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MECHANISM OF NARCOTIC ACTION AT THE FROG SKELETAL NEUROMUSCULAR JUNCTION: AN ELECTROPHYSIOLOGICAL STUDY

by

© HEATHER DALE DURHAM

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Mechanism of Narcotic Action at the Frog Skeletal Neuromuscular Junction: an Electrophysiological Study" submitted by Heather Dale Durham in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Date May 29, 1979

my grandmother

May Bassett

ABSTRACT

The action of the narcotic analgesic meperidine on the frog sciatic nerve-sartorius muscle preparation was investigated to test the hypothesis that narcotics depress neuromuscular transmission by inhibiting action potential production in the fine, unmyelinated nerve endings and/or by depressing a phase of the transmitter release mechanism.

Meperidine depressed the amplitude of muscle twitch responses to supramaximal, sciatic nerve stimulation, but potentiated responses to direct muscle stimulation.

Action potential firing (spontaneous firing or after-discharge following a nerve stimulus) was induced in the nerve terminals by TEA and recorded from the ventral root innervating the muscle. Meperidine (10^{-6} to 2×10^{-4} M) blocked both types of TEA-induced activity, an action which was prevented by naloxone in 50% of experiments. These results indicated that meperidine can affect action potential production in the nerve endings. However, this mechanism did not account for the reduction in twitch amplitude since neither the amplitude or duration of the extracellularly recorded nerve terminal action potential to sciatic nerve stimulation was affected by concentrations of meperidine (8×10^{-5} to 1.6×10^{-4} M) which reduced the amplitude of the simultaneously recorded EPP. This meperidine-induced depression of EPP amplitude (recorded intracellularly or extracellularly in the presence of curare) was partially antagonized by a low concentration of naloxone (3×10^{-8} M) which itself had no effect on neuromuscular transmission.

Meperidine depressed mepp amplitude (recorded intracellularly), but

did not significantly alter mepp frequency. The depression of mepp amplitude was not antagonised by naloxone, indicating that this effect was not mediated by opiate receptors.

Meperidine depressed the amplitude of the EPP_I evoked by iontophoresis of acetylcholine directly onto the endplate region. This action of meperidine on EPP_I was not antagonized by naloxone. Therefore, the depression of mepp and EPP_I amplitudes and part of the depression of EPP amplitude by meperidine was due to a non-opiate effect of the narcotic on the endplate. These results suggested that the naloxone-sensitive depression of EPP amplitude was due to a presynaptic effect of meperidine. However, meperidine did not alter the quantal content of the EPP measured in the presence of high [Mg^{++}] nor did naloxone antagonize meperidine-induced depression of EPP amplitude in the presence of Mg^{++}. In addition, the percentage depression of EPP amplitude by 4.2×10^{-4} M meperidine was significantly greater when curare instead of $MgCl_2$ was used to block neuromuscular transmission. It is suggested that Mg^{++}, as well as naloxone, may antagonize the opiate receptor-mediated effects of meperidine.

The anticholinesterase action of meperidine did not influence the effect of this narcotic on the EPP amplitude since meperidine was equally active in the presence and absence of physostigmine.

I is concluded that there are three mechanisms of action of meperidine operant in the frog sciatic nerve-sartorius muscle preparation; one is probably on the transmitter release mechanism under conditions of nerve stimulation which is partly, if not completely, opiate receptor mediated; a second, non-opiate receptor mediated, anaesthetic-like action on the post-synaptic membrane; and a third effect on action

potential conduction in the nerve fibers which may be partly opiate recepter mediated, but is not responsible for inhibition of neuromuscular transmission under conditions of supramaximal nerve stimulation.

The major effect of meperidine in this preparation is a non-opiate action at the endplate similar to that of local and general anaesthetics.

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CHAPTER I. INTRODUCTION

1. The Physiology of Neuromuscular Transmission

To determine how drugs such as narcotics inhibit neuromuscular transmission, it is necessary to understand the physiological mechanisms by which acetylcholine is released and how it activates the muscle fibers to contract.

Nerve fibers form special connections or synapses with the muscle fibers which they innervate. Each neuromuscular junction consists of a presynaptic motor nerve terminal and a muscular part, which is a specialized part of the muscle fiber and is referred to as the subsynaptic area or endplate region. At the skeletal neuromuscular junction, the endplate area is characterized by numerous folds of the membrane resulting in secondary clefts. These clefts are invaded by the terminal branches of the presynaptic nerve fibers (Couteaux, 1963). The presynaptic and postsynaptic elements are separated by a space of 200 to 250 Å. It is generally thought that each frog skeletal muscle synapse is innervated by only one axon. However, Vyskocil and Magazanik (1977) believe that at least 30% of fibers are innervated by two or more axons at a single endplate.

The nerve terminal contains a high density of mitochondria and numerous synaptic vesicles which contain the neurotransmitter, acetylcholine. Acetylcholine is synthesized in the cytoplasm by acetylation of choline by choline acetyltransferase (Nachmansohn and Muschado, 1943), and is then transferred to and stored in vesicles in uniform

amounts called quanta (Del Castillo and Katz, 1954a; Fatt and Katz, 1952). The contents of the vesicles can be released into the subsynaptic cleft either spontaneously or in response to nerve impulses (Fatt and Katz, 1951, 1952; Del Castillo and Katz, 1954).

The acetylcholine in the synaptic cleft is deactivated by hydrolysis to choline and acetic acid by acetylcholinesterases. The choline is returned to the nerve terminal for resynthesis into acetylcholine (Hubbard, 1973).

Not all of the acetylcholine stored in the nerve terminals is equally available for release. There is evidence for three different pools of acetylcholine; a readily releasable store enclosed in vesicles near the presynaptic membrane, a secondary releasable store, and a third nonreleasable pool of transmitter that can be converted into releasable quanta (Elmquist and Quastel, 1965).

Some synaptic vesicles appear to collect around specific foci in the presynaptic membrane in conjunction with their release sites, and in electron micrographs, they appear to be anchored to a dense material. In the frog neuromuscular junction, the dense material forms a series of transverse bands located in the terminal directly above the postsynaptic folds of the endplate (Couteaux and Pecot-Dechavassine, 1974). These filaments may represent microtubules. Thus vesicles may move by two mechanisms; "random or Brownian" motion and unidirectional displacement perhaps by electrostatic forces (Llinas and Heuser, 1977a).

The acetylcholine released spontaneously combines with specific

receptors on the endplate membrane and causes a brief depolarization of 0.5 to 1.5 mV; the miniature endplate potential (mepp). The mepp amplitude is proportional to the number of acetylcholine molecules in each packet released which is termed the "quantal size" (Fatt and Katz, 1952; Del Castillo and Katz, 1955). A nerve impulse delivered to the nerve terminal accelerates the release of quanta of acetylcholine which produces a larger depolarization of the subsynaptic membrane termed the endplate potential or EPP (Del Castillo and Katz, 1954b, Liley, 1956; Takeuchi and Takeuchi, 1959). When the amount of depolarization caused by molecules of acetylcholine reaches a certain threshold level, an action potential is initiated in the muscle fiber which, in turn, sets off the chain of events involved in muscle contraction (Bianchi, 1968).

The amplitude of the EPP is a multiple of the mepp amplitude, and the number of quanta of acetylcholine determining the EPP (quantal content) can be estimated by the ratio: EPP amplitude/ mepp amplitude (Del Castillo and Katz, 1954a). The summation of mepps is linear for small EPPs, but for large EPPs a correction for nonlinear summation was introduced by Martin (1966). However, it has been recently suggested that Martin's correction factor may over-correct by up to 50% (Bennett, Floreu, Pettigrew, 1976) and the magnitude of the correction needed for the neuromuscular junction is still uncertain (Martin, 1976; Stevens, 1976).

The effect of transmitter on the endplate can also be produced by applying acetylcholine directly to the endplate by iontophoresis (Nastuk, 1953). With this technique, application of a voltage

potential to glass microelectrodes filled with the desired electolyte causes migration of ions out the tip of the pipette (Globus, 1973). This method allows specific examination of the post-synaptic action of drugs with minimal influence by presynaptic actions.

At the skeletal neuromuscular junction, the reaction of acetylcholine with the receptors causes an increase in permeability to both sodium and potassium ions. It was originally thought that these ions moved through separate channels independently of one another in generating the endplate current (epc), but this interpretation is now considered invalid. The current theory is that combination of acetylcholine with a receptor induces a conformational change in a gating macromolecule wich opens an endplate channel to permit transmembrane fluxes of Na^+ and K^+. This synaptic channel has only two conductance states, open and closed, and transition between these two conductance states is governed by the rate constant for closing open channels and the rate constant for channel opening (Katz and Miledi, 1972; Magleby and Stevens, 1972ab; Neher and Sakmann, 1976; Dionne and Ruff, 1977; Lewis, 1979). The factor limiting the rate at which the current decays under normal conditions is a voltage-dependent conformational change in the membrane which determines the lifespan of an open channel. Increasing the extracellular calcium or magnesium concentration at the frog neuromuscular junction decreases the decay of the endplate current (Cohen and Van der Kloot, 1978) and single synaptic channel conductance (Dionne and Ruff, 1977). This action may be due to neutralization of the

negative surface charge on the membrane and thus increasing the voltage gradient within the membrane (Cohen and Van der Kloot, 1978).

Neuromuscular blocking drugs such as curare compete with acetylcholine for the receptors and thus reduce the number of acetylcholine-receptor interactions and decrease the EPP amplitude without altering the latency or time-course of the EPP. Anticholinesterase inhibitors such as physostigmine allow more acetylcholine-receptor interactions and increase the amplitude and duration of the EPP (Davson, 1970).

How does a nerve impulse accelerate the release of acetylcholine? An action potential is conducted along the myelinated nerve fibers. This transient decrease in potential is mediated by an influx of sodium ions and a subsequent efflux of potassium ions along their electrochemical gradient. These ions pass through the membrane via channels or pores which are relatively specific for either Na^+ (early channels) or K^+ (late channels), but certain other univalent ions such as Li^+ are known to substitute for Na^+. Hille has described these channels as 'filters' which allow ions to pass according to their hydrated size and to their ability to form hydrogen bonds (Davson, 1970; Luttgau, 1977). From analysis of the small 'gating currents' associated with movement of these ions (Hille, 1975; Armstrong, 1975; Ulbricht, 1974), it has been proposed that these channels are in either an open or closed state. The influx of Na^+ during the action potential is dependent on the resting membrane potential.

Calcium also affects nerve membrane excitability. Decreasing

the external calcium concentration causes an increase in both nerve and muscle fiber membrane excitability which results in a state of repetitive activity (Davson, 1970). Tetraethylammonium (TEA) causes facilitation of neuromuscular transmission and spontaneous twitching in skeletal muscle by inhibiting potassium conductance (Hille, 1967) and by its ability to release calcium from binding sites in nerve and muscle membranes (Beaulieu and Frank, 1967ab; Beaulieu, Frank and Inoue, 1967). TEA, applied to the frog neuromuscular junction, causes repetitive firing in the motor nerve terminals following a single nerve stimulus (Koketsu, 1958; Beaulieu et al., 1976) and spontaneously, in the absence of nerve impulses (Beaulieu et al., 1967). Both types of activity can be recorded antidromically from the ventral root innervating the frog sartorius muscle using extracellular electrodes (Beaulieu et al., 1967). Repetitive activity in nerves is thought to originate at the junction of the myelinated axon and the unmyelinated, smaller diameter fibers in the nerve terminal (Beaulieu et al., 1967; Standaert, 1964). The action potential in the nonmyelinated nerve terminals can be measured using extracellular microelectrodes (Katz and Miledi, 1965a).

Invasion of the action potential into the nerve terminal is associated with a brief influx of calcium. The amount of calcium crossing the presynaptic nerve terminal membrane is proportional to the amplitude and duration of the depolarization (Cooke and Quastel, 1973a). This calcium triggers the release of transmitter (Katz and Miledi, 1967, 1970) and is thought to be the coupling mechanism

between depolarisation and transmitter release. At rest, the intracellular calcium concentration is maintained at low levels compared to that in the extracellular space. Thus, the influx of calcium in response to a nerve action potential is down a steep electrochemical gradient. In the absence of extracellular calcium, the action potential invades the nerve terminal, but fails to cause transmitter release (Katz and Miledi, 1967). These investigators concluded that utilization of external calcium is restricted to a brief period which barely outlasts the depolarization of the nerve ending and which precedes transmitter release.

It has been suggested that the effect of calcium may be due to its reaction with a receptor compound X on the presynaptic terminal leading to a CaX complex formation (Del Castillo and Katz, 1954a; Gage and Quastel, 1966; Dodge and Rahamimoff, 1967; Hubbard, Jones and Landau, 1968ab). However, the identity of X and the exact mechanism of how calcium causes transmitter release remains obscure. Na^+-K^+-activated ATPase in the membrane has been postulated to play a physiological role in transmitter release (Paton, Vizi and Zar, 1971; Vizi, 1972). According to this theory, calcium fluxes are governed by the concentration of sodium, and the subsequent inhibition of Na^+-K^+-activated ATPase by Ca^{++} is involved in transmitter release. Stimulation of the membrane ATPase by termination of Ca^{++}-inhibition either by accumulation of Na^+ or Mg^{++} makes the membrane again impermeable to the transmitter (Vizi, 1978).

Standaert, Dretchen, Skirboll and Morgenroth (1967a,b) have proposed that cyclic adenosine 3',5'-monophosphate (cAMP) is involved in the excitation-secretion sequence. According to their model an

action potential activates adenyl cyclase in the nerve membrane which converts ATP to cAMP. cAMP acts via protein kinase to phosphorylate substrates that cause ion channels to open and permit Na^{+} and/or Ca^{++} to pass through the membrane. The flux of ions depolarises the nerve membrane and causes vesicles to fuse to the membrane and release transmitter. In contrast, Quastel and Hackett (1971) concluded that the cAMP concentration would have to be linearly related to the frequency of release of quanta of acetylcholine. The increase in concentration of cAMP required to fulfill this relationship after a presynaptic depolarization seemed to them to be too high to accomodate the cAMP hypothesis of transmitter release. Others have concluded that the increase in mepp frequency in the presence of phosphodiesterase inhibitors such as theophylline, an effect crucial to the cAMP hypothesis, is due to release of bound calcium from intracellular stores, and not to an effect on cAMP levels (Duncan and Statham, 1977).

The mechanism by which synaptic vesicles fuse with the presynaptic membrane and empty their contents into the synaptic cleft remains unclear. Any divalent cation, including Ca^{++} and Mg^{++}, can promote vesicle-plasma membrane contact, but only Ca^{++} triggers exocytosis (Llinas and Heuser, 1977b). Therefore, calcium must do something more than allow contact by neutralising negative surface charges. It has been suggested that exocytosis is a result of membrane phase changes induced by calcium, but this does not explain release from only specific sites on the membrane (Llinas and Heuser, 1977b).

By whatever mechanism calcium influx during depolarization of the nerve terminals is involved in release of transmitter, it must be rapidly removed from its site of action for the transmitter release to return to resting levels. Many subcellular elements bind calcium reversibly including the nerve membrane and synaptic vesicles (Rahamimoff, 1977), but the most important sequesterers in nerve endings are mitochondria (Alnaes and Rahamimoff, 1975; Rahamimoff, Lyubimov, Alnaes, Rotshenker and Rahamimoff, 1975; Blaustein, Ratzlaff and Kendrick, 1978) and some other, as yet unidentified, ATP-dependent calcium storage system (Kendrick, Blaustein, Fried and Ratzlaff, 1977). Calcium must then be extruded from the nerve terminal to restore the resting calcium concentration. There is evidence that Ca^{++} is extruded from the intracellular space in exchange for Na^{+} (Blaustein et al., 1978).

Spontaneous release of acetylcholine is much less sensitive to the calcium concentration than is evoked release (Boyd and Martin, 1956; Hubbard, 1961; Birks, Burnstock and Firth, 1968). Whereas release after a nerve impulse is dependent on the extracellular calcium , mepp frequency is dependent upon the intracellular calcium concentration (Alnaes and Rahamimoff, 1975; Duncan and Statham, 1977). Duncan and Statham concluded that under normal circumstances, the presynaptic terminals are able to maintain intracellular calcium concentration almost constant despite changes in calcium fluxes due to drug treatment or alteration in the extracellular calcium concentration.

Release of acetylcholine can be altered by other ions besides calcium. Increasing the magnesium concentration of the Ringer's

decreases the amount of acetylcholine released after depolarization of the nerve terminal, but has little effect on spontaneous release, i.e. mepp frequency (Del Castillo and Engback, 1954). In contrast to their similar effects on excitability of nerve and muscle membranes, the effects of calcium and magnesium on transmitter release are antagonistic (Del Castillo and Katz, 1954c; Del Castillo and Engback, 1954). It is thought that these ions compete for the same binding sites on the presynaptic membrane (Del Castillo and Katz, 1954c; Jenkinson, 1957; Hubbard, Jones and Landau, 1968). Decreasing the amount of acetylcholine released by each nerve action potential by decreasing the concentration of extracellular calcium and/or increasing the extracellular magnesium concentration has been used extensively in experiments measuring quantal content. This is done to allow the recording of both subthreshold EPPs and mepps.

Reducing the extracellular sodium concentration produces a small increase in the spontaneous transmitter release. During release induced by nerve terminal depolarization, this facilitation in the presence of normal calcium is balanced by reduction of the presynaptic action potential amplitude (Gage and Quastel, 1966). Transmitter release is facilitated when the external potassium concentration is raised (Cooke and Quastel, 1973b; Takeuchi and Takeuchi, 1961). In addition to the action of K^+ in depolarizing the nerve terminal membrane, Cooke and Quastel suggested that K^+ facilitates the increase in permeability of the membrane to calcium during depolarization.

In conclusion, a drug which inhibits neuromuscular transmission could act by depressing: conduction of the action potential along the myelinated axon; action potential production in the fine, unmyelinated nerve terminal fibers; synthesis of the transmitter; stimulus-secretion coupling or mobilization of transmitter; the reactivity of the endplate region to the transmitter substance; or by facilitating deactivation of the transmitter in the synaptic cleft.

2. The Pharmacology of Narcotic Drugs

The study of analgesia, tolerance and dependence produced by narcotics is one of the oldest areas of pharmacological research. However, many of the cellular mechanisms underlying the action of this class of drugs still remain obscure. The recent discovery of endogenous opioid peptides (Hughes, 1975; Terenius and Wahlstrom 1975) and the implications as to their physiological role have reinforced the importance of this research.

The major site of action of narcotic analgesics in the relief of pain is accepted generally as being in the central nervous system. A multitude of studies have been carried out to determine the effects of opiates on central nervous system electrical activity and neurotransmitters such as acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine, glycine, and γ-aminobutyric acid. These investigators have examined the effect of opiates on steady state levels, turnover and release of neurotransmitters, the effects of systemic administration and local injection of opiates upon identified neurones in the brain and spinal cord, and the effects of various pharmacological

and surgical manipulations on the mechanism by which opiates alter the above effects and the pain threshold. The results of these studies are often contradictory and vary with the species of laboratory animal and with the techniques employed. This area has been extensively reviewed by Clouet (1971), Domino, Vasko and Wilson (1976), Eidelberg (1976), Ford and Clouet (1975), Harris (1970), Kosterlitz, Collier and Villarreal (1973), Mayer and Price (1976), Mehta (1975), Messing and Lytle (1977), Way (1972) and Yaksh and Rudy (1978).

Although the importance of these studies should not be underemphasized, major problems remain unresolved because it is difficult to interpret pharmacological findings when the complex physiology of the central nervous system is not well understood. Morphine affects many neurotransmitters and biochemical pathways many of which may be involved in producing the physiological response being measured. It may be a critical balance between brain substances which is important (Way, 1972) and a treatment affecting one compound may have considerable effects on the actions of others. This means it is difficult to determine whether an effect of a narcotic on one transmitter is directly caused by the treatment or is a consequence of other pharmacological responses to the drug. In addition, the action of a pharmacological agent used to block or enhance the effects of a particular transmitter is not necessarily specific in its overall effect.

These problems have led to the use of peripheral tissues as a major tool in narcotic research to elucidate the findings from the central nervous system studies. The next section of this introduc-

tion will focus on the findings of investigators who have employed these peripheral nerve-muscle preparations with less complex physiology compared to the central nervous system.

Two general observations will become apparent. First, morphine-like drugs alter the release of a neurotransmitter without regard to the identity of the transmitter substance. Secondly, synapses in different organ systems and species of animals have different sensitivities to narcotic agonists. Morphine (10^{-8} to 10^{-6} M) depresses neuromuscular transmission very effectively in the Auerbach's plexus-guinea pig ileum and the rat or rabbit vagus nerve-SA node of the heart where acetylcholine is the transmitter, and in the cat postganglionic nerve of the superior cervical ganglion-nictitating membrane and in the mouse vas deferens where the transmitter is noradrenaline. Relaxation of the guinea pig jejunum in response to mesenteric, adrenergic nerve stimulation is also quite sensitive to morphine-blockade. A less sensitive group of preparations requires a 10^{-5} M to 10^{-3} M concentration of a narcotic to block neurotransmission. These include the rat small intestine, the cat vagus nerve-SA node of the heart, ganglionic transmission in the cat or rat superior cervical ganglion, and action potential conduction in squid or sciatic nerve axons and skeletal muscle preparations.

2.1 Preparations Sensitive to the Inhibitory Actions of Narcotics.

2.1.1 Intestinal preparations. As long ago as 1917, Trendelenberg reported that morphine inhibited the peristaltic reflex elicited by distension of isolated guinea pig ileum. However, it was not until the mid-fifties that progress was made in determining

the mechanism of this inhibition.

Schaumann (1955) investigated the action of morphine on the peristaltic reflex in guinea pig and rabbit jejunum. Although he found morphine to be ineffective in rabbit preparations, this narcotic abolished both the emptying and the preparatory phase of the reflex in guinea pig intestine. That both phases of the reflex are inhibited by morphine was reaffirmed by Kosterlitz and Robinson (1957) and Gyang, Kosterlitz and Lees (1964) using guinea pig ileum.

Since responses to nicotine, but not acetylcholine, were blocked by morphine, Schaumann suggested that morphine acts by interrupting the pathway between ganglion cells and the muscle, i.e. by preventing acetylcholine release. He tested his hypothesis by measuring acetylcholine release in the presence of morphine by the bioassay method (Schaumann, 1956, 1957). Morphine blocked acetylcholine release in the distended strips and partially inhibited release in undistended strips. In ground up tissue, morphine enhanced rather than reduced transmitter release and had no effect on the acetycholine content of the tissue, indicating that intact cells were required for the inhibitory action of morphine, and that this narcotic did not act by blocking acetylcholine synthesis.

At the same time Paton was experimenting with the transmurally stimulated guinea-pig ileum. In 1957 he concluded that the electrically induced contractions of the ileal muscle was due to excitation of postganglionic nervous structures since the contractions were blocked by atropine but not by hexamethonium and were potentiated by anticholinesterases. Morphine reduced the amplitude of both twitch

and tetanic responses, but was more effective at low rates of stimulation. The depression of contraction amplitude correlates well with the reduction in acetylcholine output per stimulus volley. Paton found the normal output of acetylcholine per pulse in the absence of drug decreased as the stimulus frequency was increased. These observations were confirmed using this preparation by Cox and Weinstock (1966) and by Cowie, Kosterlitz and Watt (1968), Paton and Zar (1968) and Greenberg, Kosterlitz and Waterfield (1970) using the transmurally stimulated Auerbach's plexus-longitudinal muscle preparation of guinea pig ileum. Greenberg et al. also found that hexamethonium did not alter the effect of morphine.

The effect of narcotics on the response of intestinal preparations to various pharmacological agents has contributed to the conclusion that the main action of opiate agonists is to decrease release of acetylcholine to nerve stimulation. Most investigators found that morphine did not block contractions of whole guinea pig ileum to exogenous acetylcholine (Schaumann, 1955; Paton, 1957; Kosterlitz and Robinson, 1957, 1958; Gyang et al., 1964) or of the longitudinal muscle of the ileum (Cowie et al., 1968). Nor did morphine affect responses to histamine in the study by Kosterlitz and Robinson (1958). Responses to ganglion stimulant drugs such as nicotine are blocked by morphine (Schaumann, 1955; Kosterlitz and Robinson, 1958). Responses to drugs which stimulate both neural and muscle elements (5-HT and barium) are partially inhibited by narcotics (Kosterlitz and Robinson, 1958; Gaddum and Picarelli, 1957).

Although most investigators have found that narcotics do not

affect the smooth muscle responses to exogenous acetylcholine, Lewis (1960) found evidence for a postsynaptic effect of morphine. He observed that morphine, in concentrations which inhibited acetylcholine release, depressed responses of the guinea pig ileum to a variety of smooth muscle stimulants such as acetylcholine, carbachol, substance P, 5-HT, barium, potassium and histamine. Waterfield and Kosterlitz (1973) found that only higher concentrations of morphine produced a nonspecific effect. Lewis concluded that morphine has a twofold depressant action on substances such as 5-HT and barium which act both directly on the muscle cells and indirectly on nervous structures, i.e. the narcotic blocks their effects on the muscle postsynaptically and prevents the increase in acetylcholine release by their indirect action. He suggested that the underlying common mechanism might be inhibition of a metabolic process common to both neuronal and muscular structures.

Meperidine also inhibits the response of ileum to exogenous acetylcholine (Paton, 1957; Gyang et al., 1964). This is not surprising in light of the local anaesthetic action of this narcotic (Gruber, Hart, Ross and Gruber, 1941; Goodman and Gilman, 1975).

From the evidence presented thus far it can be concluded that morphine prevents acetylcholine release to nerve stimulation by an action on postganglionic nervous structures. The action of narcotics on these neurones has been studied also by electrophysiological methods. Using extracellular, suction electrodes, two types of spontaneous activity have been recorded from neurones of Auerbach's plexus (Wood, 1970, 1973, 1975): a burst unit which is blocked by tetrodotoxin, but not affected by Mn^{++} or by drugs which alter

synaptic transmission including morphine (Sato, Takayanagi and Takagi, 1973; Dingledine, Goldstein and Kendig, 1974); and a single spike unit which is blocked by tetrodotoxin but not by Mn^{++} or hexamethonium. The frequency of single spike units is increased by nicotine and acetylcholine and decreased by α-adrenergic stimulants. Increase in frequency by cholinergic agents is blocked by hexamethonium and decreases in frequency by α-adrenergic stimulants are blocked by α-adrenergic blocking drugs (Sato et al., 1973). The single spike electrical activity is thought to be recorded from parasympathetic, postganglionic neurones (Wood, 1975). Spontaneous single spike activity and the increase in frequency caused by nicotinic agents are blocked by morphine and normorphine (Sato et al., 1973; Ehrenpreis, Sato, Takayanagi, Comaty and Takagi, 1976; Dingledine et al., 1974; Dingledine and Goldstein, 1975, 1976; North and Williams, 1976, 1977) and by methionine- and leucine-enkephalin (North and Williams, 1976).

Single electrical pulses applied by point stimulation near the recording electrode by a second suction electrode result in a compound or antidromic spike which is due to direct stimulation of the units under study, and a unitary spike which is synaptically driven (Dingledine and Goldstein, 1976; North and Williams, 1976). According to North and Williams (1976) normorphine has no effect on the synaptically driven spike, but hexamethonium blocks it. Dingledine and Goldstein (1976) found that morphine was virtually as effective in inhibiting spontaneous electrical activity of myenteric neurones in calcium-free Ringer's as in normal Ringer's. The synaptically driven spike was completely abolished in calcium-free

Ringer's indicating the absence of synaptic input.

These findings led both groups of investigators to conclude that it is unlikely that morphine acts by stimulating the release of an inhibitory transmitter, by blocking release of an excitatory transmitter or by blocking the postsynaptic response to released transmitter. Dingledine and Goldstein proposed that narcotics may increase the membrane threshold for electrical activity in the cholinergic motor neuron perhaps by an action at or near its terminal varicosities. North and Williams suggested that the mechanism of morphine's action was hyperpolarization or membrane stabilization of a proportion of the myenteric neurones. This theory was supported by their finding that the effects of morphine can be mimicked by passing anodal current or overcome by passing cathodal current through a stimulating electrode very close to the recording electrode. Similarly, when twitches are recorded from transmurally stimulated ileum, morphine is more effective during submaximal stimulation (Cox and Weinstock, 1966) and its effects can be antagonized by increasing the stimulus strength (Ehrenpreis, Light and Schonbuch, 1972). Also, narcotics can inhibit the increase in firing rate of extracellularly recorded spontaneous potentials due to a variety of depolarizing agents (e.g. 5-HT, nicotine, caerulein, sodium picrate or acetylcholine (Takayanagi, Sato, Takagi, 1974; North and Williams. 1977).

In the study by North and Williams (1977), the action of morphine was not affected by altering the potassium concentration in the Ringer's or by ouabain, indicating that the mechanism of action is

not likely an increase in potassium conductance or stimulation of an electrogenic Na^+-K^+ exchange.

In addition to the above findings, Ehrenpreis, et al. (1976) found that the increase in firing rate of spontaneous single spike units induced by acetylcholine was little affected by morphine. Their results suggested that acetylcholine had two actions. The primary effect was independent of the action of morphine, whereas with high acetylcholine/morphine concentration ratios, direct antagonism between the two drugs might occur in ganglia of Auerbach's plexus. On the basis of this finding these investigators postulated a secondary receptor for morphine on the preganglionic nerve terminals in addition to a postganglionic receptor site.

In contrast to findings using extracellular recording techniques, cells recorded intracellularly are not spontaneously active (North and Henderson, 1975; North and Tonini, 1976). North and Williams (1977) showed that the activity recorded extracellularly was due to mechanical deformation caused by application of suction to the electrode and not a result of synaptic input. In their studies using intracellular electrodes, North and Tonini (1976) occasionally obtained spontaneous injury potentials. These were blocked by normorphine. North and Henderson (1975) and North and Tonini (1977) found that morphine and normorphine had no effect on the amplitude or time course of the evoked epsp in Type I cells (cells which receive synaptic input) or on the action potential in Type II cells (these cells cannot be activated synaptically and may be afferent). However, in their later studies, investigators from the same laboratory

found that normorphine, added to the bath or to the neurones by iontophoresis, caused an immediate hyperpolarization of up to 20 mV in about fifty percent of Type I cells and in about ten percent of Type II cells (North, 1976; North and Tonini, 1976, 1977). This hyperpolarization was accompanied by a variable decrease in membrane resistance, was dose related, and was prevented by naloxone. The hyperpolarization often declined progressively despite the continued presence of the drug and sometimes the cells would not respond to a second dose of narcotic; this finding is reminiscent of "acute tolerance" observed in twitch experiments (Paton, 1957; Waterfield and Kosterlitz, 1973). Also, when the neurones were excited by passing brief depolarizing pulses across the soma membrane, the hyperpolarization caused by normorphine was sufficient to prevent the cell from reaching threshold for action potential firing unless the stimulus strength was increased (North and Tonini, 1976). When cell processes were stimulated, normorphine prevented conduction of the 'antidromic' action potential into the cell soma (North and Tonini, 1977).

The results of these intracellular studies provided these investigators with direct experimental evidence to support their theory (North and Williams, 1976) that narcotic drugs act in ganglion cells by hyperpolarizing the membrane. Small doses of opiates would prevent excitation of a small population of neurones. Larger concentrations would cause depression of a proportionately larger number of cells. This theory is attractive since it explains the inhibition by morphine of spontaneous activity recorded extra-

cellularly and reconciles many of the different findings obtained using intracellular and extracellular recording techniques.

According to Bülbring and Tomita (1977) their theory also explains the observation that morphine inhibits the resting release of acetylcholine in guinea pig ileum by twenty to fifty percent (Schaumann, 1957; Paton, 1957; Paton and Zar, 1968; de la Lande and Porter, 1967). De la Lande and Porter observed that the inhibition by morphine of the resting acetylcholine release was reduced or abolished by treatments which block electrical activity in nervous structures such as subdivision of the intestine into rings, cooling, anoxia, procaine, changes in electrolyte balance and glucose deprivation. They concluded that there are two mechanisms of spontaneous release in this preparation: 1) spontaneous release at nerve endings and 2) augmentation of this release by conducted impulses in nerve axons. Only the latter mechanism is inhibited by narcotics.

The role of 5-HT in the acute and chronic effects of morphine has received much attention. Gaddum and Picarelli (1957) proposed there are two kinds of tryptamine receptors in guinea pig ileum: the D receptors which are blocked by dibenzyline, LSD, etc. and mediate the smooth muscle actions of 5-HT, and the M receptors which are blocked by morphine and are located in the nerve plexus. Uptake and release of 5-HT are not affected by narcotics (Schulz and Cartwright, 1974).

Schulz and Goldstein (1973) proposed that there are excitatory, serotoninergic fibers and inhibitory adrenergic fibers impinging on and modulating the activity in cholinergic neurones innervating

the ileal longitudinal [redacted] According to their model, the acute effect of morphine could be to inhibit the seretoninergic synapse postsynaptically. Tolerance would result from an increase in the number of 5-HT sensitive receptors thus restoring the excitatory pathway. This theory was based on their finding that sensitivity to exogenous 5-HT was increased in muscles from tolerant and dependent guinea pigs and this supersensitivity paralleled the degree of tolerance to morphine.

Ginzler and Mussachio (1974) found evidence for an interaction between morphine and 5-HT in transmurally stimulated, nontolerant ileal strips. 5-HT added to the bath produced an increase in basal tone, and a decrease in the height of electrically induced twitches followed by a recovery from this inhibition. When morphine was then added in the continued presence of 5-HT, the inhibitory effect of the narcotic on twitch amplitude was considerably potentiated. This potentiation was narcotic receptor mediated since all of the effects attributed to morphine were blocked by naloxone. They argued that this effect of 5-HT was specific since 5-HT had no effect on inhibition of twitches induced by noradrenaline, and LSD reduced the inhibitory effect of 5-HT on twitch, but not the excitatory effect in resting gut. They concluded that excitatory and inhibitory effects of 5-HT are mediated by different receptors (but this does not fully explain the unusual effect of LSD), and that morphine may act on the gut and in the CNS by increasing the availability of 5-HT and potentiating its inhibitory effect. Arguing against this theory were their observations that ilea which were insensitive to 5-HT

displayed the usual sensitivity to morphine and during tachyphylaxis to 5-HT the gut still showed an augmented response to morphine in its presence.

That morphine blocks neural effects of 5-HT has been shown also by electrophysiological studies. The frequency of single unit spikes recorded extracellularly from neurones in Auerbach's plexus is increased by 5-HT. Morphine prevents this elevation in firing rate (Dingledine et al., 1974; Dingledine and Goldstein, 1975; North and Williams, 1977). In contrast, North and Henderson (1975), recording intracellularly from plexus neurones, found that 5-HT depressed the evoked epsp in most cells, had no effect on membrane potential or resistance or on the iontophoretic acetylcholine potential, and the depression of epsp amplitude was blocked by methysergide and LSD but not by morphine.

In order to reconcile the difference in the effects of 5-HT and morphine using the two techniques, North and Henderson (1975) suggested that the excitatory effects of 5-HT and inhibition by morphine occurred only at the varicose cell processes from which acetylcholine is released toward the muscle. This activity would not be measured by intracellular techniques. This hypothesis was also proposed by Dingledine and Goldstein (1976). However, North and Williams (1977) failed to record activity from the cell processes using smaller extracellular electrodes and, as discussed above, the spontaneous activity recorded is due to suction applied by the electrode.

In none of these studies was it shown that the effect of narcotics on 5-HT responses was due to an interaction of morphine with 5-HT receptors. Dingledine and Goldstein (1976) concluded that

functional 5-HT or acetylcholine receptors at nicotinic sites were not necessary for the acute, inhibitory effect of morphine in transmurally stimulated muscle preparations since the effect of the narcotic was not impaired during desensitization to 5-HT.

In ilea from guinea pigs which have been made tolerant and dependent to morphine, the spasmogenic response to exogenous 5-HT (Schulz and Goldstein, 1973; Ward and Takemori, 1976a) and the increase in spontaneous firing rate recorded extracellularly from plexus neurones (Takayanagi et al., 1974) are potentiated during the withdrawal syndrome to morphine, whereas responses to cholinergic agents are not. During tolerance, pA_2 values are increased in the central nervous system but decreased in ileum, indicating a difference in binding of the antagonist to the opiate receptor at the two sites. Thus the guinea pig ileum may not be a good model for narcotic dependence.

On the other hand, the guinea pig ileum preparation does fulfill the criteria for a good model for studying the acute effects of narcotics. These are:

1) The potency order for the various narcotics in inhibiting electrically induced twitches and for receptor binding corresponds well to their analgesic potency (Paton, 1957; Gyang et al., 1964; Kosterlitz and Watt, 1968; Creese and Snyder, 1975).

2) The inhibition of twitch amplitude is stereospecific, the levo isomers (e.g. levorphanol) being more effective than dextro isomers (e.g. dextrorphan) (Gyang et al., 1964; Cox and Weinstock, 1966; Dingledine et al., 1974; Waterfield and Kosterlitz, 1975; Ehrenpreis et al., 1976; Dingledine and Goldstein, 1976).

3) The effects of the levo isomers of narcotics are prevented or reversed by narcotic antagonists such as nalorphine and naloxone (Kosterlitz and Robinson, 1958; Cox and Weinstock, 1966; Cowie et al., 1968; Kosterlitz and Watt, 1968; Dingledine et al., 1974; Ehrenpreis et al., 1976; North and Williams, 1977).

4) Specific opiate receptor binding sites have been demonstrated by biochemical techniques (Pert and Snyder, 1973; Creese and Snyder, 1975).

5) The presence of a naturally occurring, endogenous ligand for these receptors has been demonstrated. Puig, Gascon, Craviso and Musacchio (1977) and Puig, Gascon and Musacchio (1978) reported evidence for release of endorphins from the guinea pig ileum during transmural stimulation. They stimulated Auerbach's plexus-longitudinal muscle strips for five minutes at 10 to 25 Hz. When the stimulus frequency was then reduced to 0.1 Hz, they observed an inhibition of the twitch amplitude. This inhibition was proportional to the tetanic frequency employed and, within limits, to the duration of the stimulatory period. The inhibition of twitches was potentiated by low concentrations of morphine but not by dextrorphan, and 55% to 70% of the inhibition was reversed by naloxone. However, release of endorphins has not been measured biochemically (Ehrenpreis, personal communication).

If endorphins are involved in normal neuromuscular transmission, naloxone should have an effect on intestinal responses in the absence of any narcotic agonist. Waterfield and Kosterlitz (1975) found that naloxone increased evoked acetylcholine output at low frequencies of stimulation, but this increase did not result in augmentation of twitch height to either submaximal or supramaximal stimulation. However, Van Neuten and Lal (1974) found with just threshold stimulation,

naloxone produced a dose related increase in twitch amplitude. This effect was enhanced by chronic treatment with morphine. In 1976, Van Neuten, Janssen and Fontaine demonstrated that continuous distension of the lumen of the ileum resulted in "fatigue". Tyrode from a fatigued preparation transferred to the bath containing a non-fatigued muscle caused rapid inhibition of peristalsis in the second tissue. Naloxone antagonized "fatigue" without affecting normal peristalsis.

The action of morphine on the release of catecholamines also has been investigated in guinea pig intestinal preparations. Schaumann (1958) suggested that narcotic analgesics might liberate noradrenaline or occupy the same receptors since both morphine and noradrenaline or adrenaline reduced acetylcholine output in coaxially stimulated ileum to the same extent. The similarity between the effects of morphine and catecholamines on twitch amplitude has been noted by Cowie et al. (1968), Heimans (1975a) and Ferri, Reina and Santagostino (1977). Heimans found that reserpine pretreatment rendered guinea pig ilea less sensitive to the depressant action of low concentrations of morphine, but in the study by Ferri et al., 6-hydroxydopamine pretreatment did not prevent morphine's action. In addition, α or β-adrenoreceptor blockers have no effect on morphine inhibition (Gyang and Kosterlitz, 1966; Heimans, 1975a; Ferri et al., 1977).

In a study by Henderson, Huges and Kosterlitz (1975), morphine did not alter noradrenaline output from guinea pig myenteric plexus to either twitch or tetanic frequencies of stimulation. In contrast, Heimans (1975a) found that morphine reduced the amount of tritiated

material released during stimulation of muscle strips loaded with ^{3}H-dopamine. Szerb (1961) showed that stimulation of isolated guinea pig jejunum through the periarterial, mesenteric nerves resulted in relaxation of muscles previously contracted with histamine. He believed these relaxations were due to release of noradrenaline and demonstrated that they were blocked by low concentrations of morphine. This narcotic action was partially antagonized by nalorphine, but was not dependent on the stimulus frequency. Szerb concluded that morphine prevented the release of noradrenaline due to sympathetic nerve stimulation.

Recently, Shimo and Ishii (1978) have published their evidence that morphine can antagonize the relaxation of guinea pig taenia coli produced by stimulation of non-adrenergic, inhibitory nerves. This effect of morphine is dose-dependent, varies inversely with stimulus frequency, and is almost completely reversed by naloxone.

Intestine from other species such as the rabbit or rat is much less sensitive to the inhibitory effects of narcotics. [redacted]la (1962) showed that 10^{-4} M morphine increased the tone of isolated rat small intestine, but inhibited contractions to nicotine and slightly reduced responses to 5-HT and pilocarpine. He was unable to show antagonism by nalorphine, but in the concentrations employed, nalorphine acted as an agonist.

2.1.2 The superior cervical ganglion-nictitating membrane preparation. Synaptic transmission from the postganglionic fibers of the superior cervical ganglion to the nictitating membrane of the cat is now known to be very sensitive to inhibition by narcotic analgesics, although this was not revealed by the first studies using this

preparation.

Hebb and Konzett (1949) and Cook and Bonnycastle (1953) examined the effects of several narcotics on the response of the membrane in vivo to preganglionic nerve stimulation at tetanic frequencies. Meperidine reduced responses of the nictitating membrane to preganglionic nerve stimulation and to injection of acetylcholine or KCl into the artery to the ganglion. Morphine, on the other hand, had no effect in normal preparations but potentiated responses to acetylcholine in denervated preparations because of its anticholinesterase action.

The reason for the ineffectiveness of morphine in these studies was the high frequency of stimulation employed. In 1957, Trendelenburg reexamined the effects of morphine on this preparation. He found that this narcotic depressed the height of contractions of the nictitating membrane to either preganglionic or postganglionic stimulation more at low frequencies than at high stimulus rates. Morphine also inhibited responses to the ganglion stimulant drugs nicotine, TEA, and KCl. In lower concentrations, morphine caused a long-lasting depression of the responses to histamine, pilocarpine and 5-HT. These stimulants potentiated responses of the nictitating membrane to submaximal, preganglionic stimulation, an action which was antagonized by morphine. These effects of morphine ressemble the action of cocaine on these substances and indeed, cocaine was ineffective in the presence of morphine in Trendelenburg's study.

Trendelenburg found that morphine was more effective in inhibiting responses to submaximal electrical stimulation or submaximal

concentrations of the stimulant drugs. He concluded that the depressant effect of morphine depended on the amplitude of muscle contractions and not specifically on the rate of stimulation, i.e. morphine was more effective in depressing small amplitude contractions. Cairnie, Kosterlitz and Taylor (1961) verified the frequency dependence of morphine's action, but could not correlate the degree of inhibition by morphine to the initial size of the contractions. These investigators also noted that the latent period was increased and the speed of contraction decreased in the presence of morphine. The rising phase of the contractions was most affected.

Responses to intraarterial injection of noradrenaline in this preparation are not depressed by morphine (Trendelenburg, 1957; Cowie et al., 1968; Gyang et al., 1964) indicating the absence of any postsynaptic effect. However, meperidine potentiated responses to noradrenaline and enhanced responses to low frequencies of stimulation (Gyang et al., 1964). Gyang et al. concluded that sensitization of the adrenoreceptors may have masked the depression of noradrenaline release by meperidine.

The depression of twitch amplitude by narcotics is mediated by opiate receptors since the inhibition is reversed by nalorphine (Cairnie et al., 1961; Gyang et al., 1964) and is stereospecifit; i.e. dextrorphan is ineffective (Gyang et al., 1964).

The results of these in vivo studies led to the conclusion that narcotics inhibit release of noradrenaline from postganglionic nerve endings (Trendelenburg, 1957; Cairnie et al., 1961; Gyang et al., 1964).

Morphine-like drugs have the same effect on the isolated, field-stimulated, medial smooth muscle of the cat nictitating membrane as they do in vivo (Henderson, Hughes and Kosterlitz, 1975; Knoll and Illes, 1978; Knoll, Illes and Medzihradsky, 1978). Since there are no preganglionic nerve fibers in this preparation, the main action of narcotics must by at the nerve-muscle junction. Noradrenaline output has been measured and it was observed to be reduced by morphine, especially at low frequencies of stimulation (Henderson et al., 1975; Henderson, Hughes and Thomson, 1972). The magnitude of this reduction correlates with morphine's effect on twitch amplitude.

Knoll et al. (1978) reported that a series of enkephalin analogues inhibit noradrenergic transmission in isolated nictitating membrane. The potency order agreed with the potency in guinea pig ileum except for leu^5-enkephalin which was less potent in the ileum.

In isolated rabbit superior cervical ganglion, morphine is much less effective than in the cat ganglion. Kosterlitz and Wallis (1964) found that 2.7×10^{-5} M occasionally depressed the synaptic potential recorded from the surface of the ganglion. Reducing the safety factor for ganglionic transmission by performing experiments in the presence of hexamethonium or pentolinium, increased the sensitivity to morphine. These investigators found no effect of the narcotic on axonal conduction, but state this does not preclude failure of conduction in the presynaptic terminals. Nalorphine antagonized the depression of the synaptic potential by morphine, but, in contrast to its action in other tissues, high concentrations of this antagonist did not display agonist properties.

Forbes and Dewey (1976) recorded postganglionic potentials elicited by stimulating the preganglionic nerve trunk of the rat superior cervical ganglion in vivo and in vitro. Morphine and meperidine reduced the height of the evoked postganglionic potential without affecting the time course of the compound action potential. The effect was independent of stimulus frequency. In vitro, the l- and d-isomers of pentazocine were equipotent and l-cyclazocine was slightly more potent than d-cyclazocine. Forbes and Dewey were unable to antagonize the agonist effects with naloxone and therefore concluded that this action of opiates on ganglionic transmission is nonspecific. However, in the high concentrations of naloxone (Frank, 1975a) and naltrexone (Forbes and Dewey, 1976) used these drugs have agonistic properties.

2.1.3 The isolated vas deferens preparation. In 1972, Henderson, Hughes and Kosterlitz described another preparation sensitive to low concentrations of narcotics: the mouse vas deferens. Field stimulation of this tissue results in muscle contractions due to stimulation of intramural nerve fibers and subsequent release of noradrenaline. Morphine-like drugs reduce the amplitude of these muscle contractions in a dose-dependent manner and are more effective when low frequencies of stimulation and submaximal voltage are employed (Henderson et al., 1972; Hughes, Kosterlitz and Leslie, 1975; Henderson and Hughes, 1976). These investigators showed that the output of noradrenaline in the presence of morphine is reduced in amounts and with a time couse which parallels the inhibition of coutractions. The only report contradictory to

their findings is by Jenkins, Marshall and Nasmyth (1975) who failed to measure reduction of noradrenaline output during blockade of twitch amplitude by morphine.

North and Henderson (1975) and Henderson and North (1976) recorded the excitatory junction potentials (e.j.p.) from the muscle cells of mouse vas deferens using intracellular electrodes. Morphine, normorphine and levorphanol reduced the amplitude of the e.j.p. smoothly with time. The latency of the potentials, resting membrane potential and input resistance were not affected by the narcotics.

Narcotics do not affect spontaneous release of noradrenaline in this preparation. Henderson (1976) found no change in amplitude or frequency of miniature excitatory junction potentials in the presence of normorphine or met-enkephalin. The resting outflow of tritium-labelled catecholamines is unaffected by morphine (Hughes et al., 1975) or met- or leu-enkephalin (Segawa, Murakami, Ogawa and Yajima, 1975).

Inhibition of twitch or e.j.p. amplitude was not due to post-synaptic blockade since responses to exogenous noradrenaline were not affected (North and Henderson, 1975; Jenkins et al., 1975).

The effects of opiates described above are mediated by narcotic receptors since they are all antagonized by naloxone, naltrexone or nalorphine (Henderson et al., 1972; Hughes et al., 1975; North and Henderson, 1975; Jenkins et al., 1975; Henderson and North, 1976) and because the receptors have stereospecific sensitivities (Hughes et al., 1975; North and Henderson, 1975; Henderson and North, 1976).

High sensitivity of the vas deferens to narcotics is restricted

to the mouse. Hughes et al. (1975) found that preparations from the rabbit, guinea pig, cat, rat, hamster and gerbil were not sensitive to depression by morphine. Actually, sensitivity to opiates varies with the strain of mouse. Henderson and Hughes (1976) observed that contractions of the vas deferens from TO mice were depressed by 5×10^{-8} M morphine whereas in preparations from C57/BL mice, 3 to 10 $\times 10^{-6}$ M of normorphine or morphine was required.

2.1.4 Heart preparations. Kosterlitz and Taylor (1959) studied the effect of morphine on inhibition of the sinoatrial node by vagal stimulation in vivo. In the cat, high doses of morphine slightly inhibited cardiac slowing to vagal stimulation, but had no effect in the guinea pig. However, in the rat and rabbit, doses of morphine 0.1 mg/Kg and higher produced an immediate, sharp decrease in slowing at stimulation rates of 3 to 15 pulses per second. At higher frequencies, the only action was to delay the onset of slowing. Nalorphine prevented and sometimes partially antagonized this action of morphine. The narcotic did not reduce resting heart rate except at very high doses.

Similar effects have been observed in isolated preparations. Intranodal electrical stimulation of the isolated rabbit SA node-right atrial preparation by voltages subthreshold for myocardial excitation, produces a biphasic chronotropic response (Kennedy and West, 1967): an initial increase in beat interval due to parasympathetic, cholinergic stimulation and a secondary decrease in beat interval due to sympathetic stimulation. Kennedy and West observed that 10^{-6} g/ml morphine inhibited slowing due to cholinergic stimula-

tion at low frequencies and 10^{-4} g/ml morphine reduced the adrenergic response only at high frequencies of stimulation. The latter effect was antagonized by atropine. Responses to exogenous acetylcholine or noradrenaline were not altered by morphine. These investigators concluded that the narcotic altered acetylcholine release from the nerve terminals, but could not eliminate an action on the fine, pre-synaptic terminals.

Montel and Stark (1973) focussed their attention on the effect of narcotics on adrenergic transmission in the isolated, perfused rabbit heart prepared with postganglionic nerves intact. In resting hearts both agonists such as morphine and meperidine and antagonists such as naloxone and levallorphan depressed contractile amplitude, but did not reduce resting output of noradrenaline measured flourometrically. Uptake of infused noradrenaline from the perfusate was blocked by all agonists tested and by levallorphan. This blockade of neuronal uptake of noradrenaline was potentiated by 10^{-4} M naloxone. However, this concentration of naloxone was too high for manifestation of antagonist action. Morphine and meperidine, in concentrations which blocked uptake, also reduced overflow of noradrenaline induced by sympathetic nerve stimulation.

Wong, Sullivan and Wetstone (1975) suggested that the myocardial depressant effect of morphine might be related to interference with calcium transport. In their study, decreasing the calcium concentration of the perfusate to rabbit Langendorf preparations potentiated the negative inotropic effect of morphine and inhibited the positive inotropic effect of ouabain. Also, morphine and ouabain were mutually antagonistic. Arrhythmias caused by ouabain in normal calcium were

prevented or antagonised by morphine. Wong et al. suggested that the antiarrhythmic effect of morphine might be due to prevention of accumulation of tissue calcium caused by the increase in transmembrane flux of calcium ions by ouabain.

2.1.5 Nerve preparations. The results on the effects of narcotics on nerve conduction vary with the concentrations of agonists and antagonists employed.

Cairnie and Kosterlitz (1962) found no effect of 4×10^{-5} M morphine on parameters such as conduction velocity, threshold for excitation or recovery of excitability in cat saphenous or rabbit vagus nerves in situ. Similarly Kosterlitz and Wallis (1964) found neither morphine or nalorphine to be effective in the sympathetic nerve to the cat nictitating membrane and hypogastric nerve in vivo or in vitro, or on the action potential recorded from isolated rabbit vagus. However, meperidine 10^{-4} g/ml reduced action potential amplitude in A, B, and C fibers or rabbit vagus nerves. In a study by Ritchie and Armett (1963), 5×10^{-3} M morphine or nalorphine had no effect on the action potential or membrane potential of desheathed bundles of C fibers from rabbit vagus nerve, but antagonized the depolarizing action of acetylcholine on the nerve axons.

Staiman and Seeman (1974) compared the effects of various tertiary amines (e.g. procaine, enantiomers, haloperidol, methadone and naloxone) on action potential amplitude in rat phrenic nerve and frog or rat sciatic nerve. Reduction in amplitude occurred with 10^{-3} to 10^{-4} M concentrations of these drugs. Their potency correlated with their membrane/buffer partition coefficients. Also,

smaller myelinated nerves were more sensitive to depression. Staiman and Seeman pointed out that smaller diameter fibers have a lower safety factor for cable transmission and a smaller length constant. According to Seeman, Chau-Wong and Moyyen (1972), the threshold concentration required to block impulse conduction in rat phrenic nerve did not differ for dextro- and levo-methadone and was only 1.5 to 2 times greater for dextrorphan compared to levorphanol.

Narcotics also depress conduction in squid giant axon (Simon and Rosenberg, 1970; Frazier, Murayama, Abbott and Narahashi, 1972; Frazier, Ohta and Narahashi, 1973) and in axons from walking legs of the spider crab and lobster (Simon and Rosenberg, 1970). Morphine blocks both the peak transient (g_{Na}) and the late steady-state (g_K) components of the ionic conductance during an action potential. In this respect narcotics ressemble local anaesthetics in action.

Frazier et al. (1972) observed that the onset of the action of morphine was much faster when the drug was applied directly to the internal surface of the squid axon membrane. Therefore, they concluded that the receptor for the narcotics is on the inside surface of the membrane. Simon and Rosenberg (1970) noted that levorphanol was considerably more effective at pH 8 than pH 6. Also, penetration of levorphanol into the membrane was greater at pH 8. This may have been related to the higher concentration of uncharged free base molecules at the more alkaline pH which would be expected to penetrate the membrane because of their lipophilic nature.

Lower concentrations of levorphanol blocked repetitive activity in squid axons initiated by lowering the Ca^{++} and Mg^{++} concentrations. Simon and Rosenberg (1970) suggested that levorphanol might substitute for calcium to some extent.

The above investigators concluded that the action of narcotics in nerve is nonspecific and not mediated by opiate receptors; the action is not stereospecific, i.e. levorphanol and dextrorphan are equipotent (Simon and Rosenberg, 1970); similar concentrations (10^{-3} M) of narcotic antagonists levallorphan, naloxone and M5050 also depress action potential conduction by inhibiting both g_{Na} and g_K (Frazier et al., 1973); the actions of antagonists and agonists at these concentrations are additive instead of antagonistic.

In contrast, other investigators discovered that lower concentrations of antagonists would prevent the action of narcotic agonists in nerve preparations. Krivoy (1960) found that various narcotics including meperidine and morphine decreased the response of frog sciatic nerve in vitro to tetanic stimulation. A combination of morphine and the antagonist levallorphan at equimolal concentrations (10^{-4} g/ml) was additive, but lower concentrations of levallorphan (10^{-7} g/ml) antagonized the action of morphine. Recently, Hunter and Frank (1979) have shown that meperidine reduces amplitude, the rate of rise and the rate of fall of the action potential recorded by the sucrose gap technique from isolated frog sciatic nerve. Naloxone 3×10^{-8} M antagonized the effect of meperidine on amplitude and rate of rise and potentiated the depression of rate of fall.

Jurna and Grossman (1977) also obtained evidence that the action of narcotics on the action potential of nerve axons is mediated by opiate receptors. In this study morphine increased the amplitude in Aβ fibers and depressed the amplitude in Aδ and C fibers of cat sural nerves in situ. A ten fold lower concentration of naloxone

antagonized these effects. Naloxone also antagonized the increase in amplitude of the after-hyperpolarization and the increase in refractory period induced by morphine in isolated sural nerves and rabbit and guinea pig vagus nerves.

Narcotics do not alter membrane potential in any of the nerves studied (Kosterlitz and Wallis, 1964; Simon and Rosenberg, 1970; Frazier et al., 1972; Jurna and Grossman, 1977; Hunter and Frank, 1979).

2.1.6 Skeletal muscle preparations. Pinsky and Frederickson (1971) tested the effect of morphine on isolated skeletal nerve-muscle preparations to determine whether the effect of narcotics on cholinergic synapses is universal and to look for a simpler synaptic model for determining the mechanism of this action.

In both the rat phrenic nerve-diaphragm and frog sciatic nerve-sartorius muscle preparations, morphine in concentrations greater than 5×10^{-5} M depressed the amplitude of muscle twitches induced by nerve stimulation. Similar concentrations of nalorphine were also depressant and augmented rather than antagonized the action of morphine. The depression of twitch amplitude by either drug was sometimes preceded by an increase in twitch tension. This facilitation was not due to an anticholinesterase action since it occurred in the presence of high concentrations of physostigmine.

Since the evoked release of acetylcholine was reduced in the presence of morphine, Frederickson and Pinsky (1971) concluded that the decrease in indirectly stimulated twitch amplitude was due to blockade of transmitter release.

A postsynaptic action of morphine was not likely since morphine potentiates the response of isolated frog rectus abdominis to exogenous acetylcholine. On the other hand, meperidine depresses responses of this tissue to acetylcholine (Hebb and Konzett, 1949). In addition, Frederickson and Pinsky (1971) found that the graph relating initial twitch tension to the logarithm of initial acetylcholine release was shifted to the left in the presence of morphine, indicating that the action of acetylcholine with its receptors was facilitated. Turlapaty, Rasmaswamy, Yayasunder and Ghosh (1977) observed that incubation of frog rectus preparations with morphine, for either 15 or 120 minutes, augumented contractions to exogenous acetylcholine. That is, morphine decreased the ED50 of acetylcholine; however, it also decreased the maximum contractile response to acetylcholine. In the presence of physostigmine, morphine caused no further reduction in ED50 relative to physostigmine alone. They concluded that the effect of morphine on ED50 for acetylcholine at 15 minutes was due to its anticholinesterase action. The effects on maximum contractile strength and ED50 at 120 minutes were antagonized by increasing the calcium concentration. Morphine also decreased maximal contractile responses to carbachol after 15 or 120 minutes, but decreased the ED50 for carbachol only after 120 minutes.

The inhibition of twitch amplitude observed by Pinsky and Frederickson was not likely due to an action on the muscle membrane since responses to direct stimulation of chronically denervated frog sartorii were not affected by the narcotic. According to Bell and Rees (1974), only higher concentrations of opiate drugs depress responses to direct muscle stimulation.

The amplitude of the endplate potential in the presence of morphine or nalorphine was depressed in a gradual manner with dose (Pinsky and Frederickson, 1971). Therefore, they concluded that a local anaesthetic-like action on the nerve was not likely the mechanism of action of these drugs since this type of action would at some point be all-or-none. Smaller doses of morphine or nalorphine slightly increased endplate potential amplitude (Pinsky and Frederickson, 1971).

The question remains, are these effects of narcotics on skeletal nerve-muscle preparations just discussed mediated by true opiate receptors? Bell and Rees (1974) concluded that they were not since there was no significant difference in potency between dextro- and levo-moramidine, and since naloxone did not antagonize morphine. Instead, they found naloxone to be a more potent depressor than morphine and to be synergistic with morphine in its action. However, they do not state the concentrations of naloxone used in their study. Bell and Rees also found that the potency rank of a variety of narcotic drugs was different from the order in guinea pig ileum, but their results agreed with those of Soteropoulos and Standaert (1973) using the cat soleus muscle preparation *in vivo*.

The *in vivo* cat soleus neuromuscular preparation has been used to measure drug effects on motor nerve terminals, *i.e.* small, unmyelinated nerves. The potentiation of twitch contractile strength following nerve stimulation at tetanic frequencies (PTP) is caused by post-tetanic repetitive firing in soleus nerve terminals (Standaert, 1964). In the study by Soteropoulos and Standaert (1973), morphine or naloxone injected into the popliteal artery diminished both the strength

of the initial tetanus and PTP. Both drugs also depressed the twitch height in the absence of tetanus. Smaller concentrations produced depression followed by a slowly developing potentiation of twitch tension similar to the action of other neuro-depressants such as barbiturates. When a tetanic stimulus was applied while neuromuscular blockade still persisted, the tetanus was followed by immediate recovery of transmission. Soteropoulos and Standaert suggested that morphine and naloxone depressed PTP by acting on the motor nerve terminals to suppress the [illegible]ntials of the unmyelinated nerve endings and prevent the development of generator potentials, i.e. by stabilizing the nerve membrane.

Another interesting finding from their study was that lower concentrations of naloxone administered intravenously, which had no effect on PTP or twitch tension, partially antagonized the depression [illegible]TP caused by morphine and shortened the duration of the depression [illegible] twitch amplitude after intraarterial injection of morphine. Thus, in skeletal muscle, naloxone has both agonist and antagonist actions.

This dual action of naloxone was shown clearly by Frank (1975a) and Frank and Buttar (1975), who found that morphine (10^{-3} M), meperidine (10^{-4} M), and naloxone (10^{-3} M) depressed the amplitude of the compound action potential recorded extracellularly from frog sartorius muscles. However, much lower concentrations of naloxone (3×10^{-8} to 3×10^{-7} M) antagonized the depressant effects of morphine and meperidine on action potential production. A specific action on opiate receptors was indicated since naloxone failed to antagonize the depressant effects of procaine, tetrodotoxin or dextromethorphan (Frank and Marwaha, 1978, 1979). These low concentrations of naloxone

had no effect when administered alone.

In a study using intracellular electrodes (Frank, 1975b), it was shown that meperidine depresses the action potential in frog sartorius muscle fibers by two mechanisms; one, an initial, nonspecific action which is manifested by a decrease in both sodium and potassium conductances, and a second, opiate receptor mediated mechanism causing a specific depression of the sodium conductance. Only this second mechanism is antagonized by low concentrations of naloxone.

2.2 Characteristics of Synapses Sensitive to Inhibition by Narcotics.

Why are some synapses more sensitive to inhibition by narcotics? As discussed in the previous sections, opiates are more effective when low frequency, submaximal stimulation is employed. Also the output of neurotransmitter (either cholinergic or adrenergic) per stimulus pulse differs at junctions which are very sensitive to these drugs compared to synapses which require high concentrations of opiates for manifestation of depression.

In the guinea pig ileum, the output of acetylcholine per pulse decreases considerable as frequency of stimulation is increased, insuring a high output of the transmitter at low frequencies (Paton, 1957, 1963; Cowie et al., 1968). In the rabbit ileum, which is considered an insensitive preparation, a similar relationship holds, but the total amount of acetylcholine released per pulse is 10 times less than in guinea pig and the acetylcholine content per unit weight is only 15% of that of the guinea pig (Kosterlitz et al., 1973).

In the cat nictitating membrane, the output of noradrenaline per pulse is almost constant, but high, at frequencies up to 15 Hz

(Henderson et al., 1972; Henderson and Hughes, 1976). Henderson and Hughes (1976) observed that in vas deferens of TO mice, which are very sensitive to depression by morphine, the output of noradrenaline per pulse is constant, but in vas from C57/BL mice, which is less sensitive, output per pulse increases with frequency. At adrenergic synapses considered insensitive to depression by morphine such as in guinea pig ileum or rat vas deferens and portal vein, output of noradrenaline per pulse increases about ten times over frequencies of 0.5 to 16 Hz.

It has been suggested that there are multiple mechanisms for transmitter release control at both cholinergic (Paton, 1963; Cowie et al., 1968) and adrenergic (Henderson et al., 1972) synapses. Cowie et al. proposed that evoked output of transmitter in guinea pig ileum is higher at low frequencies of stimulation because of a subsidiary, booster mechanism which operates as low frequencies. This booster mechanism is in addition to a basal component which leads to a constant output per volley at all frequencies. The booster component is depressed by morphine, adrenaline, Mg^{++} and cooling the preparation. Alternatively, Paton (1963) proposed that there are two independent mechanisms involved in the release of acetylcholine: one is common to both high and low stimulus frequencies and is morphine insensitive; the other operates only at low frequencies to provide a constant output of transmitter per unit time rather than per pulse.

However, in general it can be stated that morphine is more effective at synapses in which the output of neurotransmitter is high at low frequencies of stimulation.

Differences in receptor density or receptor characteristics also might contribute to variation in the response of synapses to narcotics. The main reason the action of narcotics often has been termed non-specific at many neuroeffector junctions is they are not antagonized by high concentrations of naloxone. It often is assumed that if the concentration of agonist must be increased to obtain an effect in these preparations, the concentration of antagonist must similarly be increased. However, from the evidence discussed in section 2.1.6 above, it appears that although these preparations are less sensitive to agonists, they display a high sensitivity to antagonists.

It is not certain whether opiate agonists, dual acting antagonists (drugs which can behave either as agonists or antagonists) and narcotic antagonists interact with a single type of opiate receptor or if there is more than one population of opiate receptors. Martin (1967) proposed that there are two types of opiate drug receptors. At one, full agonists act to produced an effect and partial agonists such as nalorphine produce only antagonism; the other only reacts to partial agonists. The more specific antagonists such as naloxone act at both sites. Smits and Takemori (1970), Takemori, Hayashi and Smits (1972) and Takemori, Oka and Nishiyama (1973) proposed that narcotic and dual acting narcotic antagonists have different binding sites on the same receptor in addition to a common site of attachment: the protonated nitrogen group. Martin, Eades, Thompson, Huppler and Gilbert (1976) proposed three types of receptor which he termed μ, κ and σ on the basis of the actions of narcotic agonists in the non-dependent and morphine-dependent spinal dog.

Knoll, Fürst and Makleit (1977) believe there are two types of narcotic receptors. According to their theory, cholinergic, peripheral synapses are prototypes for A receptors and adrenergic synapses are prototypes for B receptors. According to Creese and Snyder (1975), there are two distinct populations of binding components which have different affinities for dihydromorphine. At the moment the question of how many receptor-types exist remains open.

2.3 Postulated Mechanisms of the Inhibitory Action of Narcotics on Neurotransmission

Although it is accepted that narcotics depress release of transmitter to nerve stimulation by an action on presynaptic opiate receptors, the exact mechanism of this inhibition remains obscure. Morphine-like drugs may act either on action potential production in the nerve terminals or directly on transmitter release mechanisms.

The strongest evidence for an effect on action potential production comes from the electrophysiological studies using Auerbach's plexus-longitudinal muscle preparations of the guinea pig ileum (section 2.1.1) in which narcotics have been shown to block conduction of action potentials and to hyperpolarize the membrane potential of ganglion cells. However, these findings do not exclude an additional action on transmitter release mechanisms from postganglionic cell processes. It also is difficult to explain the frequency dependence of narcotic drug effects on the depression of transmitter release or twitch height by a mechanism on nerve conduction (North and Tonini, 1976). Also, the morphine-induced depression of the intracellularly recorded e.j.p. in vas deferens (Henderson and North, 1976) and the EPP in frog sartorius muscles (Pinsky and

Frederickson, 1971) is smooth and gradual with time. If narcotics were blocking initiation of action potential production in the unmyelinated nerve terminal fibers, this depression would be all-or-none at some point, especially in sartorius which has single synaptic connections on each of the two end-plates on each muscle fiber.

If narcotics act on a chemical process involved in transmitter release, this mechanism must be common to all sensitive preparations regardless of the identity of the transmitter. The importance of calcium ions in a wide variety of secretory systems as a link in stimulus-secretion coupling is well documented (Rubin, 1970). Thus it has been postulated that narcotics affect calcium mobilization resulting from nerve stimulation.

The sensitivity of peripheral preparations is inversely related to the calcium concentration of the Kreb's solution. In guinea pig myenteric plexus-longitudinal muscle strips, lowering the calcium concentration increases morphine's effect on the evoked twitch amplitude (Heimans, 1975; Opmeer and Van Ree, 1979). Morphine is less effective with high external calcium concentrations and adding calcium to the bathing solution, after established blockade by morphine, dose-dependently diminishes the response to morphine. This relationship between morphine and calcium ressembles competitive antagonism according to Lineweaver-Burk analysis (Opmeer and Van Ree, 1979), but these authors state that not only an effect of calcium on the interactions of morphine with its receptors, but all inhibitory actions of calcium on any process between receptor activation and the measured response which lead to an 'apparent affinity reduction' are classed

as competitive. They postulated that morphine inhibits transmitter release by altering calcium ion distribution in neurones.

In the study by Puig et al. (1978), the inhibition of twitch height by endogenous endorphins, released by tetanic stimulation of guinea pig ileum, displayed the same calcium dependency.

In a study of transmitter release in adrenergic systems, Henderson and Hughes (1974) observed that increasing the external calcium concentration preferentially increased noradrenaline output at low rates of stimulation and decreasing the amount of calcium preferentially decreased output at low frequencies. In preparations sensitive to depression by narcotics, the mouse vas deferens and cat nictitating membrane, in which the normal output of noradrenaline per pulse is constant over a large frequency range, increasing the external calcium results in a decrease in transmitter output per pulse with increasing frequency. In low calcium the output per pulse increased with frequency, an effect which is similar to the action of morphine.

In heart muscle, Wong et al. (1975) demonstrated that lowering the external calcium concentration increased the negative inotropic effect of morphine and morphine antagonized the action of ouabain, which is believed to act on calcium fluxes. In frog rectus abdominis, the decrease in contractile amplitude to exogenous acetylcholine is antagonized by increasing the external calcium concentration (Turlapaty et al. (1977).

In rabbit and guinea pig vagus nerve preparations in vitro, decreasing the external calcium slightly depolarizes the membrane perhaps by increasing sodium permeability (Jurna and Grossman, 1977). This effect was blocked by local anaesthetics, but not by morphine; however, morphine was relatively more effective in depressing the

action potential amplitude in calcium-free media (Jurna and Grossman, 1977).

In low calcium and magnesium solutions, squid axons fire repetitively to single stimuli, an action which is blocked by levorphanol (Simon and Rosenberg, 1970). These authors suggested that levorphanol might substitute to some extent for calcium and, in calcium containing solutions compete for calcium binding sites. On the other hand, Dingledine and Goldstein (1976) found morphine to be virtually as effective in inhibiting extracellularly recorded spontaneous activity in ganglion cells of Auerbach's plexus in calcium free Ringer's as in normal calcium.

Evidence for an interaction between calcium and narcotics has also emerged from studies of whole animals and brain tissue. Elevating brain Ca^{++}, Mg^{++} or Mn^{++} levels has been found to antagonize analgesia produced by narcotic drugs (Kakunaga, Kaneto and Hano, 1966; Harris, Loh and Way, 1975b) and prevent the inhibition of release of neocortical acetylcholine induced by morphine (Sanfacon, Houdi-Depuis, Vanier and Labreque, 1977).

Acute administration of morphine or levorphanol reduces rat brain calcium content (Ross, Medina and Cardenas, 1974; Cardenas and Ross, 1975; Yamamoto, Harris, Loh and Way, 1978) an action which is reduced by naloxone and is stereospecific (Cardenas and Ross, 1975). Evidence has been presented which indicates that this depletion of calcium is observed in nerve ending synaptic vesicle fractions of brain homogenates (Ross, Lynn and Cardenas, 1976; Harris, Yamamoto, Loh and Way, 1977; Yamomoto et al., 1978). This localization of the calcium

depletion correlates with the localization of opiate receptor binding material in brain homogenates (Terenius, 1973; Pert, Snowman and Snyder, 1974).

The analgesic effect of morphine is potentiated by the calcium chelator EGTA but not by EDTA which chelates both calcium and magnesium, indicating that calcium is more important than magnesium in the action of opiates (Harris et al., 1975b). The kinetics of this action also ressemble competitive inhibition. The ionophore X537A, which increases the permeability of membranes to calcium and thus promotes stimulus-secretion coupling, antagonizes analgesia to morphine in mice. This implies that the antagonistic effect of calcium is dependent upon the ion being able to penetrate the membrane and that morphine might inhibit calcium fluxes across cell membranes (Harris, Loh and Way, 1975b). In studies by Harris, Loh and Way (1976) and Iwamoto, Harris, Loh and Way (1978), the calcium antagonist lanthanum potentiated morphine analgesia in mice and was antinociceptive when administered alone. This analgesic action of lanthanum was reduced by naloxone and the neuroanatomical distribution of lanthanum in the brain was similar to morphine, i.e. the periaqueductal grey. Lanthanum displayed partial but incomplete cross-tolerance with morphine and suppressed withdrawal in dependent mice to withholding morphine or administration of naloxone (Harris, Iwamoto, Loh and Way, 1975a).

Calcium and magnesium inhibit the stereospecific binding of narcotics to rat brain homogenates (Pert and Snyder, 1973, 1974). In addition, morphine alters the binding of calcium to neuronal phospholipids and gangliosides and to synaptosomal plasma membranes (Sanghvi and Gershon, 1977). Morphine has also been shown to form complexes

with calcium and magnesium in vitro (Lin, Sutherland and Way, 1975). Calcium has higher affinity for morphine than does magnesium. These observations support a direct interaction between calcium and narcotics.

Harris et al. (1977) and Yamamoto et al. (1978) postulated that acute administration of morphine depletes vesicular calcium by inhibiting transmembrane fluxes of the ion and thus depressing transmitter release. Yamomoto et al. (1978) suggested that the sites involved should be localized on the inner surface of the nerve ending membrane. This site would be ideally suited to function as part of a "pump" mechanism to maintain very low levels of free calcium inside the nerve endings. With chronic administration, the vesicular calcium would be replenished and eventually increased above control levels by some homeostatic mechanism. This alteration in calcium balance could explain cross-tolerance with lanthanum (Yamamoto et al., 1978).

Although many antagonistic interactions between calcium and narcotics have been reported, this antagonism is not complete. In nerve and muscle fibers, decreasing the calcium concentration renders the membranes hyperexcitable (section 1), whereas narcotics depress excitability in nerve and muscle.

Ehrenpreis, Greenberg and Belman (1973), and Ehrenpreis, Greenberg and Comaty (I975) have postulated that E prostaglandins are involved in the mechanism by which acetylcholine is released during electrical stimulation and that the effect of narcotics on neurotransmission is mediated by a prostaglandin receptor.

Using the guinea pig whole ileum and longitudinal muscle preparations, Ehrenpreis et al. (1973) demonstrated that PGE_1 and PGE_2

reverse the blockade of electrically induced twitch contractions by morphine, methadone, levorphanol and meperidine. When low concentrations of prostaglandin were employed, this antagonism was competive, but with higher concentrations of morphine it became noncompetitive. PGE_1 and PGE_2 also enhanced the effectiveness of naloxone in reversing narcotic-induced blockade (Ehrenpreis et al., 1975). These investigators argued that this action of prostaglandins is specific to narcotics since these agents did not antagonize twitch-blockade induced by catecholamines, local anaesthetics or barbiturates.

Although prostaglandins can contract smooth muscle E-type prostaglandins do not likely antagonize narcotics by a postsynaptic action since responses to exogenous acetylcholine are little affected (Ehrenpreis et al., 1975) and since PGE_1 and PGE_2 only variably and minimally increase the height of electrically induced twitches (Ehrenpreis et al., 1973; Durham, 1975). Durham (1975) found that PGE_1 very effectively reversed tetrodotoxin blockade of electrically induced twitches in Auerbach's plexus-longitudinal muscle preparations of guinea pig ileum indicating a presynaptic action. Morphine (Jaques, 1969; Sanner, 1971) and met- and leu-enkephalin (Jaques, 1977) antagonize PGE-induced contractions in ileum and spontaneous contractions and increases in tone when they occur. According to Sanner (1971), prostaglandins stimulate the guinea pig ileum by both neural and direct mechanism.

Prostaglandin antagonists were also found by Ehrenpreis et al. (1973) to block contractions of guinea pig ileum. Both receptor blockers such as SC19220 and oxa-13-prostanoic acid and inhibitors of prostaglandin synthetase, i.e. indomethacin, block contractions

induced by electrical stimulation, an effect which is reversed by PGE_2. Ehrenpreis et al. also state that these actions of PGE and its inhibitors are confined to preparations which are sensitive to morphine.

Further evidence for an interaction between prostaglandins and opiates was found by Ferri, Santagostini, Braga, and Galatuas (1974) who reported that PGE_1 antagonized morphine analgesia in rats.

The link between prostaglandins, morphine and calcium must also be considered. Guanidine behaves very much like prostaglandins in the guinea pig ileum preparation (Durham, 1975). One of the similarities is that guanidine reversed morphine-induced blockade of twitches. However, it was concluded that guanidine does not affect synthesis and release of prostaglandin since the increase in twitch height in the presence of guanidine was not blocked by indomethacin. It is also interesting that both guanidine and prostaglandins can reverse tetrodotoxin blockade and that calcium is important in regulating responses to both agents (Northover, 1971; Durham, 1975). Kirkland and Baum (1972) stated that prostaglandin activity could be interpreted in terms of facilitation of movement of calcium ions into or out of biological membranes. They found evidence for marked facilitation of non-energized binding of calcium ions to mitochondrial membranes in the presence of PGE_1.

Cyclic 3',5'-adenosine monophosphate (cAMP) has been implicated as an intracellular mediator in both release of neurotransmitters presynaptically and in regulating the response of the postsynaptic membrane to stimulation (Clouet and Iwatsubo, 1975). Levels of cAMP are increased by prostaglandins which stimulate adenylate cyclase, the enzyme for its formation from ATP. Inhibition of PGE-sensitive

adenylate cyclase has been postulated as the mechanism of action of narcotics (Collier and Roy, 1974a, 1974b). These investigators reported that PGE_1 or PGE_2 stimulated the formation of cAMP in rat brain homogenate and that morphine-like drugs inhibited this stimulation without inhibiting the basal production of cAMP in the absence of prostaglandins. They found the action of narcotics was stereospecific and reversed by naloxone.

Neuroblastoma x glioma hybrid cells in culture also contain a PGE-sensitive cyclase which is inhibited by morphine (Traber, Fischer, Latzin and Hamprecht, 1974, 1975; Sharma, Nirenberg and Klee, 1975) and by enkephalins (Brandt, Gullis, Fischer, Buchen, Hamprecht, Moroder and Wunsch, 1976; Goldstein, Cox, Klee and Nirenberg, 1975) in a noncompetitive manner. Morphine also increases cGMP levels in these cells (Gullis , Traber and Hamprecht, 1975). These are all narcotic effects since they are stereospecific and antagonized by naloxone. However, morphine does not affect noradrenaline-sensitive cyclase (Traber et al., 1974).

In brain tissue, the effect of opiates on cAMP levels is not clear cut. There is no agreement in the responses reported (see Sanghvi and Gershon, 1977). Collier and Roy (1974ab) observed that morphine depressed PGE-stimulated cyclase in rat striatal slices but Katz and Catravas (1977) did not. Havemann and Kuschinsky (1978) suggested that under some experimental conditions such as with a large amount of damaged surface as occurs in homogenated tissue, endogenous activators of cAMP such as adenosine might mask the inhibitory effect of opiates on cAMP synthesis by PGE.

Clouet and Iwatsubo point out that other aspects of the regula-

tion of adenylate cyclase activity should be considered in relation to opiates such as the role of calcium. Morphine may inhibit adenylate cyclase activity either independently of its action on calcium transport or directly by reducing formation of calcium-regulator complex which may be essential for activation of cyclase (Sanghvi and Gershon, 1977).

Certain adenine nucleotides mimic the effect of morphine in that they inhibit twitch responses of electrically stimulated guinea pig myenteric plexus-longitudinal muscle preparations (Takagi and Takayanagi, 1972; Gintzler and Musacchio, 1975; Sawynok and Jhamandas, 1976). Gintzler and Musacchio suggested that noradrenaline induced inhibition of responses to field stimulation is mediated by formation of cAMP. Since morphine, ATP and adenosine potentiated the effects of noradrenaline in their study, they suggested that these agents might increase the ability of noradrenaline to stimulate cyclase or interact with cyclase in a similar manner.

Sawynok and Jhamandas (1976) found that theophylline, which inhibits adenosine and phosphodiesterase, antagonized the depression of twitches induced by adenosine, ATP, cAMP and dibutyryl-cAMP and also morphine. However, naloxone did not antagonize nucleotide-induced depression indicating the nucleotide and narcotic receptors are separate entities. These studies indicate opiates would increase, rather than decrease, cAMP levels, but as stated by Sawynok and Jhamandas (1976), other consequences of opiate action such as alteration in calcium fluxes have not been ruled out. At the moment the role of cyclic nucleotides in the action of narcotics remains controversial.

2.4 Preparations Sensitive to the Excitatory Actions of Narcotics

In contrast to the numerous inhibitory actions of narcotics, these are excitatory in some systems and species. Although morphine depresses motility of the small intestine in guinea pigs, it increases both tone and motility in the gastrointestinal tract of the dog and rat, and man resulting in delayed evacuation of the contents. Spasm in the various sphincters such as the pyloric sphincter after administration of narcotic drugs also contributes to the constipating effect (references cited in Clouet, 1971).

The effect of narcotics on intestinal motility has been extensively studied in the dog small intestine *in vivo* or *in vitro* by Burks and Long (1967ab), Burks (1973), De Oliviera and Bretas (1973) and Burks and Grubb (1974). These investigators proposed that morphine causes an increase in tone and rhythmic activity and loss of propulsive activity by releasing neuronal 5-HT. The 5-HT would act as a mediator for the effects of morphine by releasing acetylcholine which is directly responsible for the contractile effect. This action of narcotics is mediated by opiate receptors since they are significantly attenuated by naloxone (Burks and Long, 1974) and are stereospecific (Burks and Long, 1967b). Similar effects of morphine were observed by Burks (1976) in rat intestine *in vivo*.

Opiates can also cause constriction in vascular smooth muscle. Narcotics induce dose-related contractions of isolated rat aortic strips (Lee and Berkowitz, 1976). These authors concluded that the contractions were not mediated by adrenergic or cholinergic mechanisms, histamine or prostaglandins. L-isomers are 5 times more potent than d-isomers and the effects of l-pentazocine are reversed

by naloxone, by high concentrations of either isomer of methadone and also by the calcium antagonist verapamil.

The relevant finding to this thesis from these studies of the excitatory effects of opiates is that the calcium dependency of their actions is opposite to the calcium dependency found in preparations in which the morphine-like drugs are inhibitory, i.e. decreasing the calcium concentration antagonizes the excitatory actions of narcotics, but potentiates their inhibitory actions.

In the brain, morphine decreases the spontaneous release of acetylcholine in species such as the rat (Jhamandas and Sutak, 1974) but increases release from cat cerebral cortex (Jhamandas, Phillis and Pinsky, 1971). The increase in acetylcholine output is concomitant with excited motor and behavioural responses in these animals (Phillis, Mullin and Pinsky, 1973).

CHAPTER II. STATEMENT OF THE PROBLEM

As discussed in the introduction, many hypotheses have been proposed to explain narcotic analgesia and the effects of opiates on synaptic transmission. These drugs may act by inhibiting action potential production in nerve terminal fibers or by inhibiting some stage of the transmitter release mechanism.

The purpose of this thesis was to study the action of a narcotic (meperidine) on a more simply innervated preparation, in which activity on the presynaptic nerve action potential, on the transmitter release mechanism and on the postsynaptic membrane can be studied separately; the frog sciatic nerve-sartorius muscle preparation.

The questions to be answered were:

1) Is there a true narcotic receptor at the skeletal neuromuscular junction or is the depression of transmission nonspecific?
2) By what mechanism do narcotics depress neuromuscular transmission in this preparation:
   (i) by preventing the conduction of the action potential in the fine, unmyelinated nerve terminals,
   (ii) by depressing a phase of the acetylcholine release mechanism,
   or (iii) by preventing the action of acetylcholine on the subsynaptic region of the muscle?

CHAPTER III. MATERIALS AND METHODS

1. Biological Preparations

All experiments were performed on tissues isolated from frogs of the species Rana pipiens.

When the sartorius muscle alone or with sciatic nerve attached was to be employed, animals were sacrificed by a sharp blow on the head and then pithed. When, in addition, spinal roots were required, the frogs were anaesthetized by intraperitoneal injection of 1 ml of a 10% urethane solution. All frogs were stripped of the skin from the trunk and hindlimbs. Tissues were dissected with the aid of a Zeiss operating stereomicroscope. Usually two preparations were removed from each frog.

Preparations were mounted at approximately resting length in the appropriate bath containing Ringer's solution at room temperature. Forty-five minutes to one hour were allowed for equilibration of the tissues with the bath solution before experimental procedures were commenced.

1.1 Sartorius Muscle Preparation. Using a sharp scalpel, the pelvic girdle was split along its midline from ventral to dorsal surface. Care was taken to maintain the integrity of the tendon-sartorius attachment. The tendon at the distal end of the muscle was ligated and cut. Then the sartorius was dissected free by carefully cutting along the connective tissue joining it to the surrounding muscles. At the pelvic origin a sliver of bone was left attached to the tendon to give support to the preparation.

1.2 Sciatic Nerve-Sartorius Muscle Preparation. To expose the sciatic nerve on its dorsal surface, an initial cut was made with a pair of scissors along the outside edge of the coccygeo-ileacus muscles. Then all dorsal muscles and the urostyle were removed from the pelvic girdle to the level of the spinal column. The sciatic nerve was severed at its bifurcation and dissected free to its entrance into the hindlimb. The sartorius was removed up to the point of entrance of the nerve into the muscle by the method described previously. The remainder of the innervation was exposed from the ventral side by blunt dissection and, starting from the sciatic trunk, was cut free from surrounding tissue to its entrance into the muscle. Then dissection of the sartorius muscle was completed.

When electrical activity was to be recorded from the endplate region, the connective tissue on the dorsal surface of the muscle was gently teased away using extra fine jewelers forceps.

1.3 Ventral Root-Sartorius Muscle Preparation. The vertebral column was exposed by removing the muscles on the dorsal surface of the frog and removing the urostyle. The sharp point of a pair of scissors was inserted into the spinal canal and a cut was made gradually along the midline of the spinal column. The bone on both sides of this cut was chipped and cut away, exposing the spinal cord up to about the level of the fifth spinal nerves. The VIII vertebra was removed further to expose the VIII spinal roots. These roots were cut at their entrance to the spinal cord and carefully dissected from the caudal vertebral notch. The IX spinal nerve was sectioned at the level of the

sciatic nerve bifurcation. Dissection proceeded as described for the sciatic nerve-sartorius muscle preparation.

2. Solutions

All salts used in the Ringer's solution were A.C.S. or Analar Standard Reagent Grade chemicals. They were dissolved in double distilled, deionized water. The basic Ringer's solution was composed of: NaCl, 111.8 mM; KCl, 2.47 mM; $CaCl_2.2H_2O$, 1.08 mM; $NaH_2PO_4.2H_2O$, 0.44 mM; $NaHCO_3$, 2.38 mM; and, dextrose 11.1 mM. D-tubocurarine chloride or $MgCl_2.6H_2O$ were added as indicated in the experimental methods. The pH of the solution was measured at 7.1 to 7.3. Addition of drugs to the basic Ringer's altered the pH by less than 0.1 units.

3. Drugs

The drugs used in this investigation were:

(a) acetylcholine chloride, supplied by J.T. Baker Chemical Co., molecular weight 181.7.

(b) meperidine hydrochloride, supplied by Winthrop Laboratories, molecular weight 283.8.

(c) naloxone hydrochloride, supplied by Endo Laboratories Inc., molecular weight 362.9.

(d) physostigmine sulphate, supplied by Sigma Chemical Co., molecular weight 648.8.

(e) tetraethylammonium chloride, supplied by BDH Chemicals, molecular weight 183.7.

(f) d-tubocurarine chloride, supplied by Nutritional Biochemicals Corp., molecular weight 785.8.

Throughout the text, concentrations of these drugs are expressed in Molarity. A concentrated stock solution of each drug was made using the bathing solution as solvent and was refrigerated. This solution was diluted to the desired concentration during the experiment.

4. Electrodes

Glass microelectrodes were drawn from open-ended capillary tubes (1.5 mm outside diameter) using a PN-3 Narashige Scientific Instruments horizontal electrode puller. For intracellular recording the electrodes were made to have resistances of 10 to 30 MΩ when filled with a filtered 3 M KCl solution. For extracellular recording use, the electrodes were pulled in the same manner to have resistances of 5 to 10 MΩ and were filled with 0.9% NaCl.

For filling, the electrodes were mounted on glass slides using elastic bands and placed in a covered Coplin staining dish containing sufficient electrolyte to submerge the electrode tips. An electric lamp placed directly over the container resulted in filling of the tips overnight by capillary action. The following day filling was completed using a tuberculin syringe with a long, small gauge needle attached.

For ventral root recording and sciatic nerve stimulation, bipolar electrodes were constructed using platinum wire.

Iontophoresis of acetylcholine onto the endplate required construction of double-barrelled micropipettes. To facilitate filling of these electrodes, glass tubing containing a thread of fiberglass along its length was employed. Two pieces of glass, approximately 5 cm in

length, were joined together at their ends with wax. They were held over a small flame gas burner and twisted around each other in the middle approximately 360° as the glass began to melt. Electrodes were drawn using the Narashige electrode puller to have a resistance in each barrel of approximately 100 MΩ when filled with the appropriate solution. One barrel was filled with 0.9% NaCl and the other with a 1 M acetylcholine solution using a tuberculin syringe with a long, small gauge needle attached. The electrode tips filled by capillary action along the thread of fiberglass.

5. Experimental Techniques

5.1 Recording of Muscle Tension Elicited by Sciatic Nerve Stimulation. The sciatic nerve-sartorius preparation was mounted in a double compartment bath as shown diagramatically in Figure 1(A). The muscle was bathed in Ringer's solution. The nerve was led into a second chamber filled with mineral oil and rested across a pair of platinum stimulating electrodes. The nerve was stimulated at supramaximal voltage with one msec pulse duration at a rate of two pulses per minute using a Grass S44 stimulator. Muscle tension was measured isometrically using a Grass force transducer (model no. FT03C) and recorded by a Brush Oscillograph (model 16-2308-00). Approximately 10 minutes were allowed to establish stable control responses before testing with drug-containing solutions.

5.2 Recording of Tension Elicited by Direct Muscle Stimulation. The sartorius muscle preparation was mounted in the bath shown in

Figure 1B. The electrode arrangement consisted of a circular platinum ring surrounding each end of the muscle. These electrodes were connected to the output of a Grass S44 stimulator which delivered pulses of supramaximal voltage and one msec duration at the rate of two per minute. Resulting muscle contractions were recorded as described in section 5.1.

These experiments were performed with the muscles in solutions containing 1.3×10^{-4} M d-tubocurarine which was shown previously to abolish responses to nerve stimulation.

5.3 Recording of the Spontaneous Muscle Activity Induced by TEA. Mounting of the sartorius muscle preparation and the recording method were as described in section 5.2. However, the bathing fluid was Ringers's solution without d-tubocurarine.

5.4 Recording of Ventral Root Electrical Activity. The ventral root-sartorius preparation was mounted in a double compartment bath (Figure 1A). The muscle was bathed in Ringer's solution, and the nerve compartment contained mineral oil. The sciatic trunk was placed over a bipolar stimulating electrode and a ground electrode. A second bipolar electrode, which could be used for either stimulating or recording, was placed on the ventral root (Figure 2). The viability of the preparation was tested by showing that stimulation of the ventral root elicited muscle contraction. Nerve activity caused by stimulation of the sciatic trunk or by the presence of tetraethylammonium (TEA) in the muscle compartment could be recorded antidromically from the ventral root.

In experiments utilizing nerve stimulation, the sciatic trunk was

stimulated with supramaximal voltage and one msec duration at a rate of four per minute during recording periods only. The nerve was not stimulated between recording periods.

Recordings of either spontaneous or nerve-elicited potentials were made at 5, 10, 15, and 30 minutes after placing the muscle in solutions with TEA and at 30 minute intervals throughout the remainder of the experiment. Only one experiment was performed on each muscle. Ventral root action potentials were amplified by an Argonaut LRA 045 differential preamplifier and displayed on a Tektronix 502 oscilloscope. Input to the oscilloscope was AC differential in an attempt to reduce the electrical noise level. Permanent records were made on film by a Nihon Kohden camera (model PC-2A) mounted over the oscilloscope screen.

5.5 Extracellular Recording of the Nerve Terminal Action Potential and the Endplate Potential. In these experiments (Figure 3) the muscle bath was a 5.5 cm diameter petri dish with a layer of clear Sylgard resin in the bottom. The sciatic nerve-sartorius preparation was mounted by pinning the connective tissue on the outer edge of the muscle to the Sylgard layer with small insect pins (size 0.20). The nerve was supported above the Ringer's solution by stimulating electrodes and kept moist by cotton wool soaked in mineral oil.

Using the method of Katz and Miledi (1965), both the nerve terminal potential and the endplate potential (EPP) were recorded extracellularly using NaCl-filled glass microelectrodes. The electrode was inserted into a WPI Instrument probe clamped to a Leitz micromanipulator. The probe was connected to the input of a WPI Instrument

Co. model 701 microprobe system amplifier. The reference electrode was a silver chloride-coated silver wire in the bath connected to the ground input of the amplifier. The EPP output signals were amplified and displayed by a 502 Tektronix oscilloscope and the potentials were photographed for analysis.

The sciatic nerve was stimulated at supramaximal voltage and 1.5 msec duration at a rate of 12 pulses per minute throughout the experiment. The endplate region was located by following a nerve to its ending visually with a microscope and manoevering the electrode across the surface of the muscle until suitable records were obtained upon nerve stimulation.

The muscle bath was constantly perfused with the appropriate Ringer's solution at a rate of about one ml per minute by means of the flow-through system. To exchange the solution in the bath the perfusion rate was increased to approximately 10 ml per minute and at least 40 ml of solution was flushed through the seven ml volume bath. During the recording periods, the flow was stopped if there was electrical interference from the suction. Neither interference or altering the flow rate affected the amplitude of the potentials.

These experiments were performed with solutions containing 3×10^{-6} to 4×10^{-6} M d-tubocurarine which was sufficient to prevent muscle contraction to nerve stimulation, but not to abolish the EPP.

Twenty to 25 potentials were recorded during each test period and tests were made at 10 minute intervals for one hour. When muscles were exposed to meperidine, recordings were also taken at 2.5 and 5 minutes following introduction of the drug. The potentials recorded on the

photographic film were magnified using a Porst viewer and the following parameters were measured: nerve terminal potential amplitude, nerve terminal potential duration and EPP amplitude.

5.6 Intracellular Recording of Miniature Endplate Potentials.

The sartorius muscle preparation was employed for these experiments (see Figure 4 for schematic diagram). Mounting in the bath and the recording set up were as described in section 5.5 except that intracellular, 3 M KCl-filled electrodes were inserted into the endplate region for recording of miniature endplate potentials (mepp). The resting membrane potential was monitored on a second oscilloscope and used as an index of cell viability and to confirm that the electrode was still inserted in the cell properly and had not damaged the membrane excessively.

To measure mepp frequency, the signal was magnified by a Tektronix 3A3 differential amplifier of a 565 oscilloscope and fed into a Hewlitt Packard 520 IL Scaler Timer. The number of mepps occurring in each of three two minute intervals was averaged and then divided by two to obtain a mean frequency value for each test period.

Tests were made every 10 minutes for 60 minutes and the amplitude of 50 to 100 potentials was measured from each test period.

The muscle was constantly perfused with the appropriate Ringer's solution (no d-tubocurarine) and drug solutions were added by the perfusion system. Altering the flow rate of the system for a fast exchange of the bathing medium resulted in a temporary alteration of mepp frequency. Therefore, for these experiments only, the flow rate

constant throughout the experiment and the slower achievement of final drug concentration (7 to 10 minutes) was considered in interpreting the results.

5.7 Intracellular Recording of Miniature Endplate and Endplate Potentials and Derivation of Quantal Content. To determine the effect of meperidine on quantal content, miniature endplate potentials and endplate potentials were recorded simultaneously from the sciatic nerve-sartorius preparation.

Mounting of the preparation, stimulating and recording apparatus and exchange of solutions were as described in section 5.5 except that 3 M KCl-filled electrodes inserted in the endplate region were employed for intracellular recording of potentials. As in all intracellular studies, the resting membrane potential was monitored.

Muscle contractions were prevented by including 9 to 10 mM $MgCl_2$ in the perfusing Ringer's to depress neuromuscular transmission. 50 to 100 mepps and 15 to 25 EPPs were recorded during each test period. To estimate quantal content, mean EPP amplitude was divided by mean mepp amplitude for each recording period (EPP/mepp). The results in the presence of drugs were expressed as percentages of the control estimate. Since we were interested only in percentage change in quantal content caused by drug treatment, some factors common to both conditions which are usually used in quantal content determinations, were not included in the calculations.

5.8 Intracellular Recording of Potentials Induced by Iontophoretically Applied Acetylcholine. To test for a postsynaptic action

of meperidine, its effects on the endplate potential induced by iontophoretically applied acetylcholine was studied.

A Medical Systems Corp. iontophoresis unit was employed in these experiments. Its output was connected to a silver wire inserted into each barrel of the double-barrelled micropipette. One barrel contained a 1 M acetylcholine solution and served as the iontophoretic barrel. Negative current passed through this barrel caused the positive acetylcholine ions to migrate out the tip of the pipette onto the endplate region where they combined with the postsynaptic receptors and induced a potential change. A retention current, opposite in sign (i.e. positive), was continuously passed through the iontophoretic barrel to prevent leakage of acetylcholine onto the endplate between stimuli. This was important to prevent desensitization. This current was adjusted from zero to +50 nA so that no depolarization or hyperpolarization of the membrane potential occurred over time.

The second barrel of the electrode contained 0.9% NaCl and was used for current neutralization. The balance module of the iontophoretic unit automatically passed a current through this barrel equal in magnitude but opposite in direction to the sum of all currents passing through the iontophoretic barrel. This prevents current from going through the preparation to ground.

Mounting of the sartorius preparation in the bath, recording apparatus and exchange of solutions was described for other electrophysiological studies. The Ringer's solution was prepared without neuromuscular blockers. Potentials were recorded intracellularly using conventional 3 M KCl-filled electrodes inserted into the endplate

region. Electrode placement was verified by the monitoring of resting membrane potential and the presence of miniature endplate potentials. The iontophoretic pipette was placed extracellularly as close as possible to the recording electrode using a modified Prior micromanipulator which had fine adjustment controls in all directions. Its position was adjusted to obtain acceptable potential recordings upon passage of the stimulus current through the iontophoretic barrel. Pulses of 50 msec duration and -100 to -300 nA current at 12 pulses per minute were used effectively throughout the experiments.

Initially control experiments were performed to ensure that the potentials retained their initial amplitudes throughout the 30 minute experiments. In other experiments, tests were made at 5, 10, 20, and 30 minutes after exposure of the muscles to solutions containing meperidine 1.6×10^{-4} M, or meperidine 1.6×10^{-4} M plus naloxone 3×10^{-8} M.

6. Data Analysis

Action potentials were recorded on 35 mm photographic film and their amplitudes or durations measured in millimeters with the aid of a Porst film viewer. A mean value was calculated from the numbers obtained in each recording period. Each mean obtained in the presence of drug treatment was expressed as a percentage of the mean value calculated for the test period before treatment in order to convert the responses measured in millimeters to percent of control.

The results obtained from all muscles exposed to the same experimental conditions were pooled and the mean and standard error of the

mean (S.E.M.) was calculated for each recording time.

The student's t-test was used to analyze the significance of the difference between two means. If the p value was less than 0.05, the difference was considered significant.

CHAPTER IV. RESULTS

1. Experiments With Muscle Twitch Tension

1.1 The Effect of Meperidine and Naloxone on Twitches Elicited by Nerve Stimulation. These experiments were performed to determine the sensitivity of the sartorius muscles to meperidine and to see if reversal of its effects by naloxone could be produced.

Meperidine, in concentrations of 5 X 10^{-5} M to 2 X 10^{-4} M, inhibited muscle contractions to sciatic nerve stimulation [illegible] shows recordings from a muscle treated with 2 X 10^{-4} M m[illegible] The depression was rapid in onset and was maximal after one half hour. The response to a particular concentration of meperidine was quite variable in different preparations. [illegible] three muscles were tested with 5 X 10^{-5} M meperidine, the percentage depression of twitch amplitude ranged from no depression to 54% with a mean ± S.E.M. of 23 ± 16%. The effect of 10^{-4} M meperidine was tested in two muscles and caused depressions of 28% and 46% with a mean [illegible] S.E.M. of 37 ± 9%. 2 X 10^{-4} M meperidine produced a 77% ± 7% mean depression of twitch amplitude. Individual values obtained in five different muscles ranged from 52% to 96%.

To determine if this depression of twitch height involved an action on narcotic receptors, the effect of 2 X 10^{-4} M meperidine was tested in the presence of naloxone (Figure 6). Muscles were pretreated for one-half hour with naloxone in concentrations of 3 X 10^{-8} M, 3 X 10^{-7} M or 10^{-6} M. In three muscles pretreated with 3 X 10^{-8} M naloxone, meperidine depressed the twitch amplitude by 34%, 53% and

68% with a mean ± S.E.M. of 52 ± 17% after one-half hour. In the presence of 3 X 10^{-7} M naloxone, the mean depression produced by meperidine was 58 ± 17% with individual values ranging from 34% to 85% after 30 minutes exposure to 2 X 10^{-4} M meperidine (n=4). In one muscle pretreated with 10^{-6} M naloxone, the depression of twitch amplitude in the presence of 2 X 10^{-4} M meperidine was 95%.

In the concentrations employed, naloxone by itself had no obvious effect on twitch amplitude during the one-half hour pretreatment periods (Figure 7).

1.2 The Effect of Meperidine on Twitches Elicited by Direct Muscle Stimulation. To determine if the depression of nerve-induced twitches by meperidine was due entirely or in part to a direct action on the muscle fiber membrane, its effect on mechanical responses to direct muscle stimulation was investigated. Figure 8 illustrates recordings from one of the five muscles tested with 2 X 10^{-4} M meperidine. This concentration of meperidine gradually increased the twitch height to direct stimulation. The potentiation observed after one-half hour of treatment ranged from 6% to 68% above control amplitude. The mean value ± S.E.M. calculated from all experiments was 37 ± 10%.

1.3 The Effect of Meperidine on TEA-induced Muscle Contractions. The effect of TEA on the sartorius muscle preparation was tested to establish effective concentrations to produce muscle twitching and to determine if meperidine would antagonize this effect of TEA.

Each muscle (n=3) was tested with TEA in concentrations of 1, 3, 9, and 27 mM. The muscles were bathed in Ringer's solution without

TEA for fifteen minutes between each dose. All concentrations of TEA employed induced spontaneous contractions of the sartorius muscles. Figure 9A shows a recording from a muscle during exposure to 3 mM TEA. The time from introduction of TEA to the onset of twitches was inversely related to the concentration used and the effects were reversible when the muscles were returned to normal Ringer's. Neither the amplitude or frequency of these contractions could be related to effective TEA concentrations.

After control responses had been obtained to TEA as described above, the muscles were treated for one-half hour with 2×10^{-4} M meperidine. The sartorii were then retested with 1, 3, 9, and 27 mM in the presence of meperidine. No twitching was elicited by any of these concentrations of TEA. Figure 9 shows records from an experiment.

1.4 The Effect of Meperidine on Ventral Root Electrical Activity Induced by TEA. As mentioned in the introduction, Beaulieu and Frank (1967) demonstrated that TEA induces action potential firing in the nerve terminal which can be recorded from the ventral root innervating the muscle. I examined the effect of meperidine on this firing to test the hypothesis that narcotics act by inhibiting action potential production in fine nerve endings.

TEA-induced activity was observed under two conditions: repetitive firing following a single nerve stimulus or spontaneous firing without a prior electrical stimulus (Figure 10). In general, TEA-induced after-discharges to electrical stimulation could be achieved at lower concentrations than could TEA-induced spontaneous activity (Section 1.4.1 and 1.4.2).

1.4.1 Experiments on spontaneous TEA-induced activity. Since 9 mM TEA was effective in only one of three muscles tested, 27 mM TEA was used to elicit spontaneous electrical activity in the nerve terminals. Four out of 27 muscles tested were resistant to even this high concentration. When effective, 27 mM TEA caused action potential firing within 10 minutes. In all of three control muscles exposed continuously to 27 mM TEA, spontaneous activity persisted throughout the 3 hour experiments (Figure 11A). It was visually observed that spontaneous muscle contractions in the presence of TEA commenced before ventral root activity could be recorded. Both nerve and muscle had quiescent periods in the presence of TEA in which no activity could be observed. Therefore, the absence of action potentials was not attributed to a drug treatment unless no activity was recorded for at least a 15 minute period.

In experiments to determine if meperidine could block responses to TEA, muscles were exposed first to TEA. Upon the onset of activity meperidine was added to the bathing solution. This method insured that the muscles were sensitive to TEA.

Spontaneous activity induced by 27 mM TEA was blocked by concentrations of meperidine ranging from 10^{-6} M to 2×10^{-4} M. The onset of blockade was related to meperidine concentration. 2×10^{-4} M was tested in two muscles and blocked nerve terminal activity in about 15 minutes; 10^{-4} M meperidine was effective in approximately 30 minutes (n=3); 5×10^{-5} M abolished TEA activity within 60 minutes of being added to the muscle bath (n=2); 10^{-6} M meperidine blocked the effects of TEA in 1.5 to 2.5 hours in six out of eight muscles tested. Recordings from a sensitive muscle are shown in Figure 11B. In four

experiments in which 10^{-6} M meperidine completely blocked TEA-induced activity, the muscles were returned to Ringer's solution containing TEA as the only drug to determine if this action of meperidine was reversible. In two muscles no recovery of action potential firing was observed; in the other two muscles only minimal recovery of spontaneous firing was recovered.

To determine if this action of meperidine was mediated by an action on opiate receptors, the effect of naloxone on meperidine-induced depression was investigated.

In each of the experiments described above using 10^{-4} M and 2 X 10^{-5} M meperidine, 3 X 10^{-8} M naloxone was added to the bath after spontaneous activity elicited by 27 mM TEA had been abolished by meperidine. Although the experiments were continued for one hour, no return of spontaneous firing was observed.

Since the preparations did not or only partially recover from the depression of TEA activity induced by meperidine as reported above, naloxone 3 X 10^{-8} M was added to the bath at the same time as meperidine (10^{-6} M) in the next experiments to determine if the antagonist would prevent manifestation of meperidine's effects. When the concentration of meperidine was reduced to 10^{-6} M, naloxone 3 X 10^{-8} M prevented complete blockade of TEA activity by meperidine in two out of three experiments (Figure 12). When similar experiments were performed in the presence of 10^{-7} M naloxone, meperidine 10^{-6} M failed to abolish spontaneous firing in two of four experiments. The criterion for acknowledging protection by naloxone was the presence of spontaneous activity 3.5 hours after addition of meperidine and naloxone to the muscle bath.

1.4.2 Experiments on TEA-induced after-discharge. Similar results were obtained in experiments utilizing nerve stimulation.

6.8 mM TEA caused after-discharge in six out of [redacted] muscles tested within 15 minutes of being added to the muscle bath. Fifteen minutes after initiation of after-discharge, meperidine 10^{-6} M was added to the bathing Ringer's. In three experiments, meperidine completely blocked TEA-induced activity in one hour. In one muscle after-discharging still occurred even after 2 hours exposure to meperidine and in the other experiments only a few small potentials could be recorded following the stimulus after a one-half hour exposure to meperidine.

In experiments with sartorii sensitive to meperidine, naloxone 3×10^{-8} M was then added to the bath Ringer's in addition to the TEA and meperidine. In the muscles which had been blocked comple[redacted] meperidine, after-discharge did not reappear in the presence [redacted] naloxone although the experiments were continued for 2 hours. Two of these muscles were then reimmersed in Ringer's containing only TEA, but only a minimal after-discharge could be recovered.

The muscle in which meperidine did not completely block the TEA after-discharge was sensitive to naloxone. When tested one-half hour after addition of naloxone to the bath, the amount of repetitive activity had increased and was still present one hour later.

TEA 4.5 mM produced after-discharge in 6 out of eight muscles tested. 2×10^{-4} M meperidine failed to alter TEA-induced activity in three of these experiments, completely abolished after-discharge in two muscles one-half hour after being added to the bath, and partially

blocked after-discharge in one muscle (Figure 13B). When this last muscle was treated with 3 X 10^{-8} M naloxone as described above, the repetitive activity following the stimulus was increased (Figure 13C). As in experiments using 6.8 mM TEA, naloxone was ineffective when added to the bath after complete blockade of after-discharge by meperidine (n=2). In one of these experiments, reimmersing the muscle in Ringer's containing TEA as the only drug for 1.5 hours did not result in recovery of after-discharge. Since this muscle contracted to nerve stimulation, it was still viable.

1.5 <u>The Action of Meperidine on the Extracellularly Recorded Nerve Terminal Action Potential and the Endplate Potential</u>. Although the previous experiments indicated that meperidine could inhibit nerve electrical activity, it was important to determine if this mechanism was responsible for the decrease in muscle twitch tension produced by indirect nerve stimulation observed in the presence of meperidine. Therefore, the presynaptic action potential from the terminal, unmyelinated portion of the nerve and the postsynaptic response, <u>i.e.</u> the endplate potential, were recorded simultaneously from the region of the endplate. A sample recording is shown in Figure 14. The nerve terminal potential is followed by the EPP with a delay of about one msec.

In three preliminary experiments, increasing the d-tubocurarine concentration from 4 X 10^{-6} M to 1.3 X 10^{-4} M abolished the EPP, but did not affect the nerve action potential (Figure 15).

In a separate control study, the amplitude and shape of both potentials were maintained throughout the one hour experiments.

Records from such an experiment are shown in Figure 16. The control data is summarized graphically in Figure 17 and tabulated in Table 1.

To investigate the action of meperidine on these potentials, two separate studies were carried out. This was necessary because different shipments of frogs from the supply house varied in their sensitivity to meperidine. Animals obtained in the spring were less sensitive than frogs received in the fall. This has been noted by other investigators (Frank, 1957; Gibbs et al., 1971; Kosterlitz et al., 1971).

In the first study, addition of meperidine 8×10^{-5} M to the bath solution at time 0 produced a gradual depression of the EPP amplitude which reached a maximum within ten minutes (Figure 18, Table 2); however, meperidine did not reduce the amplitude of the nerve action potential sufficiently to account for the depression of EPP amplitude.

To determine if the reduction in EPP amplitude was mediated by opiate receptors or was nonspecific in nature, the experiments were repeated in the presence of naloxone 3×10^{-8} M. Naloxone was added to the bath at the same time as meperidine. In Figure 18 and Table 2 it can be seen that naloxone during the first ten minutes significantly antagonized the initial phase of the depression of EPP amplitude induced by meperidine. In other control experiments, naloxone by itself had no significant effect on nerve potential or EPP amplitude (Figure 19, Table 1).

After the studies on miniature endplate potentials (section 1.6) had been completed, the above experiments were repeated in the spring using a new shipment of frogs. Again meperidine 8×10^{-5} M depressed

the EPP amplitude without significantly affecting the nerve terminal potential (Figure 20A, Table 3); however, the depression was slower in onset and less in magnitude than in the first study. When the experiments were repeated in the presence of naloxone 3×10^{-8} M, no significant difference was found (Figure 20B, Table 3).

When the concentration of meperidine was increased to 10^{-4} M, the percentage depression of EPP amplitude was increased and was faster in onset. When these experiments were repeated in the presence of naloxone, the mean depression of EPP amplitude induced by 10^{-4} M meperidine was slightly reduced, especially during the first half hour, but this difference was not significant (Figure 21, Table 4).

Exposure of the preparations to meperidine 1.6×10^{-4} M resulted in a similar profile to that obtained with 6×10^{-5} M in the first study. The EPP amplitude was reduced by about 60%, whereas the nerve terminal potential was not significantly affected (Figure 24, Table 5). Figure 22 shows recordings from a preparation exposed to 1.6×10^{-4} M meperidine. The progressive inhibition of EPP amplitude is illustrated. It was noted also that the shape of the recovery phase of the endplate potential was altered by meperidine. An initial fast phase was followed by a slower return to baseline.

When these experiments were repeated in the presence of naloxone 3×10^{-8} M, the depression of EPP amplitude was significantly less than with meperidine alone (Figure 22, Table 5). Again, naloxone was most effective as an antagonist during the initial phase of meperidine-induced depression. Records from an experiment are shown in Figure 23.

Throughout this second study of the effect of meperidine on nerve

terminal potential and endplate potential, the nerve terminal potential duration was measured also. These results tabulated in Table 6 show that no concentration of meperidine tested altered the nerve potential duration relative to control.

1.6 The Effect of Meperidine on Miniature Endplate Potentials Recorded Intracellularly. The action of meperidine on miniature endplate potentials was investigated to determine if the narcotic could affect the spontaneous release of acetylcholine from nerve terminals or alter the responsiveness of the postsynaptic membrane to the transmitter.

Figure 25 and Table 7A exhibit the data obtained from control experiments. Mepp mean amplitude and frequency were maintained near their initial control levels if the resting membrane potential was maintained. If the resting potential became less negative by more than 10% of the value obtained in the initial recording period, the experiment was terminated.

Meperidine 8×10^{-5} M (Figure 27, Table 8A) and 1.6×10^{-4} M (Figure 28, Table 9A) produced a gradual decrease in mepp amplitude during the one hour recording period, but had no significant effect on mepp frequency or resting membrane potential. Records from an experiment are shown in Figure 26.

When these experiments were repeated in the presence of naloxone 3×10^{-8} M (added to the bath at the same time as meperidine) there was no antagonism of the depression of mepp amplitude elicited by either 8×10^{-5} M (Figure 29 and 30, Table 8B) or 1.6×10^{-4} M meperidine

(Figure 28, Table 9B).

When preparations were exposed to naloxone 3×10^{-8} M for one hour, there was no significant change in resting membrane potential, mepp amplitude or mepp frequency (Figure 31, Table 7B).

1.7 The Effect of Meperidine on Quantal Content. To test for meperidine-induced depression of acetylcholine release from the presynaptic terminal in response to a nerve impulse, the effect of meperidine on quantal content was investigated.

Figure 32 and Table 10 summarize the data from control experiments in which resting membrane potential, EPP amplitude and mepp amplitude were recorded intracellularly from sartorius endplates. These parameters and the calculated quantal content were well maintained throughout the one-half hour experiments. The concentration of $MgCl_2$ in the basic Ringer's was adjusted from 8 to 10 mM to obtain EPPs approximately ten times larger in amplitude than the mepps.

When these experiments were performed in the presence of meperidine 1.6×10^{-4} M there was no change in resting membrane potential, but both mepp and EPP amplitudes were depressed by about 35%. There was no significant percentage change in quantal content (Figure 33, Table 11A). In other experiments naloxone failed to antagonize significantly the depression of EPP amplitude induced by 1.6×10^{-4} M meperidine (Figure 34, Table 11B), although the mean depression was less during the first 10 minutes in the presence of naloxone.

This problem was reminiscent of the extracellular studies of nerve potential and EPP amplitude in which the sensitivity of the frogs to

meperidine was changeable. The percentage depression of EPP amplitude induced by 1.6 X 10^{-4} M meperidine in the quantal content studies was less than observed in the experiments using extracellular recording techniques. Therefore, additional quantal content experiments were performed using higher concentrations of meperidine.

Meperidine 4.2 X 10^{-4} M elicited a 60% reduction in mepp and EPP amplitude, but there was still no change in quantal content (Figure 35, Table 12A). When experiments were repeated in the presence of naloxone 3 X 10^{-8} M, it was again ineffective as an antagonist (Figure 36, Table 12B), i.e. its presence did not alter significantly the effect of meperidine on any of the parameters measured.

To test the possibility that magnesium was antagonizing the presynaptic narcotic action, the effect of 4.2 X 10^{-4} M meperidine on the intracellularly recorded EPP was investigated using d-tubocurarine instead of $MgCl_2$ to prevent muscle contractions. The depression of EPP amplitude was 72% by this method (Figure 38A, Table 13A). Figure 37 exhibits records from an experiment. The means from each test period were significantly different from the mean obtained in the presence of $MgCl_2$. When the experiments were repeated in the presence of naloxone, the depression of EPP amplitude was partially antagonized (Figure 38B, Table 13B).

A summary of all the data in Figure 39 reveals that the graph of EPP amplitude depression by 4.2 X 10^{-4} M meperidine in the presence of $MgCl_2$ resembles the graph illustrating partial antagonism by naloxone of the depression of EPP amplitude caused by meperidine in the presence of d-tubocurarine; indeed, there was no significant difference. Thus,

the narcotic [redacted]ptor mediated action of meperidine is not manifested in the presence of $MgCl_2$.

1.8 The Effect of Physostigmine on the Depression of EPP Amplitude Induced by Meperidine. Since some narcotics are known to have slight anticholinesterase activity, experiments were performed in the presence of the anticholinesterase physostigmine 1.3×10^{-5} M which would theoretically reduce the amount of substrate available to meperidine. D-tubocurarine was used to depress neuromuscular transmission. The concentration of d-tubocurarine was increased from 2×10^{-6} to 9×10^{-6} M to counteract the facilitatory action of physostigmine on neuromuscular transmission. The duration of the EPP was increased in the presence of physostigmine.

Depression of the intracellularly recorded EPP by meperidine 4.2×10^{-4} M was not significantly different from experiments performed in the absence of physostigmine (Fiugre 40, Table 14). If anything, physostigmine potentiated the effect of meperidine on EPP amplitude.

1.9 The Effect of Mepidine on the Endplate Potential Induced by Iontophoretically Applied Acetylcholine. To test for a non-opiate, postsynaptic action of meperidine, its effect on the intracellularly recorded endplate potential induced by iontophoretically applied acetylcholine (EPP_I) was investigated. In control experiments, resting membrane potential and EPP_I amplitude were maintained throughout the one-half hour experiments (Figure 41, Table 15A). At the end of this period the d-tubocurarine concentration was increased to 1.3×10^{-4} M

which abolished the EPP_I and verified that the measured potential was due to acetylcholine being ejected onto the endplate.

Other muscles were exposed to meperidine 1.6×10^{-4} M (Figure 43, Table 15B) which resulted in a gradual depression of mean EPP_I amplitude to 35% of the control value. Potentials recorded before and after exposure of one muscle to meperidine are displayed in Figure 42.

Naloxone 3×10^{-8} M failed to antagonize the depression of EPP_I amplitude induced by meperidine (Figure 43, Table 15C).

CHAPTER V. DISCUSSION

The advantage of using a frog's skeletal nerve-muscle preparation in investigating the mechanism of action of narcotics on synaptic transmission is that the various stages of neuromuscular transmission can be monitored separately. Although the majority of studies on the peripheral action of narcotics have been performed using the guinea pig ileum, a method of measuring electrical activity in these nerve terminals and the response of the postsynaptic membrane to the transmitter concomitantly has not been developed. Also, the question of how conduction of the action potential in Auerbach's plexus is modulated by various transmitters is unresolved. The main disadvantage in the use of frog preparations is the seasonal variation in the availability of these experimental animals from suppliers, and in the sensitivity of their sartorii to drugs. The problem with variation in sensitivity can be overcome by performing adequate control experiments. Another main disadvantage is the high concentrations of opiates required to produce an effect on frog sartorius preparations and the possibility of non-opiate actions.

The first step in this investigation was to show that meperidine depressed twitch responses to sciatic nerve stimulation, an action shown for morphine by Pinsky and Frederickson (1971). Although the depression of twitch tension by a particular concentration of meperidine was quite variable, this variation in responses to narcotic drugs also occurs between preparations of ileum from different guinea pigs (Cox and Weinstock, 1966).

Frank (1975 a,b) showed that meperidine depresses the action

potential of frog sartorius muscle fibers in similar concentrations to those employed in this study. The observation that meperidine increased the tension developed to direct muscle stimulation in the presence of sufficient curare to block synaptic transmission completely indicates that depression of action potential conduction in the muscle fiber membrane was not the main cause of the decrease in contraction amplitude to nerve stimulation observed in other experiments. This potentiation of the response to direct muscle stimulation is similar to that observed by Pinsky and Frederickson (1971) that the threshold concentration of morphine potentiated both twitch and EPP amplitudes before the onset of depression. These potentiating effects of narcotics are not likely due to their anticholinesterase action since the effects observed by Pinsky and Frederickson occurred in the presence of high concentrations of neostigmine and, in this study, the acetylcholine receptors were blocked by d-tubocurarine, eliminating the contribution of acetylcholine in production of the twitches to direct stimulation of the muscles. Frederickson and Pinsky concluded that morphine facilitates the action of acetylcholine on its receptors. In the present study, meperidine depressed the response to iontophoretically applied acetylcholine. The results in the present study suggest that narcotics potentiate the contractile mechanism in the muscle fibers, but this requires further investigation.

As discussed in the introduction, there are two major theories of the mechanism of action of narcotics on neuromuscular transmission; 1) inhibition of action potential production in nerve endings by hyperpolarizing or stabilizing the membrane potential or 2) the suppression of the acetylcholine release mechanism.

The observation that meperidine blocked TEA-induced repetitive firing following a single nerve stimulus and spontaneous activity without a prior electrical stimulus is consistent with the hypothesis that narcotics depress neuromuscular transmission by affecting action potential production in the nerve endings. Lower concentrations of meperidine were required to block TEA-induced activity than were required to reduce the amplitude of twitch responses to supramaximal nerve stimulation. This is similar to the findings in other narcotic sensitive preparations that opiates are more effective when submaximal stimulation is employed.

Beaulieu et al. (1967) found that TEA increased the excitability of the nerve terminal membrane and increased the release of acetylcholine from the nerve endings resulting in muscle twitching. They proposed that TEA displaces bound calcium from membrane sites, thus making the nerve endings hyperexcitable. Meperidine could prevent or antagonize the action potential firing induced by TEA in the nerve terminals by affecting calcium disposition in the membrane or by stabilizing the membrane by some other action. Narcotics have been shown to decrease the action potential amplitude in squid giant axon (Simon and Rosenberg, 1970; Frazier et al., 1972, 1973), in frog sciatic nerves (Hunter and Frank, 1979) and in frog sartorius muscle fibers (Frank, 1975 a,b) by depressing both the sodium and potassium conductance, but these drugs do not alter the resting membrane potential (Kosterlitz and Wallis, 1964; Simon and Rosenberg, 1970; Frazier et al., 1972; Frank, 1975b). In the study by Simon and Rosenberg, levorphanol blocked repetitive activity in squid axons

induced by lowering the Ca^{++} and Mg^{++} concentrations. These investigators suggested that levorphanol might substitute for calcium to some extent. Calcium ions also affect the membrane permeability. Lowering the external calcium concentration depolarises nerve fiber membranes by increasing the sodium permeability (Schmidt, 1960). However, Jurna and Grossman (1977) found that morphine did not reverse the depolarization of rabbit and guinea pig vagus nerves caused by lowering the extracellular Ca^{++} concentration, but morphine was more effective in depressing action potential amplitude in a calcium-free medium than in a solution with calcium. It also has been suggested that the inflow of calcium may be the natural activator of the potassium channel in nerve (Much, 1971).

To determine if the antagonism of TEA-induced firing by meperidine is mediated by opiate receptors or is non-opiate, experiments were repeated in the presence of naloxone. Since complete depression of TEA responses by meperidine was often irreversible, it is not surprising that naloxone failed to antagonize blockade of spontaneous firing or after-discharge by meperidine when added to the bath after the complete cessation of activity. Naloxone 3×10^{-8} M prevented complete depression in about 50% of all experiments when added to the bath before meperidine (spontaneous firing experiments) or before complete blockade of after-discharge by meperidine. This finding strongly suggests, but does not conclusively prove, that the action of meperidine on TEA-firing is mediated in part by combination with narcotic receptors as has been shown for morphine blockade of post-tetanic repetition in cat soleus nerve fibers (Soteropoulos and Standaert, 1964). Unfortunately, the amplitude or frequency of

spikes varies over the time course of exposure to TEA and there is no correlation between amplitude or frequency and the TEA concentration. Therefore, only the complete blockade of firing by meperidine could be considered to be a definitive response. If a partial blockade by meperidine could be measured, a naloxone-sensitive component might be detected more readily. As will be discussed later, the narcotic receptor-mediated depression of EPP amplitude by meperidine is evident only at certain concentrations and can be partially masked by the non-opiate, 'local anesthetic-like' action of the drug.

The experiments with TEA showed that meperidine can depress electrical activity in nerve endings, but it remained to be determined if this mechanism was responsible for the decrease in amplitude of indirectly elicited twitches observed in the presence of meperidine. Therefore, the presynaptic action potential from the terminal, unmyelinated portion of the nerve and the EPP were measured concurrently. A simultaneous decrease in both the nerve potential and EPP amplitudes would support the nerve terminal action potential blockade hypothesis. A decrease only in the EPP amplitude would indicate an action on the acetylcholine release mechanism and/or on the postsynaptic endplate region.

Since meperidine had no significant effect on the nerve terminal action potential amplitude or duration, but reduced the EPP amplitude, an effect on presynaptic action potential production cannot account for the depression of twitch tension developed to nerve stimulation in this preparation in the absence of TEA. The observation that depression of the EPP amplitude developed gradually with the time

of exposure to meperidine, also argues against the action potential production hypothesis and supports an action on the transmitter release mechanism.

The experiments with naloxone showed that the depression of EPP amplitude by meperidine is caused by both an action on opiate receptors and by a non-opiate effect. The onset of the narcotic receptor mediated depression is rapid and reaches a maximum after approximately 10 minutes of exposure of the preparation to meperidine. The non-opiate effect occurs with lower concentrations of meperidine than does the narcotic receptor-mediated effect. This is shown by the results of the second study using 8×10^{-5} M meperidine (Figure 20) and in the experiments using 10^{-4} M meperidine (Figure 21), in which naloxone failed to antagonize significantly the EPP amplitude depression induced by meperidine.

Although the narcotic receptor mediated effect occurred at different concentrations in sartorii from different batches of frogs, these different concentrations produced approximately the same percentage depression of EPP amplitude. In Figures 18, 24, and 38, which illustrate the narcotic receptor-mediated effect of meperidine, the depression of EPP amplitude after 30 minutes was 60% or greater. In Figures 20 and 21, which illustrate only a non-opiate effect, the maximum depression of EPP amplitude by meperidine was only 40% to 50% of control.

The fact that low concentrations of naloxone (3×10^{-8} M) antagonized the effects of much higher concentrations of meperidine (8×10^{-5} to 4.2×10^{-4} M) supports the conclusion discussed in the

introduction that preparations displaying low sensitivity to narcotic agonists, are highly sensitive to narcotic antagonists. This could reflect structural differences between narcotic receptors at sites differing in their sensitivities to agonists or a difference in accessibility of agonists to the receptors. The latter is unlikely in the present study since the onset of meperidine's action was less than one minute. These results with naloxone support the conclusion that the action of narcotics on many less sensitive preparations has been termed nonspecific because too high concentrations of narcotic antagonists were employed in attempts to antagonize the depressant effects of narcotic analgesics. The results of this investigation show that the action of narcotic analgesics in sciatic nerve-sartorius muscle preparations is not entirely non-opiate as other investigators have concluded (Bell and Rees, 1974). The reason for their conclusion was that they used naloxone in high concentrations at which naloxone either has no effect or acts like an agonist. It is possible that the action of narcotics in other preparations such as the squid axon, so far considered to be non-opiate, may have a narcotic receptor mediated component. This statement is supported by the demonstration by Hunter and Frank (1979) of a component of meperidine's action on the action potential of frog sciatic nerve which is sensitive to low concentrations of naloxone.

The results of experiments measuring the nerve terminal action potential and the EPP amplitude simultaneously indicate that meperidine depresses the mechanism for acetylcholine release and/or

inhibits the response of the postsynaptic membrane to acetylcholine. Meperidine did not affect spontaneous release as measured by mepp frequency at frog sartorius endplates (Figure 27, 28). This result is similar to the finding of Henderson (1976) that neither normorphine or met-enkephalin reduced the frequency of mepps in guinea pig ileum deferens and is consistent with the conclusion of de la Lande and Porter (1967) that in unstimulated guinea pig ileum only augmentation of spontaneous release of acetylcholine by ongoing neural activity in Auerbach's plexus is blocked by morphine-like drugs.

In this study, although the frequency of mepps was not altered significantly by meperidine, the mepp amplitude was considerably reduced in a gradual manner resembling the depression of EPP amplitude recorded either extracellularly or intracellularly in the presence of meperidine. Whereas the depression of EPP amplitude was partly narcotic receptor mediated, the depression of mepp amplitude appeared to be completely non-opiate. Naloxone failed to antagonise the depression of mepp amplitude by any concentration of meperidine tested (Figure 28, 29), including 1.6×10^{-4} M which produced a naloxone-sensitive depression of EPP amplitude in the same batch of frogs (Figure 24). The only mechanisms which can account for a depression of mepp amplitude without any change in frequency are: 1) a change in the postsynaptic action of the transmitter such as might be caused by acetylcholine receptor blockade or a change in the electrical properties of the postsynaptic membrane and 2) a reduction of the amount of transmitter packaged in each vesicle (quantal size); e.g. by decreasing acetylcholine synthesis as happens in the presence of hemicholinium. These experiments on the effect of meperidine on mepp amplitude

indicated that the narcotic receptor-mediated component of meperidine's action was not manifested in alteration of quantal size since the meperidine-induced depression of mepp amplitude was not antagonized by naloxone.

The most likely explanation of meperidine's effect on mepp amplitude is a postsynaptic mechanism. This hypothesis was supported by the observation that meperidine also depressed the amplitude of the EPP_I resulting from iontophoresis of acetylcholine onto the endplate region. This postsynaptic action of meperidine is completely non-opiate since the depression of EPP_I and mepp amplitudes by meperidine was not sensitive to naloxone antagonism. This postsynaptic, non-opiate action of meperidine explains the naloxone-insensitive component of the depression of EPP amplitude recorded both extracellularly and intracellularly.

The observation that the depression of EPP_I amplitude by meperidine did not have a narcotic receptor-mediated component, whereas depression of the EPP elicited by nerve stimulation did, suggests that the narcotic receptors in the frog sciatic nerve-sartorius preparation are located presynatically. Since meperidine did not affect the nerve terminal action potential, this narcotic must depress a stage in the release mechanism for acetylcholine.

If meperidine acts presynaptically to prevent the release of acetylcholine in addition to its postsynaptic effect, this should be reflected in the quantal content of the endplate potential. A decrease in quantal content would mean that meperidine affects release in response to nerve stimulation although not spontaneous release. When the

effect of 1.6 X 10^{-4} M meperidine on quantal content was tested, there was no change, i.e. the EPP and mepp amplitudes were depressed to the same extent, indicating that only a postsynaptic effect of meperidine was manifested in these experiments. In addition, the depression of EPP amplitude by this concentration of meperidine was not antagonized by naloxone. Since it was possible that the frogs used in these experiments displayed a low sensitivity to meperidine (note in Figure 33 and Table 11A that the maximum depression of EPP amplitude is only 38% of control) the concentration was increased to 4.2 X 10^{-4} M which produced approximately 60% reduction in EPP amplitude. However, there was still no change in quantal content and no antagonism by naloxone. The only other experimental conditions which differed from previous experiments was that EPPs were recorded intracellularly instead of extracellularly and that $MgCl_2$ was used to block partially synaptic transmission and prevent muscle contractions instead of d-tubocurarine. When EPPs were recorded intracellularly in the presence of d-tubocurarine, meperidine 4.2 X 10^{-4} M produced a greater percentage depression of EPP amplitude than it did in the presence of $MgCl_2$ and this depression was partially and significantly prevented by naloxone. These results suggest that Mg^{++} as well as naloxone antagonized the narcotic receptor-mediated effects of meperidine on frog sciatic nerve-sartorius preparations. There was no significant difference in the antagonism exerted by Mg^{++} and naloxone in these experiments (Figure 39). It is interesting that Mg^{++} also antagonizes narcotic analgesia (Kakunaga et al., 1966; Harris et al., 1975a) and prevents the inhibition of neocortical acetylcholine release induced by morphine (Sanfacon et al., 1977). This is an unusual effect of Mg^{++} since

both Mg^{++} and morphine depress neuromuscular transmission when administered separately.

The alternative explanation of the opiate action of meperidine is that the narcotic receptors are located postsynaptically, but an action of meperidine on these receptors is manifested only in the presence of curare. A naloxone-sensitive component of meperidine's action was not observed in the presence of Mg^{++} (quantal content experiments) or in the absence of a neuromuscular blocker (studies on mepps and acetylcholine potentials). An opiate action of meperidine was observed when EPPs were measured in the presence of curare. Investigation of meperidine's action on cut muscle fibers or on the acetylcholine potential in the presence of curare would test this hypothesis.

Unfortunately, quantal content cannot be measured by the direct method in the presence of d-tubocurarine since mepps are blocked by concentrations of d-tubocurarine which depress the EPP amplitude to below threshold for production of muscle fiber action potentials. Other methods of preventing muscle contraction in order to determine the quantal content of the EPP, such as using cut muscle fiber preparations, or use of the variance method for determining quantal content might reveal a decrease in quantal content by meperidine.

Thus the evidence for a presynaptic, narcotic receptor-mediated action of meperidine on the transmitter release mechanism is at this time indirect.

The depression of EPP amplitude recorded intracellularly or extracellularly was partially prevented by naloxone indicating that meperidine has both opiate receptor-mediated and non-opiate effects

on sartorius. Since the depression of mepp amplitude by meperidine was not antagonized by naloxone, the narcotic receptors are not likely situated postsynaptically nor is the narcotic receptor-mediated effect of meperidine due to a change in quantal size. The observation that the meperidine-induced depression of the EPP_I due to iontophoresis of acetylcholine onto the endplate was not naloxone sensitive is further proof that the narcotic receptors are located presynaptically. Since meperidine did not affect either the amplitude or duration of the nerve terminal action potential, blockade of action potential production in the nerve terminal fibers does not account for the action of meperidine on neuromuscular transmission. By elimination, the opiate receptor-mediated effect of meperidine should be on the acetylcholine release mechanism. Indeed, Frederickson and Pinsky (1971) showed that morphine depressed acetylcholine release in this preparation.

The only mechanism which would account for the narcotic receptors being located on the postsynaptic membrane is that the opiate action of meperidine is only manifested in the presence of curare.

When EPPs were measured extracellularly in the presence of meperidine, the rate of decay of the endplate current (epc) was often biphasic with a fast initial component and a slow, secondary component. Inhalation anaesthetics and barbiturates produce a similar action at the skeletal neuromuscular junction (Gage, 1965; Gage and Hamill, 1976; Torda and Gage, 1977). Gage and Hamill reported that at low anaesthetic concentrations, these agents increased the rate of decay of miniature endplate currents (mepc) and reduced the amplitude of mepps, perhaps by decreasing the life-time of 'open'

synaptic channels . At higher concentrations, the anaesthetics caused a reduction in the amplitude of both mepcs and mepps, and a decrease in the rate of decay of the currents. With halothane, enflurane and ethanol, the delay in the rate of decay sometimes was biphasic as reported in this thesis for meperidine. Torda and Gage (1977) suggested that halothane affected the fluidity of lipid layers of the postsynaptic membrane, ordering them at low concentrations, but increasing their fluidity at high concentrations, and that the state of the membrane might control the life-time of synaptic channels. Adams (1976) suggested that barbiturates may block 'open' receptor channels more rapidly than they can relax. Ruff (1977) concluded that the lidocaine derivative QX222 modified endplate currents by altering endplate channel gating and that the local anaesthetic interacted with the acetylcholine receptor both before and after the agonist binds to the receptor.

This non-opiate, anaesthetic-like action of meperidine on the endplate is the major action of meperidine on the frog sciatic nerve-sartorius muscle preparation. This is the first demonstration that an opiate anaesthetic has similar actions to local and general anaesthetics at skeletal muscle endplates. It is interesting that both meperidine and barbiturates can potentiate twitch tension in response to direct muscle stimulation in frog skeletal muscle (Foulks, Perry, Sanders and Washio, 1973).

It remains to be determined if narcotic analgesics other than meperidine have a non-opiate action at endplates of frog sartorius muscle. Turlapaty et al. (1977) concluded that 2.5×10^{-4} M morphine

facilitates, rather than depresses, the response of frog rectus abdominis muscle to exogenous acetylcholine. Meperidine, on the other hand, depresses responses of frogs's rectus abdominis to exogenous acetylcholine (Hebb and Konzett, 1949). Therefore, it is possible that morphine, in concentrations which depress neuromuscular transmission in frog sciatic nerve-sartorius preparations, may not have a non-opiate action on the endplate whereas meperidine does.

Turlapaty et al. concluded that part of the facilitiation of the response to acetylcholine by morphine was due to the drug's anti-cholinesterase action. Meperidine also blocks cholinesterase (Hebb and Konzett, 1950). However, in this study, the presence of sufficient physostigmine to block cholinesterases did not significantly alter the depression of EPP amplitude induced by meperidine (Figure 40). This finding indicates that the anticholinesterase action of meperidine did not significantly mask its depressant effects.

Another original contribution of this thesis is the demonstration of opiate receptors in a skeletal nerve-muscle preparation as indicated by the partial reversal of meperidine-induced depression of EPP amplitude. Further studies are suggested to determine the stereo-specificity of this action of narcotics by comparing the effects of levorphanol and dextrophan in sciatic nerve-sartorius preparations. Further study also is suggested to test the hypothesis that the receptors are located presynaptically and to determine the mechanism by which meperidine's binding to presynaptic narcotic receptors might cause inhibition of acetylcholine release. An action on calcium disposition is attractive in the light of previously reported interactions between calcium and narcotics and the analgesic property of

lanthanum discussed in the introduction. Although morphine has been shown to chelate calcium (Lin et al., 1975), a similar action of meperidine would not explain fully the effects of meperidine reported in this thesis. A reduction in extracellular free Ca^{++} might explain the inhibition of acetylcholine release induced by meperidine by reduction of the amount of free extracellular Ca^{++} available for influx during nerve terminal depolarization, but does not explain the nerve membrane stabilisation action. Reduction of extracellular Ca^{++} would increase rather than decrease membrane excitability. Nor would it explain the antagonism by naloxone. In other organ systems chelation of calcium by 10^{-8} M of a narcotic should not significantly alter the normal calcium concentration which is in the millimolar range. Further studies on the effects of narcotics on calcium disposition are required to elucidate the role of calcium in narcotic action.

The major conclusion of this thesis is that there are three mechanisms of action of meperidine operant in the frog sciatic nerve-sartorius muscle preparation: one is probably on the transmitter release mechanism under conditions of nerve stimulation which is partly, if not completely, opiate receptor mediated, a second, non-opiate, anaesthetic-like action on the postsynaptic membrane, and a third effect on action potential conduction in the nerve fibers which may be partly opiate receptor mediated, but is not responsible for inhibition of neuromuscular transmission under conditions of nerve stimulation.

The demonstration of opiate action on sciatic nerve conduction (Hunter and Frank, 1979), on transmitter release mechanisms (suggested by the present study), and on muscle fiber action potentials (Frank,

1975 a,b) in frog sciatic nerve-sartorius muscles suggests that narcotics have multiple sites of action which are mediated by opiate receptor binding. This theory is consistent with the findings in the guinea pig ileum that opiate actions are demonstrable on preganglionic nerve endings (Ehrenpreis et al., 1976), on the electrical properties of ganglion cells (Dingledine and Goldstein, 1976; North, 1976; North and Tonini, 1977), and on the mechanisms involved in release of transmitter following a nerve action potential (Paton, 1957; Cowie et al., 1968).

CHAPTER VI. SUMMARY

1) The action of the narcotic analgesic meperidine on neuromuscular transmission in the frog sciatic nerve-sartorius muscle preparation was investigated.

2) Meperidine (5×10^{-5} to 2×10^{-4} M) depressed the amplitude of twitch responses induced by supramaximal, sciatic nerve stimulation. This inhibition was not due to depression of conduction of the action potential in the muscle fibers since responses to direct muscle stimulation were potentiated by meperidine.

3) Meperidine prevented muscle twitching induced by TEA. In the presence of TEA, both spontaneous action potential firing and after-discharge following a single sciatic nerve stimulus were induced in the region of the nerve terminals and recorded from the ventral root innervating the muscle. Both types of TEA-induced firing were antagonized by meperidine (10^{-6} to 2×10^{-4} M). Naloxone (3×10^{-8} M) prevented complete blockade of TEA-induced spontaneous firing or after-discharge in about 50% of experiments, but this narcotic antagonist did not reverse complete blockade induced by meperidine. These results suggest that there are opiate receptors on the terminal, nerve fibers innervating frog sartorius muscle fibers, and that meperidine can affect action potential production in these fine, unmyelinated nerve endings.

4) An action meperidine on the receptors described in (3) does not account for the meperidine-induced depression of muscle twitch amplitude to supramaximal sciatic nerve stimulation since neither

the amplitude or duration of the extracellularly recorded nerve terminal action potential following sciatic nerve stimulation was altered by meperidine in concentrations up to 1.6×10^{-4} M.

5) Meperidine (8×10^{-5} to 4.2×10^{-4} M depressed the amplitude of the EPP recorded extracellularly or intracellularly from sartorius endplates. This depression was partially antagonized by a low concentration of naloxone (3×10^{-8} M) which itself had no effect on neuromuscular transmission.

6) Meperidine depressed mepp amplitude, but did not significantly alter mepp frequency. The depression of mepp amplitude was antagonized by naloxone, indicating that this effect was not mediated by opiate receptors.

7) Meperidine depressed the amplitude of the EPP_I due to iontophoretic application of acetylcholine directly to the endplate region. This action of meperidine was not antagonized by naloxone These results prove that the depression of mepp amplitude and part of the depression of EPP amplitude by meperidine was due to a non-opiate, postsynaptic effect of the narcotic on the endplate, and that the naloxone-sensitive depression of EPP amplitude was due to a presynaptic action of meperidine.

8) Meperidine did not alter the quantal content of the EPP measured in the presence of $MgCl_2$, nor did naloxone antagonize meperidine-induced depression of EPP amplitude in the presence of $MgCl_2$. It is suggested that Mg^{++}, as well as naloxone, antagonizes the opiate receptor-mediated effects of meperidine.

9) Meperidine had no significant effect on the resting membrane potential of the endplate region.

10) The anticholinesterase action of meperidine did not influence significantly the effect of this narcotic on the EPP amplitude since meperidine was equally active in the presence and absence of physostigmine.

11) The major conclusion of this thesis is that there are three mechanisms of action of meperidine operant in the frog sciatic nerve-sartorius muscle preparation; one is likely on the transmitter release mechanism under conditions of nerve stimulation, which is partly if not completely, opiate receptor mediated; a second, non-opiate, anaesthetic-like action on the postsynaptic membrane; and a third effect on action potential conduction in the nerve fibers which may be partly opiate receptor mediated, but is not responsible for inhibition of neuromuscular transmission under conditions of supra-maximal nerve stimulation.

12) The non-opiate action of meperidine on the endplate is the predominant effect of this drug in the frog sciatic nerve-sartorius and is similar to the action of local and general anaesthetics on skeletal muscle endplates.

Table 1. Maintenance of the mean amplitudes of A. the nerve terminal action potential and B. the endplate potential recorded extracellularly from frog sartorius muscles in the absence of a narcotic drug (control) or in the presence of naloxone.

A.

Time (min)	% control nerve terminal potential amplitude ± S.E.M.	
	control (n=5)	naloxone 3 X 10^{-8} M (n=4)
10	106.1 ± 5.8	99.7 ± 1.0
20	105.7 ± 4.8	95.6 ± 4.7
30	105.1 ± 2.8	97.6 ± 6.4
40	103.4 ± 5.0	99.8 ± 6.1
50	98.8 ± 7.8	96.5 ± 10.6
60	103.2 ± 10.1	94.1 ± 9.6

B.

Time (min)	% control endplate potential amplitude ± S.E.M.	
	control (n=5)	naloxone 3 X 10^{-8} M (n=4)
10	97.8 ± 0.7	99.5 ± 4.9
20	94.1 ± 1.1	94.1 ± 4.5
30	93.0 ± 5.9	97.2 ± 9.7
40	96.4 ± 6.9	96.1 ± 5.6
50	97.6 ± 6.8	95.9 ± 10.2
60	96.3 ± 4.6	93.3 ± 12.3

Means in the presence of naloxone were not significantly different from mean of control experiments at the same drug exposure time (unpaired t-test, $p>0.05$).

Table 2. First study of meperidine 8×10^{-5} M on the mean amplitude of the extracellularly recorded A. nerve terminal action potential and B. endplate potential of frog sartorius in the absence and presence of naloxone 3×10^{-8} M.

A.

Time (min)	% control nerve terminal potential amplitude ± S.E.M.	
	meperidine 8×10^{-5} M (n=3)	meperidine 8×10^{-5} M & naloxone 3×10^{-8} M (n=3)
3	106.6 ± 6.1	95.2 ± 8.7
5	100.5 ± 7.5	90.9 ± 9.4
10	85.5 ± 12.2	90.5 ± 9.4
20	99.5 ± 1.7	99.0 ± 9.5
30	99.9 ± 7.1	94.3 ± 12.0

B.

Time (min)	% control endplate potential amplitude ± S.E.M.	
	meperidine 8×10^{-5} M (n=3)	meperidine 8×10^{-5} M & naloxone 3×10^{-8} M (n=4)
3	68.1 ± 6.7 *	74.7 ± 4.8
5	58.1 ± 7.9 *	81.1 ± 3.8 **
10	45.4 ± 4.8 *	76.2 ± 6.4 **
20	47.0 ± 8.0 *	48.5 ± 4.3
30	40.9 ± 7.9 *	48.5 ± 4.3

* significantly different from corresponding mean of control experiments by an unpaired t-test, $p<0.05$

** significantly different from mean of meperidine experiments.

Table 3. Second study of the effect of meperidine 8×10^{-5} M on the mean amplitude of the extracellularly recorded A. nerve terminal action potential and B. endplate potential of frog sartorius and the lack of antagonism by naloxone.

A.

Time (min)	% control nerve terminal potential amplitude ± S.E.M.
	meperidine 8×10^{-5} M (n=5)
5	99.8 ± 2.7
10	98.6 ± 1.7
20	104.0 ± 3.5
30	101.9 ± 2.8
40	99.0 ± 3.3
50	98.4 ± 5.0
60	92.4 ± 3.4

B.

Time (min)	% control endplate potential amplitude ± S.E.M.	
	meperidine 8×10^{-5} M (n=5)	meperidine 8×10^{-5} M & naloxone 3×10^{-6} M (n=3)
5	84.8 ± 5.1 *	
10	82.9 ± 6.6 *	91.9 ± 10.0
20	78.6 ± 4.7 *	71.0 ± 9.0
30	71.2 ± 3.1 *	72.6 ± 6.6
40	70.3 ± 4.6 *	62.1 ± 6.0
50	68.4 ± 3.4 *	55.0 ± 11.2
60	59.3 ± 7.9 *	

* means significantly different from the mean of control experiments at the same drug exposure time (unpaired t-test, $p<0.05$).

Naloxone did not significantly alter the response to meperidine.

Table 4. The effects of meperidine 10^{-4} M on the mean amplitudes of the extracellularly recorded A. nerve terminal action potential and B. endplate potential of frog sartorius in the absence and presence of naloxone.

A.

Time (min)	% control nerve terminal potential amplitude ± S.E.M.	
	meperidine 10^{-4} M (n=4)	meperidine 10^{-4} M & naloxone 3 X 10^{-8} M (n=3)
3	100.7 ± 6.3	93.3 ± 6.3
5	98.8 ± 8.2	94.2 ± 2.9
10	96.5 ± 6.9	90.0 ± 3.2
20	94.8 ± 5.6	84.2 ± 4.2
30	93.6 ± 4.2	87.2 ± 4.1
40	86.8 ± 1.5	83.4 ± 1.3
50	81.8 ± 2.9	86.6 ± 0.4
60	87.9 ± 3.9	82.4 ± 4.6

B.

Time (min)	% control endplate potential amplitude ± S.E.M.	
	meperidine 10^{-4} M (n=4)	meperidine 10^{-4} M & naloxone 3 X 10^{-8} M (n=3)
3	65.5 ± 5.6 *	71.1 ± 4.3
5	61.1 ± 6.4 *	66.2 ± 2.5
10	55.9 ± 6.9 *	61.5 ± 1.7
20	53.5 ± 5.2 *	59.6 ± 4.3
30	50.2 ± 8.9 *	59.2 ± 2.6
40	54.3 ± 4.3 *	52.8 ± 5.2
50	49.5 ± 5.7 *	61.2 ± 3.8
60	51.6 ± 3.1 *	53.5 ± 3.4

* means significantly different from the mean of control experiments at the same drug exposure time (unpaired t-test, $p<0.05$).

Naloxone did not significantly alter the response to meperidine.

Table 5. The effect of meperidine 1.6 X 10^{-4} M on the mean amplitude of the extracellularly recorded A. nerve terminal action potential and B. endplate potential of frog sartorius and partial antagonism by naloxone.

A.

Time (min)	% control nerve terminal potential amplitude ± S.E.M.	
	meperidine 1.6 X 10^{-4} M (n=6)	meperidine 1.6 X 10^{-4} M & naloxone 3 X 10^{-8} M (n=4)
3	100.0 ± 3.4	95.6 ± 4.3
5	93.6 ± 5.7	95.3 ± 5.5
10	92.7 ± 5.6	90.7 ± 4.4
20	87.5 ± 4.8 *	90.8 ± 4.1
30	89.0 ± 4.1 *	91.6 ± 8.3
40	85.8 ± 6.8	96.5 ± 13.9
50	86.1 ± 3.9	95.9 ± 13.5
60	85.5 ± 10.6	95.9 ± 12.5

B.

Time (min)	% control endplate potential amplitude ± S.E.M.	
	meperidine 1.6 X 10^{-4} M (n=6)	meperidine 1.6 X 10^{-4} M & naloxone 3 X 10^{-8} M (n=5)
3	62.4 ± 2.9 *	79.2 ± 2.4 **
5	50.0 ± 3.2 *	65.9 ± 3.5 **
10	45.9 ± 2.6 *	59.5 ± 2.7 **
20	41.1 ± 4.0 *	55.2 ± 4.5
30	39.7 ± 2.2 *	52.7 ± 2.9
40	36.2 ± 4.3 *	51.2 ± 3.0 **
50	35.0 ± 3.9 *	48.9 ± 3.5 **
60	37.5 ± 1.4 *	48.5 ± 5.6

* significantly different from corresponding mean of control experiments by an unpaired t-test, $p<0.05$.

** means significantly different from the mean of meperidine experiments at the same drug exposure time.

Table 6. The effect of various concentrations of meperidine on the duration of the extracellularly recorded nerve terminal action potential of frog sartorius.

Time (min)	% control nerve terminal potential duration ± S.E.M.			
	meperidine concentration			
	control (n=5)	8×10^{-5} M (n=5)	10^{-4} M (n=4)	1.6×10^{-4} M (n=6)
3			97.0 ± 3.1	99.6 ± 2.9
5		99.8 ± 2.7	95.8 ± 4.2	102.2 ± 1.2
10	98.2 ± 2.0	98.6 ± 1.7	98.3 ± 1.7	102.6 ± 1.2
20	97.1 ± 1.7	104.0 ± 3.5	99.7 ± 0.3	103.5 ± 1.6 *
30	99.2 ± 0.8	101.9 ± 2.8	99.7 ± 0.3	101.7 ± 1.9
40	97.6 ± 1.6	99.0 ± 3.3	101.7 ± 1.7	102.6 ± 1.3
50	101.1 ± 2.9	98.4 ± 5.0	99.3 ± 0.7	103.9 ± 2.4
60	96.7 ± 2.8	92.4 ± 3.4	103.4 ± 3.4	103.6 ± 2.6

* mean significantly different from mean of control experiment at the same drug exposure time (unpaired t-test, $p<0.05$).

Table. 7. Maintenance of mean resting membrane potential (RMP), miniature endplate potential (mepp) amplitude and mepp frequency recorded intracellularly from frog sartorius in A. the absence of drug (n=5) and B. in the presence of naloxone 3×10^{-8} M (n=3).

A.

Time (min)	% control ± S.E.M.		
	RMP	mepp amplitude	mepp frequency
10	98.7 ± 0.8	99.0 ± 1.8	98.0 ± 2.2
20	95.4 ± 2.3	100.1 ± 2.7	87.2 ± 5.4
30	94.7 ± 2.9	103.7 ± 1.8	85.0 ± 4.8
40	95.3 ± 3.4	100.8 ± 3.9	87.0 ± 6.3
50	92.0 ± 2.8	100.1 ± 1.6	91.3 ± 4.5
60	95.0 ± 3.9	98.3 ± 2.3	92.5 ± 6.0

B.

Time (min)	% control ± S.E.M.		
	RMP	mepp amplitude	mepp frequency
10	98.7 ± 0.7	98.9 ± 2.1	114.0 ± 3.2
20	98.7 ± 0.7	98.0 ± 3.9	109.3 ± 5.5 *
30	99.0 ± 1.5	96.3 ± 1.2	104.7 ± 1.8 *
50	95.3 ± 2.3	95.1 ± 3.2	108.7 ± 7.7
60	93.3 ± 2.6	90.0 ± 5.8	96.3 ± 2.3

* means significantly different from mean of control experiments in A at the same drug exposure time (unpaired t-test, $p<0.05$).

Table 8. A. The effect of meperidine 8 X 10^{-5} M on the mean resting membrane potential (RMP), miniature endplate potential (mepp) amplitude and mepp frequency recorded intracellularly from frog sartorius (n=7) and B. lack of antagonism by naloxone (n=5).

A.

Time (min)	% control ± S.E.M.		
	RMP	mepp amplitude	mepp frequency
10	98.7 ± 1.0	89.2 ± 2.4 *	101.1 ± 2.7
20	98.0 ± 1.9	79.4 ± 3.2 *	105.1 ± 3.5 *
30	93.1 ± 2.6	68.3 ± 3.0 *	101.7 ± 5.9
40	93.8 ± 2.0	62.8 ± 5.5 *	110.6 ± 2.6 *
50	92.2 ± 2.3	59.7 ± 5.5 *	102.6 ± 5.0
60	94.3 ± 3.7	56.4 ± 12.6 *	109.0 ± 15.0

B.

Time (min)	% control ± S.E.M.		
	RMP	mepp amplitude	mepp frequency
10	99.2 ± 1.1	86.3 ± 2.2	99.2 ± 10.1
20	97.4 ± 1.2	76.1 ± 1.3	104.2 ± 10.7
30	96.2 ± 1.6	68.0 ± 2.5	88.6 ± 3.0
40	93.2 ± 2.8	63.8 ± 2.1	95.6 ± 3.8 **
50	92.6 ± 2.8	50.5 ± 2.6	98.8 ± 6.7
60	93.0 ± 3.6	58.8 ± 3.2	107.0 ± 3.6

* significantly different from corresponding mean of control experiments by an unpaired t-test, $p<0.05$.

** significantly different when means in B were compared to the mean of the corresponding time period in A.

Table 9. A. The effect of meperidine 1.6×10^{-4} M on the mean resting membrane potential (RMP), miniature endplate potential (mepp) amplitude and mepp frequency recorded intracellularly from frog sartorius (n=3) and
B. lack of antagonism by naloxone 3×10^{-8} M (n=3).

A.

Time (min)	% control ± S.E.M.		
	RMP	mepp amplitude	mepp frequency
10	96.0 ± 4.4	82.1 ± 4.8 *	94.7 ± 5.7
20	94.7 ± 4.2	64.7 ± 1.4 *	85.0 ± 8.1
30	90.7 ± 6.3	51.5 ± 2.9 *	96.3 ± 9.0

B.

Time (min)	% control ± S.E.M.	
	RMP	mepp amplitude
10	96.5 ± 0.5	67.3 ± 5.9 **
20	98.0 ± 2.0	63.2 ± 1.4
30	98.0 ± 2.0	54.4 ± 1.6

* significantly different from corresponding mean in control experiments by an unpaired t-test, $p<0.05$.

** means in B were compared to the mean of the corresponding time period in A by an unpaired t-test and found significantly different ($p<0.05$).

Table 10. Control study of the mean resting membrane potential (RMP), endplate potential (EPP) amplitude, and miniature endplate potential (mepp) amplitude, and the derived quantal content (EPP/mepp) recorded intracellularly from frog sartorius in the presence of 9 to 10 mM $MgCl_2$ (n=5).

Time (min)	% control ± S.E.M.			
	RMP	EPP amplitude	mepp amplitude	quantal content
10	98.5 ± 1.4	103.0 ± 1.2	100.3 ± 3.1	102.1 ± 2.0
20	98.4 ± 1.9	92.6 ± 6.3	99.1 ± 4.9	93.1 ± 4.1
30	97.7 ± 2.0	107.3 ± 7.7	97.0 ± 4.5	110.8 ± 6.4

Table 11. A. Lack of effect of meperidine 1.6×10^{-4} M on quantal content in frog sartorius. Resting membrane potential (RMP), endplate potential (EPP) amplitude and miniature endplate potential (mepp) amplitude were measured intracellularly in the presence of 8 to 10 mM $MgCl_2$ (n=4).
B. Experiments were repeated in the additional presence of naloxone 3×10^{-8} M (n=3).

A.

Time (min)	% control ± S.E.M.			
	RMP	EPP amplitude	mepp amplitude	quantal content
5	95.7 ± 1.1	68.4 ± 2.3 *	73.6 ± 2.0 *	93.3 ± 1.8
10	94.3 ± 2.9	61.9 ± 4.6 *	71.0 ± 5.2 *	87.7 ± 4.5 *
20	92.5 ± 3.2	62.8 ± 3.2 *	62.0 ± 4.8 *	93.1 ± 4.8
30	91.8 ± 3.7	63.3 ± 7.3 *	64.5 ± 4.5 *	98.5 ± 7.9

B.

Time (min)	% control ± S.E.M.			
	RMP	EPP amplitude	mepp amplitude	quantal content
5	96.9 ± 1.6	80.1 ± 5.2	72.9 ± 3.5	107.4 ± 2.3 **
10	93.4 ± 2.8	72.8 ± 4.3	70.5 ± 2.8	102.7 ± 10.6
20	94.1 ± 2.7	66.5 ± 9.2	64.7 ± 1.3	102.9 ± 13.9
30	94.1 ± 2.7	62.0 ± 6.7	63.2 ± 1.1	97.3 ± 12.8

* significantly different from corresponding mean of control experiments by an unpaired t-test, $p<0.05$.

** significantly different from corresponding mean in A. by an unpaired t-test, $p<0.05$.

Table 12. A. The effect of meperidine 4.2×10^{-4} M on mean quantal content in frog sartorius. Resting membrane potential (RMP), endplate potential (EPP) amplitude, and miniature endplate potential (mepp) amplitude were recorded intracellularly in the presence of 8 to 10 mM $MgCl_2$ (n=5).
B. Experiments were repeated in the additional presence of naloxone 3×10^{-8} M (n=4).

A.

Time (min)	% control ± S.E.M.			
	RMP	EPP amplitude	mepp amplitude	quantal content
5	100.0 ± 1.0	52.9 ± 2.4 *	52.4 ± 3.2 *	101.6 ± 2.0
10	95.1 ± 1.5	47.1 ± 2.3 *	46.6 ± 1.6 *	101.9 ± 5.7
20	93.5 ± 2.6	40.3 ± 4.2 *	46.6 ± 2.5 *	85.9 ± 5.0
30	94.2 ± 3.2	39.1 ± 4.2 *	43.4 ± 5.2 *	89.9 ± 0.6

B.

Time (min)	% control ± S.E.M.			†
	RMP	EPP amplitude	mepp amplitude	quantal content
5	98.1 ± 0.9	53.6 ± 4.9	55.9 ± 2.9	96.3 ± 8.8
10	97.9 ± 2.1	51.2 ± 5.5	52.3 ± 2.7	98.3 ± 10.8
20	96.3 ± 3.1	45.1 ± 5.4	44.2 ± 1.4	101.6 ± 11.6
30	99.4 ± 0.6	43.6 ± 5.7	48.2 ± 3.5	92.4 ± 17.1

* significantly different from corresponding mean of control experiments by an unpaired t-test, $p<0.05$.

† There was no significant difference between corresponding means in A and B.

Table 13. A. The effect of meperidine 4.2 X 10^{-4} M on resting membrane potential (RMP) and endplate potential (EPP) amplitude of frog sartorius recorded intracellularly in the presence of d-tubocurarine 2 to 4 X 10^{-6} M (n=7).
B. Partial reversal by naloxone 3 X 10^{-8} M (n=5).

A.

Time (min)	% control ± S.E.M.	
	RMP	EPP amplitude
5	102.4 ± 2.3	36.2 ± 2.6 *
10	101.9 ± 1.9	34.0 ± 2.3 *
20	99.6 ± 1.7	30.5 ± 1.5 *
30	97.6 ± 2.6	28.9 ± 1.4 *

B.

Time (min)	% control ± S.E.M.	
	RMP	EPP amplitude
5	97.6 ± 1.8	47.3 ± 2.8 **
10	97.1 ± 1.9	41.9 ± 3.1
20	97.1 ± 1.9	41.3 ± 3.3 **
30	97.1 ± 1.9	40.0 ± 7.0 **

* significantly different from mean depression of EPP amplitude in the presence of $MgCl_2$ by meperidine 4.2 X 10^{-4} M. (unpaired t-test, $p<0.05$)

** significantly different from the corresponding mean in A.

Table 14. The effect of meperidine 4.2 X 10^{-4} M on resting membrane potential (RMP) and endplate potential (EPP) amplitude in the presence of physostigmine 1.3 X 10^{-4} M and d-tubocurarine 9 X 10^{-6} M (n=3).

Time (min)	% control ± S.E.M.	
	RMP	EPP amplitude
5	100.9 ± 0.9	44.2 ± 2.1
10	100.9 ± 0.9	39.4 ± 0.9
20	101.3 ± 1.9	36.0 ± 0.9
30	101.3 ± 1.9	31.1 ± 1.7

+ not significantly different from corresponding mean depression in the presence of meperidine 4.2 X 10^{-4} M and d-tubocurarine 2 to 4 X 10^{-6} M by an unpaired t-test, $p<0.05$.

Table 15. A. Control study of the mean resting potential (RMP) and the amplitude of the endplate potential evoked by iontophoretic application of acetylcholine (EPP_I) recorded intracellularly from frog sartorius (n=4).
B. The effect of meperidine 1.6 X 10^{-4} M on RMP and EPP_I (n=4)
C. The lack of effect of naloxone 3 X 10^{-8} M on the depression of EPP_I amplitude induced by meperidine 1.6 X 10^{-4} M.

A. Time (min)	% control ± S.E.M.	
	RMP	EPP_I amplitude
5	99.9 ± 3.1	105.6 ± 7.7
10	102.6 ± 3.6	92.6 ± 10.7
20	105.1 ± 5.8	94.0 ± 6.0
30	106.5 ± 5.2	91.4 ± 7.3

B. Time (min)	% control ± S.E.M.	
	RMP	EPP_I amplitude
5	92.8 ± 7.7	55.4 ± 7.6 *
10	96.7 ± 7.5	50.2 ± 8.5 *
20	97.9 ± 9.5	40.2 ± 6.0 *
30	101.9 ± 4.4	35.0 ± 5.7 *

C. Time (min)	% control ± S.E.M.	
	RMP	EPP_I amplitude
5	98.2 ± 2.5	52.7 ± 3.6
10	97.3 ± 1.6	52.3 ± 3.6
20	94.6 ± 1.4	38.6 ± 5.0
30	89.9 ± 3.3	27.5 ± 5.3

* significantly different from corresponding mean in A by an unpaired t-test, $p<0.05$.

+ no significant difference between means of B and C.

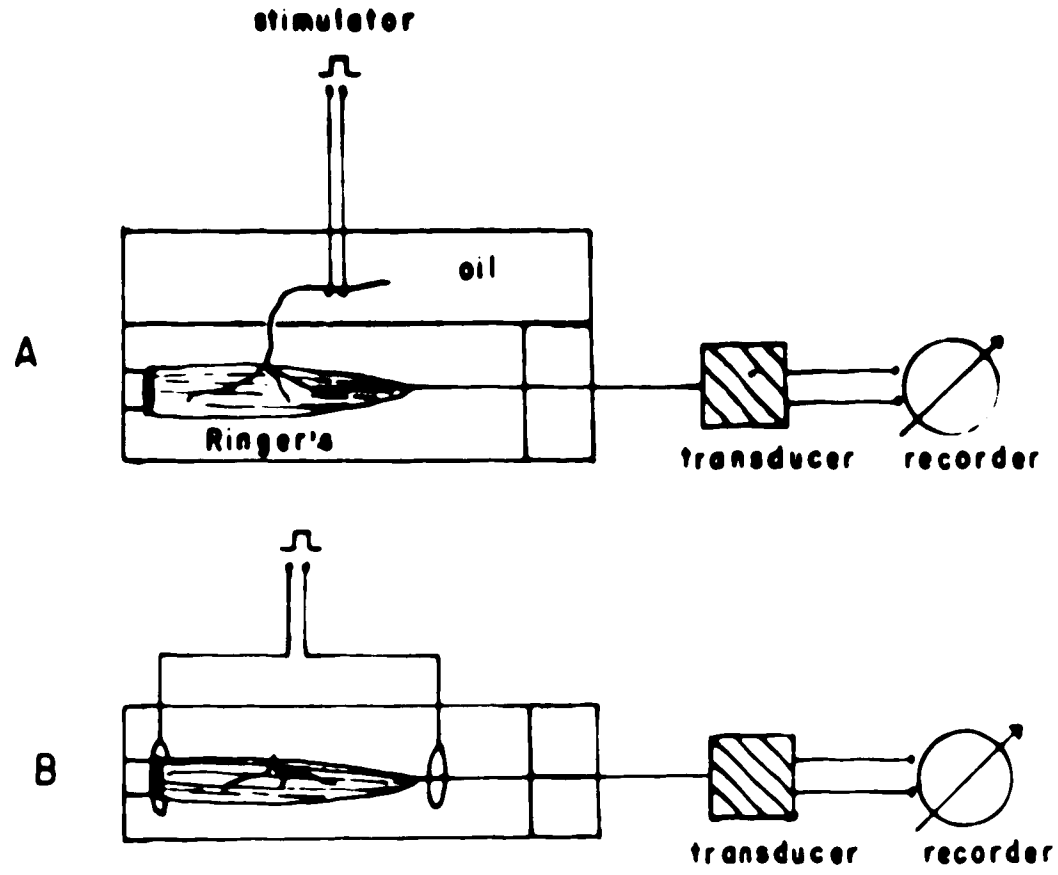


Figure 1. Schematic diagrams of apparatus used for recording muscle twitch responses: A. to sciatic nerve stimulation and B. to direct muscle stimulation.

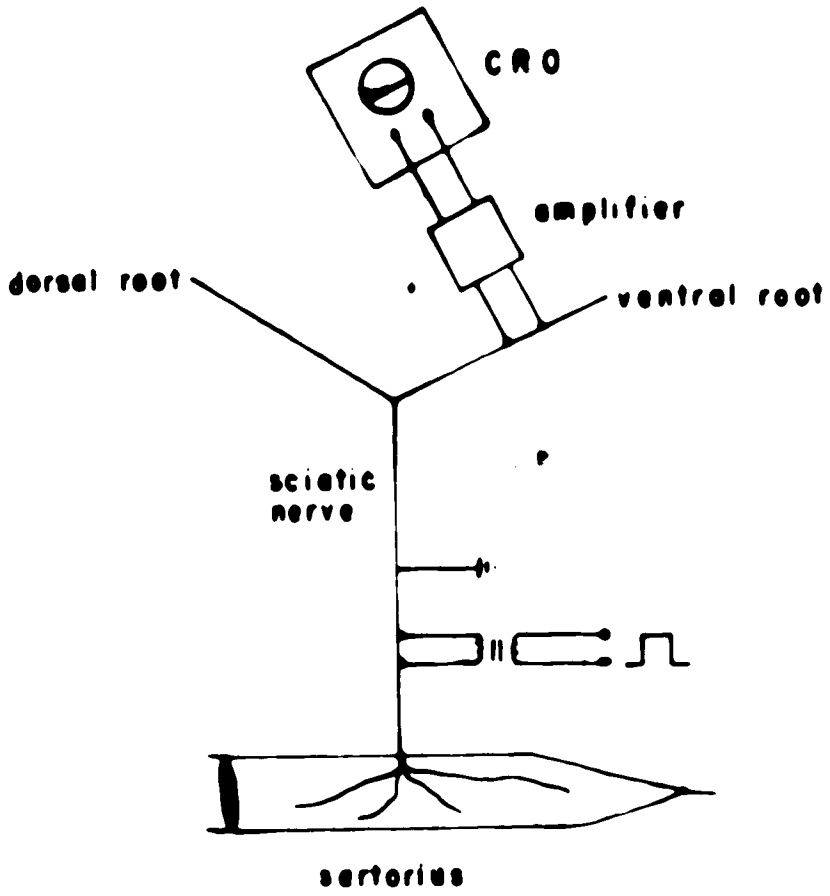


Figure 2. Experimental set up for recording motor nerve action potentials from the eighth spinal nerve. In the presence of TEA, repetitive electrical activity was generated in nerve terminals either spontaneously or after sciatic nerve stimulation. These action potentials were conducted antidromically and recorded from the ventral root using platinum, extracellular electrodes. Signals were amplified and recorded differentially on the oscilloscope.

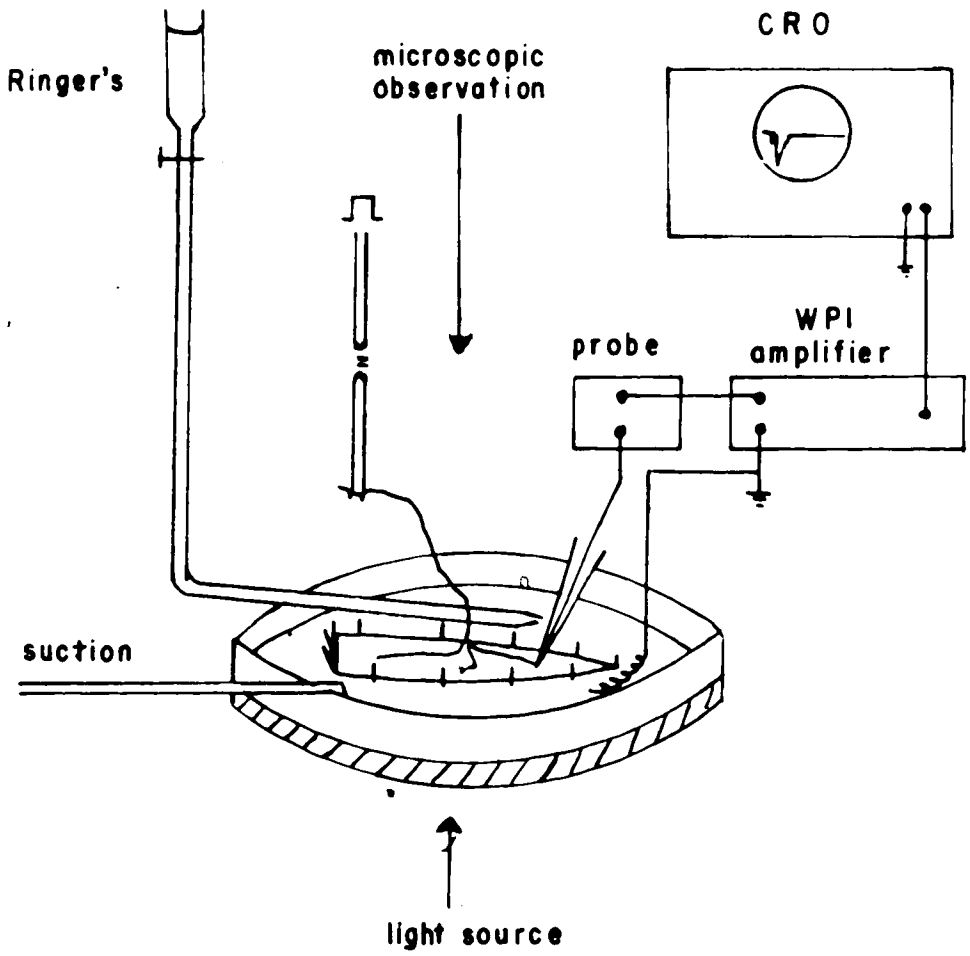


Figure 3. Schematic diagram of recording and stimulating circuits for recording simultaneously the nerve action potential and endplate potential from frog sartorius muscle using extracellular electrodes.

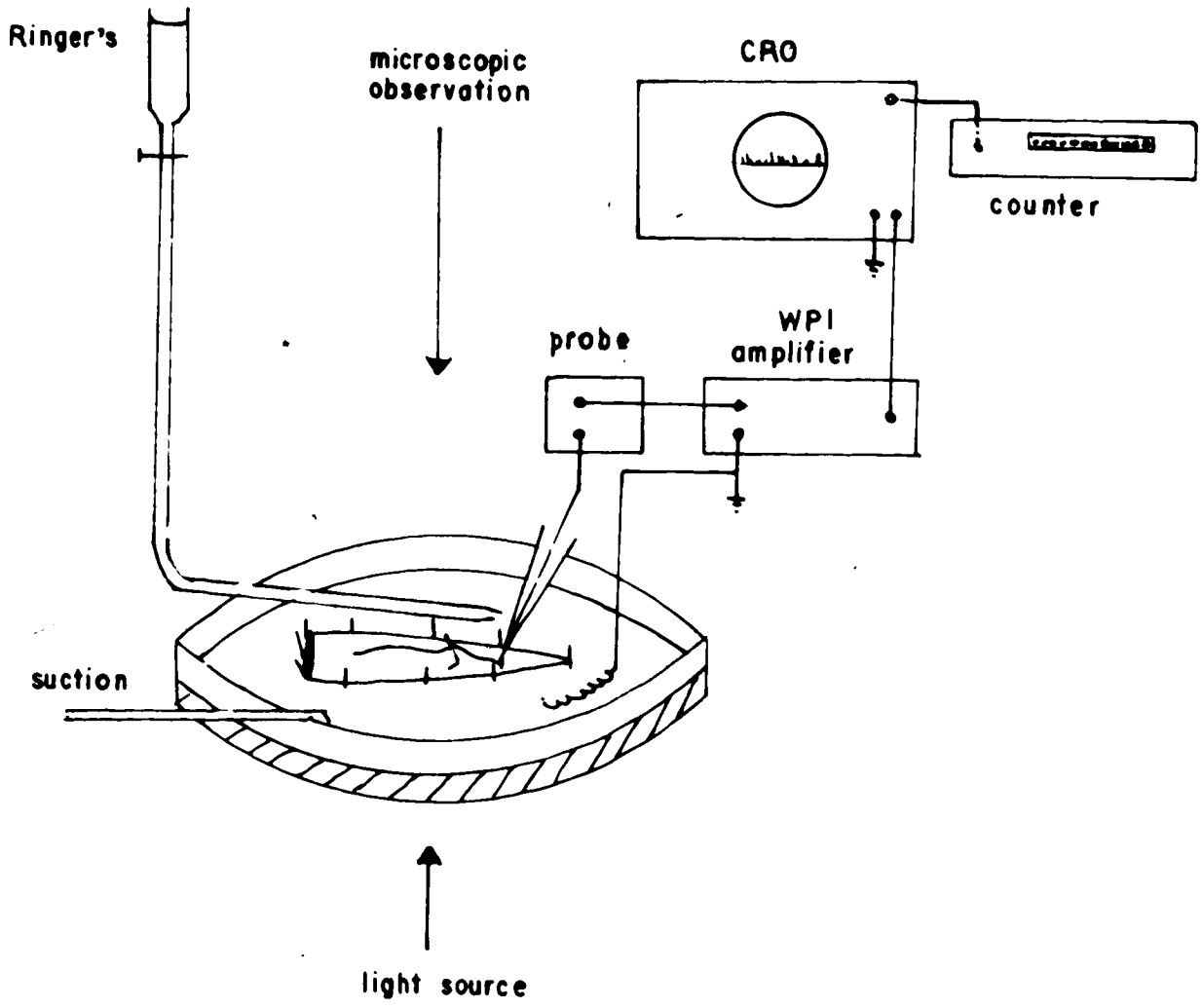


Figure 4. Schematic diagram of apparatus used for recording miniature endplate potentials intracellularly from frog sartorius muscles.

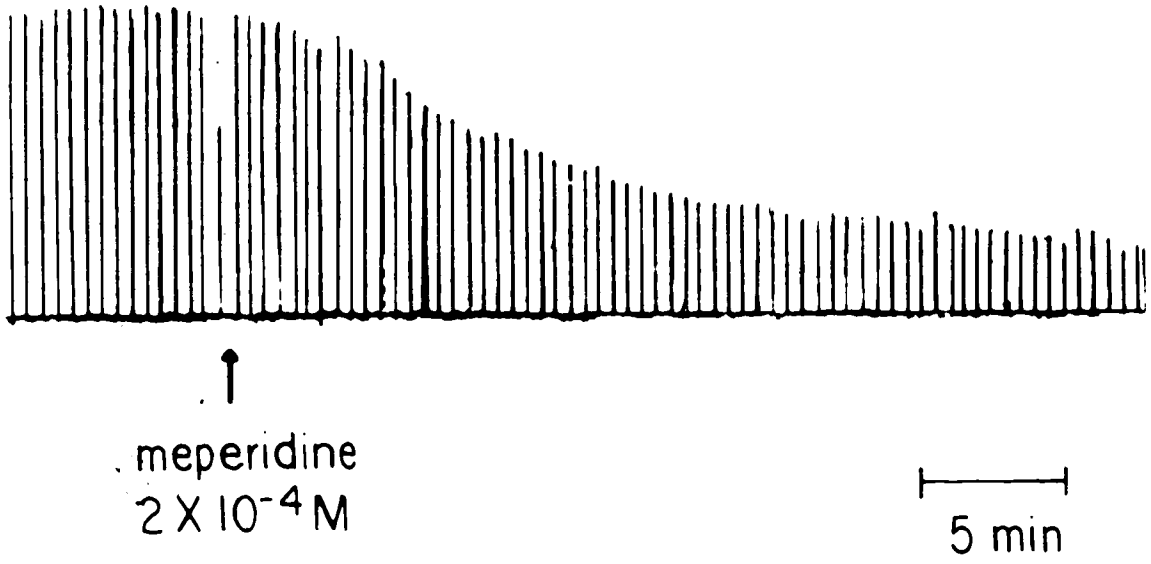


Figure 5. The action of meperidine on the response of frog sartorius muscle to sciatic nerve stimulation. The nerve was stimulated every 30 seconds by pulses of one msec duration and supramaximal voltage.

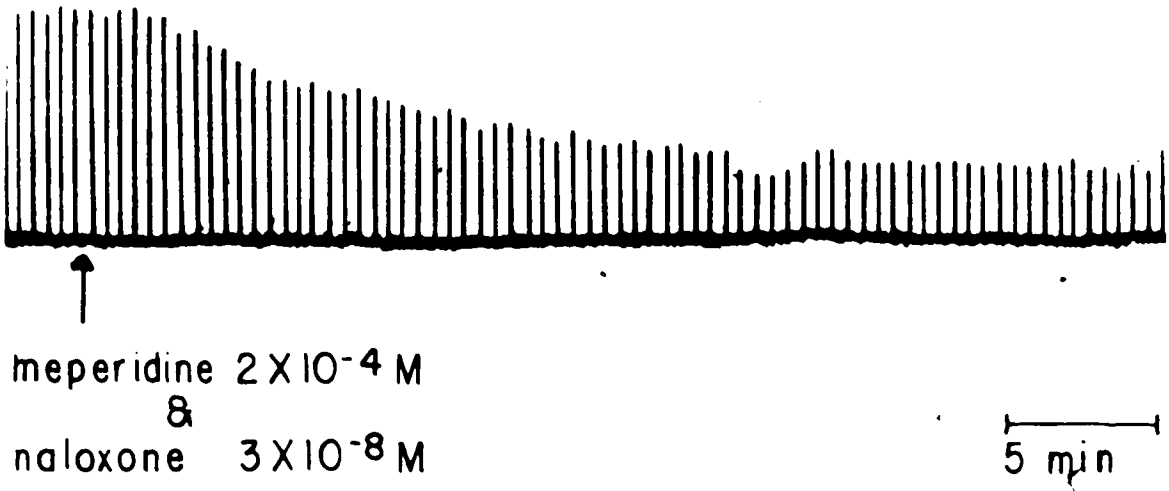


Figure 6. The action of the narcotic antagonist naloxone on meperidine-induced depression of twitch responses to sciatic nerve stimulation in frog sartorius muscle.

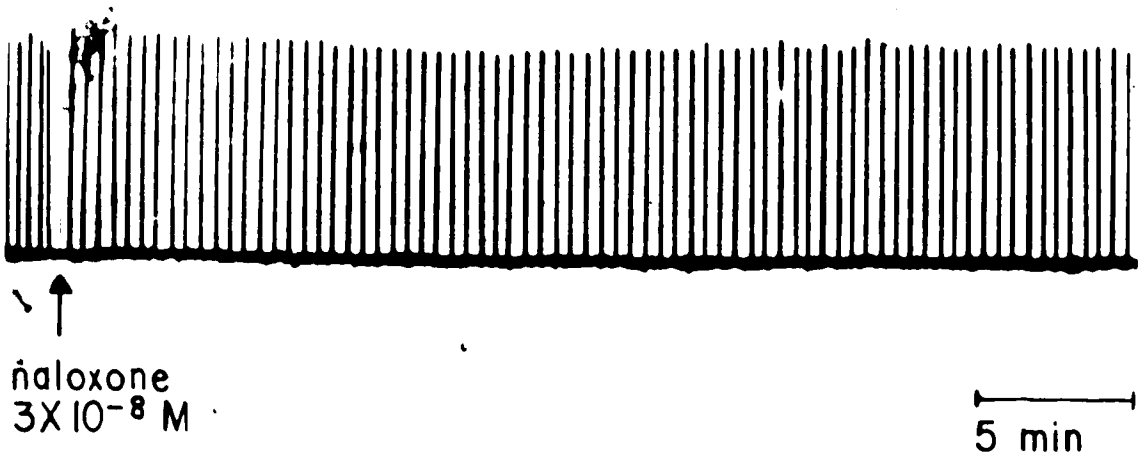


Figure 7. The action of naloxone (3×10^{-8} M) on the response of frog sartorius muscle to sciatic nerve stimulation. The nerve was stimulated every 30 seconds by pulses of one msec duration and supramaximal voltage.

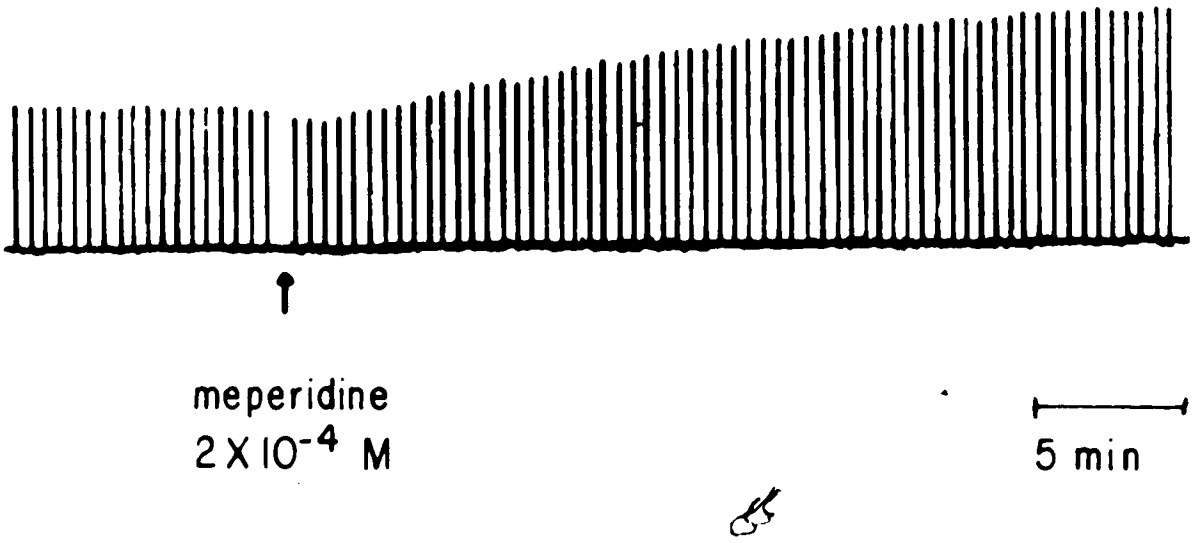


Figure 8. The potentiation by meperidine of frog sartorius muscle twitches elicited by direct muscle stimulation. The muscle was stimulated every 30 seconds by pulses of one msec duration and supramaximal voltage delivered to platinum rings surrounding each end of the muscle.

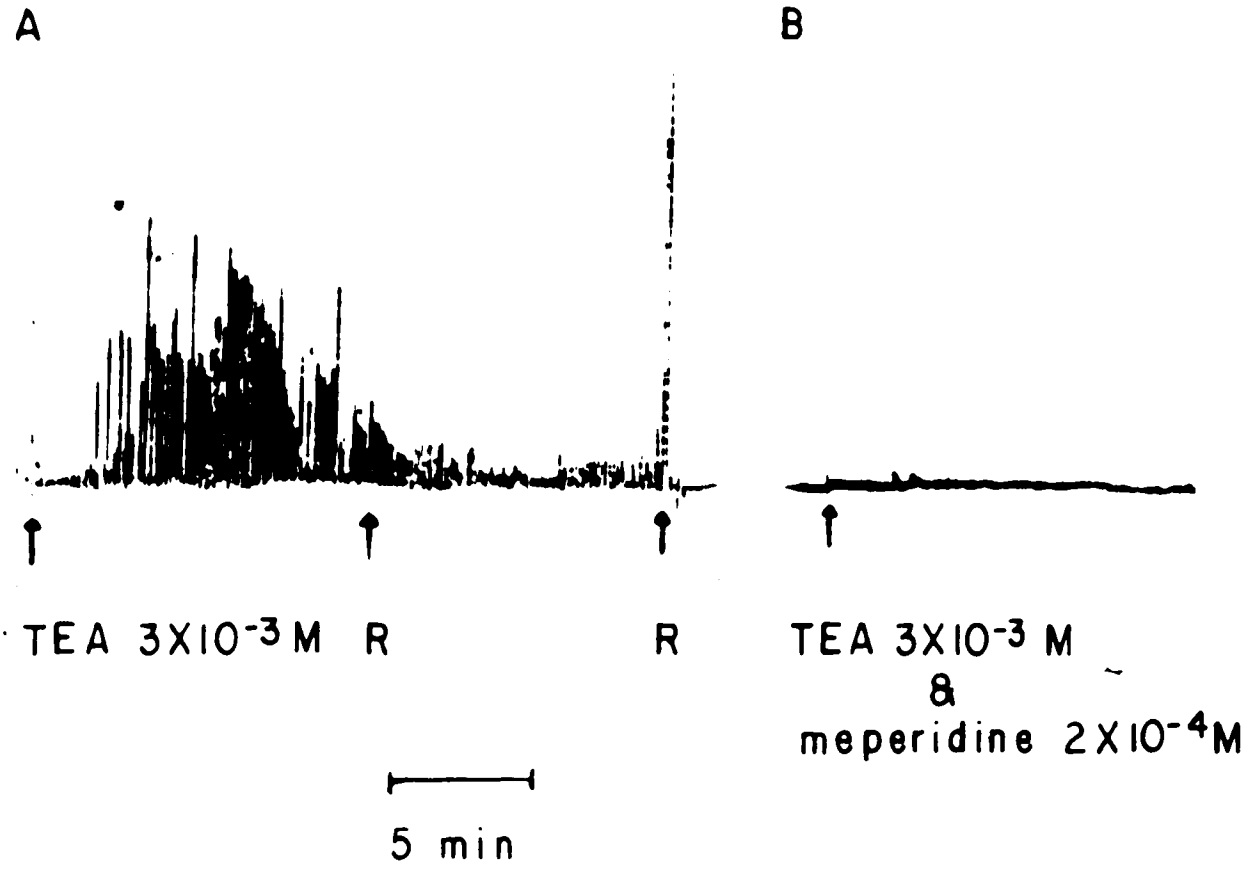


Figure 9. A. Tetraethylammonium chloride (TEA)-induced contractions in frog sartorius muscle (tracings from oscillograph records). At R the muscle was washed with Ringer's solution without TEA.
B. Prevention of TEA-induced contractions by meperidine in the same muscle.

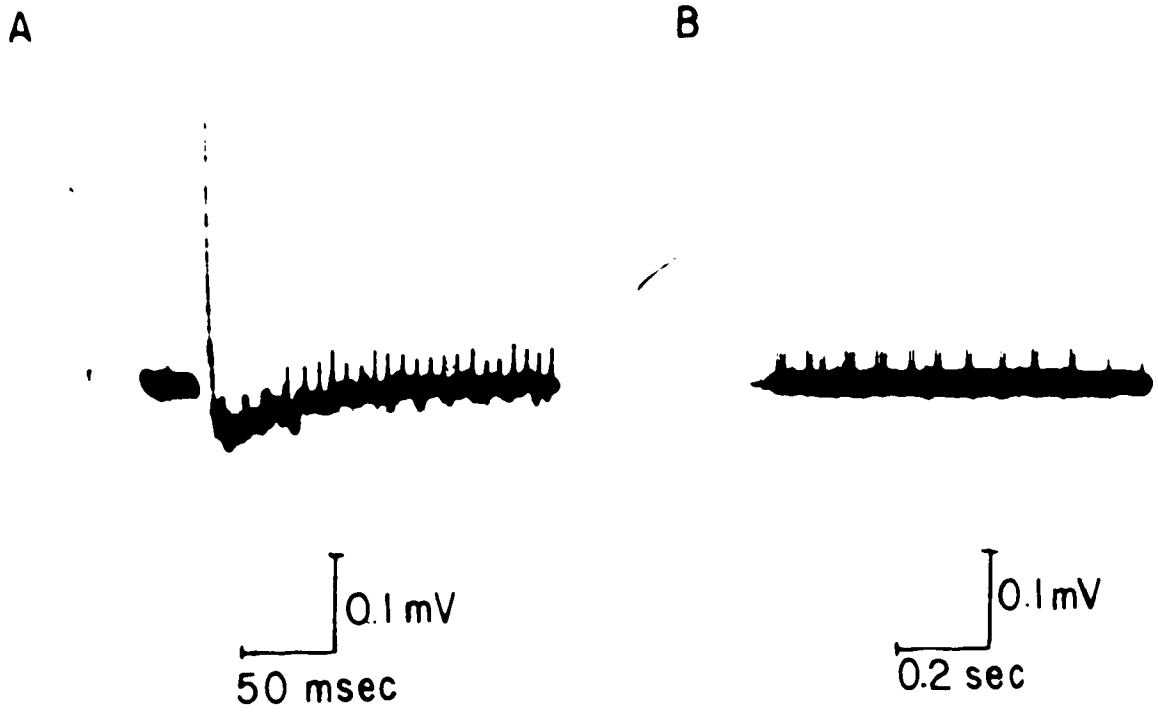


Figure 10. Frog sartorius motor nerve terminal firing during treatment with tetraethylammonium recorded extracellularly from the ventral root of the eighth spinal nerve.

A. Repetitive activity following a single sciatic nerve stimulation of supramaximal voltage, one msec duration. The nerve was being stimulated at a rate of four per minute after 20 minutes of treatment with 6.8×10^{-3} M TEA.

B. Spontaneous activity in the absence of electrical stimulation after 10 minutes treatment with 27×10^{-3} M TEA.

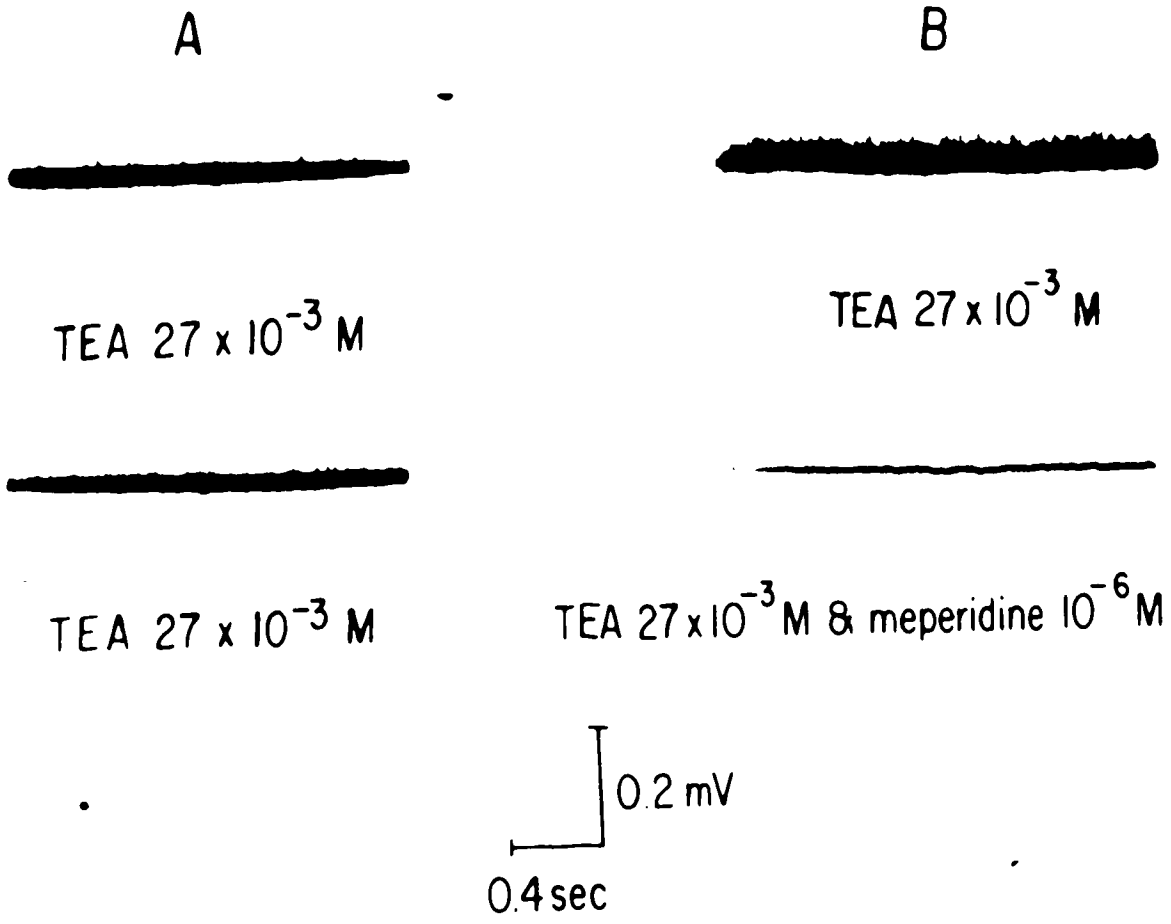


Figure 11. A. Recordings from the ventral root during a control experiment showing maintenance of tetraethylammonium (TEA)-induced spontaneous firing.
upper trace: after 10 minutes exposure of the muscle to 27 X 10^{-3} M TEA
lower trace: after 2 hours exposure to 27 X 10^{-3} M TEA
B. Blockade by meperidine of TEA-induced spontaneous activity in another preparation.
upper trace: nerve electrical activity recorded from the ventral root after 10 minutes exposure of the muscle to 27 X 10^{-3} M TEA
lower trace: 2 hours after inclusion of 10^{-6} M meperidine to the TEA-Ringer's. Meperidine was added 10 minutes after initiation of spontaneous firing.

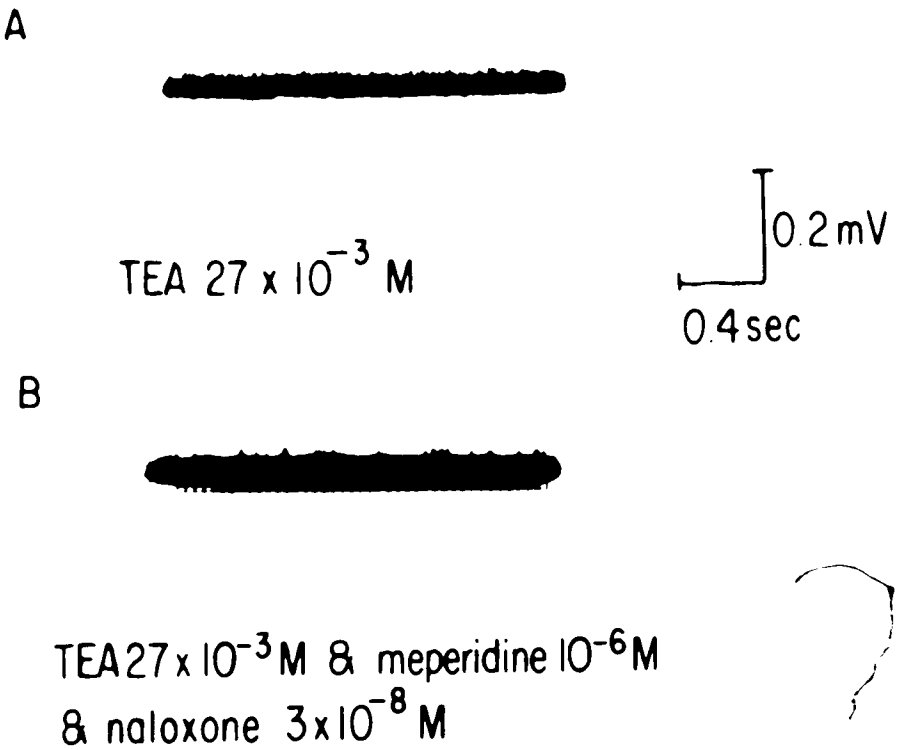


Figure 12. Recordings from an experiment in which naloxone prevented complete abolition of TEA-induced spontaneous activity by meperidine.
A. Nerve electrical activity recorded 10 min after placing the muscle in 27 X 10^{-3} M TEA.
B. Two hours after addition of 10^{-6} M meperidine and 3 X 10^{-8} M naloxone to the TEA-Ringer's.

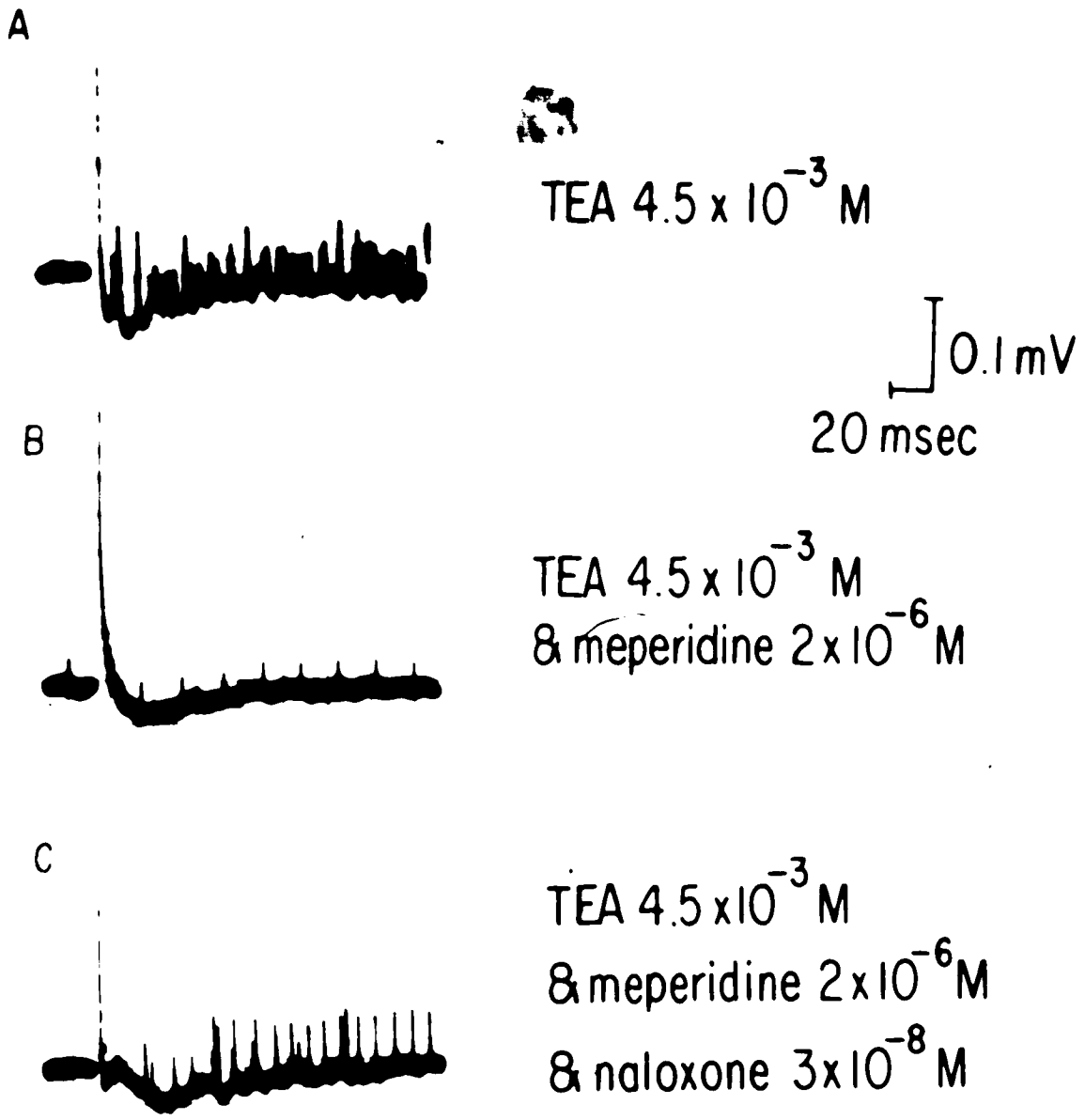


Figure 13. Meperidine blockade of tetraethylammonium (TEA)-induced activity evoked by electrical stimulation of the sciatic nerve and reversal by naloxone. The sciatic trunk was stimulated with supramaximal voltage and one msec pulse duration at a rate of four per minute. Potentials were recorded from the ventral root.

A. Recording made one-half hour after placing the muscle in 4.5 X 10^{-3} M TEA.

B. Recording made one hour after addition of 2 X 10^{-6} M meperidine to the bathing solution. Meperidine was added to the bath 15 minutes after A.

C. Recording made one-half hour after addition of 3 X 10^{-8} M naloxone to the bathing solution. Naloxone was added 5 minutes after B.

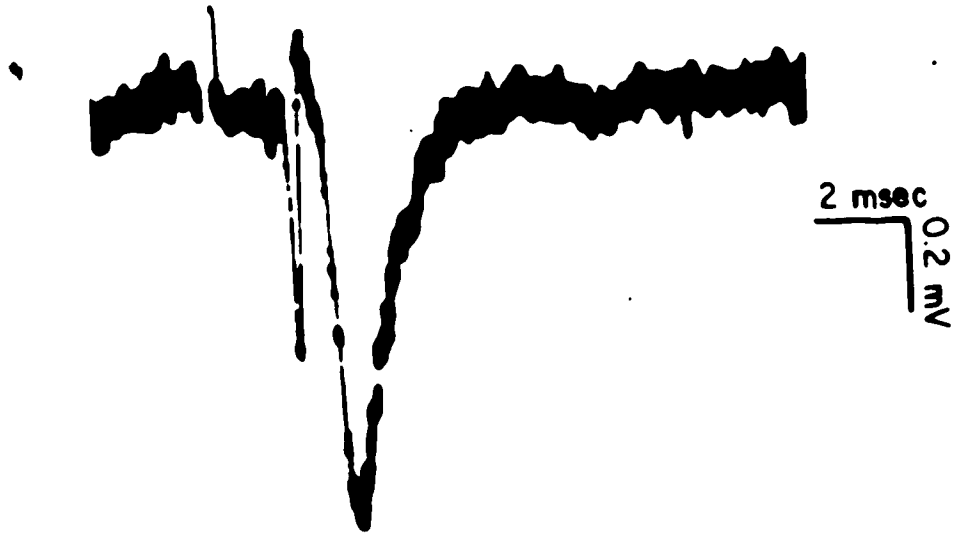


Figure 14. Representative recording of the nerve terminal action potential and endplate potential from frog's sartorius recorded extracellularly with a single electrode. The nerve terminal action potential occurred about 2 msec after the initial stimulus artifact and the endplate potential follows the nerve action potential with a delay of approximately one msec. Experiments were performed in the presence of 2 to 4 X 10^{-6} M d-tubocurarine.

d-tubocurarine 4×10^{-6} M

d-tubocurarine 1.3×10^{-4} M

0.2 mV

2 msec

Figure 15. The effect of increasing d-tubocurarine concentration on the extracellularly recorded nerve terminal action potential and the endplate potential.
Raising the concentration of d-tubocurarine from 4×10^{-6} M to 1.3×10^{-4} M abolished the endplate potentials within 5 minutes, but had no effect on the nerve potential. Both recordings are from the same endplate.

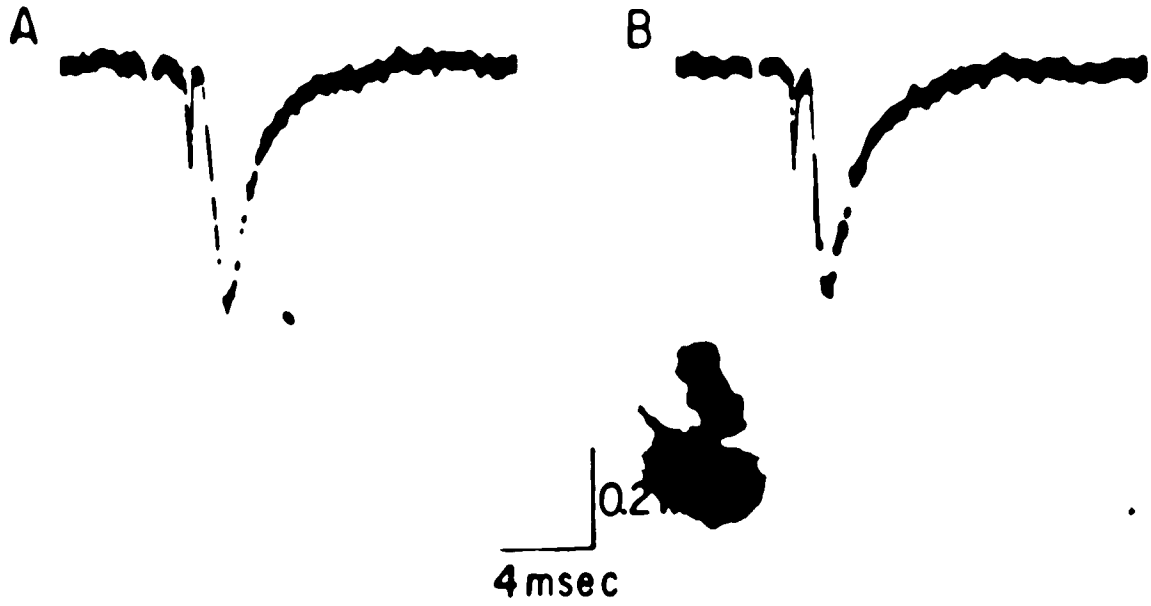


Figure 16. Representative recordings illustrating maintenance of the amplitude and time course of the extracellularly recorded nerve terminal potential and endplate potential in a control experiment: A. during the first 2 minutes of stimulation, B. 60 minutes later. The sciatic nerve was stimulated by pulses of supramaximal voltage and 1.5 msec duration at a rate of 12 per minute.

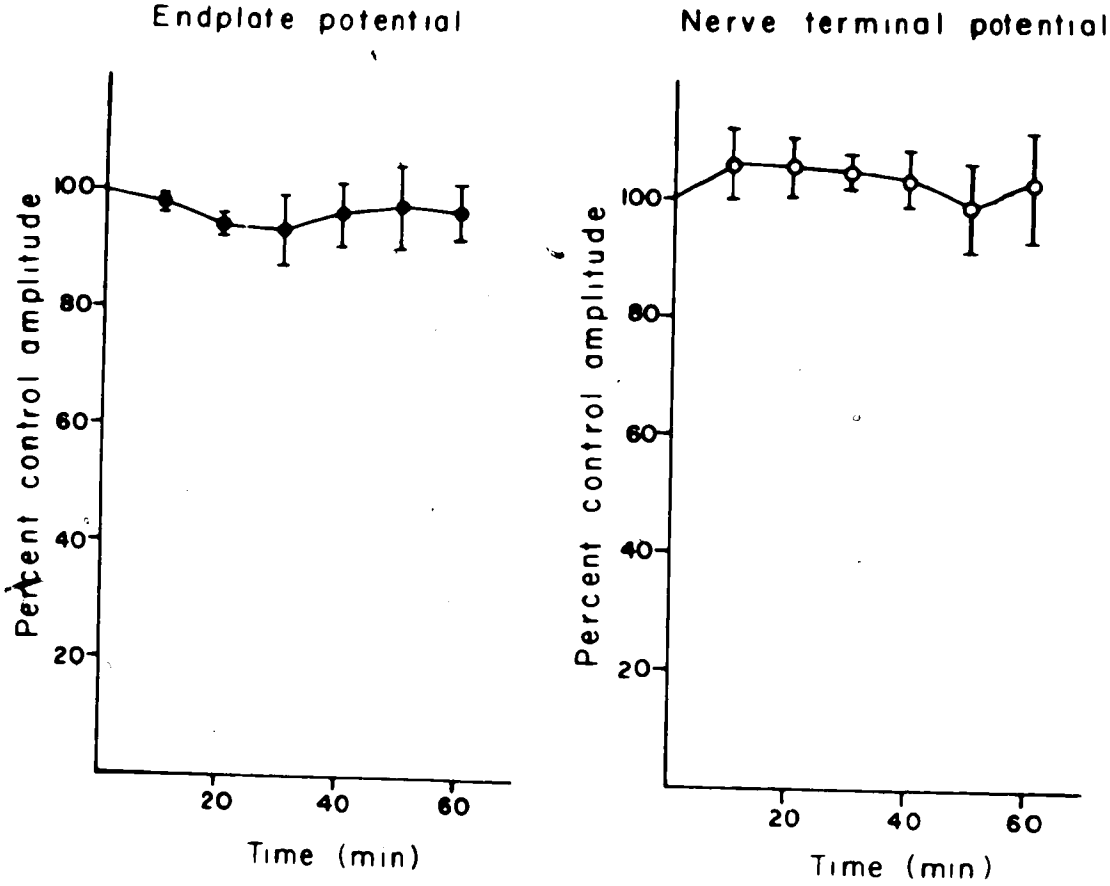


Figure 17. Control study of the amplitude of the extracellularly recorded endplate potential and nerve terminal action potential from frog sartorius muscles perfused with Ringer's solution containing 2 to 4 X 10^{-6} M d-tubocurarine (n=5).
Means ± S.E.M. were calculated from the means obtained in each experiment expressed as percentage of the initial control values.

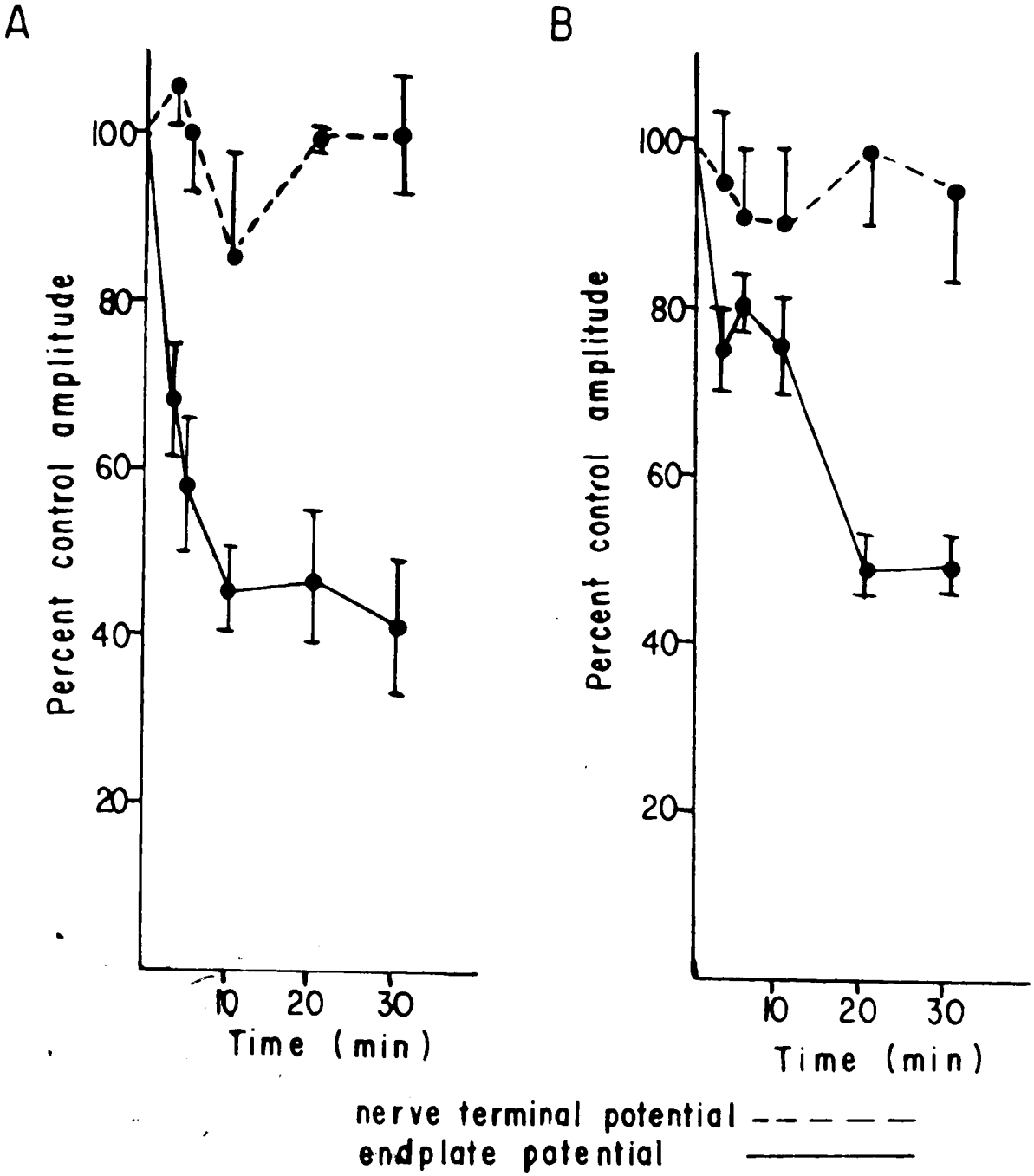


Figure 18. A. First study of the action of meperidine 8 X 10^{-5} M on the amplitude of the extracellularly recorded nerve terminal action potential and endplate potential (n=3).
B. Partial antagonism by naloxone 3 X 10^{-8} M of the depression of endplate potential amplitude induced by meperidine.
Drugs were added at time 0.
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control (absence of drug) response.

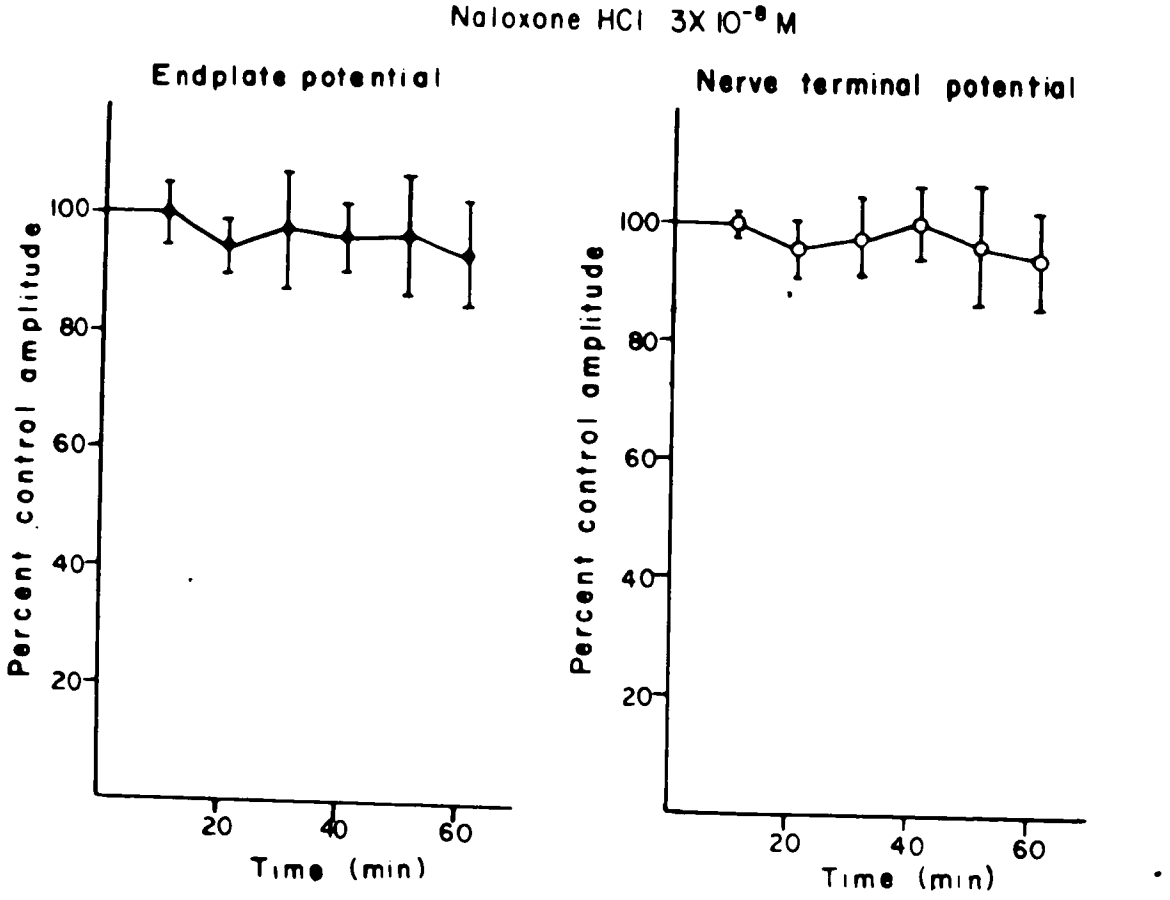


Figure 19. The action of naloxone 3 X 10^{-8} M on the amplitude of the extracellularly recorded endplate potential and nerve terminal action potential (n=4).
Naloxone was added at time 0.
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.

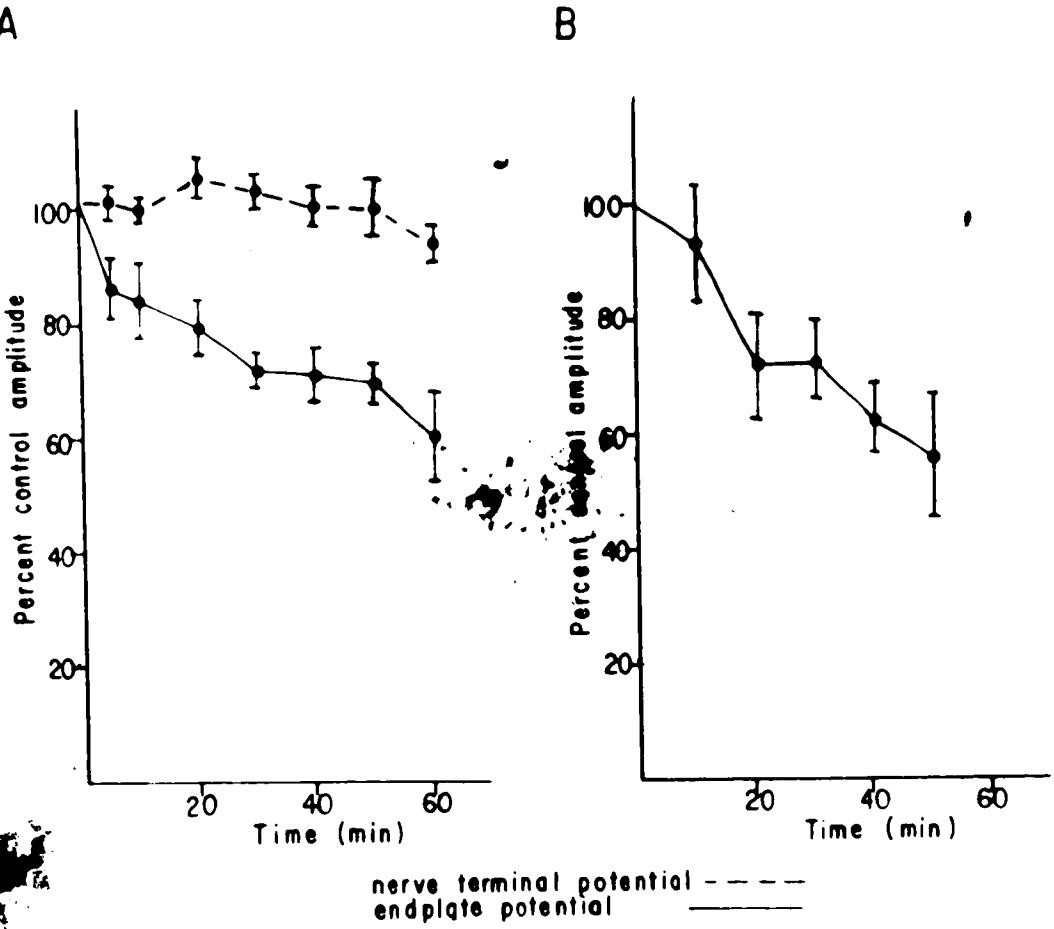


Figure 20. A. The action of meperidine 8 X 10^{-5} M on the amplitude of the extracellularly recorded nerve terminal potential and endplate potential (n=5).
B. Effect of 8 X 10^{-5} M meperidine and 3 X 10^{-8} M naloxone on the endplate potential amplitude (n=3).
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.

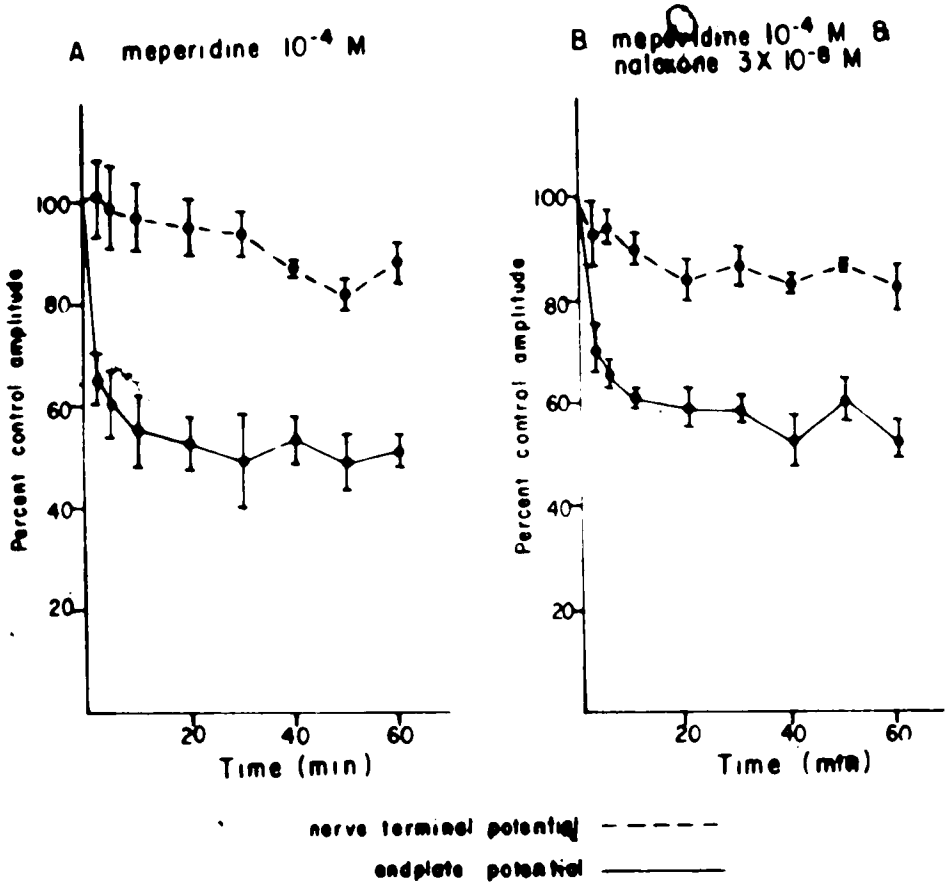


Figure 21. A. The action of meperidine 10^{-4} M on the amplitude of the extracellularly recorded nerve terminal potential and endplate potential (n=4).
B. The effect of naloxone 3×10^{-8} M and meperidine 10^{-4} M on endplate potential and nerve terminal action potential amplitudes (n=3).
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control responses.

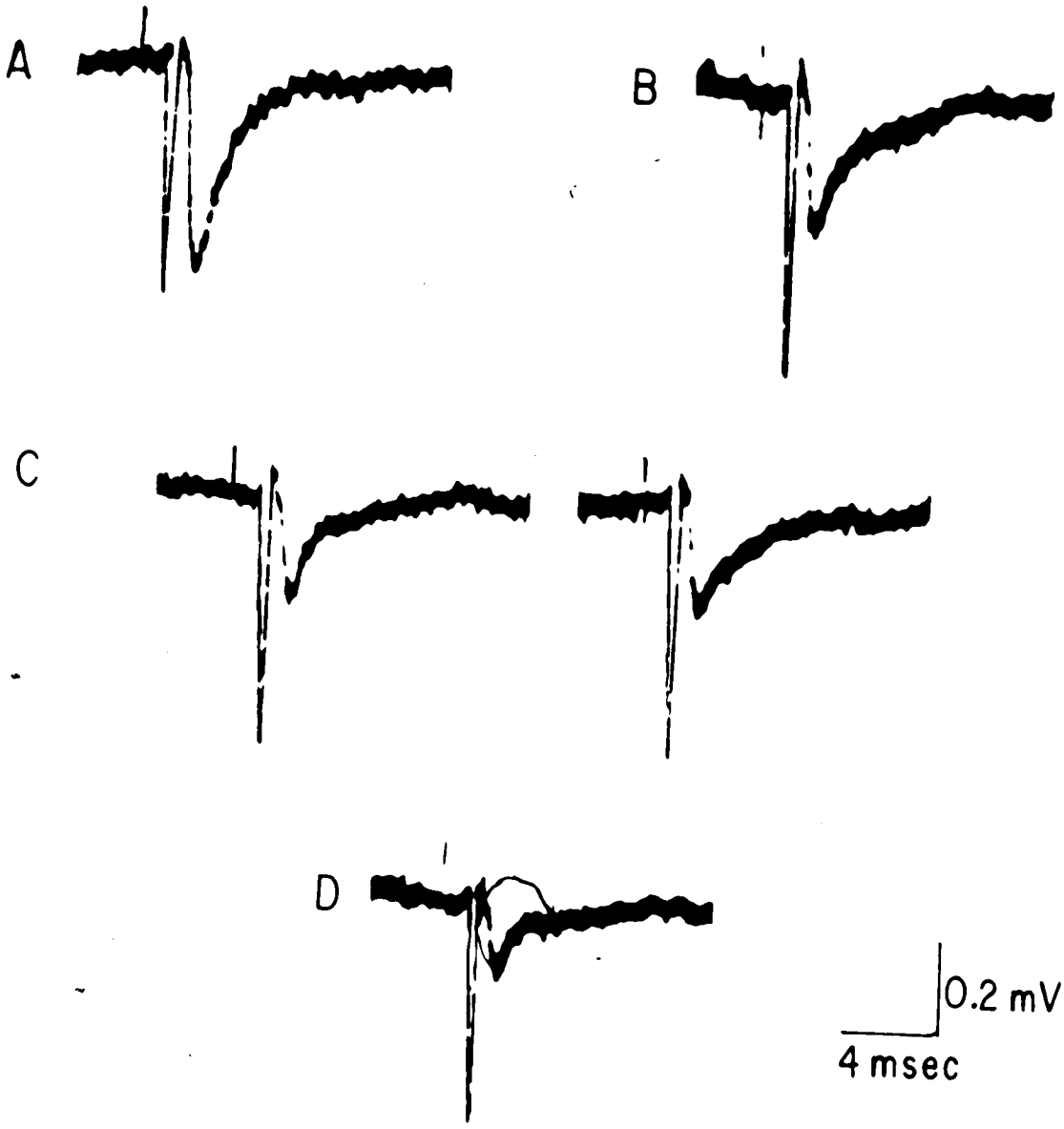


Figure 22. Recordings of extracellularly recorded nerve terminal potential and endplate potential before and during exposure to meperidine 1.6 X 10^{-4} M showing the gradual depression of EPP amplitude and alteration in its time course.

A. Control
B. Three minutes after exposure to meperidine
C. Two potentials at 10 minutes
D. At 60 minutes

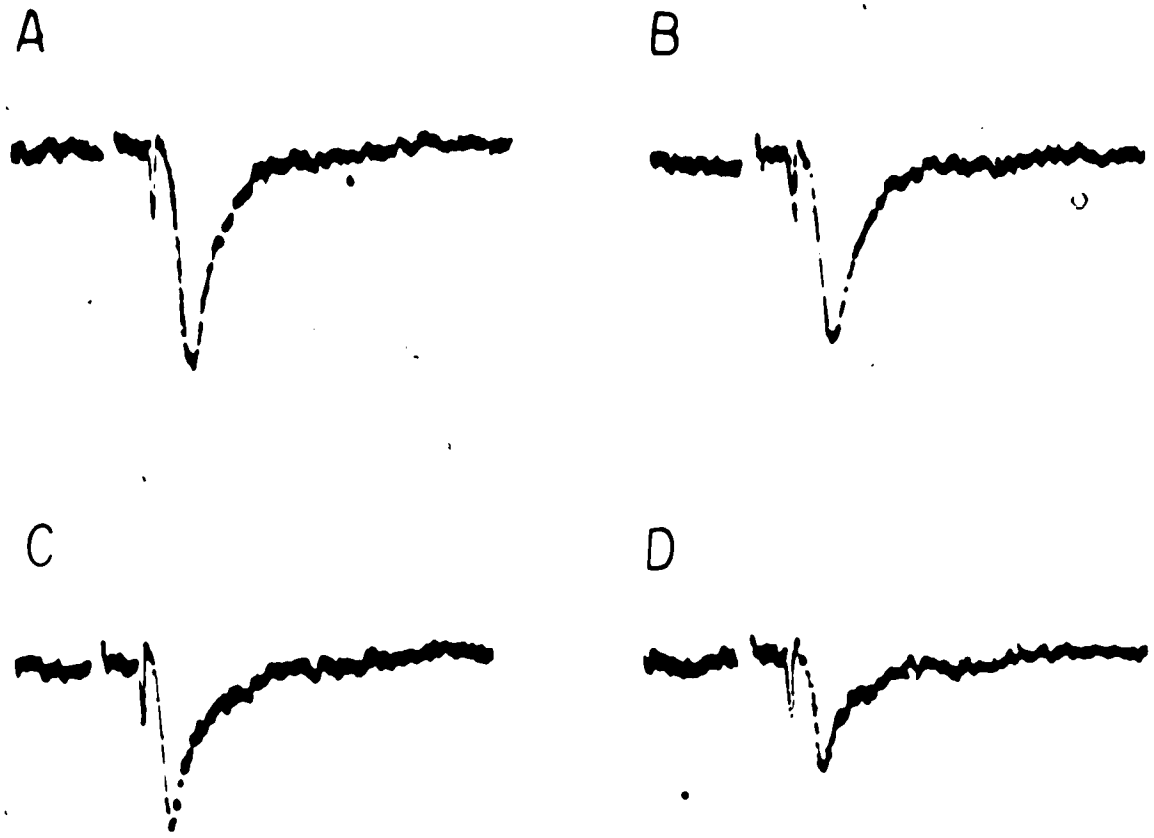


Figure 23. Recordings of the extracellularly recorded nerve terminal action potential and endplate potential before and during exposure to meperidine 1.6×10^{-4} M and naloxone 3×10^{-8} M.
A. Control
B. Three minutes after exposure to meperidine and naloxone.
C. At 10 minutes
D. At 60 minutes

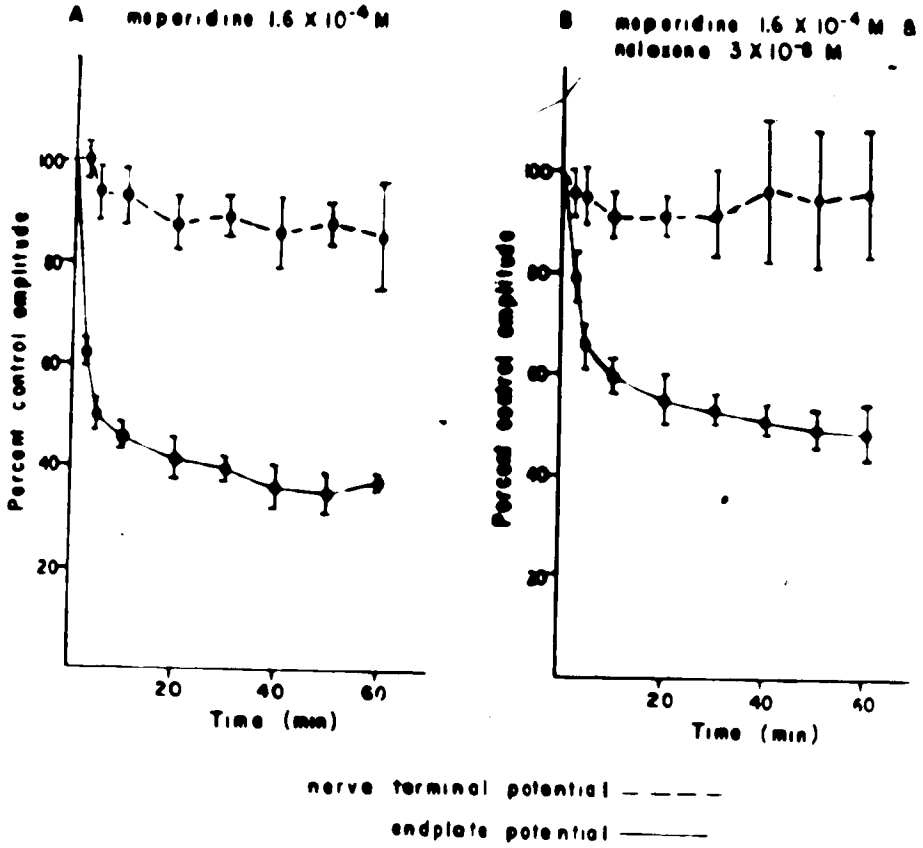


Figure 24. A. The action of meperidine 1.6 X 10^{-4} M on the amplitude of the extracellularly recorded nerve terminal potential and endplate potential (n=6).
B. The effect of naloxone 3 X 10^{-8} M on the depression of endplate potential and nerve terminal action potential amplitude induced by meperidine 1.6 X 10^{-4} M (n=5).
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.

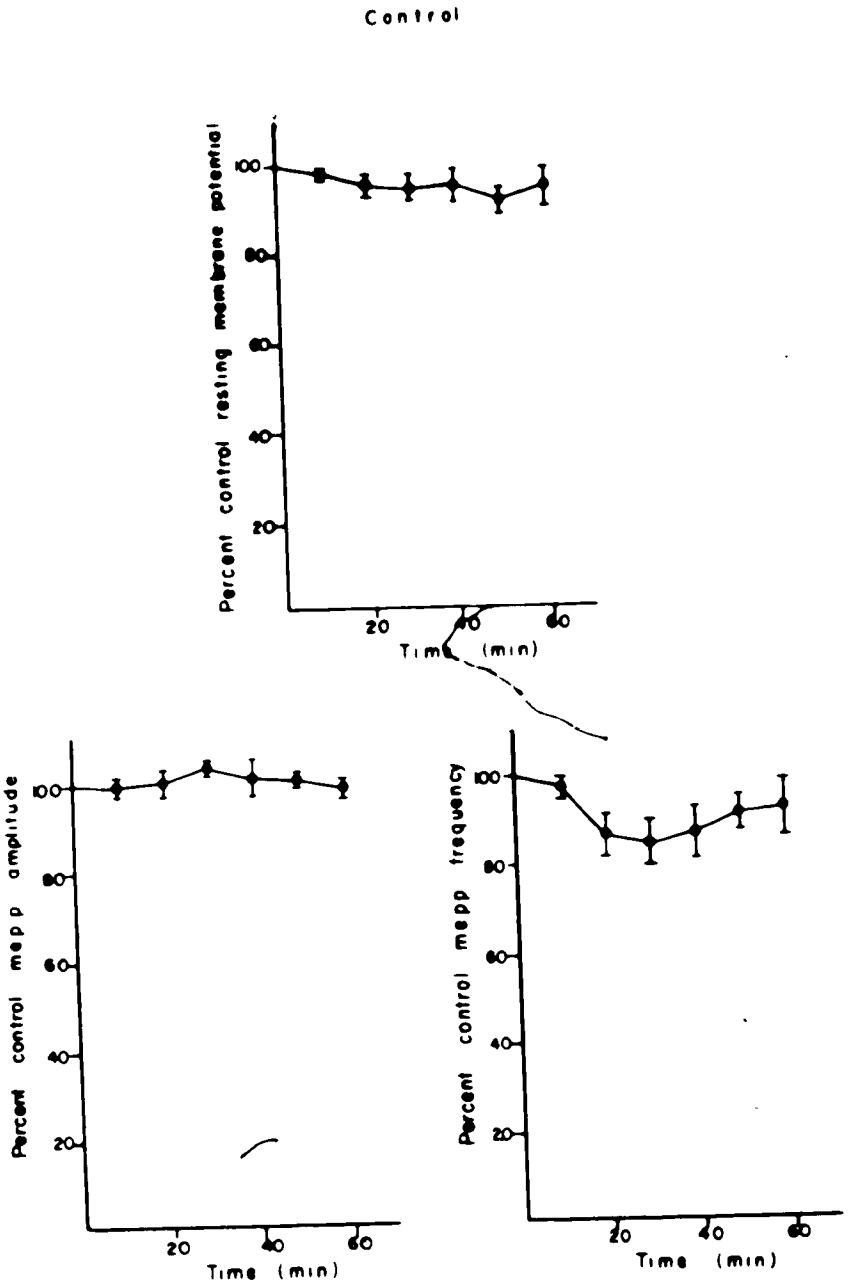


Figure 25. Control study of resting membrane potential, miniature endplate potential (mepp) amplitude and mepp frequency recorded intracellularly from frog sartorius (n=5). Means ± S.E.M. were calculated from the means obtained in each experiment expressed as percentage of the initial control value.

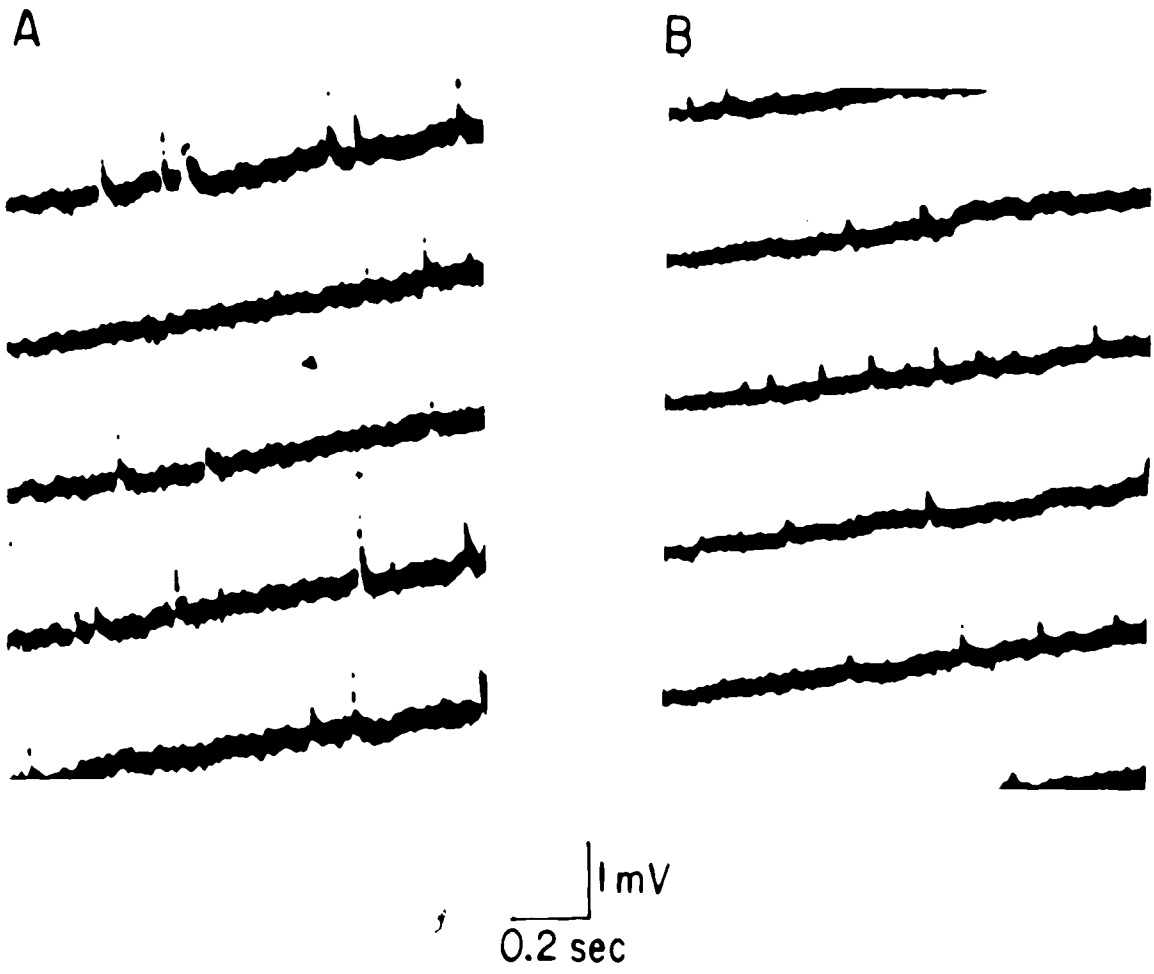


Figure 26. Miniature endplate potentials recorded intracellularly from frog sartorius A. in Ringer's solution and B. after 60 minutes exposure to meperidine 8 X 10^{-5} M.

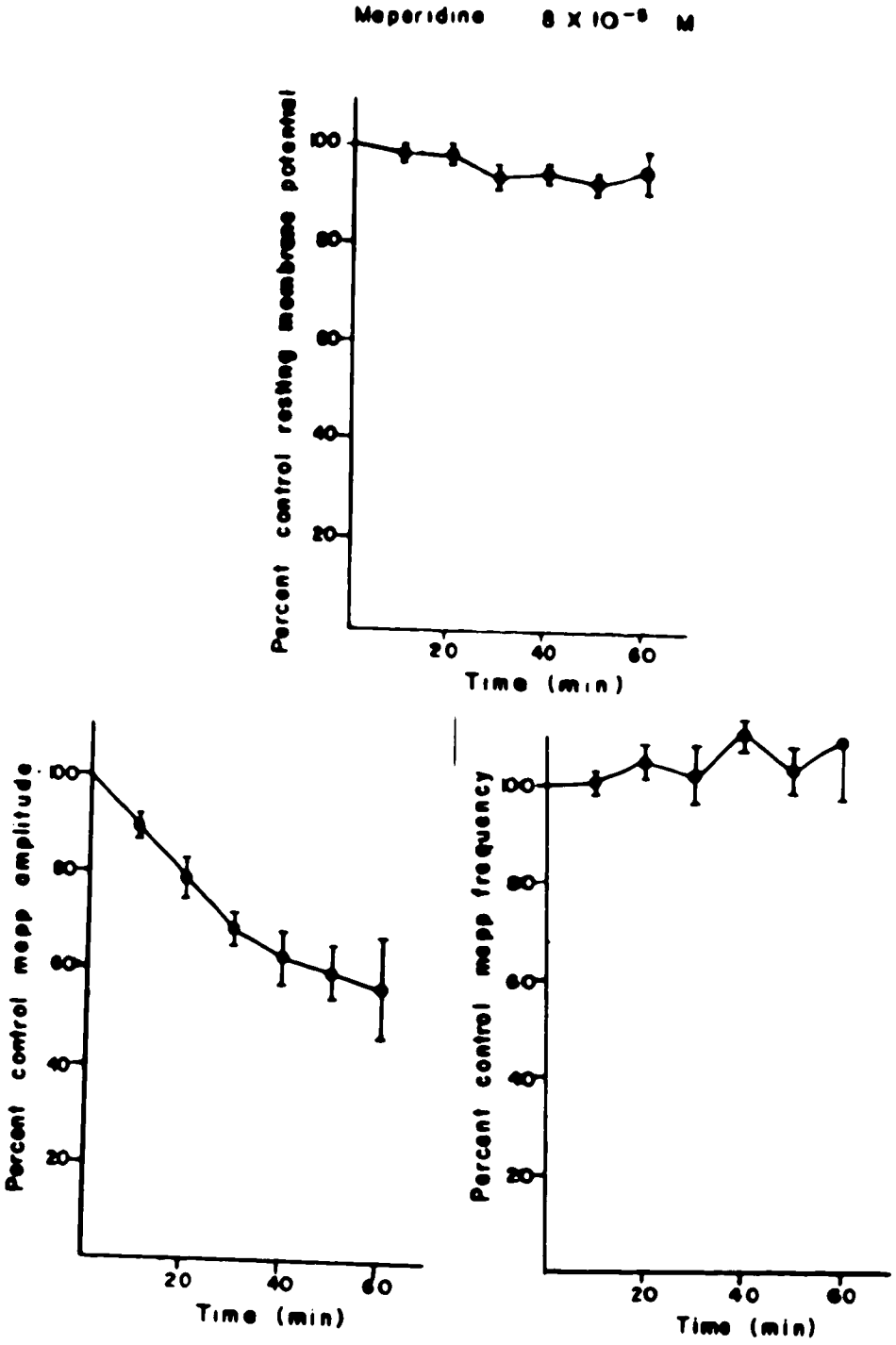


Figure 27. The action of meperidine 8 X 10^{-5} M on the resting membrane potential and miniature endplate potential (mepp) amplitude and frequency recorded intracellularly from frog sartorius (n=7).

Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control (absence of drug) response.

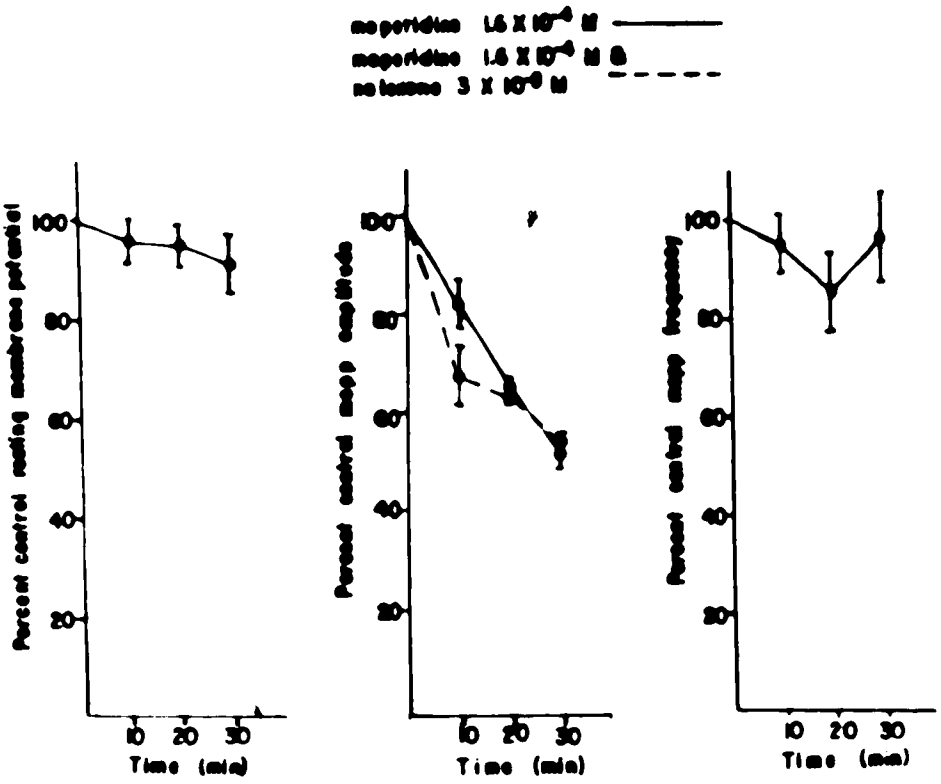


Figure 28. The effect of meperidine 1.6 X 10^{-4} M on resting membrane potential and miniature endplate potential (mepp) amplitude and frequency recorded intracellularly from frog sartorius (n=3) and the lack of antagonism exerted by naloxone 3 X 10^{-8} M on the depression of mepp amplitude (n=3). Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control (absence of drug) response.

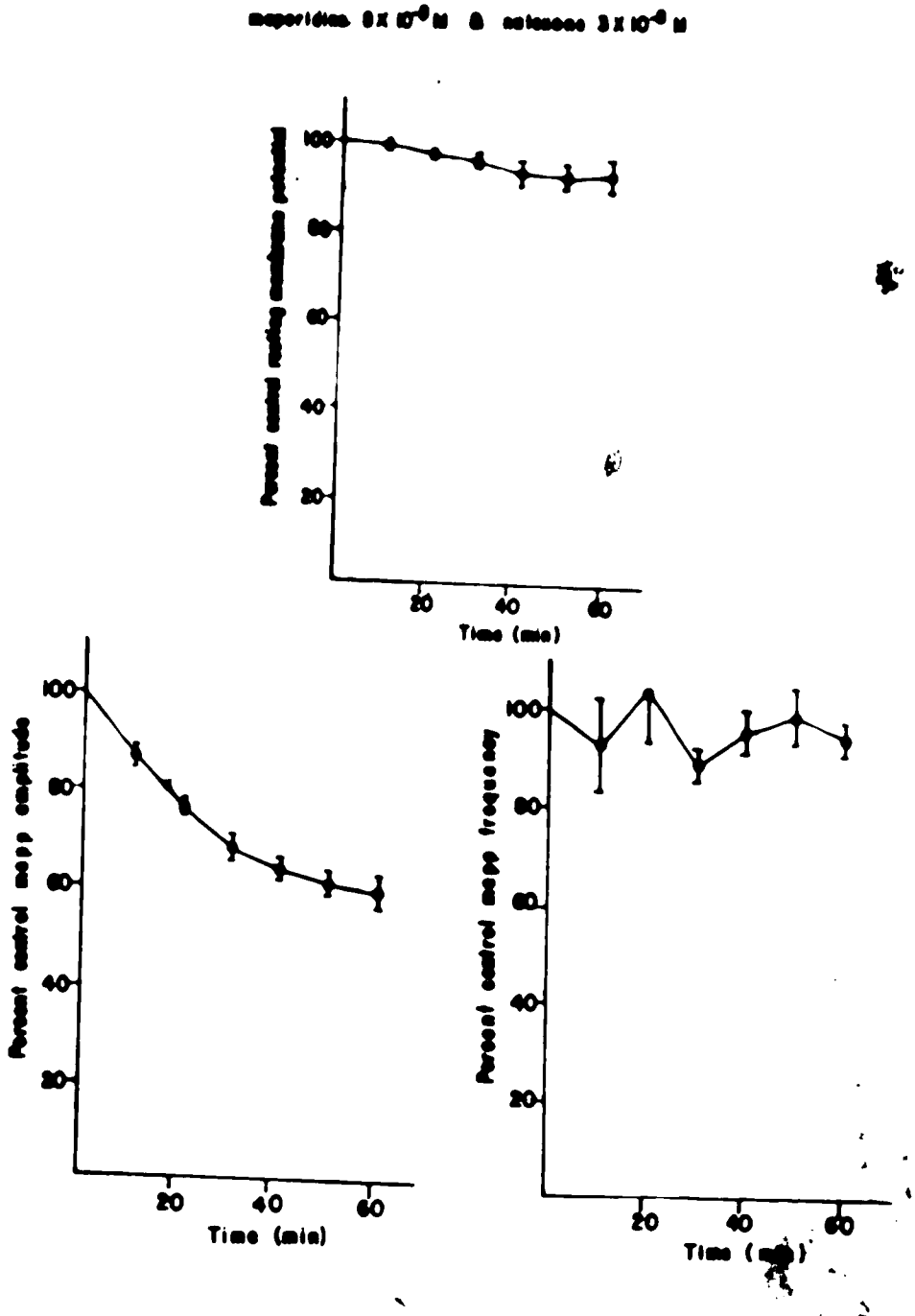


Figure 29. The effect of meperidine 8 X 10^{-5} M on the intracellularly recorded resting membrane potential and miniature end-plate potential (mepp) amplitude and frequency in the presence of naloxone 3 X 10^{-8} M (n=5).
Means ± S.E.M. were calculated from pooled results of 5 experiments expressed as percent of control response.

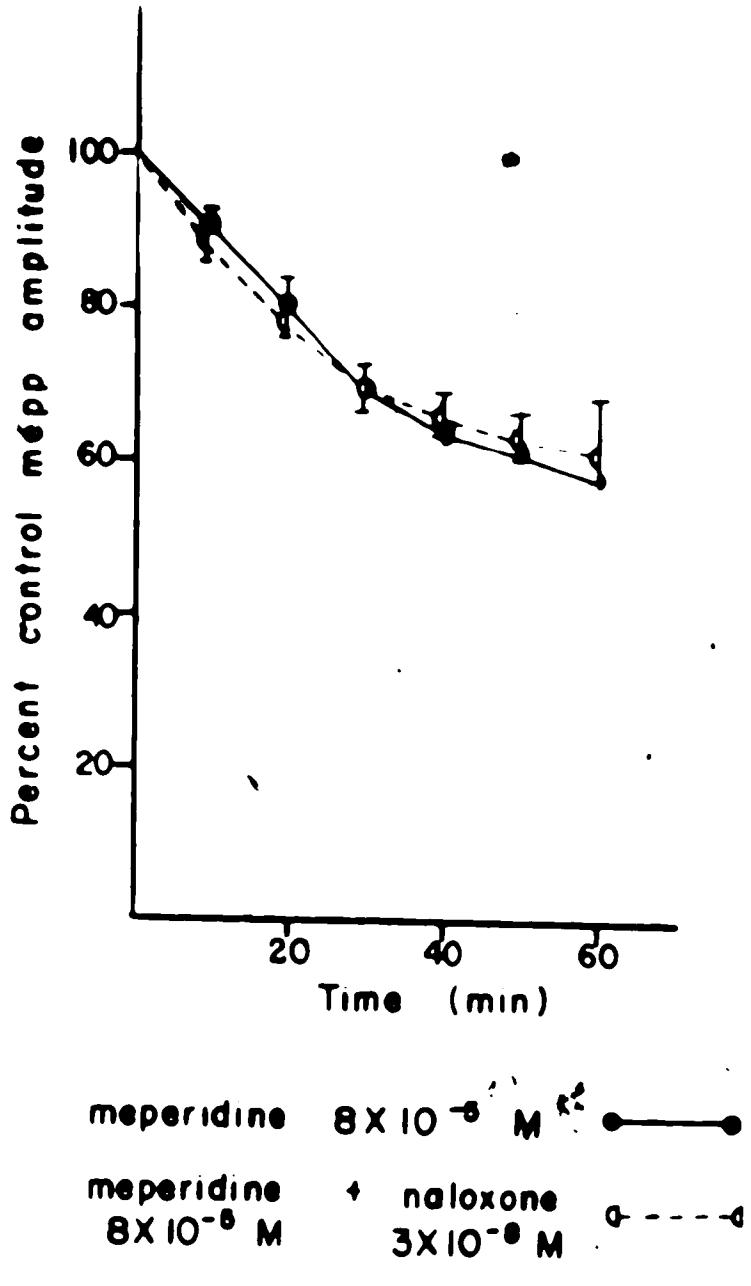


Figure 30. Summary graph showing the lack of antagonism by naloxone 3×10^{-8} M of the depression of miniature endplate potential (mepp) amplitude induced by meperidine 8×10^{-5} M.
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.

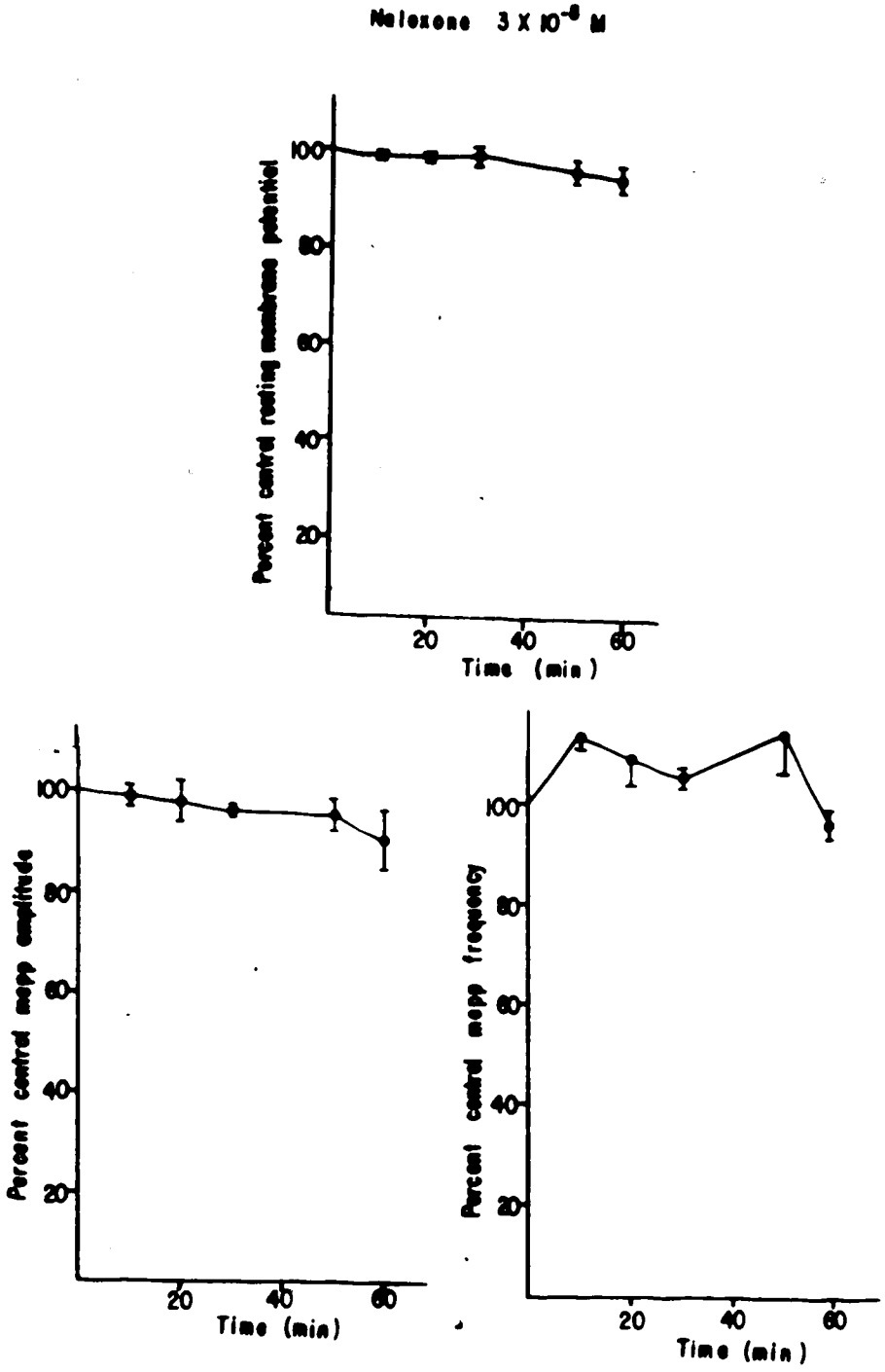


Figure 31. The effect of naloxone 3 X 10^{-8} M on resting membrane potential or miniature endplate potential (mepp) amplitude and frequency recorded intracellularly from frog sartorius (n=3).
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.

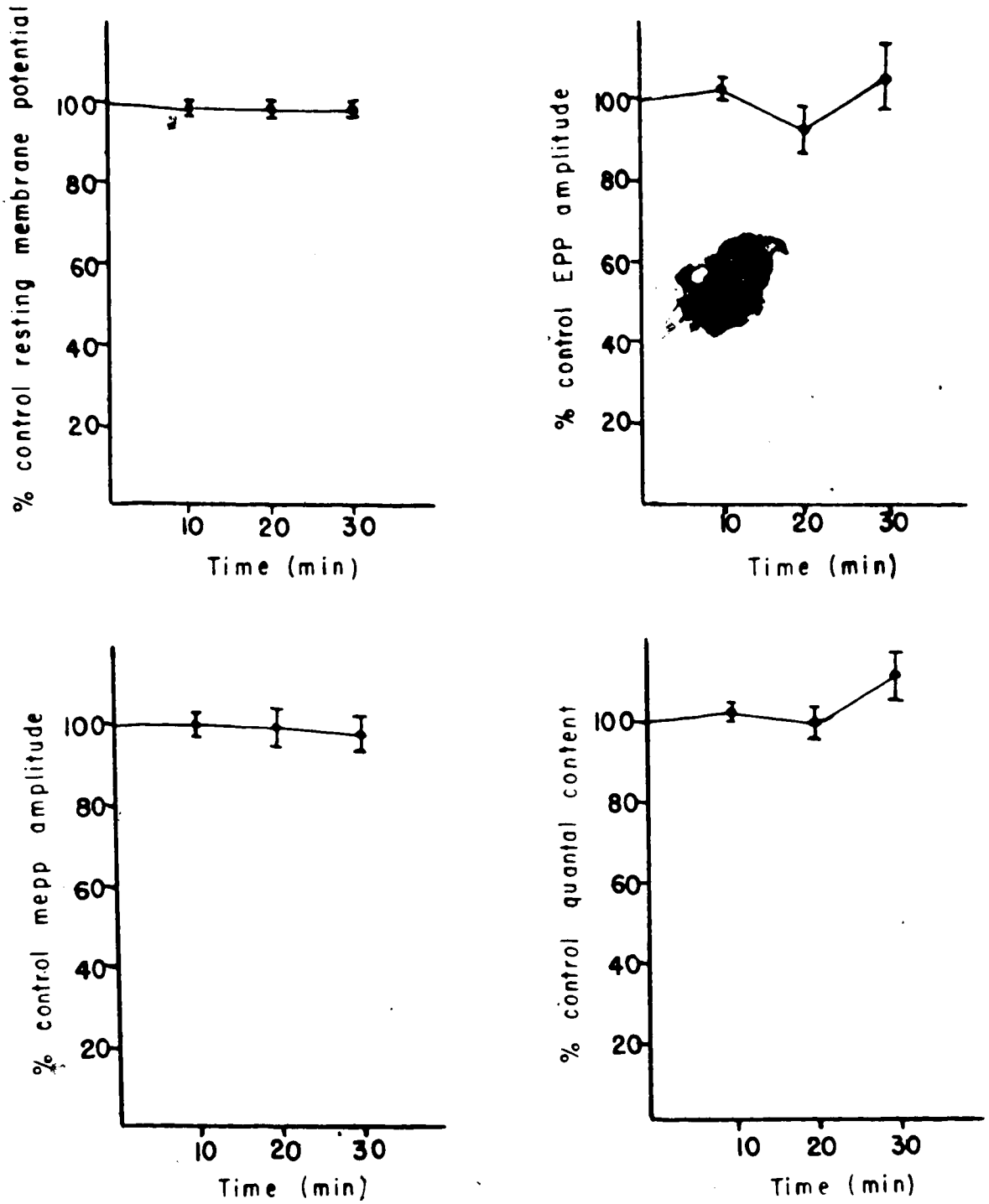


Figure 32. Control study of the resting membrane potential (RMP), endplate potential (EPP) amplitude, miniature endplate potential (mepp) amplitude and the derived quantal content (EPP/mepp) recorded intracellularly in the presence of $MgCl_2$ 8 to 10 mM (n=5).
Means ± S.E.M. were calculated from the means obtained in each experiment expressed as percent of the initial control value.

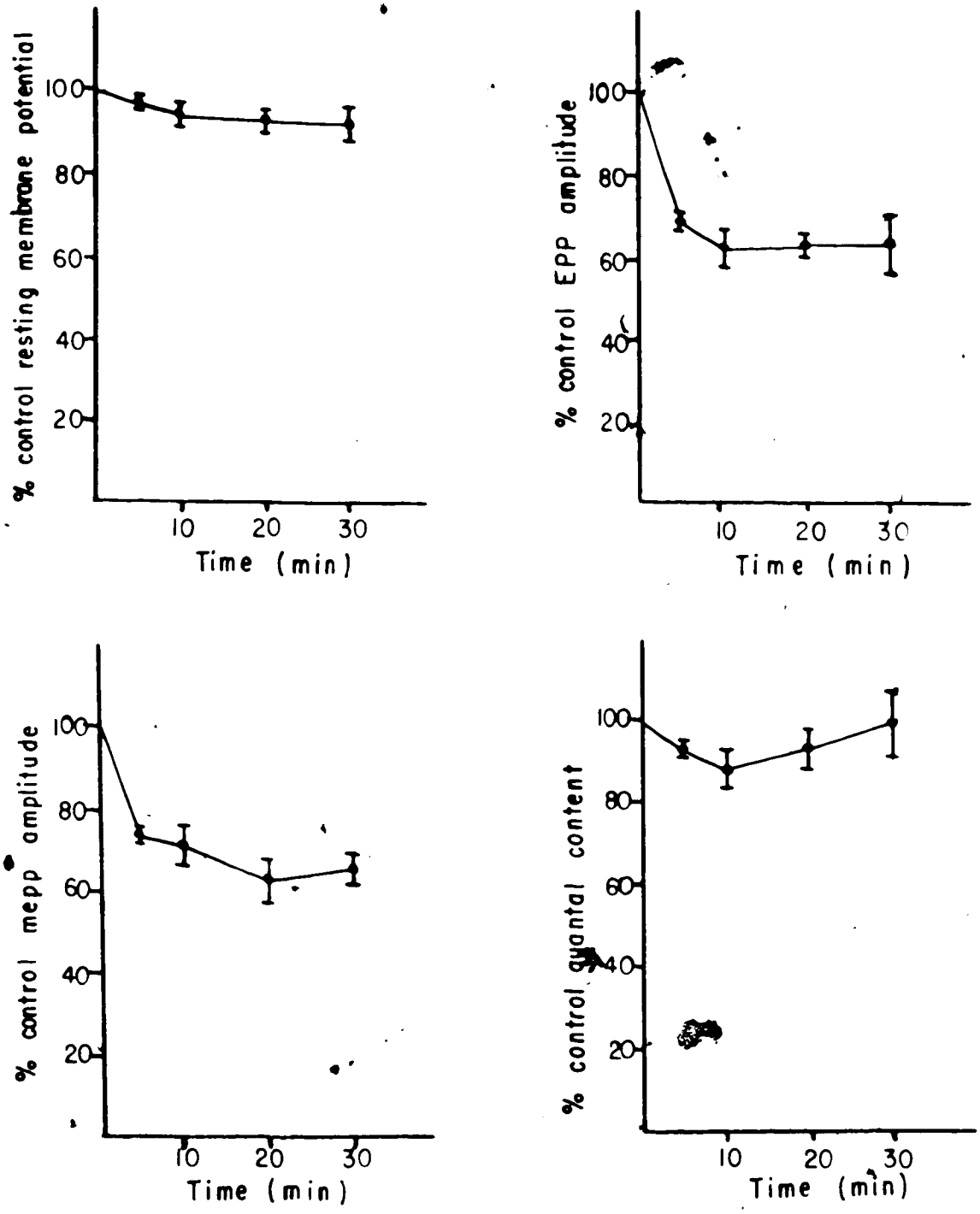
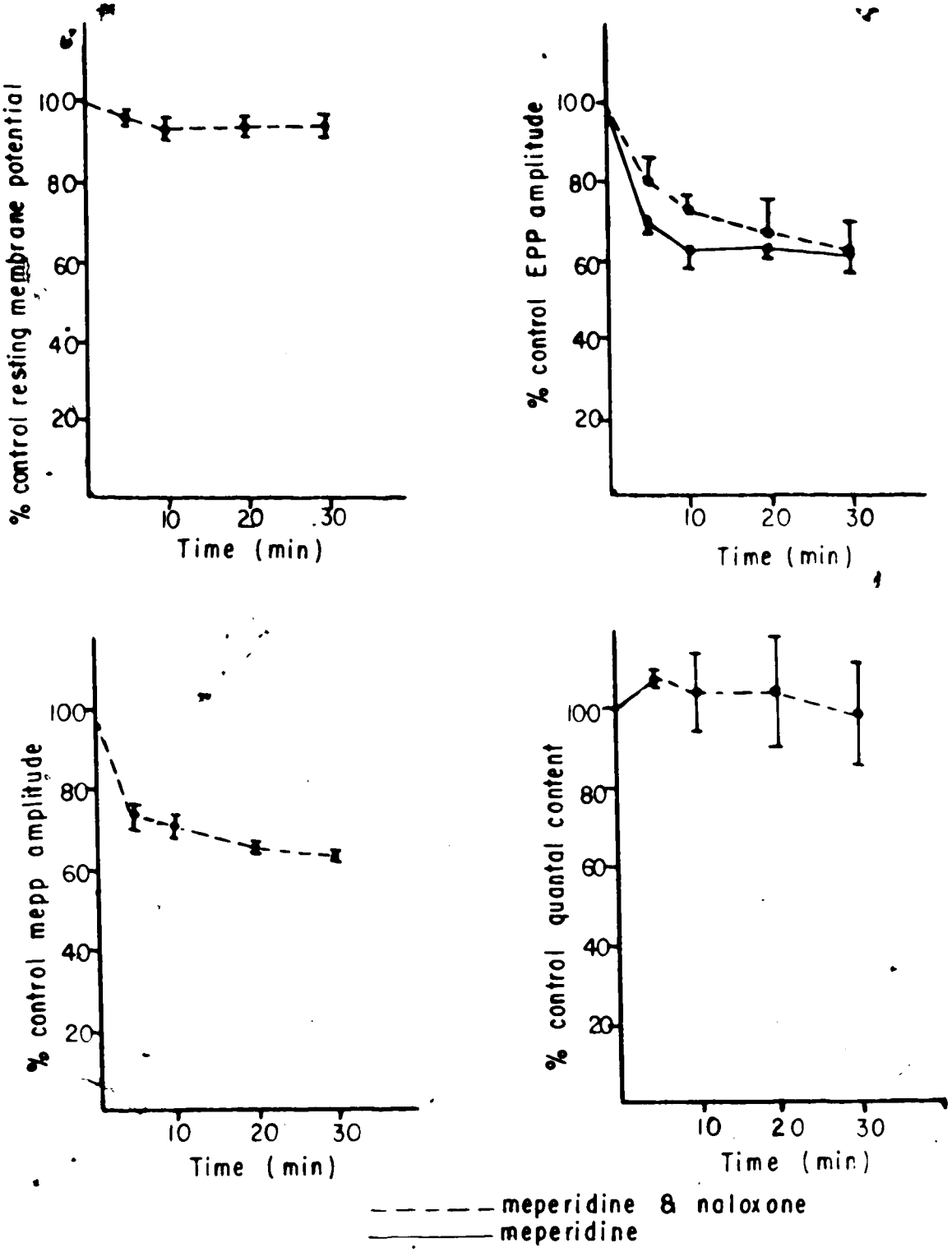


Figure 33. The effect of meperidine 1.6 X 10^{-4} M on quantal content in frog sartorius. Resting membrane potential, endplate potentials (EPP) and miniature endplate potentials (mepp) were recorded intracellularly from frog sartorius in the presence of $MgCl_2$ 8 to 10 mM (n=4).
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.

Figure 34. The effect of meperidine 1.6 X 10^{-4} M on quantal content in the presence (n=3) and absence (n=4) of naloxone 3 X 10^{-8} M. Resting membrane potentials, endplate potentials (EPP) and miniature endplate potentials (mepp) were recorded intracellularly from frog sartorius in the presence of $MgCl_2$ 8 to 10 mM.
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.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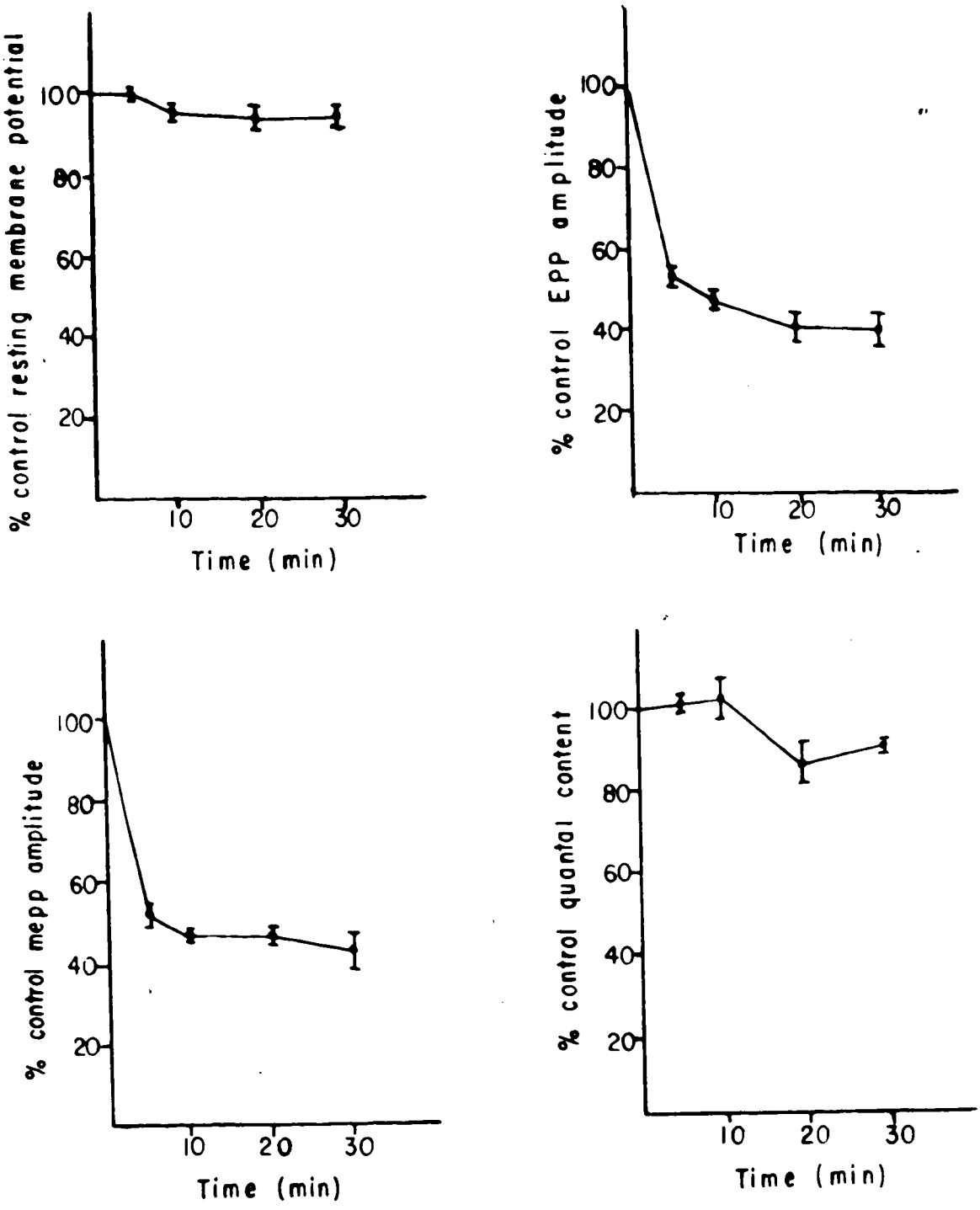
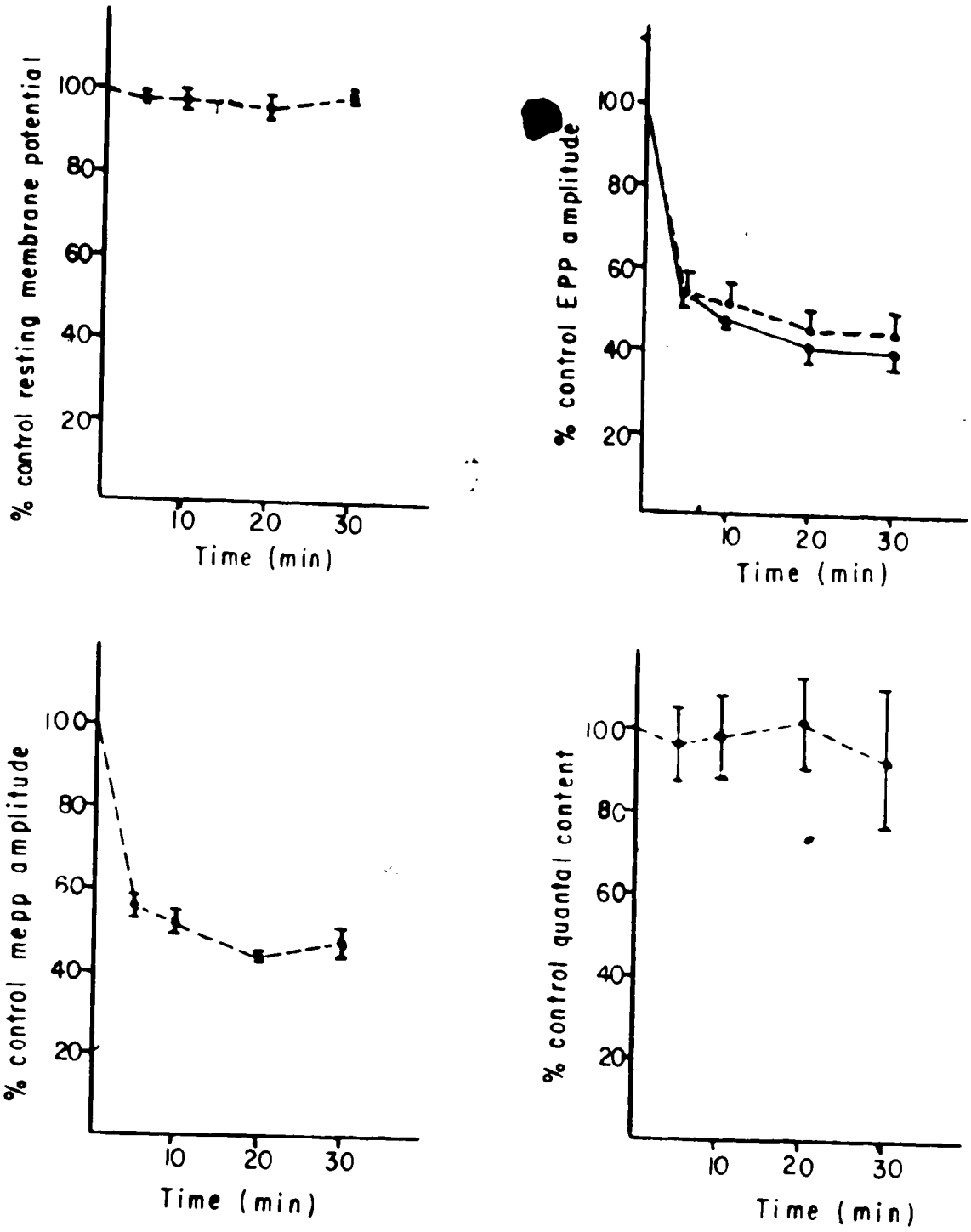


Figure 35. The effect of meperidine 4.2 X 10^{-4} M on quantal content in frog sartorius. Resting membrane potentials, endplate potentials (EPP) and miniature endplate potentials (mepp) were recorded intracellularly in the presence of $MgCl_2$ 8 to 10 mM (n=5).
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.

Figure 36. The effect of meperidine 4.2 X 10^{-4} M on quantal content in the presence (n=4) and absence (n=5) of naloxone 3 X 10^{-8} M. Resting membrane potentials, endplate potentials (EPP) and miniature endplate potentials (mepp) were recorded intracellularly in the presence of $MgCl_2$ 8 to 10 mM.
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.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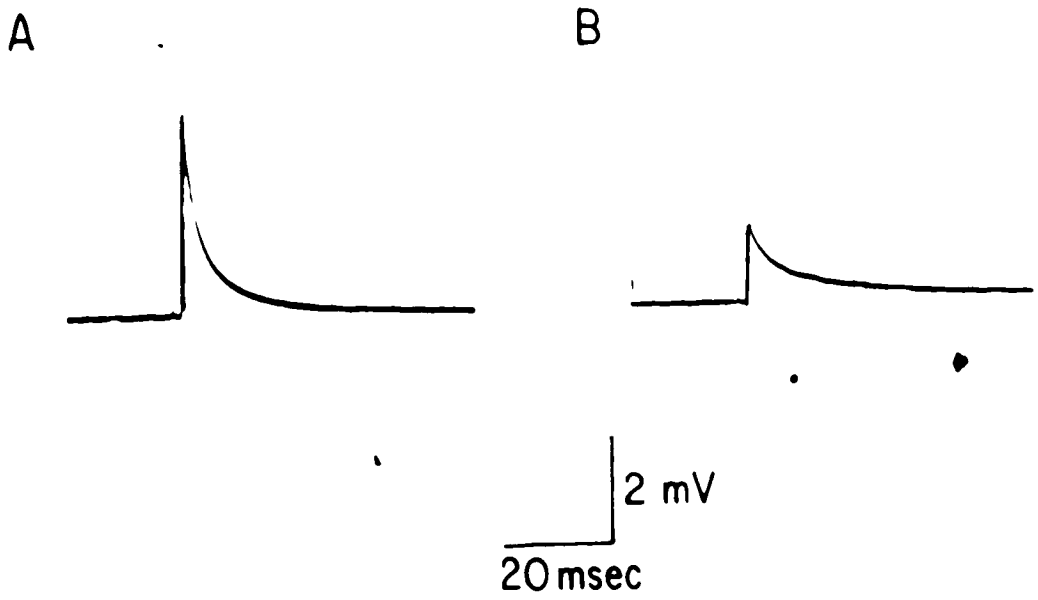
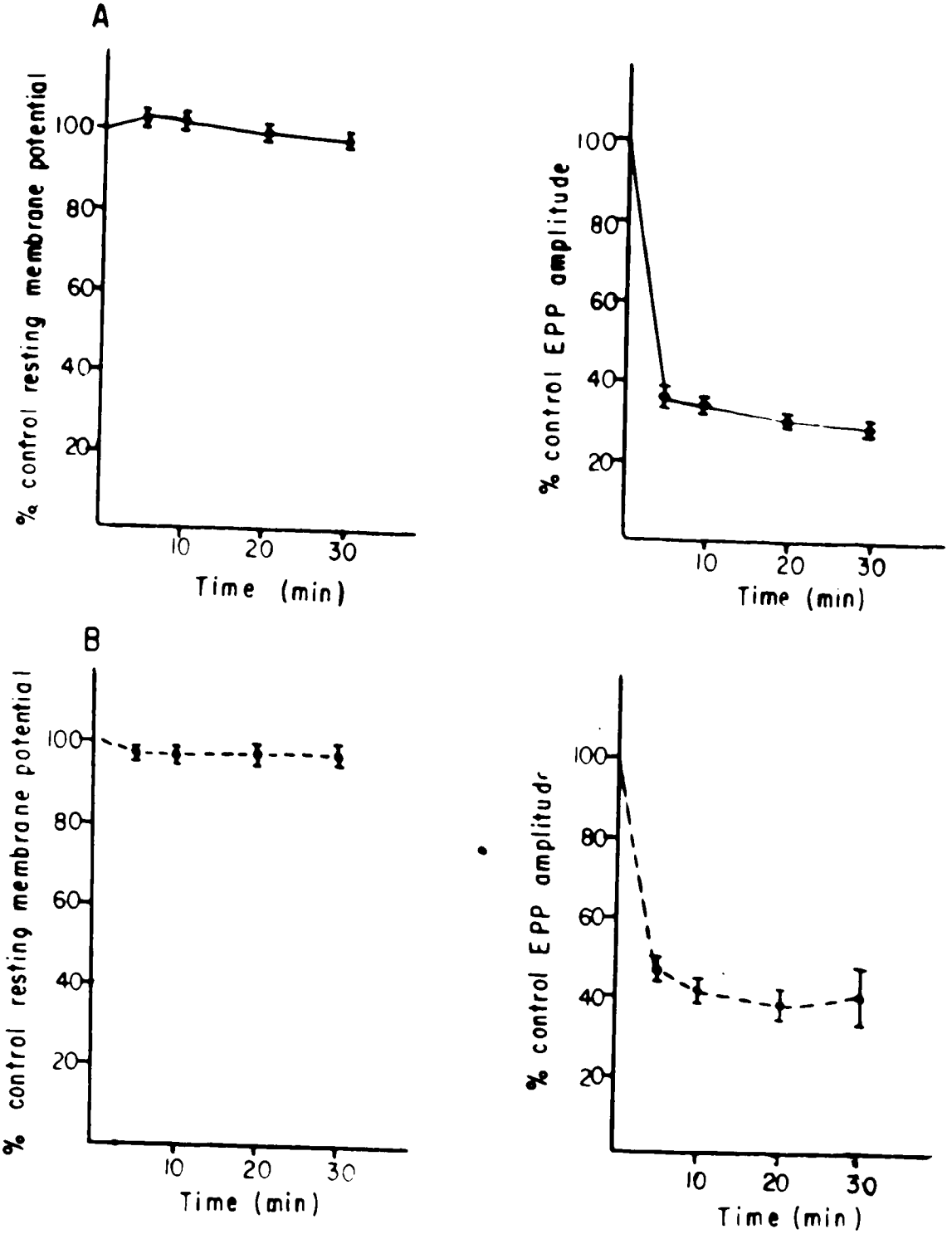


Figure 37. Endplate potentials recorded intracellularly from frog sartorius in A. Ringer's solution containing d-tubocurarine 3 X 10^{-6} M and B. after 30 minutes exposure to meperidine 4.2 X 10^{-4} M.

Figure 38. A. The effect of meperidine 4.2 X 10^{-4} M on resting membrane potential and endplate potential (EPP) amplitude recorded intracellularly in the presence of d-tubocurarine 2 X 10^{-6} M. (n=7).
B. Experiments in A were repeated in the additional presence of naloxone 3 X 10^{-8} M (n=5). Note the antagonism of EPP amplitude depression.
Means ± S.E.M. calculated from pooled results of all experiments expressed as percent of control response.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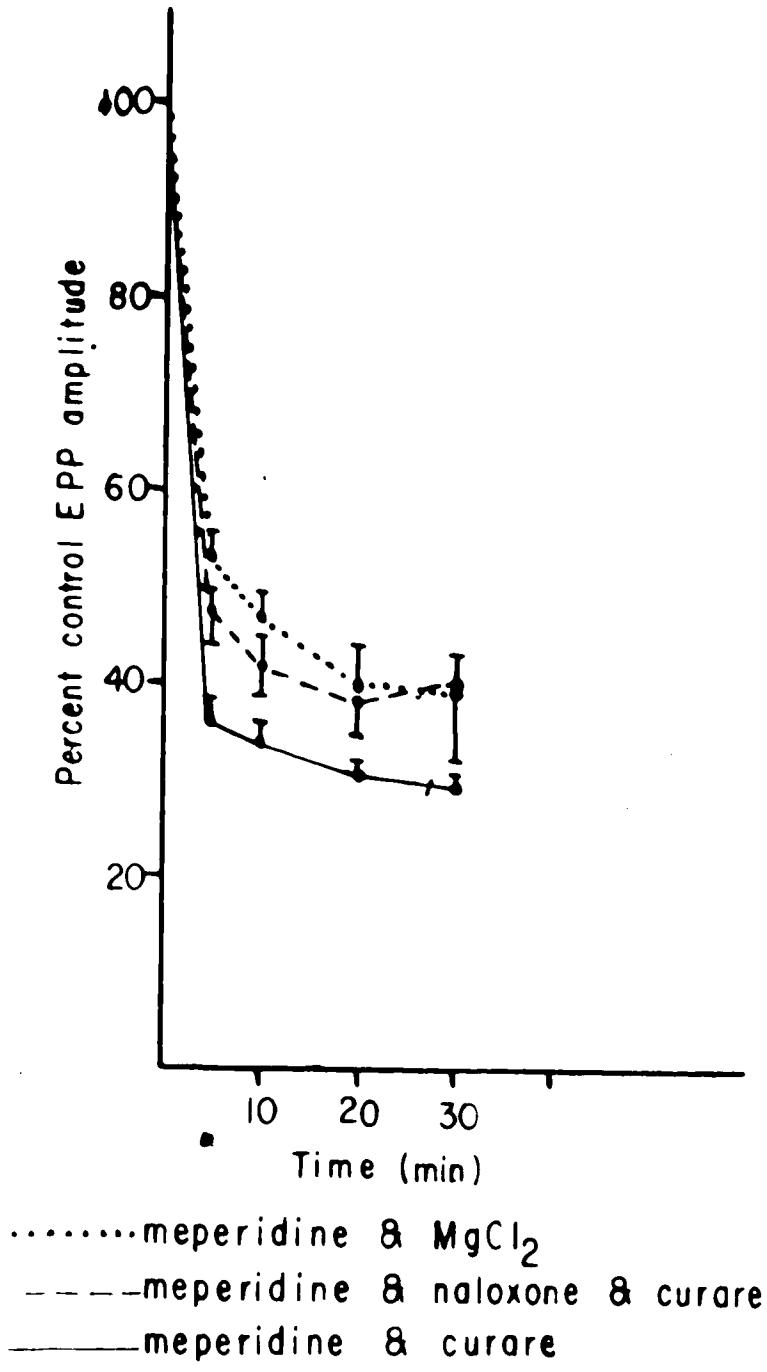


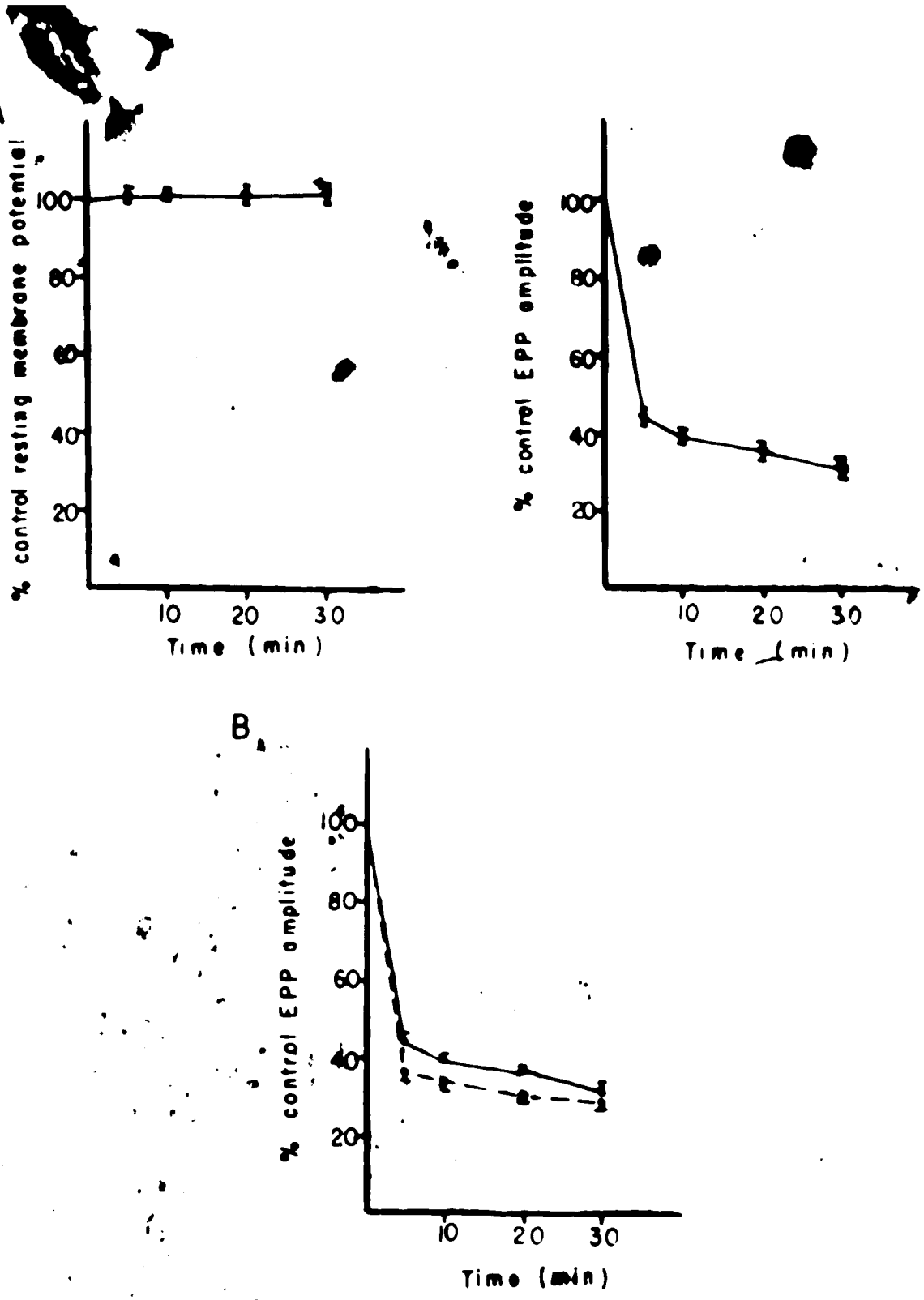
Figure 39. Summary graph comparing the depression of EPP amplitude by meperidine 4.2 X 10^{-4} M using two methods of inhibiting neuromuscular transmission and illustrating the antagonism of narcotic effect by naloxone with the d-tubocurarine method.

Figure 40. The lack of effect of physostigmine 1.3 X 10^{-4} M on the depression of endplate potential (EPP) amplitude induced by meperidine 4.2 X 10^{-4} M. All solutions contained 9 X 10^{-6} M d-tubocurarine.

A. Resting membrane potential and EPP amplitude recorded intracellularly in the presence of physostigmine and meperidine (n=3).

B. Summary graph comparing meperidine induced depression of EPP amplitude in the presence (—) and absence (---) of physostigmine.

Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of control response.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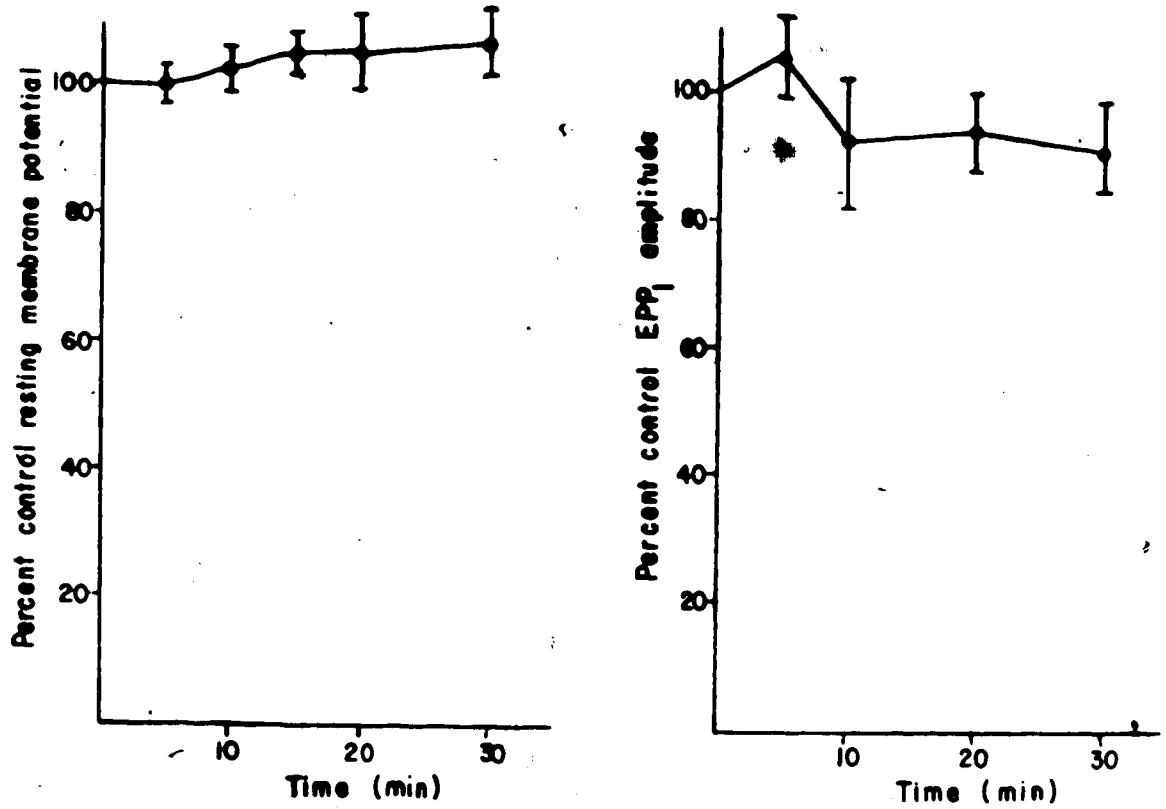

Figure 41. Control study of the resting membrane potential and amplitude of the endplate potential evoked by iontophoretic application of acetylcholine (EPP_I) recorded intracellularly from frog sartorius (n=4). Means ± S.E.M. were calculated from the means obtained in each experiment expressed as percent of the initial control response.

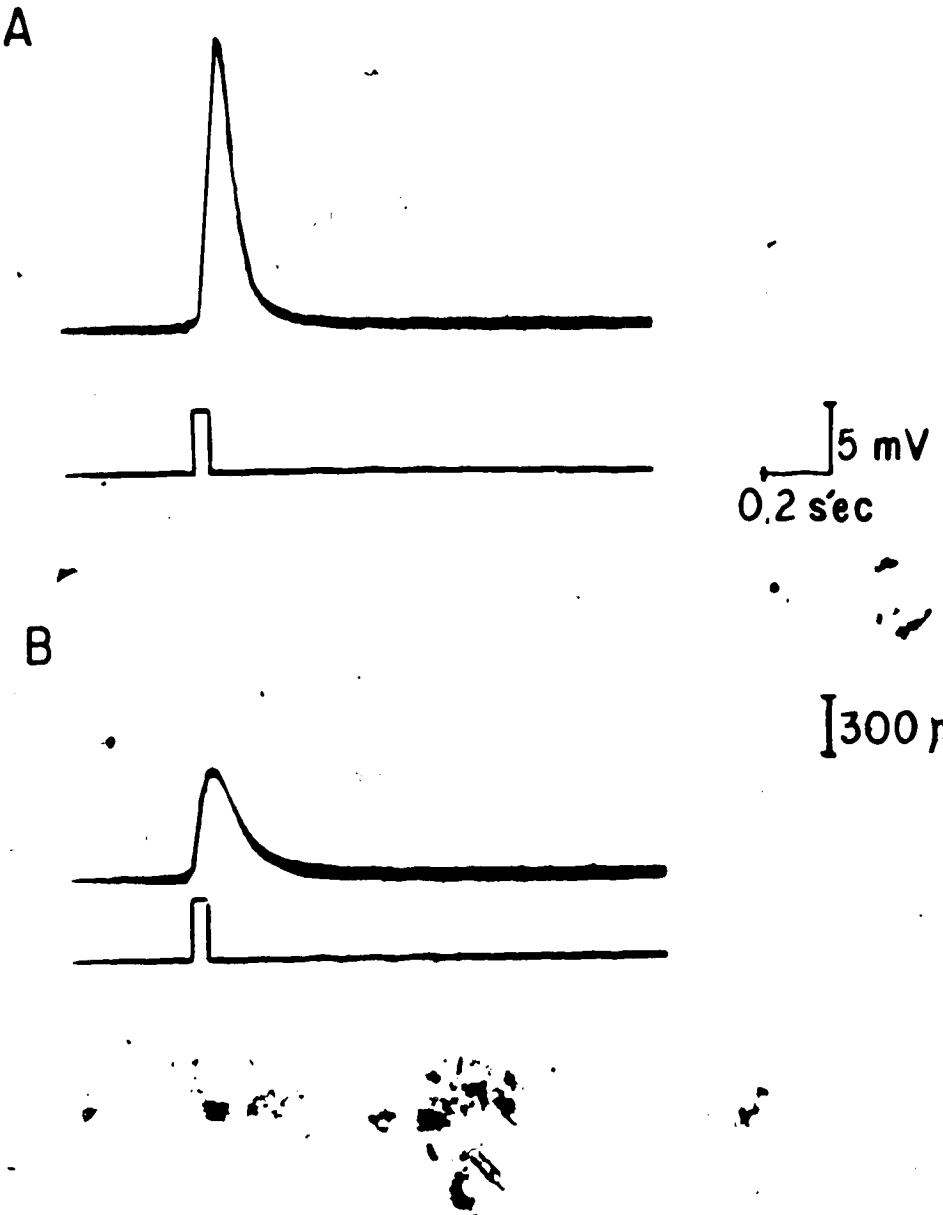

Figure 42. Endplate potential induced by iontophoretically applied acetylcholine recorded intracellularly from frog sartorius in A. Ringer's solution and B. after 30 minutes exposure to meperidine 1.6 X 10^{-4} M.

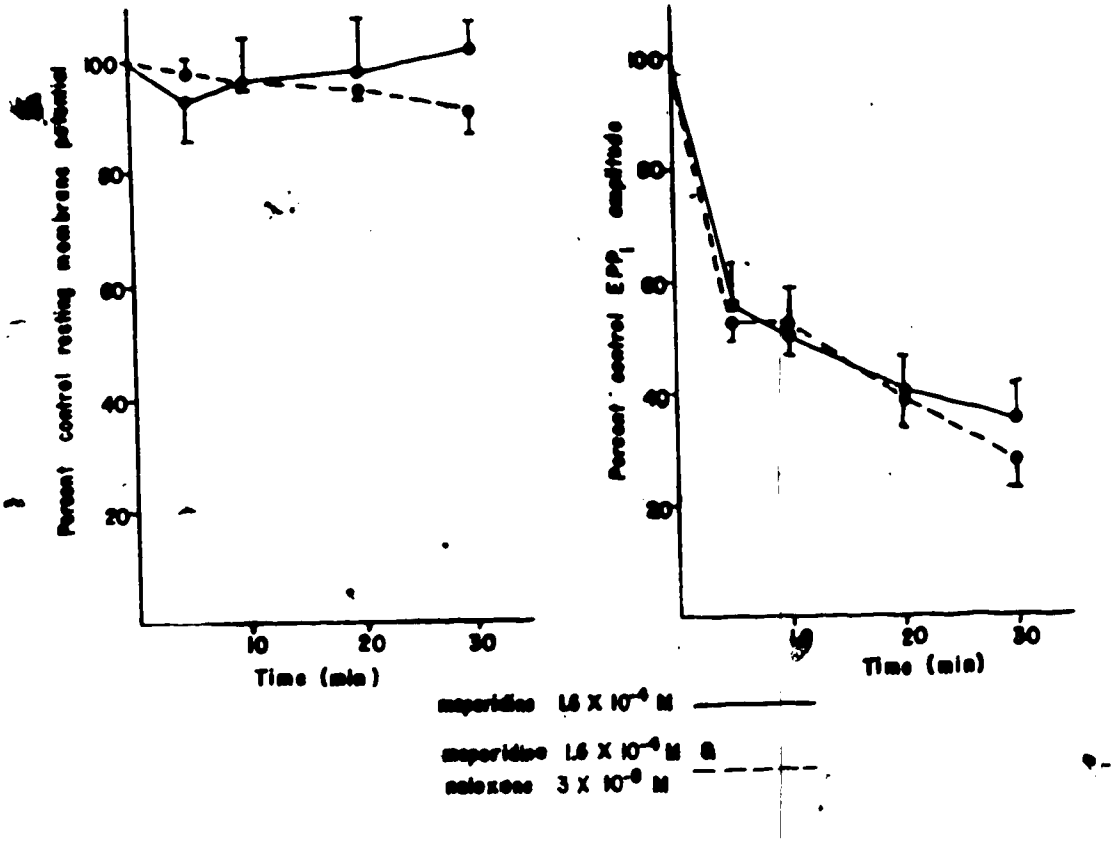

Figure 43. Depression by meperidine 1.6×10^{-4} M of EPP_I amplitude (endplate potential evoked by iontophoretically applied acetylcholine) (n=4) and comparable measurements in the presence of naloxone 3×10^{-8} M (n=3).
Resting membrane potentials and EPP_I were recorded intracellularly from frog sartorius.
Means ± S.E.M. were calculated from pooled results of all experiments expressed as percent of the initial control value.

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