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Endogenous acetylcholine modulates impulsive action via α4β2 nicotinic acetylcholine receptors in rats

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ABSTRACT

Nicotine has been well established as an impulsive action-inducing agent, but it remains unknown whether endogenous acetylcholine affects impulsive action via nicotinic acetylcholine receptors. In the present study, the 3-choice serial reaction time task (3-CSRTT), a simple and valid assessment of impulsive action, was employed. Male Wistar/ST rats were trained to detect and respond to 1-s flashes of light presented in one of three holes until stable performance was achieved. Following training on the 3-CSRTT, rats received intracerebroventricular injections of the preferential α4β2 nicotinic acetylcholine receptor antagonist dihydro-β-erythroidine (DHβE; 0, 3, 10, and 30 μg) or the selective α7 nicotinic acetylcholine receptor antagonist methyllycaconitine (MLA; 0, 3, 10, and 30 μg) 5 min before test sessions. Injection of 10 μg of DHβE significantly suppressed premature responses, an index of impulsive-like action, without changing other behavioral parameters. On the other hand, MLA infusions failed to affect impulsive-like action at any dose. These results suggest that the central α4β2 nicotinic acetylcholine receptors that enable a provoking effect of endogenous acetylcholine play a critical role in impulsive action. Substances that modulate nicotinic acetylcholine receptors, especially the α4β2 subtype, may be beneficial for the treatment of psychiatric disorders characterized by lack of inhibitory control.

1. Introduction

Activation of cholinergic pathways by nicotine has been associated with impulsive choice (Ohmura et al., 2005) and impulsive action (Yakir et al., 2007) in humans. Impulsive acts are often viewed as everyday normal behavior; however, excessive levels of impulsivity are associated with several psychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD: Solanto, 2002), schizophrenia (Enticott et al., 2008), and borderline personality disorder (Dougherty et al., 1999). In rodents, the impulsivity-provoking properties of nicotine have been well demonstrated in a delay discounting task (Dallery and Locey, 2005) and in a 5-choice serial reaction time task (5-CSRTT, Blondel et al., 2000; van Gaalen et al., 2006). Investigating central cholinergic function will contribute to further understanding of the neural correlates and neuropharmacological substrates of impulsivity.

The 5-CSRTT is one of the most prevalent animal models of impulsive action and is based on the human continuous performance test (Wilkinson, 1963; Robbins, 2002). In this task, a light in the aperture of one of five holes is briefly and randomly flashed, and animals are required to make a nose poke into the flashed hole to get a food pellet. Responses that occur before the presentation of the stimulus light are described as premature responses and result in a time-out period. They are regarded as impulsive-like action (Robbins, 2002). Thus, premature responses reflect a simple form of impulsive-like action in rodents, and the 5-CSRTT is suitable not only for screening novel treatments but also for revealing the neural basis of impulsive action.

The nicotinic acetylcholine receptor is a pentameric combination of α and β subunits. In the rat central nervous system, six α subunits (α2–α7) and three β subunits (β2–β4) have been described. Several combinations of these subunits have been detected, of which the α4β2 and α7 subtypes are the most widely distributed (Galzi and Changeux, 1995; Léna and Changeux, 1997; Cordero-Erausquin et al., 2000).

The effects of nicotine on impulsive action have been well established, though only a few studies have examined whether endogenous acetylcholine affects impulsive action via nicotinic acetylcholine receptors. Grottick and Higgins (2000) failed to detect any effects from systemic administration of nicotinic acetylcholine receptor antagonists by itself on impulsive-like action in the 5-CSRTT. However, nicotinic acetylcholine receptor antagonists blocked the effects of nicotine on impulsive-like action. It may not have been

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possible to observe a decreasing number of premature responses owing to floor effects; this study used Lister hooded rats, which rarely respond prematurely (<10 per session, Broersen and Uylings, 1999) in the 5-CSRTT. Blondel et al. (2000) reported that systemic administration of nicotinic acetylcholine receptor antagonists tended to decrease premature responses in the 5-CSRTT, but the effects were not significant. Generally speaking, systemically administered compounds have some difficulty in reaching the brain because of the blood–brain barrier, even if they are centrally acting drugs (Turek et al., 1995). Moreover, systemic administration often causes undesirable side effects due to peripheral actions (Curzon et al., 1996). Because of the disadvantages of systemic administration, the experiments by Blondel et al. (2000) may have failed to achieve significance. Direct infusions of nicotinic acetylcholine receptor antagonists into the brain may elicit effects on impulsive-like action.

To determine whether endogenous acetylcholine affects impulsive action via nicotinic acetylcholine receptors and to determine the subtype of nicotinic acetylcholine receptors responsible, we assessed the effects of intracerebroventricular (i.c.v.) injections of selective α4β2 and α7 nicotinic acetylcholine receptor antagonists on impulsive action using a 3-choice serial reaction time task (3-CSRTT), which is a simplified version of the 5-CSRTT (Tsutsui-Kimura et al., 2009).

2. Materials and methods

2.1. Subjects

Thirteen male Wistar/ST rats, supplied by Nippon SLC Co. Ltd. (Hamamatsu, Japan), were used. They were housed in groups of two to four rats under an alternating light–dark cycle (light from 7 p.m. to 7 a.m.) at approximately 21 °C and a relative humidity 40–50%. When the rats were 9 weeks old (270–290 g), we started to restrict their food intake. Thereafter their body weights were maintained at 85% of rats under free-feeding conditions. The daily food of rats in the home cages was purchased from CLEA JAPAN, Inc. (CE-2; Tokyo, Japan) and was given after each daily session. Food intake in the home cages was 10–15 g in the training period and 10–12 g (in addition to the daily food , another type of food 2–3 g in the operant box; see also Section 2.4. Three-choice serial reaction time task) in the experimental period. Water was available ad libitum. The treatment of animals complied with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of Hokkaido University.

2.2. Drugs

Dihydro-β-erythroidine hydrobromide (DHβE) and methyllycaconitine citrate (MLA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). DHβE is more selective for α3β2, α2β2, α4β2, and α4β4 (10–1000-fold) than α7-containing receptors (Harvey and Luetje, 1996; Khiroug et al., 2004). MLA is >1000-fold more selective for the α7 subtype than other subtypes (Ward et al., 1990). Both compounds were dissolved in 0.01 mol/l phosphate-buffered saline (PBS) to adjust the pH of resulting solutions to 6.9–7.0.

2.3. Apparatus

Aluminum operant chambers measuring 26×26×26 cm (Med Associates Inc., St. Albans, VT, USA) were used. The curved rear wall of each chamber contained nine 2.5-cm^2 holes, which were 2.2-cm deep and 2.3 cm above floor level. Each hole had an infrared photocell beam for detection of nose poke responses and a 2.8-W bulb at its rear. Every other hole was sealed so that only the three centrally positioned holes were accessible. A food magazine was located on the opposite wall of the chamber, and a house light was located at the top of that wall. The apparatus was controlled by a computer program written in the MED-PC language (Med Associates Inc., St. Albans, VT, USA).

2.4. Three-choice serial reaction time task

The training procedure and the task sequence employed in the 3-CSRTT were as detailed as in previous reports (Ohmura et al., 2009; Tsutsui-Kimura et al., 2009). Briefly, when the task started, the house light was illuminated. After a fixed inter-trial interval (5 s), one of the three holes was illuminated randomly and briefly (stimulus duration). Nose poking during the inter-trial interval was recorded as a premature response and resulted in the switching off of all lights (time-out: 5 s). The same trial was restarted immediately after finishing the time-out period. This parameter was regarded as an index of impulsive action. Nose poking into the lit hole while it was illuminated or within 5 s of limited hold was recorded as a correct response and was rewarded by the delivery of a food pellet (45 mg dustless precision pellets, Bio-serv, Frenchtown, NJ, USA). As an index of attentional function, accuracy (the percentage of correct responses) was calculated. Nose poking into another hole was recorded as an incorrect response and resulted in a 5 s time-out. The correct response latency, an index of motor function, and the reward latency, an index of motivation and/or appetite, were also measured. The reward latency was the time between a correct response and nose poking into the food magazine. When a rat failed to nose poke within the limited hold, the trial was recorded as an omission and resulted in a 5 s time-out. This parameter was also regarded as an index of motivation and/or appetite. After a food pellet was delivered to and collected by a rat, the house light was switched off for 2 s to allow the rat to eat the pellet before the next trial was started. The start of the next inter-trial interval was signaled by turning on the house light. Additional nose poking into any of the three holes prior to food collection was recorded as a perseverative response and resulted in a 5 s time-out. This parameter was regarded as an index of compulsive behavior. Because trials were initiated automatically, we did not set a time restriction for this task. Each session consisted of 100 trials. All rats in the present study finished 100 trials within 32 min. Training was conducted for one session per day and six sessions per week.

At the beginning of the training schedule, stimuli lasted 30 s. Depending on individual performance, the duration was progressively reduced to 1 s (by stepping to 15, 10, 5, 3, 2, 1.5, and 1 s). When a rat attained the criteria of >80% accuracy (percentage of correct responses) and <20 omissions in a session, the stimulus duration was reduced in the next session.

We used six behavioral parameters:

(a) Premature responses (no. per session)
(b) Accuracy (percentage of correct responses): [correct responses/(correct and incorrect responses)]×100
(c) Omissions (no. per session)
(d) Perseverative responses (no. per session)
(e) Correct response latency (s)
(f) Reward latency (s)

Training was completed when rats reached the target phase (stimulus duration 1 s) and showed stable performance. After the completion of training, the stimulus duration was fixed at 1 s regardless of performance. We set the criteria for determining stable performance as follows: changes in premature responses stayed within ±25%, accuracy stayed within ±5%, and percent response omissions were less than 20 for at least three consecutive sessions.

2.5. Surgery

After completing the training, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and fixed in a stereotaxic frame (Narishige, Tokyo, Japan). Stainless-steel guide cannulas (24 gauge, 9 mm long) were unilaterally implanted 2 mm above the lateral ventricle with coordinates 0.8 mm posterior to the bregma, 1.5 mm lateral to the midline, and 2.2 mm ventral to the dura (Paxinos and Watson 1996). Dummy cannulas (30 gauge, 9 mm long) were also