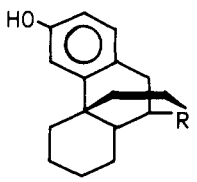
Communications to the Editor

Stereospecific Interaction of the Quaternized Opiate, *N*-Methyllevorphanol, with Opiate Receptors

Sir:

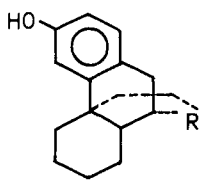
It has long been argued that one of the features necessary for the optimal interaction of drugs with opiate receptors is the presence of an electrophilic nitrogen (e.g., an amine protonated at physiological pH).[1,2] Such a moiety also exists in the quaternary salt, *N*-methylmorphine, and several groups have attempted to demonstrate its opiate properties.[3-5] However, the results of these groups, though in some cases suggestive of possible opiate activity, must be considered equivocal since (1) attempts to demonstrate in vivo activity were complicated by the untoward nonspecific effects of quaternary ammonium salts and the poor penetration of the drug to sites of action in the central nervous system, (2) the enantiomer of *N*-methylmorphine is not available and, hence, stereospecificity could not be demonstrated, and (3) blockade or reversal of the observed effects with an opiate antagonist has not been reported.

We report here the preparation of the enantiomeric quaternary iodides, *N*-methyllevorphanol [**2**, D(−)-3-hydroxy-*N*,*N*-dimethylmorphinanium iodide] and *N*-methyldextrorphan [**4**, L(+)-3-hydroxy-*N*,*N*-dimethylmorphinanium iodide], from levorphanol (**1**) and dextrorphan (**3**), respectively, and demonstrate that specific opiate effects are exerted by the ionized species **2**.



1, R = $\ddot{N}$-CH_3

2, R = $\overset{\oplus}{N}(CH_3)_2$ $\overset{\ominus}{I}$



3, R = $\ddot{N}$-CH_3

4, R = $\overset{\oplus}{N}(CH_3)_2$ $\overset{\ominus}{I}$

The methiodides **2** and **4** were prepared by treating ethereal solutions (5 mg of free base/ml) of the corresponding tertiary amines **1** and **3** with excess methyl iodide. Analytically pure **2** and **4**[6] were obtained, both in 65% overall yield, after recrystallization from ethanol. *N*-Methyllevorphanol iodide (**2**) had mp 268–269.5°; $[\alpha]^{22}D$ −48.9° (*c* 1.0, methanol). Anal. ($C_{18}H_{26}INO$) C, H, I, N. *N*-Methyldextrorphan iodide (**4**) had mp 267–268°; $[\alpha]^{22}D$ +49.5° (*c* 1.0, methanol). Anal. ($C_{18}H_{26}INO$) C, H, I, N. Both **2** and **4** appeared as a single component in two TLC systems: (1) SiO_2, concentrated NH_3–EtOH (3:7), visualized with iodoplatinate, R_f of **2** and **4** = 0.36; (2) SiO_2, EtOH–HOAc–H_2O (6:3:1), iodoplatinate, R_f of **2** and **4** = 0.47. Neither **2** nor **4** showed any trace (limit of detection <1%) of their corresponding starting amines **1** or **3** by TLC: SiO_2, MeOH–trace NH_3, iodoplatinate, R_f of **2** and **4** < 0.10; R_f of **1** and **3** = 0.39.

The properties of the quaternary morphinans have been examined on the guinea pig ileum preparation.[7] It has been shown that (1) opiate drugs inhibit the responses of the ileum preparation to electrical stimulation at concentrations similar to their concentrations in brain following administration of analgesic doses,[8,9] (2) the effect is specific to the analgetically active enantiomorph of a pair of optical isomers,[3,9] and (3) the effect is blocked or reversed by the specific opiate antagonist, naloxone, at low concentration.[3]

Strips of guinea pig ileum longitudinal muscle (with myenteric plexus) were set up and subjected to electrical stimulation as described previously.[10] *N*-Methyldextrorphan (**4**) did not affect the electrically induced contractions at concentrations up to 10^{-5} M and gave only weak depressant effect at 4×10^{-5} M. *N*-Methyllevorphanol (**2**) produced a dose-dependent inhibition of contraction with an IC_{50} of 0.61 ± 0.07 μM (mean ± SEM, $n = 7$; IC_{50} is the concentration giving a 50% inhibition of the contractile tension developed in response to the electrical stimulus). The inhibition could be completely reversed or blocked by naloxone (0.1 μM). The presence of inhibitory concentrations of **2** did not reduce the response of the muscle to exogenous acetylcholine, thus indicating that the drug was acting on the neuronal, and not the muscle, elements of the preparation.

In a second series of experiments, **2** was compared to **1** using the single dose procedure described by Kosterlitz and Watt.[11] The agonist potency of *N*-methyllevorphanol (**2**) was 0.068 ± 0.009 (mean ± SEM, $n = 10$) relative to that of levorphanol (**1**); antagonist potency was 0.032 ± 0.008 ($n = 7$) relative to levorphanol. The half time for recovery from the antagonist activity of **2** was approximately one-third that for **1**, and the rate of decline of the agonist effect of **2**, following its removal from the bathing fluid, was much more rapid than that of **1**. These differences in recovery rates exclude the possibility that the effects observed following the administration of *N*-methyllevorphanol (**2**) were produced by N-demethylation of the compound by the tissue or were due to the presence of a small quantity of **1** in its quaternized derivative.

Confirmation of the interaction of **2** with specific opiate receptors was obtained in experiments in which receptor binding was measured.[12] *N*-Methyllevorphanol (**2**) depressed the stereospecific binding of [^{3}H]etorphine (2.5 nM) by guinea pig brain homogenate, with an IC_{50} of 0.52 μM, a figure close to its IC_{50} on the ileum preparation. *N*-methyldextrorphan (**4**) was much less active, giving only 26% inhibition at 10 μM. A similar inhibition was observed with the parent compound, dextrorphan (**3**), at this concentration. Levorphanol (**1**) IC_{50} was 0.008 μM.

Clearly, the quaternary compound **2** can exert typical stereospecific, naloxone reversible effects and, hence, must interact with opiate receptors. This provides some support for the venerable, though unproved, belief first proposed by Beckett and Casy[1] that the cationic (protonated) form of the opiate drug is the active species. While the possibility exists for both **2** and protonated **1** to interact with the receptors via ionic associations, the substantially lower potency of **2** compared to its parent compound **1** might be attributed to lower receptor affinity because of steric hindrance from the additional bulky methyl substituent or, perhaps, to a loss of the hydrogen bonding potential that exists in protonated **1**, but not in **2**, through the $^+NHCH_3$ group. The opiate agonist activity of **2** is not explained by the recently proposed "clastic binding"

hypothesis of Belleau and Morgan.[13] The demonstration of the opioid activity of the quaternized compound **2**, which presumably cannot penetrate the cell membrane, implies that the opiate receptors of guinea pig ileum myenteric plexus are located on the external surface of the neuronal membrane.

Acknowledgment. We thank Dr. Avram Goldstein for helpful discussion, Rekha Padhya and Asha Naidu for expert technical assistance, Hoffman-La Roche, Inc., for levorphanol and dextrorphan tartrates, and Endo Laboratories for naloxone. This work was supported by National Institute on Drug Abuse Grants DA-972 and DA-1199 and by the Drug Abuse Council.

References and Notes

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(6) Melting points were determined on a Kofler micro-heating stage and are uncorrected. Elemental analyses were performed by the Micro-analytical Laboratory, Department of Chemistry, Stanford University; results for elements indicated are within 0.4% of theoretical values.
(7) The use of the guinea pig ileum preparation as a model for the in vivo analgesic action of opiates is well documented; see, for example, ref 3 and I. Creese and S. H. Snyder, *J. Pharmacol. Exp. Ther.*, **194**, 205 (1975).
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Received November 5, 1975

Book Reviews

Heterogeneity of Polypeptide Hormones. Edited by D. Rabinowitz and J. Roth. Academic Press, New York, N.Y. 1974. 181 pp. $16.50.

This small volume is a special issue of the *Israel Journal of Medical Sciences*, dedicated to the memory of Soloman Berson. The papers incorporated here were received for publication between Sept 1972 and Oct 1973.

This compendium of papers is not a review of the subject of polypeptide hormone heterogeneity as is suggested by the title. Rather, it is a collection of research papers each of which has as its subject one or more of the polypeptide hormones. Several of the polypeptide hormones such as insulin, proinsulin, human growth hormone, glucagon, and luteinizing hormone are the subject of more than one paper, and several of the papers are more general in nature; however, since this volume is not a good general review of polypeptide hormone heterogeneity and since the information included here is readily available from the periodical literature, this book is recommended only for those who wish to own a copy in memory of Doctor Berson.

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Amino Acids, Peptides, and Proteins. Volume 6 (Specialist Periodical Reports). Edited by R. C. Sheppard with 18 contributors. The Chemical Society, London. xviii + 514 pp. 14 × 22 cm. £16.50.

Anyone who has browsed through the Specialist Periodical Reports knows how valuable these little books are as a means of keeping pace with the latest advances in several important areas of chemistry that border on the life sciences. This sixth volume in the series on amino acids, peptides, and proteins is no exception.

With admirable terseness and modesty, the "reporters" have succeeded in condensing within the covers of this lightweight, hand-sized volume the information distilled from no fewer than 3000 journal articles, nearly all of which appeared during the year 1973 (only a few date back to 1971 and 1972). The number of papers abstracted in this book represents a 50% increase over the previous volume, which was reviewed here last year [*J. Med. Chem.*, **18**, 444 (1975)].

The organization of the material follows very closely the outline of Volume 5. Chapter One deals with amino acids; Chapter Two with primary structure, chemical modification, x-ray studies, and conformation of peptides and proteins; Chapter Three with peptide synthesis; Chapter Four with special peptide types; and Chapter Five with structure–activity correlation. Major research efforts continue to focus on new coupling reagents and blocking groups, new sequencing techniques, the use of various physical methods to elucidate tertiary structure, and the design of biologically active synthetic peptides.

There is an author index and a very detailed table of contents, but in this Reviewer's opinion the usefulness of the book (and, in fact, of the entire SPR series) would be enhanced immeasurably by the addition of a subject index. Perhaps next year a cumulative subject index covering the first seven volumes would not be amiss.

Lastly, one notes with regret that the cost of Volume 6 is almost twice that of Volume 5 (for the same number of pages). No longer incredible bargains, these books—yet still very worthwhile, even at the current higher price.

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Synthetic Peptides. Volume 3. By George R. Pettit. Academic Press, New York, N.Y. 1975. vii + 438 pp. $39.50.

This third volume of the series follows, in general, the format of its two predecessors. Thus, the bulk of the book comprises of a compilation of synthetic peptides arranged in 20 chapters of tables, each chapter having a brief introduction. The introductions to each group of tables contain selected examples from the literature in that particular area from Jan 1971 to July 1972 and complete coverage from then to Jan 1973. The tabular survey includes literature from Oct 1970 to July 1972.