EFFECTS OF DIHYDROERGOCORNINE, CCK #179 (HIDERGINE), N, N-DIBENZYL-BETA-CHLOROETHYLAMINE (DIBENAMINE), AND BENZYLIMIDAZOLINE (PRIBOLINE) ON THE ANALGESIC ACTIVITY OF MORPHINE SULFATE AND LEVO-ISOMETHADONE

by

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Dedicated to

My Mother and Dad

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INTRODUCTION

The desire to avoid pain and to protect others from pain has been one of the most important motivating forces in the history of medicine. The study of analgesia has recently been given great impetus by the development of synthetic analgesic agents such as meperidine and methadone. Morphine has been used for the standard of comparison in the field of analgesia in spite of its many disadvantages. These include the narrow margin between analgesia and respiratory depressant doses, the high incidence of side effects, particularly nausea and constipation and the dangerous property of rapid development of tolerance and addiction. In the effort to provide pain relief equal to morphine but without its objectionable side actions, two main lines of investigation have been pursued. Most promising has been the research dealing with the synthesis and pharmacologic screening of new compounds to find those providing potent analgesia together with fewer untoward effects.(1) Less explored but meriting further study is the attempt to provide more effective analgesia through the concomitant use of potentiating agents with the pain-relieving drugs. This procedure permits either an enhancement of the analgesia or, by reducing the amount of analgesic, less likelihood of untoward actions occurring. For example, such drugs as magnesium sulfate (2), prostigmine (3), d-amphetamine (4), epinephrine (5), and quinine (6) injected previous to morphine have been shown to increase its analgesic activity.

Observations in our laboratory have shown that small doses of the dihydrogenated ergot alkaloids block the rise in blood glucose caused by

single doses of morphine sulfate and l-isomethadone in rabbits.(7) Based on this, a study was undertaken to determine what effect dihydrogenated ergot alkaloids (later other adrenergic blocking agents such as Dibenamine and Priscoline) had on the analgesic activity produced by morphine and l-isomethadone.

Much attention has been devoted in recent years to methods for comparing the actions of analgesic drugs in animals and in man. This subject has been reviewed extensively by Gost al , Burrill, and Ivy (8) who have proposed the following criteria that an ideal method of testing analgesic activity should meet:

"1. It should permit quantitative determination of threshold values of the means of inducing pain.

2. It should discriminate well between graded doses of an analgesic in modifying the responses to a standard pain stimulus.

3. It should be universally applicable to both man and experimental laboratory animals.

4. It should show quantitatively the respective effects of the analgesic against different qualities of pain."

Miller (9) has added a fifth requirement in that the method should have sensitivity sufficient to reveal low grades of analgesic activity.

Since the function of analgesic drugs is to alleviate human pain, the human is the best subject for their study. But when the study concerns new and untried drugs or an extensive assay of old ones, the human subject is obviously not available. Because pain is a subjective phenomenon, the testing of analgesic drugs in animals has to be limited to the determination of and change in their threshold response to a noxious

stimulus. The stimulus applied produces a protective response of some kind which is interpreted as a pain response, and the effect upon it of the drug under investigation may then be studied.

The principal methods of testing analgesia in animals can be divided into four groups, depending on the stimulus used for producing the pain: mechanical, chemical, electrical, or thermal. The mechanical techniques of pinching the rat's tail (10) or applying graded weights to a cat's tail (11) or rat's tail (12) are not sufficiently sensitive and doubt exists as to the stimulus, i.e., whether it is pain or merely touch. The action of salicylates can be measured by their effect on the painful swollen joint of chemically induced arthritis (13), but the stimulus is difficult to control.

After reviewing 73 publications describing analgesia testing procedures, Gootal, Burrill and Ivy (8) concluded that electrical stimulation of teeth was most likely to provide objective algesimetric information. Such a method had been described by Kell and Reffert (14) who observed "the twitch of the lower lip in response to stimulation of a canine tooth through electrodes applied to amalgam fillings in the dog." The reliability and validity of this method has been reviewed by Harris and Blockus (15), wherein they have also described an improved technique and apparatus for tooth pulp algesimetry. Although they reported a significant variance between subjects' pain thresholds, the investigators preferred electrical stimulation for its precision of regulation, measurement, reproduction, and application.

Most of the currently used methods employ thermal sources of pain as modifications of the Hardy-Wolff-Goodell procedure.(16) These authors

measured the quantity of heat required to produce a painful sensation on the center of the human forehead using a constant exposure to the stimulus of three seconds' duration. After administration of a drug, threshold estimations were carried out at regular intervals of ten minutes, and any increase in the quantity of heat required to elicit pain was regarded as an analgesic effect. Application of similar techniques to animals have been uniformly satisfactory. To be sure, in animals this procedure cannot be considered a study of pain sensation, but rather of a reaction to a noxious stimulus. Andrews and Workman (17) employed thermal irradiation of the skin of the back of the dog and noted the cutaneous maximus muscle twitch response as an index of analgesic effect. This technique is also applicable to the guinea pig (18) and the white rat (19). The D'Amour and Smith modification (20) measured the duration of a constant intensity heat stimulus required to produce a tail-flick response in the rat. In the hot-plate method of Woolfe and MacDonald (21), mice were placed on a constant temperature surface, and the time interval was noted when they raised and licked their paws. Davies and coworkers (22) placed a rat's tail near a hot wire and measured the time until it was jerked away. All of these workers, and others, have regarded heat as the most accurately measurable stimulus offering results with consistent reproducibility. However, of all the methods described, none is sufficiently sensitive. The effects of mild analgesics, such as acetylsalicylic acid, are not usually detectable and the maximum possible analgesic effect is limited. Nevertheless, "comparisons of equi-analgesic doses of the more powerful analgesic drugs show that thermal tests in animals correlate quite well with

clinical observations."(23)

In the present study, a modification of the D'Amour and Smith method (20) was used for determination of the pain-threshold response in rats. This procedure was chosen because it is convenient for small animals, and thus facilitates the use of adequate numbers to allow for individual variation. The technique avoids the tedious stepwise process of locating the "pain threshold" in successive trials, several of which are needed to determine the exact threshold intensity, as required in the method of Hardy, Wolff, and Goodell (16). This is accomplished by fixing the intensity of the stimulus and allowing it to act until a response results. This eliminates the cumulative effect from one exposure to the next. The D'Amour and Smith method, in the author's experience, is less vulnerable to the adverse influence that emotional and psychological distractions have upon the "pain threshold" as determined by Hardy, Wolff, and Goodell in human subjects. This observation has recently been confirmed by DeJongh and Knoppers.(24)

Following these studies, it was found that certain adrenergic blocking agents potentiated the analgesic activity of morphine sulfate and l-isomethadone in the rat. The question then arose as to whether other systemic actions of the analgesics were modified by the previous administration of an anti-adrenergic drug. Observations were therefore made of the respiratory rate and rectal temperature of adult rabbits before and after the injection of CCK #179 and l-isomethadone.

METHODS AND MATERIALS

Analgesic activity was determined by the radiant thermal stimulus method of D'Amour and Smith.(20) Sprague-Dawley rats, weighing from 100 to 250 grams, were used in groups of 10 to 15 animals with one-half of the group serving as controls for each trial. Only one sex was studied in any one trial. The animals were housed and fed under uniform conditions. All tests were made in a quiet room kept at a uniform temperature of about 25°C.

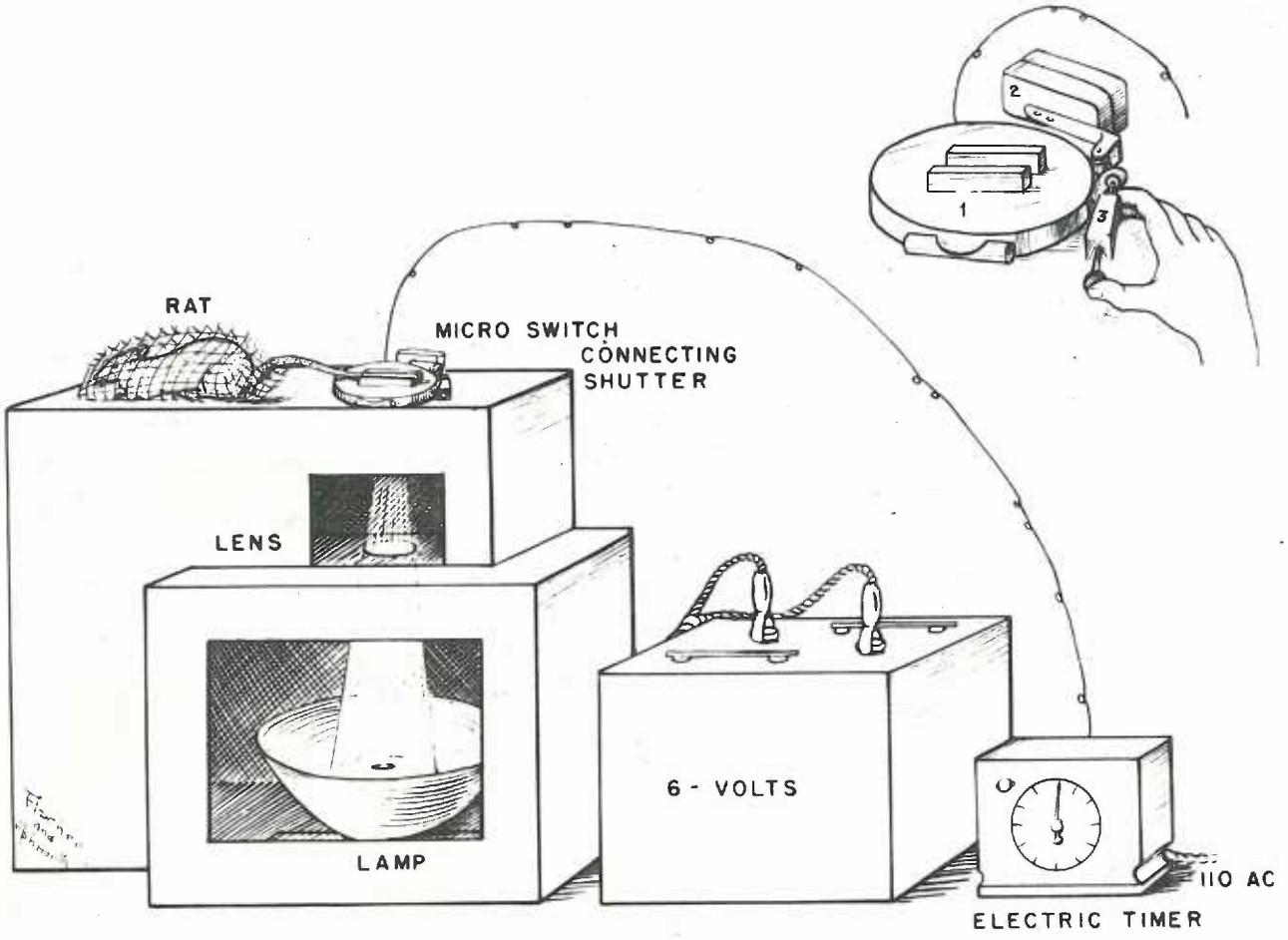
The method, as illustrated in Figure 1, requires focusing a beam of light of constant intensity on the tip of the rat's tail by means of a converging lens until the animal "flicks" its tail away. To open or close the pathway of the light beam, an automatic shutter was connected to an electric timer so that it was possible to start or stop the timer simultaneously with operation of the shutter. The time between the beginning of the exposure and the tail-flick response is the "reaction time". (25) Only animals having a normal reaction time of from 3 to 5 seconds were used. Administration of an opiate analgesic, like morphine, delays this response to the noxious stimulus, or in appropriate doses, abolishes it altogether. A severe burn may be inflicted without any response although the animal is completely conscious. Thus, the term "analgesia" is used in this discussion to refer to the apparent loss of nociceptive reaction, as indicated by the slowing or loss of response shown by the rat when his tail is burned. When analgesia was deep enough to prevent a tail-flick at the end of 12 seconds, exposure was discontinued to avoid

Figure 1

Specifications of the D'Amour and Smith Analgesimeter

The source of radiation is a prefocused lamp of 32 candle-power and of 6 to 8 voltage (G. E. #2530). The biconvex lens, with a diameter of 10.2 cms. and a focal distance of 15.5 cms., concentrates the beam of light on the 1.5 cm. circular opening in the center of the asbestos platform. The shutter (1) is opened and closed by a plunger (3). A microswitch (2) connects the shutter to the electric timer.

D'AMOUR AND SMITH ANALGESIMETER



burning the tail and the animal was considered as having a reaction time of 12 seconds.

Before testing, the animals were placed in individual conical cages made of wire mesh, molded to prevent much movement. Following determinations of the normal reaction time, intraperitoneal injections of 1.0 ml. of 0.85 per cent saline were given to all the animals, and control readings were taken at twenty-minute intervals for 120 to 150 minutes. The mean reaction time was determined from the average of these five or six control responses. By thus subjecting the animals to a preliminary conditioning period, we have found that the individual variations among rats of a group decreased. This allows a more reliable base-line for the comparison of drug effects and was previously reported by Irwin et al (26) and Bonnycastle and Leonard (27).

Morphine sulfate and levo-isomethadone (l-isoamidone) were the analgesic drugs studied. The dihydrogenated ergot alkaloids used were dihydroergocornine methanesulfonate (DHO #130) and CCK #179 (Hydergine) which is a combination of equal parts of methanesulfonate solutions of dihydroergocornine, dihydroergocristine and dihydroergokryptine. The other adrenergic blocking agents employed were N, N-dibenzyl-beta-chloroethylamine hydrochloride (Dibenamine), and 2-benzyl-imidazoline hydrochloride (Priscoline). For injection, Dibenamine was diluted with isotonic saline and the other drugs in distilled water to a concentration that would allow the injection of a total volume of 0.5 to 1.0 ml.

To compare the effects on analgesic activity, the adrenergic blocking agent was injected subcutaneously to one half of the group. Thirty minutes later the analgesic drug was given intraperitoneally to all the

rate. Reaction times were tested at 15-minute intervals during the first hour after the injection of the analgesic, and then at thirty-minute intervals until these readings returned to near the previously determined control base-line results. Care was taken during the tests, by having an assistant select animals at random, to see that the observer had no knowledge of which animals had received the particular compounds studied, or the sequence in which the rats were tested.

Effect of CCK #179 alone and in combination with l-isomethadone on the respiratory rate was determined in the rabbit. Forty-two male and female white rabbits, weighing from 2.3 to 4.2 kg., were selected at random with three animals in each group. Using tally counters, control observations of the respiratory rate were made for at least 50 minutes prior to the intravenous injection of the drugs into the marginal ear vein. CCK #179 was then administered in dosages of 0.05, 0.15, and 0.30 mgm./kg. fifteen minutes before the l-isomethadone hydrochloride (0.50 mgm./kg.) was injected. Other groups of animals received single intravenous injections of either CCK #179, l-isomethadone, or isotonic saline. Following drug administration, observations were determined at 15-minute intervals for a period of 120 minutes. Rectal temperatures were also recorded before and after the drugs were injected. All tests were performed in a quiet room having a temperature of 25° ± 0.5°C.

RESULTS

Control Responses. Control tail-flick responses to a radiant thermal stimulus were obtained from 33 rats after the intraperitoneal injection of 1.0 ml. saline. Based on the average of five readings for each rat, tested at twenty-minute intervals, the individual rat showed a mean reaction time of 4.33 seconds with a standard deviation of ± 0.66. Differences in the control responses between the male and female rat were negligible.

Changes in Analgesic Activity Following Adrenergic Blocking Agents. In assessing the results of this assay procedure, we have considered the reaction of the animal to the stimulus in the presence of an analgesic drug as a graded response. A curve of the analgesic response can be constructed by plotting as ordinates the differences between pre-injection and post-injection reaction times; and as abscissa, time after injection of the drug studied. Figure 2 shows a comparison of the effect of morphine given alone on the tail-flick response and when preceded by an injection of CCK #179. The increase in the duration of the mean reaction time is interpreted as the index of analgesic activity, which is considerably greater when the combination of drugs is used. A portion of a typical protocol is presented in Table 1-A.

The alteration in mean reaction time may be presented as the percentage change from the control threshold by comparing the difference between the peak response time and the control response time as shown in Table 1. The effects of the adrenergic blocking agents given alone on the tail-flick response (Table 1) did not differ appreciably from

isotonic saline.

Both of the dihydrogenated ergot alkaloids, CCK #179 and DHO #180, were found to provide a considerable elevation of the mean per cent increase in peak reaction time when injected previously to l-isomethadone (Table 2) or morphine sulfate (Table 3). Statistical evaluation, using the Student's 't' test (28), showed these differences to be significant when P was less than 0.05. This data appears more significant when the results of the total number of groups given the drug combinations are compared with the groups given only the analgesic drug.

Figure 3 demonstrates the greater increase in mean reaction time and duration of effect of l-isoamidone given after CCK #179 than when the same dose of l-isoamidone was administered alone. Similarly, when Dibenamine is injected before morphine (Figure 4), there is a larger increase as well as a prolongation in the mean reaction time for morphine. The additional data provided in Table 4 further supports this observation and shows that Priscoline likewise enhances the analgesic activity of morphine. Table 5 shows the increase in mean reaction time of l-isomethadone was higher following the administration of Dibenamine or Priscoline than when l-isomethadone was given alone.

Effect of CCK #179 on the Respiratory Depression Produced by l-isomethadone in the Rabbit. Figure 5 shows that the l-isomethadone produced a marked decrease in the mean respiratory rate within 15 minutes after intravenous administration. However, previous administration of CCK #179 did not increase the depression of the respiratory

rate caused by l-isomethadone; rather, the depression was less. A slight rise in rectal temperature was produced by each drug tested. All animals survived the large dosage of CCK #179 (0.30 mgm./kg.) used. As shown in Table 6, the change in mean respiratory rate of rabbits administered CCK #179 alone did not differ significantly from the animals injected with isotonic saline. The depression of the mean respiratory rate produced by l-isomethadone (0.50 mgm./kg.) was significantly greater ($P < 0.001$) than with CCK #179 or isotonic saline, but was not augmented by the prior administration of CCK #179.

Figure 2

Mean-response curves of rats to a radiant heat stimulus following the subcutaneous administration of CCK #179 thirty minutes before morphine sulfate (i.p.) at 0 time. The number in brackets indicates the total number of animals that received the compound.

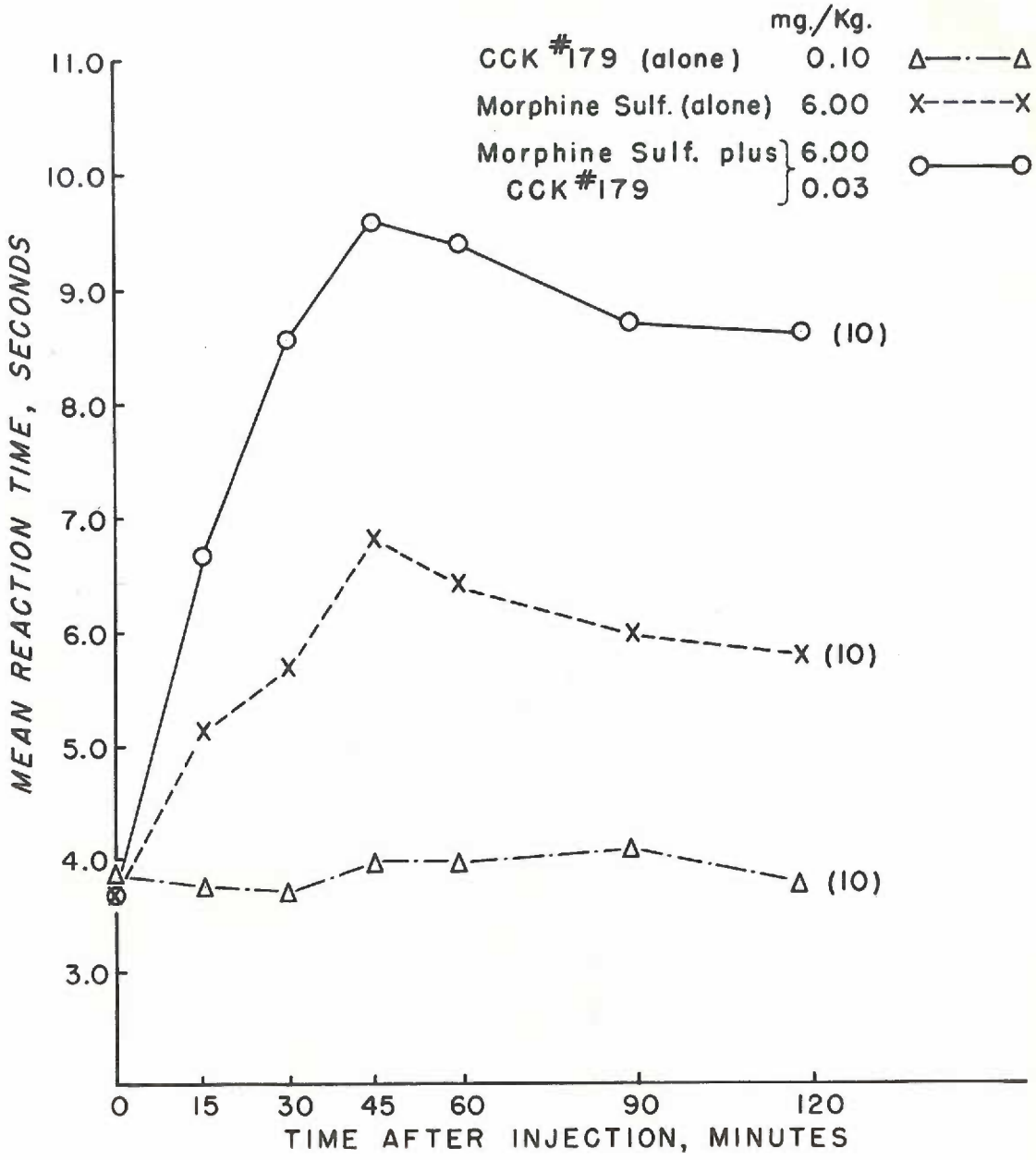

mg./Kg.
CCK #179 (alone) 0.10
Morphine Sulf. (alone) 6.00
Morphine Sulf. plus CCK #179 6.00 0.03
(10)
(10)
(10)
MEAN REACTION TIME, SECONDS
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
0
15
30
45
60
90
120
TIME AFTER INJECTION, MINUTES

TABLE 1-A. A PORTION OF A TYPICAL PROTOCOL

Group I: Dihydroergocornine: 0.10 mg./kg. plus l-isomethadone: 4.0 mg./kg.

RAT NO.	MEAN CONTROL	15'	30'	45'	60'	90'	120'	150'	180'
2	4.23	A	A	A	A	A	A	11.40	11.00
6	3.96	8.65	6.50	9.50	5.75	4.80	5.50	5.00	3.30
10	3.96	6.10	8.95	A	10.65	3.80	6.80	4.60	3.80
16	4.75	A	A	A	A	A	A	10.70	9.55
18	3.20	5.10	5.75	6.35	5.80	2.90	3.10	2.60	2.20
20	4.45	A	A	A	11.00	A	A	5.00	6.60
MEAN:	4.09	9.31	9.53	10.64	9.53	7.92	8.56	6.55	6.07
% INCREASE* :		128	133	160	133	93.6	109	60.1	48.4

Group II: L-isomethadone: 4.0 mg./kg. (alone)

RAT NO.	MEAN CONTROL	15'	30'	45'	60'	90'	120'	150'	180'
1	3.85	4.70	4.60	3.15	3.00	2.35	2.60	2.70	2.00
4	3.15	3.80	4.00	3.10	3.60	3.80	1.95	2.70	3.45
7	4.30	6.80	6.10	7.75	8.50	2.70	5.40	7.50	4.05
14	4.60	5.50	5.30	9.05	3.30	2.55	4.45	4.85	3.20
17	4.06	7.00	6.85	4.30	4.50	3.85	2.30	2.85	2.10
19	5.18	6.40	6.20	7.05	4.60	1.95	2.70	2.30	3.40
MEAN:	4.19	5.70	5.51	5.73	4.58	2.87	3.23	3.82	3.03
%INCREASE* :		36.0	31.5	36.7	9.3	-55.3	-20.5	-8.8	-27.7
P for difference** :		>0.60	<0.05	<0.01	<0.01	<0.01	<0.05	<0.03	>0.10	>0.05

Mean Control represents the average of 5 or 6 readings at 20 minute intervals prior to the injection of the drugs.

"A" refers to no response by the rat after 12.0 seconds exposure to stimulus.

* Mean per cent increase in reaction time.

** P is the probability of random selection calculated from 't' values for the differences between the mean responses of groups I and II.

TABLE 1

EFFECTS OF ADRENERGIC BLOCKING AGENTS
ON THE TAIL FLICK RESPONSE OF THE RAT

DRUG	DOSE mgm./kgm.	NO. OF RATS	MEAN % INCREASE IN PEAK REACTION TIME*	TIME AFTER INJECTION (minutes)
0.85% Saline	1.0 cc.	10	7.8	60
CCK #179 (Hydergine)	0.10	10	6.6	90
Dibenamine HCL	3.00	10	12.3	15
	6.00	9	18.0	30
Priscoline HCL	20.00	10	15.2	90

$$* = \frac{\text{Mean Peak Response, After Drug (Seconds)} - \text{Mean Control Time (Seconds)}}{\text{Mean Control Time (Seconds)}} \times 100$$

TABLE 2
ANALGETIC ACTIVITY OF L-ISOMETHADONE FOLLOWING THE ADMINISTRATION OF CCK #179 OR DHO #180

DRUG	DOSE	NO. OF RATS	MEAN % INCREASE IN PEAK REACTION TIME	TIME AFTER INJECTION	P
	mg/kg			(minutes)	
L-Isomethadone (alone)	4.00	40	67.4	45	
CCK #179 plus L-Isomethadone	0.03 / 4.00	7	138.1	30	<0.005
CCK #179 plus L-Isomethadone	0.06 / 4.00	14	95.2	15	>0.10
CCK #179 plus L-Isomethadone	0.10 / 4.00	6	86.8	60	>0.10
DHO #180 plus L-Isomethadone	0.05 / 4.00	5	158.0	15-45'	<0.005
DHO #180 plus L-Isomethadone	0.10 / 4.00	6	160.1	45	<0.01
DHO #180 plus L-Isomethadone	0.40 / 4.00	6	69.5	45	>0.10

TABLE 3
ANALGETIC ACTIVITY OF MORPHINE SULFATE FOLLOWING THE ADMINISTRATION OF CCK #179 OR DHO #180

DRUG	DOSE	NO. OF RATS	MEAN % INCREASE IN PEAK REACTION TIME	TIME AFTER INJECTION	P
	mg/kg			(minutes)	
Morphine (alone)	4.00	20	44.5	30	
CCK #179 plus Morphine	0.03 4.00	8	64.3	30	>0.10
CCK #179 plus Morphine	0.06 4.00	7	98.4	45	>0.10
CCK #179 plus Morphine	0.10 4.00	5	48.7	30	>0.10
DHO #180 plus Morphine	0.05 4.00	5	95.8	30	<0.10
DHO #180 plus Morphine	0.10 4.00	9	83.6	15	<0.10

Figure 3

Comparison of the mean-response curves of rats following the subcutaneous administration of CCK #179 thirty minutes before l-isoamidone with animals receiving single injections of l-isoamidone at 0 time.

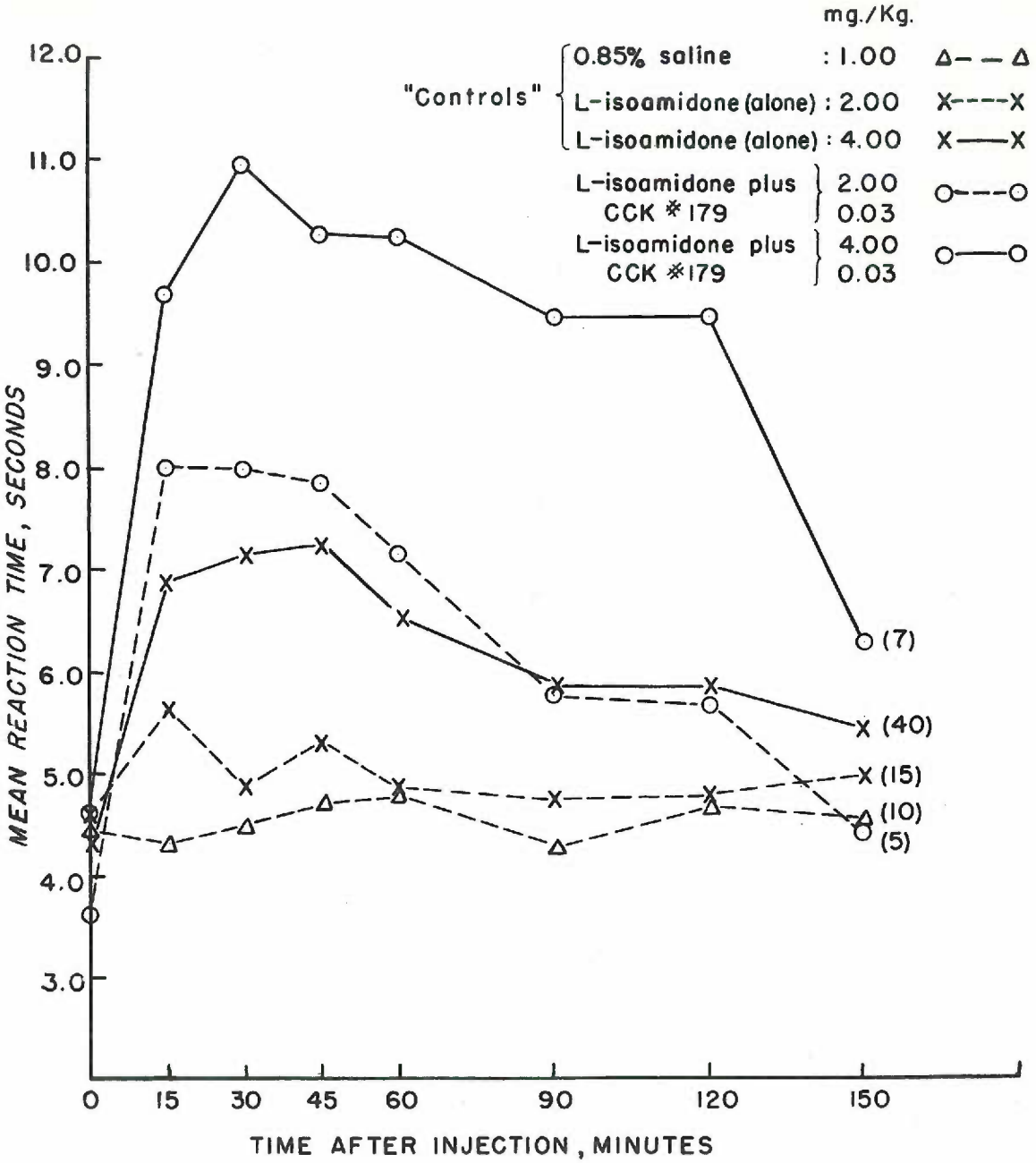

mg./Kg.
"Controls"
0.85% saline : 1.00
L-isoamidone (alone) : 2.00
L-isoamidone (alone) : 4.00
L-isoamidone plus CCK *179 2.00 0.03
L-isoamidone plus CCK *179 4.00 0.03
MEAN REACTION TIME, SECONDS
TIME AFTER INJECTION, MINUTES
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
0
15
30
45
60
90
120
150
(7)
(40)
(15)
(10)
(5)

Figure 4

Effect of single injections of morphine sulfate and Dibenamine hydrochloride as compared with their combined effect on the rat tail-flick response to a radiant thermal stimulus.

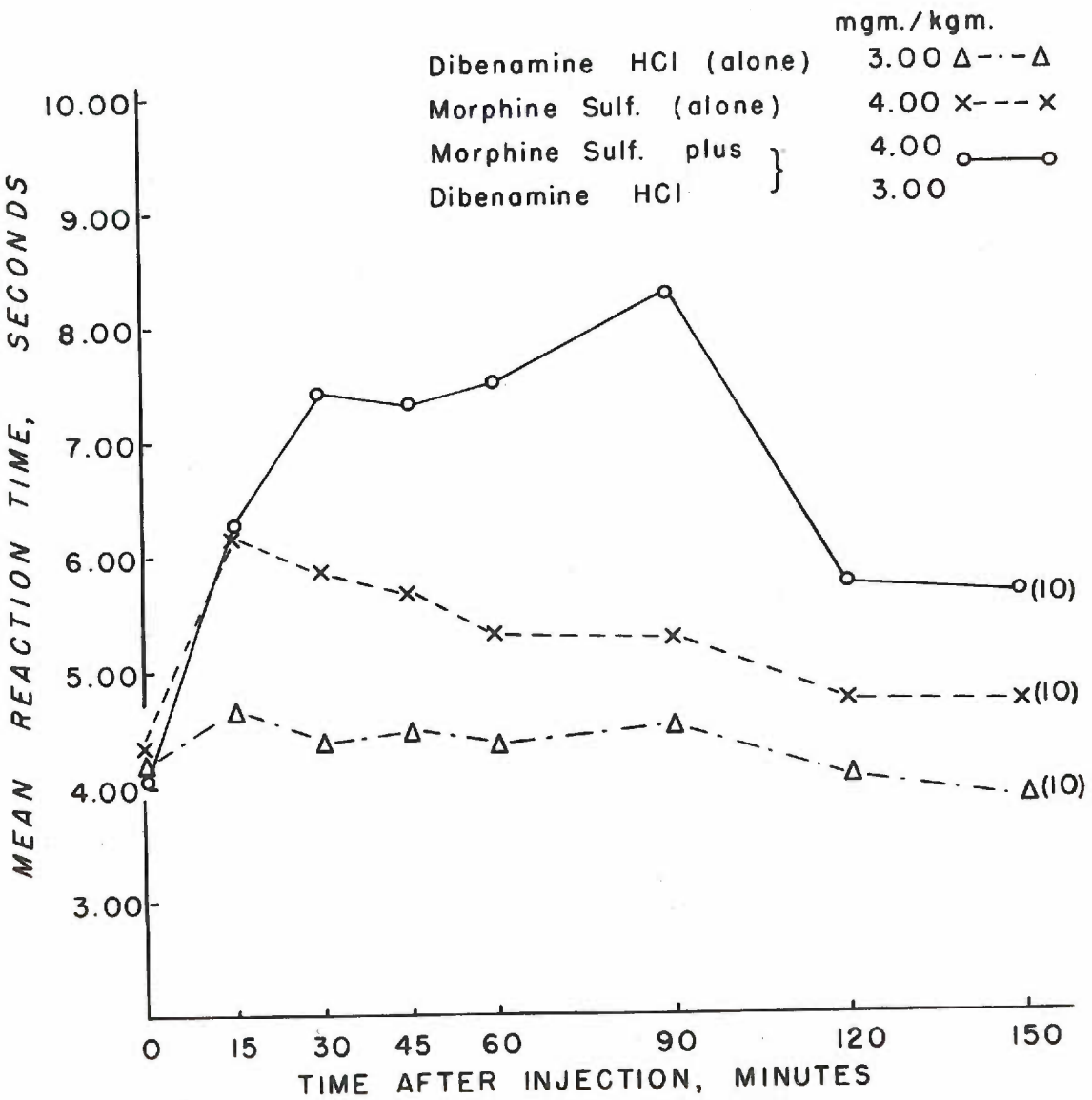

mgm./kgm.
Dibenamine HCl (alone) 3.00 Δ-·-Δ
Morphine Sulf. (alone) 4.00 ×---×
Morphine Sulf. plus 4.00
Dibenamine HCl 3.00
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
MEAN REACTION TIME, SECONDS
(10)
×(10)
Δ(10)
0
15
30
45
60
90
120
150
TIME AFTER INJECTION, MINUTES

TABLE 4

ANALGESIC ACTIVITY OF MORPHINE SULFATE FOLLOWING THE ADMINISTRATION OF DIBENAMINE HCL OR PRISCOLINE HCL

DRUG	DOSE mgm./kgm.	NO. OF RATS	MEAN % INCREASE IN PEAK REACTION TIME*	TIME AFTER INJECTION (minutes)
Morphine (alone)	4.00	60	40.6	30
Dibenamine plus Morphine	1.00 4.00	5	125.9	30
Dibenamine plus Morphine	3.00 4.00	10	100.2	90
Dibenamine plus Morphine	6.00 4.00	5	115.9	15
*Dibenamine plus Morphine	6.00 4.00	5	73.2	60
Priscoline plus Morphine	10.00 4.00	11	55.6	45
Priscoline plus Morphine	20.00 4.00	12	127.9	90

*Morphine sulfate was injected 60 minutes after Dibenamine HCL in this trial.

TABLE 5

ANALGESIC ACTIVITY OF L-ISOMETHADONE FOLLOWING THE ADMINISTRATION OF DIBENAMINE HCL OR PRISCOLINE HCL

DRUG	DOSE	NO. OF RATS	MEAN % INCREASE IN PEAK REACTION TIME	TIME AFTER INJECTION
	mgm./kgm.			(minutes)
L-isomethadone (alone)	2.00	24	15.3	15
Dibenamine plus L-isomethadone	1.00 2.00	5	35.2	15
Dibenamine plus L-isomethadone	3.00 2.00	9	72.9	15
Dibenamine plus L-isomethadone	12.00 2.00	6	71.9	45
Priscoline plus L-isomethadone	15.00 2.00	5	64.9	90
Priscoline plus L-isomethadone	20.00 2.00	5	68.0	60

Figure 5

Mean respiratory rate and rectal temperature of rabbits administered CCK #179 fifteen minutes before the injection of l-isomethadone at 0 time. Each point represents the mean for six animals.

DRUG I.V.	mg/Kg
0.85% Saline:	1.00cc.
CCK #179:	0.30
L-isomethadone:	0.50
CCK #179 plus L-isomethadone	0.30 0.50

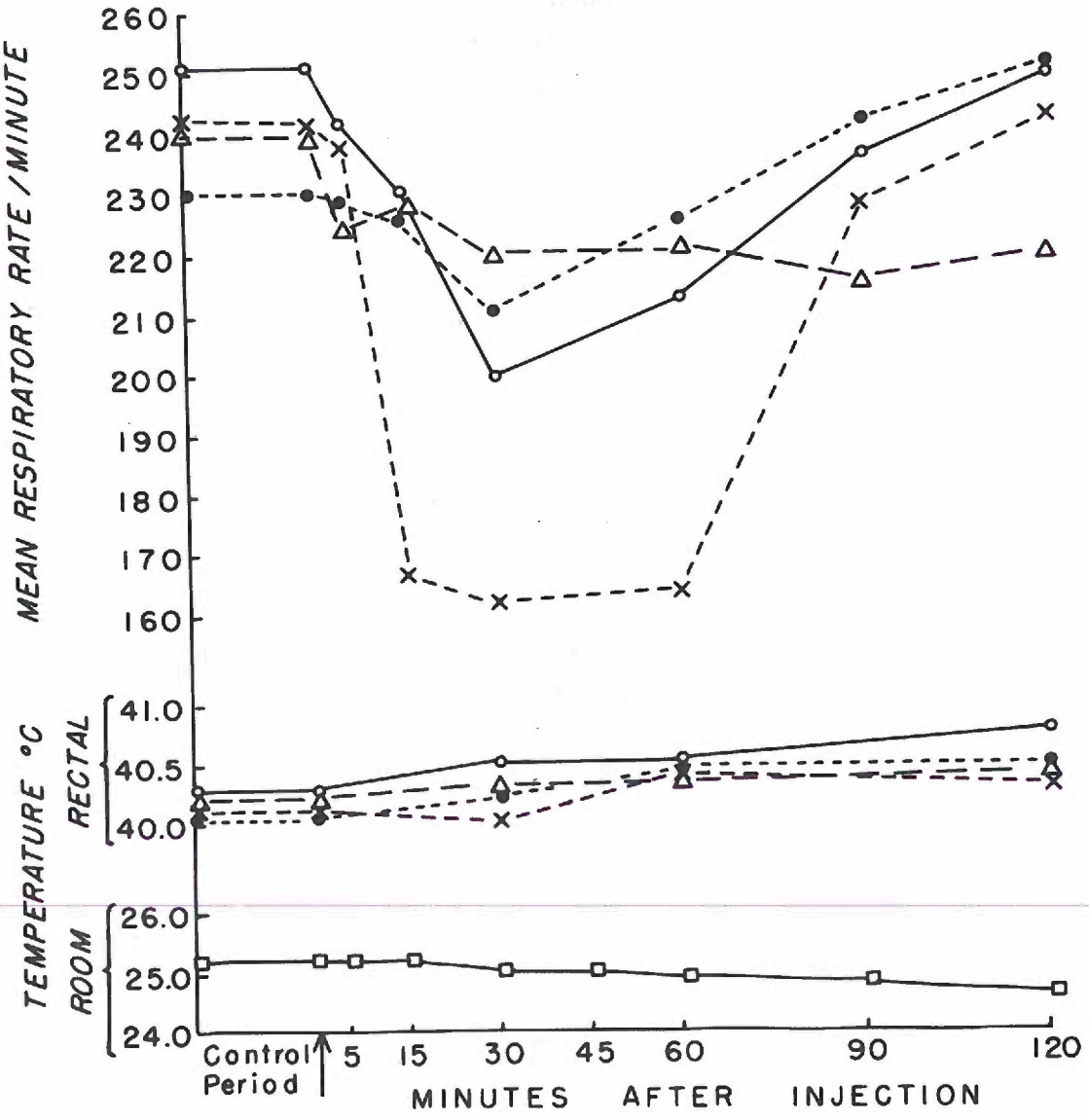


TABLE 6

EFFECTS OF CCK #179 ALONE AND IN COMBINATION WITH L-ISOMETHADONE ON THE RESPIRATORY RATE.

COMPARISON	NO. OF RABBITS	DIFFERENCE OF THE MEANS (S.D.)*	99% CONFIDENCE LIMITS ON DIFF.	P (DIFF.)‡
Saline vs.	6			
CCK #179	12	4.1 ± 10.10	-25.4 to +33.6	> .70
L-isomethadone vs.	12	72.3 ± 8.66	+47.0 to +97.6	< .001
Saline	6			
L-isomethadone vs.	12			
CCK #179	12	76.4 ± 10.48	+46.9 to +105.9	< .001
L-isomethadone vs.	12			
CCK #179 & l-isomethadone	12	10.2 ± 17.00	-37.0 to +58.1	> .10
CCK #179 & l-isomethadone	12			
vs. CCK #179	12	66.2 ± 17.77	+16.1 to +116.3	< .001

*Difference between the means of the maximum decrease in respiratory rate produced by each drug.

$$s = +\sqrt{\frac{\Sigma x^2 - (\frac{\Sigma x}{n})^2}{n-1}} \qquad S.D._{Diff.} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

‡Probability for random selection calculated from t values for the differences of the means.

DISCUSSION

It has been stated elsewhere that the magnitude of pain threshold elevation produced by a drug may be interpreted as a measure of the analgesic action of the drug.(8) However, one may doubt the validity of interpreting the human experience of analgesia in terms of the objective response of an animal. In answer to this question, Jackson has recently evaluated analgesics both in man and the rat.(29) He stated that the results do appear to show some parallel between relative analgesic potency as estimated in animals and clinical experience in the use of the morphine group of drugs. His data indicated that thermal stimulation tests in rats do in fact measure "analgesia".(30)

The potent analgesic agents, such as morphine and l-isomethadone, have been assumed to exert their pain-relieving action through a depression of the thalamic region of the central nervous system.(31) In support of this theory, the barbiturate sedatives have been reported to supplement the effects of morphine when administered together at the same time.(32,33) It has been postulated that the dihydrogenated alkaloids of ergot exert a central depressant action on the sympathetic nervous system.(34-36) Hence, one should expect to find a fall in respiratory rate and body temperature following the administration of CCK #179. In the present study no significant decrease in respiratory rate or sedative effect was observed in rabbits administered CCK #179. Nor can a possible latent action of CCK #179 on the respiratory center be considered because of its inability to further increase the respiratory depression produced by l-isomethadone. In further disagreement is the observation that when the adrenergic blocking agents were administered

alone to the rats, no increase in the mean reaction occurred. It was therefore thought that depression of the central nervous system activity does not adequately explain the enhancement of opiate analgesia by these adrenergic blocking agents.

Realising that the subject of pain is a complex and controversial one, the author has attempted to formulate a possible mechanism to explain the increase in analgesic effect of morphine produced by the adrenergic blocking agents.

A clearer understanding of the relief of pain can be achieved by a discussion of a general theory of pain, recently advocated by Good.(37) He distinguished the following five different mechanisms known to produce or be responsible for pain under clinical or experimental conditions:

"1. Impaired blood circulation: coronary occlusion, angina.

2. Prolonged and/or sustained contraction of striped or smooth muscle: intestinal and other visceral colics, labor pains.

3. Pressure from within a hollow visceral organ: gall stone.

4. Vascular pain: intermittent claudication.

5. Inflammatory pain: in spite of visible hyperemia, there is in inflammation, a stagnation of the blood present by reason of pathologically dilated capillaries."

The common denominator of the five different mechanisms of pain is diminished blood flow, i.e. the quantity of blood passing through the unit of tissue per minute is decreased. Good has therefore advanced the theory that "diminished blood flow leading to an oxygen deficiency, relative to the momentary function of the tissues concerned, is the cause of pain, wherever it may occur".(37) The present author does not agree that hypoxia is the cause of all pain, but will accept it as an explanation for the above-mentioned mechanisms.

It has been shown by Lewis and Kesa (38) that when skin has been injured and thus rendered hyperalgesic but not actually painful, simple arrest of the circulation to this injured area may induce pain. After citing several experiments, Lewis states, "there is abundant evidence that pain may arise out of malnutrition of tissues consequent upon reduced blood flow".(39)

The blood supply of nerves has been demonstrated to be very abundant by Roberts (40) who has reported that every nerve is nourished by small blood vessels, termed vasa nervorum. The nutrient arteries and some of those within the nerve have a well-defined muscular media, suggesting that the flow of blood through the vasa nervorum is subject to vasomotor autonomic control.(40). Acute occlusion of the vasa nervorum produced severe pain, paresthesias, and progressive nerve disintegration, depending on the duration of time the neural ischemia persisted.(40) On the basis of his experimental findings and observations on patients, Roberts proposed that spasm of the vasa nervorum with subsequent ischemia of the nerves causes painful impulses to ascend over the spinothalamic tracts resulting in the sensation of pain. This pain has often been relieved by sympathetic nerve blocks, vasodilating drugs, and other efforts aimed at improving the decreased blood supply of nerves.(41)

Among the most significant factors governing local circulation are the autonomic nerves, which control the arteriolar tonus. Good claims the most important factor responsible for pain as a leading symptom of many diseases is the autonomic nervous system, especially an imbalance of the sympathetic and parasympathetic.(37) He has attributed

hyperactivity of the sympathetic division as performing the greatest role in the production of pain.

Appreciating the fact that one of the chief functions of the sympathetic nervous system is vasoconstriction, Leriche had long taught the extremely important part that vasoconstriction has in the production of pain.(42) Based on this, Trimble and Morrison studied patients suffering from intractable visceral pain due to advanced malignant disease.(43) They found that great relief of pain was obtained by interruption of the sympathetic nerve paths involved, through the use of alcoholic nerve blocks or surgical denervation. Their conclusions were that the production of pain was the result of vasoconstriction, consequential to the deficient blood supply in the region of the nerve endings.

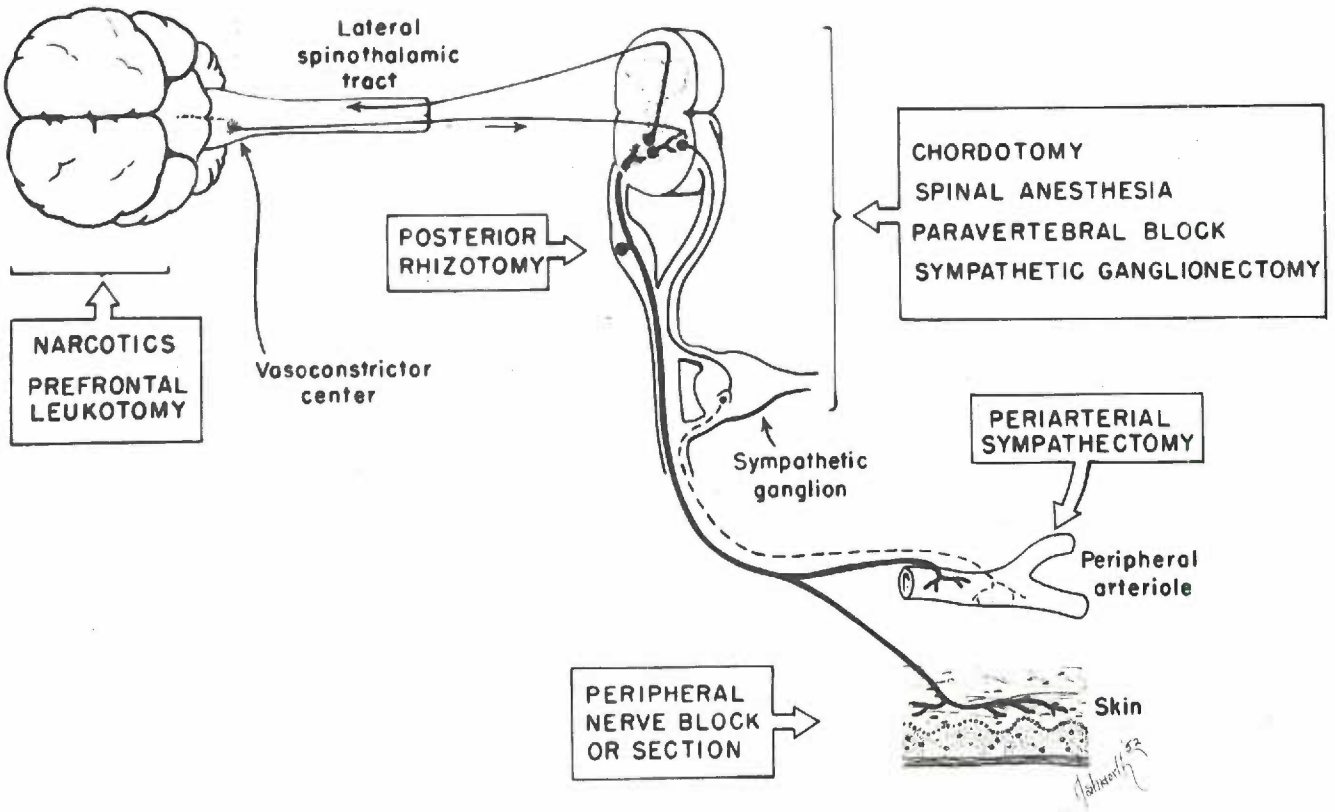
In the present study, the noxious stimulus was radiant-heat. When applied temperature to skin is raised to a point where it becomes painful, vasoconstriction occurs.(44)

As illustrated in Figure 6, Kuntz has described in the walls of the blood vessels of an extremity, afferent fibers which transverse the sympathetic trunk and enter the spinal cord through the dorsal roots of the nerves which convey the corresponding efferent fibers.(45) He stated that these afferent nerve fibers which reach the extremity through the sympathetic trunk not only conduct impulses which result in painful sensations, but also impulses which reflexly activate the sympathetic nerves to that extremity. Such reflex stimulation tends to increase vasomotor tonus in the extremity and thereby aggravate the pain. Sympathectomy in the treatment of these patients not only

Figure 6

Schematic location of the various methods for altering the neuro-anatomical pathway of noxious impulses.

THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN ANALGESIA



interrupts the afferent nerve fibers through which the pain is mediated but also abolishes the reflex vasomotor tonus, thus insuring improvement in the circulation of the limb.(45)

In patients with vascular occlusion, Livingston has postulated that a similar reflex arc, including the internuncial centers of the spinal gray matter, acts, at times, like a reverberating circuit.(46) He has stated that the afferent painful impulses initiate vasoconstriction; this, in turn, increases the pain which then reflexly aggravates the vasoconstriction.

Gross (5), Friend and Harris (47) have presented evidence that the liberation of epinephrine from the adrenal medulla by morphine (48) is an important component of the analgesic action produced by morphine. Epinephrine has been shown to participate in and enhance the analgesic action of morphine.(4,5,49,50) Vasodilatation of the blood vessels of the skeletal muscles occurs in response to small doses of epinephrine, but ordinarily it is obscured by the more powerful vasoconstriction of the splanchnic blood vessels.(51,53) White and Smithwick have also found that small doses of epinephrine regularly cause an increase of limb volume, due to vasodilatation of voluntary muscle.(54) They reported that the vasodilator action of epinephrine is increased after sympathectomy.

The adrenergic blocking agents, CCK #179, DMO #180, Dibenamine, and Priscoline all possess a common action, i.e. they block and reverse the vasopressor response to epinephrine.(55-57) Because they block the responses of smooth muscle to sympathetic nerve stimulation (58), these agents may be likened to a "chemical sympathectomy".(63) The vaso-

constrictor action of epinephrine is blocked, but epinephrine is not destroyed and continues to circulate.(56,59) The reversal of the pressor response to epinephrine by the adrenergic blocking agents represents a blocking of the vasoconstrictor action. Consequently, there is an unmasking of the vasodilator effect of epinephrine.(56,60) This results in marked dilatation of the peripheral blood vessels, and increases the blood flow through the tissues.(61-65) The increase in blood flow following the injection of CCK #179, DHO #180, Dibenamine, and Priscoline is suggested as the factor concerned in their potentiating the analgesic activity of morphine and l-isomethadone in the rat. However, caution should be urged in the interpretation of data obtained from laboratory animals, since the ultimate usefulness of analgesic effects must be determined by actual clinical experience.

SUMMARY

Following a review of the literature on the numerous methods of testing analgesic effects, a modification of the radiant thermal stimulus algesimeter of D'Amour and Smith was constructed. Analgesic activity was determined in Sprague-Dawley rats by measuring the time between exposure of the rat's tail to the noxious stimulus and the tail-flick response, i.e. the reaction time.

Control tail-flick responses were obtained from 334 rats after the intraperitoneal injection of 1.0 ml. isotonic saline. Based on the average of five readings for each rat, tested at twenty-minute intervals, the individual rat showed a mean reaction time of 4.33 seconds with a standard deviation of ± 0.66. Following control readings, one of the adrenergic blocking agents was injected subcutaneously to one half of the group of rats. Thirty minutes later, morphine sulfate or l-isomethadone was given intraperitoneally to all of the animals in the group.

The effects of the adrenergic blocking agents, CCK #179, dihydroergocornine, Dibenamine, and Priscoline administered alone on the tail-flick response did not differ appreciably from isotonic saline. The administration of CCK #179, dihydroergocornine, Dibenamine, or Priscoline thirty minutes before the injection of morphine sulfate or l-isomethadone significantly enhanced the intensity and duration of analgesic activity compared to the effects obtained from the same dose of morphine sulfate or l-isomethadone given alone.

Observations of adult rabbits administered l-isomethadone showed a marked decrease in the mean respiratory rate within fifteen minutes

after intravenous injection. The intravenous administration of CCK #179 fifteen minutes before l-isomethadone did not increase the degree of respiratory depression produced by l-isomethadone. The change in mean respiratory rate and rectal temperature of rabbits administered CCK #179 alone did not differ significantly from isotonic saline.

Possible mechanisms of explaining the potentiation of analgesic effect of morphine sulfate and l-isomethadone by the adrenergic blocking agents were discussed. A general theory of pain has attributed the decrease in blood supply of nerve tissue, relative to the momentary function of the tissue, to be responsible for the production of painful impulses. This would imply relief of pain when the blood flow was restored or increased. The increase in blood flow following the injection of CCK #179, dihydroergocornine, Dibenamine, and Priscoline is suggested as the factor concerned in their potentiating the analgesic activity of morphine sulfate and l-isomethadone.

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