modern
pharmacology—toxicology
volume 14

developments in opiate research

edited by
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Developments in Opiate Research

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Y075149


MARCEL DEKKER, INC. New York and Basel

LIBRARY OF CONGRESS CATALOGING IN PUBLICATION DATA

Main entry under title:

Developments in opiate research.

(Modern pharmacology-toxicology ; v. 14)
Includes bibliographies and index.
1. Narcotics--Physiological effect. 2. Neuropharmacology. 3. Pharmacology, Experimental. I. Herz, Albert (Date)
RM328.D48 615'.782 78-9745
ISBN 0-8427-6762-4

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MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):
10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

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PREFACE

After so many centuries, it might seem pretentious to laud the recent developments in opiate pharmacology. They have, however, been quite astounding and are, for the most part, directly attributable to the initial demonstration of opiate receptors in vertebrate nervous tissue. Although this happened only five years ago, it has allowed many new insights to be gained into both the location and cellular basis of opiate actions. Perhaps most exciting of all has been the discovery of endogenous ligands for these opiate receptors, substances which comprise a whole new class of putative neurohumoral substances. Opiate research is, therefore, emerging as a central field in neurobiology and will, no doubt, continue to fascinate investigators in a wide variety of disciplines for many years to come.

What, then, has this book to offer, coming as it does at a time of such rapid expansion in knowledge? At the outset, I should emphasize that an extensive coverage of the existing literature is not provided. Instead, attention has been focused on a few selected topics, in which the most significant recent progress has been made. Nevertheless, the fact that the authors work in close proximity and collaboration has, I hope, enabled us to produce a consistent appraisal of the most exciting areas of opiate research, at least as they stood until the middle of 1977.

Production of this book owes much to the dedicated secretarial assistance of Miss M. McGeeney, Mrs. L. Müller, Frau H. Roth and Frau I. v. Unruh. Thanks are also due to Frau U. Gramsch for preparing the diagrams, to Drs. J.P. Fry, H. Osborne, M. Reddington, J. Traber and I.F. Tulloch for their help in the preparation of the text and last, but by no means least, to Marcel Dekker, Inc., for the initial impetus to start writing.

Research reported from this laboratory was supported by the Deutsche Forschungsgemeinschaft and by the Bundesministerium für Jugend, Familie und Gesundheit.

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Chapter 1

THE OPIATE RECEPTORS

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1.0 INTRODUCTION

It has long been assumed that opiates exert their pharmacological action via the occupation of receptors in central and peripheral nervous tissue. Indirect evidence in support of this hypothesis has been obtained from research into the specificity of pharmacological action of opiates. For example, opiates have been found to induce characteristic effects, such as analgesia, changes in mood, respiratory depression and constipation. Such effects are evoked by some narcotics, e.g. etorphine or sufentanyl, after the administration of extremely low doses (in the range of 1 nmole/kg) which suggests interaction with a minute population of recognition sites. Specific recognition sites for narcotics are also implicated by the finding that "opiate activity" is elicited by molecules with common structural features,

which, in turn, would argue for the necessity for a specific complementary receptor.

Despite these observations, initial experiments aimed at directly demonstrating opiate receptors, either by studying the binding of opiates to nervous tissues in vitro (van Praag and Simon, 1966; Scrafani et al., 1969; Höllt and Teschemacher, 1972; Seeman et al., 1972) or by examining the brain distribution of opiates after injection into animals in vivo (Mulé and Woods, 1962; Ingoglia and Dole, 1970; Clouet and Williams, 1973) were unsuccessful. The main drawback of these studies was the inability to demonstrate a high affinity binding component in brain tissue, which is taken as a necessary criterion of receptor binding (see section 2.1). A major reason for this failing was that the degree of non-specific binding observed in the earlier experiments tended to overshadow the small fraction of specific interactions with receptor sites. Although this problem was not totally overcome by Goldstein et al. (1971), these workers were able to demonstrate a specific opiate binding component in mouse brain, which accounted for only 2% of the total binding.

The breakthrough in identification of opiate receptor binding came in 1973. The availability of tritiated opiates of high specific radioactivity enabled the use of extremely low concentrations of opiates in the binding determination. With these highly labelled compounds, three groups independently (Pert and Snyder, 1973a; Simon et al., 1973; Terenius, 1973a) demonstrated a high affinity binding of opiates to rat brain tissue, which appeared to reflect the interaction of the compounds with pharmacologically relevant receptor sites. As a result of these findings, there has been a rapid proliferation of data in this field as reflected by the numerous reviews on the subject of opiate receptor research (Goldstein, 1974; Snyder, 1975a,b; Simon, 1975a,b,c; Pert, 1976; Snyder, 1977; Snyder and Simantov,1977). It is the aim of this review to discuss several aspects of these results and to emphasise some areas not previously reviewed in detail.

It should be noted that the term "receptor", as traditionally conceived in pharmacology, describes a putative macromolecular entity, which possesses both the ability to bind a ligand and the capacity to initiate a pharmacological response. It has become common practice to use the term "receptor(s)" when referring to binding sites of high affinity and specificity, and

has been generally adhered to in this review except where specifically indicated.

2.0 GENERAL METHODOLOGY

2.1 Principle of measuring high affinity binding

In practice, the binding of ligands is measured to relatively crude tissue preparation rather than to purified receptors. Therefore, the binding of a ligand in such a system has to be considered to consist of several components. In the simplest case, besides the binding to the receptor sites which is of a high affinity and saturable, another binding component exists, which is non-saturable and has commonly been termed non-specific. Hydrophobic interactions between the ligands and a wide variety of lipidic tissue components are responsible for the latter type of binding. This had been shown for the non-specific binding of several opiates (Höllt and Teschemacher, 1975).

Figure 1 shows the result of an experiment, in which the interactions of an opiate with specific and non-specific binding sites were investigated. Increasing concentrations of the opiate agonist etorphine (tritium labelled to high specific activity) was incubated together with rat brain homogenate. The upper curve shows the results obtained for the binding of ^{3}H-etorphine, whilst the lower curve represents the binding of ^{3}H-etorphine together with a large excess of non-labelled etorphine - high enough to displace the labelled compound from the saturable sites. This latter type of etorphine binding is characteristic of non-specific, non-saturable binding. The difference curve obtained by subtracting the values of non-specific binding from those of total binding is hyperbolic indicating the increasing saturation of a finite population of binding sites. This figure also illustrates the importance of using low concentration of ligands in performing the binding assay, since it can be seen that the ratio of high affinity to non-specific binding is highest at very low concentrations of etorphine. In general, for such experiments radiolabelled opiates with a high affinity for receptors should be preferred to compounds of lower affinity, since receptor occupation can be achieved at concentrations at which non-specific binding is minimal.

Further improvements in optimising the high affinity/non-specific binding ratio has been made by a filtration method, which separates bound from free ligand while the non-specific binding is lowered by several washings (Pert and Snyder, 1973a). In order to prevent washing out of the specifically bound drug, washing is carried out rapidly (only for seconds) and at low temperature. These steps ensure that ligand dissociation from specific sites (half-life in the range of minutes; Pert and Snyder, 1973b) is kept to a minimum.

Although this technique has been successfully used with most radiolabelled opiates, there are occasions

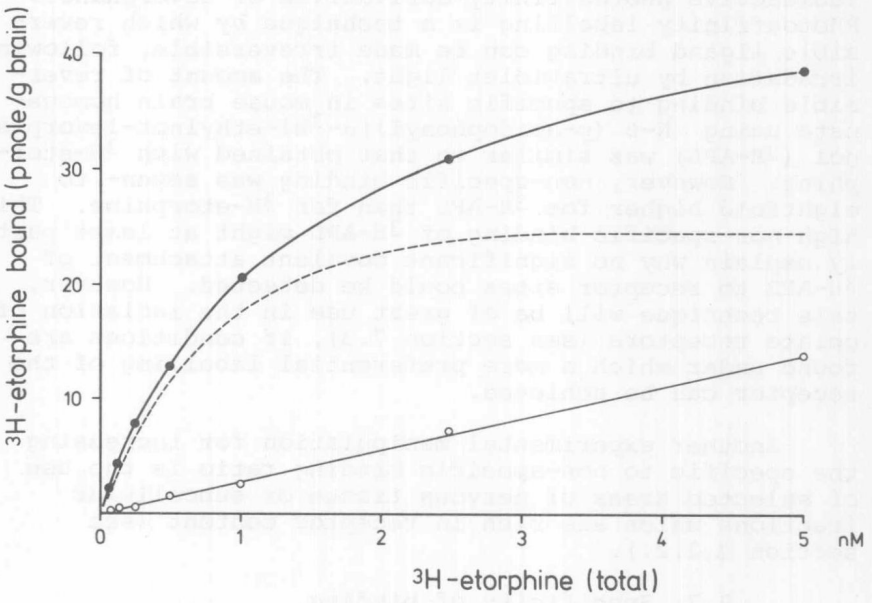

Fig. 1. Saturation curve obtained by binding of increasing amounts of ^{3}H-etorphine to crude rat brain membranes diluted 1 : 100 with 50 mM sodium phosphate buffer (pH 7.4 at 25°C). Total binding is graphed by o———o. The unspecific binding is represented by the lower curve, measured as the binding in the presence of 1 µM unlabelled etorphine (o———o). The difference curve represents the calculated amount of specifically bound etorphine (x-----x).

where difficulties can be encountered. For instance, the non-specific binding of ^{3}H-loperamide - an antidiarrheal agent with a high affinity for opiate receptor sites - is not significantly reduced by the filter washing procedure (Wüster, unpublished). Since the non-specific binding of this compound is very high (presumably due to its high surface avtivity), it is almost impossible to measure specific opiate binding with ^{3}H-loperamide.

Difficulties in separating specific binding from non-specific binding have also been reported by Cox et al. (1976) who attempted to label opiate receptors by radioactive photoaffinity derivatives of levorphanol. Photoaffinity labelling is a technique by which reversible ligand binding can be made irreversible, following irradation by ultraviolet light. The amount of reversible binding to specific sites in mouse brain homogenate using N-ß (p-azidophenyl)[α-^{3}H]-ethylnor-levorphanol (^{3}H-APL) was similar to that obtained with ^{3}H-etorphine. However, non-specific binding was seven- to eightfold higher for ^{3}H-APL than for ^{3}H-etorphine. This high non-specific binding of ^{3}H-APL might at least partly explain why no significant covalent attachment of ^{3}H-APL to receptor sites could be detected. However, this technique will be of great use in the isolation of opiate receptors (see section 7.3), if conditions are found under which a more preferential labelling of the receptor can be achieved.

Another experimental manipulation for increasing the specific to non-specific binding ratio is the use of selected areas of nervous tissue or subcellular fractions which are rich in receptor content (see section 3.2.2.).

2.2 Specificity of binding

It has been pointed out (Dole et al., 1975; Goldstein et al., 1974) that the establishment of a saturable high affinity opiate binding component is insufficient proof of binding to the pharmacologically relevant receptors, since "silent" receptors (Goldstein et al., 1974) or "acceptors" (Birnbaumer et al., 1974) might also bind opiates with a high affinity without initiating a pharmacological response.

In order to ensure that the high affinity binding of ligands represents binding to opiate receptor sites, it has to be shown that pharmacological activity of the drugs results from their binding. The specificity of

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