Communications to the Editor

N-Methylnalorphine: Definition of N-Allyl Conformation for Antagonism at the Opiate Receptor

Sir:

The fundamentally important question of whether morphine and its congeners adopt a conformation in the drug–receptor complex with the nitrogen substituent in an equatorial or axial configuration is not yet resolved. The answer to this question has great significance for the rational development of novel agents that interact with the opiate receptor.

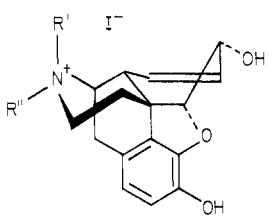
The apparent requirement for an equatorial configuration of the nitrogen substituents in morphine-like agonists was noted by Belleau et al.[1] and also by Feinberg et al.,[2] who further suggested that antagonists are distinguished by having their nitrogen substituents equatorially oriented. However, recent evidence indicates that both axial and equatorial substituents (as defined by solid-state crystal structures) can lead to active agonists.[3] Other theories have postulated that levels of agonism/antagonism may be related to various conformational minima attainable by antagonist nitrogen substituents[4] or that more subtle conformational changes in the piperidine ring might lead to different interactions with the amine binding site.[5] While more recent work has shown that potent antagonists may be generated in series not possessing an antagonist pharmacophore on the N atom,[6,7a,b] all antagonists or agonist/antagonists of current clinical importance possess such a substituent.[8]

Recent quantum mechanical calculations reveal that the axial–equatorial minimum-energy differences for protonated and neutral morphine are only of the order of 0.4 and 2.9 kcal/mol, respectively, and these barriers do not increase significantly in the antagonist N-allylnormorphine (nalorphine).[9] Since, according to estimates by Ariens,[10] the amount of binding energy available for a ligand–receptor interaction assuming high complementarity of fit is also of this order of magnitude, the relevance of any specific conformation of an isolated flexible molecule to the drug–receptor interaction is unclear. In principle, sufficient energy is available to cause an induced fit in an agent with either an axially or an equatorially confined susbstituent.

We undertook an experimental test of the importance of the conformation at nitrogen in opiate antagonists by a comparison of the pharmacology of the diastereoisomeric quaternary compounds A and B which have their N-allyl substituents confined to the axial and equatorial positions, respectively. These were prepared by the method of Koczka and Bernath[11a] by alkylation of morphine with allyl iodide and N-allylnormorphine with methyl iodide, respectively.

The two compounds[11b] were examined for their ability to displace [^{3}H]diprenorphine (1 nmol) from rat brain homogenate by the procedure of Pert and Snyder[12] and for both μ and δ agonist and antagonist effects[13] in the electrically stimulated mouse isolated vas deferens (mvd)[14] and for μ and K agonist effects in the guinea pig isolated ileum (gpi).[15]

Quaternization is seen to lead to very reduced antagonist potency against both μ and δ agonists and to a decreased binding affinity in competition with [^{3}H]diprenorphine. The axial isomer A has approximately one-hundreth the



isomer A, R' = $CH_2CH{=}CH_2$; R'' = CH_3
isomer B, R' = CH_3; R'' = $CH_2CH{=}CH_2$

antagonist potency of nalorphine in the mvd assay, which very closely parallels the decrease in binding affinity both in the presence and absence of Na^+ to approximately one-hundreth that of nalorphine. Isomer A is completely ineffective in antagonizing the effects of Met^{5}E at a concentration of 22 μmol (approximately 5 times the pA_2 concentration of isomer B at this receptor and approximately 20 times its own pA_2 concentration at the μ receptor).

(1) Belleau, B.; Conway, T.; Ahmed, F. R.; Hardy, A. D. *J. Med. Chem.* **1974**, *17*, 907.
(2) Feinburg, A. P.; Creese, I.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 4215.
(3) Shiotani, S.; Kometani, T.; Iitaka, Y.; Itai, A. *J. Med. Chem.* **1978**, *21*, 153.
(4) Loew, G. H.; Berkowitz, D. S. *J. Med. Chem.* **1978**, *21*, 101.
(5) Kolb, V. M. *J. Pharm. Sci.* **1978**, *67*, 999.
(6) Zimmerman, D. M.; Nickander, R.; Horng, J. S.; Wong, D. T. *Nature (London)* **1978**, *275*, 332.
(7) (a) Leysen, J. E.; Laduron, P. M.; Neimegeers, C. J. E. "Characteristics and Function of Opioids"; Elsevier/North Holland: Amsterdam, 1978; p 479. (b) Michne, W. F.; Lewis, T. R.; Michalec, S. J.; Pierson, A. K.; Gillan, M. G. C.; Patterson, S. J.; Robson, L. E.; Kosterlitz, H. W. in ref 7, p 197.
(8) See, for example, discussions in *Adv. Biochem. Physchopharmacol.* **1974**, *8*.
(9) Loew, G. H.; Berkowitz, D. S. *J. Med. Chem.* **1975**, *18*, 656.
(10) (a) For discussion, see Ariens, E. J. "Drug Design"; Academic Press: New York, 1971; Vol. I, Chapter 1, pp 194–233. (b) For estimate, see Ariens, E. J. "Trends in Pharmaceutical Sciences", Elsevier/North Holland: Amsterdam, 1979; p 14.
(11) (a) Koczka, K.; Bernath, G. *Acta Chim. Acad. Sci. Hung.* **1967**, *51*, 393. (b) Isomers A and B were prepared exactly as described in ref 11a. The samples of A and B were recrystallized six times from water and 90% aqueous methanol, respectively, and then had melting points 240–241 and 255–256 °C, respectively. The IR spectrum of the two pure materials, according to the criteria of ref 11a, showed none of the other isomer as a contaminant. An HPLC analysis on Spherisorb 5 μm ODS, 25 × 0.46 cm i.d., at 60 °C with 0.1% (w/v) pentanesulfonic acid (sodium salt) in acetonitrile/water (96:4) at a flow rate of 1 mL min^{-1} with UV detection at 227 nm gave retention times of 21.6 and 23.6 min, respectively, for isomers A and B. A quantitative assay of very low levels of impurities proved difficult, but samples of A and B were >98% pure.
(12) Pert, C. B.; Snyder, S. H. *Mol. Pharmacol.* **1974**, *10*, 868.
(13) Lord, J. A. H.; Waterfield, A. A.; Hughes, J.; Kosterlitz, H. W. *Nature (London)* **1977**, *267*, 495.
(14) Henderson, G.; Hughes, J.; Kosterlitz, H. W. *Br. J. Pharmacol.* **1972**, 764.
(15) Gyang, E. A.; Kosterlitz, H. W. *Br. J. Pharmacol. Chemother.* **1966**, *27*, 514.

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Table I. Results of in Vitro Pharmacological Evaluation of Diastereoisomers A and B

compd	opiate antagonist action in the mouse isolated vas deferens: concn, nmol, required to give a dose ratio of 2		IC_{50},[a] nmol	
	vs. Met⁵E	vs. normorphine	$+Na^+$	$-Na^+$
nalorphine	286 ± 52 (3)	22.4 ± 3.1 (3)	160	59
A	>22 000 (2)[b]	1247 ± 268 (2)	11 500	8500
B	4 925 ± 1 435 (5)	59.2 ± 5.3 (5)	510	440

[a] IC_{50} is that concentration necessary to inhibit the stereospecific [³H]diprenorphine (1 nmol) binding at 36 °C. [b] At 22 μmol, no antagonism of the effects of Met⁵E was apparent.

The reduced binding affinity of the equatorial isomer B is approximately one-third that of nalorphine, and this again closely parallels the reduced antagonist potency in reversing the effects of normorphine in the mvd assay to approximately one-third that of nalorphine. The antagonist potency in reversing the effects of Met⁵E is less, however, being approximately one-twentieth that of morphine, and isomer B therefore possessing 80-fold higher affinity for the μ receptor (cf. pA_2 vs. normorphine with pA_2 vs. Met⁵E). The effects of quaternization on reduced affinities and potencies are broadly in line with the results of previous studies of quaternized opiates.[16-19] The quaternization of nalorphine with allyl bromide has been shown to reduce both agonist and antagonist potencies, respectively, to 2.1 and 12% that of nalorphine.[20] That study, however, did not examine the importance of configuration about the N atom to agonist or antagonist effects. In line with the conclusions from previous work,[12] the sodium index IC_{50} $(+Na^+)/IC_{50}$ $(-Na^+)$ for isomer B as a pure antagonist is expected to be <1, and that for isomer A as a partial agonist should be >1. It is found that indexes for both are marginally >1 (A, 1.26; B, 1.16). Although the relative order is as expected, it is difficult to assess the importance of such small differences, as some other partial agonists have indexes close to 1.[12] The value of the sodium index for A is probably a reflection of the fact that its intrinsic agonist activity is low in relation to its antagonist activity.

Neither isomer possessed any agonist activity (<0.05% normorphine) in the mvd preparation. In the gpi, however, while isomer B was devoid of any agonist activity in concentrations up to 44 μmol, isomer A possessed definite agonist activity manifested by a depression in the twitch height that was completely reversed by naloxone (0.09 μmol). The agonist potency was very weak, being only 0.26 ± 0.01% (n = 6) that of normorphine. Displacement of the dose–response curve to A with low concentrations 1.5–9 nmol of naloxone indicated that the compound is 4–5 times more resistant than normorphine to the antagonist effects of naloxone. This finding is consistent with the idea that isomer A may be acting on a κ receptor in the gpi (see Hutchinson et al.[21]).

The marked differences in pharmacological behavior between isomers A and B warranted unequivocal determination of their three-dimensional structure. Their configurations have previously been assigned on the basis of chemical rationale and spectroscopic evidence.[11] However, lack of selectivity in the quaternization reactions with alkylation of both morphine and *N*-allylnormorphine giving differing proportions of A and B and the essential ambiguity of the original structural assignments[22] induced us to examine the structure of the isomer with the higher antagonist potency by X-ray diffraction.

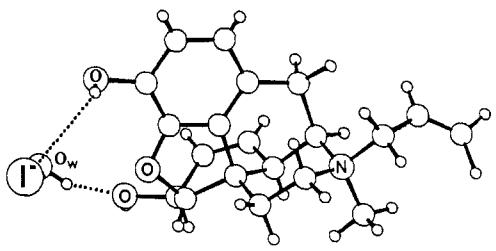

Figure 1. Observed structure of isomer B, showing equatorial configuration of the allyl group about the N atom, and the hydrogen-bonding system linking hydroxyl groups, water, and the iodide ion.

The structure determined by this analysis (depicted in Figure 1) represents the first unequivocal demonstration that the equatorial position is the preferred configuration for the antagonist N-substituent (the allyl group)[23] and confirms the original structure assignment of Koczka and coworkers. The configuration of the chiral N atom is *R*. The bond angles and distances in this structure are as expected and generally correspond closely to those in nalorphine. The torsion angle defining the orientation of the allyl group N(17)–C(19)–C(20)–C(21) is −104.2 (5)° and that in nalorphine is 136.5°.[24] Although this angular distortion appears large, the torsion angle found for the identical fragment in naloxone is −97.9°.[25] The partial

(16) Foster, R. S.; Jenden, D. J.; Lomax, P. *J. Pharmacol. Exp. Ther.* **1967**, *157*, 185.
(17) Opheim, K. E.; Cox, B. M. *J. Med. Chem.* **1976**, *19*, 858.
(18) Kosterlitz, H. W.; Lord, J. A. H.; Watt, A. J. "Agonist and Antagonist Actions of Narcotic Analgesic Drugs"; Macmillan: London, 1972; p 45.
(19) See also Valentino, R. J.; Herling, S.; Woods, J. H.; Medzihradsky, F.; Merz, H. *J. Pharmacol. Exp. Ther.* **1981**, *217*, 652.
(20) Kosterlitz, H. W.; Waterfield, A. A.; Berthoud, V., paper presented to the Committee on Problems of Drug Dependence, Mexico City, 1974.
(21) Hutchinson, M.; Kosterlitz, H. W.; Leslie, F. M.; Waterfield, A. A.; Terenius, L. *Br. J. Pharm.* **1975**, *55*, 541–546.
(22) Bernath, G.; Szabo, J. A.; Koczka, K.; Vinkler, P. *Acta Chim. Acad. Sci. Hung.* **1967**, *51*, 339.
(23) A sample of isomer B was crystallized as a monohydrate by evaporation of an aqueous methanol solution. The crystals are othorhombic, space group $P2_12_12_1$ with a = 9.136 (1), b = 18.677 (2), and c = 11.323 (1) Å, with Z = 4 molecules of $C_{20}H_{24}NO_3^+I^-\cdot H_2O$; 2108 intensity data with $2\theta < 130°$ were measured with graphite monochromatised Cu Kα radiation and were corrected for absorption (μ = 133 cm⁻¹, Cu Kα). Merging equivalent reflections yielded 1945 unique reflections; 1753 had $F > 3\sigma$ and were treated as observed. The structure was solved by the heavy-atom method refined by the method of cascade blocked full-matrix least squares to a final residual R_W = 0.038, corresponding to a weighted residual R_W = 0.038 ($\sum w\Delta^2$ was minimized, where $w = 1/(\sigma^2F + 0.0003F^2)$. In the final cycles of refinement, the hydrogen atoms were refined isotropically and the other atoms anisotropically. All calculations subsequent to data reduction were performed with SHELX. Atomic coordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Cambridge, CB2 1EW, England. Any request should be accompanied by the full literature citation for this communication.
(24) Gelders, Y. G.; De Ranker, C. J.; van Rooijen-Reiss, C. *Cryst. Struct. Commun.* **1979**, *8*, 995.

opiate agonist effects of nalorphine have been related to variations in this torsion angle,[9] but its variability may simply serve to emphasize the obvious flexibility of such a substituent; indeed, from published data, the conformation of the allyl group in the quaternary derivative is of somewhat lower energy than that found in nalorphine hydrobromide.[9] The possibility that this torsion angle is influenced by ionic interactions should not be discounted, since the iodide ion is found 5.32 and 5.34 Å, respectively, from the quaternary nitrogen atoms of two adjacent drug molecules. in addition, the iodide ion is part of the chain of hydrogen bonds, O(6)–H···O(H_2O)–H···I^-···H–O(3) linking the 6-hydroxy and 3-phenolic substituents of the same molecule. The remaining water hydrogen atom interacts with the 6-hydroxy group of a symmetry-related drug molecule, resulting in an infinite chain of hydrogen bonds extending along the a-axial direction.

Other interesting observations emerge from the pharmacological evaluation of A and B. The fact that isomer A with an axial N-substituent possesses some agonist activity whereas B with an equatorial N-substituent is a pure opiate antagonist lends support to the hypothesis of Feinberg et al.[2] relating to N-substituent conformations. It must be stressed, however, that isomer A has but a low intrinsic agonist activity and still possesses a very substantial (in comparison to its agonist activity) antagonist component. Both isomers A and B possess affinity for the μ rather than the δ receptor. Since isomer B has greater affinity at both μ and δ receptors than isomer A, it is apparent that the equatorial configuration of the N-allyl group is the favored conformation for interaction at both types of opiate receptor.

To our knowledge this is the first unequivocal experimental determination of the contribution of configuration about the N atom to antagonist effects in opiates.

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Supplementary Material Available: Atomic coordinates, anisotropic temperature factors, bond distances, and bond angles for isomer B (3 pages). Ordering information is given on any current masthead page.

(25) Karle, I. L. *Acta. Crystallogr., Sect. B* **1974**, *30*, 1682.

(26) Pfizer Central Research, Sandwich, CT13 9NJ, England.
(27) The Cancer Institute, Peter MacCullum Hospital, Melbourne, Australia.

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Articles

Potential Neuroleptic Agents. 2,6-Dialkoxybenzamide Derivatives with Potent Dopamine Receptor Blocking Activities

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A series of some novel *N*-(1-ethyl-2-pyrrolidinylmethyl)benzamides was synthesized and tested for dopamine receptor blockade in vivo by the ability to block the apomorphine syndrome in the rat. Several compounds were considerably more potent than sulpiride as dopamine receptor blockers and displayed low liability to induce extrapyramidal side effects (catalepsy) in the rat. The blockade of dopamine receptor activity in vivo was mainly confined to the levorotatory isomers having the *S* absolute configuration. The structure–activity relationships are discussed.

The substituted benzamides sulpiride, metoclopramide, and tiapride display pharmacological properties characteristic for neuroleptic drugs.[1-6] Sulpiride has been reported to be an effective antipsychotic agent that at therapeutic doses produces less marked extrapyramidal side effects than most antipsychotic compounds.[7,8] Animal investigations have demonstrated that many of the pharmacological effects of this compound are associated with blockade of (DA) receptors in both striatal, mesolimbic, and tuberoinfundibular DA containing neurons. Thus, sulpiride blocks some of the behavioral effects of the DA agonist apomorphine that are mediated via activation of striatal and mesolimbic DA receptors[4,10] and inhibits the binding of radiolabeled dopamine receptor antagonists,

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(1) L. Justin-Besancon, Cl. Laville, J. Margarit, and M. Thominet, *C. R. Hebd Seances Acad. Sci.*, **279**, 375 (1974).
(2) P. Jenner and C. D. Marsden, *Life Sci.*, **25**, 479 (1979).
(3) M. Stanley and S. Wilk, *Life Sci.*, 24, 1907 (1979).
(4) K. Fuxe, S. O. Ögren, B. B. Fredholm. L. F. Agnati, T. Hökfelt, and M. Perez de la Mora, in "Rhinencephale Neurotransmetteurs et Psychoses", J. de Ajuriagerra and R. Tissot, Eds., Georg & Cie, Geneva, 1977, pp 253–289.
(5) P. Borenstein, C. Champien, P. M. Cujo, P. Gekiere, C. Olivenstein, and P. Karmarz, *Sem. Hop.*, **19**, 1301 (1969).
(6) A. Tagliamonti, G. De Montis, M. Olianas, L. Vargiu, G. U. Corsini, and G. L. Gessa, *J. Neurochem.*, 24, 707 (1975).
(7) D. H. Mielke, D. M. Gallant, and C. Kessler, *Am. J. Physchiatry*, 134, 1371 (1977).

0022-2623/82/1825-1280$01.25/0