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Merperidine in in vitro bioassays

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Meperidine in *in vitro* bioassays

by

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DISSERTATION

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DEDICATION

I dedicate this dissertation, and the degree to follow, to the gentleman who first introduced me to the scientific method and taught me the proper way to approach an experiment, Professor Russell F. Doolittle.

PREFACE

I would like to acknowledge the wonderful input of my major preceptor, Professor Edward Leong Way, without whose encouragement I might never have completed this research. I also wish to thank Dr. Ahmad Rezvani, whose advice and help were indispensable. Other members of Professor Way's laboratory, particularly Donald Rhoads, my fellow graduate student and sufferer, and Jacqueline Carnes, our secretary, supported my efforts in every way, and deserve my heart-felt thanks. In addition, I want to thank the other two members of my dissertation committee, Professors Horace Loh and Edward Wei; their kindness and forbearance were considerable.

Finally, I want to acknowledge the love and support of my entire family.

Meperidine in *in vitro* Bioassays

Carol Elizabeth Glasgow

ABSTRACT

An apparent dichotomy between the effects of morphine and meperidine in humans and some animal bioassays prompted this research. The project's direction resulted from three factors: first, observation of poor *in vitro* tolerance development of meperidine, a well-known and much used analgetic believed to have a mechanism of action similar to morphine; second, minimal and ambiguous information in the literature on tolerance development of meperidine; and, third, studies of other opiate effects showing a number of differences between morphine and meperidine.

Three *in vitro* bioassay systems were utilized to evaluate meperidine; myenteric plexus-longitudinal muscle of the guinea pig ileum (GPI), mouse *vas deferens* (MVD), and rat *vas deferens* (RVD). Tissues attached to an electrode were bathed in the appropriate physiologic saline bubbled with a mixture of O_2 and CO_2, and field stimulation was generated by a stimulator and monitored by a polygraph. Chemicals to be tested were added to the bath at various times and conditions of treatment.

Findings were as follows:

- Meperidine, although generally described as a morphine-like compound, has many actions that are not morphinomimetic.

- Like morphine, meperidine reduces the electrically generated response in the GPI and the effect is blocked by opiate inhibitors.
- Although meperidine acts as an opiate it is also atropinergic. However, it affects the electrically generated GPI at concentrations below which an atropinergic response is observed.
- Unlike morphine, meperidine *in vitro* induces only minimal tolerance, and no physical dependence.
- Meperidine's responses are reduced by calcium concentrations, but to a much lesser degree than with morphine.
- Meperidine's actions were only affected by hexamethonium in the GPI and yohimbine in the MVD.
- Meperidine reduced the electrically generated twitch and induced tolerance in the MVD, but the effect is not considered opioid, as it is not affected by opiate inhibitors.
- Research on phencyclidine (PCP), indicated areas in which meperidine and PCP had similar responses, but others where the results were diverse.

In conclusion, although meperidine possesses morphine-like activity, its effects are mediated at different sites, perhaps by a different mechanism.

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INTRODUCTION

Meperidine, 1-methyl-4-phenyl-4-piperidine carboxylic acid ethyl ester hydrochloride, also known as Demerol™, Dolantin™, isonipecaine and pethidine, is one of the most widely used analgetics. When absorbed orally, it is only 50 percent as effective as when given parenterally (Jaffe and Martin, 1985). This first synthetic opioid compound was introduced by Eisleb and Schaumann as an analgetic in 1939. Although originally investigated as an atropinergic, its analgetic effect was discovered serendipitously. When first put on the market it was advertised as an analgetic, a sedative and a spasmolytic (see Eddy et al., 1957). Early reports claimed that many of the severe side effects of morphine, such as respiratory depression, urinary retention and constipation, were rarely or never seen with meperidine (Batterman, 1943). Unfortunately, with increased usage, most of meperidine's side effects do not appear to differ qualitatively from morphine's, only quantitatively. Meperidine is roughly one-tenth as potent an analgetic as morphine, but it has approximately the same pharmacologic profile in man, having most of the effects that are blocked by classic opiate antagonists such as naloxone (Jaffe and Martin, 1985).

The literature indicates that little work has been done on tolerance development to meperidine and the published results are ambiguous. Furthermore, studies

published in other areas show a number of differences in the effects of morphine and meperidine both *in vivo* and *in vitro*.

Model Systems for Assessing Opiate Action

A number of *in vitro* model systems have been used to study the pharmacology of meperidine. In general, those used are the classic ones utilized for assessing opiate effects. The usefulness of opium in treating diarrhea by reducing peristaltic activity has long been known. In 1917, Trendelenburg was the first to demonstrate morphine's paralysis of the peristaltic reflex in the isolated guinea pig ileum (GPI) (see Schaumann, 1955). Schaumann *et al.* (1952) demonstrated that meperidine and other analgesics had the same effect on the ileum, and the rank order paralleled the analgetic ability. In 1955, Schaumann suggested that morphine acted on the peristaltic response by preventing the release of acetylcholine, and by 1956, he demonstrated this effect in intestinal tissue.

The best known preparation for the bioassay of opiate activity is the electrically stimulated GPI assay developed by Paton (1955). The contractile response of the ileum to electrical stimulation appears mediated by the release of acetylcholine (ACh) and is blocked by anticholinesterases. In 1957, Paton used it to measure the effects of morphine,

as well as other opiates and opioids, of which meperidine was one. He discovered that meperidine, like morphine, caused depression of the electrically induced contractions. Although he recognized that meperidine was also atropinergic and had some direct blocking action on the ACh which caused the contraction, he also concluded that there was a morphine-like effect. His ranking of potencies of the drugs he tested correlated well with their order of analgetic potency in man. He reported that meperidine was roughly half as potent as morphine in the gut. Creese and Snyder (1975) tested a number of compounds for activity in the GPI preparation, and obtained an IC_{50} (the concentration needed to produce a 50 percent inhibition of contraction height) of approximately 2 micromolar (uM) for meperidine, while morphine's IC_{50} was approximately ten times lower, or 0.2 uM. The relative difference in morphine/meperidine potencies may be due in part to the different preparations used by the experimenters. Paton (1957) used a section of whole GPI, while Creese and Snyder (1975) utilized only the myenteric plexus-longitudinal muscle (MPLM).

The response to morphine of many other tissues and organs has been examined *in vitro*. Investigators have looked at the nictitating membrane, colon, *vas deferens*, red blood cells, brain slices, etc., in a number of different species, only to find the response to morphine is

extremely species- and tissue-specific. For example, while the GPI is quite sensitive to morphine, the rabbit ileum is non-responsive (Greenberg et al., 1970).

The mouse vas deferens (MVD) is another isolated organ which has been shown to respond to morphine, although at relatively higher concentrations than the GPI (Henderson et al., 1972). Further research on vasa deferentia by the same group of investigators led them to conclude that, among the usual laboratory animals, only the mouse was sensitive. The rabbit, cat, guinea pig, rat, hamster and gerbil vasa deferentia did not respond to morphine (Hughes et al., 1975). These investigators also tested a number of other opiates and opioids, including meperidine in the mouse preparation, and they obtained an IC_{50} of 16 uM, while Rhodes (1983) recorded an approximate IC_{50} of 3 uM. In contrast to the cholinergic response seen with electrical stimulation in the GPI (Paton, 1955), the MVD responds via adrenergic transmission (Henderson et al., 1972).

In the rat vas deferens (RVD) Lemaire et al. (1978) demonstrated a slight decrease (10 to 20 percent) in the electrically evoked response with 100 uM morphine, but meperidine reduced the twitch height up to 40 percent at concentrations from 1 to 50 uM. In a later set of experiments by Huidobro et al. (1980), the investigators did not observe a depression of twitch height in the RVD

with meperidine, but rather an increase in the twitch height with both morphine and meperidine.

Meperidine Classification

It is well-recognized that there are multiple opiate receptors, but there has been very little research into the categorization of meperidine. The concept of multiple morphine-like receptor classes is a relatively recent development proposed originally by Martin *et al.* (1976). The authors suggested the nomenclature of mu, kappa and sigma, based on the syndromes seen in the chronic spinal dog following administration of morphine (mu), ketocyclazocine (kappa) and SKF 10,047 (sigma). Further research has revealed the presence of additional receptor classes, the delta receptor (named from the *deferens*, Lord *et al.*, 1976), while investigation in the RVD yielded a specific response to the endorphins (Lemaire *et al.*, 1978; Schulz *et al.*, 1979), which led to the naming of the epsilon receptor after endorphins (Wuster *et al.*, 1979). Subclasses have also been identified (e.g., mu_1 and mu_2, Wolozin and Pasternak, 1981).

In 1978 Martin *et al.* tested meperidine in the chronic spinal dog and found that it did not suppress the abstinence syndrome in the morphine-dependent dog, one of the methods they used to distinguish between the various

classes. In addition, in looking at a number of other effects in this preparation following administration of morphine and meperidine, qualitatively different responses were seen in three tests, and quantitative differences in others. Despite this evidence, the authors considered meperidine to be a mu agonist. However in a more recent discussion on this area, Jaffe and Martin (1985) suggested that, compared with morphine, meperidine may interact more strongly with the kappa receptor. Other researchers who investigated a number of different morphine-like compounds for classification, revealed meperidine as having a limited relationship to other members of the class. Cowan et al. (1979) classified chemicals on the basis of change in the seizure threshold in rats. Of 17 compounds tested, meperidine demonstrated the same type of activity as two other compounds, pentazocine and normeperidine. Geller et al. (1983) classified 22 compounds on the basis of body temperature changes in rats, and assigned meperidine, d-pentazocine and normorphine to the same group.

Categorizing Opiate Action via Tolerance and Cross-tolerance

Tolerance

Tolerance is a reduced sensitivity in certain physiologic responses to a chemical substance after prior

exposure. Unfortunately, tolerance development to the analgetic effect of opiates is very common. This reduces the clinical usefulness of an opiate because tolerance to all effects does not develop equally and toxic responses may not be as affected. Therefore much research is on-going to determine both the mechanism of tolerance development, and to discover analgetics to which tolerance does not develop.

There are several types of tolerance; pharmacokinetic or metabolic alteration, cellular tolerance or behavioral tolerance. In the case of morphine, tolerance can be developed to the measured effect with *in vitro* model systems, which seems to indicate that this form of tolerance is cellular.

Using the isolated GPI to investigate morphine's action, Paton (1957) was the first to report the development of tolerance *in vitro* following a brief incubation with the drug. Fennessy *et al*. (1969) repeated the work but reported that tolerance to meperidine was not observed. He stated that "this agrees with the observation of Paton who attributed the failure of development of tolerance to pethidine (meperidine) to the atropine-like action of this drug". However, a close reading of Paton's 1957 paper does not reveal any statement where he specifically says that tolerance to meperidine did or did not develop.

The lack of tolerance development to meperidine is not surprising, as there are practically no instances of tolerance development to meperidine reported in the literature, in either clinical or experimental studies. General texts such as Goodman and Gilman (Jaffe and Martin, 1985) or Kolodny and McLaughlin (1966) stated categorically that tolerance develops in humans (presumably to the use of meperidine as an analgetic). Eddy et al. (1957), in their review of synthetic opiate-like drugs, described many clinical studies with a total of several hundred patients, with treatments in some cases for several weeks. However only one patient required an increase in dosage, from 100 milligrams (mg) to 150 mg after 50 doses.

In a more experimental milieu, Andrews (1942a) tried to demonstrate tolerance to meperidine in persons who were abusing the drug. The four subjects had been addicted to opiates but had taken no drugs for nine months before the experiment began. They were allowed as much meperidine as they wished, starting with 300 mg every one and a half hours. (The recommended medical dose is 80 to 100 mg every two to four hours [Jaffe and Martin, 1985].) As reported in a companion paper by Himmelsbach (1942), one of the subjects used a total of 150 grams (g) of meperidine over a ten week period with a mean dose of 200 mg every two hours, or approximately 2.4 g per day. Using an electrical stimulus for determining pain thresholds, Andrews (1942a),

ascertained that tolerance developed to meperidine, although, because of a concomitant decrease in the time to the threshold raising effect, he concluded that the tolerance is either accompanied by, or is the result of, changes in the rate of drug utilization.

That the latter supposition is more likely correct is confirmed by the results of Glynn and Mather (1982), who followed the course of meperidine pharmacokinetically in three patients from three to twelve months. Two of the three patients developed tolerance to meperidine, i.e., they claimed that they needed more drug to kill the pain, but when the minimum effective analgetic concentration of drug was measured directly from the bloodstream, the actual meperidine concentration needed to block pain was not changed over the course of the experiment. In one patient, a reduced oral absorption was noted after extended treatment, yet given adequate time, the previously noted blood concentration to achieve analgesia was attained and was still analgetic. In these circumstances, it appears that the tolerance observed is not cellular tolerance such as that seen with morphine <u>in vitro</u>.

Experimental tolerance to meperidine in laboratory animals is also difficult to demonstrate consistently. In 1948, acute vascular tolerance to meperidine was checked in several species. A partial tolerance developed in some animals, complete tolerance in others, with the effect

being altered by the presence of anesthetic in the treated animals (Shideman and Johnson, 1948). Knapp (1968) reported tolerance development to the local anesthetic effect of meperidine. Cox et al. (1968) also investigated the development of acute tolerance to meperidine, but, in this case, tolerance to an analgetic effect (antinociception) was measured. Following infusion of meperidine in the rat, analgetic activity increased for about two to four hours, and then declined; the decline was blocked by concomitant administration of actinomycin D. This same effect was seen in animals treated with morphine, and investigation of the effect of actinomycin D on already developed tolerance was checked. In this instance actinomycin D did not reverse the tolerance already developed, suggesting that tolerance development was mediated by some form of protein production. Cowan et al. (1979) investigated the changes in seizure threshold following administration of various opiates or opioids. The effect of meperidine on the seizure threshold was not altered by previous exposure to the drug, i.e., no tolerance developed, although tolerance developed with several others, such as morphine and levorphanol.

Cross-tolerance

One way to distinguish between members of the various receptor classes is to assess their cross-tolerance.

Cross-tolerance is the ability of a chemical, to which tolerance has developed, to reduce the effect of a subsequently administered chemical. If two chemicals act on the same receptor, and tolerance to the first chemical reduces the receptor's response, then the second chemical's response will also be similarly altered.

Leander and McMillan (1977) reported that low doses of meperidine generally increased and higher doses generally decreased pigeons' responding under a fixed-interval (FI) component of schedule-controlled responding. In birds on a daily dose of methadone (which had previously demonstrated a dose-dependent response to the compound), morphine showed a ten-fold shift of its dose-response curve, but no tolerance to meperidine was exhibited. In monkeys on a FI multiple schedule (Witkin et al., 1983), both morphine and meperidine produced dose-related decreases in the rates of responding, but only meperidine caused small changes in the temporal pattern of responding. Tolerance to morphine reversed the rate-decreasing effects of meperidine, indicating some development of cross-tolerance for this particular parameter. While the behavioral effects evaluated were similar in each case, test species were different which may account for the differing responses. Moreover, cross-tolerance development to meperidine was looked for, but not found in pigeons tolerant to methadone, whereas tolerance in the monkeys was observed in animals

rendered tolerant to morphine. Cowan et al. (1979) reported no cross-tolerance with respect to changes in seizure threshold between meperidine and pentazocine, cyclazocine or etorphine. The authors considered it very unusual that no cross-tolerance between meperidine and etorphine developed; however the fact should not be too surprising, as both cyclazocine and etorphine shifted the seizure threshold one way, and meperidine the other. Meperidine and pentazocine responded in similar ways, yet developed no cross-tolerance to each other.

In the opossum lower esophageal sphincter (LES), meperidine caused a dose-dependent reduction in internal pressure. Both ketocyclazocine and buprenorphine also reduced internal pressure, but no cross-tolerance developed between any of these three compounds when used at their maximally effective dose (Rattan and Goyal, 1983).

Su et al. (1981) investigated cross-tolerance in an in vitro GPI preparation using an ileum from an animal made tolerant to morphine in vivo. They tested for cross-tolerance in this excised tissue to normorphine, ketocyclazocine and SKF 10,047, three agonists presumed to bind to differing populations of receptors. Indeed, the authors concluded that there were three different receptor types in the ileum, because the relative cross-tolerance indices for these chemicals at the kappa and sigma receptors were different from that of the mu agonist,

normorphine. In addition, the sensitivity of the compounds to the opiate antagonist naloxone were significantly different, indicating different receptor populations for each compound. Schulz et al. (1980) performed a similar experiment to differentiate between mu and delta receptors in the MVD as well, on the basis of cross-tolerance studies both in vivo and in vitro. A literature search has revealed no information showing that meperidine has been evaluated in an in vitro system for tolerance or cross-tolerance development.

Use of Physical Dependence to Assess Opiate Action

Although the evidence for tolerance development to meperidine in humans is equivocal, that for physical dependence and addiction is not. As early as 1940, there were reported cases of addiction, and in the 1940's the foreign literature was filled with reports (see Eddy et al., 1957). Himmelsbach (1942) was among the first to investigate meperidine's dependence liability and ability to substitute for morphine in morphine addicts. He discovered that meperidine would help reduce the signs and symptoms of morphine withdrawal, although the subjects expressed a preference for morphine. In postmorphine addicts given meperidine, it was fairly easy to induce dependence, and when these subjects were withdrawn, a

morphine-like withdrawal syndrome was seen, though not as severe as that seen following withdrawal from morphine.

Abuse liability however, does not depend entirely on the ability to induce physical dependence. Wieder (1946) described three cases of addiction to meperidine in persons who denied receiving any pleasant feeling following morphine administration, but noted great pleasure and relief with meperidine. One patient stated that he "preferred its effects to those of morphine and when given a choice he would always take meperidine". Jaffe (1970) states that "a high percentage of doctors and nurses who become addicts select meperidine over other agents to which they have equal access". There is also a feeling that meperidine is less addicting than morphine, because it is less potent (see Eddy et al., 1957), although this has not been supported by its history.

It has been more difficult to induce physical dependence to meperidine in animals, with early experiments (Barlow and Lewis, 1951) yielding negative results. In later studies, when meperidine was given in higher doses and more frequently either by infusion (Teiger, 1974) or in the drinking water (McMillan et al., 1976), investigators were able to demonstrate physical dependence to the compound in rats, which as seen in humans, was less severe than dependence to morphine (Himmelsbach, 1942). No

studies evaluating the voluntary maintenance of meperidine addiction in animals were found in the literature.

Ehrenpreis *et al*. (1972) was the first to report that morphine-tolerant ileal strips respond to naloxone differently from control strips, responding with a contracture to the naloxone challenge. Collier *et al*. (1981) attempted to quantitate the degree of physical dependence development on opiates in the GPI *in vitro*, and found with prior exposure to agonists both a time dependence and a concentration dependency on the opiate for inducing the naloxone contracture. There appears to be no information in the published literature on the potential of naloxone to induce contractions in meperidine-tolerant tissues.

Use of Opiate Antagonists to Assess Opiate Action

Because so many different compounds have morphine-like activity even though they are structurally dissimilar, the usual way of defining an opioid is not by structure or by its actions *per se*, but by its reaction to an opiate antagonist -- a reversal or reduction in agonist response which might imply that both agonist and antagonist work on the same receptor. The failure of an opiate antagonist to reverse an agonist would indicate that the agonist response was non-opioid-like.

Naloxone was developed and investigated in the early 1960's, a member of a class of compounds that showed antagonistic effects beginning with N-allylcodeine in 1915. The N-allyl substituted compounds range in effect from N-allylnoroxymorphone (naloxone), which has little or no agonist activity, to N-allylnormorphine (nalorphine), with both agonist and antagonist effects.

The response of various chemicals to an opiate antagonist can yield other information, including evidence of the type of receptor subpopulation with which the opiate is reacting. The pA scale first proposed by Schild in 1947 is a way of measuring the potency of the antagonist. The pA_2 is the most used part of the scale, though any ratio can be measured. The pA_2 is the amount of an antagonist which reduces the effect of an agonist by one-half, so that twice as much agonist is needed to give the same effect as seen in the absence of the antagonist. Arunlakshana and Schild (1959) discussed the use of the measurement of pA_x of different agonists which act on the same receptors. They also suggested the use of the pA scale to determine whether or not the antagonist is competitive.

The pA scale was developed in the GPI bioassay, which is the most commonly used procedure for evaluating opiate and opioid agonists and antagonists (e.g., Kosterlitz and Watt, 1968). However, pA_2's have been determined *in vivo* for antagonism to analgetic effects as well (Takemori *et*

al., 1969). Su et al. (1981) used pA_2 values to distinguish between various agonists in the GPI. Using the prototypic mu (morphine), kappa (ketocyclazocine) and sigma (SKF 10,047) drugs, the investigators found that the pA_2 of naloxone for each of the three agonists was significantly different, suggesting that they did represent binding to different receptors.

Meperidine's respiratory depressant effect has been shown to be antagonized by naloxone in vivo in humans (Foldes et al., 1963). However, in a number of different types of animal assays, quite varied responses occur. Meperidine studied in experimental behavioral models has been found to affect these systems and to be affected by antagonists in a number of ways. Papers from Leander's group of researchers exemplify the difficulties in interpreting the various results. In 1976, McMillan and Leander produced schedule-induced polydipsia in rats and, with it, a decrease in the responding rate for various food-pellet rewards. In the FI lever-pressing (LP) schedule, meperidine showed an unusual response, with low doses of meperidine yielding a small increase in the rate of licking and the amount of water consumed, in contrast with the other class members. However, at high doses of meperidine, both the rate of licking and the rate of responding to the food-pellet reward were decreased. In the fixed-time (FT) and FI licking schedules, both licking

and water consumption were decreased at all dose levels. Naloxone blocked the rate-increasing response of low doses of meperidine in the FILP schedule, but not the rate-decreasing effects of higher doses of meperidine. Naloxone had no effect on either licking or water consumption in the other two schedules, and, in fact, the depressant effects of the highest dose of meperidine may have been intensified by naloxone, although morphine rates were completely reversed. In 1977, Leander and McMillan studied the effects of meperidine on pigeons in a multiple fixed-ratio (FR), FI schedule of food presentation. The FI component in this experiment also responded with a biphasic response, with low doses of meperidine increasing and higher doses decreasing response rates, while the FR component also responded with decreased rates at higher doses, although lower doses generally had no effect. Naloxone again blocked the rate-increasing effects of meperidine, but had no effect on the rate-decreasing effects of higher doses and, as in the rat, the highest dose of naloxone seemed to enhance the rate-decreasing effects under both the FI and FR schedules of responding.

In contrast, although squirrel monkeys responded biphasically to meperidine in a similar multiple FI schedule, in that meperidine slightly increased the rate of response at the lowest dose, while decreasing it at higher doses, naloxone reversed the rate-decreasing effects

(Witkin et al., 1983). Moreover, in the squirrel monkey naloxone induced a totally different effect to that seen with morphine, disrupting temporal patterns of response and with higher doses of naloxone not only blocking the decreased rate response, but actually increasing it to levels above the control.

There are other cases in which naloxone, when added to meperidine, not only does not antagonize meperidine's effects, but, in fact, potentiates it. These effects are normally seen when the meperidine response is not the same as morphine's. Cowan et al. (1979) measured seizure thresholds to flurothyl in rats and alterations in response to a number of different morphine-like compounds. Morphine was among a group of chemicals which raised the seizure threshold, tolerance to the chemical was seen, and naloxone attenuated the effect. On the other hand, meperidine decreased the seizure threshold, no tolerance was noted, and the decrease was potentiated rather than attenuated by naloxone. Geller et al. (1983) examined the effects of a number of compounds on body temperature in rats. Morphine caused hyperthermia at lower doses and hypothermia at higher ones, and both effects could be blocked by naloxone. Meperidine, on the other hand, had little direct effect on body temperature, but induced hypothermia in combination with naloxone.

In those instances where naloxone does block meperidine's action, the antagonistic effect of naloxone on meperidine frequently differs in quality from that seen on opiates or other opioids, probably reflecting the different percentages of morphine-like activity needed for each of these effects. Aceto et al. (1969) investigated the use of the Straub tail reaction in mice to measure morphine-like action in a number of chemicals, and the agonists' response to antagonists. They found that with this particular opiate effect, the ED_{50} of naloxone needed to antagonize 64 mg/kilogram (kg) meperidine (84 percent effect) was only 0.006 mg/kg, while 0.014 mg/kg of naloxone was needed to cause a 50 percent antagonism of the effects of 16 mg/kg morphine (88 percent effect). In contrast to the two-fold difference seen in this assay, in a bioassay in the opossum LES, the dose of naloxone needed to antagonize meperidine was ten-fold higher than that required for buprenorphine. Gilbert and Martin (1975) looked at the dose of various class members needed to induce convulsions and the ability of naloxone to counteract the effect. Pretreatment with naloxone was able to block meperidine's convulsant effect requiring approximately 140 percent of control levels to induce convulsions, while the dose of heroin needed to cause a seizure increased more than 200 percent in the presence of naloxone. In this case, meperidine was better able to counteract naloxone's effect than heroin,

indicating that the opiate effect is probably not the major cause of the convulsive response being measured.

Naloxone has been tested as an antagonist of meperidine in both the mouse and rat vasa deferentia. Rhodes (1983) observed a very unusual biphasic response in the electrically stimulated MVD with naloxone. One uM naloxone had no significant effect on low doses of meperidine which induced less than a 50 percent decrease in the twitch height, but at higher doses of meperidine, the same dose of naloxone reversed the effect completely. Lemaire (1978), achieving only a partial inhibition of the electrically stimulated contraction in the RVD with meperidine, assumed that it was not a morphine-like effect, because it was not reversed by naloxone.

It requires 17.5 uM meperidine to inhibit naloxone binding to the extent of 50 percent in the guinea pig intestine, while the dose of morphine needed is one-hundredth less, 0.17 uM (Creese and Snyder, 1975). In the rat brain, the ratio is even greater. With 100 millimolar (mM) sodium chloride (NaCl), the ratio is approximately 450:1; without additional NaCl, a thousand times more meperidine than morphine is required to inhibit naloxone binding.

Interactions with Calcium for Assessing Opiate Activity

There has been much evidence that calcium is involved intimately with the actions of opiates and opioids (Chapman and Way, 1982). Manipulations of calcium concentration alter the response to opiates. The converse is also true -- exposure to opiates, both acutely and chronically, can affect calcium distribution and movement throughout the cell.

In the guinea pig model system, many investigators (Nutt, 1968; Opmeer and Van Ree, 1979, 1980; Huidobro-Toro et al., 1981) have shown that alteration of calcium concentration changes the response to morphine or other opiates; an increase in calcium antagonizes morphine and causes a shift of the morphine dose-response curve to the right while a decrease shifts the curve to the left. As little as a four-fold increase in the calcium concentration in the incubating buffer shifts the dose-response curve of normorphine more than a hundred-fold to the right, while the same change in calcium shifts the ACh dose-response curve only about four-fold to the right (Huidobro-Toro et al., 1981). This effect is ion-specific, as magnesium at comparable concentrations shows no antagonism of morphine (Nutt, 1968; Opmeer and Van Ree, 1980; Huidobro-Toro et al., 1981). Calcium does not appear to be involved in the induction of tolerance, as omitting calcium from the

incubating buffer did not affect tolerance development (Opmeer and Van Ree, 1980).

An interaction between an opiate (normorphine) and calcium can also be demonstrated in the MVD. Increasing the calcium concentration in the incubation buffer of a normorphine-treated preparation reversed the effect of the drug, while addition of calcium to the vas deferens enhanced the excitatory junction potential amplitude directly and normorphine reduced the amplitude (Illes et al., 1980). The release of norepinephrine from electrically stimulated tissue increased with increasing calcium concentration, and normorphine interfered with the calcium non-competitively, contrasting with the antagonism of magnesium (Illes et al., 1982).

Increasing the calcium levels also antagonizes opiate effects in the RVD (Nicolaou and Ziodrou, 1985). The difference in the response of various morphine-like compounds to calcium in this system may reflect the relative concentration of different receptors, as morphine responded only slightly (two-fold) to the same calcium changes that caused a hundred-fold shift in the IC_{50} of DALAMID.

Meperidine has also been shown to be affected by calcium changes. In an in vitro system with frog sartorius muscle, 35 mM meperidine completely blocked the rate of rise of the action potential over time. If A23187, a

calcium ionophore, is added simultaneously with the meperidine, complete depression does not occur and only a partial blockade is obtained (Rohani and Frank, 1983). However, at this concentration of meperidine, a different effect of the compound might be occurring. As early as 1945, blockade of both sensory and motor function in the frog sciatic nerve was noted with one percent meperidine (Way, 1945). Later studies of meperidine's effect on the isolated vagus nerve of the rabbit (Kosterlitz and Wallis, 1964) indicated that meperidine (100 microgram[ug]/milliliter[ml]) caused a decrease in the size of the action potential and reduced the conduction velocity, but this effect was seen in a few minutes, in contrast to the hours it took for the complete response of the frog muscle (Rohani and Frank, 1983). The nerve effects seen in the rabbit vagus nerve are not seen with morphine, and Kosterlitz and Wallis attributed them to the local anaesthetic effect of meperidine.

Manipulation of the internal calcium concentration can be achieved in another way. It had been known that 4-aminopyridine (4-AP) increased neurotransmitter release in response to depolarization of the nerve terminal, and Lundh and Thesleff (1977) postulated that this was mediated through an inward current carried by calcium ions. However, Vizi et al. (1977) found that 4-AP was able to increase ACh release in the absence of external calcium in

the incubating medium, although upon addition of a calcium-chelating agent (EDTA), 4-AP was ineffective in increasing ACh release. These investigators suggested that 4-AP lowered the need for calcium in the excitation-secretion coupling process. 4-AP has been shown to antagonize opiates in the inhibition of peristalsis in the GPI (Kromer et al., 1980), in the inhibition of the electrically generated contraction of the GPI (Rezvani et al., 1983) and the MVD (Illes et al., 1980).

Neurotransmitter Interactions for Studying Opiate Actions

Morphine is known to reduce the electrically generated contractions of the GPI by reducing the amount of ACh available for stimulation (Schaumann, 1956), and this effect is rapidly reversed by anticholinesterase drugs (Paton, 1957). This opiate effect is not apparent with cholinergic transmission in other intestinal strips, e.g., the rabbit ileum (Greenberg et al., 1970). On the other hand, neurons in both the CNS and the peripheral tissues with other neurotransmitter mechanisms have been shown to respond to morphine.

Serotonergic facets of meperidine action

Serotonin (5-hydroxytryptamine) is known to affect morphine in many ways, and has been postulated in the past

to be a mediator of morphine's central nervous system (CNS) activity. However, the evidence supporting this is contradictory (Way, 1972), and points to the possibility that serotonin has a controlling or inhibitory function rather than a first messenger role.

While meperidine has been found to have interactions with several neuroamines in the CNS, one of the most deeply studied has been with serotonin; in part because there have been several reports of toxic effects in persons taking meperidine and monoamine oxidase (MAO) inhibitors. This response may include excitation, delirium, hyperpyrexia, convulsions or severe respiratory depression (Jaffe and Martin, 1985). No other potent morphine-like compound shows this effect to such a degree. However, in mice, lethality of several such compounds was potentiated to the same extent as meperidine's by pretreatment with MAO inhibitors (Rogers and Thornton, 1969). Increased killing by tranylcypromine (an MAO inhibitor) showed a close correlation in time with the rise of brain serotonin concentration, and the dose-response curves for these effects were parallel. The authors suggested that the interaction between meperidine and MAO inhibitors was related to the serotonin concentration, as changes in dopamine ($r=0.56$), and adrenaline ($r=0.44$) did not show the same significant correlation as did serotonin ($r=0.957$, $P<0.001$). However, when using concentrations analogous to

the human situation, and utilizing the rabbit as the experimental model (the rabbit has a response to the meperidine/MAO combination like man), the potentiated toxicity is seen only with meperidine, not morphine or pentazocine (Penn and Rogers, 1971). Meperidine is known to block re-uptake of serotonin in both the CNS and the peripheral nervous system, while morphine and methadone have little or none of this type of activity (Carlsson and Lindqvist, 1969), but this apparently is not sufficient to account for the lethality of the meperidine/MAO combination, as a potent inhibitor of serotonin uptake, when given with tranylcypromine, did not cause death (Fuller and Snoddy, 1975). However Fahim et al. (1972) found that pretreatment with p-chlorophenylalanine, a serotonin synthesis inhibitor, protected against the lethal combination. Whatever the mechanism, it is non-morphine-like, as treatment with nalorphine had no effect on the fatal hyperpyrexia seen with the combination in the rabbit (Fahim et al., 1972).

Meperidine also interacts with phenothiazines to cause an enhancement of respiratory depression. Chlorpromazine concurrently administered with meperidine exaggerates and extends the respiratory depressant effect of meperidine (Lambertsen et al., 1961). Chlorpromazine is a drug with varied effects on neurotransmitters, being known to act as

an antihistaminic, have alpha-adrenergic antagonist activity, and be a serotonin blocker (Jarvik, 1970).

Serotonin-containing neurons appear to be present in the guinea pig myenteric plexus. Various nerve cell bodies and fibers in this system demonstrated immunoreactivity to antibody preparations raised to serotonin (Furness and Costa, 1982). The immunoreactive material was depleted by treatment with reserpine, and after depletion, immunoreactivity could be restored by *in vitro* application of serotonin or 5-hydroxytryptophan, but the 5-hydroxytryptophan restoration was blocked by an L-amino acid decarboxylase inhibitor (Costa *et al.*, 1982). Furthermore, meperidine induces release of serotonin from the intestinal tract (Burks and Long, 1967).

Adrenergic aspects

While most adrenergic junctions are insensitive to opioids, both the cat nictitating membrane (Trendelenberg, 1957) and the MVD (Henderson *et al.*, 1972), are among those which respond to morphine with a depression in the electrically generated contractions. In both these tissues (Henderson *et al.*, 1972, 1975), morphine is not a direct adrenergic antagonist, but alters the release of norepinephrine at the nerve-smooth muscle junction. Meperidine also acts on the MVD (Hughes *et al.*, 1975), but the mechanism of action has not been elucidated. There are

also adrenergic neurons in the GPI, although these are insensitive to morphine (Henderson et al., 1975).

Purinergic aspects

Purinergic nerves are a fairly recent discovery (see Burnstock, 1980), but from the first a resemblance between the actions of opiates and the action of various purinergic analogues has pointed to the possibility that purinergic nerves may participate in the body's response to opioids (Gintzler and Mussachio, 1975). Morphine's effect in the GPI has also been shown to be influenced by compounds which alter the purinergic response. The inhibitory response of morphine in the ileum is potentiated by tolazoline, a known antagonist of adenosine triphosphate, and by phosphodiesterase inhibitors (Gintzler and Mussachio, 1975). Adenosine can induce a form of drug-dependence similar to that seen with opiates. Withdrawal abstinence from adenosine dependence in the isolated GPI can be blocked by normorphine, but the addition of naloxone to an adenosine-dependent ileal strip does not produce contractures. However, 8-phenyltheophylline, a potent P_1-purinoceptor antagonist (Griffith et al., 1981), and caffeine do induce contractures (Collier and Tucker, 1983). Gintzler and Mussachio (1975) postulated that cyclic adenosine monophosphate (cAMP) may play a role in mediating some of the inhibitory effects produced by morphine, and

cAMP also seems to be involved in the production of tolerance to morphine both *in vivo* (Ho *et al.*, 1973) and *in vitro* (Rezvani *et al.*, 1983).

Substance P interactions

Substance P was discovered as early as 1973 to substitute for morphine in chronically morphinized mice (Stern and Hadzovic, 1973). Further experiments indicated that substance P was a potent analgetic in mice when administered intracerebrally (Stewart *et al.*, 1976; Frederickson *et al.*, 1978). This analgetic activity was antagonized by naloxone, and animals made tolerant to morphine were cross-tolerant to substance P (Stewart *et al.*, 1976).

However, substance P did not bind to morphine receptors in the central nervous system (Terenius, 1975), nor did it act like morphine in the field-stimulated GPI (Cox *et al.*, 1975) or in the MVD (Frederickson *et al.*, 1978). Frederickson postulated that substance P does not act directly, but causes release of endorphins, which then induce analgesia. The same reticular formation neurons are stimulated by both substance P and morphine (Collingridge and Davies, 1982).

Substance P does have a direct effect on the GPI (Cox *et al.*, 1975) and MVD (Frederickson *et al.*, 1978), causing a contractile response in these tissues. Substance P-

containing neurons have been shown to be present in the GPI (Franco et al., 1979; Costa et al., 1980) and the contractile response to capsaicin (Chahl, 1982) and serotonin (Chahl, 1983) in this tissue is due in part to substance P release. In GPI strips from animals made tolerant to morphine, pre-treatment with capsaicin, known to deplete the substance P-containing neurons (Jessell et al., 1978; Nagy et al., 1980), and desensitization of the tissues to substance P, blocks the non-hyoscine sensitive component of naloxone-contracture (Tsou et al., 1982).

Following substance P desensitization of the GPI, capsaicin no longer caused any contractile response (Tsou et al., 1982). While substance P does not have an opioid-like effect in blocking the electrically induced contractions on the GPI or the MVD, capsaicin does (Szolcsanyi and Bartho, 1982). Doses of 1-3 ug/ml capsaicin had no effect but, at higher concentrations, a reversible (by washing out) inhibition by capsaicin of the electrically induced contractions was seen.

Summary of Introduction and Basis for Research

Based on the above review of the literature, there is ample evidence that meperidine does not always resemble morphine in its pharmacologic profiles. The original data on excised organs and tissues presented in the following pages were obtained in an attempt to clarify the response for some of the differences between morphine and meperidine. The sensitivity of the GPI to opiates is considered to be predictive of the analgetic effects seen in the CNS (see Kosterlitz and Waterfield, 1975 and Schultz, 1978), and the experiments were performed in this preparation to learn why meperidine does not act on the ileum in the same way that morphine does, although it certainly is an analgetic.

MATERIALS AND METHODS

Chemicals

N-Allylnormetazocine (SKF 10,047) was a gift from Dr. Edgar Iwamoto, naloxone hydrochloride from ENDO Laboratories (Garden City, NJ), l-methadone from Eli Lilly Co. (Indianapolis, IN) and ethylketocyclazocine methanesulfonate (EKC), l-pentazocine and meperidine hydrochloride from Sterling-Winthrop Research Institute (Rensselaer, NY). The delta antagonist ICI 174,864 was a gift from the Imperial Chemical Industries (Macclesfield, Cheshire, U.K.), and normorphine, phencyclidine and naltrexone were gifts from the National Institute for Drug Abuse, while the morphine sulfate was purchased from Mallinckrodt Inc. (St. Louis, MO). Ketamine hydrochloride came from Parke-Davis (Detroit, MI), and chlorpromazine from Smith, Kline and French (Philadelphia, PA). Hexamethonium bromide was supplied by K & K Laboratories, Inc. (Hollywood, CA) and atropine sulphate by Merck & Co. (Rahway, NJ), while D-ala, D-leuenkephalin was purchased from Peninsula Laboratories (Belmont, CA). Sigma Chemical Co. (St. Louis, MO) was the purveyor of pargyline, capsaicin, 8-phenyltheophylline, acetylcholine chloride, cyclic adenosine monophosphate, yohimbine and 4-aminopyridine.

The following chemicals for the preparation of the physiologic saline solutions were analytic grade. Glucose, sodium bicarbonate ($NaHCO_3$) and sodium chloride (NaCl) were bought from Mallinckrodt Inc., potassium chloride (KCl) and potassium phosphate monobasic (KH_2PO_4) came from J.T. Baker Chemical Co. (Phillipsburg, NJ). Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) was purchased from Sigma Chemical Co., and choline chloride from Nutritional Biochemicals Co. (Cleveland, OH).

Preparation of Excised Tissues

Studies were performed mostly on the isolated myenteric plexus of the guinea pig ileum (GPI). More limited studies were made on the mouse and rat vasa deferentia.

Guinea pig ileum

Male Hartley guinea pigs were purchased from EZH Laboratory (Williams, CA) and were generally between 300 and 400 g at time of sacrifice, although weights varied from 180 to 800 g. The animals were supplied with Purina Guinea Pig Chow (Ralston Purina Co., St. Louis, MO) and water ad libitum. Animals were killed by a blow to the neck. The ileum was excised about 10 centimeters (cm) above the distal end and kept in a physiologic saline of

the following composition (mM): NaCl, 154; KCl, 5.66; $CaCl_2 \cdot 2H_2O$, 2.54; $NaHCO_3$, 5.95; glucose, 2.77; choline chloride, 0.002. Adjoining pieces of ileum were cut about 30 millimeters (mm) long, starting at the distal end, and the longitudinal muscle was gently slit along the grain with the tip of a forceps to facilitate separation from the circular muscle, and then teased from the muscle with a cotton tipped swab.

Vasa deferentia

Adult male ICR mice were obtained from the Simonsen Laboratory (Gilroy, CA); adult male Sprague-Dawley rats from Simonsen or Charles River (Wilmington, MA). The animals were maintained on Purina Laboratory Chow (Ralston Purina Co.) and water ad libitum. Mice were killed by cervical dislocation or decapitation, while the rats were anesthetized with CO_2 and killed by exsanguination. The vasa deferentia were removed, cleaned of extraneous membrane and fat and placed in physiologic saline of the following composition (mM): NaCl, 118; KCl, 4.7; $CaCl_2 \cdot 2H_2O$, 2.54; KH_2PO_4, 0.93; $NaHCO_3$, 25; glucose, 11.

Experimental Set-up

A length of cotton-covered polyester thread was fastened to each end of the excised ileum or vas, one piece

of thread fastened to the bottom ring of the electrode, and the other thread strung through the upper ring and fastened to the transducer. The strips were mounted in a tissue bath (Van Waters and Rogers, South San Francisco, CA) in the appropriate physiologic saline at 37° C and bubbled with a mixture of 95 percent O_2 and 5 percent CO_2 (Ohio Medical Products, Madison, WI). The tissues were allowed to equilibrate for one hour while under 0.5 g of tension and washed every 20 minutes with fresh buffer, at least 10 volumes, throughout the experiment, and both before and after any drug addition, unless otherwise specified.

Field stimulation of the tissues was effected with a S44 Grass stimulator (Grass Instrument Co., Quincy, MA), through two platinum rings serving as electrodes, using repeated pulses of 70 volts (V), 5 milliseconds (ms) in duration, with a delay of 1.5 ms, and at a frequency of 0.15 hertz. Contractions were recorded by a FT .03 transducer attached to a Grass polygraph, Model 7 (both instruments from Grass Instrument Co.).

Estimation of the IC_{50} of Opiate Agonists to Field Stimulation

After one hour of equilibration, the tissues were electrically stimulated until a relatively constant response was obtained. The tissue's response to an agonist

was measured by adding a quantity of the substance to the tissue bath, waiting until the contractions reached a new level, and calculating the decrease or increase of response to the compound as a simple percentage. The concentration of each agonist needed to induce a 50 percent inhibition of the twitch height (IC_{50}) was estimated by plotting the percent of inhibition against the concentration of compound used, and interpolating the IC_{50} from the curve.

In those cases where increases in the concentration of the agonist did not yield a depression of the twitch height above 50 percent, no IC_{50} could be obtained, and results are given as the dose at which maximal response was achieved.

Assessment of Anticholinergic Effects

To determine the relative anticholinergic response of an agent, the inhibitory effect on ACh-induced contracture in the GPI was evaluated by obtaining the dose-response curve of ACh in the presence and absence of differing doses of inhibitor. The tissue was initially washed, and when the baseline returned to normal, ACh was added, and after the contraction peaked, the tissue washed again. Following administration of ACh, spontaneous activity tended to decrease for a short time, and the response to the next dose could be more easily read. The inhibitor, when

tested, was added shortly before the ACh (2-5 min). The height of the ACh contraction was measured and plotted against the concentration, to give a dose-response curve, in the absence and presence of inhibitor. The effective concentration of ACh to achieve 50 percent of the maximal height (EC_{50}) was interpolated from the graph. The ratio of the EC_{50} after/before inhibitor was used to help determine the anticholinergic effect of meperidine (on chemically induced contracture) related to the opioid effect (on electrically induced contracture).

Assessment of the Effects of Calcium

To assess the effect of calcium on the response of the tissue to a drug, varying concentrations of $CaCl_2 \cdot 2H_2O$ were added to the physiologic saline in the tissue bath, and allowed to equilibrate with the tissue for one hour. The dose-response curve of the tissue to the drug was obtained and plotted. The calcium concentrations tried ranged from 0.63 mM to 10.16 mM, or from one-quarter to four times the usual concentration.

Assessment of Tolerance

To induce tolerance to opiates in the various <u>in vitro</u> systems, the dose-response curve of the agonist was

determined in the usual manner. The preparation was then incubated with a fraction of the IC_{50} of each agonist for various times, and its IC_{50} was re-determined, following the method of Rezvani et al. (1983).

Usually the tissues were incubated with one IC_{50} of an agonist for two hours, although various other concentrations and times were used at times. The tissues were washed every twenty minutes during the incubation period with physiologic saline containing the agonist at the incubating concentration. At the end of the incubation the tissues were stimulated as previously described until a steady response was obtained. The IC_{50} of the agonist was then re-determined in the presence of the incubating chemical. The degree of tolerance developed was expressed as a ratio, the IC_{50} after/before incubation. Since tolerance development indicates a loss of sensitivity to the compound, the dose-response curve is shifted to the right and the ratio should be a number greater than one.

Cross-tolerance in these in vitro systems is evaluated by inducing tolerance to one drug and seeing if the tissue also shows a reduced sensitivity to a second compound.

Assessment of Physical Dependence

Using the method of Collier et al. (1981) to quantify the response and compare individual tissues, each tissue

was calibrated by measuring its response to a maximal dose of ACh before rendering the preparation tolerant-dependent by incubation with the agonist for at least 2 hours. A contractural response induced by the addition of naloxone (100 ug) to the tissue bath was then expressed as a percentage of the maximal response elicited by the ACh.

Sensitivity to Antagonism

In determining the effect of the antagonist naloxone on the agonist, a dose-response curve for the agonist was obtained, after which the curve was re-determined in the presence of differing concentrations of naloxone. The naloxone was added at least five minutes before the agonist to ensure equilibrium with the tissue. The IC_{50}'s of the various dose-response curves were read from the plotted curves and transformed. The transformation follows the formula of Schild and Arunlakshana (1947): x = log (IC_{50} in the presence of antagonist/IC_{50} without antagonist) - 1. From the plot of the transformed IC_{50}'s against the log of the antagonist concentration it is possible to derive the pA_2 (the concentration of antagonist at which twice as much agonist is needed to achieve the same effect), and the slope of the line, which can indicate whether or not the antagonist is acting competitively.

Other opiate antagonists were also tested against the agonist, but these were not evaluated to the same extent as naloxone. One method used in trying to determine if any of these compounds were more effective against the agonist, responses of a constant concentration of agonist with increasing concentrations of antagonist, were measured. However at times, alterations in the dose-response curve of the agonist using one concentration of antagonist were also evaluated.

This method was also used with several other chemicals, in the attempt to determine by what mechanism the effects of the agonist were mediated. The specific inhibitors and concentrations used will be reported in the section on results.

Potentiation of the Tolerance Response by Cyclic Adenosine Monophosphate (cAMP)

After the IC_{50} of the agonist was determined, the tissue was incubated with 12 uM cAMP for one hour, and two hours additional incubation with 12 uM cAMP and one IC_{50} of the agonist, and the dose-response curve re-calculated. Incubation of cAMP with the agonist used to induce tolerance has been reported to increase the level of tolerance developed (Rezvani et al., 1983).

Desensitization of Guinea Pig Ileum to Capsaicin

Ileal strips for which a dose-response curve for the agonist had been obtained were incubated with 2.6 uM capsaicin (a known depletor of substance P) for two hours, after which another dose-response curve was produced (Tsou et al., 1982).

Statistical Methods

P values calculated by the paired Student's t-test.

RESULTS

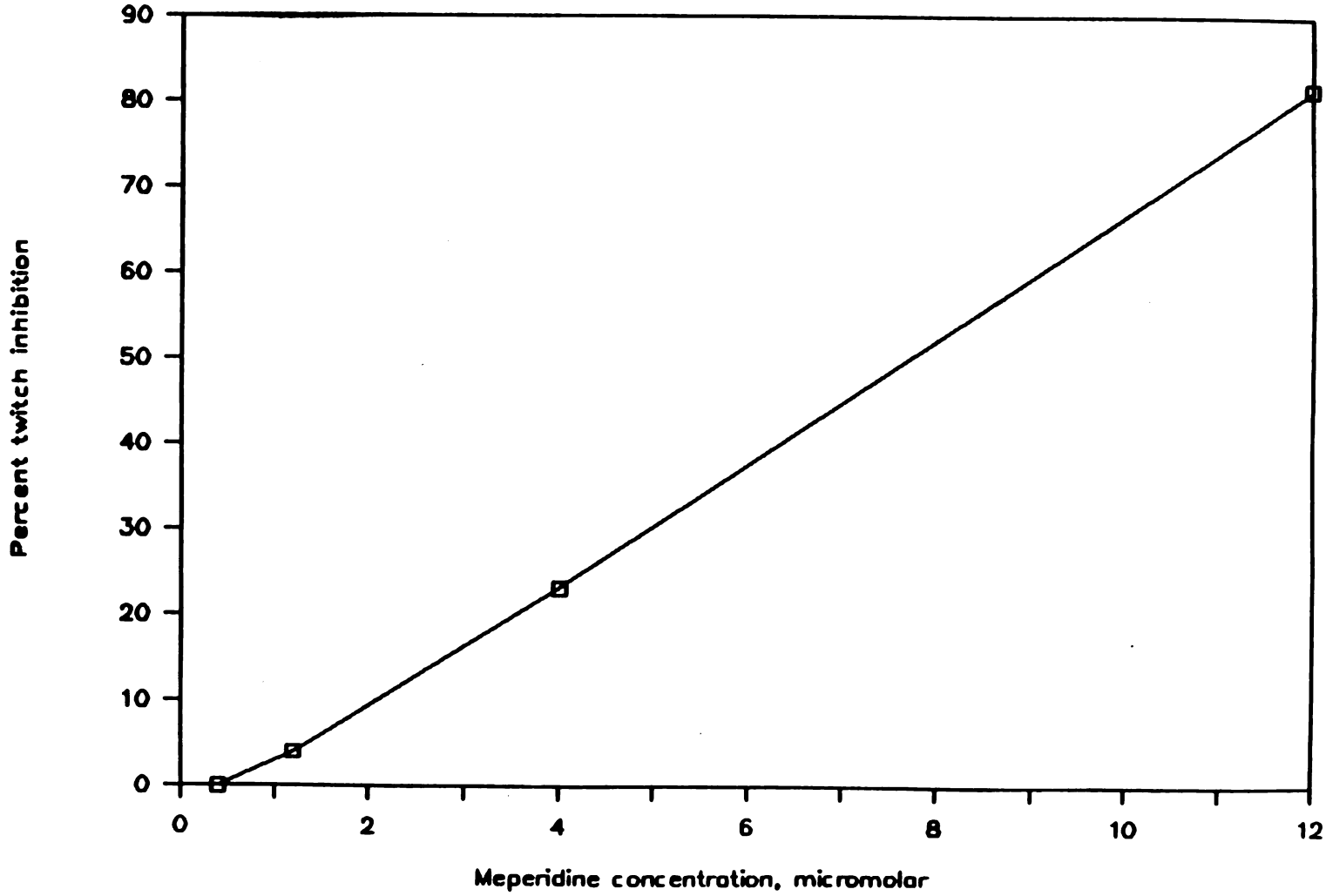
Meperidine and Morphine Effects on the Guinea Pig Ileum

Meperidine caused depression of the electrically induced stimulus contraction in a dose-dependent manner (Figure 1). Generally a good dose-response curve was obtained, with a fairly steep slope, and plateauing did not occur. While there was some variation in the IC_{50} of the drug in individual tissues, it remained usually around 5 uM.

FIGURE 1, Legend

Dose-response relationship of meperidine in the guinea pig ileum. A representative example of meperidine's inhibition of electrically evoked contractions in guinea pig ileum myenteric plexus-longitudinal muscle preparation. Abscissa represents meperidine's concentration in micromolar; ordinate, the percent inhibition of electrically generated stimulus contraction induced by meperidine.

FIGURE 1

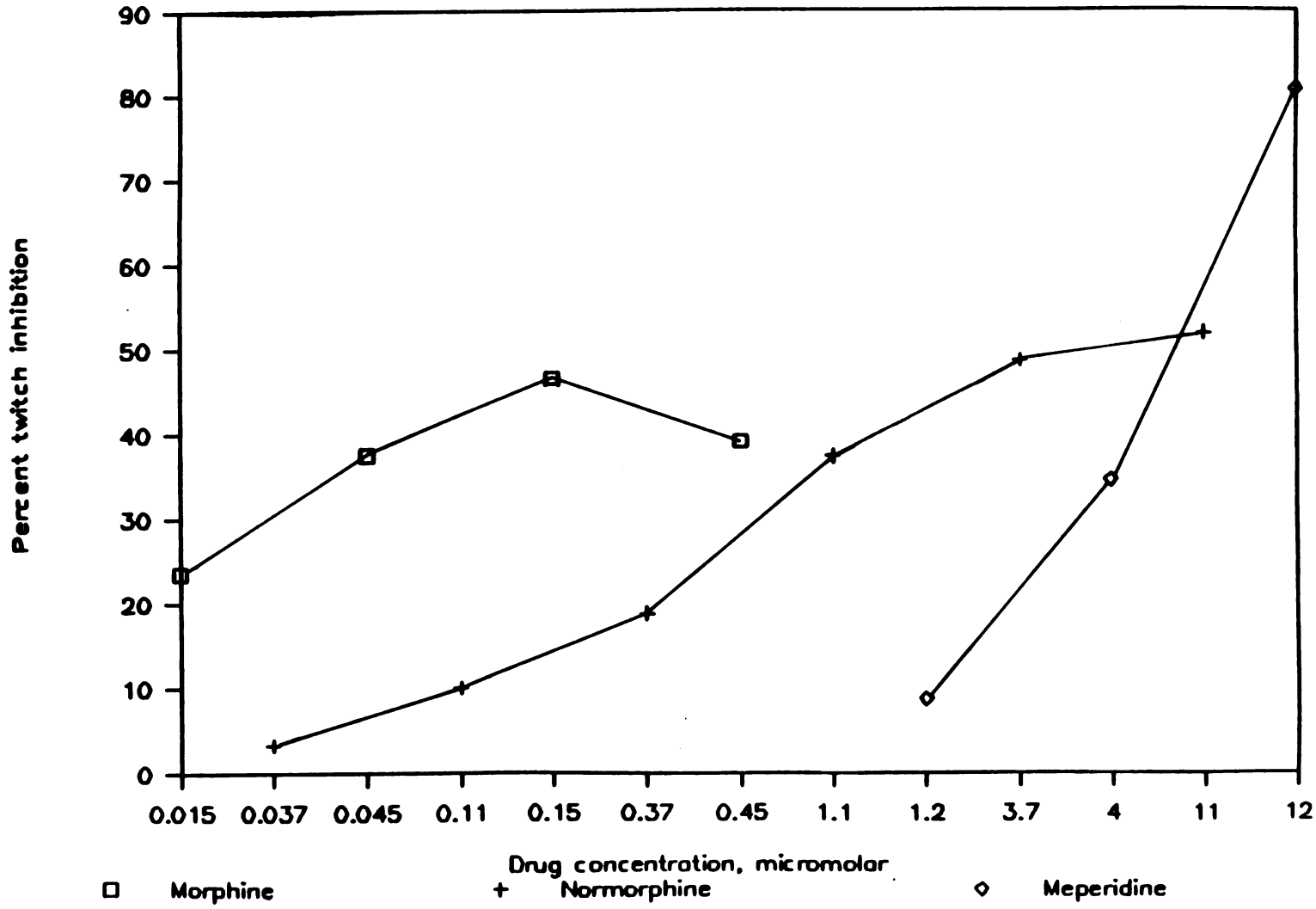

90
80
70
60
50
40
30
20
10
0
Percent twitch inhibition
0
2
4
6
8
10
12
Meperidine concentration, micromolar

Although these effects of meperidine appeared to be morphine-like, the response to morphine was less uniform. A full blockade of the electrically induced stimulus contraction was not always achieved and some preparations failed to show a 50 percent depression of the twitch height in response to morphine. Morphine may produce inconsistent responses because it is difficult to remove by washing. For this reason most experimenters prefer to use normorphine rather than morphine. However, even in the same tissues, the response to meperidine and other opioids could be quite different (Figure 2).

FIGURE 2, Legend

Comparison of inhibitory effects of meperidine with other opiate agonists. Illustration shows three chemicals which inhibit electrically evoked contractions in the guinea pig ileum myenteric plexus-longitudinal muscle preparation; morphine (MS), meperidine (M) and normorphine (NM). Comparisons with meperidine were performed using the same tissue preparations. Abscissa represents the concentration of the compounds in micromolar; ordinate, the percent inhibition of the electrically induced stimulus contraction. Points are means (standard error of means calculated, but not illustrated).

FIGURE 2


Percent twitch inhibition
90
80
70
60
50
40
30
20
10
0
0.015
0.037
0.045
0.11
0.15
0.37
0.45
1.1
1.2
3.7
4
11
12
Drug concentration, micromolar
Morphine
Normorphine
Meperidine

Assessment of the Anticholinergic Response of Meperidine

Although meperidine possesses anticholinergic activity, it does not have significant effects on the ACh-induced contraction at or below concentrations that cause approximately a 40 percent inhibition of the electrically induced stimulus (Table 1).

TABLE 1

Anticholinergic Actions of Meperidine
in the Guinea Pig Ileum

Meperidine (uM)	ACh DR	Percent Inhibition
0	1.4±0.2	
1.2	1.2±0.1	11.9±3.2
4.0	1.8±0.2	38.4±2.7
12.0	7.4±0.6*	88.5±3.1
40.0	8.0±0.5*	80.7±5.3

By plotting the dose-response curve of acetylcholine (ACh), the effective concentration of ACh needed to induce 50 percent of the maximal height (EC_{50}) in a guinea pig ileum myenteric plexus-longitudinal muscle preparation can be determined. With this information for ACh in the presence of varying micromolar (uM) concentrations of meperidine, a dose ratio (DR) or the EC_{50} after meperidine/EC_{50} before meperidine may be calculated. Comparing these calculations with percent inhibition of the twitch height induced by meperidine, indicates that at meperidine concentrations which induce below approximately 40 percent inhibition of the twitch height, there does not appear to be a significant anticholinergic effect.

*) $P<0.001$

Repeated exposure to exogenous ACh reduced the tissue's contractile response. The dose-response curve of ACh was shifted to the right (EC_{50} went from 2.2±0.4

nanomolar [nM] to 3.7±0.7 nM) and the maximal response to ACh was reduced by 14.0±3.6 percent. Therefore, to minimize the effect of the lowered response to repeated exogenous ACh exposure, no more than two ACh dose-response curves were performed on any one tissue (one control and one in the presence of the morphine-like compound).

Effect of Calcium on the Actions of Meperidine

Calcium significantly antagonized the inhibitory effects of meperidine on the ileum. Over a range of calcium concentrations from 0.63 to 10.16 mM, the IC_{50} of meperidine increased in a dose-dependent manner from 1.8±0.2 to 10.3±0.4 uM (Figure 3).

FIGURE 3, Legend

Effects of increasing calcium on meperidine potency. Increasing calcium concentrations in the guinea pig ileum myenteric plexus-longitudinal muscle preparation decreases meperidine's potency significantly, as shown by inhibition of electrically induced contractions. Calcium ion concentrations of incubation bath in millimolar (mM) on horizontal axis. Meperidine IC_{50}'s in micromolar, interpolated from dose-response curves, in the presence of calcium concentrations can be read from the vertical axis. Bars represent means of the IC_{50}'s (standard error of means calculated, but not illustrated).

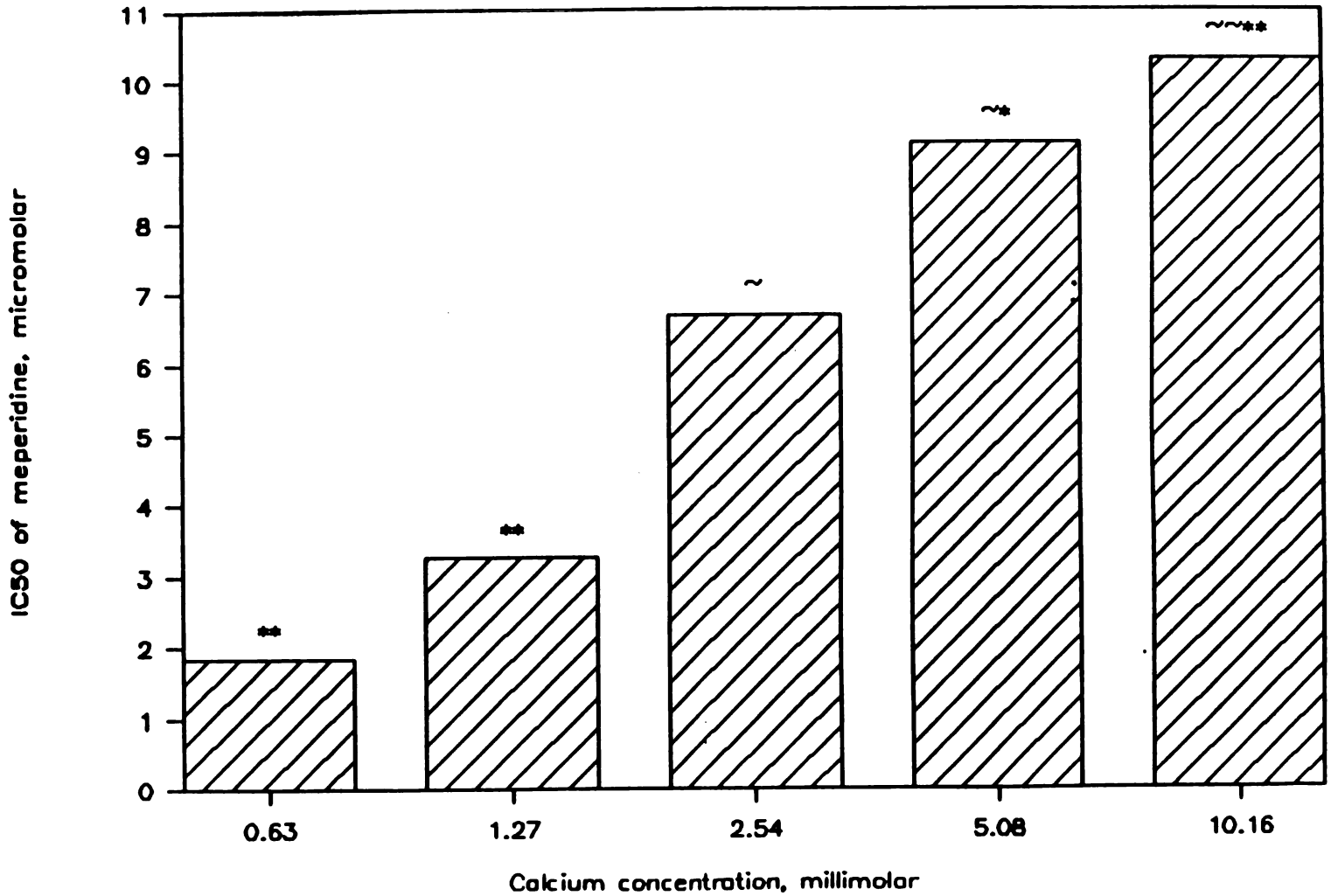
*) P<0.025 compared with 2.54 mM calcium.

**) P<0.001 compared with 2.54 mM calcium.

~) P<0.005 compared with 0.63 mM calcium.

~~) P<0.001 compared with 0.63 mM calcium.

FIGURE 3

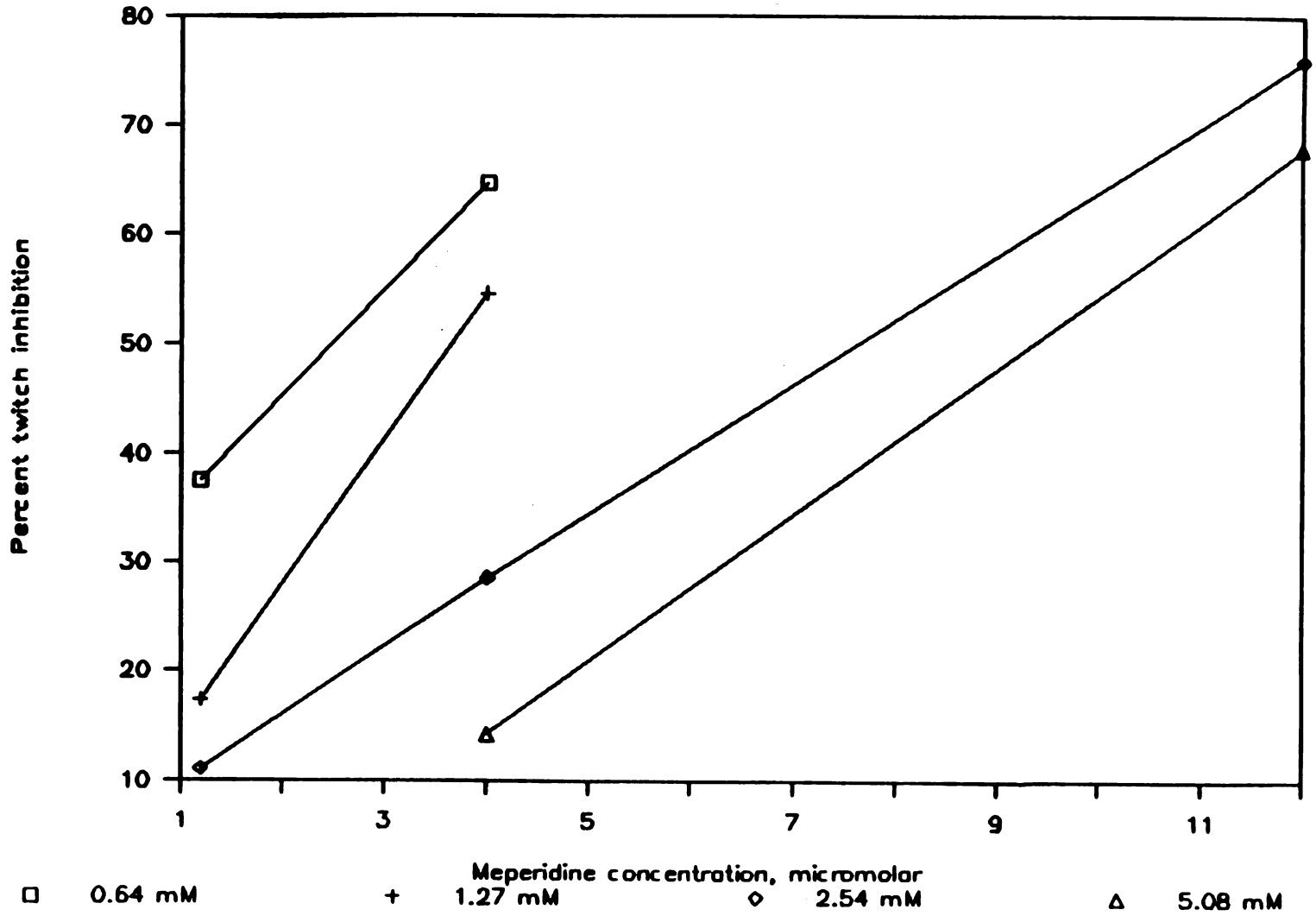

IC50 of meperidine, micromolar
11
10
9
8
7
6
5
4
3
2
1
0
0.63
1.27
2.54
5.08
10.16
Calcium concentration, millimolar

In one experiment the effects of calcium on the dose-response curve of meperidine can be seen as the concentration of calcium in the media increased from 0.63 to 5.08 mM (Figure 4). The slopes of the curves are not exactly parallel, with those at the lower calcium concentrations being steeper, and becoming shallower with increasing calcium.

FIGURE 4, Legend

Effects of increasing calcium on meperidine responsivity. Varying calcium concentration significantly affects meperidine dose-response curves of electrically generated stimulus contractions in the guinea pig ileum myenteric plexus-longitudinal muscle preparation. At sufficiently low calcium concentrations, tissue will not react to the stimulus at all. As calcium concentration increases, response to the electrical stimulus grows stronger, although less reactive to meperidine. Responses were more unstable at the lower concentrations used in this set of experiments. Increasing the calcium concentration further continued to reduce meperidine's effect. Abscissa represents meperidine concentration in micromolar; ordinate, the percent twitch inhibition. Dose-response curves shown from one representative experiment with meperidine in the presence of 0.64 millimolar (mM), 1.27 mM, 2.54 mM and 5.08 mM calcium.

FIGURE 4


80
70
60
50
40
30
20
10
Percent twitch inhibition
1
3
5
7
9
11
Meperidine concentration, micromolar
□ 0.64 mM
+ 1.27 mM
◇ 2.54 mM
△ 5.08 mM

The antagonistic effect of calcium against meperidine differs considerably from that against ACh and morphine. When compared with a limited range of calcium changes from 1.27 to 5.08 mM, shifts in the EC_{50} of ACh and the IC_{50}'s of meperidine and morphine respectively can be seen to be quite different (Table 2).

TABLE 2

Effects of Increasing Calcium (Ca) Concentrations on the Response to Acetylcholine (ACh), Meperidine (M) and Morphine (MS) in the Guinea Pig Ileum

Ca (mM)	ACh EC_{50}[a]	M IC_{50}[b]	MS IC_{50}
1.27	2.3±0.1	3.3±0.6	0.9±0.0
2.54	3.2±0.4	6.7±0.5*	5.2±0.6*
5.08	3.1±0.8	9.1±0.9*	

a) The median dose of ACh (10^{-8} molar) to effect a contractile response.

b) The median dose of meperidine (10^{-6} molar) or morphine (10^{-7} molar) to inhibit electrically evoked contractions.

*) $P<0.001$ compared with lowest calcium concentration for that compound.

Although the ACh EC_{50} increased slightly, the increase was not at a level of statistical significance. However, calcium significantly increased the meperidine IC_{50} two to three-fold ($P<0.001$), while morphine showed an even greater increase. The morphine IC_{50} increased five-fold ($P<0.001$)

when the calcium concentration was increased from 1.27 to 2.54 mM. When the calcium concentration was again doubled to 5.08 mM, the morphine dose-response curve shifted to the right again, but the degree could not be measured because the maximal response was obtained at a concentration lower than its IC_{50}.

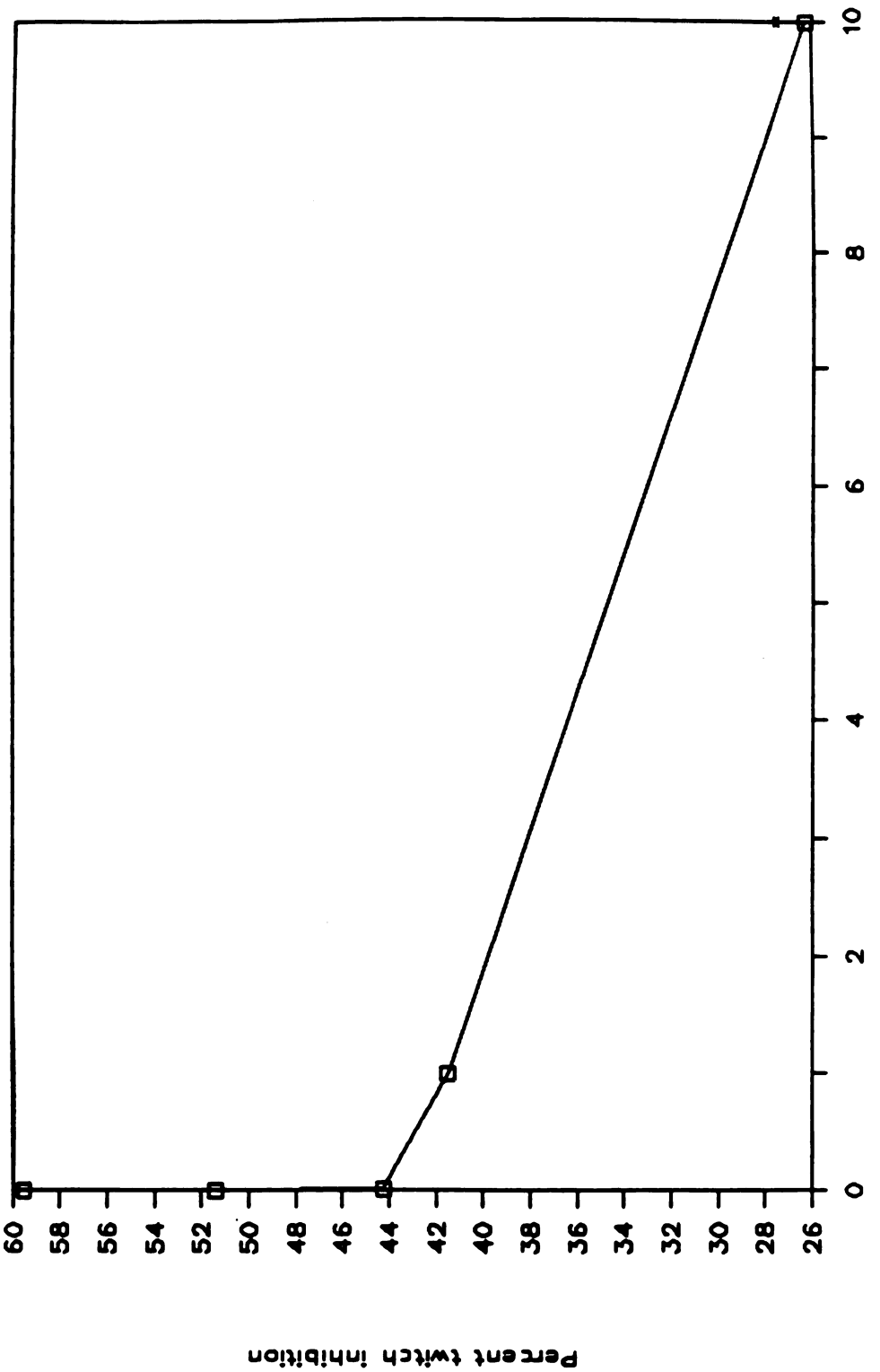
4-AP, a potassium channel blocker which reportedly increases the internal availability of calcium ions, also antagonized the effects of meperidine. Over a thousand-fold range, from 0.1 nM through 0.1 uM, 4-AP reduced the effect of meperidine on the twitch height in a dose-dependent manner (Figure 5). At 0.1 uM, the depression became statistically significant ($P<0.005$), but at 1.1 uM 4-AP depressed the ileum. An attempt was made to evaluate the inhibitory action of 4-AP on meperidine by observing the antagonist's effect on meperidine's dose-response curve. At 0.1 uM, 4-AP shifted the meperidine dose-response curve to the right, and the IC_{50} was increased from 3.9±0.6 to 5.3±0.6 uM. When the concentration of 4-AP was raised to 0.3 uM, a concentration which caused a 26.5±3.3 percent depression of the twitch height, the inhibitory effect of meperidine was antagonized even further, and the meperidine IC_{50} was increased to 9.0±0.6 uM, at a level of significance of $P<0.001$.

FIGURE 5, Legend

4-Aminopyridine's effect on the response of the guinea pig ileum to meperidine. 4-Aminopyridine (4-AP) added to the myenteric plexus-longitudinal muscle preparation incubation mixture before meperidine reduced the effect of meperidine in a dose-dependent manner which was statistically significant at 10 micromolar (uM) (P<0.005). Abscissa represents 4-AP concentration in uM; ordinate, the percent inhibition of the electrically induced stimulus contraction caused by meperidine in the presence of 4-AP. Points are means (standard error of means calculated, but not illustrated).

*) P<0.005

FIGURE 5


Percent twitch inhibition
60
58
56
54
52
50
48
46
44
42
40
38
36
34
32
30
28
26
0
2
4
6
8
10
4-Aminopyridine, micromolar

Tolerance Studies on Meperidine Compared with Other Agonists on the Guinea Pig Ileum

Only a low degree of tolerance developed to meperidine with the *in vitro* procedures. Incubating the guinea pig ileal strips with buffer for two hours may have sensitized the tissue slightly as there was a shift in the dose-response curve to the left (0.8±0.1-fold, $P<0.005$). But incubating the tissues with meperidine caused small but significant shifts to the right.

As can be seen from the data presented in Table 3, incubation with meperidine produced a modest degree of tolerance.

TABLE 3

Tolerance Development to Meperidine
in the Guinea Pig Ileum Preparation

Meperidine (uM)	Tolerance[a]
0	1.0±0.1
1	1.8±0.2**
2	1.6±0.2**
3	2.5±0.1**
4	1.4±0.1*
5	1.8±0.1**
6	1.1±0.1

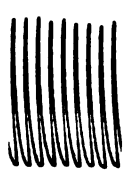
[a]) The degree of tolerance development to meperidine in the guinea pig ileum myenteric plexus-longitudinal muscle preparation following two hours incubation with various concentrations of meperidine is measured by the mean of the IC_{50} of meperidine post/preincubation ± standard error of the mean.

*) $P<0.05$ compared with incubation in physiologic saline.

**) $P<0.001$ compared with incubation in physiologic saline.

Although the data were statistically significant, the maximum tolerance obtained with 3 uM meperidine was only 2.5-fold. Moreover, the response to meperidine appeared to be biphasic in that tolerance decreased with further increases in meperidine concentration. When the meperidine concentration was greater than 5 uM, no tolerance was demonstrated; perhaps because of a non-opioid-like response that tended to oppose the opioid effects of meperidine. Also, as the concentration of meperidine in the incubation buffer was increased, the stability of the preparation decreased. This deterioration was evidenced by diminished responsivity measured by recovery of twitch height. Following incubation with 7 uM meperidine, twitch height was depressed a mean of 69.1±2.4 percent, and measurements were rendered difficult to read because of the irregular response (Figure 6).

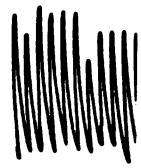
FIGURE 6



A-1

A-2

B-1



B-2

Effect of two hours incubation in eight micromolar meperidine. These polygraph recordings demonstrate the negative effect of high concentrations of meperidine on the response of the guinea pig ileum myenteric plexus-longitudinal muscle (MPLM) preparation to an electrically generated stimulus. Tracings show MPLM's response in physiologic saline before two hours incubation in the saline (A-1), and after (A-2). (B-1) and (B-2), respectively, show the corresponding response in another MPLM in saline before incubation, and following two hour incubation in 8 micromolar meperidine. The polygraph is set to the same readings before and after incubation. As shown in (B-2), the twitch height has been reduced and the stability of the tissue compromised following exposure to high levels of meperidine.

Incubating the tissues with 12 uM cAMP and 5 uM meperidine did not significantly alter the tolerance development to meperidine (1.5±0.1-fold as compared with 1.4±0.1-fold without cAMP). However, one of the appropriate controls, incubation of the tissues with 12 uM cAMP for three hours in the absence of meperidine, did induce a slight, but non-significant shift of the meperidine IC_{50} (1.2±0.1-fold), and appeared to prevent development of meperidine sensitivity seen when tissues were incubated in buffer alone (0.8±0.1-fold).

Cross-tolerance

Meperidine exhibited little cross-tolerance to mu and none to kappa agonists. After incubating tissues with 43 nM morphine virtually no response to morphine could be detected and even high concentrations (1.5 uM) only depressed the twitch height a few percent. However, the response of morphine-tolerant preparations showed only a slight tolerance (1.9±0.3-fold) to meperidine. Increasing the incubation time with 43 nM morphine from two to four hours increased the tissue's tolerance to meperidine slightly (2.2±0.3-fold), while doubling the level of morphine in the incubation buffer to 85 nM did not increase tolerance development to meperidine (1.5±0.0-fold) and may have even reduced the response.

Meperidine exhibited cross-tolerance to various opioid agonists to varying degrees. Table 4 lists the degree of cross-tolerance exhibited by meperidine to each agonist after incubation of the GPI MPLM preparation with 1 x IC_{50} or more of each compound.

TABLE 4

Degree of Cross-tolerance Exhibited by Meperidine Rendered Tolerant to Various Opiate Agonists in the Guinea Pig Ileum

Class	Agonist	Conc.[a]	Degree of Tolerance to Meperidine[b]	Degree of Tolerance to Self[c]
mu	morphine	43 nM	1.9±0.3*	10.8
			2.2±0.3*+	
		85 nM	1.5±0.0*	
	l-methadone	39 nM	2.2±0.1*	
		80 nM	1.5±0.0*	
		130 nM	2.0±0.1*	
kappa	EKC	0.87 nM	1.2±0.1	>1000
		8.7 nM	1.0±0.1	
delta	DADL	1 uM	2.1±0.3*	62-450
sigma	SKF 10,047	380 nM	3.0±0.7*	
		770 nM	3.5±0.6*	
		1500 nM	4.4±0.5*	
	pentazocine	80 nM	8.4±1.8*	8-69
	phencyclidine	41 uM	1.3±0.4	1.6
unknown	ketamine	390 uM	8.0±0.9*	2.1

a) Concentration.

b) Mean of the IC_{50} of meperidine post/preincubation ± standard error of the mean. The meperidine IC_{50} was determined before and after two hours incubation with each agonist. The concentration of agonist used was at least one IC_{50}.

c) Mean of the IC_{50} of agonist post/preincubation. The agonist IC_{50} was determined before and after two hours incubation. The concentration of agonist used was at least one IC_{50}, although not necessarily the concentration given in the concentration column.

*) $P<0.001$ compared with incubation in physiologic saline.

+) Incubation was for four hours.

The treatment produced significant but varying degrees of tolerance with each compound to itself. The following compounds showed high tolerance, morphine: 10.8-fold; EKC: at least 1000-fold; D-ala, D-leuenkephalin (DADL): 61.6 to 450-fold; pentazocine: 8.2 to 68.8-fold. Some compounds did not convey much tolerance to themselves -- phencyclidine: 1.6-fold; and ketamine: 2.1-fold. The response of these tolerant tissues to meperidine was mixed. Tissues tolerant to morphine and l-methadone, both mu agonists, also exhibited tolerance to meperidine (1.9±0.3-fold and 2.2±0.1-fold), but the level of tolerance could not be significantly enhanced by increasing the concentration of the agonist. No tolerance to meperidine was seen after tolerance development to the kappa agonist EKC. DADL produced some degree of cross-tolerance to meperidine (2.2±0.3-fold). Tissues made tolerant to the classic sigma agonist SKF 10,047 were also tolerant to

meperidine and to a greater degree (3.0±0.7-fold) than with either morphine or l-methadone, and the tolerance could be increased to 4.4±0.5-fold by a four-times increase in the incubating concentration. The response of meperidine to other sigma agonists was mixed, with one IC_{50} of pentazocine-incubated tissues producing a much greater tolerance development (8.4±1.8-fold) than SKF 10,047, while phencyclidine (PCP) induced no cross-tolerance to meperidine (1.3±0.4-fold). Perhaps the most interesting response was ketamine's, with ketamine-incubated tissues exhibiting tolerance to meperidine (8.0±0.9-fold) to nearly the same extent as to pentazocine.

With SKF 10,047, interesting but inconsistent findings were noted. At 380 nM, which is the dose at which the maximum depression of the twitch was obtained to the compound, the degree of tolerance development was 3.0±0.7-fold. However, the results were somewhat variable. While two of the eight tissues evinced greater than a five-fold shift in the dose-response curve to the right, in most of the tissues the shift was less than two-fold. When the concentration of SKF 10,047 in the incubation buffer was increased to 770 nM, although four of the 27 tissues tested showed nearly ten-fold tolerance development, the mean degree of tolerance was only 3.4±0.6-fold. However, increasing the concentration of SKF 10,047 to 1.5 uM increased the degree of tolerance to meperidine to

4.4±0.5-fold and decreased the variability of the response. Other manipulations did not materially enhance tolerance development to meperidine. Neither increasing the incubation period with SKF 10,047 to four hours, nor adding 12 uM cAMP to the incubation buffer with the agonist had any effect.

Cross-tolerance development between meperidine and morphine-like compounds could not be demonstrated. In tissues which had been incubated with concentrations of meperidine that produces about a two- to threefold tolerance to meperidine little or no effect on the IC_{50} of either morphine or ketamine was observed. After incubating with 5 uM meperidine for two hours the morphine dose-response curve did not shift significantly (1.0±0.3-fold). Higher doses of meperidine rendered the tissues so insensitive to morphine that a post-incubation dose-response curve of morphine could not be obtained. Incubation with 2 uM meperidine did not result in cross-tolerance development to ketamine; the ratio of the IC_{50} of ketamine after and before incubation with meperidine was 1.1±0.1.

Physical Dependence

Attempts to demonstrate physical dependence on meperidine <u>in vitro</u> in the guinea pig ileal strips were

unsuccessful. Two hours incubation with meperidine at doses of up to 16 uM did not produce a supersensitive response after removal of the meperidine by washing with buffer. Nor could a consistent contractile response to naloxone be obtained which was significantly different from that seen in the controls, whereas incubation of tissues with morphine generally produced a contraction in the tissue in response to naloxone. Moreover, increasing the incubation time with meperidine to four hours did not enhance the acquisition of a contractile response to naloxone.

Effect of Various Pharmacologic Agents on the Responsivity to Meperidine in the Guinea Pig Ileum

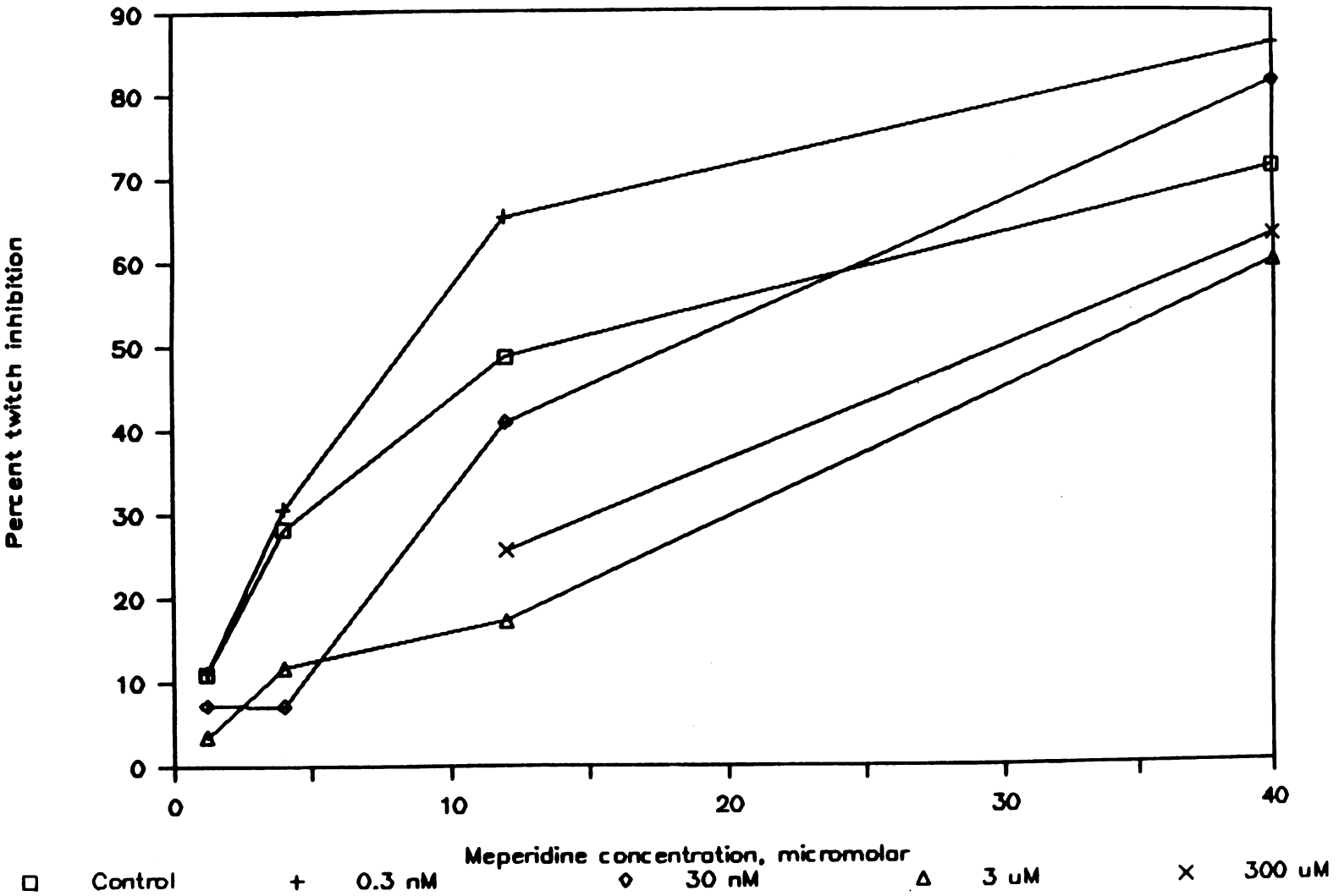
Naloxone and naltrexone

Naloxone significantly antagonized the effect of meperidine in the GPI. At the lowest doses of naloxone tested (0.3 and 3.0 nM), the meperidine dose-response curve was not significantly affected. However, at a dose of naloxone of 0.3 uM, the increases in the IC_{50} ratio became significant at the $P<0.05$ level, but did not show any further dose-dependent increase. The slope of the curves did not deviate significantly from parallel (see Figure 7). A Schild plot of naloxone's antagonism of meperidine gave a calculated pA_2 of 5.56, and a slope of -0.22.

FIGURE 7, Legend

Effects of naloxone on meperidine potency. Dose-response curves of meperidine on guinea pig ileum myenteric plexus-longitudinal muscle preparation displayed in the presence of varying concentrations of naloxone, from 0.3 nanomolar to 0.3 millimolar. Abscissa represents meperidine concentration in micromolar; ordinate, the percent inhibition of electrically induced stimulus contraction in the presence of naloxone. Steepest portions of curves are roughly parallel for those with naloxone, but the control curve without naloxone has a slightly shallower slope. Points are means (standard error of means calculated, but not illustrated).

FIGURE 7


Percent twitch inhibition
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
Meperidine concentration, micromolar
□ Control
+ 0.3 nM
◊ 30 nM
Δ 3 uM
× 300 uM

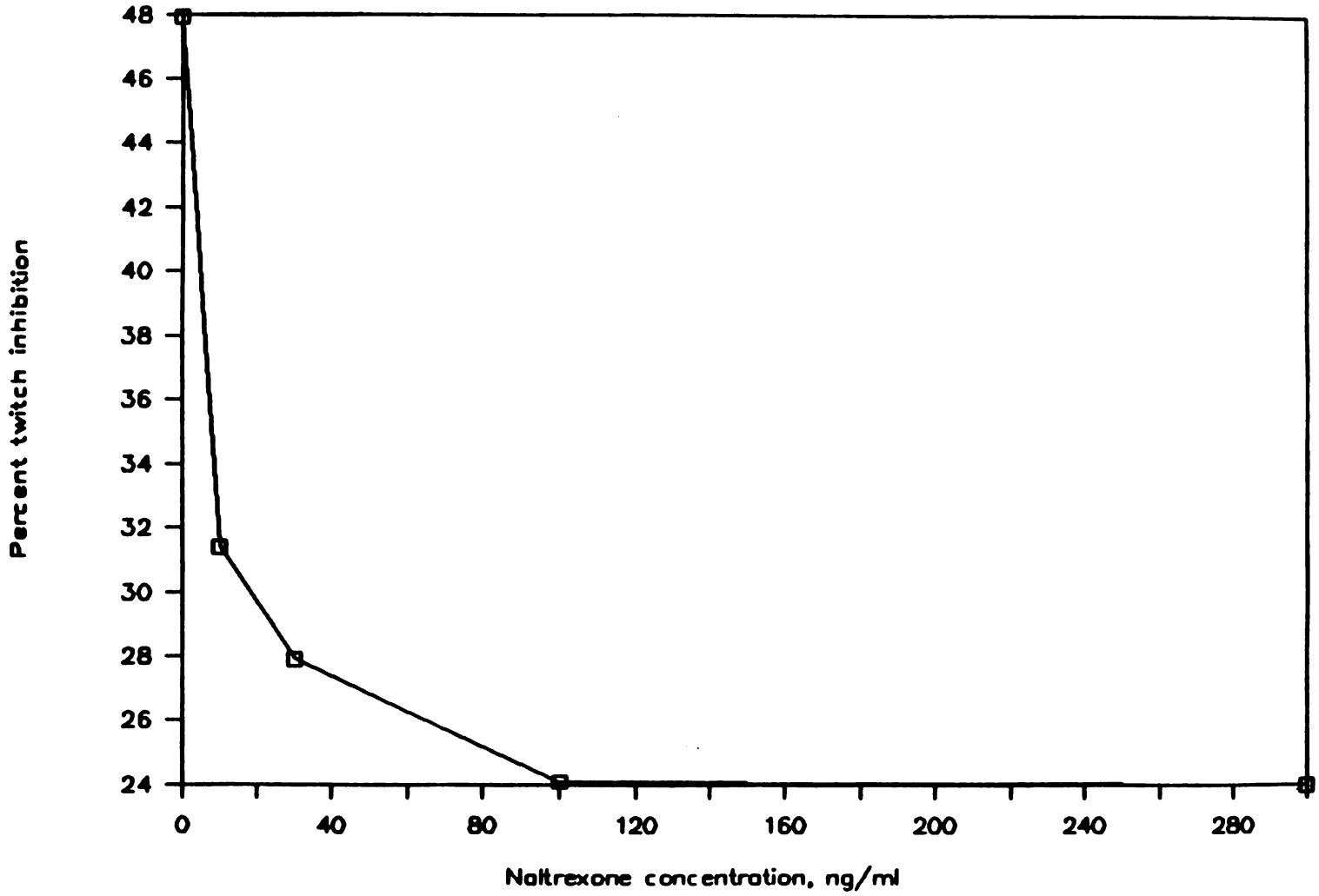
Naltrexone also antagonized meperidine's effect in the GPI to the same extent as naloxone. A detailed dose-response curve of naltrexone antagonism of meperidine is shown in Figure 8. At 0.01 ug/ml, naltrexone demonstrated a statistically significant antagonism of meperidine ($P<0.05$), reaching a maximum at 0.3 ug/ml, after which no greater degree of antagonism could be demonstrated, even to 30 ug/ml.

FIGURE 8, Legend

Naltrexone and meperidine's effect on the guinea pig ileum. At lowest dose used (0.01 microgram/ milliliter) antagonistic effect was statistically significant ($P<0.05$). However, while increasing the dose caused slight, variable increase in antagonism, the increase was not significant. Abscissa represents the naltrexone dose; ordinate, the percent inhibition of twitch height by 4 micromolar meperidine in presence of naltrexone. Points are means (standard error of means calculated, but not illustrated).

*) $P<0.05$

FIGURE 8

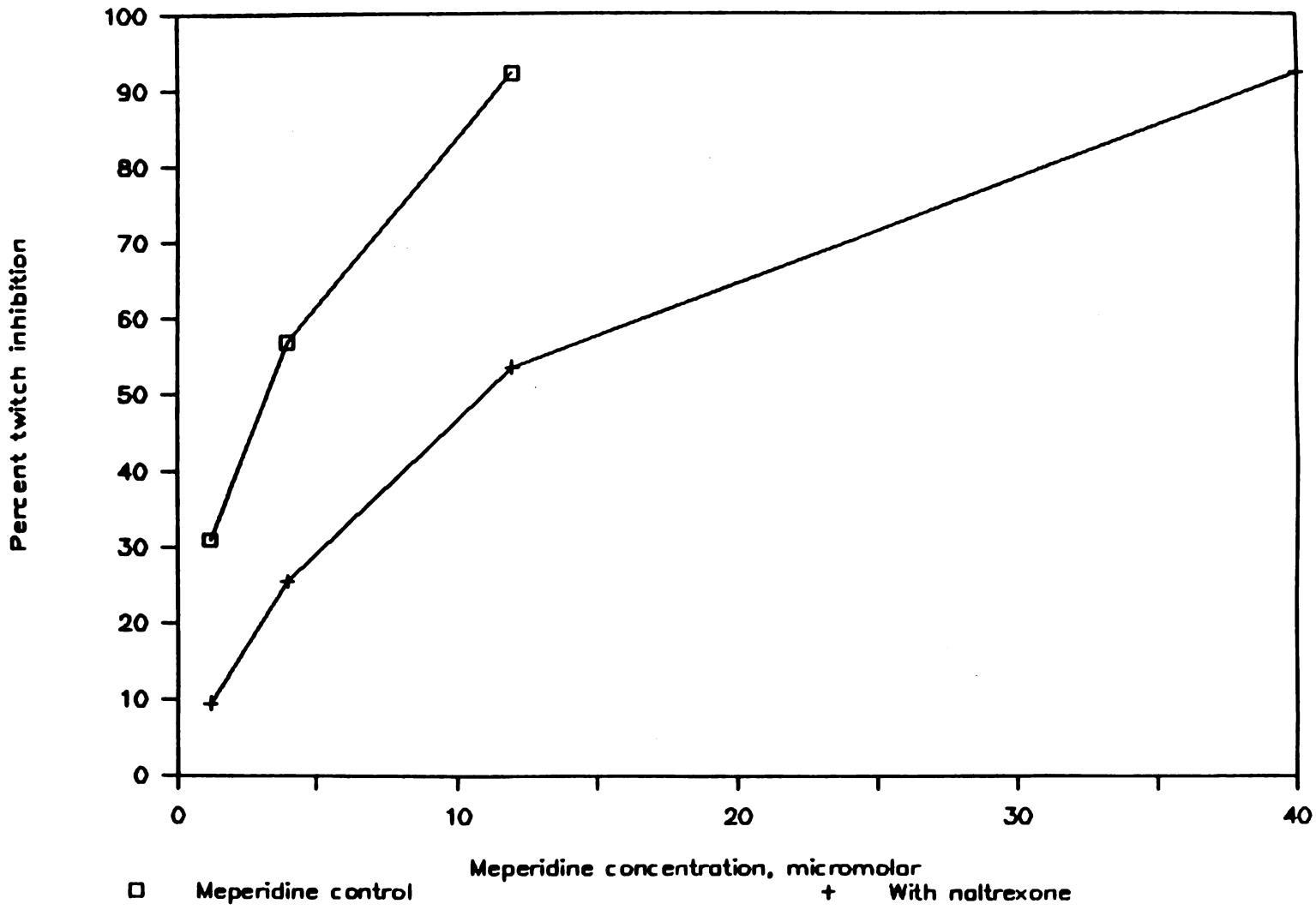

Percent twitch inhibition
48
46
44
42
40
38
36
34
32
30
28
26
24
0
40
80
120
160
200
240
280
Naltrexone concentration, ng/ml

Plotted out as a dose-response curve, Figure 9 shows the shift in the electrically generated MPLM's response to meperidine in the presence of 3 ug/ml naltrexone. The shift demonstrates the antagonism of naltrexone to meperidine, but the plot also indicates the strong non-opioid aspect of the meperidine/naltrexone interaction. Figure 8 showed that the maximum effect that naltrexone had on a meperidine dose was reached at 0.3 ug, but a plot at 3 ug/ml still shows that meperidine can inhibit greater than 90 percent of the electrically evoked twitch response.

FIGURE 9, Legend

Potency of meperidine in the presence of naltrexone. Even at 3 micrograms/milliliter naltrexone, twitch height reduction of guinea pig ileum in response to meperidine is at maximum over 90 percent, although with a significant shift to right, indicating strong atropinergic presence. Abscissa represents meperidine concentration in micromolar; ordinate, the percent inhibition induced by meperidine in electrically generated stimulus contraction in guinea pig ileum myenteric plexus-longitudinal muscle preparation. Points are means (standard error of means calculated, but not illustrated).

FIGURE 9


100
90
80
70
60
50
40
30
20
10
0
Percent twitch inhibition
0
10
20
30
40
Meperidine concentration, micromolar
Meperidine control
With naltrexone

Other opiate inhibitors

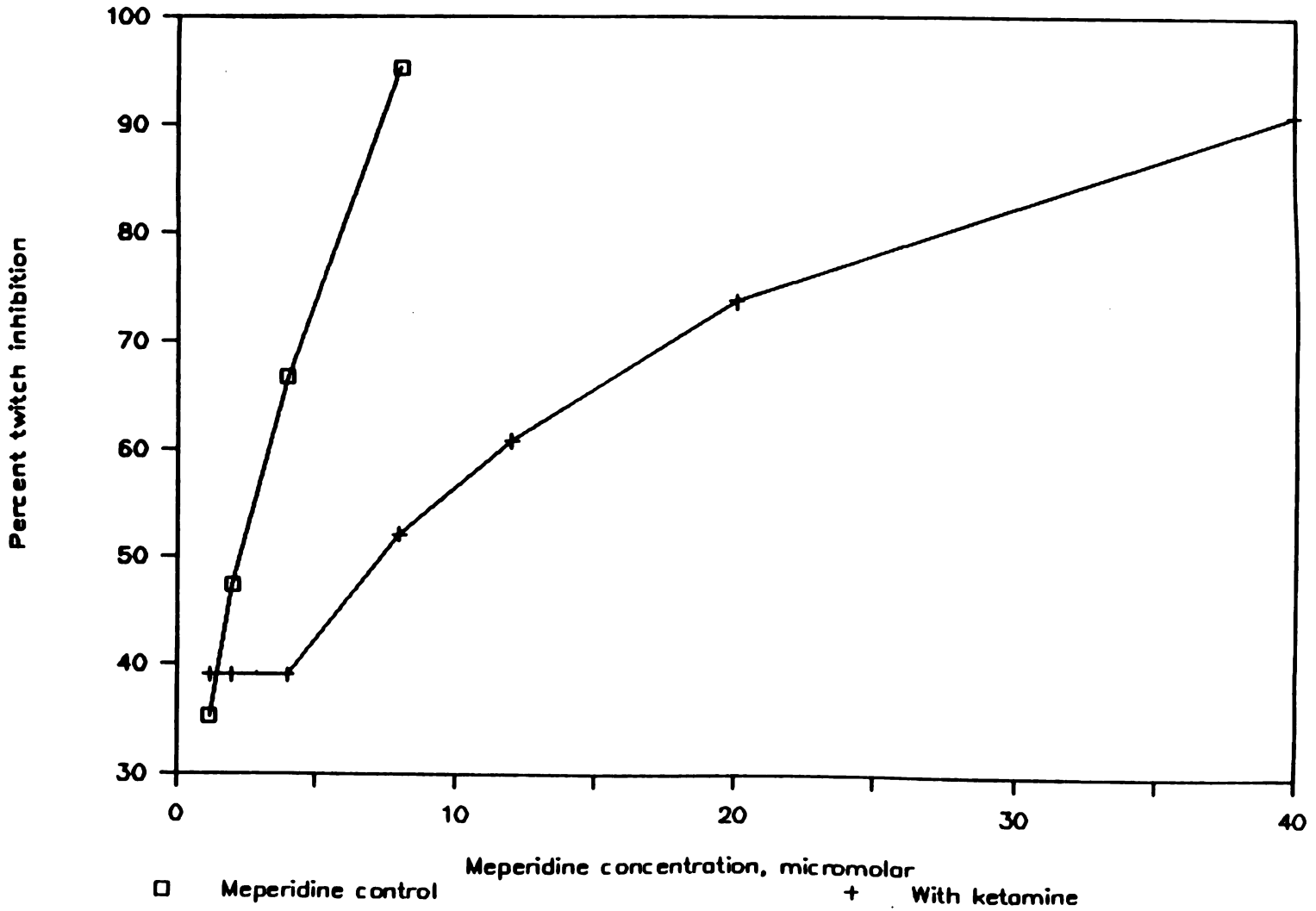
Because SKF 10,047 is an opiate antagonist as well as an opiate agonist, and not all the putative sigma agonists showed cross-tolerance to meperidine, it was considered necessary to test for SKF 10,047's possible antagonism of meperidine. Addition of SKF 10,047 at a concentration of 1.5 uM in the tissue bath before adding meperidine shifted the IC_{50} of meperidine to the right, from 1.8±0.1 to 3.6±0.5 uM, a significant change ($P<0.05$).

The other compounds which appeared to convey cross-tolerance to meperidine were also tested to see if antagonism might be a factor in reducing meperidine action. Ketamine produced a biphasic response at 360 uM with the shift in the IC_{50} of the meperidine dose-response curve going from 4.2±0.6 to 15.2±2.2 uM ($P<0.001$). However, at higher concentrations of ketamine (550 uM), the shift to the right was less when compared with the control values, the IC_{50} only moving from 4.3±0.1 to 11.1±1.5 uM, but still significant ($P<0.005$). Increasing the ketamine concentration even higher to 730 uM, the shift was only from 4.4±0.8 to 7.2±0.4 uM, still less significant ($P<0.025$). In most cases, the meperidine dose-response curve following the addition of these antagonists started out not at zero, but at a depression of the twitch height which indicated the agonist response of the antagonist (Figure 10).

FIGURE 10, Legend

Ketamine and meperidine's effect on the guinea pig ileum. This figure demonstrates antagonism of ketamine to meperidine in a guinea pig ileum myenteric plexus-longitudinal muscle preparation. Abscissa represents concentration of meperidine in micromolar; ordinate, the percent inhibition of electrically induced stimulus contraction produced by meperidine alone or with 0.36 millimolar ketamine. Clearly, addition of ketamine to meperidine reduces meperidine potency. Ketamine caused a 39.1 percent inhibition of twitch height in itself. But when the dose is added to a dose of meperidine (4 micromolar) which can cause up to 66.7 percent inhibition, there is no additive or synergistic effect. Above that dose, the percent inhibition starts mounting with increasing meperidine, probably indicative of the atropinergic effect.

FIGURE 10


100
90
80
70
60
50
40
30
Percent twitch inhibition
0
10
20
30
40
Meperidine concentration, micromolar
Meperidine control
With ketamine

Ketamine also antagonized other opiates, with a concentration of 0.36 mM ketamine almost completely antagonizing 45 nM morphine (98.4 percent), and 0.55 mM ketamine shifting the dose-response curve of PCP 1.8-fold to the left.

The meperidine IC_{50} was shifted over to the right from 1.8±0.2 to 3.0±0.5 uM ($P<0.01$) after exposure to one uM pentazocine. However, the direct antagonistic effect of pentazocine on meperidine was difficult to evaluate, as the dose-response curve changed slope.

When PCP was tested to see if it acted as an antagonist to meperidine, there was again a shift of the meperidine dose-response curve from an IC_{50} of 3.8±0.6 to 5.4±0.2 uM in the presence of 12 uM PCP, statistically significant ($P<0.025$), but of a smaller significance than those seen with the other sigma compounds tested.

Morphine, on the other hand, did not inhibit meperidine in the GPI preparation.

Capsaicin

Following two hours incubation with 2.6 uM capsaicin, a depletor of substance P, the dose-response curves of meperidine were not significantly altered. At this concentration, capsaicin had no effect on the meperidine response, as the meperidine IC_{50} after incubation with capsaicin only shifted 1.1±0.1-fold.

Atropine

Meperidine's inhibitory effect on the electrically stimulated MPLM preparation of the GPI was enhanced by atropine in a synergistic fashion, at doses below those where atropine had a direct effect. Atropine depressed the GPI at 42 nM and above, but had no direct effect at lower concentrations. However, at 14 nM a statistically significant potentiation (87.4±18.9 percent, $P<0.05$) of meperidine's response was seen. Concentrations down to 0.42 nM (77.8±29.8 percent) also showed significant potentiation of meperidine's antagonistic effect, but the response was not dose-dependent.

Hexamethonium

In contrast to atropine, hexamethonium bromide reduced meperidine's effect on the electrically generated contraction in a dose-dependent manner (Figure 11). At a concentration of 280 nM, only an insignificant reduction of 11.5±6.3 percent was achieved, but at 280 uM a significant reduction of 24.7±7.0 percent was obtained ($P<0.05$). At 2.8 mM the reduction was even more pronounced (59.4±6.9 percent, $P<0.001$), but hexamethonium's action at that concentration was not confined solely to reduction of meperidine's effect, but acted directly on the GPI preparation to slightly increase (9.1±1.5 percent) the twitch height.

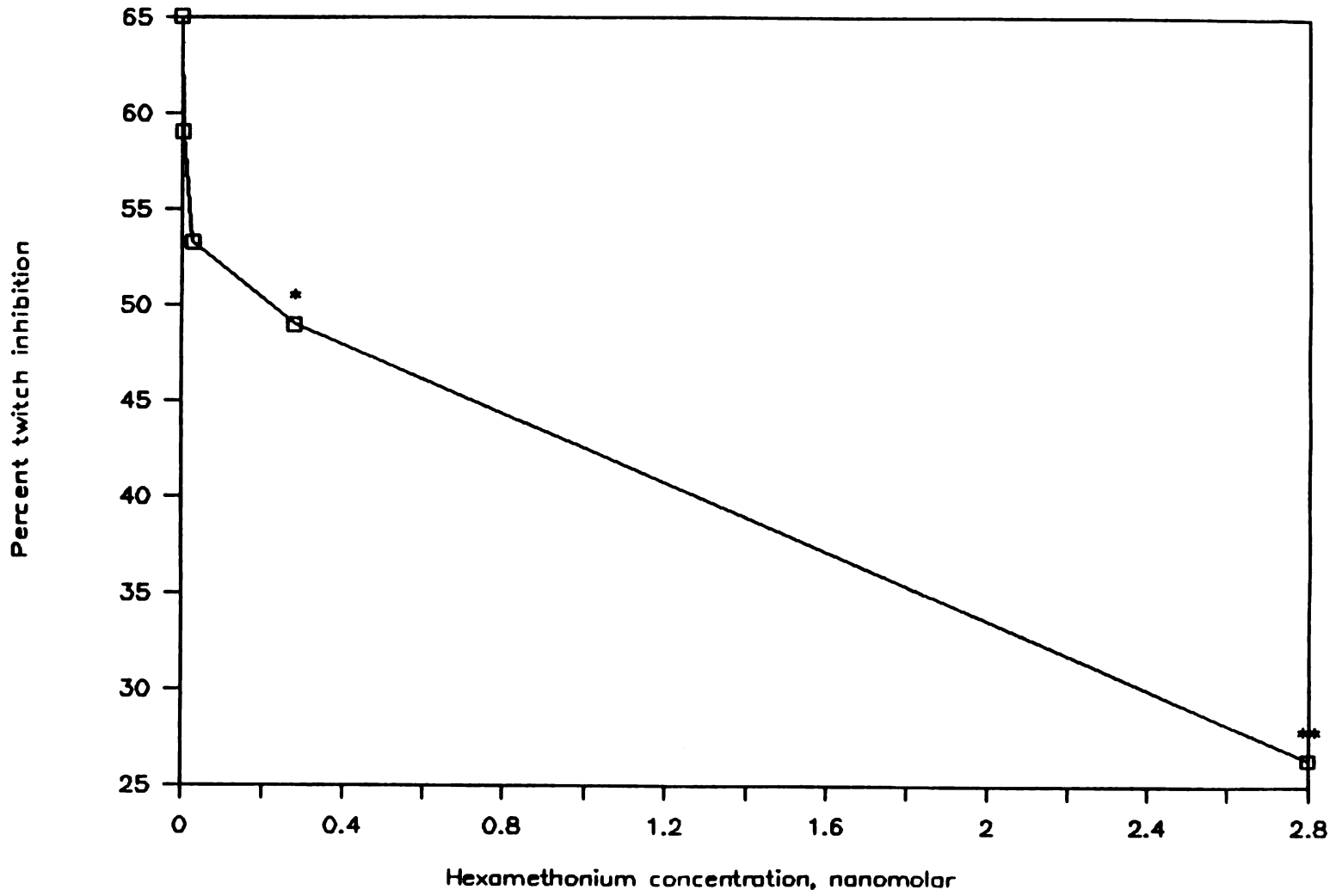
FIGURE 11, Legend

Hexamethonium bromide and meperidine's effects on the guinea pig ileum. Hexamethonium bromide added to guinea pig ileum myenteric plexus-longitudinal muscle preparation incubation mixture before meperidine reduced meperidine's effect in a dose-dependent, statistically significant manner. Abscissa represents hexamethonium bromide concentration; ordinate, the percent inhibition of electrically induced stimulus contraction of preparation caused by meperidine in presence of hexamethonium bromide. Points are means (standard error of means calculated, but not illustrated).

*) $P<0.05$ compared with control.

**) $P<0.005$ compared with the control.

FIGURE 11


65
60
55
50
45
40
35
30
25
Percent twitch inhibition
*
**
0
0.4
0.8
1.2
1.6
2
2.4
2.8
Hexamethonium concentration, nanomolar

Other Compounds

A number of other compounds were investigated to determine if they affected meperidine's response and could give insight on its mechanism of action. Chlorpromazine, pargyline, 8-phenyltheophylline and yohimbine were all tested in conjunction with meperidine in an attempt to find a class of enhancing or inhibiting compounds which would affect the response.

At concentrations of 7 and 70 nM, chlorpromazine had no significant effect of meperidine's action in the GPI. At 700 nM and above the drug directly depressed the electrically induced twitch height.

Following pretreatment of the ileal strips with pargyline (from 5.1 nM through 51 uM), the response of 4 uM meperidine was not altered significantly.

8-Phenyltheophylline had little or no effect on the action of meperidine in the GPI. There was a slight antagonism of meperidine's inhibition of the electrically induced contraction in the presence of 19 uM 8-phenyltheophylline (7.0±3.4 percent reduction). At 58 uM, the compound demonstrated increasing antagonism to meperidine to a level of 20.1±5.5 percent (still not significant), but increasing the dose to 190 uM decreased 8-phenyltheophylline's reduction of meperidine's effect on the GPI to 14.3±3.5 percent.

Yohimbine, at 2.82 uM, depressed the electrically generated contraction slightly (4.6±1.7 percent), and caused a significant reduction (21.2±3.8 percent, $P<0.01$) of the effect of 4 uM meperidine. But at a ten-fold higher concentration of yohimbine (28.2 uM), addition of meperidine yielded a depression of the twitch height not statistically significant from that in the absence of yohimbine. Moreover, the twitch height was directly depressed an average of 20.3±2.5 percent.

Meperidine Effects on the Mouse vas deferens

The MVD exhibited a mixed response to meperidine. A few tissues responded with nearly complete cessation of action of the electrically induced contraction, while others exhibited what is usually considered partial agonism in that a maximal effect was not elicited. Under these circumstances, with the lower doses, it was possible to achieve a dose-dependent depression of the electrically generated response. The percent inhibition increased to 44 percent with a mean of 34.0±2.0 percent, but at higher doses, a downward trend rather than a plateau was seen. When the concentration of meperidine was increased further, a dose-dependent increase in the electrically induced contraction was noted. In those tissues which did not

peak, the mean IC_{50} was 6.1±0.6 uM, while in those tissues which did, the maximal response was observed at 5.3±0.6 uM.

The response to morphine is also mixed in the MVD, with some tissues giving a greater than 50 percent response (IC_{50} = 25.1±13.9 uM), but a few showing a peaking response, with a maximal response at 12.5±2.5 uM, and a mean depression of the twitch height of 35.5±2.5 percent.

A two hour incubation with 4 uM, or one hour with 12 uM meperidine (the dose which caused maximal effect in that experiment), induced considerable tolerance. Most tissues exhibited no decrease in the twitch height to electrical stimulation in response to meperidine at doses that gave an effect before incubation. Moreover, as the doses of meperidine increased in the second dose-response curve, all the tissues exhibited hypersensitivity development, indicated by the several-fold increased twitch height in the presence of meperidine.

Partial cross-tolerance to morphine was induced with meperidine. Although incubation with 1.2 uM meperidine for two hours failed to alter the response of the MVD to morphine, increasing the incubation concentration to 4 uM meperidine did induce cross-tolerance to morphine. Although a complete dose-response curve with an IC_{50} was achieved before incubation, there was plateauing at 31.3±3.3 percent.

Incubation with 4 uM meperidine for two hours did not alter the IC_{50} of DADL as the shift of the dose-response curve was 1.0±0.0-fold.

Naloxone concentrations up to 0.3 mM caused no reversal of the response to 4 uM meperidine in the MVD. In some tissues in which a dose-response curve of meperidine was measured, subsequent addition of 100 ug of naloxone caused irreversible increase of 146.1±1.7 percent in the twitch height of the tissues without affecting the subsequent response to meperidine.

Doses of delta antagonist ICI 174,864 from 10 nanograms(ng)/ml to 1 ug/ml had no significant effect on meperidine's response. At 10 ug/ml, the effect was still not statistically significant, but indicated possible antagonism with a depressed twitch height of only 52.0±7.7 percent instead of 69.8±7.6 percent. This concentration of antagonist caused a 25.9±10.3 percent depression in the twitch height before the addition of meperidine. This same dose of ICI 174,864 significantly antagonized DADL ($P<0.01$). At concentrations of ICI 174,864 up to 0.5 mg/ml, no dose of the antagonist had any effect whatsoever on reversing meperidine's depression of the electrically generated response.

Yohimbine had a greater effect on the action of meperidine in the MVD (Figure 12). Meperidine's effect was reduced in a dose-dependent manner with 2.82 nM (39.7±16.6

percent, $P<0.025$) through 282 nM (74.4±7.8 percent, $P<0.001$) yohimbine.

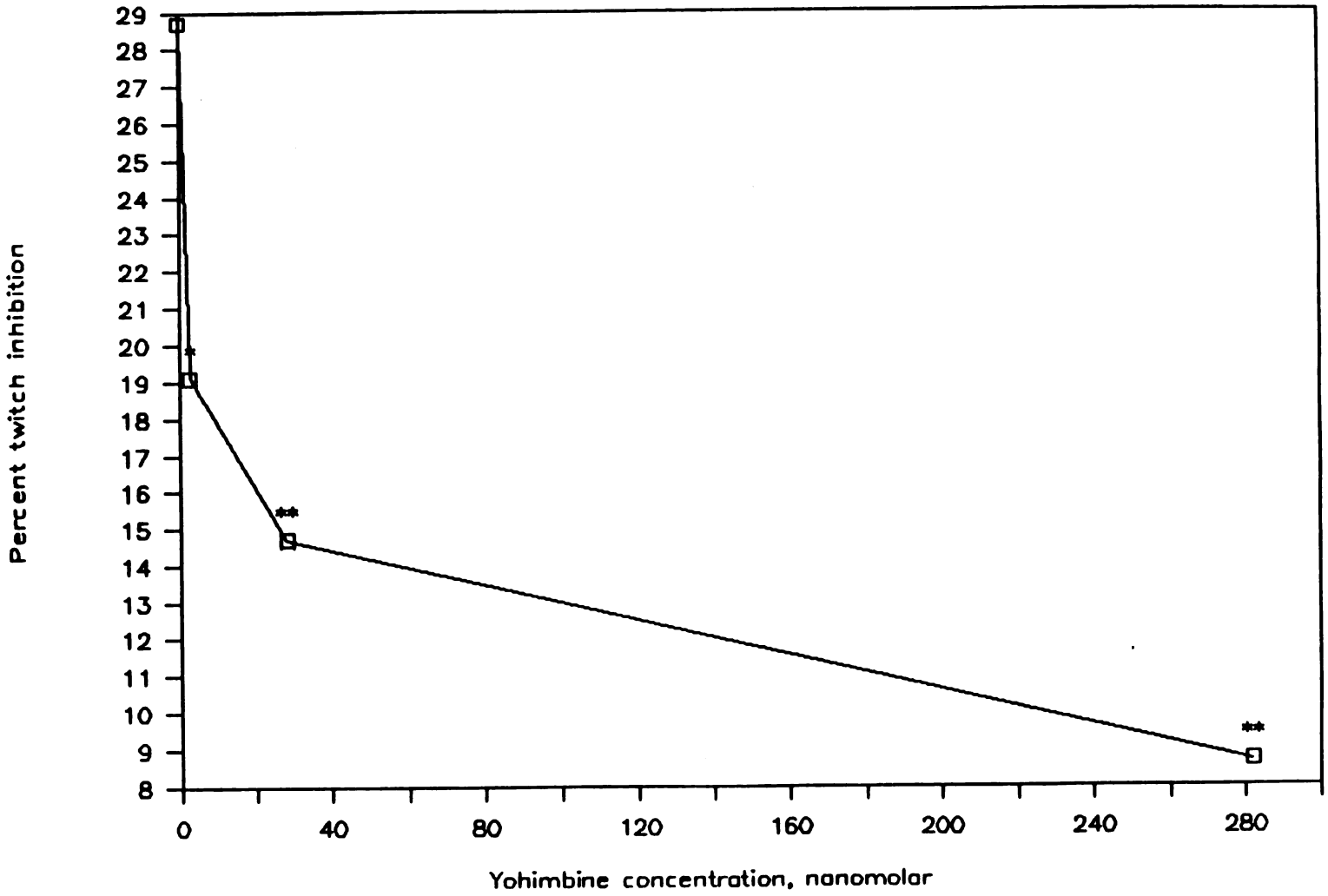
FIGURE 12, Legend

Yohimbine antagonism of meperidine in the mouse vas deferens. Yohimbine acts as antagonist of meperidine's effects on mouse vas deferens in dose-dependent fashion. Prior addition of 2.82 through 282 nanomolar (nM) yohimbine reduced the effects of 4 micromolar meperidine significantly. Abscissa represents yohimbine concentration in nM; ordinate, the percent inhibition of electrically generated stimulus contraction caused by meperidine. Points are means (standard error of means calculated, but not illustrated).

*) $P<0.025$ compared with control.

**) $P<0.001$ compared with control.

FIGURE 12


Percent twitch inhibition
29
28
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
*
**
**
0
40
80
120
160
200
240
280
Yohimbine concentration, nanomolar

Meperidine Effects on the Rat vas deferens

Below 50 uM meperidine a slight decrease in the twitch height (18 percent in some tissues) was seen, but between 50 and 500 uM, a dose-dependent increase in contraction height (up to 300 percent) was observed. This was similar to that seen in the MVD. However, at the highest concentration of meperidine tested (1200 uM), following an initial increase, a decrease in the twitch height was seen. The tissue contractions were quite variable at this concentration under electrical stimulation, and, at rest, spontaneous contractions were frequent and abrupt.

Comparison of Meperidine with Phencyclidine

1-(1-Phenylcyclohexyl) piperidine (phencyclidine, PCP) reacts in many ways similar to meperidine in the GPI, although it is less potent. The IC_{50} of PCP is 12.1±1.1 uM and tolerance development can be induced. Incubation with one IC_{50} for two hours shifted the dose-response curve to 18.7±1.8 uM ($P<0.005$).

Like meperidine, PCP is atropinergic and inhibits the contractile action of exogenously applied acetylcholine. A dose of PCP which inhibited the electrically generated stimulus contraction 94.5±2.1 percent, significantly

increased the EC_{50} of the acetylcholine dose-response curve from 5.9±0.3 to 10.7±2.4 nM ($P<0.05$).

Unlike what is seen with meperidine, PCP is not greatly affected by alterations in calcium levels, as increasing the calcium concentration from 1.27 to 5.08 mM in the buffer only shifted the IC_{50} from 46.2±5.7 to 54.0±11.2 uM, a non-significant amount. Pre-treatment with 4-AP did, however, reduce the effect of PCP on the GPI. 4-AP (0.1 uM) reduced the effect of twelve uM PCP 19.8±5.5 percent, and by 0.3 uM 4-AP, a statistically significant 73.2±9.8 percent ($P<0.001$). However, at 0.3 uM 4-AP, the twitch height was depressed by 29.9±8.9 percent.

Naloxone has a minimal effect on PCP in the GPI, 0.3 mM naloxone inhibiting PCP a mean of 14.6±2.0 percent. Yohimbine caused no statistically significant change of PCP's effects in the GPI at concentrations up to 28.2 uM. However, this concentration of yohimbine by itself caused a 34.5±1.2 percent depression in the twitch height.

Ketamine also proved to be a mild inhibitor of PCP as well as meperidine. At 550 uM ketamine, which caused a 27.3±2.7 percent reduction of the twitch height, ketamine shifted the IC_{50} of the PCP dose-response curve from 65.8±4.6 to 117.0±14.3 uM ($P<0.005$). In the same preparation, 550 uM ketamine antagonized completely a concentration of meperidine which caused a 31.3±1.9 percent inhibition of the twitch height in the absence of ketamine.

DISCUSSION

Précis

The GPI *in vitro* bioassay was developed by Paton (1955), who later (1957) suggested that it seemed a suitable model for predicting analgetic activity. After comparing a number of opioid-like compounds with morphine, he found a rank order of twitch inhibition potency that correlated well with their analgetic potency in man. Tolerance of the ileum to morphine could be produced, and even a state he termed "morphine-dependence". As this class of agents grew larger and more diverse, the usefulness of the bioassay as a model for the prediction of analgetic activity was further substantiated, and it became increasingly used as a tool for studying mechanisms of action.

The data presented in this dissertation demonstrate that meperidine has opioid activity in the MPLM preparation of the GPI in addition to its anticholinergic effect. Meperidine antagonizes the ileum's response to electrical stimulation at concentrations below those which block the response to exogenous acetylcholine. This effect is opioid in nature as it is blocked by naloxone and various other narcotic antagonists.

There are, however, differences in the activity of meperidine which distinguish it from other opioids acting on the GPI. Unlike most opioids, meperidine induced very little *in vitro* tolerance in this system. Nor was physical dependence development on meperidine demonstrable *in vitro*. Some degree of cross-tolerance to other opiates was exhibited by meperidine, but the pattern was unusual. Only slight cross-tolerance was exhibited by meperidine in ilea rendered tolerant to classic mu opiates, and none to the kappa agonist EKC, while the response to sigma compounds was mixed. In preparations made tolerant to putative sigma compounds, meperidine showed cross-tolerance to pentazocine and SKF 10,047, but not to PCP. However, with ketamine, a compound closely related to PCP, meperidine did show cross-tolerance. In the GPI tolerant to DADL, a delta agonist, meperidine also exhibited cross-tolerance. Finally, although the response to meperidine was antagonized by calcium, the effect was modest, and the alterations were much less than those seen with morphine at similar changes in calcium content.

The mechanism by which meperidine blocks the electrically stimulated contractions of the GPI remains to be established. While it may reduce acetylcholine release, it doesn't seem to mediate its effect via the same receptor as morphine. Hexamethonium bromide, a ganglionic blocker, which almost completely inhibits the effect of meperidine,

was the only chemical agent tested which had a major effect.

In the MVD, meperidine blocked the response to electrical stimulation but this did not appear to be demonstrably opioid, since the effect was not antagonized by the opiate antagonist naloxone. Although meperidine's mechanism of action in this system may be via adrenergic neurons as is morphine's, it might also be serotonergic, like yohimbine. The latter compound has been shown to inhibit both types of neurons, and it proved to be meperidine's most potent antagonist in the vas deferens, even though it had no effect against meperidine in the GPI. Incubation with meperidine does induce potent tolerance development in the MVD.

Of the many morphine-like compounds to which meperidine did not demonstrate cross-tolerance in the GPI, PCP was the most interesting as its pattern of action was similar in some aspects to that of meperidine. For example, with PCP, also an anticholinergic, very little tolerance was induced following in vitro incubation; naloxone and ketamine showed minor antagonistic effects, and calcium concentration alterations had no significant consequence, although addition of 4-AP did modify PCP's effect on the GPI.

Opiate Actions

Meperidine was originally intended for use as an anticholinergic, but its analgetic properties were discovered serendipitously by Eisleb and Schaumann (see Jaffe and Martin, 1985). Although Paton (1957) attributed a portion of the drug's activity in the GPI to its atropinergic effect, he indicated that meperidine behaved like a drug "having a mild atropinic action and a distinct morphine-like effect". It is clear from the results presented in Table 1 that meperidine is atropine-like, but it also demonstrates nonatropinergic blockade of the electrically induced contraction of the ileum at doses lower than those that are anticholinergic. Apart from the small nondose-dependent shift to the right of the acetylcholine dose-response curve at low concentrations of meperidine due to a reduction in the response of the tissue to acetylcholine (i.e., simply repeating the acetylcholine dose-response curve without adding any drug to the incubating buffer shifted the curve to the right), no anticholinergic response was seen at meperidine concentrations which inhibited the electrical stimulus up to 40 percent. That this response was most likely opioid in nature is demonstrated by the evidence that narcotic antagonists reduce the effect of low doses of meperidine on the GPI.

Both naloxone and naltrexone inhibit meperidine in the ileum to the same extent, while some less specific opiate antagonists showed only modest responses. The standard opiate antagonist naloxone is only able to act as a partial blocking agent probably because of meperidine's dual action in the ileum, as it is unlikely that any narcotic antagonist is affecting the anticholinergic effect of meperidine. The anticholinergic aspect of meperidine's action in the GPI would explain the rather unusual findings seen in the Schild plot. Thus other than as an indication that meperidine does have some opiate activity in the GPI, the plot cannot be used to derive precise quantitative data for interpretation of opioid-like actions.

However, the measurement of the direct opiate effect can be accomplished another way, as demonstrated by Creese and Snyder (1974) when they determined the K_D values of a series of opiate agonists and antagonists in the guinea pig intestine, and plotted that against the IC_{50} concentration for the agonists. As the points plotted showed a good correlation, the authors concluded this indicated that the receptor binding sites investigated by them were "pharmacologically relevant". However, in meperidine's case, the K_D was 100-fold higher than morphine, while the IC_{50} was only 10-fold higher. The difference is probably due to the fact that meperidine exerts some of its effect in the GPI through the anticholinergic response.

Unusual Opiate Responses

A high degree of tolerance is usually quite easy to induce with morphine-like drugs both in humans and in animals, but meperidine may be an exception to the rule. Fennessy et al. (1969) working in the GPI with many of the same chemicals as Paton (1957), reported that neither tolerance nor dependence could be observed with meperidine, although Paton had apparently observed such.

In the present experiments, a value of 2.5 times the baseline was the maximum tolerance achieved, which, while statistically significant, did not compare with results seen with morphine and its surrogates tested with this procedure. Furthermore, while Rezvani et al. (1983) were able to increase in vitro tolerance development by increasing the concentration of drug in the incubation buffer, the time of incubation or adding cAMP to the incubation buffer, none of these manipulations increased tolerance development to meperidine.

Tolerance to atropine and the other belladonna alkaloids has been seen to some extent in man (Innes and Nickerson, 1970), and the tolerance to meperidine noted in the ilea may reflect tolerance to its atropinergic action. This may explain why the tolerance observed was not potentiated by any of the maneuvers that increase tolerance for other opiates. It may also explain why the degree of

tolerance to the various concentrations of meperidine, while statistically significant, did not approach the level of tolerance seen with other opiates. But it does not account for cross-tolerance seen to those compounds.

The low degree of tolerance development to meperidine was disappointing, but not totally unexpected. As discussed in the Introduction, tolerance to meperidine is more difficult to demonstrate than with most opioids. Only four papers on tolerance to meperidine *in vivo* have been published, and none using *in vitro* methods. The two papers which gave an opinion on the mechanism of tolerance to the analgetic effects in meperidine in humans concluded that changes in drug utilization are partially or wholly responsible (Andrews, 1942a, Glynn and Mather, 1982). These published reports indicate that tolerance to meperidine may be due to pharmacokinetic alterations, and not cellular tolerance, as seen with morphine. This makes it probable that the mechanism by which tolerance develops to opiates may not be applicable to meperidine, which seems to work on the GPI via a different mechanism.

The inability to demonstrate physical dependence development on meperidine *in vitro* is also not consistent with the usual response displayed by a morphine-like compound. Although dependence on meperidine can be demonstrated in both animals and man *in vivo*, it is difficult to establish in the laboratory. In contrast, it

is very easy to induce with morphine, both *in vivo* and *in vitro*. Although concentrations of meperidine up to 67 uM were incubated with the ileum strips, no consistent contractile response could be evoked after adding naloxone. It is unlikely that a contractile response to naloxone would have been induced by incubation of higher concentrations of meperidine, since the anticholinergic effects of meperidine would have become manifest. Thus, Ehrenpreis *et al*. (1972) found that naloxone contracture could be completely blocked by atropine, and he concluded that naloxone induced the contracture by releasing acetylcholine. Although later Tsou *et al*. (1982) discovered that there is also a non-atropinergic component of the response that is due to substance P release, the greatest part of the response is attributable to acetylcholine. Hence, at high concentrations, the anticholinergic properties of meperidine would affect the contractile response of naloxone; the higher the meperidine concentration in the buffer, the less response to naloxone would be seen, even if physical dependence were present.

There was some variability in the responses to naloxone that were seen in both the controls and the treated tissues. This has been reported previously and may be due to cyclic changes in the guinea pig (Rodriguez *et al*., 1980). When viewed over a period of a year, the investigators found a peak in the control tissues to

naloxone in the summer, with no response in the winter months. The response to naloxone in morphine-incubated tissues was much greater, but showed a similar variation depending on the time of year. Earlier, Weinstock and Shoham (1974) demonstrated that the GPI had a seasonal response to exogenously administered ACh and serotonin, greater in the summer than the winter. However, release of endogenous ACh, both spontaneous and from field-stimulation, increased in the summer (Hazra, 1975). Tolerance development to morphine *in vitro* was also affected by the season, with a greater percentage of ilea developing tolerance in the summer (Shoham-Moshonov and Weinstock, 1977). This variability in response to naloxone does not alter our conclusions.

Of the many opioid agonists to which meperidine was tested for cross-tolerance, it exhibited the greatest response to three compounds; pentazocine, SKF 10,047 and ketamine. However, while the initial assumption was that meperidine acts on a sigma receptor in the ileum, research using another putative sigma compound, PCP, demonstrated no cross-tolerance between meperidine and PCP. Because pentazocine and SKF 10,047 are narcotic antagonists as well as kappa agonists and ketamine has also been shown to antagonize morphine (Little *et al.*, 1983), it seemed appropriate to assess their antagonistic properties for a

possible explanation of the shifts in the meperidine dose-response curve.

While meperidine, pentazocine, SKF 10,047 and ketamine all inhibited the electrically induced response in the ileum, combining meperidine with any of the others did not yield an additive or synergistic response. As can be seen in Figure 9, the curve shows a biphasic response which is basically the response of the other agonist for the early part of the curve, and then a slope parallel to the meperidine curve but showing less potency. Because the response in the initial portion of the curve is identical to that seen from the added compound's effective dose, it seems to indicate that the opiate effects of meperidine were being antagonized, at least partially. Also, the increased twitch inhibition in the second phase of the curve was yielding primarily the anticholinergic response. It is unlikely that tolerance development would cause the shift, because the compounds were added almost simultaneously.

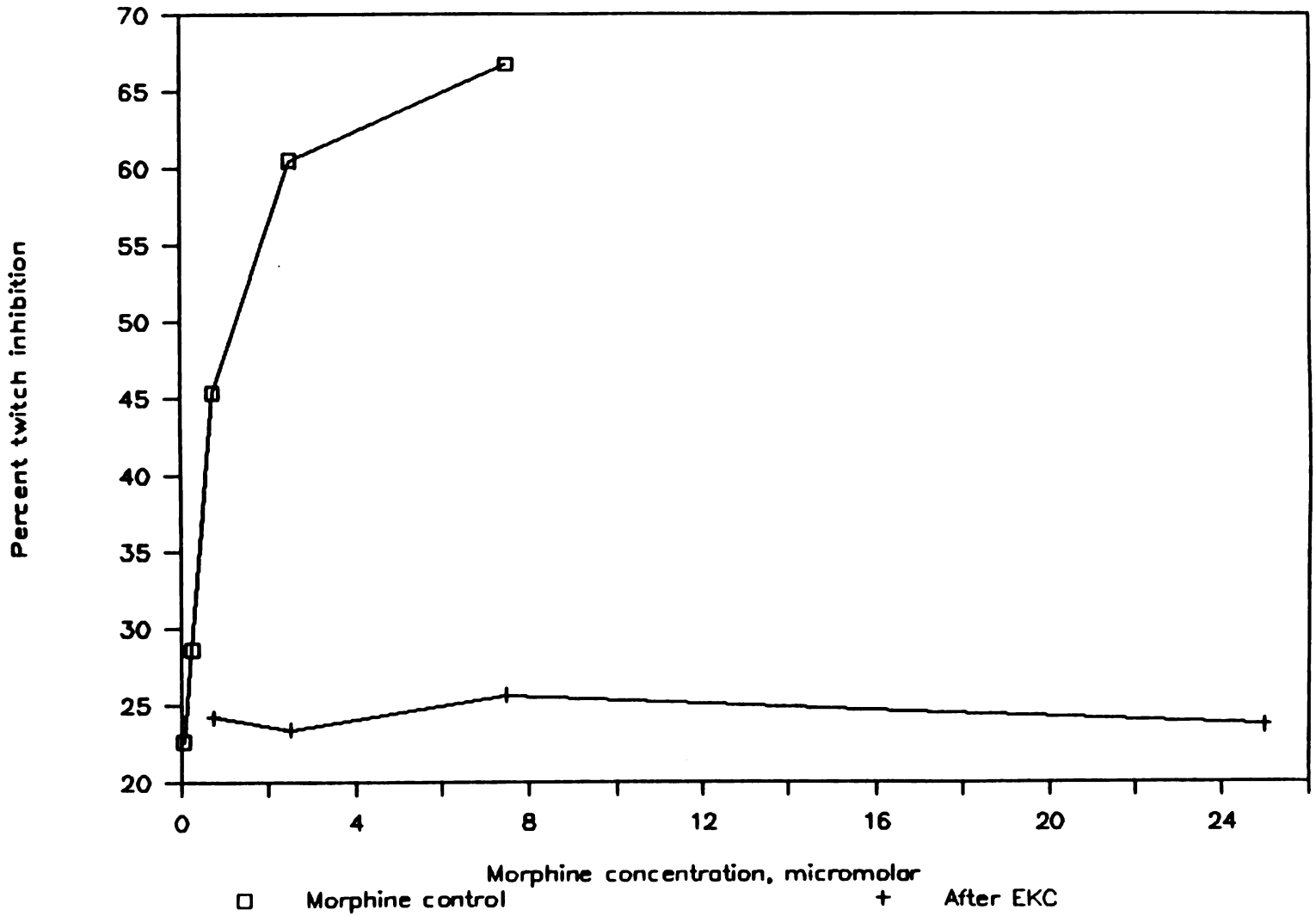
As discussed in the Introduction, meperidine, while frequently assumed to be a mu compound (e.g., Martin et al., 1978), does not fit into this classification at all times (Cowan et al., 1979, Geller et al., 1983). In fact, in these two citations it resembles more closely pentazocine. But Jaffe and Martin (1985) list pentazocine as a kappa and sigma agonist and a mu antagonist, and

meperidine seems not to fit into any of those general classifications either. Other opiate antagonists which antagonize morphine -- naloxone, naltrexone, SKF 10,047, and ketamine, also antagonize meperidine to a variable extent, but meperidine exhibited little cross-tolerance to any of the selective mu agonists tested in the GPI. Maneuvers to increase tolerance by increasing the concentration of morphine or its time of incubation did not further enhance cross-tolerance of meperidine to morphine. Nor does meperidine show cross-tolerance to the kappa agonist, EKC, although morphine, a classic mu agonist, clearly does (Figure 13). Considering the fact that meperidine did not act like a mu compound in a number of model systems, it seems unlikely that antagonism of meperidine by mu antagonists would justify considering its effect in the GPI to be moderated by a mu receptor.

FIGURE 13, Legend

Cross-tolerance of morphine to ethylketocyclazocine (EKC). Two hour incubation of the guinea pig ileum myenteric plexus-longitudinal muscle with a concentration of EKC (2×10^{-10} molar), which induces tolerance to itself, induces almost complete tolerance to morphine as well. Abscissa is concentration of morphine in micromolar; ordinate, the percent twitch inhibition. Curve to left is morphine dose-response curve before incubation, while the curve paralleling the abscissa is response following incubation with EKC. Points are means (standard error of means calculated, but not illustrated).

FIGURE 13


70
65
60
55
50
45
40
35
30
25
20
Percent twitch inhibition
0
4
8
12
16
20
24
Morphine concentration, micromolar
Morphine control
After EKC

However, recent research by Takemori and Portoghese (1985) has demonstrated a new receptor for DADL in the GPI, termed mu', which is neither typically mu nor delta. In fact, meperidine, which demonstrated very little cross-tolerance to classical mu opiates, did exhibit somewhat greater cross-tolerance to DADL in the GPI. These experiments may indicate a closer relationship between the two components particularly since meperidine, like DADL, is also active in the MVD with similar potency to that seen in the guinea pig. This may also be the same as the mu_2 receptor originally identified by Wolozin and Pasternak (1981) that was later found to be the major mu receptor in the GPI (Gintzler and Pasternak, 1983).

The multiplicity of opioid receptors were identified following observation of dissimilar pharmacological effects, lack of cross-tolerance or specific effects and differential sensitivity to antagonism by naloxone or other opiate antagonists. Some of those factors seem to hold true with meperidine when compared with morphine in the GPI. Other investigators have noted meperidine's unusual responses in _in vivo_ systems. The same lack of cross-tolerance is seen in other model systems, e.g., changes in seizure threshold in which cross-tolerance development between meperidine and pentazocine, cyclazocine or etorphine (Cowan _et al._, 1979) was looked for, but not seen, in a behavioral system (Leander and McMillan, 1977)

no cross-tolerance between methadone and meperidine developed, and in the opossum LES there was no cross-tolerance between meperidine and buprenorphine or ketocyclazocine (Rattan and Goyal, 1983). However, in at least one case, in another behavioral system (Witkin et al., 1979), some cross-tolerance between morphine and meperidine was demonstrable.

Rattan and Goyal (1983) postulated that meperidine's effect in the LES was due to a previously unidentified opiate receptor on noncholinergic, nonadrenergic inhibitory neurons. In this system, mu, kappa, delta and sigma receptors are readily identified and the authors came to the conclusion that this was a new receptor because meperidine's effect in this system was not altered by muscarinic (atropine) or nicotinic (hexamethonium) anticholinergics, or propranolol, a beta-adrenergic blocker. Nor did haloperidol or pyrilamine, drugs which affect other neuraminergic systems, affect meperidine's response, a decrease in LES pressure. Tetrodotoxin, the only chemical which did block meperidine, had no effect on the other two opiates tested, buprenorphine and ketocyclazocine, which also caused dose-dependent reductions in LES pressure and were antagonized by naloxone.

Mechanism of Action

In the GPI, morphine acts to block cholinergic activity by causing a decrease in the amount of acetylcholine released in response to a stimulus (Schaumann, 1956). Although it is not known whether or not meperidine acts to block the release of acetylcholine in the GPI, this seems possible. However, it does not seem to work at the same site. So how is its effect of blockade achieved? It may be that the opiate mechanism of action is mediated additionally through a noncholinergic, nonalpha-adrenergic, nonserotonergic system and that meperidine acts to excite an inhibitory system, rather than directly inhibiting. Rattan and Goyal's contention (as discussed above) is that in the opossum, meperidine may activate a new opioid receptor in the LES, present on the nonadrenergic inhibitory neurons, causing inhibition of the sphincter. But this "new" receptor does not seem to be the same one responsible for meperidine's effects in the GPI, since hexamethonium, which had no effect on the LES pressure reduction caused by meperidine, was the only chemical affecting meperidine's blockade of contractions in the GPI caused by an electrical stimulus. However as tetrodotoxin was the only chemical affecting meperidine's action in the LES, this does indicate that the effect was mediated through the nerve, rather than the muscle.

As morphine has the same effects that meperidine does in both man and the *in vitro* ileum preparation, it has been assumed that they act on the same receptors, which the preliminary data presented here contradicts. At lower doses, meperidine reduces, and at higher doses it blocks the excitatory effect of an electrical stimulus on the GPI MPLM *in vitro*. In as much as only the ganglionic blocker hexamethonium seems to affect the meperidine action, meperidine may be acting to block an excitatory neuron by stimulating an inhibitory one. Hexamethonium, like morphine, depresses acetylcholine release from the ileum (Greenberg *et al.*, 1970), but at a much higher concentration than that which blocks the effect of meperidine. In the GPI, as in the LES, atropine had no effect on meperidine's actions -- nor did chlorpromazine, 8-phenyltheophylline, pargyline, capsaicin, yohimbine or cAMP.

Morphine is generally more potent than meperidine in the GPI assay by a factor of ten to one hundred, and it is more potent as an analgetic in man by a factor of three to ten. These disparities could be due to differences in absorption, metabolism, etc., in man, as well as the possibility of a different receptor mediating the two responses. It is known that various receptors are responsible for morphine's differing responses which can be dissociated, such as analgesia and respiratory depression

(Ling et al., 1983), as can analgesia and physical dependence (Ling et al., 1984). However, analgesia appears to be mediated primarily through the mu_1 receptor (Pasternak, 1981), while in the GPI, binding studies do not demonstrate the presence of any appreciable mu_1 sites (Gintzler and Pasternak, 1983). But Wood et al. (1982) report that in the CNS mu_1 receptors appear to regulate cholinergic neurons. Some investigators have tested whether the major metabolic products of morphine and meperidine contribute to the parent compound's effects (Miller and Anderson, 1954; Fennessy et al., 1969). Miller and Anderson (1954) found that normorphine was only one tenth as potent an analgetic in mice as morphine, while normeperidine was only slightly less potent than meperidine. On the other hand, Fennessy et al. (1969) found normorphine to be about 50 percent more potent than morphine in the GPI, while Creese and Snyder (1975) found them to have roughly the same potency. Since N-demethylation of morphine and meperidine probably does not occur in the GPI, the effects of these two compounds in this system are probably reflective of their direct action on the receptor. Even in vivo the probability is that the nor-compound would not greatly alter the effect of meperidine as an analgetic, but metabolism of morphine to normorphine in vivo would tend to reduce analgetic effectiveness. This may explain the relative difference in

potency between morphine and meperidine in the two test systems.

Mouse vas deferens

Henderson et al. (1972) found the dissociation constant for morphine in the MVD was very similar to that found in the guinea pig myenteric plexus (Kosterlitz and Watt, 1968) although the IC_{50} of 0.47 uM (Henderson et al., 1972) is higher than that seen in the guinea pig (68.2 nM, Kosterlitz and Watt, 1968). The same laboratory (Hughes et al., 1975) expanded its research in the MVD and obtained an IC_{50} of 16 uM for meperidine. Unfortunately, although the authors report an IC_{50}, they do not report whether meperidine achieved 100 percent inhibition, as much of their data was extrapolated from a concentration which gave a depression of 23 percent.

As it is unlikely that the inhibition by meperidine of the electrically induced contraction and stimulation of the same event are mediated by the same mechanism, it is possible that the reason for the appearance of partial agonism in so many tissues is due to competing effects, i.e., at lower doses the inhibitory effect is stronger, while at higher doses the stimulation is greater and overpowers the inhibition. One indication that this might be true can be observed following the induction of

tolerance in the MVD. Of eight tissues incubated with meperidine, all showed complete tolerance to the inhibitory effect of meperidine, while at the dose where the before-incubation dose-response curve reached a maximum, following tolerance development the tissues started to show a dose-dependent increase in twitch height.

Morphine is more potent in the GPI than in the MVD, but meperidine is equally potent in both. The MVD contains the delta opiate receptor, but meperidine does not seem to be acting primarily on the opiate receptor in this system. While tolerance to meperidine can be developed in the tissue, cross-tolerance to the specific delta agonist DADL was not achieved, nor were the effects of meperidine antagonized by the delta antagonist ICI 184,764. However, if naloxone was added to the tissue bath after exposure of the tissue to meperidine, the response of the ileum to the electrical stimulus was increased and the change in the response persisted in that washing the tissue for 30 minutes in buffer did not result in the loss of the hypersensitive response which is indicative of the dependent state.

The only compound which seemed to act as an antagonist to meperidine's effect in the MVD was a non-opiate, yohimbine. Yohimbine acts on alpha$_2$-adrenergic neurons as well as affecting serotonergic systems, and meperidine may be acting on the same adrenergic system that morphine does

in this tissue, in contrast to what is seen in the GPI, although perhaps not on the same receptor. Naloxone is known to antagonize morphine in the MVD (Henderson et al., 1972), but in these experiments meperidine was not antagonized by naloxone in the MVD. However Rhodes et al. (1983), have demonstrated that both yohimbine and naloxone act as antagonists to meperidine in the MVD. As meperidine is slightly more potent than morphine in this tissue, it should be of great interest to follow up this possibility, and discover if they do act on the same receptor.

Phencyclidine

Phencyclidine was discovered by an investigator testing a number of meperidine derivatives for opiate activity. PCP antagonizes exogenous ACh in the GPI smooth muscle as well as being an anti-acetylcholinesterase drug (Kloog et al., 1977). PCP also blocks electrically evoked contractions in the GPI, and partial antagonism of PCP's effect could be obtained with naloxone (see Itzhak et al., 1981). Such a response was not noted in these experiments, with 100 uM of naloxone inhibiting PCP only 14.6 ± 2.0 percent. Another researcher found that the sigma compounds PCP and SKF 10,047 were not antagonized by naltrexone in the chronic spinal dog (Vaupel, 1983). Because PCP and meperidine have a piperidine base with a

phenyl substituent, and the similar pharmacologic response evoked by both in the GPI, it was postulated that the two compounds acted on the same receptor. Furthermore, PCP and SKF 10,047 are psychotomimetics, and meperidine also has CNS effects not seen to the same degree in other opiates. Meperidine is known to cause hallucinations and dysphoria (Andrews, 1942b) and delirium (Eisendrath et al., 1987) which might bear a relationship to "sigma" effects. The psychotogenetic effect in SKF 10,047 is found in both the (+) and (-) isomers, but the opioid effects are found only with the (-) isomer (Khazan et al., 1984). Meperidine also causes excitatory effects to which tolerance does not develop (Jaffe, 1970). But all these are CNS effects, and there may not be a corresponding receptor in the GPI. Moreover, Eisendrath et al. came to the conclusion that the atropinergic aspects of meperidine, or its metabolite, normeperidine, were the cause of meperidine's delirium. And, as PCP is also an anticholinergic, it is quite likely that their so-called "sigma" effects are totally non-opiate in origin. For example, PCP does not show cross-tolerance to meperidine in this system, and SKF 10,047's blockade of meperidine's action in the electrically stimulated GPI is not seen with PCP. However, the antagonistic effect of SKF 10,047 on meperidine is likely a non-sigma response and should probably not be considered as an issue.

CONCLUSIONS

The GPI is a suitable model for the prediction of the analgetic effect of opiate-like compounds. This has been extensively discussed by several investigators (e.g., Cox and Weinstock, 1966; and Kosterlitz and Waterfield, 1975). Despite the suitability of the GPI bioassay for assessing the effects of morphine and its surrogates, this study questions whether meperidine is acting on the same receptor as morphine to inhibit electrically generated contractions, or for that matter, to mediate analgesia. The effect of meperidine and morphine in the MVD indicate that it does not. In this system the effects of the two compounds differ markedly and a true opioid effect was not apparent with meperidine. Meperidine exhibits some common properties with putative sigma compounds, but it does not appear to have true sigma activity.

It is concluded that although meperidine possesses opioid-like activity, its effects are mediated at different sites, perhaps by a different mechanism than morphine. Because the responses of meperidine and morphine in these models are not identical, further research distinguishing the effects in the ileum of the two compounds to discern their respective mechanisms of action may lead to a better understanding of the receptor involved in the analgetic response.

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GLOSSARY

Acronym	Term
ACh	Acetylcholine
4-AP	4-Aminopyridine
cAMP	Cyclic adenosine monophosphate
DADL	D-ala, D-leuenkephalin
EC_{50}	Concentration to cause 50 percent of the effect
EKC	Ethylketocyclazocine
FI	Fixed-interval
FILP	Fixed-interval lever-pressing
FR	Fixed-ratio
FT	Fixed-time
GPI	Guinea pig ileum
IC_{50}	Concentration to cause 50 percent inhibition of the effect
LES	Lower esophageal sphincter
MAO	Monoamine oxidase
MPLM	Myenteric plexus-longitudinal muscle
MVD	Mouse vas deferens
PCP	Phencyclidine
RVD	Rat vas deferens
SKF 10,047	N-allylnormetazocine