# THE INVOLVEMENT OF CHOLINERGIC MECHANISMS IN MORPHINE DEPENDENCY

A Thesis Presented to The University
of Manitoba in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

by

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Winnipeg

September, 1979

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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## ABSTRACT

Everyone is motivated by the search for pleasure and for the relief of pain. Narcotic use and abuse probably date to the ancient Egyptians. Despite their popularity and lengthy history, a clear understanding of their mechanism of action has not yet been forthcoming. There has, however, been no shortage of theoretical speculation. Prominent amongst these hypotheses is the tenet that cholinergic pathways play an important role in the actions of the opiates. In the present work an attempt has been made to further elucidate the former's participation in the phenomena of morphine dependency and withdrawal. In addition, work presented here was amongst the first studies performed in search of endogenous opiates and the methodology provided the basis for many future investigations.

Choline chloride, a partial cholinergic agonist, had previously been shown to ameliorate the withdrawal syndrome in rats. A more objective, all-or-none quantitation was used here to further delineate this beneficial effect. Antagonist-precipitated jumping in mice, as well as in rats, was significantly reduced in choline pretreated animals. Also, the optimal dose of this partial agonist differed as a function of the rate and extent of development of morphine dependency.

The toxicity of choline, given acutely, as determined by its LD50, displayed the classical sigmoid shape. Successive sub-lethal doses appeared to have cumulative toxicity, with animals exhibiting increased sensitivity during nighttime hours. This agrees with results of other investigators who have shown a diurnal sensitivity for other cholinergic

agents.

In an attempt to monitor changes in cholinergic sensitivity during the development of morphine dependency as well as during the abstinence syndrome, the tremorigenic agents, oxotremorine and harmine, were injected into animals at various times during and after chronic morphine administration. There was a marked variation in tremor response throughout the morphine cycle with oxotremorine (classically cholinergic in nature) whereas no variation occurred with harmine (noncholinergic in action). In addition, a choline-oxotremorine interaction was observed, with modification by atropine, whereas no such relationship was observed between choline, harmine, and propranolol.

Although it is difficult to confidently extrapolate the beneficial effects of choline chloride from rats to man, in spite of a suggestion of such an effect seen in a small unblinded study conducted during the course of this work, it appears to be a worthwhile venture to search for longer-acting partial cholinergic agonists and assess their value in larger-scale human studies.

Towards the end of these studies, interest was mounting in the search for endogenous opiates. Experiments conducted here were based on the hypothesis that such a substance might be a gonadal or adrenal steroid and that they may be mobilized during sustained, mildly noxious stimuli. The effects observed after gonadectomy and/or adrenalectomy are compatible with such a possibility. Furthermore, using similar testing procedures, an anomalous, antinociceptive effect of naloxone was observed at very low doses. This result represents one of the earliest demonstrations of an interaction between an exogenous opiate antagonist and a deliberate experimental provocation of endogenous opioid release.

ACKNOWLEDGEMENTS

The author is extremely grateful to his supervisor, Dr. Carl Pinsky, for his unabating enthusiasm, encouragement, and guidance throughout these studies.

The author is also indebted to Dr. Ian Innes for his understanding and patience and for his constant willingness to participate in informal conversation.

The contributions of Departmental Staff as well as of fellow graduate students to the author's education are deeply appreciated and will be cherished always.

Sincere thanks are also due to: Egon Brockhausen for his competent technical assistance as well as for his art work; Ortensio Gigliotti for his skillful design and construction of experimental apparatus; Barbara Donahue and Marlene McBean for their very efficient typing of this manuscript; Tom Hopper and Roy Simpson for their assistance with the photography; and Marge Turner for her motherly advice and for a constant liaison with Staff.

The author is forever indebted to his wife, Jacki, for her patience, understanding, and never-ending support throughout these long years.

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# I INTRODUCTION

## (A) HISTORY AND GENERAL PHARMACOLOGY OF MORPHINE

The search for pleasure and for the relief of pain motivates everyone. From the beginning of recorded history, drugs have played a prominent role in pleasurable recreational activities and in relieving various forms of discomfort, both mental and physical (Sapira, 1975). Ancient Sumeria has often been credited with the origin of opium, referred to by the ideograph "hul-gil" or "joy plants" (Jaffe and Martin, 1975), but its use and abuse probably date to the more ancient Egyptians as evidenced by the decoration of tombs with the poppy (Sapira, 1975).

Morphine is the prototype of a family of chemicals generally referred to as the opiate narcotics. It is one of many alkaloids extracted from the juice of the unripe poppy plant, Papaver somniferum, and is a member of the class phenanthrenes. Morphine is readily absorbed from the gastrointestinal tract but its effects are less after oral administration than after parenteral administration. Only small quantities pass the blood-brain barrier under normal conditions. It is conjugated with glucuronic acid and most of it is excreted in the urine along with small amounts of free morphine (Jaffe and Martin, 1975).

A variety of semisynthetic drugs are made from morphine by simple substitution. Thus, agonists such as codeine and heroin, and antagonists such as nalorphine and naloxone are easily derived from the parent compound.

Morphine exerts its actions on virtually every organ system in man. Centrally, it causes analgesia, mood changes, drowsiness, mental clouding and ultimately, sleep. These effects are dose-dependent so that it is possible to achieve significant analgesia without loss of consciousness. The analgesic effect is selective in that the responsiveness of other

sensory modalities is not diminished. The type of pain is differentially affected by morphine, continuous dull pain being alleviated more easily than sharp intermittent pain. The mechanism of action of analgesia is still unknown but it appears that it, is the reactive component, i.e. the "appreciation" of pain, that is altered and not the pain sensation itself.

The effects of the opiates on the gastrointestinal tract have been recognized for centuries. These drugs cause an increase in the tone of the antral part of the stomach as well as of the first part of the duodenum in addition to a decrease in motility of the former. This results in a delay of passage of gastric contents and constitutes a major component of the constipation observed with morphine administration. There are similar effects on intestinal tone where increases are seen in the small and large intestine, ileocecal valve and anal sphincter. In addition these periods of hypertonicity may be followed by periods of atony. Propulsive contractions are diminished in all areas of the intestine and this further contributes to the constipating actions. Large doses of atropine can antagonize the spasmogenic effects; extrinsic nerve resection and ganglion blocking drugs do not, suggesting that it may be nerve plexuses within the bowel wall which are being affected (ibid). The smooth muscle contraction produced by morphine-like drugs may exacerbate an attack of biliary colic instead of alleviating the pain in this condition. Ureteric tone and contractions are increased as are detrusor tone in the urinary bladder and vescical sphincter, the latter two phenomena causing difficulty in urination.

The most serious consequences of excessive morphine administration are those of respiratory depression. All phases of respiratory activity

are decreased even with therapeutic doses and at least part of this effect is due to a direct action on the brainstem resulting in a diminished response to plasma $PCO_2$ and to a disruption in the regular rhythmicity of respiration (ibid).

Direct stimulation by morphine of the chemoreceptor trigger zone (CTZ) in the area postrema of the medulla causes nausea and vomiting, evidence of which is more common in ambulatory than recumbent patients, suggesting that there is also a vestibular component to these effects. Morphine and its derivatives also depress the cough reflex at least partly by a direct effect on the cough center in the medulla (ibid).

Pupillary constriction in man persists even after tolerance has developed to other actions. There is considerable species variation in the pupillary response to morphine but these responses do not change with repeated drug administration (ibid).

The cardiovascular system is little affected by therapeutic doses of morphine although orthostatic hypotension and fainting do occur. There is evidence from animal studies that this effect may involve morphine-induced inhibition of central noradrenaline neurotransmission (Gomes et al., 1976).

(B) DEFINITION OF TERMS: THEORIES OF MORPHINE DEPENDENCY

(a) General. The mechanisms of the development of tolerance to and physical dependence on the opiate narcotics are still unknown but there certainly has been no shortage of theoretical speculation. Although the terms tolerance and dependence are often mentioned together, implying that they are similar in definition, they are, in fact, quite different phenomena.

Tolerance, as defined by Seevers and Woods (1953), is "cellular

adaptation to an alien chemical environment characterized by diminished biological response". Tolerance to a drug, therefore, is demonstrated only during repeated administration of that drug (or of a similar drug - "cross tolerance"). Physical dependence, on the other hand, is a state which is inferred from the signs of physiological dysfunction that occur only after discontinuation of drug administration; these signs are reversed upon re-administration of the drug (Schuster and Balster, 1973). Almost all drugs that cause dependence also produce tolerance. It is important, however, to point out that not all drugs which produce tolerance will necessarily cause dependence.

In addition to the foregoing definitions, the two phenomena have also been differentiated by their time course for development. Tolerance was considered to have a shorter time course for development than did physical dependence. Several papers, however, have shown that physical dependence can occur very quickly and that a single low dose of morphine is sufficient to cause measurable dependence (Barthelemy and Jacob, 1972; Kosersky et al., 1974; Frumkin, 1974; Smits, 1975). These results suggested that the classical concept, which holds that a prolonged period of drug administration is necessary for the induction of opiate narcotic dependence, may no longer be tenable.

Several theories have been advanced to explain the phenomena of opiate tolerance and dependence, they are not necessarily mutually exclusive but may merely be dealing with the phenomena in different terms. There are two major approaches, however, that have emerged as contending theories and have received considerable attention. The first of these postulates a continuous and unchanged interaction between the drug and