Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 1, pp. 67–71, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0090-5

Nicotine Self-Administration and Locomotor Activity Are Not Modified by the 5-HT_3 Antagonists ICS 205-930 and MDL 72222

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Received 15 July 1993

CORRIGALL, W. A. AND K. M. COEN. *Nicotine self-administration and locomotor activity are not modified by the 5-HT_3 antagonists ICS 205-930 and MDL 72222.* PHARMACOL BIOCHEM BEHAV **49**(1) 67–71, 1994.—The subtype-selective serotonin 5-HT_3 antagonists MDL 72222 and ICS 205-930 were tested for their ability to modify nicotine self-administration and locomotor activity in rats. In self-administration experiments, MDL 72222 produced no statistically significant changes over a dose range of 1 to 30 μg/kg, nor at the considerably higher dose of 1 mg/kg. MDL 72222 was similarly without effect in nicotine-produced locomotor activity, except at the 1 mg/kg dose, which reduced scores. In an initial test on nicotine self-administration, ICS 205-930 produced a small decrease in drug-taking behavior at 1 and 3 μg/kg which just reached statistical significance, but had no effects at higher doses. However, these low-dose effects could not be replicated. In addition, ICS 205-930 was without effect on nicotine locomotor activity, even at the two low doses that had reduced self-administration. We conclude that these 5-HT_3 antagonists do not modulate nicotine reinforcement or behavioral arousal.

5-HT_3 antagonists Nicotine reinforcement Locomotor activity MDL 72222 ICS 205-930

AS FOR other drugs of abuse, research into the mechanisms by which nicotine reinforces self-administration behavior has focussed extensively on the mesolimbic dopamine system. To this end, we have recently established that nicotine self-administration is reduced a) following treatment with selective D_1 and D_2 dopamine antagonists (9), b) after dopaminergic lesions of the ascending projection from the ventral tegmental area produced by neurotoxin infusions into the nucleus accumbens (11), and, c) by microinfusions of a nicotinic antagonist into the ventral tegmental region (10). These data provide good evidence that nicotine reinforcement depends, in part, on the interaction of the drug with the mesolimbic dopamine system. In this aspect of its reinforcing action nicotine is similar to cocaine. Unlike cocaine, however, which acts at the level of the synaptic terminals in the accumbens region [see review in (19)], nicotine activates the mesolimbic system through the ventral tegmental area and not via the accumbens (10).

Given the seemingly ubiquitous involvement of the mesolimbic dopamine system in reinforcement processes, an important research question has become the identification of neurochemical systems which might selectively modulate the action of the mesolimbic dopamine system. One candidate neurochemical system is serotonin (5-HT). In particular, evidence that manipulations of the 5-HT_3 receptor subsystem can alter behaviors that are believed to result from elevated mesolimbic dopamine (12), and also modify dopamine release in the nucleus accumbens (4,18) has focussed attention on this subsystem as a possible modulator of mesolimbic dopamine in drug reinforcement. Furthermore, a report that 5-HT_3 antagonists attenuate the conditioned place preference produced by nicotine (3) adds to speculation that this receptor subtype may play a role in some aspect of nicotine reinforcement. In contrast, studies have shown that 5-HT_3 antagonists have no effect on cocaine (20) or heroin (16) self-administration. However, because nicotine apparently targets the dopamine system differently than these drugs (6), 5-HT_3 manipulations could be differentially effective in nicotine self-administration. Therefore, we have examined the effects of treatment with the 5-

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HT_3 antagonists ICS 205-930 and MDL 72222 on intravenous nicotine self-administration in rats. As a comparison behavioral test, we have also examined the effects of these antagonists on nicotine-produced locomotor activity, a behavioral measure that also depends on a mesolimbic dopamine substrate (5,23,25).

METHOD

Subjects were drug-naive male Long-Evans rats (Charles River, Lachine, Quebec). Animals were housed in a reversed light : dark cycle colony room (lights off between 0700 and 1900 h). Prior to the start of experimental procedures, animals had ad lib access to food and water and weighed approximately 300 g. Separate groups of animals were used for each of the following experiments:

1. two groups of eight animals each in which the effects of ICS 205-930 and MDL 72222 were initially examined for effect on nicotine self-administration;
2. a group of 15 animals in which the same doses of the 5-HT_3 antagonists were examined in nicotine locomotor activity;
3. a group of 17 animals in which a high dose of MDL 72222 was examined for effect on nicotine self-administration;
4. a group of 16 animals in which the same high dose of MDL 72222 was examined in locomotor activity; and,
5. a group of 12 animals for replication of low dose treatments with ICS 205-930 on nicotine self-administration.

Techniques for drug self-administration studies were similar to those we have used previously (7,8). Animals were deprived of food for a short period (24 h), and trained to press a lever on a continuous reinforcement (CRF) schedule for food pellets (45 mg). Once trained, each animal was surgically prepared with a chronic intravenous catheter implanted in the jugular vein; the catheters exited between the scapulae. Surgery was performed under anesthesia induced by acepromazine maleate (10 mg/kg IP) and ketamine hydrochloride (100 mg/kg IM). A single dose of penicillin (30,000 units IM) was administered at the completion of surgical procedures. Animals were allowed to recover for a period of 1 week before drug self-administration sessions were begun.

Drug self-administration was initiated on a CRF schedule with a 1-min signalled time-out (TO) period following each drug infusion. Over an approximate 3-week period the response requirements were increased to the final value of fixed-ratio 5 (FR5); the TO remained at 1 min. During the TO, responding was recorded but did not lead to drug delivery. For both acquisition and maintenance of self-administration, the unit dose of nicotine was 0.03 mg/kg/infusion. This is a midrange dose (7,8).

Self-administration sessions were carried out in operant chambers equipped with two levers. Responding on one of the levers resulted in drug delivery when schedule requirements were met, while responding on the other lever was recorded but was never reinforced. Self-administration sessions were 60 min. in duration and occurred once daily Monday through Friday.

For locomotor activity studies, animals were habituated to activity boxes equipped with infrared sensors, as previously described (9). Boxes were housed in sound-attenuating cubicles in a quiet room. During habituation, each animal received a SC (SC) injection of saline (1 ml/kg), and was placed in a separate activity box for a 1-h period; cumulative locomotor scores were measured during habituation to provide a baseline against which nicotine-produced locomotor activity could be judged. Following habituation, daily nicotine treatments were begun. Nicotine was administered SC at a dose of 0.3 mg/kg; each animal was placed in a separate activity box immediately following nicotine treatment, and cumulative locomotor scores were again measured over a 1-h period. Nicotine treatment continued once per day for 4 weeks. After this time nicotine injections were continued once daily, preceded on occasion by antagonist treatments, as described below.

Treatments with the serotonin antagonists were carried out not more than twice each week, typically on Tuesday and Friday. Antagonists were injected subcutaneously 60 min prior to the start of self-administration or locomotor activity sessions. Control injections were made in each case with the vehicle used to dissolve the particular 5-HT_3 antagonist (described below). A nonordered sequence of administration was used for the various doses.

The following drugs were used: [−]-nicotine bitartrate (Sigma Chemical Co., St. Louis, MO), and ICS 205-930 and MDL 72222 (both from RBI Inc., Natick, MA). Nicotine solutions were prepared in saline and the pH adjusted to 7.0 ± 0.1 with sodium hydroxide. Solutions for IV self-administration were passed through a 0.22 μm filter to sterilize them prior to use. For nicotine self-administration, the solution concentration was 0.3 mg/ml (delivered in a volume of 0.1 ml/kg over an infusion time of approximately 1 s). Serotonin antagonists were dissolved in dilute organic acids; MDL 72222 was dissolved in 0.1 ml of 0.4% lactic acid and made up to volume with isotonic saline, and ICS 205-930 was dissolved in 0.1 ml of 0.1 N tartaric acid and similarly made up to the required volume. Values for doses and concentrations for all compounds (except anesthetics) refer to the base.

Data consisted of the number of nicotine infusions that were self-administered, or the number of locomotor counts, on treatment sessions. These data were analyzed with analysis of variance for a repeated measures design, or *t*-tests, as appropriate. Post hoc analyses were done with Duncan's multiple range test.

RESULTS

As in our previous studies, nicotine maintained reliable self-administration behavior. Acquisition typically required 3

TABLE 1

DEVELOPMENT OF NICOTINE LOCOMOTOR ACTIVITY

	Saline Habituation	Nicotine	Saline Substitution
Group 1 (n = 15)	256 ± 30	889 ± 75*	455 ± 50†
Group 2 (n = 16)	220 ± 21	818 ± 72‡	440 ± 26§

Values are group means ± standard errors of the mean for three phases in the development of nicotine locomotor activity, specifically, a) habituation to the activity boxes accompanied by saline injections, b) locomotor activity produced by nicotine after 4-weeks of treatment, and c) locomotor activity following saline substitution after nicotine-produced activity has developed.

*Significantly different from saline habituation; $t(14) = 9.92, p < 0.001$. †Significantly different from nicotine; $t(14) = 7.88, p < 0.001$. ‡Significantly different from saline habituation; $t(15) = 8.33, p < 0.001$. §Significantly different from nicotine; $t(15) = 5.61, p < 0.001$

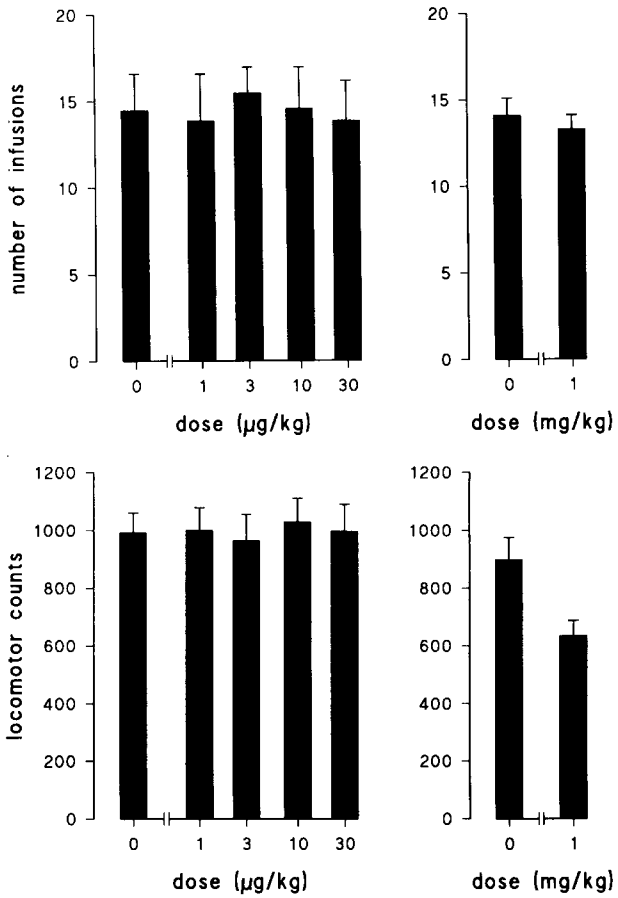

FIG. 1. (A) Effects of the 5-HT$_3$ antagonist MDL 72222 on nicotine self-administration. Sample sizes are 8 in the left-hand panel and 17 in the right. Note that the dose axis is in milligrams in the right-hand panel. In this and subsequent figures, the zero-dose points represent treatment with the vehicles used to inject the antagonists. Data are shown as the mean + standard error of the mean in this and all other graphs. (B) Effects of MDL 72222 on nicotine-produced locomotor activity. Sample sizes are 15 in the left-hand panel and 16 in the right.

weeks; at this time responding was stable in most subjects. Only animals in which there was no sustained trend, over a 1-week period, to increase or decrease self-administration after acquisition were used in these experiments. Responding of the animals was focussed on the active rather than the inactive lever (the average ratio of inactive-to-active lever responding in these animals at the time testing was begun was 0.16, with a standard error of 0.02).

Following the 4-week treatment period with nicotine, locomotor scores after nicotine injection were substantially and significantly increased compared to habituation (Table 1). In addition, single-session substitution of saline for nicotine produced a significant decrease in locomotor activity scores (Table 1). To reduce locomotor scores to values comparable to those observed in habituation, extinction with multiple saline trials is necessary. Reduction in nicotine-produced locomotor activity by serotonin antagonists would, therefore, be expected to result in scores between those for nicotine alone and those after single-session saline substitution.

The effects of treatment with the 5-HT$_3$ antagonist MDL 72222 on nicotine self-administration and locomotor activity are shown in Fig. 1. In self-administration experiments (Fig. 1A), MDL 72222 produced no statistically significant changes over a dose range of 1 to 30 μg/kg, $F(4, 28) = 0.65$, NS. Because of the difference in potencies between MDL 72222 and ICS 205-930, an additional much higher dose of MDL 72222 was tested later in a separate group of animals. This dose (1 mg/kg) was also without effect on nicotine self-administration, $t(16) = 0.77$, NS. In locomotor activity experiments (Fig. 1B) MDL 72222 was similarly without effect over the original dose range tested, $F(4, 56) = 0.62$, NS, but did reduce activity in a highly significant way at the 1 mg/kg dose, $t(15) = 6.06$, $p < 0.001$.

The effects of ICS 205-930 are shown in Fig. 2. ICS 205-930 produced a small decrease in nicotine self-administration at the lowest doses tested, but had no effects at higher doses (Fig. 2A). These effects of ICS 205-930 just reached statistical significance, $F(4, 28) = 3.28$, $p < 0.03$. Post hoc tests indicated that, for doses of 1 and 3 μg/kg, the number of infusions differed from saline treatment values at the $p = 0.05$ level. In addition, at each of these two doses seven of the eight subjects showed a decrease after ICS 205-930. However, in a replicate test of the effects of the two lowest doses of the ICS compound on nicotine self-administration, there was no

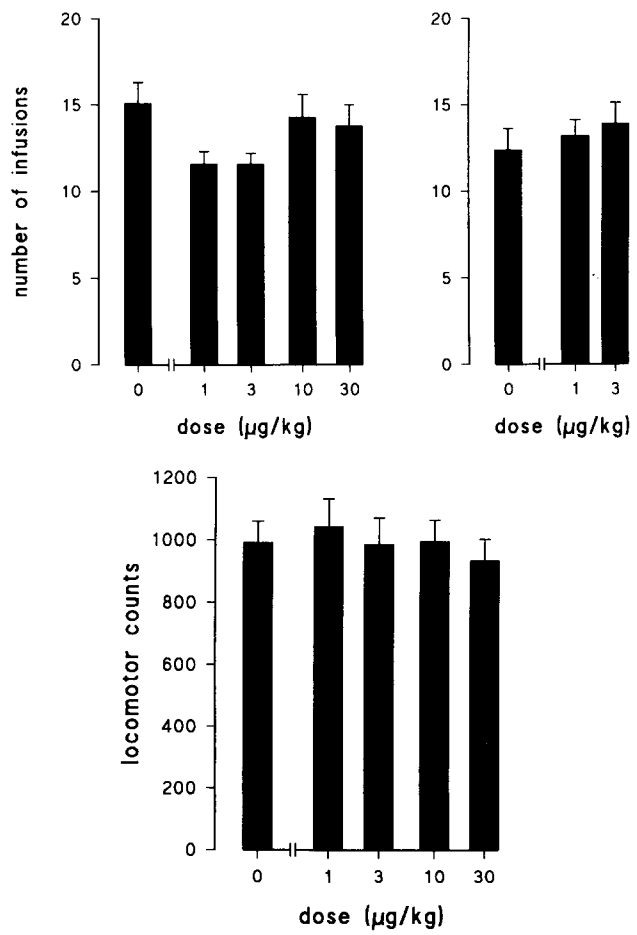

FIG. 2. (A) Effects of the 5-HT$_3$ antagonist ICS 205-930 on nicotine self-administration. Sample sizes are 8 in the left-hand panel and 12 in the right. (B) Effects of ICS 205-930 on nicotine-produced locomotor activity. Sample size is 15.

apparent or statistically significant effect [Fig. 2A, right-hand panel; $F(2, 22) = 1.49$, NS]. ICS 205-930 was without effect on nicotine-produced locomotor activity (Fig. 2B), even at the two low doses that had reduced nicotine self-administration, $F(4, 56) = 2.13$, NS.

DISCUSSION

The 5-HT_3 antagonist MDL 72222 was without effect on nicotine self-administration over a dose range from 1 μg/kg to 1 mg/kg. Similarly, there was no effect of the compound on nicotine-produced locomotor activity, except at the highest dose. Obviously, the reduction in locomotor activity at 1 mg/kg is not due to a generalized impairment of the subjects' ability to respond; if it were, a parallel reduction in operant responding would be anticipated. In addition, it seems unlikely that the reduction is due to an effect on mesolimbic dopamine, because parallel effects on self-administration did not occur. The effect of MDL 72222 on locomotor activity at 1 mg/kg is more likely due to an effect on mechanisms specific to spontaneous activity which do not play a role in incentive motivational behavior, or to a nonspecific effect of the compound.

ICS 205-930 produced no alteration of nicotine locomotor activity over the dose range tested. However, the effects of ICS 205-930 on nicotine self-administration were not as clearly negative as those of MDL 72222. In the original test of ICS 205-930, small reductions in self-administration occurred at the two lowest doses of the antagonist. However, the small magnitude of these effects and the curious dose-effect relationship (loss of the effect at higher doses) led us to attempt to replicate the findings. In a retest of the two lowest doses of ICS 205-930, we did not observe any tendency toward a reduction in nicotine self-administration.

It should be noted that biphasic effects of 5-HT_3 antagonists, observed here with ICS 205-930 in one of two self-administration tests, have been reported in previous research with this compound [e.g., (2,13,21,26)], and also with other 5-HT_3 antagonists (21). It is possible, therefore, that the doses of 1 and 3 μg represent maximal doses for a specific effect of ICS 205-930 on nicotine self-administration. That is, perhaps at doses of ICS 205-930 lower than 1 μg, more replicable effects would have been observed. On the other hand, tests with a full dose range of MDL 72222 showed no effects on nicotine self-administration. As a result of this observation and the lack of replicability of the original ICS 205-930 effect, we did not pursue the effect of ICS 205-930 at lower doses.

Previous research has produced a mixed picture of 5-HT_3 antagonist effects. For example, the 5-HT_3 antagonist zatosetron has been shown to decrease the number of spontaneously active dopamine cells after acute or chronic administration (24), but different effects on dopamine cells were observed with MDL 73,147EF (27) and granisetron (1). In a similar vein, the discriminative stimulus effects of ethanol were attenuated by ICS 205-930 and MDL 72222, but not by zacopride (14). Specifically for drug reinforcement and related processes, the 5-HT_3 antagonist MDL 72222 has been reported to block the conditioned place preference produced by morphine (3,15) and nicotine (3) but is without effect on heroin (16), cocaine (20), or nicotine (this study) self-administration. ICS 205-930 has been shown to reduce ethanol self-administration by rodents (17) but in the present study was without effect on nicotine self-administration. Ondansetron reduces alcohol consumption in humans (28) but is without effect on cigarette smoking (29). In general, therefore, it may be that the presence or absence of a 5-HT_3 antagonist effect depends on the particular antagonist chosen, the dose range employed, and the particular test being used, amongst other factors.

As for the interaction between 5-HT_3 antagonists and nicotine, there has been little work, and that which has been done may be compromised in part by the above conclusion. The original suggestion that nicotine reward might be under the control of 5-HT_3 systems, based on observations of conditioned place preference (3), has not been substantiated here using the same antagonists but tested in a self-administration paradigm. Also, cigarette smoking by humans is unaffected by treatment with a 5-HT_3 antagonist (29). A recent report showed that ondansetron did not facilitate brain stimulation reward (BSR), nor did it modify nicotine's facilitation of BSR (22). To the extent that facilitation of BSR is related to the rewarding properties of nicotine, this lack of effect is consistent with our observations for nicotine self-administration. In the same BSR study, ondansetron's ability to attenuate the depressant effects of nicotine was interpreted with a hypothesized neuronal network in which a serotonergic neuron was assumed to be interposed between the midbrain dopamine neurons and the nicotinic cholinergic input to them. However, this scheme does not fit with our recent observations with respect to the site at which exogenously self-administered nicotine targets mesolimbic dopamine cells (10) nor with the lack of effect of 5-HT_3 antagonists on nicotine self-administration. At present, we conclude that nicotine self-administration is not under the control of a 5-HT_3 system, at least not one sensitive to ICS 205-930 or MDL 72222.

ACKNOWLEDGEMENTS

We are grateful to Paul Goldbach for conducting some of the locomotor activity experiments in this report. This study was supported by the Addiction Research Foundation of Ontario.

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