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# Interaction Between Nicotine and Endogenous Opioid Mechanisms in the Unanesthetized Dog[1]

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KAMERLING, S. G., J. G. WETTSTEIN, J. W. SLOAN, T.-P. SU AND W. R. MARTIN. *Interaction between nicotine and endogenous opioid mechanisms in the unanesthetized dog.* PHARMAC. BIOCHEM. BEHAV. 17(4) 733–740, 1982.—Nicotine produced a distinct reproducible syndrome in the conscious dog when injected intravenously or intracerebroventricularly. Intravenously administered nicotine (40 μg/kg/min for 20 minutes) increased cardiac and respiratory rates and produced analgesia, miosis, hypothermia, behavioral restlessness and emesis. When microinjected into the third cerebral ventricle, nicotine (100–200 μg) similarly increased cardiac and respiratory rates and pupillary diameter; and produced behavioral restlessness, emesis, erratic analgesia and maintained wakefulness and a desynchronized EEG. Microinjection of nicotine (5–25 μg) into the periaqueductal gray failed to alter any of the parameters studied. Intravenous pretreatment with the opioid antagonist naltrexone (2 mg/kg) influenced the action of intravenous nicotine on certain physiological systems. While naltrexone alone produced a significant degree of tachycardia, miosis, and analgesia, it potentiated the tachypnea and antagonized the miotic response evoked by nicotine. Methionine-enkephalin was detected in perfusates obtained from the lateral cerebral ventricles of conscious dogs. Nicotine produced a non-significant decrease in enkephalin levels. These observations suggest that there are interactions between endogenous opioid and nicotinic processes. However, they are complex and may differ from one functional system to another.

Nicotine Naltrexone Conscious dog Analgesia Cardiac and respiratory rates Pupillary diameter Body temperature EEG Behavior Intracerebroventricular

RECENT studies from a number of laboratories suggest an interaction between opiates (and opioids) and nicotine on several behavioral and physiological processes. The narcotic antagonist, naloxone, reduces both the frequency and amount of tobacco smoking in chronic smokers [21] and decreases the intake of other positive reinforcers such as ethanol [2]. Mecamylamine (a centrally acting nicotinic antagonist) exacerbates the behavioral severity of early withdrawal from morphine in rats [43]. Opioids can reduce the number of functional nicotinic receptor sites on adrenal chromaffin cells and decrease nicotine-induced secretion of catecholamines [23]. Cross-tolerance to the analgesic effects of chronically administered morphine and nicotine in the mouse has been suggested [38]. These data suggest that there may be interactions between nicotine and endogenous opioid mechanisms. This study explores interactions between nicotine and enkephalinergic processes in the dog central nervous system. The actions of nicotine on the skin twitch reflex and autonomic function were characterized in the intact dog in the presence and absence of naltrexone. Studies were conducted to establish which of these actions of nicotine were of central origin. Further, the effect of a nicotine infusion on the release of methionine-enkephalin into lateral ventricle perfusates was studied. The unanesthetized dog was chosen because the pharmacology of the opioid analgesics have been extensively investigated in this species [34,35] and [2] methods have been developed for simultaneous measurement of several neurochemical, physiological and behavioral parameters. There are relatively few reports of the action of centrally or peripherally administered nicotine in the unanesthetized dog [15, 16, 24, 28, 29].

## METHOD

Female beagle type dogs weighing between 8.5 and 11.0 kg were used in studies of the effects of nicotine in the intact dog. Approximately 10 days prior to experimentation two 10 mm stainless steel guide cannulae (18 gauge, 10 mm in length) were placed in the brain stereotaxically (Anterior 15, Lateral 8) approximately 5 mm below the dura which allowed the painless introduction of perfusion cannulae into

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the right and left lateral ventricles of the unanesthetized dog. Before surgery and during the surgical recovery period, animals were acclimated to both the laboratory personnel and environment and trained to remain quietly in a sling support for two or more hours. Following conditioning, 60 minute experimental sessions were conducted at weekly intervals. Electrocardiogram (ECG), respiratory rate, and body temperature were recorded continuously and sampled every 5 minutes for analysis. The skin twitch reflex (a nociceptive response) was evoked and pupillary diameter measured at 5 minute intervals. Heart rate was obtained from electrocardiograms while respiration was measured using a chest bellows and a pressure transducer (Statham P 23BC). Respiratory and cardiac rates were quantified by counting the respective number of breaths and beats per minute displayed on strip chart recordings (Grass Polygraph Model 7D). Rectal temperature was monitored using a thermister probe and digital thermometer (Bailey, Model BAT 8). Autonomic, motor and behavioral signs were evaluated continuously by the presence or absence of lacrimation, salivation, rhinorrhea, vomiting, shivering and relaxation of the nictitating membrane and recorded on a check list. State of sleep or arousal was classified on the following scale: (1) sleep; eyes closed, (2) quiet; calm, eyes open, no body movements, (3) restless; repetitive movements of head, forelimbs or hindlimbs without vocalization. The skin twitch reflex was evoked by application of radiant heat focused upon a blackened 3 $cm^2$ area of depilated skin [17]. Its latency was the time interval between the onset of the light and the twitch. A stimulus cutoff of 10 seconds was used. Pupillary diameter (mm) was measured from Polaroid photographs of the eye [32].

In the first experiment each animal participated in three treatment conditions; (1) a 20 minute intravenous infusion of nicotine (40 $\mu$g/kg/min), (2) a 20 minute intravenous infusion of physiological saline at a volume and rate equal to that of nicotine and (3) no treatment. Each animal was concomitantly subjected to ventricular perfusion for 40 minutes.

A twenty minute infusion was selected since it was desirable to have a sustained nicotine effect during the cerebral perfusion. The selection of the dose and infusion was based on several theoretical and practical considerations. These included the short half life of nicotine, the obtaining of a marked but not overtly toxic response to nicotine and the attainment of nicotine levels which would approximate those achieved by the smoking of several high nicotine cigarettes. During the course of infusion the effects of nicotine increased and for some effects did not become maximal until after the end of the infusion. Initially 9 animals were employed using a $3 \times 3$ (dogs and treatments) Latin square crossover design. Since some animals died during the course of the experiment and were replaced, a balanced design was obtained in only six animals. For these six animals, data was analyzed using an analysis of variance (ANOVA) in which between dogs, weeks and treatments variance was segregated. Since neither between weeks variance nor between saline and no treatment condition variance were significant, a two-way analysis of variance (treatments and dogs) comparing the saline and nicotine treatment conditions was done for data from 8 or 9 animals for all parameters. Observations were grouped into three, 20 minute epochs. The first epoch consisted of 4 pre-drug observations which were averaged for each animal and each treatment. Nicotine or saline was administered only during the second (20 minute) epoch. Responses for each parameter obtained during the second and third epochs were expressed as a percent of the pre-drug means.

Prior to ventricular perfusion two 23 gauge stainless steel perfusion cannulae (fitted with 29 gauge stylets) were lowered into the lateral ventricles to a depth at which cerebrospinal fluid (CSF) flowed freely from both cannulae. The ventricles were perfused with artificial cerebrospinal fluid (ASF) warmed to 37°C [37] at a rate of 1.1 ml/min, using a Harvard Apparatus model 475 pump. The perfusate was administered into one lateral ventricle and collected from the contralateral ventricle under a slight vacuum. Separate perfusate samples were collected during the 20 min infusion epoch ($T_0$–$T_{20}$) and a 20 min post infusion epoch ($T_{20}$–$T_{40}$). Perfusion experiments were conducted between the hours of 1:00 and 5:00 p.m. The methionine-enkephalin (ME) RIA procedure used was a modification of methods previously described [7, 12, 39]. Antiserum was generated in rabbits from a ME-poly-L-lysine conjungate prepared according to a previously described procedure [48]. The cross reactivity of the antiserum was 1.05% with LE and 0.12% with $\beta$-endorphin. The antisera had a sensitivity greater than 0.1 pmol of ME per RIA tube, and was used in a final dilution of 120:1.

All intravenous infusions were administered through an indwelling hypodermic needle inserted into the radial vein prior to the control period. Anhydrous (−)nicotine base was prepared in sterile saline at a concentration of 1 mg/ml, just prior to administration.

In another experiment 8 dogs participated in 4 treatment conditions (1) a 20 minute intravenous infusion of nicotine (NIC) at a dose/rate of 40 $\mu$g/kg/min, (2) a 2 minute intravenous infusion of 2 mg/kg naltrexone (NAL), (3) a 2 minute intravenous infusion of physiological saline (SAL) at a volume and rate equivalent to that of the naltrexone injection and (4) a nicotine infusion (40 $\mu$g/kg/min for 20 minutes), 20 minutes after a 2 mg/kg dose of naltrexone (NN). The dose of naltrexone (2 mg/kg) selected was one which would occupy most of the $\mu$ and $\kappa$ receptors and which can be presumed to antagonize the physiologic effects of endogenous $\mu$ or $\kappa$ agonists. The animals participated in these 4 treatments at 7 to 10 day intervals according to a Latin square crossover design, but did not undergo concomitant ventricular perfusion. Control and post-treatment observations were calculated according to the method described above. The areas under the time action curve was calculated for each parameter according to the sampling intervals indicated in Table 2. Using an analysis of variance, the between sessions or between animals variance was not significant. Treatments (NAL, NIC, NN and SAL) were compared using a paired $t$-test. The NN-(NAL + NICO) comparison (Table 2) was made to determine if the treatment effects which were significantly greater than (i.e., potentiation) or less than (i.e., antagonism) would have been expected if the treatments were independently additive. Naltrexone HCl was prepared in sterile physiological saline at a concentration of 10 mg/ml.

In a third experiment dogs were stereotaxically implanted with third ventricle (lateral 1.0 mm, anterior 14.0, 24.0 mm below dura) and/or periaqueductal gray cannulae (lateral 1.5 mm, anterior 8 or 11 mm, 26 mm below dura). Beginning ten days after surgery each animal received 4 injections into the third ventricle at weekly intervals according to a crossover design: (1) nicotine 50 $\mu$g/10 $\mu$l/2 min, (2) nicotine 100 $\mu$g/10 $\mu$l/2 min, (3) nicotine 200 $\mu$g/10 $\mu$l/2 min and (4) 0.9% saline 10 $\mu$l/2 min. The 50 $\mu$g dose of nicotine did not alter any of the physiologic parameters studied and was therefore not included in statistical analyses. Doses of 100 and 200 $\mu$g are

within the range of those reported which produced pharmacologic effects in the conscious dog [24], cat [13] and monkey [14]. Nicotine (5, 10 and 25 μg) was administered into the periaqueductal gray at a rate of 1 μl/min over one minute through a 30 g injector cannula. Prior to sacrifice 1.0 μl of 1% bromophenol blue was injected into the PAG for histologic localization of the cannula. The pH of the saline solutions was adjusted to 9.2 with NaOH. Prior to third ventricular microinjection free flow of cerebrospinal fluid was obtained. Pre- and post-treatment observations were made according to methods described above. In this experiment cortical EEG was obtained from skull screws chronically implanted bilaterally over the parietal lobe. EEG electrogenesis was determined using a Drohocki integrator (Grass Model 7P1DC). The mean electrogenesis for the pretreatment condition (control) was obtained by averaging the electrogenesis of 20 consecutive one minute intervals prior to treatment. Following treatment, electrogenesis was measured for consecutive minute intervals and was expressed as percent mean electrogenesis. Since no significant difference between the effects of the two nicotine doses were found, areas under time action curves for both doses were pooled and compared with saline. The significance of saline-nicotine differences was assessed using a paired replicate analysis.

TABLE 1

EFFECTS OF NICOTINE (40 μg/kg/min) AND SALINE INFUSIONS ON RESPIRATORY AND CARDIAC RATES, SKIN TWITCH REFLEX LATENCY, PUPILLARY DIAMETER, RECTAL TEMPERATURE

| | | 2nd Epoch | | 3rd Epoch | |
|---|---|---|---|---|---|
| Parameters | N | Nicotine $T_0$–$T_{20}$ | Saline $T_0$–$T_{20}$ | Nicotine $T_{25}$–$T_{40}$ | Saline $T_{25}$–$T_{40}$ |
| Respiratory Rate | 9 | 517 ± 66* | 334 ± 39 | 293 ± 32 | 337 ± 42 |
| Heart Rate | 9 | 603 + 39† | 401 ± 10 | 485 ± 49 | 391 ± 8 |
| Skin Twitch Latency | 9 | 581 · 59* | 395 + 19 | 511 ± 39 | 430 ± 35 |
| Pupil Diameter | 8 | 325 ± 19 | 375 ± 12 | 302 ± 25* | 360 ± 15 |
| Temperature | 8 | 397 ± 1 | 399 ± 0 | 394 + 1† | 398 + 0 |

Areas under time action curves of nicotine and saline are compared during the second (treatment) and third (post-treatment) epochs. Statistically significant differences are indicated at the *$p<0.05$ and †$p<0.01$ levels. N = the number of experimental animals. Numerical values indicate mean areas (±S.E.M.).

## RESULTS

### *Effects of Nicotine Administered Intravenously*

The time course of the effects of intravenous nicotine in the intact dog on 5 physiological parameters are presented in Fig. 1. Data in Table 1 presents the comparisons between nicotine and saline effects on areas under the time-action curves for the second (nicotine and saline infusion) and third (post-treatment) epochs. No statistically significant differences in variance in areas under time-action curves were found for animals (between dogs), sessions (between weeks) or between the saline and the no treatment conditions for any parameter. As can be seen in Fig. 1 the nicotine infusion produced a rapid acceleration in respiratory and heart rates and increased in skin twitch latency. Pupillary constriction and hypothermia had a slower onset of action. The effects of nicotine infusion in comparison to a saline infusion were also determined in the naltrexone-nicotine interaction experiment. Nicotine infusion produced behavioral restlessness. Panting and hyperventilation generally preceded these signs. Two or more episodes of vomiting and retching occurred consistently in each animal 13 to 18 minutes after the start of the nicotine infusion and did not occur thereafter. Salivation, lacrimation and rhinorrhea usually preceded and followed emesis. Although the nictitating membrane relaxed when the animal appeared to sleep, it was retracted during the second and third epochs in the nicotine treated group.

### *Effects of Nicotine Administered into the Periaqueductal Gray and Third Cerebral Ventricle*

Nicotine, (5, 10 and 25 μg) was microinjected into the periaqueductal gray of five dogs and failed to alter any physiologic parameters studied. Dye studies showed that the tip of the chemotrode was in the periaqueductal gray of all dogs. Nicotine injected into the third ventricle produced a significant increase in respiratory and heart rate and in pupillary diameter and a significant decrease in EEG electrogenesis relative to saline treatment (Table 2). Nicotine evoked tachycardia, tachypnea and miosis were apparent within 5 minutes after injection and persisted for 20 to 30 minutes and prevented the increase in EEG electrogenesis seen in the saline treated dogs. None of the dogs slept or demonstrated any episodes of drowsiness or sleep spindles after nicotine. Most dogs were aroused and restless following nicotine administration. One or more episodes of vomiting and slight amounts of accompanying rhinorrhea and salivation were also seen in the subjects. Three of six dogs showed marked analgesia (200–300% above control) while the others showed none. Dogs which demonstrated analgesia were those in which nicotine produced marked changes in the other parameters. In addition, increaaes in skin twitch reflex latency occurred erratically over the time course of drug action.

### *Naltrexone-Nicotine Interaction in the Intact Dog*

Naltrexone produced significant increases in heart rate and skin twitch latency and decreases pupillary diameter (Table 3). It did not alter respiration rate or body temperature. When the dogs were pretreated with naltrexone, nicotine also significantly increased heart rate and skin twitch latency and decreased pupil diameter. As can be seen from Table 3 nicotine produced a greater increase in respiratory rate and lesser degree of miosis in the naltrexone treated dogs than is predicted by assuming that naltrexone and nicotine exert their effects independently. There was a nonsignificant trend for the effect of nicotine on heart rate to be less in the naltrexone treated dog. Animals receiving naltrexone prior to nicotine showed copious, watery salivation during the nicotine infusion. Salivation of this magnitude was never observed after either nicotine or naltrexone. Naltrexone pretreatment failed to alter the frequency of nicotine-induced emesis. Naltrexone did not produce vomiting.

### *Methionine-Enkephalin in Ventricular Perfusates*

The volume of perfusates collected over a twenty minute

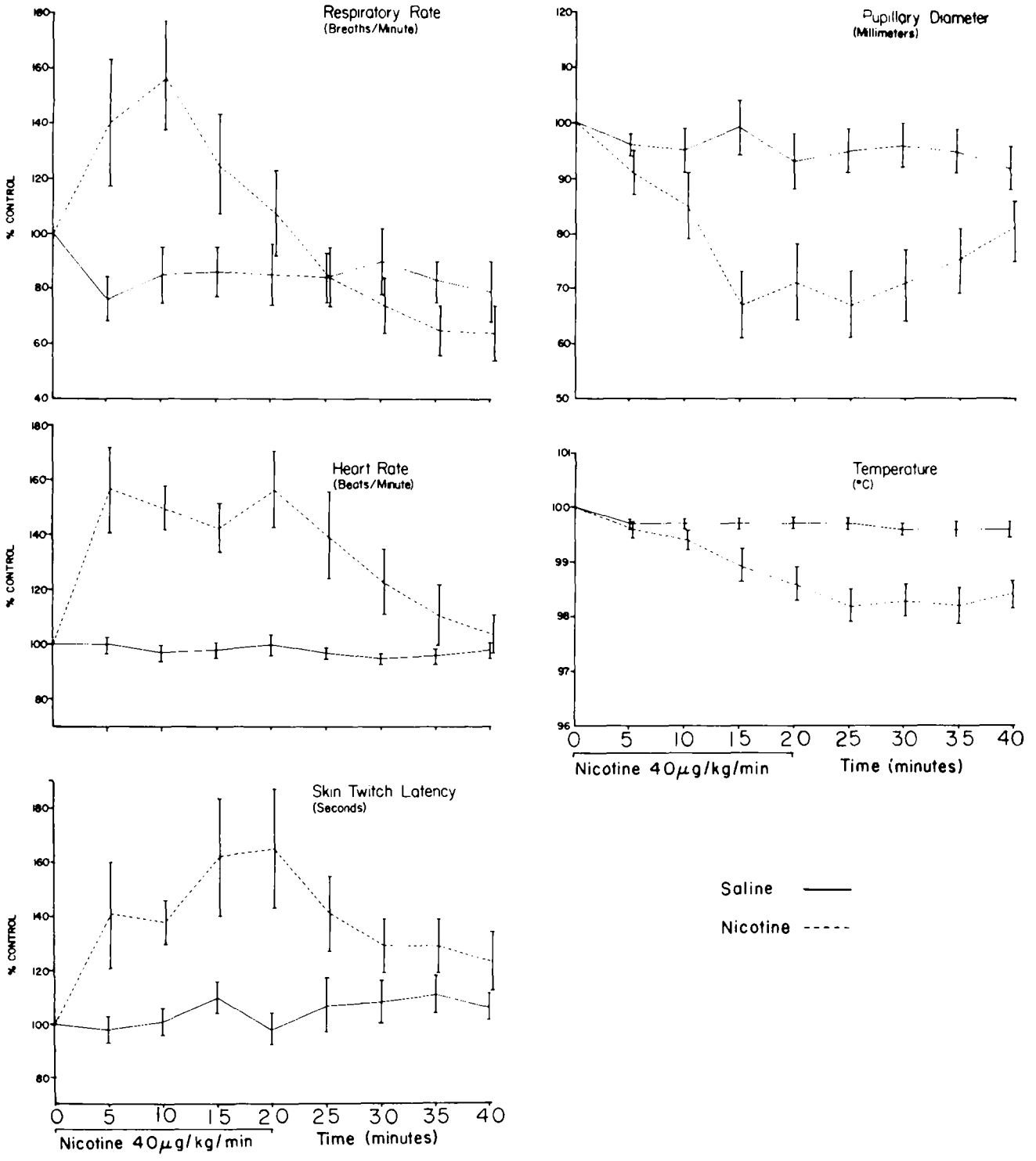


FIG. 1. The time course of the effects of a 20 minute infusion of nicotine (40 μg/kg/min) and saline on respiratory and cardiac rates, skin twitch reflex latency, pupillary diameter and rectal temperature. Each point represents the mean response (±S.E.M.) of 8 or 9 animals.

TABLE 2

EFFECTS OF NICOTINE ADMINISTERED INTO THE THIRD VENTRICLE ON RESPIRATORY AND CARDIAC RATES, SKIN TWITCH REFLEX LATENCY, PUPIL DIAMETER, RECTAL TEMPERATURE AND EEG ELECTROGENESIS

| Parameter | Sampling Interval | Nicotine minus Saline | $p$ Value | Level of Significance | Sample size (n) |
|---|---|---|---|---|---|
| Respiration Rate | $T_{12-22}$ | 434 ± 113 | 3.82 | <0.025 | 6 |
| Heart Rate | $T_{8-20}$ | 201 ± 42 | 4.78 | <0.005 | 6 |
| Skin Twitch Latency | $T_{10-20}$ | 109 ± 55 | 1.99 | <0.20 | 6 |
| Pupil Diameter | $T_{10-25}$ | 36 ± 11 | 3.18 | <0.025 | 6 |
| Temperature | $T_{2-32}$ | 39.5 ± 38.1 | 1.04 | <0.40 | 6 |
| EEG | $T_{8-24}$ | 244 ± 61 | 4.00 | <0.025 | 6 |

The sampling interval includes the time after injection during which nicotine produced significant changes in a given parameter. No significant differences between 100 μg and 200 μg doses of nicotine were found. Therefore, differences in areas under the time-action curve between saline and nicotine were averaged for both doses, for each animal. Mean differences (±SEM) are indicated in the nicotine minus saline columns. $p$ Values and levels of significance were determined using a paired $t$-test. The same 6 animals participated in all treatment conditions.

period ranged from 6 to 25 ml (Mean=15 ml) which is on the average 70% of the artificial spinal fluid administered. The concentration of methionine-enkephalin in the perfusate was not related to the volume collected as determined by both a regression analysis and calculating a correlation coefficient. The combined volume of the lateral and third ventricles of the dog is 1.6 to 2.4 ml [5]. These figures mean that the fluid in the ventricular system was changed 10 to 20 times during the 40 minute perfusion. There is no significant differences between the concentration of methionine-enkephalin in the perfusates collected during the two periods (Table 4). The data obtained in the Latin square crossover experiments were analyzed using a three-way analysis of variance. The variance attributable to treatments, animals or time epochs was not significant. The within animal variance was less than the between animals variance. Further, paired comparisons for time epochs were made. As can be seen from Table 4 there was no difference in concentration of ME between the

TABLE 3

EFFECTS OF NALTREXONE AND NICOTINE ALONE AND IN COMBINATION ON RESPIRATORY AND CARDIAC RATES, SKIN TWITCH REFLEX LATENCY, PUPILLARY DIAMETER AND RECTAL TEMPERATURE

| Parameter | N | Sampling Period | NAL | NICO | NN | NN-(NAL+NICO) |
|---|---|---|---|---|---|---|
| Respiratory Rate | 7 | $T_{10-20}$ | −13 ± 15 | 173 ± 82 | 394 ± 164 | 234 ± 90* |
| Heart Rate | 8 | $T_{5-20}$ | 67 ± 25* | 226 ± 42* | 195 ± 33* | −98 ± 67 |
| Skin Twitch Latency | 6 | $T_{5-20}$ | 108 ± 35* | 149 ± 45* | 248 ± 88* | 9 ± 71 |
| Pupil Diameter | 7 | $T_{5-20}$ | −54 ± 19* | −88 ± 16* | −53 ± 8 | 89 ± 27* |
| Temperature | 8 | $T_{5-20}$ | −6 ± 13 | 19 ± 7 | −16 ± 11 | 9 ± 18 |

Nicotine (40 μg/kg/min for 20 min IV) was administered alone (NICO) and 20 minutes after naltrexone (2 mg/kg IV) (NN). Naltrexone (2 mg/kg IV) was also administered alone (NAL). Sampling periods refer to the time after nicotine infusion began (NICO,NN). NN-(NAL + NICO) is the difference between the sum of the nicotine and naltrexone treatments alone and the nicotine-after-naltrexone treatment. Numerical values in the treatment columns are differences in areas under time action curves (±SEM) between each treatment and saline for the given sampling period.

TABLE 4

EFFECT OF TIME AND TREATMENT ON METHIONINE-ENKEPHALIN IN BRAIN PERFUSATE

| Treatment | Time Epoch $T_0$–$T_{20}$ | $T_{20}$–$T_{30}$ | Combined Mean |
|---|---|---|---|
| None | 0.46 ± 0.18 (6) | 0.45 ± 0.17 (6) | 0.45 ± 0.12 (12) |
| Saline | 0.85 ± 0.28 (6) | 0.94 ± 0.31 (6) | 0.90 ± 0.20 (12) |
| Nicotine | 0.60 ± 0.17 (6) | 0.56 ± 0.34 (6) | 0.58 ± 0.18 (12) |
| Combined Mean | 0.64 ± 0.12 (18) | 0.65 ± 0.16 (18) | |

Endogenous methionine-enkephalin was analyzed by RIA. Mean nanograms of methionine-enkephalin per ml of perfusate ±SEM are reported. The number of observations is shown in parentheses.

first and second periods. Although there was a trend for ME to be higher in the saline-treated dogs than in the untreated dogs this was not statistically significant. There also was a trend for nicotine treatment to decrease ME levels in comparison with saline treatment but this was not significant.

## DISCUSSION

Intravenous nicotine produces a syndrome in the conscious dog consisting of behavioral arousal, tachycardia, tachypnea, miosis, hypothermia, analgesia and emesis. The ability of nicotine to evoke some of these signs is well known while its ability to evoke others is somewhat controversial. The sites of action of nicotine are complex and involve effects upon autonomic ganglia, peripheral chemoreceptors, chromaffin tissue and the central nervous system, however the importance of the contribution of these different sites to the actions of nicotine in the intact animal is not completely understood.

The observations that nicotine produces tachycardia and tachypnea when administered intravenously and into the third ventricle confirm and extend other studies. Nicotine (20–60 μg) administered into the lateral cerebral ventricles of the unanesthetized dog caused tachycardia and an increase in blood pressure [24]. When administered into the fourth ventricle nicotine produced tachypnea in the anesthetized dog [41] and panting in the unanesthetized cat [47]. In low doses (1.0 μg) nicotine increased ventilation and blood pressure when applied to the ventral surface of the medulla of anesthetized cats. Doses greater than 2 μg produced a depressor response [9,11]. Wu and Martin (in preparation) have found that nicotine administered in the vicinity of the nucleus ambiguus slows heart rate and decreases blood pressure. The effect of nicotine on hypothalmic vasomotor and cardioaccelerator regions have not been studied. It is possible that nicotine administered into the third ventricle is producing its cardiovascular effects by acting on the hypothalamus. Further, nicotinic processes may exert diverse effects on the medulla. It is unlikely that the effects on respiration or pulse rate of intraventricularly administered nicotine are due to a peripheral action because when 100 and 200 μg were administered in a bolus intravenously they produced only transient gasping and a brief tachycardia (15 sec or less). Further Mandel *et al.* [29] found that doses of less than 200 μg of nicotine administered into the circulation of unanesthetized dog were without effect. Responses were most often observed at intravenous doses of around 25–50 μg/kg. Thus the central effects of nicotine contribute to its respiratory and cardiovascular effects.

Prolongation of the skin twitch reflex in the dog observed after intravenous nicotine has not been previously reported. The maximum degree of analgesia produced by the nicotine infusion was modest and roughly equivalent to 0.1 to 0.2 mg/kg of morphine [35]. Nicotine failed to produce consistent prolongation of the skin twitch latency when administered into the third ventricle of the dog. Some dogs however showed profound analgesia while others did not. These erratic results may reflect the variability in distribution or penetration of nicotine into caudal brain structures. Nicotines' ability to produce analgesia in rats has been studied and is controversial. Parenterally administered nicotine prolongs tail flick and hot plate latency in mice and rats [30, 38, 45], however little or no analgesia was reported using the flinch jump or tail pinch assays which are sensitive methods for assessing analgesia [20, 44, 45]. Intraventricular nicotine increases tail flick latency in the rat [45]. Mecamylamine but not hexamethonium blocks nicotine-induced analgesia in the mouse [38] and rat [45]. The periaqueductal gray is not involved in nicotines analgesic action in the dog since injection of nicotine into this site had no effect on skin twitch latency.

Intravenously administered nicotine produced miosis while intraventricularly administered nicotine produced mydriasis. The effects of nicotine on pupillary diameter of the dog have not apparently been studied systematically. Studies of the actions of nicotine on the isolated cat iris support the idea that some of its effects are at the ganglia [46]. Further, both sympathetic and parasympathetic fibers have some potential for producing both miosis and mydriasis in the dog [26]. Injection of nicotine into the third ventricle produces mydriasis which may be part of its more general activating effect and is probably due to an action on the central nervous system. Miosis observed during intravenous infusion probably is a consequence of the dominance of nicotines' effect on the ciliary ganglion, however, this speculation is far from established.

Nicotine produces hypothermia in the mouse when given subcutaneously [31] and intraventricularly [3] and in the febrile mouse, rabbit and dog [31]. Hall [13] found that nicotine (50–100 μg) given into the lateral ventricle of the unanesthetized cat produced hypothermia. When nicotine (50 to 800 μg) was injected into the lateral ventricle of the rhesus monkey an erratic hypothermia was produced, however when the ventricular system was perfused from the lateral to the fourth ventricle at a rate of 5 to 10 μg/min, a slow onset dose related hypothermic response was produced [14]. Although nicotine causes hypothermia in the dog its site of action has not been identified. The failure to see hypother-

mia following administration into the third ventricle may have been a consequence of the fact that it was injected into the caudal part of the third ventricle and did not gain access to the anterior hypothalamus.

A single bolus or short infusion of low doses of nicotine (around 5 to 20 μg/kg, IV) typically produces EEG desynchrony (cortical activation) a decrease in EEG amplitude and behavioral arousal in the dog [16,22] and in other species [1, 4, 6, 27, 42, 47, 50]. However, the effects of longer term infusions (e.g., 20 minutes) of higher doses (greater than 25 μg/kg) of nicotine on EEG parameters and behavior is variable. An intravenous dose of nicotine (4 μg/kg) administered every minute for 20 minutes produced both an increase and decrease in EEG activity in the cat [4]. Large doses of nicotine (250 to 500 μg) injected into the vertebral artery produce arousal followed by sleep in the unanesthetized dog [15]. Intraventricular nicotine (2–10 μg) produces a prostration-immobilization syndrome in the rat [1]. In our studies nicotine administered into the third ventricle did not alter EEG electrogenesis in the awake dog however, it prevented sleep and drowsiness as well as the associated increase in electrogenesis.

Although naltrexone has been characterized as a competitive opioid antagonist it produced tachycardia, analgesia, and miosis. The tachycardia observed after naltrexone is consistent with the hypothesis that endogenous opioids tonically modulated cardiovascular function and that the actions of these peptides can be antagonized by naloxone or naltrexone. Graded doses of naltrexone have been studied in the chronic spinal dog [33] and tended to increase pulse rate, prolong the skin twitch reflex latency but did not alter pupillary diameter. Wu and Martin (in press) have shown that naloxone (1 mg/kg) increases blood pressure as well as pulse and respiratory rate and minute volume in the unanesthetized acutely decerebrated dog. Laubie *et al.* [25] found that β-endorphin administered intracisternally produced a naloxone antagonizable bradycardia in the chloralose anesthetized dog. The effects of naloxone and naltrexone on pain and nociceptive reflexes and on pupillary diameter are both small and complex and are not readily reconciled by assuming that they are producing their effect by antagonizing an endogenous opioid ligand involved in the production of analgesia or by acting as a partial opioid agonist.

Nicotine produced significantly less miosis and a greater increase in respiratory rate after naltrexone than was predicted by adding the miotic or respiratory effects of nicotine and naltrexone. There was also a nonsignificant trend for naltrexone to antagonize nicotine induced tachycardia. Although it was not quantitated naltrexone markedly enhanced nicotine induced salivation. These data suggest that endogenous opioids enhance nicotines ability to produce miosis and antagonize its ability to evoke tachypnea and tachycardia and that there are interactions between the physiologic actions of endogenous opioid peptides and nicotinic processes. These interactions are complex however and seem to vary from one functional system to another. It is well known that opioid analgesics and opioid peptides will inhibit the release of acetylcholine from both peripheral tissues and the brain of several species and that this inhibitory effect can be antagonized by opioid antagonists [10, 19, 49]. Feedback mechanisms have been postulated for cardiovascular, respiratory and nociceptive reflexes. If endogenous opioid ligands are involved in feedback mechanisms regulating reflex pathways containing nicotinic synapses, the nature of the feedback process (positive or negative) will determine whether the interaction would be greater or less than additive. Further nicotinic and opioid processes could be serially related and be either facilitatory or inhibitory to each other. Wu and Martin (in preparation) have shown that the nucleus ambiguus is activated by both nicotinic and opioid processes which function independently and may be redundant parallel pathways.

Methionine-enkephalin was identified in lateral ventricle perfusates. It was estimated that the ventricular volume was replaced 10 to 20 times during the course of the experiment. Since the concentration of methionine-enkephalin did not change appreciably during the perfusion these results suggest that tonic release of methionine-enkephalin into the lateral ventricular system must have been significant. Nicotine produced a nonsignificant decrease in ventricular perfusate levels of methionine-enkephalin when compared to the appropriate control (saline). It is not possible to exclude a possible interaction between methionine-enkephalin and nicotinergic mechanisms. Although methionine-enkephalin as well as opioid and nicotinic binding sites have been identified in forebrain, it has not been possible to identify functions that are unequivocally mediated by forebrain structures which involve nicotinic processes. Failure to obtain a statistically significant change may indicate that nicotine may both increase and decrease enkephalinergic processes, as the naltrexone data suggests, with little change in the net release.

In conclusion, nicotine evokes a reproducible syndrome in the intact unanesthetized dog involving several functional systems. The actions of nicotine on the central nervous system contributes to the manifestation of the signs of the syndrome. Many of the effects produced by nicotine may reflect its actions on the hypothalamus, mesencephalon and medulla. The data further suggests that there are interactions between enkephalinergic or endorphinergic and nicotinic mechanisms in the dog and that these interactions may differ from one functional system to another. Finally methionine-enkephalin has been identified in the dog ventricular fluid perfusate and seems to be continuously released. The data on the influence of nicotine on the release of methionine-enkephalin into the ventricular fluid is ambiguous.

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