Psychopharmacology (1982) 77:317–321

Psychopharmacology

Behavioral Effects of Intraventricularly Administered (–)-Nicotine on Fixed Ratio Schedules of Food Presentation in Rats

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Abstract. The behavioral effects of intraventricularly (IVT) administered (–)-nicotine on food-maintained behavior were studied. Rats responded by pressing a lever under various fixed ratio (FR) schedules. Infusion of 5 µg of (–)-nicotine suppressed responding under an FR 16 schedule for 11–13 min. The effect was inversely related to the ratio size (16, 32, 64 responses per food delivery), but it was directly related to the infused (–)-nicotine dose (0.312, 0.624, 1.25, 2.5, 5.0, 10.0 µg) when ratio size was held constant. Response rates following the (–)-nicotine-induced suppression were similar to those obtained prior to infusion. The behavioral effects of (–)-nicotine were blocked, in a dose-related manner, by the centrally acting nicotinic-cholinergic antagonist, mecamylamine (0.05–3.0 mg/kg) but not by the peripherally acting antagonist, hexamethonium (0.5–3.0 mg/kg), suggesting that the behavioral effects of IVT infusions of (–)-nicotine are mediated by central nicotinic-cholinergic receptors.

Key words: (–)-Nicotine – FR Schedules – Mecamylamine – Hexamethonium – Nicotinic-cholinergic – Intraventricular

Nicotine is one of the most widely used compounds, but basic research on its mode of action in the brain and on its effects on animal behavior has lagged far behind other research on commonly used substances. Most previous studies have investigated the behavioral effects of systemically administered nicotine in rats or monkeys. In rats nicotine increases responding maintained under fixed-interval (FI), variable-interval, and differential-reinforcement of low rate schedules of food or water presentation and under schedules of electric shock postponement (Bovet and Bovet-Nitti 1965; Morrison and Stephenson 1969; Pradhan 1970; Pradhan and Dutta 1970; Ando 1975) and decreases responding under fixed-ratio (FR) schedules of food or water presentation (Morrison and Stephenson 1969; Pradhan 1970). Qualitatively similar results on responding have been reported in squirrel monkeys maintained under a multiple FI-FR schedule of either presentation of food or termination of a stimulus associated with electric shock (Davis et al. 1973; Spealman et al. 1981).

There are no reports, to our knowledge, of the effects of intraventricular (IVT) administration of nicotine on schedule-controlled behavior. Intraventricular administration is a means of studying nicotine with the relative absence of peripheral effects. Abood and co-workers (1978, 1979) reported that an IVT infusion of nicotine (2–10 µg) into the lateral ventricle resulted in a prostration-immobilization syndrome in rats. This was also found by C. Levy (personal communication). This prostration syndrome was prevented or antagonized by IVT pretreatment with *N'*-benzyl-nornicotine and some piperidine derivatives, but not by a variety of neurotransmitters or psychotropic agents.

The main purpose of the present study was to establish a more detailed profile of the behavioral effects of IVT administration of (–)-nicotine. The second purpose of the study was to examine the effects of two nicotinic-cholinergic blocking agents, mecamylamine and hexamethonium on the bahavioral changes induced by the IVT infusion of (–)-nicotine.

Experiment 1

Fixed Ratio Schedules of Food Presentation

The effects of IVT infusion of (–)-nicotine were tested on behavior maintained under FR schedules of food presentation.

Materials and Methods

Eight experimentally naive male albino rats (Holtzman Co., Madison, Wisconsin), between 90 and 120 days old and weighing between 190 and 230 g were used. Animals were housed individually and were allowed food continuously for 3 weeks, during which time weights were recorded daily. The mean weights were calculated from the last 5 days of the 3-week period, after which the rats were reduced to 80% of their free-feeding weights. These weights were periodically adjusted to control for growth rate.

Rats were anesthetized with ketamine (70 mg/kg/IM) and sodium pentobarbital (18 mg/kg/IP) and a stainless steel cannula (No. 220 DK rat cannula, David Kopt Co.) was stereotaxically inserted into the left lateral ventricle (posterior = 1.1. mm from bregma, lateral = 1.7 mm, vertical = 5.1 mm from the skull surface). The cannula was attached to the skull by acrylic cement and three small set screws.

At the conclusion of all the experiments animals were sacrificed, perfused intracardially with 0.9% saline, 10% formalin and then infused intraventricularly with 2 µl of Evans Blue Dye. Brains were removed and sliced for verification of cannula placement.

Four identical operant conditioning chambers (Lehigh Valley Electronics No. 143-25), each contained in a sound-

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0033-3158/82/0077/0317/$01.00

attenuated cubicle (LVE No. 132-02), were used. Located at one end of the chamber were two levers (LVS No. 121-05), a pellet receptacle, six cue lights (lever lights), a speaker, and a house light.

With each operation of the pellet dispenser, a single 45-mg Bio Serve food pellet was delivered to the receptacle. White noise was constantly present and an exhaust fan provided ventinlation.

Procedure. Each rat was trained to lever press under an FR 1 schedule for a single delivery of food. Over a 2-week period the ratio size was increased to 16. Daily sessions (Monday–Friday) consisted of two successive 15-min periods with a 5-min time out (TO) after the first 15-min period. During the TO the rats were placed in a holding cage. When response rates during the two 15-min periods were stable (less than 10% variance in daily response rate for both 15-min periods over five sessions) IVT infusions were begun. All infusions were given during the last minute of the TO and the rats were immediately placed back in the operant chamber. Data from repetitive 15-min sessions were collected until the response rates were within preinfusion levels. All infusions were separated by 3–5 days of stable response rates. Under FR 16 the rats were infused as follows: 1) 5 µl of 0.9% saline, 2) 5 µg of (–)-nicotine in 5 µl of 0.9% saline. Following this the ratio size was increased to 32 and after stabilization of response rates the rats were infused with: 1) 5 µg of (–)-nicotine in 5 µl of 0.9% saline, 2) 5 µl of 0.9% saline and 3) 5 µg of (–)-nicotine in 5 µl of saline.

Subsequently, lever pressing was maintained under FR 64 and the rats were infused with 5 µg of (–)-nicotine in 5 µl of 0.9% saline.

Infusion Procedure. The infusion cannula was attached by polyethylene tubing to a 10 µl Hamilton syringe. The tubing and cannula were flushed with 95% ethanol prior to being filled with (–)-nicotine. A microliter syringe was filled with 95% ethanol and was attached to the tubing by an 18-gauge needle. All infusions were given in a volume of 5 µl. Rats were restrained by wrapping them in a cloth towel, leaving their heads exposed. The stylus was removed from the cannula and the infusion cannula inserted. Solutions were infused in less than 1.0 s. Following the infusion the stylus was replaced and the rats were immediately placed into the operant chambers.

Results and Discussion

During non-infusion and saline control sessions, characteristic FR response patterns occurred. That is, a brief pause was followed by an abrupt transition to a high rate of responding that was maintained until the ratio was completed (top panel Fig. 1). Response rate varied directly as a function of ratio size (mean ± SE responses per s under FR 16, 1.78 ± 0.11; FR 32, 2.72 ± 0.23; FR 64, 3.23 ± 0.67). The latency to complete the first ratio following an IVT saline infusion under FR 16 and 32 was less than 30 s; however, IVT infusions of (–)-nicotine (5 µg/5 µl) increased the latency to complete the first ratio (Fig. 2). The effect of IVT infusions of nicotine on the latency depended primarily on the FR size, which resulted in different response rates. The two nicotine infusions under FR 32 (7-day interinfusion interval) did not differ significantly from each other ($df = 5$ $t = 0.64$ $P > 0.1$) in the latency, suggesting that the decrease in latency with increasing FR sizes was not due to repetitive testing.

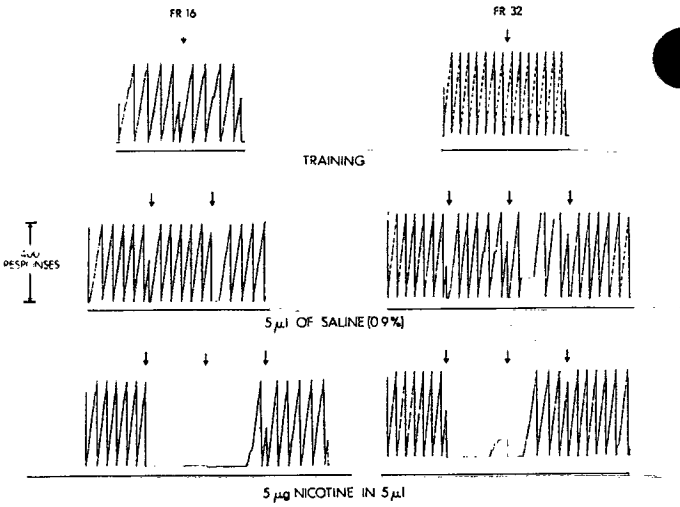

Fig. 1. Cumulative records for a single rat maintained under FR 16 and 32. The stepping pen recorded lever presses, and each downward deflection of the stepping pen indicated a pellet delivery. The stepping pen reset automatically after 400 responses. Arrows at the top of each record indicate the end of a 15-min period. Note the difference in latency to complete the first ratio between FR 16 and 32 following a nicotine infusion

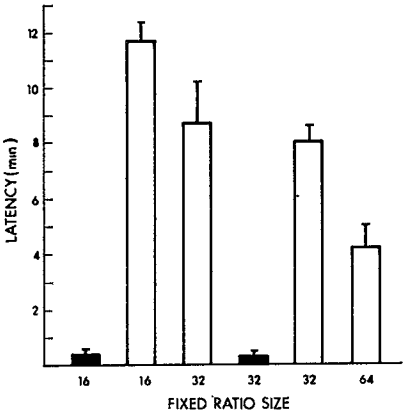

Fig. 2. Effects of saline and nicotine (5 µg) infusions on the average latency to complete the first ratio under FR 16, 32 and 64 schedules of food presentation. Each bar represents the average latency ($N = 8$) and vertical lines show the standard error. Solid bars show saline infusions and open bars nicotine infusions

Figure 1 contains cumulative records that show the pattern of responding under FR 16 and 32, and the time course of the nicotine-induced response suppression for a single rat. All rats showed similar patterns. Characteristic responding can be seen under both ratios during baseline and saline control conditions. Note the longer latency to the first completed ratio under the FR 16 schedule.

Experiment 2

Behavior Maintained Under an FR 32 Schedule of Food Presentation

In this experiment the ratio was held constant and various doses of (–)-nicotine were administered.

Eight experimentally naive albino rats were maintained under the same conditions and tested in the same apparatus as described in Experiment 1.

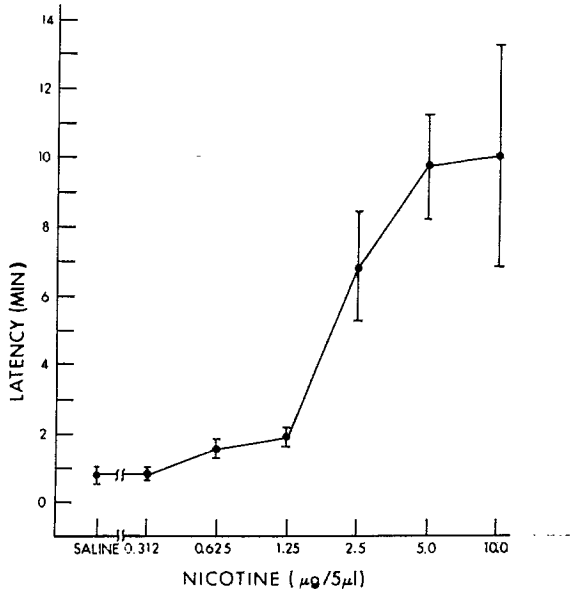

Fig. 3. Latency to complete the first ratio as a function of nicotine dose. Each point from 0.312 to 2.5 μg is a mean of eight animals. At doses of 5.0 and 10.0 μg the points are means of five animals. Brackets indicate the standard error

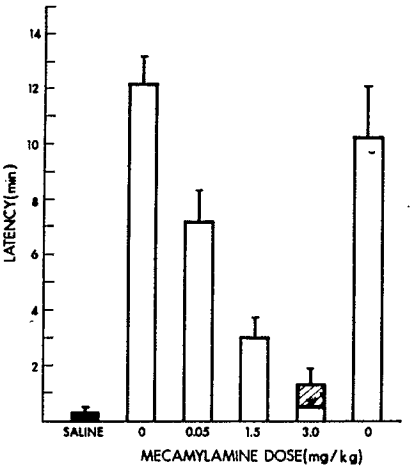

Fig. 4. Antagonism by mecamylamine of the effects of nicotine on the latency to complete the first ratio under FR 32 schedule of food presentation. Solid bars show the mean ($N = 5$) latency following a saline infusion with and without mecamylamine pretreatment. Open bars show nicotine infusions. Vertical lines show the standard error. The hatched bar shows the mean latency with aberrant animal included

Procedures. The rats were trained to lover press for a 45 mg food pellet under an FR 32 schedule. When response rates were stable (less than 10% variance in daily rate for both 15-min periods over five sessions) IVT infusions were begun. All infusions were given during the 5-min TO period and separated by 7 days. Rats were tested with nicotine doses as follows: 2.5, 1.25, 0.625, 0.312, 5.0, and 10 μg of (–)-nicotine in a constant volume of 5 μl of saline.

Results and Discussion

Increases in nicotine dose led to increases in the latency to complete the first ratio (Fig. 3). At the lowest dose tested response latencies were not significantly different from saline-

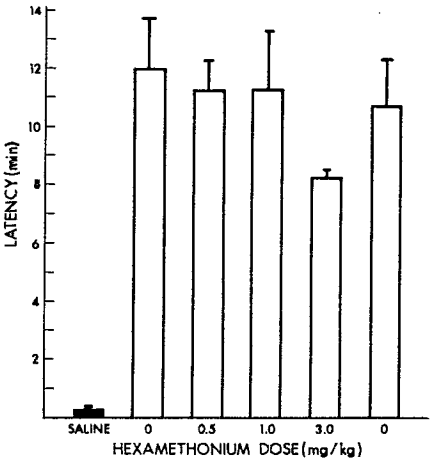

Fig. 5. Antagonism by hexamethonium of effects of nicotine on the latency to complete the first ratio under FR 32 schedule of food presentation. Solid bars show the mean ($N = 5$) latency following a saline infusion with and without hexamethonium pretreatment. Open bars show nicotine infusions. Vertical lines show the standard error

infusion values. At the next two doses (0.625 and 1.25 μg) the response latency increased to above saline-infusion levels. When the 2.5 μg dose was infused, the mean latency increased to 6.8 min (± 2.2). The two highest doses (5.0 and 10.0 μg) produced the longest latencies. Three animals were not tested at these doses due to blockage in the cannulae.

Experiment 3

Nicotine in Combination with Mecamylamine or Hexamethonium

In a number of studies of discriminative stimulus properties of nicotine in rats pretreatment with mecamylamine consistently blocked the nicotine effect, whereas hexamethonium did not block the effect at any dose tested (Morrison and Stephenson 1969; Schecter and Rosecrans 1971; Hazell et al. 1978). In addition, mecamylamine but not hexamethonium blocked the behavioral effect of nicotine in monkeys maintained under a multiple FI-FR schedule of either termination of a stimulus associated with electric shock or presentation of food (Spealman et al. 1981). Our third experiment compared the behavioral effects of IVT administration of nicotine in combination with either mecamylamine or hexamethonium on responding maintained under an FR 32 schedule of food presentation.

Ten experimentally naive rats were maintained under the same conditions and tested in the same apparatus as described in Experiment 1.

Procedure. The rats were trained to lever press for a 45 mg food pellet under an FR 32 schedule. When response rates were stable (less than 10% variance in daily response rate for both 15-min periods over five sessions) IVT infusions were begun. All infusions were given during the 5-min TO and separated by 7 days. Five rats were administered the following sequence of injections: 1) saline, 5 μl of 0.9%, 2) saline with a presession injection of mecamylamine, 1.5 and 3.0 mg/kg SC 5 min prior to the first 15-min period, 3) (–)-nicotine, 10 μg in 5 μl, 4) (–)-nicotine, 10 μg in 5 μl with a presession injection of

mecamylamine, 0.05, 1.5 and 3.0 mg/kg SC, and 5) (–)-nicotine in 5 µl of saline.

The remaining five rats were maintained under FR 32 and tested with presession injections of hexamethonium chloride (0.05, 1.5 and 3.0 mg/kg SC given 10 min prior to a 10 µg infusion of (–)-nicotine. All tests were separated by a 7-day interval.

Results and Discussion

Saline infusions with or without mecamylamine pretreatment had little effect on the latency to complete the first ratio (Fig. 4). There was no significant difference between saline and saline-mecamylamine combinations (at 1.5 mg/kg SC, $df = 4$, $t = 1.29$ $P > 0.1$; at 3.0 mg/kg SC, $df = 4$, $t = 1.97$ $P > 0.1$). The average latency to the first completed ratio following a 10 µg IVT infusion of (–)-nicotine was 13 min (± 1.5 min). Presession injections of mecamylamine blocked the effect of nicotine in a dose-related fashion (Fig. 4). Injections of mecamylamine (0.05 and 1.5 mg/kg SC) decreased the latency by 40 and 84% respectively. In four of the five animals tested, mecamylamine (3.0 mg/kg SC) completely blocked the effect of IVT nicotine (saline vs 3.0 mg/kg mecamylamine and nicotine, $df = 3$, $t = 1.58$ $P > 0.1$). For one rat mecamylamine at this dose did not completely block the effect and the latency was 4 min, 30 s. No explanation is apparent as to why this rat's behavior differed from the others. All rats were given a nicotine retest and their latencies did not differ from the original nicotine test value (Fig. 4).

In contrast to the nicotine-induced changes in latency, subsequent response rates did not show any systematic changes following any experimental manipulations, suggesting that when recovery occurred it was complete.

Unlike mecamylamine, hexamethonium at doses of 0.5 and 1.0 mg/kg SC failed to block the latency to lever press (Fig. 5). However, at a dose of 3.0 mg/kg SC there was a partial antagonism of the nicotine-induced latency changes. Penetration of hexamethonium into the brain is limited (Taylor 1980) but not excluded, and it is likely that at the highest dose given, amounts sufficient to produce a partial antagnoism did cross the blood brain barrier. Nicotine retest values without preinjection of hexamethonium did not differ from the original nicotine test values.

General Discussion

Responding by rats was maintained under various FR schedules of food presentation. Under these conditions the duration of the effects of IVT administration of nicotine extended far beyond the observed time course previously reported (Abood et al. 1978, 1979). The latency to complete the first ratio following a nicotine infusion was inversely related to the ratio size. The similarity between the latencies observed from the nicotine infusion under the FR 32 schedule suggests that the effect of ratio size on the nicotine-induced latency change was not secondary to repetitive nicotine testing. This finding is similar to that previously reported (Abood et al. 1979). These authors showed that tolerance to the behavioral effects of IVT nicotine would develop after chronic nicotine infusions (i.e., infusions on 6 consecutive days), but that behavior ratings returned to initial levels within 2 days following the last infusion. The interinfusion interval in the present study was 7 days, and tolerance to the effects of IVT nicotine was not observed. During the non-infusion and saline control sessions, characteristic FR response patterns were obtained. Rates and patterns of responding following recovery from a nicotine infusion did not differ from control values. One major difference between each of the ratio schedules was that the response rate was directly related to the FR size. This would suggest that the effects of IVT nicotine are largely dependent upon the rate of emitted behavior.

As the dose of nicotine was decreased the latency decreased and corresponding changes were noted in the observed prostration to a dose of 1.25 µg. The present data is in contrast with that of Abood and co-workers (1979) which demonstrated a monotonic dose-response function for 2–10 µg of IVT nicotine. An explanation of the difference between the two sets of data is that in the present study a more sensitive measure of behavior was used from which the dose response curve was obtained and doses as low as 0.312 µg were tested.

The behavioral effects of systemically administered nicotine in combination with nicotine antagonists have been examined previously in both monkeys (Spealman et al. 1981) and rodents (Morrison et al. 1969; Stitzer et al. 1970). In the present study, doses of mecamylamine that had little or no effect on responding when given alone blocked behavioral effects of IVT nicotine. Presession treatment with 0.05 mg/kg of mecamylamine reduced the nicotine-induced latency changes by 40%, and as the dose was increased to 1.5 and 3.0 mg/kg, latency measures approached control values. When nicotine was again administered alone the latency values returned to the previous high levels. Since mecamylamine produces ganglionic blockade by occupying cholinergic receptors, our results suggest that the effects of IVT infusions of nicotine may be mediated by nicotinic-cholinergic mechanisms. These results are not compatible with previous studies that have examined different behaviors. Abood et al. (1978, 1979) have suggested that the prostration syndrome seen following IVT infusions of nicotine may not be mediated by cholinergic mechanisms. An explanation for the different results between the studies is not apparent from a comparison of the procedure.

Unlike mecamylamine, hexamethonium (0.5, 1.0, 3.0 mg/kg) failed to block the effect of IVT nicotine on the latency changes in the FR schedule. It should be noted that at the highest dose tested (3.0 mg/kg) a partial antagonism of the effects of nicotine on FR responding did occur. Although hexamethonium does not readily penetrate the central nervous system (McIssac 1962), it is likely that at this high dose enough is penetrating to produce a partial effect. Since presession treatment with mecamylamine blocked the behavioral effects of nicotine and hexamethonium did not, it would appear that the effects of IVT nicotine infusions on behavior maintained under FR schedules reflect central effects of nicotine on cholinergic sites.

The present findings are compatible with previous reports that mecamylamine is effective in antagonizing the discriminative stimulus effects of nicotine (Morrison and Stephenson 1969; Schecter and Rosecrans 1971; Hazell et al. 1978), and that high doses of hexamethonium (10.0–20.0 mg/kg) can partially block the effects of 0.4 mg/kg of nicotine in rats responding under an FI schedule of water presentation (Stitzer et al. 1970).

Acknowledgement. The assistance of Frank Ryan and Jan Jones in preparing the manuscript is greatly appreciated and we thank Mary

Satterfield for typing the manuscript. A preliminary report of these experiments was presented to the Society for Neuroscience 11th Annual Meeting (1981).

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Received December 2, 1981