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Isradipine Inhibits Nicotine Intravenous Self-Administration in Drug-Naive Mice

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MARTELLOTTA, M. C., A. KUZMIN, E. ZVARTAU, G. COSSU, G. L. GESSA AND W. FRATTA. *Isradipine inhibits nicotine intravenous self-administration in drug-naive mice.* PHARMACOL BIOCHEM BEHAV **52**(2) 271-274, 1995. – The effect of isradipine, a dihydropyridine calcium antagonist, on intravenous self-administration of nicotine in naive mice has been investigated. When nicotine injections were made contingent upon nose-poke response by naive mice, they increased their rate of nose poking with respect to animals receiving contingent saline injections or yoked control animals receiving noncontingent nicotine injections. Pretreatment of mice with mecamylamine (2.4 mg/kg) inhibited self-administration of nicotine contingent upon a nose-poke response. The same effect was observed with isradipine (0.5-1.0 mg/kg) in a dose-related manner and stereospecifically. These data suggest that isradipine suppresses the reinforcing properties of nicotine and might be useful for treatment of nicotine abuse.

Nicotine Isradipine Mecamylamine Self-administration by naive mice

OF ALL drugs of abuse, nicotine, in the form of tobacco smoking, is the most popular worldwide. A clear pattern of nicotine self-administration has been demonstrated in rats, dogs, and primates (17). In common with other drugs of abuse such as cocaine, morphine and amphetamine, the positive reinforcing effect of nicotine has been associated with a stimulation of dopamine release in the mesolimbic system (5,7,14). Like cocaine and morphine, nicotine-induced dopamine release is calcium dependent (1). Pharmacological effects of nicotine, such as antinociception and locomotor activity, are reduced by administration of calcium antagonists of the dihydropyridine type (4). It has also been shown that nicotine discrimination in rats is blocked both by the calcium antagonist isradipine and the dopamine release inhibitor CGS 10746B, suggesting that calcium influx and dopamine release are important conditions for nicotine discrimination (15). We have recently shown that calcium channel blockers of the dihydropyridine class, and, in particular, isradipine, are able to inhibit the positive reinforcing effects of cocaine and morphine under different experimental conditions such as place preference (9,12), intravenous (IV) self-administration in rats (6,10) and IV self-administration in drug-naive mice (9). This latter self-administration procedure was first described by Criswell and Ridings (3). They have shown that morphine was self-administered by naive mice. We have shown that cocaine was also self-administered by naive mice (9). The aim of the present study was to establish whether nicotine could show a clear pattern of self-administration in drug-naive mice and to determine the ability of isradipine to interfere with the reinforcing properties of nicotine.

METHOD

Animals

Male Swiss albino mice (Charles River, Italy), weighing 20–25 g, were used. Upon arrival, animals were housed six per cage and acclimatized to laboratory conditions (12L : 12D cycle, lights on at 0800 h, 21 ± 1°C room temperature, and 60% relative humidity) for at least 1 week prior to use. Food and water were available ad lib until the time of testing.

Drugs

(−)Nicotine bitartrate (Sigma, St. Louis, MO) was freshly dissolved in saline. Doses are referred to the salt. (±)Isradipine and its isomers (Sandoz, Italy) were dissolved in 9% Tween-80 solution in distilled water and injected subcutaneously 80 min prior to the experiment in a volume of 1 ml/kg.

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Mecamylamine-HCl (Sigma) was injected IP in a volume of 1 ml/kg 10 min prior to the experiment. Dose is referred to the salt.

Apparatus and Procedures

As previously described (3,9), mice were tested in pairs of identical test cages (8 × 8 × 8 cm): the "active" mouse was placed in one cage and the "yoked passive" mouse confined to the other. Each test cage presented a frontal hole provided with an infrared detector that activated a cumulative recorder (Colburn Instruments, Basile, Como, Italy) and operated a syringe pump (Life Science Instruments, CA) to deliver solution contingent on a nose-poke response (NPR). A rear vertical chink was made on the opposite wall through which the mouse's tail was extended outside the box and taped to a horizontal surface allowing access to the lateral tail veins with a 27 ga winged needle, connected with the syringe through a Teflon tubing. Each nose poke of the active mouse resulted in a contingent injection of 1.0 μl of either saline or the drug dissolved in saline, both to the active and yoked passive mouse. Therefore, the passive yoked mouse received the same amount of drug at the same time intervals as the active mouse. Nose pokes of the yoked control were counted but had no programmed consequences. No particular stimuli (food, light, etc.) was presented to the animals as a consequence to nose-poke response. A background white noise was on during the whole experimental period. Experiments were performed between 1300 and 1600 h. Mice were first placed in the test cage for 10 min of habituation, during which the tail was taped but no needle was inserted. Mice adapt rapidly to this form of immobilization, as previously described (3,11). Pairs of animals were selected on the basis of approximately equal levels of nose poking during 10 min of habituation and randomly allocated to the different experimental groups. Each mouse was used only once. After this time, needles were inserted in lateral tail veins and an IV injection was made contingent upon each nose poke of the active animal. As a measure of the reinforcing effect of a drug, the ratio (R) between the cumulative number of NPRs of the active and passive mouse during 30-min session was used. The effect of the drug was considered reinforcing, neutral, and aversive when R was higher, equal and smaller than 1, respectively (9).

Statistics

Significance was determined with one-way analysis of variance followed by Newman-Keuls test.

RESULTS

As shown in Fig. 1 no statistically significant difference was observed in the mean number of NPRs of active and passive mice when saline injections were contingent upon NPRs. Therefore, R in these conditions was not different from unity. Nicotine influenced R in a concentration-dependent manner. Thus, the lowest concentration tested, 0.025 mg/kg/inj, failed to modify R because it did not affect NPRs in either the active or passive mouse. Concentrations of 0.050 and 0.075 mg/kg/inj significantly increased NPRs of active mice, but had no significant effect on the nose poking of passive animals; thus, these concentrations increased R and are considered reinforcing. The highest nicotine concentration tested, 0.1 mg/kg/inj, failed to increase NPRs both of active mice and passive ones. Therefore, R was not different from unity. Our results have shown that the reinforcing effect of nicotine in drug-naive mice is concentration dependent according to a bell-shaped curve.

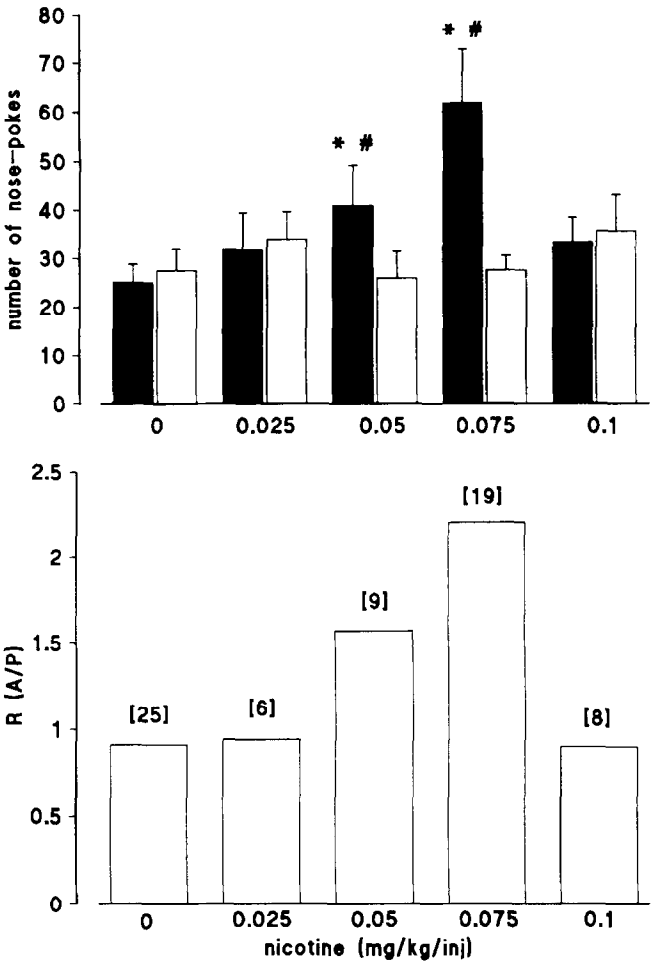

FIG. 1. Concentration-dependent nicotine self-administration. Top: solid bars = A (active mice); open bars = P (passive mice). Each bar represents the mean ± SEM of cumulative NPRs in a 30-min session. *$p < 0.01$ with respect to mice self-administering saline. #$p < 0.01$ with respect to the yoked passive mice. Bottom: each bar represents the ratio (R = A/P) between the cumulative number of NPRs of the active and passive mice. Number of pairs are indicated in parentheses.

Pretreatment with mecamylamine, the selective antagonist of nicotinic central receptors, at the dose of 2.4 mg/kg was able to antagonize the reinforcing effect of nicotine (0.075 mg/kg/inj) withouth affecting nose poking of saline self-administrating mice (Fig. 2).

The calcium antagonist, isradipine, was tested against the concentration of nicotine producing maximal reinforcement (0.075 mg/kg/inj). As shown in Fig. 3, (±)isradipine inhibited the reinforcing effect of nicotine in a dose-dependent manner. Doses of 0.5 and 1 mg/kg selectively decreased the number of NPRs in active mice without affecting the number of NPRs in passive ones. Isradipine, up to the dose of 2.5 mg/kg, did not modify nose poking of saline self-administering mice (data not shown). Testing of both isomers of isradipine revealed that the inhibition of the nicotine reinforcing effect obtained with the (+)isomer at the dose of 0.5 mg/kg was

similar to that obtained with the racemic compound at equimolar doses (1 mg/kg). On the contrary, the equimolar dose of the (−)isomer was not statistical different from vehicle-treated mice.

CONCLUSIONS

The addictive properties of nicotine have been demonstrated both in humans, especially in the form of tobacco smoking, and in laboratory animals using different experimental techniques such as conditioned place preference, IV self-administration (16). In the present study we have shown for the first time that nicotine is self-administered in drug-naive mice. This method of IV self-administration in naive mice is not comparable with the "conventional" IV self-administration in rats, nor is it the aim of the present study to consider this methodology as alternative to the more classical chronic self-administration methodologies. However, naive mice have been shown to self-administer drugs of abuse such as morphine, cocaine (3,9) and, as we show in the present study, nicotine. In this light, self-administration in naive mice might be considered as an additional test for studying drugs of abuse and treatments interfering with drug self-administration. Validity of the method is confirmed by the fact that, in spite of the large individual variabilities in naive animals, the mean number of NPRs of active and passive animals was not statistically different when saline injection was made contingent upon the response, whereas NPRs of the active mouse selectively increased when injection of a proper concentration of nicotine was contingent upon nose poking. The same feature was shown for cocaine and morphine (9). Furthermore, administration of the central nicotinic receptor antagonist mecamylamine antagonizes the reinforcing effect of nicotine. Pretreatment with isradipine, an L-type calcium channel antagonist of the dihydropyridine class, is able to prevent the positive reinforcing effect of nicotine. This action of isradipine is dose dependent and stereospecific. Indeed, pretreatment with (±)isradipine significantly reduced nicotine-induced NPRs in the active mouse without affecting NPRs in the passive one. Furthermore, at equimolar doses this effect is shown only by (+)isradipine. The mechanism of action by which isradipine exerts this effect is not clear. However, the possibility exists that inhibition of the reinforcing effect of nicotine by isradipine might be consequent to an inhibition of nicotine-induced dopamine release. A large amount of indirect evidence might support this hypothesis. An increased meso-

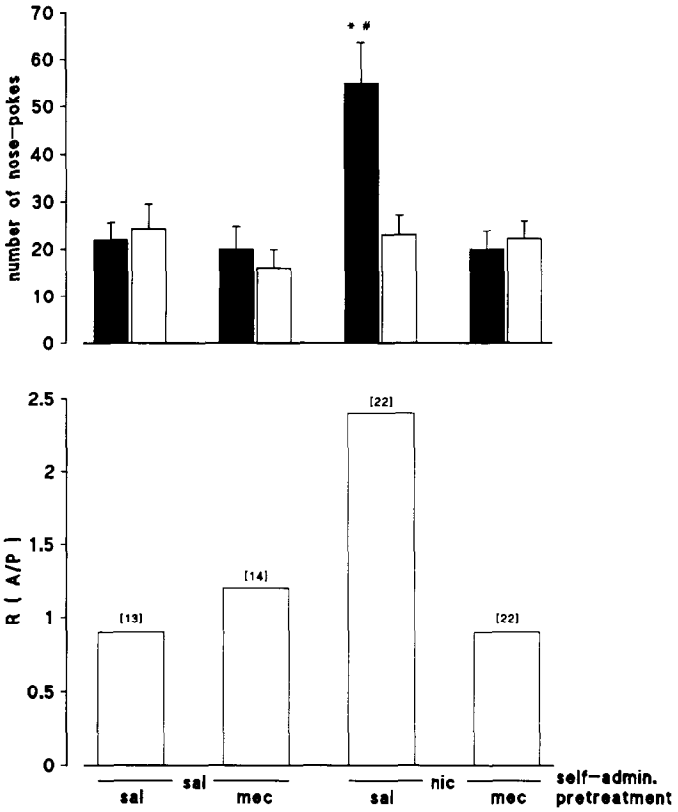

FIG. 2. Antagonism by mecamylamine (2.4 mg/kg) of nicotine self-administration (0.075 mg/kg/inj). Top: solid bar = A (active mice); open bars = P (passive mice). Each bar represents the mean ± SEM of cumulative NPRs in a 30-min session. *$p < 0.01$ with respect to mice pretreated with mecamylamine. #$p < 0.01$ with respect to the yoked passive mice. Bottom: each bar represents the ratio (R = A/P) between the cumulative number of NPRs of the active and passive mice. Number of pairs are indicated in parentheses.

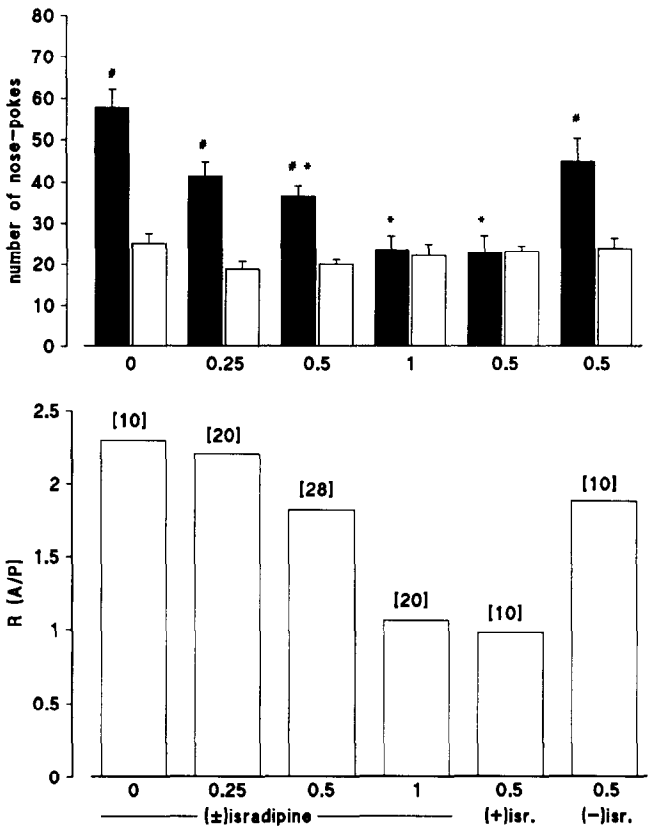

FIG. 3. Antagonism by isradipine of nicotine (0.075 mg/kg/inj) self-administration. Top: solid bar = A (active mice); open bars = P (passive mice). Doses are expressed as mg/kg. *$p < 0.01$ with respect to mice pretreated with vehicle. #$p < 0.01$ with respect to the yoked passive mice. Bottom: each bar represents the ratio (R = A/P) between the cumulative number of NPRs of the active and passive mice. Number of pairs are indicated in parentheses.

limbic dopamine-release is thought to be responsible for the positive reinforcing effect of nicotine, as well as of cocaine and morphine (2,7,14). This release is calcium dependent (1). It has been shown that calcium antagonists of the dihydropyridine class, especially isradipine, antagonize cocaine- and morphine-induced dopamine release (12). We have also shown that isradipine antagonizes cocaine- and morphine-induced place preference (9,13), IV self-administration in rats (6,10), as well as in drug-naive mice (9). This evidence suggests the hypothesis that the antagonism of isradipine on the positive reinforcing effect of nicotine might be due to an inhibition of nicotine-induced dopamine release. In conclusion, irrespective of the mechanism involved, the present results might indicate the possibility that isradipine or structurally related compounds could be used against nicotine abuse.

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