Nicotinic Acetylcholine $\alpha 4\beta 2$ Receptor Regulates the Motivational Effect of Intracranial Self Stimulation Behavior in the Runway Method

Hidenori Sagara1*, Yoshihisa Kitamura2, Tetsuji Yae1, Kazuhiko Shibata1, Katsuya Suemaru3, Toshiaki Sendo4, Hiroaki Araki5, and Yutaka Gomita5

1Department of Pharmaceutical Information Sciences, Matsuyama University, 4-2 Bunkyo-cho, Ehime 790-8578, Japan
2Department of Pharmaceutical Care and Health Sciences, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1, Tsushima-naka, Okayama 700-8530, Japan
3Division of Pharmacy, Ehime University Hospital, 454 Shiitsukawa, Shigenobu-cho, Onsen-gun, Ehime 791-0295, Japan
4Department of Hospital Pharmacy, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama 700-8558, Japan
5Shijitsu University, 1-6-1, Nishigawara, Okayama 703-8516, Japan

Received July 1, 2008; Accepted October 7, 2008

Abstract. Recently, it was demonstrated that the priming stimulation effect (PSE) of intracranial self-stimulation (ICSS) with the runway method can be used as a model system to study the motivation that contributes to specific behaviors. It was postulated that these behaviors could be used to compare the effects of various drugs on the mechanism of motivation. In the present study, the influences of nicotine, methyllycaconitine ($\alpha 7$ nicotine-receptor antagonist), and dihydro-$\beta$-erythroidine ($\alpha 4\beta 2$ nicotine-receptor antagonist) on motivation were examined using the runway method for ICSS. Electrodes were implanted into the medial forebrain bundle of Wistar rats. The rats ran to the goal lever to get the reward (50 – 200 $\mu$A, 0.2 ms, 60 Hz) and pretrial electric stimulation (priming stimulation) in the medial forebrain bundle was performed. The experiment measured the running time from the start box until the rat pressed the goal lever for the reward stimulation. Under these reward and priming stimulation conditions, nicotine (0.2 mg/kg) induced a significant increase in running speed. The nicotine receptor antagonist $\alpha 4\beta 2$ rather than $\alpha 7$ showed a dose-dependent antagonistic action on the effect of nicotine on running speed. These results demonstrate that nicotine enhances the running speed towards the goal lever via $\alpha 4\beta 2$ nicotinic receptors and suggest that $\alpha 4\beta 2$ nicotinic receptors influence the brain mechanism of motivation.

Keywords: nicotine, methyllycaconitine, dihydro-$\beta$-erythroidine, intracranial self-stimulation, motivation

Introduction

It is well recognized that nicotine is a dependence-inducing substance. Nicotine dependence is concerned with the reinforcing effects of nicotine and the strong behavioral components and environmental cues associated with smoking (1 – 3). In essence, cigarettes provide, with each inhalation, individualized control over the amount and frequency of nicotine that is delivered to the brain, and thus over mesolimbic dopamine (DA) neurotransmission – a crucial element in the response to addictive substances (4).

It has been established that $\alpha 7$ and $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChR) are related to nicotine dependence and the reward effect in the brain (5 – 7). It was reported that the blockade of $\alpha 7$ nicotine receptors in the ventral tegmental area (VTA) attenuates the reward-facilitating effect of systemic nicotine (7). Moreover, the $\beta 2$ subunit of nAChR knock-out mice did not exhibit nicotine conditioned place preference (CPP) while the $\alpha 7$ subunit of nAChR knock-out mice did (5). This result indicates that $\alpha 4\beta 2$ nAChR is instrumental in nicotine dependence. Clinically, varenicline, a $\alpha 4\beta 2$ nAChR partial agonist, has been shown to reduce...
the free base. NaOH solution. Drug doses are expressed in terms of nicotine solution was further adjusted to pH 7.0 with sodium chloride) and were administered s.c. in an injection volume of 0.1 ml per 100 g body weight. The drugs were dissolved in physiological saline (0.9% sodium chloride) and were purchased from Research Biochemicals (Natick, MA, USA). All drugs were dissolved in physiological saline (0.9% sodium chloride) and were administered s.c. in an injection volume of 0.1 ml per 100 g body weight. The nicotine solution was further adjusted to pH 7.0 with NaOH solution. Drug doses are expressed in terms of the free base.

Recently, it was demonstrated that the priming stimulation effect (PSE) of intracranial self-stimulation (ICSS) with the runway method is a model system that can be used to study the motivation that contributes to specific behaviors (15–18). The runway method is an experimental methodology that could separately study the reward and motivational system of ICSS behavior (15–18). Moreover, the runway method of ICSS can evaluate the motivational effect by using neither the dependence drug nor the dependence animal models. Therefore, the runway method has priorities in this respect compared with the method of experimenting on CPP and intracranial self-administration. Furthermore, nicotine was reported to significantly enhance the motivational effect in ICSS with the runway method (18). However, the influence of the nAChR subtype on the motivational effect of nicotine is unclear.

In this research, the role of α4β2 and α7 nAChR in nicotine’s motivational effect was studied using the runway method for ICSS. Studying the effect in the runway method for ICSS could lead to a better understanding of the effect of nAChR on motivation.

Materials and Methods

Subjects

Male Wistar rats (Charles River, Tokyo), weighing 250–300 g at the time of surgery, were used. Three animals were housed in each plastic cage (26 × 36 × 25 cm) in a room maintained at 22 ± 2°C with a 12-h light/dark cycle (lights on at 19:00). Food and water were provided ad libitum. The experimental protocol was conducted according to the Guidelines of the Ethics Review Committee for Animal Experimentation of Okayama University Medical School.

Drugs

(−)-Nicotine tartrate was purchased from Sigma Chemical (St. Louis, MO, USA). Dihydroxy-β-erythroidine and methyllycaconitine were purchased from Research Biochemicals (Natick, MA, USA). All drugs were dissolved in physiological saline (0.9% sodium chloride) and were administered s.c. in an injection volume of 0.1 ml per 100 g body weight. The nicotine solution was further adjusted to pH 7.0 with NaOH solution. Drug doses are expressed in terms of the free base.

Surgery

Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg, Nembutal; Dainippon Sumitomo Pharma Co., Tokyo). Stainless steel electrodes consisting of a twisted pair of stainless steel wires (tip diameter: 0.2 mm), which were insulated except at the top 0.5 mm of the tips, were stereotaxically implanted (SR-5; Narishige Scientific Instrument Lab, Tokyo) into the medial forebrain bundle (MFB) at the level of the posterior hypothalamus (flat skull coordinates: 2.8 mm posterior to the bregma; 1.8 mm lateral to the sagittal suture; and 8.5–9.0 mm below the skull surface) (19). After the electrodes were inserted into the MFB, they were connected to the pins of a small socket fixed to the skull using dental cement, and 2 screws were driven into the skull. At least 7 days were allowed for the rats to recover before beginning training for ICSS behavior in a Skinner box.

Apparatus

A Skinner box (30.8-cm-wide, 25.4-cm-long, and 27.7-cm-high) and runway apparatus (Neuroscience, Tokyo) were used in this experiment. The runway apparatus was made from 5-mm acrylic board and consisted of a start box (26.5-cm-wide, 26-cm-long, and 30-cm-high), controlled start door (26.5-cm-wide, 30-cm-high) that opened by dropping down, runway (18-cm-wide, 180-cm-long, 30-cm-high), and priming box (30-cm-wide, 30-cm-long, and 30-cm-high). A retractable lever was set at the end of the runway 7-cm above the floor (the goal lever). Stimulation was from constant current stimulators with 0.2-ms pulses of 60-Hz alternating current. The stimulation current was individually adjusted for each rat.

Experimental procedures

One week after surgery the rats were tested for self-stimulation in the Skinner box. The rats that pushed the lever at a stable rate for three days in the Skinner box (50 presses per minute) were used for the experiment. Each rat was trained in the runway until its running speed stabilized. Upon reaching the goal end and pressing the lever, they received a reward stimulation of 0.2-ms pulses of 60-Hz alternating current. The current was set at 50–200 μA to produce a maximal priming stimulation effect (PSE: a maximal difference between the running speeds on primed versus unprimed trials). In a trial, the rat was removed from the runway as soon as it received reward stimulation and placed in the priming box that stood beside the runway, where 25 s later it received 10 priming stimulations (1 stimulation per second, same parameters as the reward). When priming stimulation ceased, the rat was transferred from the
priming box to the start box of the runway. Five seconds after cessation of the priming stimulation, the start box door opened. If the rat ran to the goal lever and pushed it, the rat received reward stimulation. The running time from the opening of the door to pressing the goal lever was recorded by microcomputer. The runway method of Gallistel et al. was performed in this experiment (20, 21).

**Experiment 1: Measurement technique for determining the motivational effect of nicotine on ICSS in the runway method**

This experimental procedure involved 30 trials and consisted of pre-sessions, baseline sessions, and test sessions (Fig. 1). Each session comprised 10 trials. In the pre-session, the rat received 10 priming stimulations and a reward stimulation for pushing the goal lever. In the baseline session, the rats received 5 priming stimulations and a reward stimulation for pushing the goal lever. In the test session, after the administration of saline or nicotine, rats received 5 priming stimulations and a reward stimulation for pushing the goal lever. Saline and nicotine were administered 30 min before the baseline or test session. The motivational effect of the nicotine was determined as the ratio of the baseline running time to the test-session running time. When the time for the test session was significantly lower than the time of the baseline session, it was determined that the motivational effect of nicotine was positive.

**Experiment 2: Effect of nicotine and nicotinic acetylcholine–receptor subtype antagonists on priming stimulation effects in rats**

This experimental procedure involved 30 trials and consisted of pre-sessions, baseline sessions, and test sessions. Each session comprised 10 trials. In the pre-session, the rat received 10 priming stimulations and a reward stimulation for pushing the goal lever. In the baseline session, rats received 5 priming stimulations and a reward stimulation for pushing the goal lever. In the test session, after the administration of nicotine and nicotine antagonist, rats received 5 priming stimulations and a reward stimulation for pushing the goal lever. Saline (1 ml/kg, s.c.) was administered 30 min before the baseline session, and nicotine was administered 30 min before the test session. Nicotinic receptor antagonists (dihydro-β-erythroidine and methyllycaconitine) were administered 45 min before the test session.

**Experiment 3: Measurement of spontaneous locomotor activity**

The spontaneous locomotor activity was measured using the ANIMEX Apparatus (Muromachi Kikai Inc., Tokyo). The level of spontaneous activity in a novel environment was measured for 10 min after each rat was placed individually in a clear cage (40-cm-wide, 35-cm-long, 38-cm-high). The level of activity was estimated as the number of interruptions of magnetic field by the animal’s horizontal movements. Saline (1 ml/kg, s.c) was administered 30 min before the measurement. Nicotine was administered 30 min before the drug session. Nicotinic receptor antagonists (methyllycaconitine and dihydroxy-β-erythroidine) were administered 45 min before the measurement.

**Data analysis**

Values are shown as a group mean and as standard errors of the mean. The drug session values were expressed as a percentage of the control session values. The results were evaluated statistically using Student’s t-test or one-way analysis of variance (ANOVA) followed by the Scheffé’s test. The significance level was set at P<0.05.
Histology

At the end of the experiment, all subjects were given an overdose of chloral hydrate and perfused intracardially with saline and formalin (4%). Coronal brains sections were generated and were stained with crystal violet to determine the placement of electrodes.

Results

The experimental design for measuring the motivational effect using the runway method is shown in Fig. 1A. The running speed in the pre-session was significantly higher than that seen during the baseline or test sessions \[F(2,15)=49.834, P<0.01\]. There were no significant differences in the running speeds of the baseline and test sessions (Fig. 1B). The motivational effect of nicotine is shown in Fig. 2. Nicotine (0.2 mg/kg) produced an increase (115% – 136%, mean value = 126.5%) in the running speed with ICSS behavior \( P<0.01 \). Figure 3 shows the effect of vehicle (saline), methyllycaconitine \( \alpha_7 \) nicotine receptor antagonist; 1, 2, and 5 mg/kg), and dihydro-\( \beta \)-erythroidine \( \alpha 4/2 \) nicotine receptor antagonist; 1, 2, and 5 mg/kg) on the running speed for ICSS behavior of rats that received priming stimulation. Saline had no effect on the running speed for ICSS behavior of rats that received priming stimulation. Similarly, there were no effects of methyllycaconitine \( F(3,20)=0.3, \text{NS} \) or dihydro-\( \beta \)-erythroidine \( F(3, 5)=0.7, \text{NS} \) injection on the running speed for ICSS behavior of rats that received priming stimulation. Figure 4 shows the effect of methyllycaconitine (1, 2, and 5 mg/kg) and dihydro-\( \beta \)-erythroidine (1, 2, 5, and 10 mg/kg) with nicotine (0.2 mg/kg) on the running speed for ICSS behavior of rats that received priming stimulation. Nicotine (0.2 mg/kg) produced an increase in the running speed for ICSS behavior. The administration of methyllycaconitine did not significantly block this facilitation \( F(3,0)=0.8, \text{NS} \). On the other hand, the administration of dihydro-\( \beta \)-erythroidine significantly blocked this facilitation \( F(3,25)=5.7, P=0.002 \). Post-hoc analysis with Scheffé’s test showed that this effect was significant with dihydro-\( \beta \)-erythroidine at doses of 2, 5, and 10 mg/kg per side.

None of the doses of nicotine, methyllycaconitine and dihydroxy-\( \beta \)-erythroidine affected locomotor activity \( F(8,45)=0.9 \text{NS} \) (Table 1).
In the present study, 0.2 mg/kg of nicotine enhanced the running speed due to PSE in the runway method with ICSS. In addition, it was demonstrated that the nicotine-receptor antagonist $\alpha_4\beta_2$ shows a dose-dependent antagonistic action on running speed enhancement by nicotine, but this effect was not observed with nicotine-receptor antagonist $\alpha_7$. Running speed enhancement by PSE in the runway method is due to the motivational effect of ICSS (22). These present results suggest that nicotine has an effect on enhancing motivation. Furthermore, these results suggest that nicotine’s motivational effect is via $\alpha_4\beta_2$ nicotine receptors.

Table 1. Effect of nicotine and nicotine-receptor antagonists on locomotor activity in rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg), n = 6</th>
<th>Locomotor activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>137.2 ± 18.6</td>
</tr>
<tr>
<td>Nicotine</td>
<td>0.2</td>
<td>143.1 ± 11.8</td>
</tr>
<tr>
<td>Methyllycaconitine</td>
<td>1</td>
<td>134.9 ± 21.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>141.8 ± 14.9</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>126.1 ± 24.7</td>
</tr>
<tr>
<td>Dihydro-β-erythroidine</td>
<td>1</td>
<td>131.9 ± 11.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>128.7 ± 17.3</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>144.0 ± 22.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>124.0 ± 25.9</td>
</tr>
</tbody>
</table>

Nicotine and saline were administered subcutaneously 30 min before the measurement. Methyllycaconitine and dihydro-β-erythroidine were administered subcutaneously 45 min before the measurement. Each value represents the mean of six rats. (one-way ANOVA followed by Scheffé’s test).

The running speed in the runway method with ICSS can be regulated by priming stimulation strength (23, 24). Priming stimulation strength is the variable factor for the motivation after the goal lever is pressed in the runway method (25). Therefore, running speed indicates the scale of the motivational effect with ICSS behavior. In this study, drug efficacy was evaluated using a method comparing the running speed of the control and drug sessions with five priming stimulations. The running speed of five priming stimulations was able to confirm the increase/decrease in drug efficacy (15, 18). Thus, the drug efficacy of nicotine and nAChR antagonists was tested with this experimental setup.

It was reported previously that nicotine induced a significant increase in running speed (18). This study confirmed that nicotine (0.2 mg/kg) significantly enhanced the motivational effect on ICSS behavior using the runway method. Thus, this study suggests that nicotine acts on a motivational neuromechanism in the brain. Although the details of this neuromechanism are currently unclear, the increase in running speed is thought to indicate that nicotine influences motivational neural circuitry. It has been confirmed that impulses along the mesolimbic dopamine neural circuitry projecting to the thalamus have a significant association with the expression of motivation (26, 27). There are many nicotine receptors, including $\alpha_7$ and $\alpha_4\beta_2$ nicotine receptors, in the DA nervous in the VTA (28 – 30). Nicotine directly acts on the DA nervous system in the VTA (31 – 36). The rapid and transient increases in DA release in the nucleus accumbens (NAC) by VTA stimulation that inhaled nicotine produces was thought to be the major factor of compulsive substance-seeking behavior.
behavior and nicotine dependence in humans (37 – 43). Moreover, it is recognized that the craving of smoking is provoked by decreases in DA release from the NAC (44 – 47). Therefore, it is thought that the enhancement effect on the running speed of nicotine is due to the effect of the direct action on the DA nucleus in VTA. Recently, it has been reported that the α4β2 nicotine receptors influence DA release from the VTA. In contrast, the α7 nicotine receptor does not influence the acceleration of DA release (48, 49). In the present study, the administration of dihydro-β-erythroidine, an α4β2 nicotine–receptor antagonist, demonstrated a dose-dependent antagonistic effect on running speed. However, the administration of methyllycaconitine, an α7 nicotine–receptor antagonist, did not demonstrate an antagonistic effect on running speed enhancement due to nicotine. There were no independent influences on the running speed of PSE due to the α7 nicotine receptor and α4β2 nicotine–receptor antagonists. Therefore, it is suggested that the enhancement effect on running speed by nicotine is via the α4β2 nicotine receptor. These results suggest that the enhancement effect on running speed by nicotine involves DA release via α4β2 nicotinic receptors in the mesolimbic DA neurons. Moreover, varenicline, an α4β2-nAChR partial agonist, reduced DA release from the NAC following nicotine administration (8, 50). Varenicline has a smaller maximal effect than nicotine. When not smoking, varenicline has a mild nicotine-like effect and can relieve craving and withdrawal symptoms, which is a result of its relative functional efficacy versus nicotine (50). These clinical results support the role of α4β2 nAChR in the motivational effect of nicotine.

The runway method is an experimental methodology that could separately study the reward and motivational effect of ICSS behavior (15 – 18). The methodology of this present study is a technique of measuring the motivational effect. It has been reported that the blockade of α7 nicotine receptors in the VTA attenuates the reward-facilitating effect of nicotine (7). Consequently, it is thought that the α7 nicotine receptor is concerned with not only the motivational effect but also the reward effect. This present study suggests that nicotine enhances running speed in the runway method with ICSS and the α4β2 receptor plays an important role in this enhancement. These findings suggest that nicotine, via the α4β2 nicotinic receptor, affects motivation in the runway method with ICSS.

Acknowledgment

This study was supported by a grant from the Smoking Research Foundation of Japan.

References

17. Sagara H, Kitamura Y, Sendo T, Araki H, Gomita Y. Effect of diazepam to the runway method using priming stimulation of