SELECTIVE INHIBITION OF CENTRAL SYMPATHETIC NEURONS

BY MORPHINE AND ITS REVERSAL BY NALOXONE

by

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ABSTRACT

Recent investigations clarifying the monoaminergic influences

on sympathetic preganglionic neurons in the cat have allowed a

direct assessment of the effects of narcotic analgesics and anta-

gonists on central transmission. Intravenous morphine, metha-

done, meperidine or codeine produced an immediate and sustained

depression of discharges evoked from these spinal sympathetic

neurons. The potency ratios for production of similar degrees of

depression by these four agents compared favorably to the potency

ratios reported for equianalgesic doses. The depression appeared

to be specific in that it was rapidly reversed by very low doses of

naloxone or nalorphine and that a non-analgesic stereoisomer re-

lated to these drugs produced qualitatively different effects on the

sympathetic preganglionic neurons. The depression of these neu-

rons offers an adequate and sufficient mechanism to account for

the centrally mediated vasodepressor effect produced by morphine

and other narcotic analgesics. The pharmacological characteriza-

tion of the morphine and naloxone actions strongly suggests that

morphine functions as a 5-HT agonist in this system, while nalo-

xone can both block 5-HT receptors and also stimulate these

spinal neurons. The significance of these findings relative to other acute and chronic effects of the narcotic analgesics are discussed.

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INTRODUCTION

The involvement of norepinephrine (NE) and 5-hydroxy-
tryptamine (5-HT) in the central actions of the narcotic analgesics
has been amply demonstrated[13, 52, 87]. However, the exact nature
and extent of monoamine participation remains unclear. To date,
the vast majority of studies have been concerned with correlations
between the gross physiological effects of these drugs and major
alterations in the concentrations of the putative central transmitters.
Relatively few studies have attempted to assess directly the ac-
tions of the narcotics on discrete, yet functional NE or 5-HT com-
ponents of the central nervous system.

Current developments in understanding the role of endogen-
ous monoamines in the activity of sympathetic preganglionic
neurons suggest that these neurons could serve as a model system
for delineating the mechanisms of action of drugs affecting central
NE and 5-HT transmission. Histochemical and anatomical studies
have shown that these preganglionic neurons, with cell bodies loca-
ted in the intermedolateral columns of the spinal cord, receive a
rich supply of NE and 5-HT terminals from neurons originating in
the vasomotor centers of the caudal medulla oblongata[16, 29, 95].

Electrophysiological and pharmacological investigations have dem-

onstrated that the NE neurons are excitatory and the 5-HT neurons

are inhibitory to the sympathetic preganglionic neurons[28,34,66].

By monitoring evoked discharges from these preganglionic neurons,

coupled with the appropriate use of monoamine precursors and

blocking agents, drug actions mediated through the NE or 5-HT

synapses can be assessed and characterized.

This paper describes the effects of several narcotic analge-

sics and related compounds on activity evoked from sympathetic

preganglionic neurons. The experiments were conducted in unanes-

thetized spinal cats to confine the site of drug action to synapses at

the spinal level. The narcotics consistently depressed evoked

sympathetic activity, an effect that could be rapidly antagonized by

naloxone or nalorphine. Characterization of the effect of morphine

on these neurons not only suggests its site of action in this system

but also offers a mechanism that can adequately account for the

centrally mediated vasodepressor effect produced by this drug[20,58,59].

METHODS

Surgical procedure

Results were obtained from 44 adult cats of either sex weighing between 2.1 and 5.8 kg. During brief ether anesthesia a tracheal cannula was placed, both common carotid arteries were ligated (one of which was cannulated for continuous monitoring of arterial pressure) and the vertebral arteries were clamped and occluded at C2. Anesthesia was discontinued after the spinal cord was transected at C1. Ventilation was maintained with a positive pressure respirator adjusted to hold the end expiratory CO_2 concentration between 4 and 5% (Spinco Medical Gas Analyzer). Arterial pressure usually stabilized between 70 and 85 mm Hg following spinal transection. In the few experiments in which blood pressure tended to fall below this level, adequate perfusion was maintained by wrapping the hind limbs and abdomen with an elastic bandage to reduce peripheral pooling of blood and by intravenous administration of small volumes (5 to 10 ml) of 10% dextran-40. Body temperature was monitored and maintained in the range of 36 to 38 °C by an automatically controlled heating plate. A peripheral vein, usually the left cephalic, was cannulated for

administration of drugs. Gallamine triethiodide (Flaxedil) was administered as needed to provide muscle paralysis throughout the experiment.

The left thoracic sympathetic preganglionic rami communicantes and intercostal nerves of segments T2, T3, and T4 were approached by a dorsal midline incision, reflection of the skin to the left, and removal of overlying muscle. The proximal portions of ribs 2, 3, and 4 and adjacent intercostal muscles were removed. Care was taken to leave the parietal pleural membrane intact to serve as the floor of the recording pool. An ipsilateral pneumothorax and a rigid blunt retainer positioned against the exposed parietal pleural membrane minimized respiratory movement in the vicinity of the exposed nerves.

Dissection was continued under a binocular dissecting microscope (3-25 magnification). Intercostal nerves were sectioned approximately 20 mm distal to the vertebral column and were dissected free of adjacent tissue to a point just proximal to the sympathetic trunk. The sympathetic preganglionic rami were identified by their location and appearance, their distal entry into the sympathetic chain or gangion, and their proximal juncture with

appropriate spinal nerves. The intercostal nerves and pregang-

lionic rami were then covered with warm mineral oil. The rami

were carefully cleaned of remaining connective tissue between

their merger with the sympathetic trunk and their juncture with a

corresponding spinal nerve; rami were then sectioned proximal

to the sympathetic chain.

In experiments depending upon intraspinal stimulation, a

dorsolateral laminectomy was performed to expose the spinal cord

at either the C2 and C3 or the C5 and C6 segments. The dura

was opened and reflected to expose the dorsal roots which were

sectioned distally and displaced medially. This exposed dorso-

lateral surface of the spinal cord was covered with warm mineral

oil.

Recording and stimulating procedures

Evoked sympathetic discharges were recorded with a bipolar

silver-wire electrode from two of the three exposed sympathetic

preganglionic rami. Evoked responses were amplified (Tektronix

2A61; frequency response 6-600 Hz) and displayed on a dual-beam

oscilloscope for continuous monitoring. Evoked activity was quan-

titated on-line by integrating the area above baseline of 16 or 32

consecutive responses with a signal averaging computer (Nicolet

1072). Photographs of the analog computer output and representa-

tive individual discharges served as permanent records for the

complete course of each experiment.

Sympathetic preganglionic neurons receive excitatory input,

both from the periphery, through small myelinated afferent fibers

in the intracostal spinal nerves [27,34], and from supraspinal cen-

ters via tracts descending in the dorsolateral funiculus of the

spinal cord [41,66]. Both pathways were utilized in the present

study to evoke sympathetic preganglionic discharges. Fig. 1 pre-

sents schematic representations of the relevant structures involved

in the peripherally activated spinal reflex pathway (A) and in the

centrally activated intraspinal pathway (B).

Stimulation of one of the freed intercostal nerves with su-

pramaximal rectangular pulses (0.2 msec) delivered through bi-

polar silver-wire electrodes from an isolated stimulator (Devices,

Mark IV) at a frequency of 15/min evoked maximal sympathetic dis-

charges. Reflex responses were evoked either within the same

segment or from one or two segments distant.

Activation of the thoracic sympathetic preganglionic neurons through the intraspinal pathway (B of Fig. 1) was produced by stimulating descending tracts in the dorsolateral funiculus of the cervical spinal cord with bipolar tungsten microelectrodes (tip exposure, 25 μ; 3-9 Mohm; separation, 1 mm). The electrodes, held in a micromanipulator, were oriented to enter the spinal cord vertically in the sagittal plane. Maximal responses were evoked with biphasic pulses (0.2 msec) at a frequency of 6/min and a stimulus intensity between 100 and 150 μA. Electrodes were positioned under microscopic control to provide the largest sympathetic discharges upon stimulation (Devices, Mark IV) at intensities just above threshold; the intensity was then raised to evoke maximal responses. The use of biphasic pulses and a low rate of stimulation conferred good stability of responses for many hours. In all experiments, a digital master control timing unit (Devices, Digitimer) was used to trigger stimulation and recording equipment.

Experimental procedure

A minimum of five hours elapsed between spinal cord transection and the start of recording. During a control period of at least one hour prior to drug administration, evoked responses

were sampled to assess the stability of the preparation and to generate data from which to calculate a mean control response and its variance. In the absence of drug treatment, evoked sympathetic discharges routinely remained within ± 2 Standard Deviations of the mean control value for many hours. Therefore, changes occurring after drug administration were considered significant when consecutive readings fell outside this range.

Following drug administration, evoked activity was sampled at 5 or 10 min intervals, depending upon the rapidity of drug effect and the time required to make measurements. Absolute sizes of evoked discharges generated by the averaging computer were converted to percentages of the mean control value to standardize data for comparison among different experiments. The duration of individual experiments depended upon the physiological stability of the animal and the time required to complete the experimental procedures. Some experiments required up to 15 hours following cord transection for completion.

Although some quantitative differences among individual experiments were noted, drug effects were generally qualitatively consistent and variations were minor. Some of the variability

among individual experiments may have been due to relative differences in sensitivity or the proportion of preganglionic neurons available for discharge.

With the exception of parachlorophenylalanine (PCPA), all drugs were administered intravenously. PCPA was suspended in water with the aid of a small amount of Tween-80 and was injected intraperitoneally. D, L-5-HTP was dissolved in warm, normal saline at a concentration of 10 mg/ml. All other drug solutions were prepared with distilled water or normal saline or commercially available parenteral forms were used.

RESULTS

Drug effects on evoked sympathetic activity

Morphine. The effects of low doses of morphine SO_4 on activity evoked from sympathetic preganglionic neurons were assessed in spinal cats. At doses of 0.5, 1, or 2 mg/kg, morphine consistently produced marked depression of transmission through either the spinal reflex (Fig. 2A) or the intraspinal pathway (Fig. 2B). The onset of depression was rapid, became significant within 5 min, and reached a maximum within 20-30 min. Although the full duration of the depression was not established, little or no recovery occurred within 3 hrs. and only partial recovery was observed in several experiments that were followed up to 5 1/2 hrs.

Responses to the same dose of morphine were somewhat variable among different experiments, but the degree of depression was generally dose-dependent. Although the effects of small doses of morphine were usually additive, little or no further depression could be produced by doses in excess of 2 mg/kg. The minimum effective dose of morphine appeared to be between 0.2 and 0.3 mg/kg; 0.25 mg/kg produced only marginal reductions in discharges evoked through the intraspinal pathway which was significantly

more sensitive than the reflex pathway to depression by morphine

$(P < 0.05)$. Intraspinally evoked responses were reduced by an

average of 43.2% (S.E., $\pm$ 4.5%; N = 14) by 1 mg/kg of morphine,

whereas the same dose reduced spinal sympathetic reflexes by an

average of only 27.5% (S.E., $\pm$ 4.0%; N = 6).

Naloxone. Morphine-induced depression of sympathetic dis-

charges evoked by either pathway was rapidly and completely an-

tagonized by very small doses of naloxone HCl (Fig. 2). The

effects of 1 or 2 mg/kg of morphine were completely reversed by

10 or 20 μg/kg of naloxone. The ratio between the dose of naloxone

required for complete antagonism and the dose of morphine given

was about 1:100. Submaximal doses of naloxone that only partially

reversed the depression by morphine were additive. Antagonism

by full doses of naloxone was apparent within several minutes and

total reversal of depression was usually achieved within 10 min.

Following reversal of morphine-induced depression by naloxone,

evoked responses were maintained at or above control levels for

1-2 hrs before depression gradually intervened. Upon its reappear-

ance this secondary morphine depression was equally susceptible

to naloxone reversal, indicating that the duration of action of this

antagonist was shorter than that of morphine. Prior adminis-
tration of an appropriate dose of naloxone was also effective in
preventing depression by morphine, but the antagonism was sur-
mountable.

In approximately two-thirds of the experiments, antagonism
of the depressant effect of morphine by naloxone produced an en-
hancement of evoked sympathetic responses by as much as 30%
above control levels (Fig. 2). This enhancement was unrelated
to the initial degree of depression by morphine or to the interval
between administration of morphine and naloxone. Naloxone,
alone and in small doses, frequently, but not consistently, caused
an increase in evoked preganglionic activity to as much as
130-150% of control values; enhancement was maintained with
gradual decrement for up to 2 1/2 hr. The apparent excitatory
effect of naloxone, either alone or after morphine, occurred with
about the same frequency and to about the same degree in respon-
ses evoked by either pathway. Further analysis of this effect of
naloxone is presented below.

Nalorphine. A second narcotic antagonist, nalorphine HCl,
was tested in several experiments for its ability to counteract the

depressant effect of 1 mg/kg of morphine on evoked sympathetic

discharges. Depression was readily antagonized by 200 μg/kg of

nalorphine, responses being restored to control values within

10 min and maintained for more than 1 hr. The effects of nalor-

phine were not characterized further.

Methadone, meperidine and codeine. The effects of several

other narcotic analgesics on activity evoked from sympathetic

preganglionic neurons were determined for comparison with mor-

phine. As illustrated in A, B and C of Fig. 3, methadone HCl

(1 mg/kg), meperidine HCl (10 mg/kg), and codeine PO_4 (12 mg/kg)

each produced a significant reduction in sympathetic responses

evoked by stimulation of the intraspinal pathway. The depression

produced by each of the three drugs was obvious within minutes,

had reached a maximum by 15-20 min, and was stable at that re-

duced level for at least 1 hr. Although depression was consistently

produced by each agent, some variability in the intensity of effect

was noted among different experiments. In all experiments, nalo-

xone (10-20 μg/kg) promptly reversed the depression induced by

each narcotic and frequently caused a transient increase in evoked

activity above control values.

The effects of these narcotic analgesics on evoked sympathetic activity were qualitatively similar to those observed for morphine. The time required for onset and full development of depression and the susceptibility of that depression to naloxone antagonism were comparable for all four agents. The doses required for equivalent effect provided the major difference in the profile of action by these drugs. Although 1 mg/kg of methadone usually produced a reduction commensurate with that seen with the same dose of morphine, production of a corresponding degree of depression by meperidine and codeine required 10-12 times that amount. These differences in potencies, however, compare favorably to the ratios reported by other investigators for equianalgesic doses in both man and animals[53, 65].

Dextromethorphan. Dextramethorphan is the d-isomer of a codeine analog of levorphanol. As such, it is structurally related to the narcotic analgesics but is devoid of analgesic, euphoric and addictive properties[42]. At low doses an antitussive action is its most prominent central effect; however, larger doses can produce generalized CNS depression[10, 42]. Dextromethorphan HBr was studied with the intention of further defining the mechanism

mediating the action of the narcotic analgesics on evoked sympa-

thetic activity. Preliminary experiments showed intravenous

dextromethorphan to have a serious vasodepressor action, an ef-

fect that could be prevented by prior treatment with pyrilamine

maleate. Pyrilamine alone had no effect on evoked responses and

did not alter the actions of morphine or naloxone.

Fig. 3D shows a representative experiment in which intra-

spinally evoked discharges were modestly reduced by the admin-

istration of 5 mg/kg of dextromethorphan. At this dose, the degree

of depression was variable, ranging from 20-40%. Its onset was

notably less rapid than that routinely observed after morphine and

the other narcotic analgesics. The reduction in evoked activity

produced by dextromethorphan could not be reversed by naloxone.

As illustrated in Fig. 3D, the depression produced by 5 mg/kg re-

mained relatively unchanged for up to 3 hrs despite an additional

5 mg/kg dose or the administration of 40 μg/kg of naloxone.

In the cat, a selective antitussive action is obtained at doses

of dextromethorphan in the range of 0.25 to 0.50 mg/kg; slightly

higher doses produce a more generalized CNS depression[10]. The

characteristics of the dextromethorphan effect on evoked

sympathetic activity indicate that it is different than that produced by the narcotic analgesics and may represent this non-selective depression. The observed differences suggest that the action of the analgesic isomers on sympathetic preganglionic neurons is structurally selective.

Pharmacological characterization of the morphine effect and naloxone antagonism

The bulbospinal serotonergic and noradrenergic neurons terminating in lateral columns of the thoracic spinal cord exert respective inhibitory and excitatory influences on sympathetic preganglionic neurons[28,34,66]. The intraspinally evoked responses referred to in this study are evoked by stimulation of the descending axons of these noradrenergic neurons in the cervical spinal cord[28,66]. The pathway involved in spinal reflex responses, however, contains no monoaminergic neurons[16]. The ability of the narcotic analgesics to depress activity in both pathways, cannot, therefore, be explained by postulating an interference with noradrenergic transmission. Of the known synaptic mechanisms influencing sympathetic preganglionic neuron responses, only an action involving stimulation of inhibitory 5-HT receptors could

account for this depression[28]. Accordingly, a series of experiments were conducted to assess the role of 5-HT in the morphine-induced depression of the sympathetic preganglionic neurons. In addition, aspects of the naloxone antagonism of morphine were investigated.

PCPA. To assess whether endogenous 5-HT was necessary for the action of morphine, morphine was administered to animals pretreated with parachlorophenylalanine (PCPA). PCPA, a depletor of central and peripheral 5-HT[50], was administered intraperitoneally (100 mg/kg/day) for 3 days prior to the experiment. This dosage schedule has been reported to greatly reduce spinal levels of 5-HT without altering catecholamine levels[50]. The results from two such experiments are shown in Fig. 4. Depletion of central 5-HT did not affect the ability of morphine to depress evoked sympathetic activity. Both spinal reflex (Fig. 4A) and intraspinal responses (Fig. 4B) were rapidly reduced to levels normally achieved with these doses of morphine. Similarly, PCPA pretreatment did not hamper naloxone reversal of the morphine-induced depression. In fact, it appeared that naloxone reversal was not only more rapid but also produced a greater enhancement

of responses above control values than was usually observed in

cats not treated with PCPA. Acute depletion of central monoamines

with reserpine (10 mg/kg) also failed to modify the actions of

morphine or its antagonism by naloxone. These experiments in-

dicate that morphine-induced depression of sympathetic pregan-

glionic neurons and its antagonism by naloxone are not dependent

on intact 5-HT stores.

Tolazoline. The drug tolazoline, a 5-HT and α-adrenergic

receptor antagonist in the periphery[32, 67], also has been found to

block the inhibitory 5-HT receptors on sympathetic preganglionic

neurons[28]. In a series of five experiments tolazoline was tested

for its ability to antagonize the effect of morphine on evoked sym-

pathetic discharges. Tolazoline HCl (15-45 mg/kg) proved effec-

tive in both preventing (Fig. 5A) and reversing (Fig. 5B) the

morphine-induced depression of either pathway. Although tolazo-

line consistently antagonized the depressant effect of morphine,

the reversal was less rapid than that usually seen with naloxone.

5-HTP. The serotonin precursor, 5-HTP, produces an im-

mediate and sustained depression of sympathetic preganglionic

neurons[28, 34, 66]. The depression following 5-HTP has been

shown to depend on its conversion to 5-HT and subsequent leakage

from the serotonin terminals adjacent to these sympathetic

neurons [28,66]. The antagonism of morphine by tolazoline provided

presumptive evidence of a 5-HT-like action for this narcotic anal-

gesic. It was reasoned, therefore, that if morphine stimulates

5-HT inhibitory receptors in this system, then naloxone should

similarly oppose the depressant effect of 5-HT.

Two representative spinal reflex experiments are shown in

Fig. 6. When given during the depression produced by 5-HTP

(Fig. 6A), naloxone reveresed the depression and returned the

size of evoked responses to control levels. Naloxone, given prior

to 5-HTP (Fig. 6B), prevented the development of depression ty-

pically seen following the administration of this precursor. The

dashed curves in these figures represent the expected degree and

duration of inhibition normally produced by the respective doses

of 5-HTP. As a rule, somewhat larger amounts of naloxone were

required for complete antagonism of the 5-HTP-induced depression

than were required to counteract morphine. Although the onset of

naloxone reversal of depression by 5-HTP was almost immediate,

it required 30-40 min for complete antagonism.

Clonidine. Clonidine, a drug primarily known for its centrally mediated hypotensive effect,[48, 49, 68, 75, 79], has also been reported to possess certain properties in common with the narcotic analgesics[17, 69, 74, 88]. The effect of clonidine on activity evoked from the sympathetic preganglionic neurons appears to be very similar to that of morphine[28]. Small doses of clonidine (15-25 μg/kg) consistently produced marked depression of both spinal reflexes and intraspinally evoked responses. This depression is rapid in onset and graded according to dose. Depletion of central 5-HT with PCPA pretreatment does not prevent this effect. Tolazoline, however, is an effective antagonist against the actions of clonidine in this system[28]. Based on these previous observations it was concluded that clonidine depresses the sympathetic outflow by stimulation of inhibitory 5-HT receptors on the sympathetic preganglionic neurons[28]. Therefore, the ability of naloxone to antagonize clonidine-induced depression was assessed. Results from a series of six experiments demonstrated that naloxone (20-40 μg/kg) was capable of opposing the action of clonidine on evoked sympathetic activity. Naloxone given immediately before the administration of clonidine prevented the expected reduction in evoked

activity. Intraspinally evoked responses which were usually de-

pressed by clonidine for more than 3 hrs could be restored to

control levels by naloxone at any stage of the depression (Fig. 7).

The rate of reversal was similar to that seen against morphine

but the amount of naloxone needed was usually slightly greater

than that required for the antagonism of a comparable degree of

morphine-induced depression.

Chlorpromazine. Naloxone alone or during the course of

reversing morphine-induced depression frequently increased

evoked sympathetic discharges above control values. Although

observed in more than a dozen experiments, naloxone did not

consistently produce this effect. The augmented responses fol-

lowing naloxone reversal of morphine appeared to be unrelated

to either the degree or duration of the depression. On a number

of occasions in which naloxone was given first and no enhance-

ment occurred, subsequent challenge with a depressant dose of

morphine showed that the naloxone blockade was effective. The

lack of correlation between the appearance of this excitation and

the naloxone antagonism of morphine suggested that naloxone may

exert additional effects on the sympathetic preganglionic neurons

apart from its morphine blocking action. Therefore, further characterization of the naloxone actions on these neurons was undertaken.

Chlorpromazine HCl (CPZ), a peripheral and central catecholamine receptor antagonist[1, 94], has been shown to block the excitatory catecholamine receptors on sympathetic preganglionic neurons[28, 34, 66]. Naloxone enhancement of sympathetic responses evoked through the spinal reflex pathway was rapidly blocked by CPZ at doses of 5-8 mg/kg (Fig. 8A). This blockade was effective immediately and lasted for several hours. Although capable of antagonizing the naloxone excitatory effect, further investigation showed that CPZ did not influence either the morphine-induced depression or the ability of naloxone to reverse that depression (Fig. 8B). These data demonstrate conclusively that this excitation, which may be mediated through the catecholamine component of the sympathetic preganglionic neuron system, involves an action of naloxone separate from its ability to antagonize morphine.

DISCUSSION

Morphine can produce bizarre motor and behavioral effects in cats at doses greater than 4 mg/kg; below this dose, morphine produces typical sedative and analgesic effects [62]. The present experiments demonstrate that in spinal cats, morphine, at 0.5-2 mg/kg, produces a prolonged depression of activity evoked from sympathetic preganglionic neurons. This effect occurs rapidly and persists for at least 5 1/2 hr. The depression by morphine appears to be specific since the depression can be antagonized by very low doses of naloxone or nalorphine. Demonstration of identical patterns of depression by three other narcotic analgesics and their antagonism by naloxone indicate that this effect is not peculiar to morphine but represents an action common to this class of drugs. The degree of depression produced by the narcotic analgesics was routinely significant, but exhibited some variability among experiments. However, their relative potency for producing comparable depression of preganglionic neurons (morphine, 1 mg/kg; methadone, 1 mg/kg; meperidine, 10 mg/kg; codeine, 12 mg/kg) is equivalent to their relative anagesic potency [53, 65]. Dextromethorphan, a nonanalgesic stereoisomer related

to levorphanol, produces only a limited and nonspecific depression of evoked sympathetic activity; the particular characteristics of this effect indicates an action different from that of morphine and related drugs.

The observed effects of the narcotic analgesics on activity evoked from sympathetic preganglionic neurons generally fulfill the criteria for defining useful opiate analgesic models[31]. The identical effects of four narcotic analgesics, their relative potency ratios, the rapid and specific antagonism by naloxone and nalorphine, and the evidence for stereospecificity indicate that these neurons are a potentially useful neuronal system for studying the central actions of the narcotic analgesics. Furthermore, the involvement of monaminergic neurons in this system presents the possibility of evaluating their role in the central actions of these drugs.

Bulbospinal 5-HT and NE neurons terminate adjacent to the sympathetic preganglionic neurons in the lateral columns of the thoracic spinal cord[16, 29, 95]. Physiological and pharmacological evidence indicates that these monoaminergic neurons exert opposite effects on spinal sympathetic neurons, 5-HT being inhibitory

and NE being excitatory[28, 34, 66] . In the present study, discharges from this pool of spinal neurons were evoked through two separate pathways. Transmission through the intraspinal pathway involves the descending NE neurons [28, 66] whereas the spinal reflex pathway contains no monoaminergic neurons [16]. Although these two pathways both terminate with the sympathetic preganglionic neurons, the actions of drugs at monoaminergic synapses affect these pathways differently. For example, blockade of NE synapses interrupts transmission through the intraspinal pathway but leaves the spinal reflex pathway intact[28, 34, 66]. On the other hand, activation of 5-HT receptors, either through the release of presynaptic 5-HT[34, 66] or by a direct postsynaptic action[28] produces inhibition of both pathways.

Of the known monoaminergic influences impinging upon evoked spinal sympathetic discharges, the only action of morphine that is consistent with its ability to depress both pathways is activation of inhibitory 5-HT receptors on sympathetic preganglionic neurons either directly or indirectly through release of 5-HT. In addition to the classical narcotic antagonists, tolazoline was also found to be an effective antagonist of morphine on these neurons. Although

tolazoline is generally regarded as a peripheral α-adrenergic receptor blocking agent[67], it also antagonizes 5-HT both in the periphery[32] and on spinal sympathetic neurons[28]. However, the tolazoline blockade of morphine cannot distinguish between a pre-synaptic or postsynaptic site of action. The failure of 5-HT deple-tion to alter the ability of morphine to depress evoked sympathetic activity indicates that morphine does not cause release of 5-HT but strongly suggests that morphine directly stimulates inhibitory 5-HT receptors in this system. The observations that naloxone antagonizes the depressant effects of both 5-HTP and clonidine, two agents that also appear to produce stimulation of inhibitory 5-HT receptors on these neurons[28], further strengthens this con-clusion. Full understanding of the anatomy, physiology and bio-chemistry of the spinal sympathetic neurons is incomplete. Con-sequently, the possibility that morphine acts through some as yet unidentified process cannot be totally excluded, nor can the unlike-ly possibility be ruled out that morphine depresses the two path-ways by different mechanisms. However, the most direct inter-pretation of the available evidence points to a site of action on sympathetic preganglionic neurons that strongly resembles the

the postsynaptic inhibitory 5-HT receptors and is consistent with the hypothesis of a direct action on those receptors. No precise explanation can be given for the greater sensitivity of the intra-spinal pathway than of the spinal reflex pathway to morphine. However, the same phenomenon has been observed for depression of these pathways by clonidine[28] and may represent differences in the positional relationship between the locations of the 5-HT receptors and the respective terminations of the two excitatory pathways.

Naloxone rapidly and reversibly blocks the morphine-induced depression of sympathetic preganglionic neurons in the microgram dosage range. Based on these observations and on a considerable amount of other available evidence, the naloxone antagonism of morphine is most likely a result of direct competition at the morphine receptor, which in the present system appears to be an inhibitory 5-HT receptor; the ability of naloxone to antagonize the effects of 5-HTP and clonidine on these neurons also strongly supports this probability. Although the naloxone blockade of 5-HTP-induced depression could conceivably result from a presynaptic action, no such mechanism can account for naloxone reversal of

the depression by clonidine. The slightly greater amounts of

naloxone needed to reverse the depressant effects of 5-HTP and clo-

nidine than needed to antagonize morphine may reflect slight dif-

ferences in affinity of these agents for the 5-HT receptors. The

less rapid antagonism of the effect of 5-HTP by naloxone may in-

dicate a greater specificity of the antagonist against compounds

with related molecular structures. Aside from these small quan-

titative differences, the mechanisms for naloxone antagonism of

5-HTP, clonidine, and the narcotic analgesics all appear to be

qualitatively identical.

In addition to its ability to block the effect of narcotic anal-

gesics on sympathetic preganglionic neurons, naloxone frequently

produces increases in evoked activity. The relative irregularity

in the appearance of this secondary effect when contrasted against

the regularity with which naloxone antagonized the effect of mor-

phine rules out the possibility that naloxone stimulation of pre-

ganglionic neurons is responsible for its antagonism of morphine-

induced depression. Furthermore, chlorpromazine can block this

excitation without affecting the ability of naloxone to reverse the

depression by morphine. Excitation following the administration

of narcotic antagonists either alone or in the course of antagoniz-

ing narcotic depression has been reported frequently in both

clinical and experimental situations, and several authors have

noted the irregularity of its appearance [25, 44, 46, 55, 58, 59, 61].

The rapid blockade of naloxone-induced excitation by chlorproma-

zine indicates that this effect is mediated through the excitatory

NE input to the sympathetic preganglionic neurons [28, 34, 66]. If

the excitation was due to direct stimulation of NE receptors, then

evoked sympathetic responses should have been increased in

every experiment [28, 34, 66]. However, a presynaptic action de-

pendent upon some residual catecholamine leakage from decen-

tralized NE terminals in some experiments could account for the

irregular appearance of the excitatory effect. Indeed, blockade of

synaptosomal NE reuptake by naloxone has been demonstrated

in vitro [12]. To the degree that this secondary effect is present,

the capacity to stimulate sympathetic neurons in addition to its

direct morphine blocking action suggests that naloxone may act as

a physiological antagonist as well as a pharmacological antagonist

of narcotic analgesics in this system.

Interpretation of the respective roles of catecholamine and

5-HT systems in the acute central effects of the narcotic analge-

sics is controversial. Many studies are available, but large var-

iations in the doses of narcotics and antagonists used, species

differences, and the diverse methods employed for assessing the

acute effects all combine to make reconciliation of conflicting

interpretations very difficult. Evidence is available to suggest

that central NE mechanisms either may mediate [51, 73, 80, 85] or

may antagonize [8, 9, 64, 78, 84] the effects of morphine and other

narcotic analgesics. With regards to 5-HT, some investigators

have found that decreasing central 5-HT tone does not influence

acute morphine-induced analgesia [6, 38, 60, 91] while others claim

that analgesia does require functional 5-HT neurons or that 5-HT

and morphine produce additive analgesic effects [70, 71, 72, 90]. The

sympathetic preganglionic neurons almost certainly play no dir-

ect role in mediating nociception, but, insofar as these neurons

can serve as a central model, the present results may help to re-

solve these controversies. Some caution should be used when

comparing results from whole animal studies with those of the

present study in which spontaneous bulbospinal 5-HT tone has

been compromised by spinal transsection. Nevertheless, in light of the apparent 5-HT agonist action of morphine on sympathetic preganglionic neurons, it can be suggested that morphine could augment central 5-HT activity in the intact animal, in which case PCPA pretreatment might appear to reduce the effect of morphine whereas activation of 5-HT activity might appear to enhance the effect of morphine.

A number of investigators have concluded that reactions to nociceptive stimuli and their alterations by the narcotic analgesics are dependent not on a single monoamine but on the relative balance of several endogenous compounds[77,78,84,86]. Takemori et al.[86] have classified the 5-HT system as a positive modulator and the dopaminergic system as a negative modulator of the analgesic action of morphine. The observations that 5-HT markedly potentiates whereas NE attenuates the actions of narcotic analgesics have led others to suggest that the central effects of these drugs are intimately involved with opposed 5-HT and NE systems[8,9,77,78,84]. Since the sympathetic preganglionic neurons are inhibited by 5-HT and excited by NE[28,34,66], the present demonstration that they are also inhibited by morphine is

consistent with the hypothesis that 5-HT systems are synergistic with its central actions. The lack of dopamine terminals in the spinal cord curtail any judgment on its role in the actions of morphine. The ability of NE to excite preganglionic neurons, however, presents a provocative example supporting contentions that noradrenergic activity opposes the central effects of the narcotic analgesics.

Morphine and other narcotics depress cardiovascular function through an action at both central and peripheral sites [20, 21, 22, 58]. The inhibition of central cardiovascular structures that contributes to reduction in blood pressure, heart rate and sympathetic activity can be blocked by narcotic antagonists [18, 20, 54, 58], shows rapid development of tolerance [7, 58] and appears to be involved in some of the manifestations of dependence and withdrawal [58]. A medullary site mediating this central inhibition has been suggested [7, 18, 21, 54], but an action at the spinal level has not been excluded [54]. Since the sympathetic preganglionic neurons are the final central units for integrating sympathetic outflow and vasomotor control, inhibition of these neurons may account for the central component of narcotic-induced depression of cardiovascular tone. This

explanation is consistent with the observations that 5-HT precursors and clonidine, both of which produce vasodepression[2,3,43,49,68] and inhibition of sympathetic outflow[4,39,56], also depress spinal sympathetic neurons[28,34,66]. Although depression of these spinal neurons may be sufficient to account for the centrally mediated hypotension, demonstrations by other investigators that supraspinal cardiovascular centers are inhibited by narcotics[18,21,54] indicate actions at several levels along the neuraxis.

Although the present study was concerned only with the acute actions of the narcotic analgesics on the sympathetic preganglionic neurons, the unequivocal results prompt some speculation about the chronic effects of these agents on CNS activity. In addition to spinal integration of sympathetic outflow, other central systems may function under reciprocal catecholamine and 5-HT control. For example, these putative transmitters have been shown to produce opposite effects on the central control of motor rhythmicity[89], respiration[24,25], sleep[63], certain aspects of animal behavior[5,30,57,92] and temperature regulation[40]. The normal functioning, adjustments and derangements of such central systems may reflect changes in balance between

opposing monoaminergic influences rather than isolated changes

in the activity of a single element. Responses to disturbances in

functional balance and the reestablishment of centrally controlled

homeostasis required by the presence of a narcotic analgesic have

been offered as explanations for the phenomena of tolerance,

dependence and withdrawal[15, 36, 47]. Depression of the autono-

mic nervous system by administration of the narcotic analgesics

disappears during the course of continuous treatment[7, 36]. If,

following the development of tolerance, drug treatment is discontin-

ued, narcotic withdrawal with overt manifestations of sympathetic

hyperactivity develop [36, 45].

Both clonidine and 5-HT, which acutely depress the excita-

bility of sympathetic preganglionic neurons, also share other

properties with the narcotic analgesics. Precursors of 5-HT pro-

duce vasodepression and inhibition of sympathetic outflow[2, 3, 4, 23].

Continuous administration of 5-HTP produces tolerance to many of

its central actions, and abrupt withdrawal produces signs of sym-

pathetic activation[93]. Clonidine, a centrally acting drug used for

anti-hypertensive therapy, also has been shown to possess both

anti-nociceptive and sedative properties[17, 19, 68, 69, 74]. In

addition, clonidine can antagonize some of the manifestations of

naloxone precipitated withdrawal from narcotics in animals[88],

and abrupt discontinuance during chronic therapy can produce ab-

stinence signs similar in many respects to those seen during

narcotic withdrawal[33, 39]. Therefore, it appears that prolonged

exposure to clonidine or 5-HT precursors can produce forms of

both tolerance and dependence that are similar to those produced

by narcotic analgesics.

Whether continuous stimulation of inhibitory 5-HT recep-

tors could elicit an increase in noradrenergic activity to balance

the depression and thereby lead to the reduced narcotic effect is

hypothetical, but considerable evidence is available to suggest

this possibility. A number of studies have shown that chronic

administration of narcotic analgesics does cause an increase in

central norepinephrine turnover[14, 17, 80, 83]. A lessening of some

of the symptoms of narcotic withdrawal has been achieved either

by decreasing central noradrenergic stores[35, 76] or by admin-

istering the catecholamine antagonists, chlorpromazine or phen-

oxybenzamine[11]. Increasing the levels of central catecholamines,

however, augments certain manifestations of withdrawal[35].

Enhanced development of tolerance and intensified withdrawal can also be achieved by raising central levels of cyclic adenosine monophosphate[15, 26, 37]; this observation takes on added significance from recent evidence that this cyclic nucleotide mediates the central postsynaptic actions of NE[81, 82]. If an increase in central NE tone response to continuous narcotic-induced inhibition is responsible for the development of tolerance, the augmented NE excitatory tone might also account for the increase in central activity seen upon withdrawal.

The development of an endogenous mechanism opposing the actions of narcotic drugs could also account for the development of latent hyperexcitability in which decreasing amounts of antagonist are required to precipitate a withdrawal reaction as tolerance increases[44, 87]. In response to a sustained narcotic-induced depression and in an effort to reestablish functional homeostasis, excitatory neuronal components (perhaps NE neurons in the spinal sympathetic system) may gradually increase their activity above normal levels. Termination of the narcotic action, either by withdrawing the drug or by displacing it from opiate receptors would unmask the enhanced excitatory activity. As the opposing

excitatory tone progressively increases to enhance the level of

tolerance, it would require lessening amounts of antagonist to

precipitate a withdrawal reaction because the excitatory system

would be operating at progressively higher levels. Considered in

this context, equivalent partial displacement of narcotic analgesics

molecules from their receptors by an antagonist would precipitate

greater withdrawal reactions in highly tolerant subjects than in

subjects with lower levels of tolerance. An intriguing observa-

tion of the present study was the ability of naloxone not only to block·

the narcotic action but frequently to increase the excitability of

sympathetic preganglionic neurons, apparently by augmenting the

NE tone to these cells.

Although the findings of this study pertain only to the acute

actions of narcotic analgesics and the suggestions for their long-

term effects are only speculative, they are in agreement with

the observations of many other investigators and they do form a

basis for testable hypotheses to explain a number of the central

effects of these drugs.

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Fig. 1. Schematic representations of the two pathways used for

evoking sympathetic preganglionic neuron (SPGN) discharges.

A, Stimulation of small myelinated afferent fibers in adjacent in-

tercostal nerves evokes SPGN discharges through the polysynaptic

spinal reflex pathway. B, Stimulation with bipolar microelectrodes

positioned in the dorsolateral funiculus of the cervical spinal cord

evokes SPGN discharges through the intraspinal pathway. Dis-

charges are recorded from upper thoracic preganglionic rami.

SPINAL REFLEX

A.

NE
SPGN
5HT

B. INTRASPINAL

C2

NE
SPGN
5HT
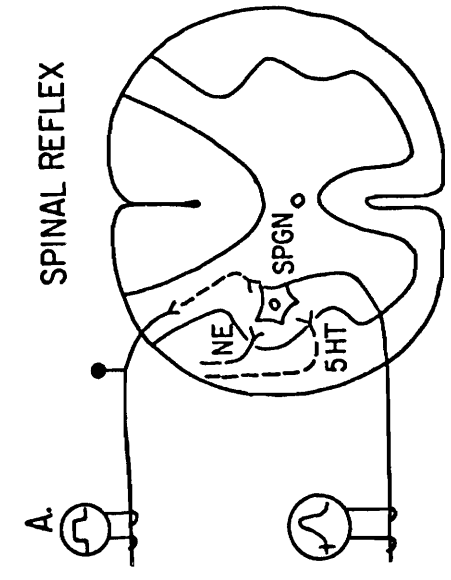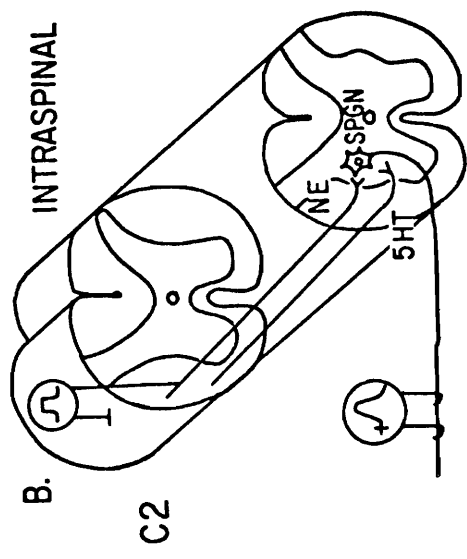
Fig. 2. Effects of morphine and naloxone on transmission through spinal reflex and intraspinal pathways. A, Morphine-induced reduction in spinal sympathetic reflex and its antagonism by naloxone. Activity was evoked by stimulating T2 intercostal nerve and recorded from T4 preganglionic ramus. B, Morphine-induced depression of intraspinally evoked responses (recorded from T2 ramus) and its antagonism by naloxone. Representative traces are shown for each phase (C, control; M, morphine; and N, naloxone) of the two experiments. Upper traces are individually evoked responses; lower traces represent computer averages of 32 (spinal reflex) or 16 (intraspinally evoked) consecutive responses. Calibrations: vertical, 40 µV in A, 100 µV in B; horizontal, 20 msec in both. Graphs below show time courses of the respective experiments; individual points were calculated from computer averages. Dashed lines indicate $\pm$ 2 Standard Deviations (S.D.) of the mean control value obtained during the control period.
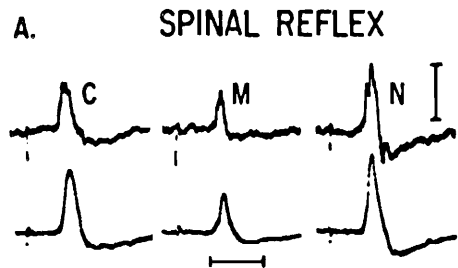
A. SPINAL REFLEX   B. INTRASPINAL
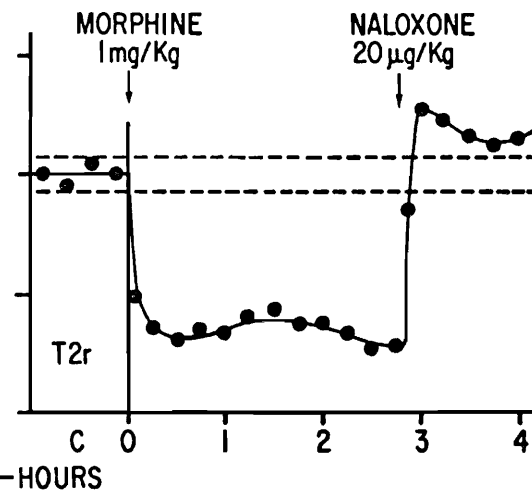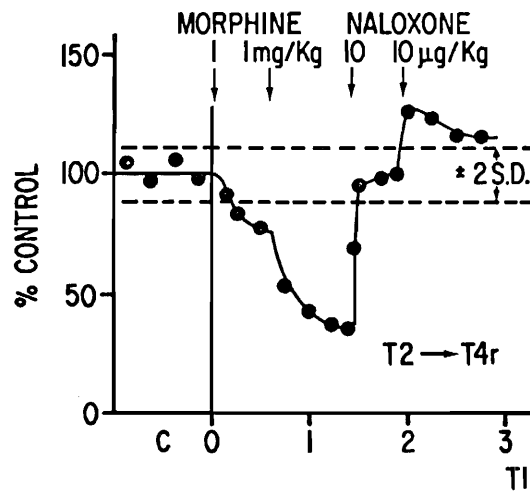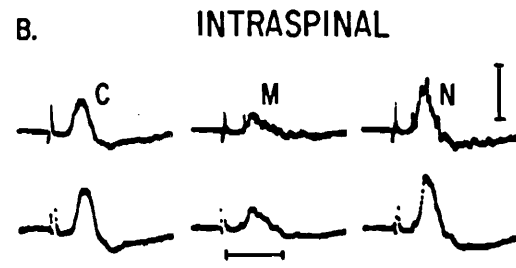
Fig. 3. Rapid depression of intraspinally evoked responses by

methadone (A), meperidine (B), and codeine (C) and antagonism

by naloxone. Limited depression by dextromethorphan (D) is unre-

sponsive to naloxone. Pyrilamine was given in D to prevent the

severe vasodepressor effect of dextromethorphan. Dashed lines
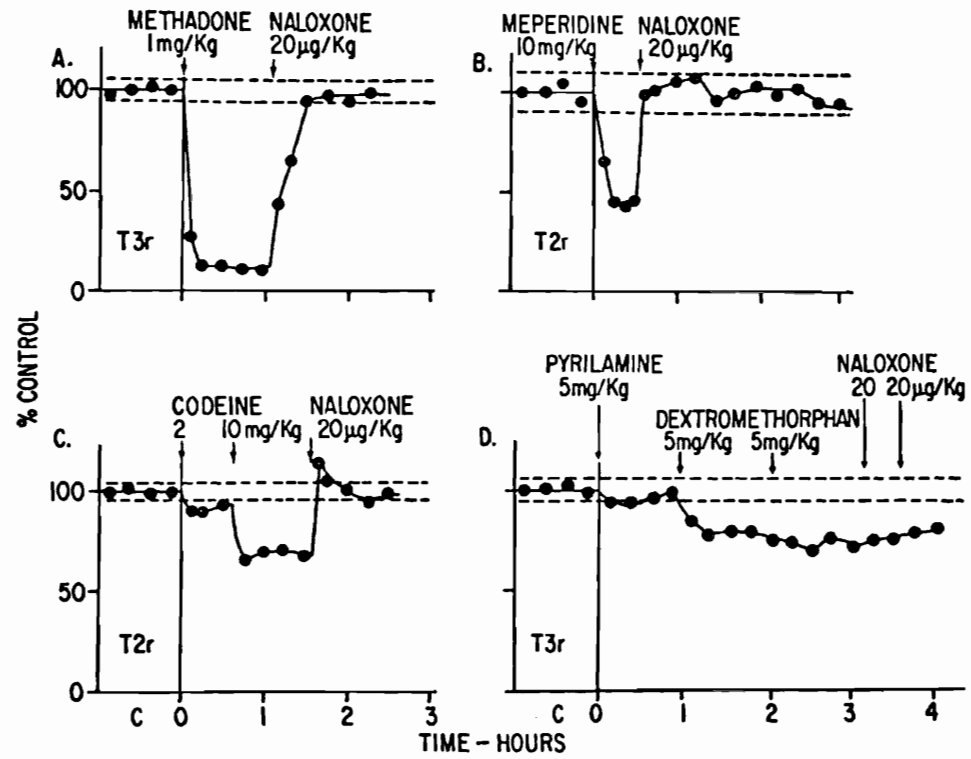
represent $\pm$ 2 S.D.

INTRASPINAL RESPONSES

Fig. 4. Morphine-induced depression of evoked sympathetic dis-

charges and naloxone reversal after pretreatment with parachloro-

phenylalanine (PCPA, 100 mg/kg/day, i.p., for 3 days prior to the

day of the experiment). Pretreatment did not alter the depressant

effect of morphine or its reversal by naloxone in either the spinal

reflex (A) or the intraspinally evoked response (B). Dashed lines,
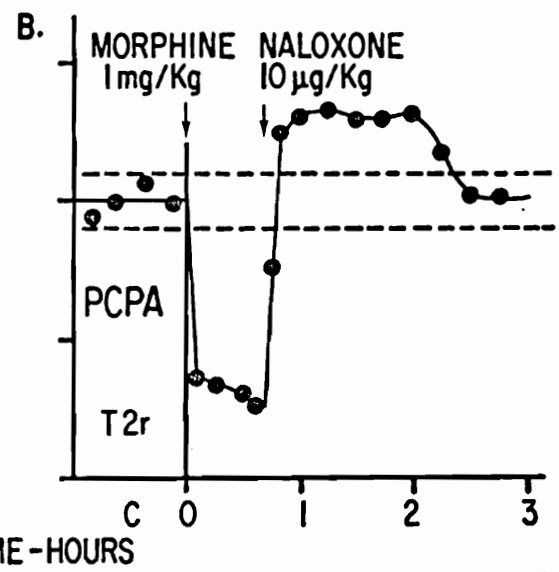
$\pm$ 2 S.D.

SPINAL REFLEX
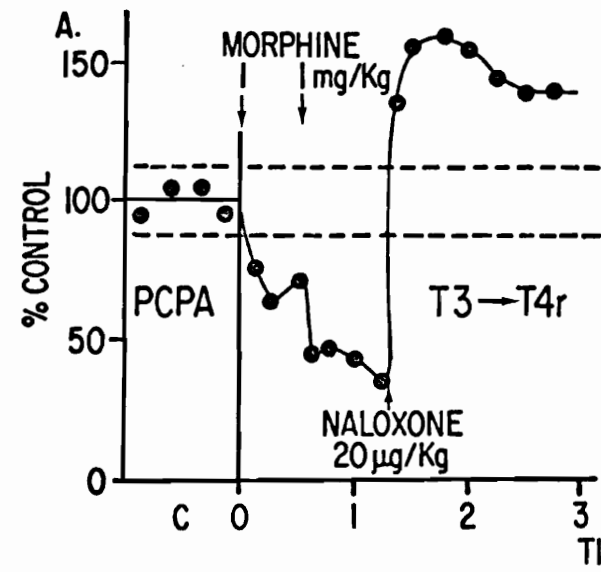
INTRASPINAL

Fig. 5. Antagonism of morphine-induced depression by tolazoline. A, Prevention of morphine-induced depression of spinal sympathetic reflexes by tolazoline. B, Reversal of morphine-induced depression of intraspinally evoked responses by tolazoline. Dashed lines, $\pm$ 2 S.D.
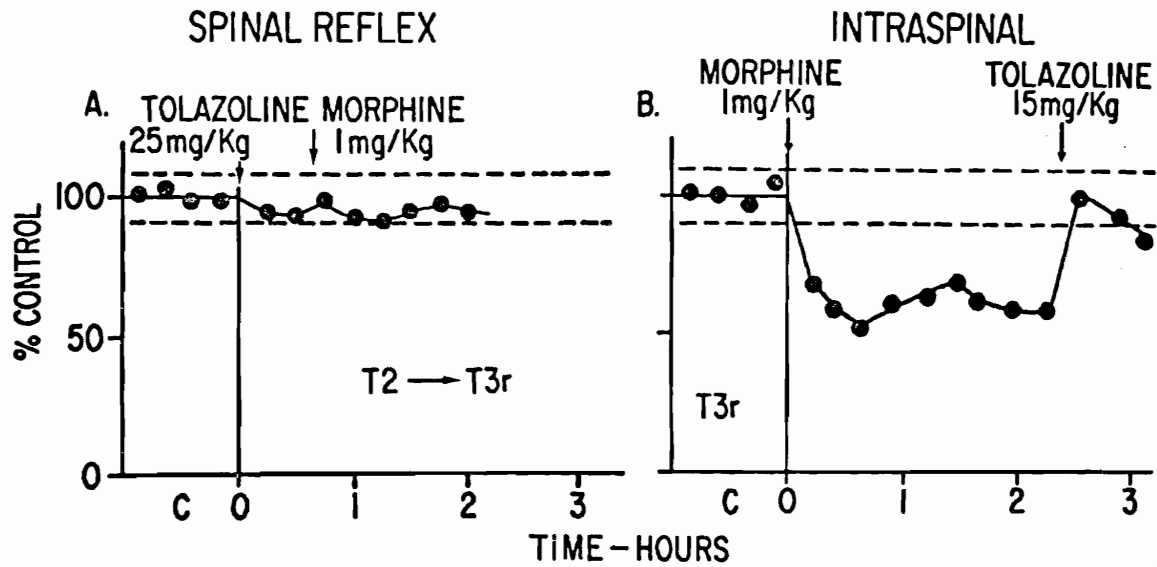
SPINAL REFLEX

INTRASPINAL

A. TOLAZOLINE MORPHINE
25mg/Kg ↓ 1mg/Kg

B. MORPHINE
1mg/Kg

TOLAZOLINE
15mg/Kg

% CONTROL

100

50

0

T2 ⟶ T3r

T3r

C 0   1   2   3

C 0   1   2   3

TIME – HOURS

Fig. 6. Antagonism (A) and prevention (B) of 5-HTP-induced depression of spinal sympathetic reflexes by naloxone. Dashed curves represent the normal responses to 5-HTP. Dashed lines, $\pm$ 2 S.D.
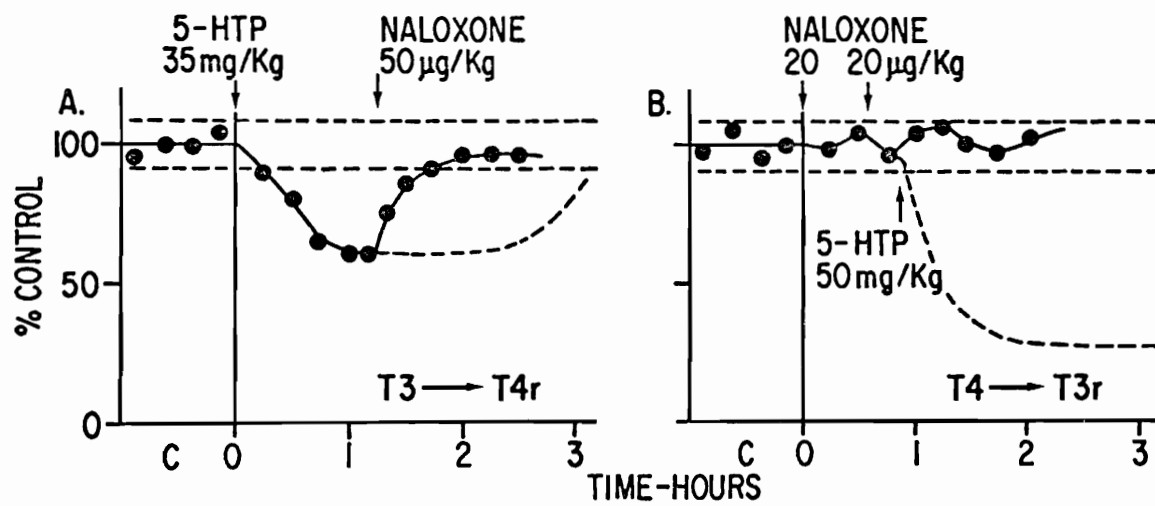
SPINAL REFLEXES

Fig. 7. Antagonism by naloxone of clonidine-induced depression

of intraspinally evoked responses. Dashed curve represents the

normal response to clonidine. Dashed lines, $\pm$ 2 S. D.

CLONIDINE 20μg/Kg

NALOXONE 20 20μg/Kg

% CONTROL

100
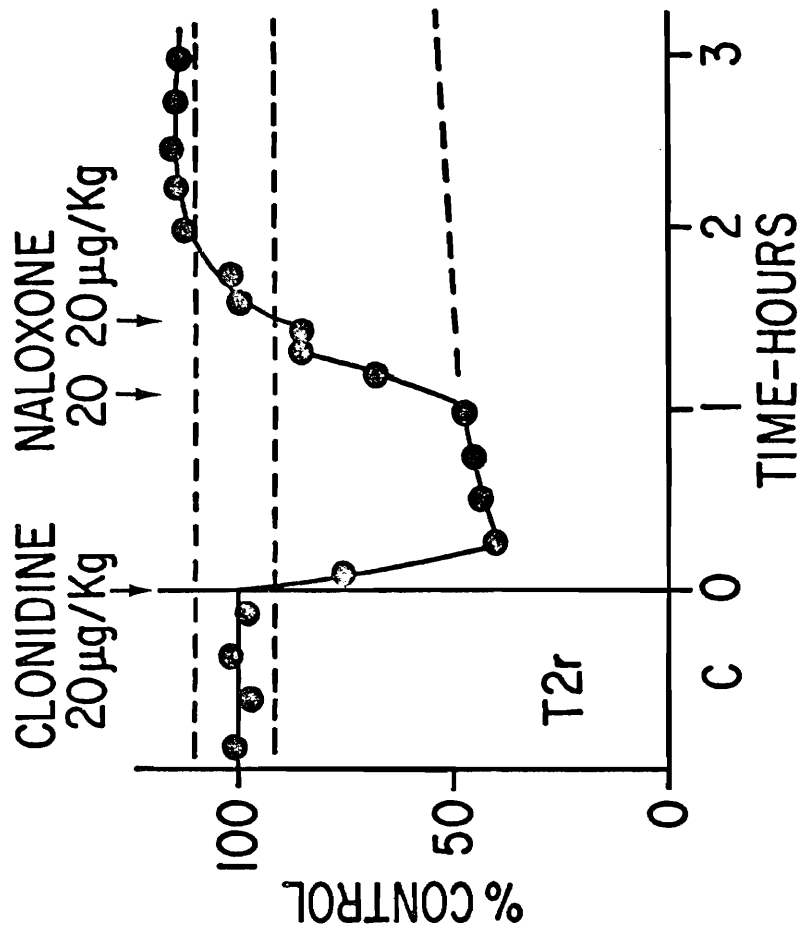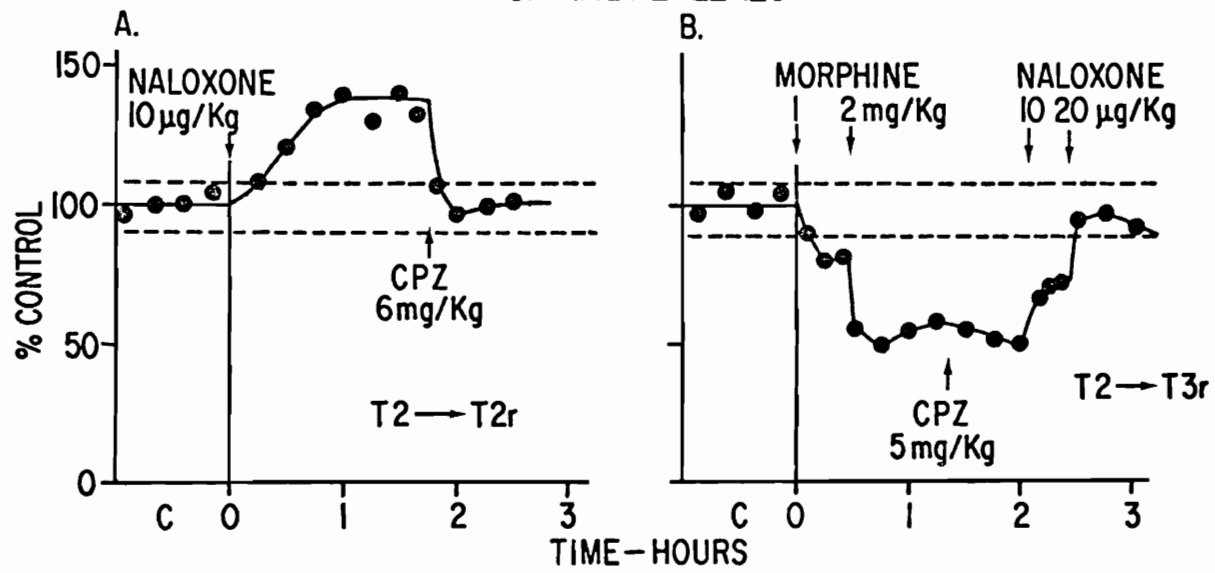
50

0

T2r

C 0 1 2 3

TIME—HOURS

Fig. 8. Effects of chlorpromazine (CPZ) on the actions of naloxone and morphine in the spinal reflex pathway. A, Rapid antagonism of naloxone enhancement of spinal reflex discharges by CPZ. B, Morphine-induced depression of evoked activity and its reversal by naloxone is unaffected by CPZ. Dashed lines, $\pm$ 2 S.D.

SPINAL REFLEXES

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Publications

McCloskey, K. L., Muscillo, G. C., and Noordewier, B., Prostatic acid phosphatase activity in the post-coital vagina, Journal of Forensic Sciences, 20 (1975) 630-636.

Finkle, B. S., McCloskey, K. L., Kiplinger, G., and Bennett, I., A national assessment of propoxyphene in post-mortem medico-legal investigations, in press. Journal of Forensic Sciences.

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