Pharmacology Biochemistry & Behavior, Vol. 29, pp. 725–731. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Opioid and Nicotinic Medullary Hyperalgesic Influences in the Decerebrated Rat

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Received 28 July 1987

MARTIN, W. R., S. KUMAR AND J. W. SLOAN. *Opioid and nicotinic medullary hyperalgesic influences in the decerebrated rat.* PHARMACOL BIOCHEM BEHAV 29(4) 725–731, 1988.—The effects of ethylketazocine (EKC) administered intraperitoneally and the nicotinic ligands (−)- and (+)-nicotine, (−)-cytisine, (−)-lobeline, and (+)-2-methylpiperidine administered into the 4th ventricle on the latency of the thermally evoked withdrawal reflex of the decerebrate rat were investigated. EKC administered intraperitoneally produced both hyperalgesia and analgesia. (−)-Nicotine administered into the 4th ventricle produced a biphasic dose related effect on the latency of the withdrawal reflex; low doses produced a dose related analgesia while higher doses produced hyperalgesia. (−)-Cytisine and (−)-lobeline administered into the 4th ventricle produced biphasic effects. (+)-2-Methylpiperidine administered into the 4th ventricle produced a significant degree of hyperalgesia. Both the analgesic and hyperalgesic effects of (−)-nicotine were antagonized by mecamylamine (1 mg/kg) and naltrexone (5 mg/kg). The hyperalgesic action of (+)-2-methylpiperidine was antagonized by naltrexone but not by mecamylamine. These observations suggest that there are both medullary opioidergic and nicotinic cholinergic mechanisms for modulating both analgesic and hyperalgesic processes and that nicotinic ligands have multiple mechanisms of action in the brain.

Nicotine Ethylketazocine Lobeline Cytisine Analgesia Hyperalgesia

SEVERAL investigators have shown that naloxone can produce hyperalgesia under appropriate circumstances in patients suffering from pain [6,7] and in experimental animals [4]. Wu *et al.* [20] obtained evidence for a kappaergic medullary hyperalgesic center in the decerebrate dog. In the acutely decerebrated dog ethylketazocine (EKC) (0.5 mg/kg IV) significantly shortened the latency of the skin twitch reflex while naloxone (1 mg/kg IV) produced a modest but significant prolongation. Fentanyl (50 μg/kg IV) did not produce any significant changes in the latency of the skin twitch reflex in this preparation. When EKC (0.5 mg/kg IV) was administered to the acutely decerebrated spinal (C-1) dog, it significantly prolonged the latency of the skin twitch reflex. Naloxone (1 mg/kg IV) significantly antagonized both the hyperalgesic action of EKC in the acutely decerebrated dog and its analgesic action in the spinalized acutely decerebrated dog. These observations suggested that both the analgesic and the hyperalgesic actions of EKC involve opioid processes and that the hyperalgesic action of EKC is mediated by a medullary center while its analgesic action is mediated at a spinal cord level. Previous studies in the intact dog with an indwelling fourth ventricle cannula have demonstrated that the intraventricular administration of both (+)- and (−)-nicotine produced a prolongation of the skin twitch reflex suggesting the existence of a nicotinic medullary analgesic mechanism [9]. (±)-2-Methylpiperidine on the other hand produced hyperalgesia.

The present studies were conducted to determine if nicotinic and opioid drugs modulate the heat evoked hindlimb withdrawal reflex in the decerebrated rat at a medullary site. To this end EKC and five nicotinic ligands with different binding specificities were studied; (−)-nicotine, (+)-nicotine, (−)-lobeline, (−)-cytisine and (+)-2-methylpiperidine. Sloan *et al.* [16], on the basis of binding studies employing rat brain synaptosomes, has characterized four binding sites: (I) an upregulatory site; (II) a (−)-nicotine high affinity binding site; (III) a (+)-nicotine high affinity binding site; and (IV) a low affinity binding site. (−)-Nicotine was found to bind to Sites I, II, and IV; (+)-nicotine to Sites I, III, and IV; lobeline to Site IV; cytisine to Site II; and (+)-2-methylpiperidine to Site I. Studies were also conducted to determine the mechanism of action of (−)-nicotine in modulating the withdrawal reflex.

METHOD

These experiments were conducted in female Sprague-Dawley rats weighing between 200 to 300 grams. They were anesthetized with diethyl ether, their trachea was cannulated and they were placed in a rat stereotaxic instrument (Kopf). The skin, fascia, and muscles were reflected from the calvarium. A horizontal opening was made in the calvarium with a drill at about AP-O which allowed the brainstem to be transected with a pair of electrodes using electro-cautery.

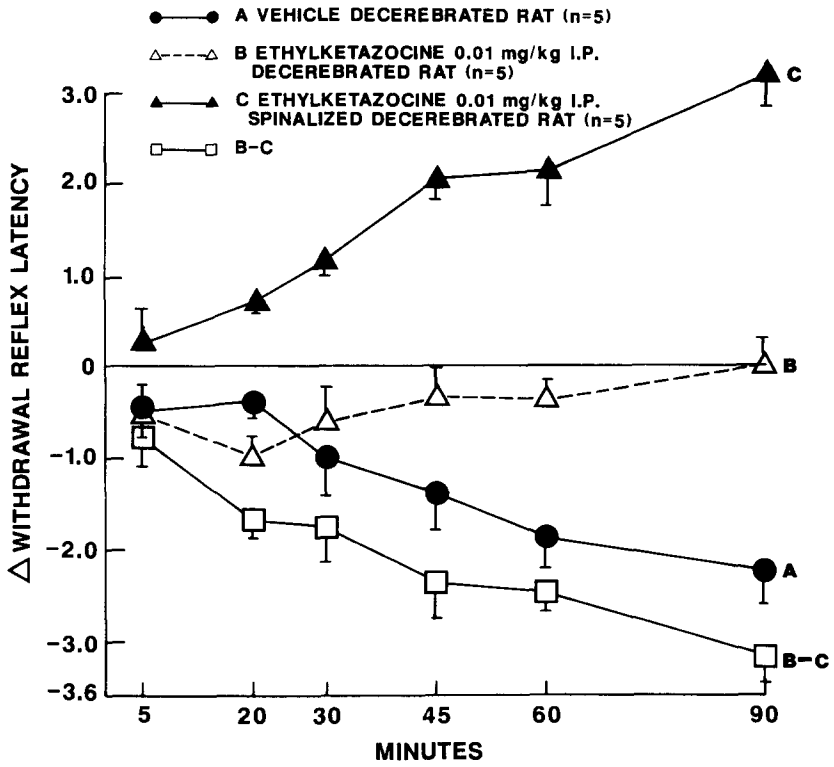

FIG. 1. The time action course of ethylketazocine on the latency of the thermally evoked withdrawal reflex in the acutely decerebrated and spinalized decerebrated rat. Δ latency is expressed in seconds.

The calvarium over the posterior fossa on one side was removed to relieve intracranial pressure and a hole was drilled in the midline for the introduction of a chemotrode. Although this preparation exhibits spontaneous respiration, respiratory movements frequently became progressively more shallow after treatment with EKC. For this reason rats were placed on artificial respiration (60 breaths/min) using a Harvard Instrument Co. small animal respirator. Body temperature was maintained at 37±1°C with a heat lamp which was controlled by a needle thermister placed in the upper foreleg and a temperature controller (YSI Model 63). A chemotrode was placed in the fourth ventricle (4th v) (AP −2.3; L 0; V −3.8). The hindquarters of the animal were placed on its side and the right superior foot was blackened with a Dura-Ink Marker. Ether was then removed and thirty minutes later experiments were initiated. In another series of experiments with the decerebrated rat the spinal cord was transected between C-1 and C-2.

A heat lamp was focused on a card placed over the blackened foot under a low level of illumination. The temperature of the heat lamp was adjusted to yield a withdrawal reflex with a latency of approximately 7 seconds. Four control observations were made at 15 minute intervals and the latency of the hindlimb withdrawal reflex was measured. A 20 second cut-off was used. Following the obtaining of the last control observation the experimental drug was administered into the fourth ventricle in a volume of 1 μl or intraperitoneally. Observations were repeated 5, 20, 30, 45, 60 and 90 minutes after administration of drug intraperitoneally or at 5 minute intervals if the drug was administered into the fourth ventricle.

At the end of each experiment, 1 μl of fast green was injected into the fourth ventricle and the brain was removed following sacrifice, fixed in formalin and subsequently examined to determine the extent of diffusion of the dye.

The mean latency of the last three predrug observations was calculated for each experiment and this value was subtracted from all subsequent observations. These differences were used to calculate time action curves. Statistical calculations were made for individual points on the time action lines or for areas of segments calculated using the trapezoidal rule of the time action lines. In experiments involving nicotinic ligands the 0–30 minute area under the time action curve was used for statistical calculations. All studies were controlled for the vehicle and cations of salts and the mean changes produced by the vehicle (or cations) were subtracted from the drug effect. Data were analyzed using a one-way ANOVA and the between doses variance was partitioned into a linear, quadratic and residual components or analyzed using group comparisons and a *t*-test.

(−)-Nicotine hydrogen (+)-tartrate and (−)-cytisine were obtained from Research Plus (Bayonne, NJ); (+)-nicotine and (+)-2-methylpiperidine resolved by Drs. W. T. Smith and Amy Howell (Chemistry Department, University of Kentucky); (−)-lobeline and (+)-tartaric acid, Sigma Chemical Company (St. Louis, MO); lactic acid certified A.C.S., Fisher Scientific (Louisville, KY).

RESULTS

In this manuscript prolongation of the latency of the withdrawal reflex will be referred to as analgesia and the shorten-

TABLE 1

THE EFFECTS OF GRADED DOSES OF ETHYLKETOCYCLAZOCINE (EKC) AND ITS LACTIC ACID VEHICLE (0.01 mg/kg IP) ON THE LATENCY OF THE WITHDRAWAL REFLEX

Decerebrated Rat	
EKC (0.005 mg/kg IP)	-18.6 ± 34.0 (5)
EKC (0.01 mg/kg IP) (curve B)	-38.6 ± 20.4 $(5)_{0.0025}$ (B–A)
EKC (0.04 mg/kg IP)	91.0 ± 12.3 (5)
EKC Vehicle (IP) (curve A)	-121.2 ± 22.4 $(5)^{0.02}$
Saline (IP)	-81.2 ± 39.2 $(5)^{0.06}$
Spinalized Decerebrate Rat	
EKC (0.01 mg/kg IP) (curve C)	151.8 ± 22.0 $(5)_{0.001}$ (C–A)
B–C	-190.4 ± 20.4 $(5)_{0.05}$

Superscript indicates the p value of the change from predrug control. Subscript is the p value of the comparison indicated by the letters in parentheses. All values are the mean and SE of the areas under the 0–90 minutes time action curve. The letter designations for the curves refer to Fig. 1.

The effects of EKC (0.01 mg/kg IP) in the decerebrated rat and in the spinalized decerebrated rat were compared.

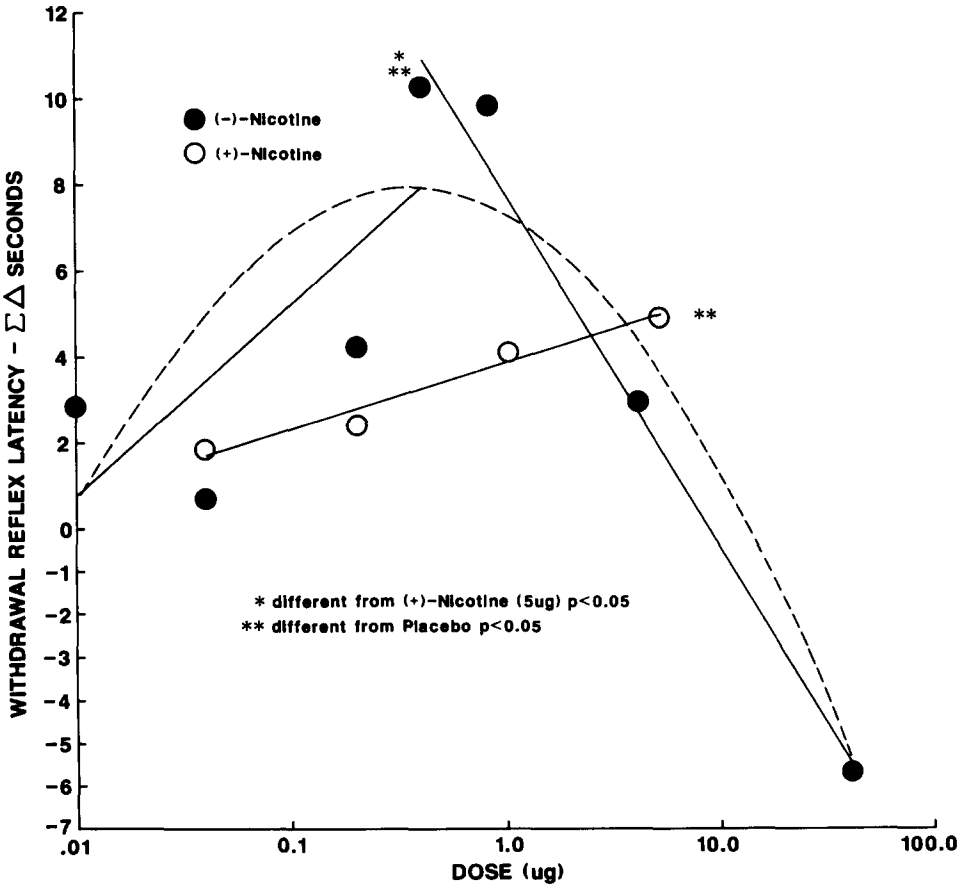

FIG. 2. Dose-response relationships of (−)- and (+)-nicotine in altering the latency of the thermally evoked withdrawal reflex in the acutely decerebrated rat. The ordinate is the mean area under the 0–30 mm segment of the nicotine time action curves minus the mean area of the appropriate vehicle time action curve. The solid lines are calculated linear regressions of the indicated segments of the dose-response curve. The dashed line is the calculated parabola which best and significantly ($p<0.0001$) fit the (−)-nicotine data.

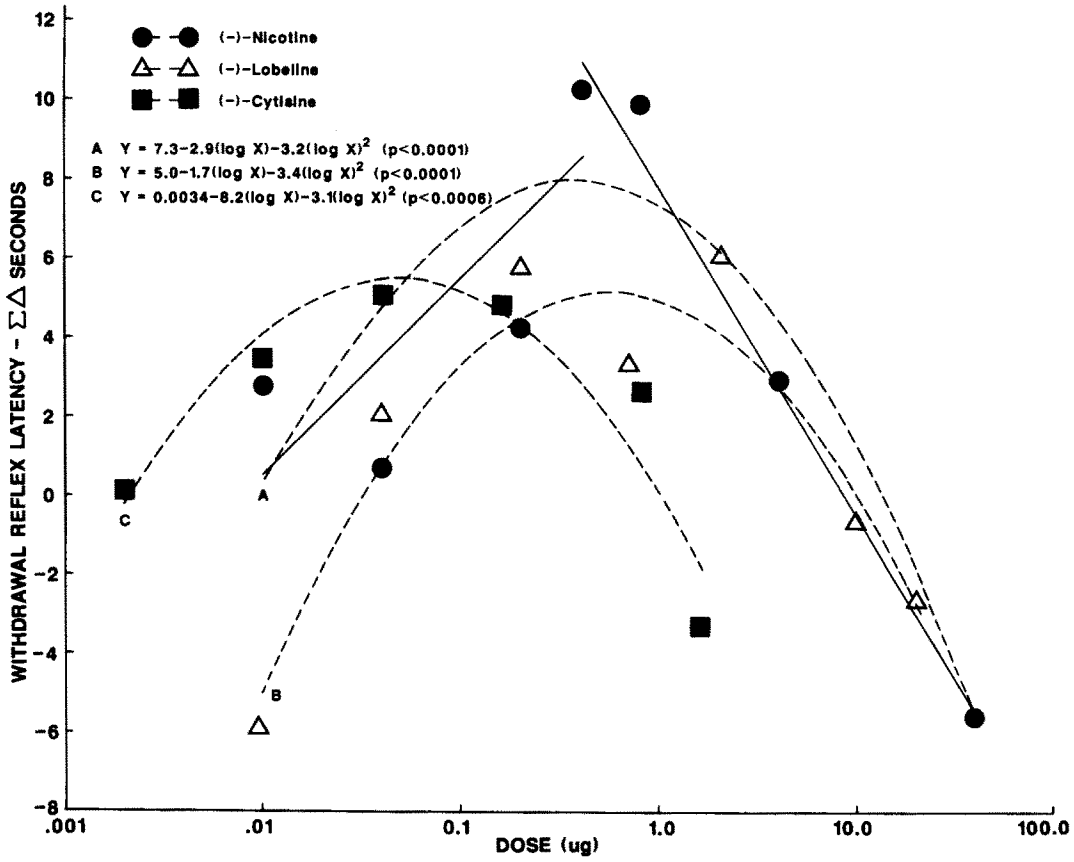

FIG. 3. Dose-response relationships for (−)-nicotine, (−)-cytisine and (−)-lobeline. The ordinate is the same as in Fig. 2. Curve A is the best fit parabola for the (−)-nicotine data; curve B for (−)-lobeline and curve C for (−)-cytisine.

ing of its latency as hyperalgesia. This is done for convenience. There is no intent to equate the changes in latency of the withdrawal reflex to pain in man.

The withdrawal reflex gradually decreases in amplitude during the course of the experiment, however its latency remained constant during the control period as assessed by a two-way analysis of variance (rats × replications) and for over twenty minutes thereafter. As can be seen from Fig. 1 (line A) the latency of the withdrawal reflex decreased progressively thereafter in the EKC vehicle (lactic acid) treated groups and this hyperalgesia was statistically significant (Table 1).

Figure 1 (line B) also illustrates the effect of EKC (0.01 mg/kg IP) on the latency of the withdrawal reflex. A modest degree of hyperalgesia was observed twenty minutes after the administration of EKC. Thereafter a significant analgesic effect became evident (Fig. 1, line B). Table 1 shows the effect of several doses of EKC on the latency of the withdrawal reflex. No dose-response relationship was established. Since it is known that EKC depresses nociceptive reflexes at the spinal cord level the magnitude of this effect was assessed in the acutely spinalized decerebrated rat. In this preparation EKC (0.01 mg/kg) produced a marked and progressive prolongation of the latency of the withdrawal reflex throughout the ninety minute observation period (Fig. 1, line C). When the latency of the withdrawal reflex in the decerebrated rat is corrected for the spinal cord depressant effect of EKC (Fig. 1, line B–C; Table 1) it is apparent that EKC has a statistically significant hyperalgesic effect at the medullary level, findings which are similar to those in the dog [20].

Studies have also been conducted on the effects of several nicotinic drugs on the latency of the withdrawal reflex. (−)-Nicotine's effect on the latency of the withdrawal reflex was biphasic with lower doses (0.01 to 0.8 μg) producing a dose related prolongation of the latency of the withdrawal reflex while higher doses (0.8 to 40 μg) produced a dose related shortening of the latency (Fig. 2). (+)-Nicotine (0.4 to 5 μg) produced a significant prolongation of the latency of the withdrawal reflex however the slope of the dose-response line was less than that seen with (−)-nicotine. Further (+)-nicotine did not produce any hyperalgesia (Fig. 2). Figure 3 compares the effects of (−)-lobeline and (−)-cytisine with the effects of (−)-nicotine on the latency of the withdrawal reflex. As can be seen both (−)-lobeline and (−)-cytisine also produced a biphasic dose-response line however the maximum prolongation of the latency was less for both drugs than that seen with (−)-nicotine. (−)-Cytisine was approximately 5 times more potent than (−)-nicotine in producing analgesia while (−)-lobeline was about half as potent. (−)-Cytisine was about ten times more potent than (−)-nicotine in shortening the latency of the withdrawal reflex while (−)-lobeline was about equipotent or somewhat more potent.

The analgesic action of (−)-nicotine is clearly antagonized by both mecamylamine and naltrexone (Table 2). Naltrexone, which produced a modest but non-significant de-

TABLE 2

THE EFFECTS OF (−)-NICOTINE AND (+)-2-METHYLPIPERIDINE AND THEIR INTERACTIONS WITH MECAMYLAMINE AND NALTREXONE ON THE LATENCY OF THE RAT HIND LIMB WITHDRAWAL REFLEX LATENCY

Drugs (Doses and Routes of Administration)	Mean AUC (30 min) ±SE (n)
(−)-Nicotine (0.4 μg IV v)	$10.3 \pm 0.6\ (6)^{-5}$
(−)-Nicotine (0.4 μg IV v) + Mecamylamine (1.0 mg/kg IP)	$3.2 \pm 0.5\ (3)^{-3}_{4}$
(−)-Nicotine (0.4 μg IV v) + Naltrexone (5.0 mg/kg IP)	$3.9 \pm 1.3\ (5)^{0.05}_{3}$
(−)-Nicotine (40 μg IV v)	$-5.6 \pm 1.5\ (5)^{-2}$
(−)-Nicotine (40 μg IV v) + Mecamylamine (1.0 mg/kg IP)	$2.7 \pm 0.6\ (5)^{-2}_{5}$
(−)-Nicotine (40 μg IV v) + Naltrexone (5.0 mg/kg IP)	$3.1 \pm 0.4\ (4)^{-3}_{3}$
(+)-2-Methylpiperidine (1 μg IV v)	$-3.1 \pm 0.9\ (8)^{-2}$
(+)-2-Methylpiperidine (4 μg IV v)	$-2.3 \pm 0.8\ (6)^{0.05}$
(+)-2-Methylpiperidine (1 μg IV v) + Mecamylamine (1.0 mg/kg IP)	$-0.3 \pm 1.6\ (6)_{-1}$
(+)-2-Methylpiperidine (1 μg IV v) + Naltrexone (5.0 mg/kg IP)	$2.2 \pm 1.0\ (9)^{0.05}_{2}$
Mecamylamine (1.0 mg/kg IP)	$-0.3 \pm 1.7\ (4)$
Naltrexone (5.0 mg/kg IP)	$1.7 \pm 1.8\ (5)$

Superscripts indicate the p value of the mean difference from vehicle control. Subscripts are the p values of the mean differences between the drug interaction study and the effects of (−)-nicotine alone. p Values other than 0.05 are expressed as exponents.

IV v—drug administered into the IVth ventricle; IP—drug administered intraperitoneally.

gree of analgesia also antagonized the analgesic action of (−)-nicotine. Mecamylamine and naltrexone antagonized (−)-nicotine's hyperalgesic action which is striking in view of the fact that the same dose of these drugs antagonized the analgesic action of (−)-nicotine and hence would have been predicted to enhance the hyperalgesic action of (−)-nicotine assuming that the interaction between the medullary analgesic and hyperalgesic mechanisms are physiologically antagonistic.

(+)-2-Methylpiperidine, a drug which shows selectivity in upregulating the binding of (−)-nicotine to rat brain synaptosomes (Sloan *et al.* [13]), also produced hyperalgesia which was significantly antagonized by naltrexone but not by mecamylamine.

DISCUSSION

The withdrawal reflex in the decerebrate rat is a nociceptive reflex whose latency is prolonged by morphine [8]. Using the experimental paradigms described the latency of the reflex decreased across time. Repeated thermal stimuli may sensitize the peripheral receptors, thus decreasing the latency of the reflex. The liminal analgesic effect of ether may slowly dissipate during the course of the experiment which may also play a role in sensitizing the reflex. The intraperitoneal administration of the lactic acid vehicle may have further decreased the latency of the withdrawal reflex. These data confirm the previous observation of Wu *et al.* [20] and Kamerling *et al.* [5] in the dog which indicated that there is a medullary kappaergic hyperalgesic center.

These observations are largely confirmatory of observation made in dogs with indwelling fourth ventricle cannulae in that both (−)- and (+)-nicotine produced analgesia [9]. No dose-response relationship was obtained in the dog, however the distribution of the drugs was different in the two species because of differences in the size of the ventricles and because of the method of administration of the drugs into the fourth ventricle. Dye studies in the fourth ventricle dog indicated that the drug distributed along the cannula which lay in the midline floor of the caudal half of the fourth ventricle. The floor of the fourth ventricle was stained about 1 mm on either side of the midline. In the rat the entire floor of the fourth ventricle and in some rats the inferior surface of the flocculus was stained (lobule I and X). Others have also reported that (−)-nicotine produces analgesia in the rat [12,19]. To our knowledge this is the first study identifying nicotine's hyperalgesic action.

(+)-Nicotine differed from (−)-nicotine in that its maximum analgesic effect and the slope of its dose-response line was less than that of (−)-nicotine. (+)-Nicotine's analgesic activity has also been observed in the mouse [2] and the dog [9]. No hyperalgesia was observed after the administration of (+)-nicotine into the fourth ventricle. It is possible that higher doses may have produced hyperalgesia but because of the limited supply of this isomer these studies were not done. Binding studies with (+)-nicotine indicate that it probably binds to a different high affinity site (Site III) than (−)-nicotine (Site II) [13] although both isomers may occupy the same low affinity site. Another possibility which may explain the differences between the two isomers of nicotine is that they may differ in pharmacologic activity.

Only preliminary experiments have been done exploring the mechanisms underlying the analgesic and hyperalgesic actions of (−)-nicotine. The analgesic action of (−)-nicotine appears to involve both a nicotinic cholinergic and an opioidergic mechanism since both mecamylamine and naltrexone, in doses which suggest specificity, decreased the analgesic action of nicotine. Further, both mecamylamine and naltrexone antagonized the hyperalgesic action of (−)-nicotine. The degree of antagonism of the hyperalgesic effect of (−)-nicotine by both mecamylamine and naltrexone may have been underestimated since these same drugs antagonized the analgesic action of (−)-nicotine.

(−)-Cytisine and (−)-lobeline appear to have similar effects on the latency of the withdrawal reflex, producing both a prolongation and a shortening except that (−)-cytisine is approximately 10 times more potent than (−)-lobeline. These are the first reports of these drugs' analgesic action in the rat.

The present studies with (−)-cytisine are in part similar to previous observations made in the dog where (−)-cytisine, administered into the 4th ventricle, exhibited a trend in producing hyperalgesia [9]. However, in dog studies, (−)-cytisine did not produce analgesia. As previously indicated, the distribution of dye in the dog was restricted to the caudal half of the medulla.

Previous studies of (±)-2-methylpiperidine indicated that it produced hyperalgesia when administered into the 4th ventricle of the dog [9]. It was demonstrated that the ability of 2-methylpiperidine to up-regulate the high affinity binding site resided in the (+) isomer [15]. The fact that naltrexone but not mecamylamine antagonized the hyperalgesic action of (+)-2-methylpiperidine indicates that this action is mediated in a different way than (−)-nicotine's hyperalgesic action.

TABLE 3

THE RELATIVE POTENCIES OF (−)- AND (+)-NICOTINE, (−)-LOBELINE AND (−)-CYTISINE IN PRODUCING ANALGESIA, HYPERALGESIA, CARDIOVASCULAR AND RESPIRATORY CHANGES, CONTRACTING THE RAT COLON AND GENERALIZING TO (−)-NICOTINE AS A DISCRIMINATIVE STIMULI IN THE RAT

	Rat Analgesia (1)	Rat Hyperalgesia (1)	Rat Cardiovascular and Respiratory Changes (2)	Rat Discrimination Studies (3)	Rat Colon Studies (4)
(−)-Nicotine	1 (1)	1	1	1 (1)	1 (1)
(+)-Nicotine	10 (20)	Inactive	7–12.5	14 (13)	11 (20)
(−)-Lobeline	5 (16)	0.7	10–50	Inactive	4 (16)
(−)-Cytisine	0.1 (0.6)	0.1	1.4–8	14 (0.2)	1 (0.2)

The number in parentheses under the column heading indicates the citation (1) present study; (2) [16]; (3) [18]; (4) [10,11]. The values in parentheses after the potency estimates are the relative potencies of the drugs in inhibiting binding of [^{3}H](−)-nicotine or [^{3}H](±)-nicotine to the rat brain P_2 fraction obtained by the above indicated investigator.

As previously discussed, binding studies have suggested that nicotinic ligands interact with several binding sites [17]. The binding properties of (−)- and (+)-nicotine, (−)-cytisine, and (−)-lobeline have been reviewed and their actions on blood pressure, pulse rate and respiration of the anesthetized rat have been presented [14]. These data, including the results of the present study, are summarized in Table 3 with the end of relating binding and pharmacologic data. As can be seen from Table 3, (−)-nicotine is 10 times more potent than (+)-nicotine and about one tenth as potent as (−)-cytisine in producing analgesia, values that are in good agreement with estimates of their ability to inhibit the binding ^{3}H-(−)-nicotine based on IC_{50}s and on estimates of their K_Ds for the (−)-nicotine high affinity site (Site II) [14]. This hypothesis is not consistent with the observation that nicotine's analgesic action is antagonized by mecamylamine. Mecamylamine does not appear to interact with the high affinity binding site (Site II) of (−)-nicotine [3, 11, 17] but does bind to the low affinity site (Site IV) [14]. The fact that the (+)-nicotine dose-response line has a gentler slope than the (−)-nicotine dose-response line may be consistent with the hypothesis that (+) nicotine produces its effects by interacting with another receptor (Site III). The (−)-lobeline data present problems in trying to reconcile its action with the above interpretation and binding data. (−)-Lobeline does not appear to interact with high affinity sites (Sites II and III) but only with the low affinity site (Site IV) [16]. The similarity of its dose-response line to that of (−)-cytisine's which appears to bind to only the high affinity site (Site II) is also difficult to relate to the binding data. These data can be reconciled by assuming that both Sites II and IV have similar actions in modulating the withdrawal reflex and would be consistent with the view that Sites II and IV are different recognition sites on the same receptor. The pattern of effects of (+)-2-methylpiperidine is different from the other prototypic drugs [e.g., (−)-nicotine] in several regards: (1) It produced hyperalgesia but not analgesia; (2) further its hyperalgesic effect was antagonized by naltrexone but not by macamylamine. (−)-Nicotine, (−)-lobeline and (−)-cytisine had different patterns of action on cardiovascular and respiratory function in the anesthetized rat (Table 2). Taken together these data support the hypothesis that nicotinic ligands have multiple modes of action on brain function as first suggested by Abood *et al.* [1].

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