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RAPID COMMUNICATION

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Medial prefrontal cortex is involved in the discriminative stimulus effects of nicotine in rats

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Abstract *Rationale*: Central nicotinic receptors have been reported to be involved in the discriminative stimulus (DS) effects of nicotine. *Objectives*: The purpose of the present study was to investigate the role of the medial prefrontal cortex (mPFC) and the medial habenular nucleus (mHb) in the DS effects of nicotine. *Methods*: Substitution tests with nicotine administered into mPFC and mHb were conducted in rats trained to discriminate nicotine (0.5 mg/kg, SC) from saline in a two-lever, food reinforced, operant task. *Results*: Nicotine (40 µg) administered into mPFC substituted for nicotine (0.5 mg/kg, SC), whereas nicotine administered into mHb did not. *Conclusions*: Together with our previous study indicating that the nucleus accumbens and the ventral tegmental area are partially involved in the DS effects of nicotine, the present study suggests that mPFC is primarily involved in the DS effects of nicotine.

Key words Nicotine · Discriminative stimulus effect · Medial prefrontal cortex · Medial habenular nucleus

Introduction

Much work has been done to show that the discriminative stimulus (DS) effects of nicotine are mediated through central nicotinic receptors (Hirschhorn and Rosecrans 1974; Stolerman et al. 1984). However, only two regions, the dorsal hippocampus and the mesencephalic reticular formation, were reported to be involved in the DS effects of nicotine (Meltzer and Rosecrans 1981; Shoaib and Stolerman 1996). We have studied the implication of brain regions at which are located the mesolimbocortical dopaminergic neurons in the DS effects of nicotine (Miyata et al. 1991; Ando et al. 1993), because various lines of neurochemical studies have revealed that nicotinic receptors are located on the mesolimbocortical dopaminergic neurons (Clarke 1985), and that stimulation of these receptors leads to activation of the same dopaminergic neurons (Imperato et al. 1986). Our previous studies have shown that both the nucleus accumbens and the ventral tegmental area are partially involved in the DS effects of nicotine (Miyata et al. 1991; Ando et al. 1993). The present study was designed to investigate the role of the medial prefrontal cortex, which is the cortical terminal field of the mesolimbocortical dopaminergic neurons, in the manifestation of the DS effects of nicotine. In addition, among the central regions that possess high densities of nicotinic cholinergic receptors, the medial habenular nucleus was investigated regarding its role in manifestation of the DS effects of nicotine. This region was selected based on the possible role that the medial habenular nucleus may play in nicotine discrimination by receiving neural projections from the hippocampus (Nieuwenhuys et al. 1988).

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Materials and methods

Male Sprague-Dawley rats (234–290 g) were obtained from Clea Japan Inc. (Tokyo). Rats were individually housed and fed 15 g food per day (water freely available) throughout the experiment, except for a period of 3 days before and 7 days after cannulation surgery. This experiment was performed in accordance with the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985). The experiment was performed in a standard two-lever operant chamber. The operant schedule and recording of data were controlled by an LSI-11 microcomputer (Digital Equipment Co., Maynard, Mass., USA) using software developed at this laboratory.

The procedure for establishing nicotine discrimination has been described in detail (Ando et al. 1993). In summary, after acquisition of lever-pressing behavior under a fixed ratio 10 sched-

ule (FR-10), nicotine discrimination training was instituted: pressing one of two levers (randomly assigned) was reinforced with food pellets (50 mg) following nicotine (0.5 mg/kg of free base, SC) on certain days, while pressing the other lever was reinforced following saline (1 ml/kg, SC) on other days. (–)-Nicotine (Tokyo Kasei Co., Tokyo) was diluted in 0.9% saline. Rats were given injections of either nicotine or saline 10 min prior to daily training sessions (4 days a week) in a double alternation sequence (i.e., nicotine, nicotine, saline, saline, nicotine, ...). Sessions were terminated after 30 min or 100 reinforcements, whichever occurred first. The discrimination criterion to determine establishment and maintenance of drug discrimination behavior was that the appropriate lever was chosen for (a) at least 80% of the responses for the first food pellet in a session, and also for (b) at least 80% of the responses in a session overall, for at least five consecutive training sessions.

After discrimination between nicotine (0.5 mg/kg, SC) and saline was successfully established, guide cannulae were bilaterally implanted into the medial prefrontal cortex (n=7) and the medial habenular nucleus (n=7) under sodium pentobarbital (Nembutal, 40 mg/kg, IV) anesthesia. The coordinates for each region were as follows: the medial prefrontal cortex: 3.4 mm anterior (A), 0.8 mm lateral (L) and 5.0 mm ventral (V) to bregma; the medial habenular nucleus: 3.5 mm (P), 0.25 mm (L), 5.5 mm (V) according to the atlas of Paxinos and Watson (1986).

After the cannulation surgery, the substitution of nicotine administered directly into each cerebral region for nicotine (0.5 mg/kg, SC) was tested. In each substitution test, nicotine was tested in doses ranging from 10–40 µg (5–20 µg per side) for the medial prefrontal cortex, and 5–40 µg (2.5–20 µg per side) for the medial habenular nucleus. Saline in a volume of 0.4 µl per side was also administered into each region as control. The substitution tests were interspersed with daily discrimination training sessions, and were only performed again after the discrimination criterion described above had been satisfied for at least three consecutive daily discrimination training sessions. In the substitution test sessions, the procedure was identical with that in the discrimination training sessions except for the duration of a test session (2 min) and the fact that every tenth consecutive response on either lever elicited reinforcement. Following the experiment, the rats were killed under deep pentobarbital anesthesia and the injection sites were identified histologically. Data were discarded from rats in which the injection sites were not found to be clearly within the expected regions. The percent of nicotine-appropriate responses data and the response rate data were analyzed with a one-way repeated measures analysis of variance (ANOVA) followed by post hoc Dunnett's tests (P<0.05) where appropriate.

Results

Rats attained the training criterion for the establishment of nicotine discrimination within 62 sessions (about 4 months) from the beginning of the experiment. Two rats were excluded from the study on the basis of inaccurate placements of cannulae for the medial habenular nucleus.

The results of the substitution tests are presented in Table 1. With saline administered into each cerebral region, the mean percent of nicotine-appropriate responses ranged between 1.0 and 2.3%, and the mean total response rate per min ranged between 27.1 and 29.5 across groups.

Nicotine administered into the medial prefrontal cortex increased the mean percent of nicotine-appropriate responses in a dose dependent manner [F(3,18)=24.481, P<0.001]. The maximum percent of nicotine-appropriate responses was 88.2% at 40 µg (P<0.05), and four out of seven rats showed 80% or more nicotine-appropriate responses at this dose. Nicotine administered into the medial prefrontal cortex did not change the mean total response rate per min [F(3,18)=0.793, P>0.05].

Nicotine administered into the medial habenular nucleus failed to increase the mean percent of nicotine-appropriate responses [F(3,12)=0.845, P>0.05]. The maximum percent of nicotine-appropriate responses was 3.2% at 10 µg, and none of five rats showed 80% or more nicotine-appropriate responses. On the other hand, nicotine administered into the medial habenular nucleus decreased the mean total response rate per min [F(3,12)=19.995, P<0.001], and a significant decrease was observed at 40 µg (P<0.001).

Discussion

The present study showed that nicotine (40 µg) administered into the medial prefrontal cortex substituted for nicotine (0.5 mg/kg, SC). Together with our previous investigation of the role of the nucleus accumbens and the ventral tegmental area in mediating the DS effects of nicotine

Table 1 Results of substitution tests with nicotine administered into each brain region in rats discriminating nicotine (0.5 mg/kg, SC) from saline

Brain region and drugs	Doses (µg/rat)	Nicotine appropriate responses (%)[a]	Response rate per min[a]	n/N[b]
Medial prefrontal cortex				
Saline	0.4 µl/side	1.0±1.0	27.1±2.3	0/7
Nicotine	10	1.0±1.0	33.6±3.3	0/7
Nicotine	20	37.1±17.8	26.4±5.7	2/7
Nicotine	40	88.2±7.0	26.6±5.2	4/7
Medial habenular nucleus				
Saline	0.4 µl/side	2.3±2.3	29.5±1.9	0/5
Nicotine	5	2.2±2.0	32.7±1.6	0/5
Nicotine	10	3.2±2.1	35.5±3.0	0/5
Nicotine	40	2.2±1.2	10.1±4.5*	0/5

[a] Mean and SE during each 2-min test session

[b] n/N indicates the number of rats showing 80% or more nicotine-appropriate responses (n) out of the total number of rats tested (N)

* Significantly different from saline control (P<0.001, Dunnett's tests)

(Miyata et al. 1991; Ando et al. 1993), these results indicate the involvement of fronto-striatal circuitry. However, the fact that the dose of nicotine for substitution was higher in the nucleus accumbens (77.6% of nicotine-appropriate responses at 100 µg) than in the medial prefrontal cortex (40 µg) indicates that the nucleus accumbens is less sensitive to nicotine than the medial prefrontal cortex in manifesting the DS effects of nicotine. These results thus suggest that the involvement of the medial prefrontal cortex is more significant than that of the nucleus accumbens. On the other hand, a lesser degree of substitution (58.7% and 60.6% of nicotine-appropriate responses at 40 µg and 60 µg of nicotine, respectively) was observed in the ventral tegmental area than in the above two regions. However, the sensitivity to nicotine in the ventral tegmental area was higher than in the nucleus accumbens and was almost equivalent to that in the medial prefrontal cortex in terms of the dose range of nicotine tested. These results suggest that the ventral tegmental area is also partially involved in the DS effects of nicotine. These findings may indicate that the medial prefrontal cortex is primarily involved in the DS effects of nicotine, whereas the nucleus accumbens and the ventral tegmental area are only partially involved. Among these three regions, involvement of the nucleus accumbens is controversial, because Shoaib and Stolerman (1996) reported that nicotine administered into the nucleus accumbens did not substitute for the training dose of nicotine (SC). The contradictory results are difficult to explain, but several differences in experimental method between the two studies should be taken into consideration. For example, (1) the test doses (20–140 µg) of nicotine were quite high in our previous study compared with those (2–8 µg) used in their study; (2) the training dose (0.5 mg/kg) of nicotine was also higher in our study than that (0.2 mg/kg) used in their study; and (3) the discrimination criterion for the substitution tests was set more strictly in our study than in their study, that is, at least 80% nicotine-appropriate responses both for the first food pellet in the session and for the session overall were required for at least three consecutive training sessions in our previous study, while a difference of greater than 60% between the percentage of nicotine-appropriate responses after SC administration of nicotine and saline was required in their study. These factors may have contributed to the inconsistency in the results, but the precise reason remains unclear. As for the medial habenular nucleus, which possesses high densities of nicotinic cholinergic receptors, nicotine administered into the region did not substitute for nicotine (0.5 mg/kg, SC). These results suggest that the medial habenular nucleus is not involved in the DS effects of nicotine.

A problem in both the present and our previous studies (Miyata et al. 1991; Ando et al. 1993) was that the doses of nicotine administered into each cerebral region were relatively high in comparison with other studies (Meltzer and Rosecrans 1981; Shoaib and Stolerman 1996). For example, with nicotine administered into the dorsal hippocampus, nicotine-appropriate responses were 44.1% and 30.5% at 10 µg and 20 µg, respectively, in rats trained to discriminate nicotine (0.5 mg/kg, SC) from saline in our previous study (Ando et al. 1993), whereas the corresponding value was 54.5% at 8 µg in rats trained to discriminate nicotine (0.2 mg/kg, SC) from saline (Shoaib and Stolerman 1996). However, the dose range of nicotine in the present study was wide enough to cover both the lower doses producing no changes in the total response rate and the higher doses producing either obvious effects on nicotine-appropriate responses or decreases in the response rate. Thus, the selection of the drug doses seemed to be appropriate. It is unclear, however, why such high doses of nicotine were necessary for the substitution in the present study. One possibility is that the training criterion for nicotine discrimination was reached in 62 sessions (about 4 months) in the present study, which is a longer period than reported in other studies (Meltzer and Rosecrans 1981; Shoaib and Stolerman 1996). Therefore, tolerance to certain effects of nicotine may have developed more markedly during the training sessions in the present study than in other studies. Another more likely possibility is that cerebral regions other than those tested in the present study, or that complex neural networks rather than a single neural system are involved in mediating the DS effects of nicotine. These questions await resolution.

In conclusion, the present findings together with those of our previous studies suggest that the medial prefrontal cortex is primarily involved in the DS effects of nicotine.

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