Notes

Correlation of Opiate Receptor Affinity with Analgetic Effects of Meperidine Homologues

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The affinity for opiate receptor sites in brain tissue in a series of N-substituted meperidine homologues has been compared with the analgetic potency of these compounds in mice. There is a good correlation between affinity for opiate receptor binding sites assayed in the presence of sodium and analgetic potency for homologues whose N-substituent has six or fewer carbons. The apparent discrepancy between the weak affinity of these drugs for opiate receptors and their fairly potent analgetic effects in vivo can be explained by meperidine's efficient penetration into brain.

Opiate receptor binding has been characterized biochemically[1-4] in terms of its subcellular,[5] regional,[6,7] and phylogenetic[8] distribution, as well as the in vitro influence of sodium,[9] temperature,[10] and protein-modifying reagents,[11] which predict and differentiate opiate agonist and antagonist activity. A close correlation between biochemical binding in vitro and activity in vivo has been a prerequisite for demonstrating the pharmacological relevance of opiate receptor binding.[1,2,9,12] In the guinea pig ileum, the proportion of the maximal response evoked by opiate agonists and antagonists on contractions in vitro is closely related to the fraction of the receptors occupied.[12] Similar calculations based on the analgetic response are more difficult to establish because drug concentrations at the receptor sites, which are influenced by metabolic and distributional variables, are more difficult to control rigorously. Nonetheless, for a wide variety of opiates, affinity for the opiate receptor does generally correlate with analgetic activity.[1,2,9,13] Homologous series of drugs reduce the extent of chemical variation and, in some cases, minimize differences in drug metabolism and distribution. Correlations between opiate receptor affinity and analgetic activity have been reported for homologous series of ketobemidones,[13] benzazocines,[14] and benzomorphans.[14,15]

In the present study we have evaluated opiate receptor affinity for a series of meperidine homologues for which detailed information regarding their analgetic activity,[16,17] metabolism, and penetration into brain[18] is available.

Results and Discussion

One of the apparent discrepancies in the relationship of opiate receptor affinity to analgesia has been the surprisingly low affinity of meperidine[2,9] which requires a concentration of 40 μM to inhibit receptor binding of [^{3}H]naloxone by 50% in the presence of sodium ion. Thus, the affinity of meperidine for the opiate receptor is only 0.2% of that of morphine, whereas its pharmacological potency in vivo in the mouse hot-plate analgetic test[17,19] is about 10% of that of morphine.[16]

The apparent discrepancy probably derives from meperidine's ability to penetrate the brain readily and achieve 600-fold higher brain levels relative to morphine. Five minutes after intraveous injection of the ED_{50} dose of meperidine, when analgesia is at its peak, mouse brain levels are 60 μmol/g.[18,19] This corresponds closely to the concentration of meperidine required to occupy half of the opiate receptors in the in vitro assay of brain homogenate performed in the presence of physiological concentrations of sodium. After an ED_{50} dose of morphine of 4 μg/kg, peak analgesia occurs about 1 h later when 0.1 μmol/g of brain concentration has accumulated.[19]

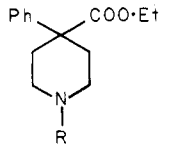
This concentration is sufficient to occupy half of the opiate receptor sites in the presence of sodium in the in vitro assay.[9] Thus, for both morphine and meperidine, the concentration of drug required to occupy half of the receptors in the presence of sodium in vitro is very similar to the brain concentration achieved when a half-maximal analgetic response can be evoked.

In proceeding from the *N*-methyl (meperidine) to ethyl homologue, there is a tenfold decrease in affinity for the opiate receptor assayed in the absence of sodium, while assays in the presence of sodium indicate a similar affinity for the two compounds. Since methyl and ethyl homologues have essentially the same analgetic potency,[19] assays in the presence of sodium predict analgetic activity better than assays in its absence. While ethyl and propyl homologues do not differ markedly in affinity for the opiate receptor or in analgetic potency, the butyl homologue is two to three times more potent than the propyl derivative both in inhibiting opiate receptor binding in the presence of sodium and in analgetic activity.

N-Pentylnormeperidine has about twice the affinity of *N*-butylnormeperidine for the opiate receptor, whether assayed with or without sodium, and has about twice the analgetic potency of *N*-butylnormeperidine. *N*-Hexylnormeperidine, with twice the analgetic activity of the *N*-pentyl compound, has twice and seven times the affinity of the pentyl homologue for the opiate receptor in the absence and presence of sodium, respectively.

As the chain length of the nitrogen substituent extends beyond six carbons, opiate receptor affinity and analgetic potency correlate less well. The heptyl and octyl derivatives possess less analgetic potency in vivo than would be expected from their in vitro affinities for opiate receptor sites, though the nonyl derivative fits in the sequence of correlations of methyl through hexyl derivatives. The

Table I. Comparison of Opiate Receptor Affinity and Analgetic Potency of a Homologous Meperidine Series



Homologue	Opiate receptor inhibn[a] (IC_{50}, μM) −NaCl	Opiate receptor inhibn[a] (IC_{50}, μM) +NaCl	Sodium response ratio[b]	Analgesia[c,d] (ED_{50}, μmol/kg)
Methyl (meperidine)	0.50	40	80	33
Ethyl	5.00	50	10	41
Propyl	4.00	100	25	46
Butyl	0.90	30	33	20
Pentyl	0.40	15	38	10
Hexyl	0.20	2.0	10	5
Heptyl	0.055	0.65	12	5
Octyl	0.03	0.80	27	10
Nonyl	0.15	8.0	53	15

[a] Inhibition of opiate receptor binding is presented as the concentration of drug which reduced specific [^{3}H]naloxone binding by 50%, the mean of two determinations which varied less than 30%. [b] The ratio of IC_{50} concentrations for incubations conducted in the presence of 100 mM NaCl to those conducted in its absence. [c] Analgetic potency in mice was assessed by the hot-plate technique.[17,19] [d] The equations correlating ED_{50} doses with IC_{50} in the presence (+NaCl) and absence (−NaCl) of sodium are $ED_{50} = 0.44\ IC_{50} + 8.39$ ($n = 9$; $r^2 = 0.86$; $s = 6.24$) and $ED_{50} = 7.09\ IC_{50} + 11.70$ ($n = 9$; $r^2 = 0.73$; $s = 8.76$), respectively.

discrepancies may derive from a decreased ability of these highly lipid-soluble compounds to accumulate in the brain if they instead accumulated in body fat. Larson and Portoghese[18] found that *N*-methyl-, propyl-, butyl-, and hexylnormeperidines entered the brain to similar extents at the time of peak analgesia.

Analgetic potencies of the meperidine homologues correlate better with affinity for the opiate receptor in the presence of sodium ($r^2 = 0.86$) than in its absence ($r^2 = 0.73$). The better correlations in the presence of sodium presumably reflect the sodium-containing physiologic environment of the opiate receptor.

The ability of over 20 opiates to inhibit [^{3}H]naloxone binding in the presence relative to the absence of sodium ("sodium response ratio") is closely correlated with the agonist–antagonist properties of these drugs.[9] Opiates with a sodium response ratio above 12 are all "pure agonists", i.e., they mimic the action of morphine in almost all pharmacological test situations and show no antagonist properties in vivo. Opiate antagonists, which block or reverse the action of morphine and other opiate agonists, have a sodium response ratio of about 3 or less. Mixed agonist–antagonists, which show variable pharmacological properties depending on the test situation employed, have intermediate responses to sodium.[9,15]

All of the meperidine homologues show sodium response profiles which would place them in the category of "pure opiate agonists", which is consistent with the known pharmacology of these drugs. Reasons for the eightfold variation in sodium response ratios among the homologues are unclear. In this study affinity of meperidine homologues for the opiate receptor correlates in general with their analgetic potency. Since the extracellular space in the brain is bathed in sodium, it is not surprising that this correlation is better for opiate receptor assays conducted in the presence rather than in the absence of sodium. Previously we found that opiate receptor affinity and analgetic potency were correlated in a homologous series of ketobemidone derivatives.[13] In general, ketobemidones are considerably more potent, both analgetically and in affinity for the opiate receptor, than comparable meperidine homologues.

The unique metabolic pattern, pharmacodynamics, and weak opiate receptor affinity of meperidine may explain why it is among the most clinically useful analgetics. Meperidine enters the brain much more rapidly than morphine. With intravenous tracer doses in rodents, about 2% of an administered dose of meperidine accumulates in the brain[18] while less than 0.05% of administered morphine is localized in the brain. One would anticipate that, at equianalgesic doses, meperidine should elicit fewer undesirable side effects that are mediated by peripheral opiate receptors than morphine. Indeed, at equianalgesic doses, meperidine is less spasmogenic and causes less constipation.

Experimental Section

Opiate receptor binding was measured as previously described.[9] Rats (150–250 g) were decapitated and their whole brains with cerebella removed were homogenized in 200 vol of ice-cold Tris–HCl buffer (pH 7.4 at 25+ °C). After centrifugation at 10000g, the supernatant fluid was discarded and the brain membranes were reconstituted in the original volume of Tris buffer with or without NaCl (100 mM). Aliquots (1.9 ml) of this freshly prepared homogenate were incubated with [^{3}H]naloxone (1 nM), obtained from New England Nuclear Corp. (24 Ci/mmol), for 30 min at 25 °C. Samples were placed on ice for 30 min, rapidly filtered individually by low pressure over Whatman glass fiber filters (GFB), and washed with two 7-ml portions of cold sodium-free Tris buffer. Membrane-laden filters were transferred to counting vials containing 12 ml of detergent scintillation cocktail (Hydromix, Yorktown Products) and counted the following day by liquid scintillation spectrometry at 35–40% efficiency. The concentration of nonradioactive drug required for 50% inhibition (IC_{50}) of stereospecific [^{3}H]naloxone binding (binding occurring in the presence of 100 nM levallorphan subtracted from radioactivity bound in the presence of 100 nM dextrallorphan) was determined by plotting percent inhibition of the mean of triplicate incubations of seven to ten concentrations of drug on log probit paper. IC_{50} values represent the mean from two separate determinations in which values varied less than 30%.

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Charge Distribution of Histamine Monocation in Its "Essential" Conformation

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With the atomic nuclei in positions earlier defined as the "essential" conformation for activity of the histamine monocation, the charge distribution is obtained by integrating the square of the molecular wave function. The results with ab initio wave functions indicate that the positive charge is evenly dispersed over the molecular skeleton and that pictures of receptors involving localized negative sites may be invalid. A detailed description of electron distribution is given.

A comparison of the conformational flexibility of the histamine monocation, which is active at both H_1 and H_2 receptors, with that of the 4-methyl compound (H_1 activity half the activity of histamine and H_2 activity $^1/_{500}$th) has led to the definition of a possible H_1 "essential" conformation.[1] The inclusion of further active species such as *N,N*-dimethylhistamine suggests restriction of attention to the single trans (antiperiplanar) conformation. These conclusions were reached using potential surfaces generated using the crude extended Hückel method which was justified by experimental criteria.[1] For the defined essential conformation we now use the more reliable ab initio molecular orbital method to compute the charge distribution.

This defined conformation may be essential in producing activity for one of two reasons. The precise spatial arrangement of atoms may be that which is required to bind to the receptor, in which case properties of the receptor binding site may be inferred. The alternative rationale for a conformation which is available to active compounds but denied to inactive species might be that the essential conformation is one through which the molecule must pass in order to gain access to the receptor site.

In either case, but particularly if the former alternative in fact represents the molecular situation, a precise definition of the relative atomic positions of the various nuclei is obviously helpful. Far more useful, however, would be a clear indication of the detailed electronic structure of the histamine monocation in its essential conformation. Any interaction between the active molecule and its receptor must involve electrons rather than nuclei which are merely positive centers to which the electronic cloud is held.

Crude theoretical methods[2] which give an indication of electronic distribution have been available for many years. Recently a method giving an accurate value for the number of electrons in any defined volume of space and suitable for pharmacological species has been devised.[3] Here we apply this technique to the histamine monocation with the nuclei in positions indicated earlier as "essential".

Charge Densities. Since the earliest days of quantum mechanics it has been realized that the square of a molecular wave function, ψ, yields a probability or electron density. The value of ψ^2, or $\psi^*\psi$ if ψ is complex, integrated over a defined region of space will give the number of electrons in that volume. The reason why this obvious source of electron density information has not been applied to molecules which are not highly symmetrical has been the practical problem of evaluating $\int\psi^*\psi d\upsilon$ over volumes of space which are removed from the origin of the molecular coordinate system.

The recent advance has been made possible by the fact that computational methods are now so powerful that it is possible to compute an accurate molecular wave function so quickly that it is not too extravagant to choose each individual atom in the molecule successively as the origin of the coordinate system and to repeat the quantum mechanical calculation for each case. Integrating $\psi^*\psi d\upsilon$ when the volume $\int d\upsilon$ is a sphere centered at the origin is relatively simple so that in this way the charge density in any sphere centered on each individual nucleus may be obtained.

There remains the problem of the arbitrary definition of the volume elements which are considered. Spheres are mathematically simplest and also sensible, although a