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Mechanisms Involved in the Respiratory Depressant Actions of Nicotine in Anesthetized Rats

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SLOAN, J. W., W. R. MARTIN AND M. BOSTWICK. *Mechanisms involved in the respiratory depressant actions of nicotine in anesthetized rats*. PHARMACOL BIOCHEM BEHAV **34**(3) 559–564, 1989.—In the urethane-pentobarbital anesthetized rat, the respiratory depressant and lethal effects of intravenously infused (−)-nicotine (120 μg/kg/min) or (+)-nicotine (600 μg/kg/min) were effectively prevented by pretreatment with the opioid antagonist, naltrexone, whereas the lethal effect of (−)-nicotine (120 μg/kg/min) was not altered by bilateral adrenalectomy. Further, pretreatment with either the nicotinic ganglion-blocker, mecamylamine, a secondary amine, or the quarternary nicotinic ganglion-blocker, hexamethonium, completely prevented the lethal effects of (−)-nicotine (120 μg/kg/min). These data suggest that central opioidergic and nicotinic processes are involved in nicotine's respiratory depressant and lethal effects.

Nicotine Opioidergic and nicotinic processes: nicotine lethality Nicotine's respiratory depressant actions in the rat
Naltrexone prevention of nicotine lethality Hexamethonium prevention of nicotine lethality
Mecamylamine prevention of nicotine lethality Adrenalectomy and nicotine lethality

BOTH (−)- and (+)-nicotine are known to produce death through respiratory depression (26, 30, 31, 44, 58). Other nicotinic ligands such as cytisine and lobeline also cause death (6,36) due to respiratory depression (13,18). Studies have shown that the respiratory responses to the application of nicotine to the ventrolateral surface of the medulla, its injection intravenously or into the lateral ventricles of the cat, can be prevented by the application of hexamethonium to the ventrolateral medullary surface (16,28). Numerous studies have also demonstrated that nicotinic antagonists such as mecamylamine, which act both centrally and peripherally, alter patterns of nicotine intake in both humans and animals (33).

Since the isolation of endogenous opioid peptides in the brain (32), high concentrations of opiate receptors as well as enkephalins have been found in the solitary nuclei and other medullary areas that affect respiration (4, 5, 8). One of the consequences of acute intoxication by exogenous opioids in man is respiratory depression, a condition which is now routinely treated with narcotic antagonists (43, 46, 47). Endogenous opioids also produce naloxone antagonizable respiratory depression when applied to the ventral surface of the brainstem; when injected into the lateral ventricles and into the pontomedullary subarachnoid space; depression of spontaneous discharge; and depression of L-glutamate induced firing of respiration-related units in the pontine and bulbar respiratory centers (14, 20, 22, 49, 51, 74). Further, it has recently been shown that nicotine can increase endogenous opioids centrally. After the repeated short-term administration of nicotine (0.1 mg/kg/IP six times at 30-minute intervals) increased levels of methionine enkephalin are found in the rat striatum (52). A number of studies have suggested that opioid mechanisms are involved in the pharmacologic effects of nicotine (12, 34, 53, 54). The purpose of this paper is to explore nicotinic and opioidergic mechanisms in nicotine's respiratory depressant actions in the urethane-pentobarbital-anesthetized rat.

METHOD

Experimental Techniques

These studies were conducted in the urethane (1 g/kg, IP)-pentobarbital (20 mg/kg/IP) anesthetized female Sprague-Dawley rat (200–300 g). Respiration (inspiratory flow rate) was measured via a tracheal cannula using a Rudolph valve and Gould pneumograph flow transducer. During the course of the experiment, body temperature was maintained at 37 ± 1°C by a heat lamp (actuated by a YSI Model 63RC temperature controller connected to the rat by a rectal thermister probe). Minute volume was obtained using a Grass 7P10E polygraph integrator. Tidal volume was calculated from the minute volume and respiratory rate. Respiratory rate and minute volume were recorded continuously on a polygraph (Grass,

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Model 7D). These measures were also recorded on tape and subsequently digitized (Apple IIe Micro Computer equipped with an ISAAC Data Acquisition and Lab Control Module) for computer analysis.

Drugs were dissolved in normal saline and administered as the free base. (−)- and (+)-nicotine were administered via a cannula inserted into the right external jugular vein at a rate of 0.025 ml/minute for 16 minutes following a 10-minute predrug and a 10-minute saline or drug pretreatment period. The doses of (−)-nicotine (120 μg/kg/min) and (+)-nicotine (600 μg/kg/min) were selected on the basis of dose ranging. These doses were chosen because they reproducibly depressed respiration and caused death in the urethane-pentobarbital anesthetized rat. Doses of hexamethonium, mecamylamine and naltrexone were also chosen by dose ranging. Doses were selected which reproducibly prevented respiratory depression and death in the (−)-nicotine- (120 μg/kg/min) and (+)-nicotine- (600 μg/kg/min) infused rat. Saline, naltrexone, hexamethonium and mecamylamine were administered as an IV bolus over a 1-min period in a volume of approximately 0.16 ml. Residual drug or saline was flushed into the vein with approximately 0.2 ml of saline and observations were made for 10 minutes prior to the administration of nicotine.

Data Analysis

The data are presented as the change in response from the appropriate control. The mean change in response for each drug treatment for the different parameters (±S.E.M.) was estimated for each minute and time-response curves were constructed. The data were further analyzed by appropriate unpaired comparisons and by Chi-Square analysis.

Drugs and Chemicals

(+)-Nicotine was resolved by Dr. Amy Howell and Dr. W. T. Smith of the University of Kentucky, Department of Chemistry (60). (−)-Nicotine was obtained from Research Plus (Bayone, NJ); hexamethonium bromide, Sigma Chemical Co. (St. Louis, MO); mecamylamine hydrochloride, Dr. Clement Stone, Merck Sharp and Dohme (West Point, PA). Sources of other drugs and chemicals have been identified previously (59).

RESULTS

Figure 1 shows that in the urethane-pentobarbital anesthetized rat an IV injection of normal saline had no effect on respiratory measures, whereas naltrexone enhanced tidal volume, but did not alter respiratory rate or minute volume relative to the pretreatment control. Hexamethonium tended to increase all respiratory measures, but these changes were not statistically significant. In contrast, mecamylamine produced a significant enhancement of respiratory rate and minute volume.

The continuous IV infusion of either (−)-nicotine (120 μg/kg/minute) or (+)-nicotine (600 μg/kg/minute) depressed respiratory rate and produced respiratory arrest in 4 of 5 rats. (−)-Nicotine differed from (+)-nicotine in that severe respiratory depression occurred faster and 4 of 5 rats were dead within 4 minutes. Although (+)-nicotine produced some depression of respiratory rate within the first 10 minutes, both minute volume and tidal volume were enhanced during this period. After 10 minutes of infusion, however, the respiratory depressant actions of (+)-nicotine predominated and 4 of 5 rats were dead at 16 minutes (Fig. 2). When rats were pretreated with naltrexone the survival rate was significantly increased after both (−)-nicotine (5 of 6 rats survived, $\chi^2=4.41$, $p>0.05$) and after (+)-nicotine (5 of 5 rats survived, $\chi^2=6.67$, $p<0.05$). In this regard, it can be seen that

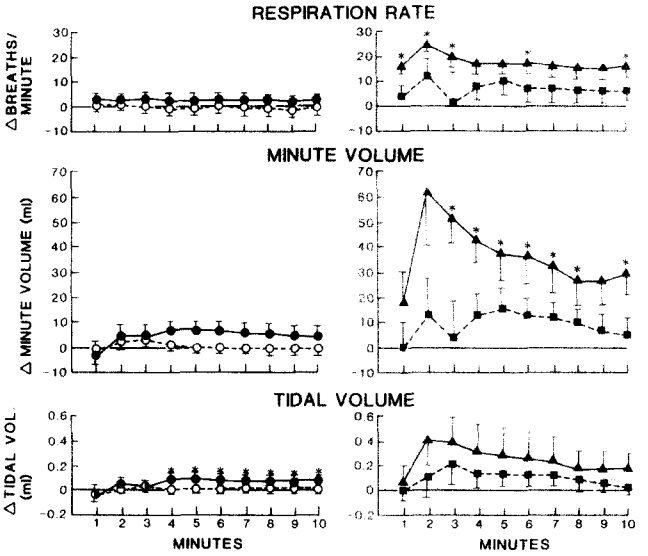

FIG. 1. The effects of naltrexone, saline, mecamylamine and hexamethonium on respiratory rate, tidal volume and minute volume in the urethane-pentobarbital anesthetized rat. Drugs (or saline) were given as an IV bolus in a volume of 1.6 ml of normal saline and flushed into the vein with 0.2 ml of normal saline. Values represent the mean change (±S.E.) from the pretreatment control for the number of animals (shown below) for each minute during the 10-minute observation period. ○: Saline (bolus IV) (n=4); ●: naltrexone (5 mg/kg, bolus IV) (n=6); ■: hexamethonium (1 mg/kg, bolus IV) (n=5); ▲: mecamylamine (1 mg/kg, bolus IV) (n=5). *Significant change from pretreatment control, $p<0.05$.

after naltrexone pretreatment a greater enhancement of minute volume and tidal volume was produced in the presence of (+)- and (−)-nicotine than in their absence (Figs. 1 and 2). Further, this stimulation of respiration in the presence of naltrexone was greater after (−)-nicotine infusion than after (+)-nicotine infusion. Bilateral adrenalectomy did not alter the lethality of (−)-nicotine infusion (120 μg/kg/min). Three of 6 rats survived compared to sham-operated rats where 2 of 4 rats survived (−)-nicotine infusion ($\chi^2=0$).

Respiratory rate and minute volume were significantly enhanced following the infusion of (−)-nicotine (120 μg/kg/min) in both the mecamylamine- and hexamethonium-pretreated rats compared to saline pretreatment and the lethal effects of (−)-nicotine were completely abolished (Fig. 3). It should be pointed out, however, that the changes induced by mecamylamine and hexamethonium after (−)-nicotine were different. Whereas the marked stimulation of respiration induced by mecamylamine was blunted by (−)-nicotine infusion, tidal volume and minute volume were enhanced by (−)-nicotine in the hexamethonium-pretreated rats. This was similar to the effects produced by (−)-nicotine infusion after naltrexone pretreatment (Figs. 1 and 3).

DISCUSSION

Although nicotine can stimulate respiration in man (67) and laboratory animals (1, 15, 34, 57), it can also depress respiration (17, 23, 24, 57, 65, 68). These studies present evidence which suggest that nicotine depresses respiration through both nicotinic and opioidergic mechanisms which have a major central component. As previously reported, the urethane-pentobarbital anesthetized rat shows little evidence of the ganglion-stimulant actions of nicotine, while the depressant effects predominate (59,61).

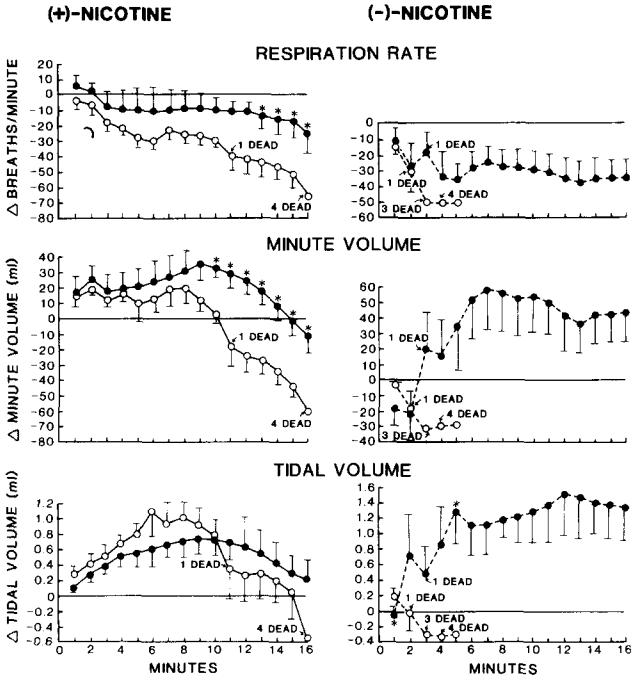

FIG. 2. Comparison of the effects of the continuous IV infusion of (+)-nicotine (600 μg/kg/min, solid lines) and (−)-nicotine (120 μg/kg/min, dashed lines) on respiratory measures and lethality in saline- and naltrexone-pretreated rats. Each point represents the mean change (±S.E.) from the appropriate pretreatment condition for each minute for the number of rats indicated below in parentheses. ○-○: Saline pretreatment (n=5); ●-●: naltrexone pretreatment (5 mg/kg IV) (n=5); ○--○: saline pretreatment (n=5); ●--●: naltrexone pretreatment (5 mg/kg IV) (n=6). *Different from saline pretreatment, $p<0.05$.

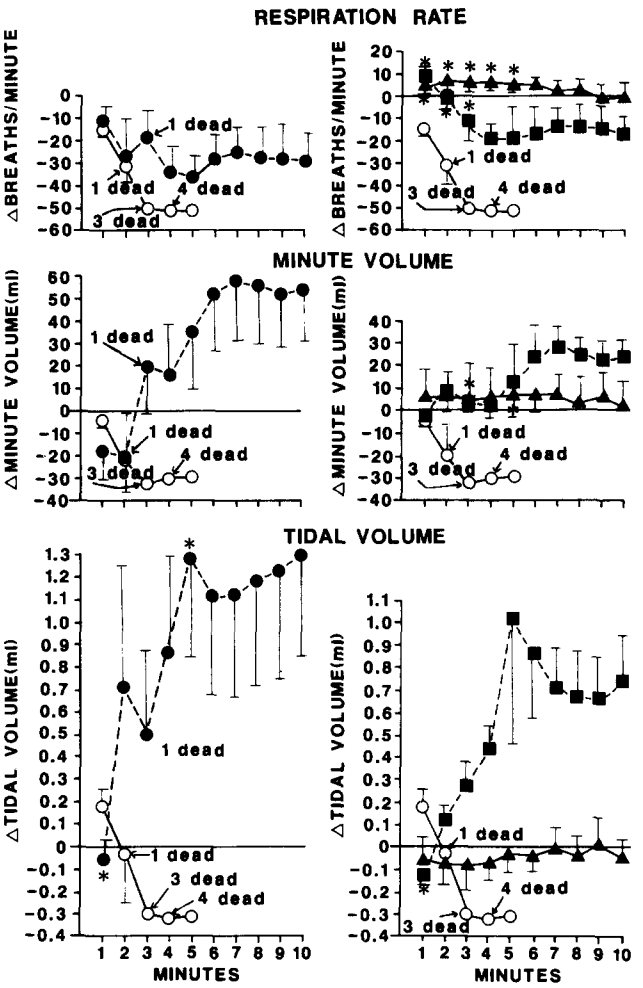

FIG. 3. Comparison of the effects of the continuous IV infusion of (−)-nicotine (120 μg/kg/minute) on lethality and respiratory measures in the saline-, naltrexone-, hexamethonium- and mecamylamine-pretreated anesthetized rat. Each point represents the mean change (±S.E.) from the appropriate pretreatment condition for each minute for the number of experiments indicated. ○: Saline pretreatment (n=5); ●: naltrexone pretreatment (5 mg/kg) (n=6); ■: hexamethonium preatment (1 mg/kg) (n=5); ▲: mecamylamine pretreatment (1 mg/kg) (n=5). *Different from saline preatment, $p<0.05$.

The effects of naltrexone and other opioid antagonists on respiration are complex and may be the consequence of several modes of action. It has generally been reported that both naltrexone and naloxone are devoid of significant effects on respiration in healthy men and animals, but have been shown to play a role in altering respiration brought about by several pathologic conditions, presumably by antagonizing endogenous opioids (27, 43, 47). In this regard, naltrexone has been shown to partially reverse the depressed level of consciousness and the depressed flexor reflex response, but not respiration in the pentobarbital anesthetized chronic spinal dog. These data were interpreted as suggesting that some of naltrexone's actions are mediated through κ receptors where the effects of pentobarbital are antagonized directly, or, alternatively, indirectly by reversing the effects of pentobarbital-released endogenous substances (25). In the present experiments, naltrexone alone produced a slight enhancement of respiratory measures in the urethane-pentobarbital anesthetized rat. The ability of naltrexone to stimulate respiration may be related to the level of endogenous opioid tone. In the present acute studies endogenous opiates may have been released in response to the surgical procedures, whereas in the chronic spinal dog studies (25) the animals had recovered from surgery.

Naltrexone pretreatment prevented the lethal effects of both (−)- and (+)-nicotine suggesting the involvement of opioidergic processes in nicotine's respiratory depressant effects. These data are consistent with evidence obtained in man which have demonstrated that naloxone pretreatment reversed postsmoking respiratory depression (67). It has been shown that nicotine can release opioid peptides from the perfused dog and bovine adrenal gland and from adrenal medulla chromaffin cells in primary culture (19, 35, 70, 72). If the release of opioid peptides from the adrenal gland by nicotine is responsible for its respiratory depressant actions, then adrenalectomy should abolish this effect. The adrenal gland does not appear to be the essential component involved in nicotine's respiratory depressant effects since bilateral adrenalectomy did not alter the lethality of nicotine in the anesthetized rat. This observation argues against a peripheral opioidergic site for nicotine's respiratory depressant effects. Endogenous opioids and opioid receptors are found in the medulla of the rat (4) as are nicotine binding sites (38,45) in areas closely associated with central respiratory control in the rat (29). Moreover, it has been demonstrated that after intracisternal administration in dogs, beta-endorphin induced a naloxone-reversible respiratory depressant action (49). Met-enkephalin applied to the ventral surface of the cat brainstem depressed both tidal volume and respiratory rate (21). Thus, there is substantive data indicating the existence of a medullary opioid respiratory depressant mechanism.

Both mecamylamine and hexamethonium tended to enhance respiration, although this was a statistically significant effect for mecamylamine only. Hexamethonium, however, has been reported previously to produce an initial increase in tidal volume when applied to the chemoreceptive zones of the medulla oblongata of the chloralose-urethane anesthetized cat (16). The mechanism by which mecamylamine and hexamethonium enhance respiration is unknown. One possibility with regard to mecamylamine is that this effect may be related to mecamylamine's ability to stimulate presynaptic nicotine receptors to release endogenous substances that in turn stimulate respiration (40). Another hypothesis is that both mecamylamine and hexamethonium are antagonizing endogenous nicotinic tone. Mecamylamine is a secondary amine that penetrates the blood-brain barrier (7, 9, 48) and acts both pre- and postsynaptically (56). It has been shown to have selectivity in antagonizing both the peripheral and central effects of nicotine (10).

Hexamethonium is a quarternary ammonium ganglion-blocking agent that acts only at postsynaptic nicotinic receptors (10) and is generally thought to penetrate the blood-brain barrier poorly and, thus, its blocking actions have been used to distinguish the peripheral from the central actions of nicotine (11, 50, 62, 63). Other investigators, however, have found that hexamethonium does enter the brain in pharmacologically significant amounts (3,41). Further, many of the central effects of systemically administered nicotine such as antinociception in the rat (69); convulsions in the rat and mouse (39, 64, 73); emesis in the cat (37); the prostration immobilization syndrome induced by centrally administered nicotine in the rat (56); the ventilatory response to nicotine microinjected into the third ventricle of the cat (42); the response to its intravenous administration in the dog, cat and rabbit (17); and the discrimination of intracerebroventricularly administered nicotine in the rat (55) can be prevented by either systemically or centrally administered hexamethonium.

Although hexamethonium and mecamylamine differed in their effects on nicotine-induced respiratory depression in that hexamethonium-treated rats showed more nicotine-induced respiratory stimulation, both drugs completely prevented the lethal effects of (−)-nicotine in the urethane-pentobarbital anesthetized rat. The respiratory stimulant actions of mecamylamine, hexamethonium and naltrexone may be due to their respective abilities to antagonize endogenous nicotinic cholinergic and opioidergic tone in the respiratory centers. In this regard, mecamylamine was more effective than hexamethonium and naltrexone. In the presence of either hexamethonium, mecamylamine or naltrexone, (−)-nicotine produced an increase in tidal volume and minute volume. This stimulatory effect of (−)-nicotine was greater in hexamethonium- and naltrexone-pretreated rats than in mecamylamine-pretreated rats. These observations can be reconciled with the hypothesis that these drugs are blocking endogenous and exogenous nicotinic- and opioidergic-mediated respiratory depression. Further, the data are entirely consistent with the idea that this blockade is mediated by competitive antagonism of endogenous and exogenous agonists. The studies, however, were not designed to address this issue.

These data taken together suggest that central sites are involved in nicotine's lethal effects and support the concept that central opioidergic and nicotinic processes probably act in concert to modulate nicotine's effects on respiration. Further studies are necessary, however, to locate the possible sites where this modulation occurs.

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