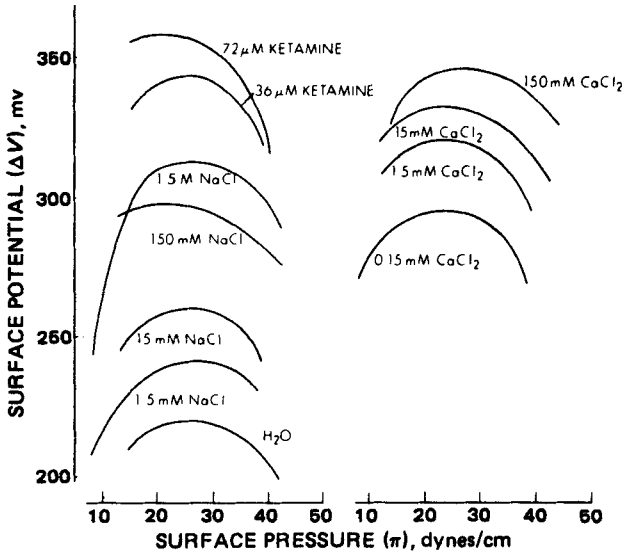


Figure 5—*Surface potential of ganglioside films spread on various sodium chloride, calcium chloride, or ketamine hydrochloride concentrations. The surface potential values are related to the values of the pressure of the ganglioside film.*

onto the surface of two different ketamine hydrochloride concentrations, 10 and 20 μg/ml, or 36 and 72 μ*M*, respectively. The ganglioside film pressure was practically the same for the two ketamine concentrations. The surface potential values, however, were greater for the greater ketamine concentration (curve B) and much greater than the values on distilled water (Fig. 2). Although the greater surface potential of the ganglioside on ketamine was probably due to interaction of the ketaminium cation with the anionic fixed charge of the ganglioside (6, 7), a contribution may come from ketamine penetration into the ganglioside film, as was recently suggested for the interaction of Ca^{2+} with dipalmitoyl lecithin films (14). As expected, the effect of ketamine on the dipalmitoyl lecithin surface potential was only a few millivolts, suggesting little interaction or penetration.

A comparison of ketamine with Na^+ and Ca^{2+} is shown in Fig. 5, which summarizes experiments where the ganglioside was spread on various concentrations of sodium chloride, calcium chloride, and ketamine hydrochloride. The surface potential values were related to the pressure of the ganglioside film on each electrolyte. In all cases, the ΔV values had a maximum at film pressure slightly above 25 dynes/cm, a maximum that appears to be a characteristic of the ganglioside.

The effectiveness of the electrolyte's cation was clearly in the order water < sodium chloride < calcium chloride < ketamine hydrochloride. The ΔV–π curve on distilled water was much lower than on 150 m*M* NaCl, which was near that on 150 μ*M* $CaCl_2$; this result indicates that the cation is important in causing a rise of the ganglioside surface potential. On an equivalents basis, Ca^{2+} was about 500 times as effective as Na^+. Since the surface potential on 150 m*M* $CaCl_2$ was about the same as on 50 μ*M* ketamine, on an equivalents basis, ketamine appears to be about 6000 times as effective as Ca^{2+}; *i.e.*, 6000 times the equivalent concentration of calcium chloride must be used to obtain the effect produced by 50 μ*M* ketamine.

Mitochondrial Lipids—Unlike the mixed gangliosides, the surface potential effect of ketamine on mitochondrial lipid was in the order water < ketamine = sodium chloride < calcium chloride (Fig. 6). Although it can distinguish between Ca^{2+} and Na^+ with a difference in ΔV that is customary with acidic phospholipids, namely $Ca^{2+} > Na^+ > H_2O$ (7), the anionic mitochondrial lipid apparently not only had no preference for ketamine, but the latter's effect was the same as that of Na^+, well below that of Ca^{2+}. This finding suggests a marked specificity and selectivity of the ketamine for the ganglioside.

This conclusion is supported by the fact that, in the interaction with cardiolipin, ketamine was markedly less effective than Ca^{2+} in raising the surface potential. Therefore, the diminished effect of ketamine with mitochondrial lipid is not caused just by a dilution of cardiolipin by lecithin and other nonionic lipids and is consistent with the fact that, in mixed films of dipalmitoyl lecithin and either dipalmitoyl phosphatidic acid or sodium dipalmitoyl phosphate, saturation of specific charge effects by Ca^{2+} occurred with 10–20 mole % of acidic lipid (14, 16), with ≥80 mole

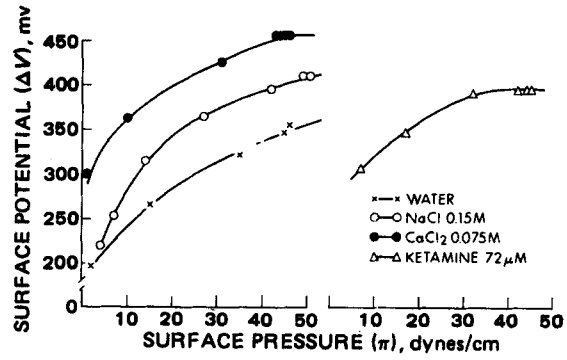


Figure 6—*Ketamine interactions with spread mitochondrial lipid films. The surface potential is related to the sodium chloride, calcium chloride, and ketamine hydrochloride concentrations in the aqueous phase.*

% of lecithin still in the film. Similarly, the large ΔV effect of the ganglioside–ketamine system was markedly reduced as the lecithin content was gradually increased.

This ionic behavior of cardiolipin, a mitochondrial lipid, and ganglioside could suggest that specificity of either interaction or penetration of ketamine requires homogeneous ganglioside rather than a mixed ganglioside–lecithin surface. To what extent this condition is realized in the biological system is still unknown; however, the biological significance of the experimental ganglioside–ketamine system is indicated by the fact that the ganglioside content is unusually high in gray matter, where ketamine is supposed to act (1–5).

Ketamine Effect on Rat Liver Mitochondrial Respiration—At a concentration of 10 μg/ml (37 μ*M*), at which it interacted strongly with the ganglioside, ketamine did not affect the oxidative phosphorylation coupling, measured by the rate of oxygen uptake[4].

This finding may indicate that unlike Ca^{2+}, which is known to affect the mitochrondrial respiration (21), ketamine did not interfere with either the surface potential of the mitochondrial lipid or intact mitochondrial respiration. Such results are consistent with the belief that mitochondria are probably not the site of action of ketamine.

Molecular Perspectives and Biological Significance—Two important properties of ketamine were shown: (*a*) a large surface potential (at zero pressure), which is relatable either to a unique orientation of ketamine's positive charge at the air–water interface or to a ± orientation of the dipoles of especially ionized surface water (18, 19), since ketamine pressure is zero or near zero and (*b*) the ability of micromolar ketamine concentrations to increase markedly the surface potential of ganglioside films, suggesting a unique ketamine interaction with ganglioside, indeed at concentrations at which Ca^{2+} and monovalent ions such as choline and acetylcholine hydrochloride had no comparable effect.

Although the large surface potential of ketamine solutions, about 500 mv in the absence of a film pressure, could be attributed to a large ketaminium$^+Cl^-$ dipole moment in the absence of film pressure, another contribution to the surface potential could derive from a ± orientation of ionized interfacial water on the ketamine solution surface (19).

Since ketamine was 6000 times more effective than Ca^{2+} in raising the surface potential of ganglioside, whereas the effect was comparably nil with dipalmitoyl lecithin films, and since Ca^{2+} plays a central role in acetylcholine discharge from the synapses during nerve function (5), invasion of the synaptic surfaces by ketamine may perhaps reversibly eliminate Ca^{2+} action. The experiments also suggest that the action of ketamine may be preferentially on synaptic surfaces and not on mitochondria.

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Long-Range Substituent Effects in Morphine-Type Agonists and Antagonists: A Possible Explanation for Some Opiate Anomalies

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Received December 27, 1978, from the *Department of Chemistry and Biochemistry and the School of Medicine, Southern Illinois University, Carbondale, IL 62901.* Accepted for publication April 11, 1979.

Abstract □ Anomalous variations in the pKa values of variously substituted morphine-type agonists and antagonists are interpreted as a reflection of long-range substituent effects operating in these molecules. Based on the operation of long-range effects, a mechanism is proposed by which substitution into the *N*-normorphine portion of morphine-type agonists and antagonists changes the activity of the parent molecule. Thus, a remote substituent would distort the whole molecule *via* a conformational transmission effect and thereby (*a*) change the fit between the opiate and its receptor; (*b*) change the electron density distribution throughout the molecule and, therefore, at the nitrogen; (*c*) modify the directionality of the lone-electron pair on the nitrogen; and (*d*) affect the pKa of the drug. The operation of long-range effects as proposed here could account for some of the anomalous changes in opiate activity effected by substitution into the parent molecule.

Keyphrases □ Morphine-type agonists and antagonists—substituents, long-range effects, structure–activity relationships □ Opiates—substituents, long-range effects, structure–activity relationships □ Opiate receptors—effect of opiate substituents, structure–activity relationships □ Structure–activity relationships—substituted morphine-type agonists and antagonists

The analgesic activity of morphine-type opiate agonists as well as the antimorphine activity of morphine-type opiate antagonists is influenced by substitution into the *N*-normorphine portion of these molecules (1). In that study (1), the activities of several series of closely related compounds were obtained by means of standard whole animal testing[1].

The data (1) did not lead, however, to an understanding of the process by which a substituent changes the opiate activity of the parent molecule. Often, the direction of the change was unexpected, in which cases the observed behavior of the substituted opiate seemed to be "anomalous."

Table I—Substitution Effect on the Antimorphine Activity of a Series of *N*-Allylnormorphines and *N*-Propylnormorphines [a]

Normorphine Moiety	Relative Antimorphine Activity of *N*-Allyl-normorphines	Relative Antimorphine Activity of *N*-Propyl-normorphines
Unsubstituted	1.0	1.0
3,6-Diacetyl	0.5	0.9
7,8-Dihydro	0.7	1.9
6-Desoxy	2.2	1.9
7,8-Dihydro-6-desoxy	1.8	0.2
7,8-Dihydro-6-keto	1.3	2.3
7,8-Dihydro-3,6-diacetyl	0.1	0.1
3-Methoxy-7,8-dihydro-6-desoxy	1.4	0.4

[a] Taken from Ref. 1.

BACKGROUND

Winter *et al.* (1) measured the antimorphine activities of two series of substituted opiate antagonists: *N*-propylnormorphines and *N*-allylnormorphines. Identical substituents were introduced into the same positions of the *N*-normorphine moiety of these two parent antagonists so that the observed differences in activity between these two series did not reflect differences in the transport rate caused by the substituent *per se*. An examination of the data (Table I) reveals no apparent correlation between the structure (or position) of the substituent and the direction of antimorphine activity change caused by the substituent.

These investigators (1) also found that the introduction of a common substituent into the *N*-normorphine moiety of morphine agonists and the corresponding antagonists (*N*-allylnormorphines) changed respective agonist and antagonist activities which were, as often as not, unrelated (Table II). These data are difficult to rationalize, especially if one accepts the hypothesis that the same cavity of the opiate receptor accommodates morphine-type agonists and antagonists, as suggested in the opiate receptor models of Goldstein (3) and Kolb (4) and as implied in some other opiate receptor models (5).

Within the category of the compounds covered in Table II, the following example is intriguing: 3,6-diacetylmorphine (heroin) exhibits 2.4 (6) or 2.5 (7) times the *in vivo* analgesic activity of morphine[2], and 1.45

[1] Analgesic and antimorphine activities were tested by the tail flick response in rats by the method of D'Amour and Smith (2). Drugs were injected subcutaneously.

[2] Ratio of ED_{50} (milligrams per kilogram) relative to morphine from mouse hot-plate experiments.

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