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Correlation Between Behavioral Alteration to Chronic Cocaine Treatment and G-Protein ADP-Ribosylation in Mice

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Abstract The role of Gi-proteins on cataleptic responses induced by SCH23390, a dopamine D_1 receptor antagonist, and haloperidol, a mainly dopamine D_2 receptor antagonist, one day after chronic cocaine treatment in mice was examined by injecting intravenously (i.v.) pertussis toxin, which catalyzes adenosine diphosphate (ADP)-ribosylation of Gi-proteins. SCH23390- and haloperidol-induced catalepsy was potentiated 3-24 h after administration of a single dose (5 and 10μg/kg i.v.) of the toxin, but not at 1 μg/kg. It was apparent that the longer the time interval between pertussis toxin and dopamine antagonists treatments, and the higher the dose of pertussis toxin, the greater were the cataleptic responses. Mice given subcutaneous administration (s.c.) of cocaine (10mg/kg) once every other day for 15 days (a total of 8 injections) exhibited an attenuated SCH23390-induced catalepsy (SCH23390 catalepsy) and an enhanced haloperidol-induced catalepsy (haloperidol catalepsy) one day after the last cocaine injection. The inhibitory effect of chronic cocaine treatment on SCH23390 catalepsy was reversed by a single pretreatment with pertussis toxin (1 and 5μg/kg i.v.), whereas the enhancing effect of that on haloperidol catalepsy was further enhanced with same dose of toxin. These results suggest that there may be an interrelationship between Gi-protein ADP-ribosylation and D_2 receptor subsensitivity (enhanced haloperidol catalepsy) induced by chronic cocaine treatment, whereas an opposite relationship exists between D_1 receptor supersensitivity (attenuation of SCH23390 catalepsy) induced by chronic cocaine treatment and the ribosylation. Accordingly, behavioral sensitization (reverse tolerance) seen one day after chronic cocaine treatment, which results in D_1 receptor supersensitivity and D_2 receptor subsensitivity may not involve Gi protein ADP-ribosylation.

Key words : Cocaine, SCH23390, Haloperidol, Pertussis toxin, Catalepsy, Gi-protein,

Introduction

Chronic exposure to psychostimulants, such as cocaine and amphetamine, produces a progressive augmentation of the acute behavioral effects of these drugs[1,2]. These behavioral supersensitive responses in animals have been considered as analogous to the psychostimulant - induced psychosis and schizophrenia-like symptoms in humans. The motor-stimulant effects of cocaine and amphetamine are believed to result from activation of the mesolimbic dopamine system[3], which projects from A10 dopamine neurons to

innervate nucleus accumbens and a number of limbic brain nuclei[4,5]. The desensitization of presynaptic striatal dopamine receptors is one of several putative mechanisms thought to be involved in the development of this behavioral sensitization to cocaine[6,7].

On the other hand, chronic treatment of mice with cocaine results in attenuated and enhanced sensitivities to the cataleptic actions of SCH23390 and haloperidol, respectively, one day after a 15 day cocaine pretreatment[8]. The attenuated cataleptic response to SCH23390 15 days after exposure to chronic cocaine treatment could be interpreted as a development of supersensitivity of dopamine D_1 receptors, which may correspond to sensitization to cocaine. The enhanced haloperidol catalepsy may represent a state of subsensitivity of dopamine D_2 receptors, which corresponds to tolerance development to cocaine. This would indicate that the dopamine D_1 receptor may be the receptor involved in psychostimulant - induced sensitization[8].

There is substantial evidence for the existence of multiple dopamine receptor subtypes, most commonly classified as D_1, which stimulates production of cyclic AMP, and D_2, which either inhibits or has no effect on c-AMP production[9,10]. Inhibitory regulation of dopamine neurons is mediated by dopamine autoreceptor (D_2 receptor) and $GABA_B$ receptor-mediated opening of potassium channels. Increased potassium conductance by either receptor is Gi protein dependent[11]. Gi-protein activated by D_2 receptor stimulation decreased cyclic AMP synthesis. Recently, an action of Gi-proteins in the ventral tegmental area has been implicated in behavioral sensitization to cocaine[12,13]. The purpose of this study is to investigate whether or not D_1 receptor supersensitivity and D_2 receptor subsensitivity induced by chronic cocaine treatment are implicated in the Gi-protein ADP-ribosylation.

Methods

Animals

Healthy male ddY albino mice (5 weeks, 25 -30g), purchased from Kyudo Animal Laboratory (Saga, Japan), were allowed free access to food and water. The mice were housed and all trials were carried out at an environmental temperature of 23±1℃, with a 12-h light-dark cycle (light on 7 : 00 a.m. -light off 7 : 00 p.m.). We used 6-week-old mice for a 15-day pretreatment with cocaine at the start of the study. All experiments were thus performed with 8-week-old mice weighing 35-40g.

Measurement of catalepsy

Cataleptic responses were measured by means of the bar method by placing mice individually on a plastic board (25×35cm) with a horizontal wire bar (diameter 3mm, sealed with vinyl) suspended 5cm above the floor. The observers were blinded with respect to treatment. The animal's front paws were placed gently on the bar, and the time taken for the mouse to remove both paws from the bar was recorded. We observed cataleptic responses 15min after SCH23390 (0.3mg/kg) and 30min after haloperidol (0.3 mg/kg) (3, 6 and 24h, respectively after pertussis toxin).

Administration of drugs

To examine the dose-related effects of catalepsy, intravenous injection (i.v.) of pertussis toxin (1, 5 and 10μg/kg) or saline was administered 2.75, 5.75 and 23.75h before intraperitoneal administration (i.p.) of SCH23390 (0.3mg/kg), or 2.5, 5.5 and 23.5h before haloperidol (0.3mg/kg i.p.). Furthermore, mice (6 week-old, 30-32g) received subcutaneous administration (s.c.) of cocaine (10mg/kg) or saline (5ml/kg s.c.) once every other day for 15 (8 injections) days. Pertussis toxin (1 and 5μg/kg i.v.) was administered immediately after last injection of cocaine or saline. The cataleptic responses induced by SCH23390 and haloperidol were observed 24h after pertussis toxin and last cocaine treatments.

Drugs

The used drugs were pertussis toxin (Sigma, St. Louis, MO), cocaine hydrochloride (Takeda, Osaka, Japan), SCH23390 hydrochloride (RBI, Natick, MA, USA) and haloperidol hydrochloride (Dainippon, Osaka, Japan). All drugs were dissolved in

saline and an equal volume of vehicle (5ml/kg) was injected.

Statistics

The data are expressed as mean ± S.E.M. Each group consisted of 6-10 animals. Dose responses of pertussis toxin were evaluated by one-way ANOVA (Table 1) and the data from the combined treatment trial were analyzed by the two-way ANOVA (Table 2). Differences between two data points were compared by the Newman-Keuls test. A difference at $P<0.05$ was considered statistically significant.

Results

Effects of pertussis toxin on cataleptic responses induced by SCH23390 or haloperidol

Pertussis toxin at 1μg/kg i.v. did not affect SCH23390- and haloperidol-induced cataleptic responses at all time periods (3, 6, 24h). At 5μg/kg i.v. of pertussis toxin did not affect either SCH23390 or haloperidol catalepsy at 3h, but increased it during the subsequent 24h periods, whereas it enhanced SCH23390 catalepsy at the 6 and 24h periods. At 10μg/kg i.v. of the toxin cataleptic responses induced by SCH23390 or haloperidol were enhanced at all time periods (3, 6 and 24h) tested (Table 1).

Single administration of pertussis toxin (i.v.) inhibited motor activity (by Animex) in a dose dependent manner (data not shown).

Effects of pertussis toxin on altered catalepsy induced by dopamine receptor antagonists in chronic cocaine pretreatment.

Data of effects of the different groups on SCH23390-induced cataleptic responses are shown in Table 2. In chronic cocaine treatment, pertussis toxin (single), or chronic cocaine + pertussis toxin, two way ANOVA showed significant chronic cocaine treatment effect [$F(1,24)=21.947$ ($P<0.0001$)], pertussis toxin effect [$F(1,24)=20.601$ ($P<0.0001$)], and chronic cocaine x pertussis toxin interaction effect [$F(1,24)=18.036$ ($P<0.0005$)]. Data of effect of these groups on haloperidol-induced cataleptic responses

Table 1. The effects of pertussis toxin on SCH23390- or haloperidol-induced cataleptic responses.

Pretreatment (μg/kg)	Cataleptic responses (min) 3h	(N)	6h	(N)	24h	(N)
			SCH23390-catalepsy			
Saline	6.2±0.4	(7)	5.6±0.6	(6)	5.3±0.7	(7)
Pertussis toxin (1)	5.7±0.4	(7)	6.0±0.5	(6)	5.4±0.4	(7)
Pertussis toxin (5)	8.3±0.4	(6)	10.3±1.0*	(6)	12.3±1.3**	(6)
Pertussis toxin (10)	12.6±1.9**	(6)	14.3±2.2**	(6)	15.0±2.5**	(6)
	$F(3,22)=10.753$		$F(3,20)=10.695$		$F(3,22)=13.191$	
	$P<0.0001$		$P<0.0002$		$P<0.0001$	
			Haloperidol-catalepsy			
Saline	2.0±0.3	(10)	2.5±0.3	(10)	2.3±0.3	(10)
Pertussis toxin (1)	2.2±0.3	(10)	2.7±0.4	(10)	2.7±0.2	(10)
Pertussis toxin (5)	2.2±0.1	(10)	5.4±0.6*	(10)	6.4±0.6*	(10)
Pertussis toxin (10)	3.2±0.2*	(10)	8.2±1.2**	(10)	8.9±1.7**	(10)
	$F(3,20)=6.243$		$F(3,20)=14.871$		$F(3,22)=13.372$	
	$P<0.005$		$P<0.0001$		$P<0.0001$	

SCH23390 (0.3mg/kg i.p.)-or haloperidol (0.3mg/kg i.p.)-induced catalepsy was observed 3, 6 and 24h after pertussis toxin (1, 5 and 10μg/kg i.v.) or saline. We administered SCH23390 and haloperidol 15 and 30min, respectively before the observation. *$P<0.05$, **$P<0.002$ as compared to saline-group (N) : Number of animals

Table 2. Effects of chronic cocaine on cataleptic responses induced by SCH23390 or haloperidol, and effects of pertussis toxin : case of early withdrawal period (1 day)

Pretreatment	Cataleptic responses (min) SCH23390 (N)	Haloperidol (N)
Saline (5ml/kg×8)	5.3±0.7 (7)	2.3±0.3 (7)
Cocaine (10mg/kg×8)	0.9±0.2* (7)	5.0±0.4* (7)
Pertussis toxin (1μg/kg)	5.4±0.4 (7)	2.7±0.2 (7)
Cocaine (10mg/kg×8)+ Pertussis toxin (1μg/kg)	5.2±0.8† (7)	9.4±1.2‡†§ (7)

Groups of mice received saline (5ml/kg s.c.) and cocaine (10mg/kg s.c.) once every other day for 15 day (8 injection). Pertussis toxine (1μg/kg i.v.) was administered immediately after the last injection of saline or cocaine. SCH23390- or haloperidol-induced catalepsy was observed 24 h after pertussis toxin or saline. Further explanation as in Table 1. *$P<0.05$‡, §$P<0.002$ as compared to saline (*,‡), cocaine (†) and the corresponding pertussis toxin (§)

are also indicated in Table 2. There was a significant chronic cocaine treatment effect [$F(1,24)=55.801$ ($P<0.0001$)], pertussis toxin effect [$F(1,24)=14.078$ ($P<0.001$)], or chronic cocaine x pertussis toxin interaction effect [$F(1,24)=10.291$ ($P<0.005$)].

The Newman-Keuls test comparing results between two treatment groups showed that on day 1 after chronic cocaine treatment (10mg/kg s.c. for 15 days, total of 8 injections), SCH23390 (0.3mg/kg i.p.) inhibited cataleptic responses (Saline vs. cocaine, $P<0.0001$), and that of haloperidol (0.3mg/kg i.p.) enhanced (Saline vs. cocaine, $P<0.05$). The attenuated SCH23390 cataleptic response was reversed 24 h after 1 μg/kg of pertussis toxin (Saline vs. Cocaine+purtussis toxin, $P=0.9187$, not significantly different), whereas the enhanced haloperidol catalepsy was further potentiated 24h after 1μg/kg of pertussis toxin (Cocaine vs. Cocaine+pertussis toxin, $P<0.001$) (Table 2).

Discussion

In this study, SCH23390-induced catalepsy was attenuated and haloperidol-induced catalepsy was enhanced 24h after the last injection of chronic cocaine treatment for 15 days (8 injections). These results were compatible with the previous report[8]. Dopamine D_1 and D_2 receptor activities interact synergistically to stimulate locomotor activity[14]; locomotor activity mainly depends on the activation of the dopamine D_1 receptor[15]. It has been reported that chronic dopamine D_2 receptor stimulation by dopamine D_2/D_3 receptor agonist such as quinpirole[16] and bromocriptine[17] produces subsensitization, whereas chronic dopamine D_1 receptor agonist, SKF38393, administration produces supersensitivity[16]. Furthermore, chronic administration of dopamine precursor, L-dopa, enhances dopamine-sensitive adenyl cyclase activity[18], suggesting that the sensitivity of the dopamine D_1 receptor-coupled cyclase might be increased. The behavioral sensitization induced by chronic exposure to an indirect dopamine receptor agonist such as cocaine may be dependent on the role of dopamine D_1 receptors. If that was the case it would indicate that the D_1 receptor may be mainly involved in psychostimulant-induced sensitization, which in man is manifested as psychostimulants-induced psychosis and schizophrenia-like symptoms[1,19,20]. Sensitization to psychostimulants is associated with decrease in the capacity of dopamine receptor agonists to inhibit the firing frequency of dopamine neurons[21,22]. A decrease in inhibitory regulation of the dopamine cells by daily exposure to psychostimulants augments mesolimbic dopamine transmission and behavioral sensitization[23,24].

Somatodendritic D_2 autoreceptors are coupled to an adenosine triphosphate (ATP)-

sensitive potassium channel by a pertussis toxin sensitive Gi-protein[11,25,26]. We had expected that in this study, if Gi-protein ADP-ribosylation is implicated in the behavioral sensitization induced one day after chronic cocaine treatment, pretreatment with pertussis toxin would result in the potentiation of either an enhanced haloperidol catalepsy or an attenuated SCH23390 catalepsy. However, in this study, the intravenous pretreatment with pertussis toxin further increased the enhanced haloperidol catalepsy and antagonized the attenuated SCH23390 catalepsy. Intracerebroventricular injection effects of pertussis toxin also were simillar to these resulsts (unpublished observation). Thus, there may be an interrelationship between dopamine D_2 receptor subsensitivity induced by chronic cocaine treatment and Gi-protein ADP-ribosylation, but an opposite relationship between dopamine D_1 receptor supersensitivity and the ribosylation, despite dopamine D_1 receptors is pertussis toxin-insensitive.

It has been postulated that neuroleptic-induced catalepsy results from the blockade of dopamine receptors in the neostriatum and nucleus accumbens[27]. Dopamine, acting as an inhibitory transmitter, functions to regulate the activity of cholinergic interneurons in neostriatum. Neuroleptics, such as haloperidol, by blocking dopamine receptors on cholinergic cell bodies and/or dendrites, reduce dopamine's inhibitory control of cholinergic neuron activity[28]. Dopamine receptors not only influence the cholinergic muscarinic receptors, but muscarinic M_1 and M_2 receptors also might mediate dopamine D_1 and D_2 receptor responses, respectively. There are, at least, some relationships between muscarinic M_1 receptors and dopaminergic D_1 receptors, and between muscarinic M_2 receptors and dopamine D_2 receptors in cataleptic responses. Dopamine D_1 and D_2 receptors may interact in a synergistic fashion on dopaminergic systems, but act independently of each other in influencing other system such as cholinergic neurons[29]. Furthermore, activation of the muscarinic M_2 and M_4 receptor subtypes, or $GABA_B$ receptors causes a pertussis toxin-sensitive inhibition of adenylate cyclase[30-32]. It has been suggested that there may be a chain of GABA neurons within the nucleus accumbens[33,34]. The GABA neurons may be inhibitory interneurons on the pathway subserving locomotor activity which receive dopaminergic, cholinergic and serotonergic neurons within the nucleus accumbens[35]. Stimulation of D_2 and $GABA_B$ receptors results in an increased efflux of K^+ ions[11,36]. The coupling of these receptors to K^+ channels is via GTP-binding proteins[11]. The G proteins which couple D_2[37] and $GABA_B$[38] receptors to K^+ channels are purtussis toxin-sensitive. However, there is also the evidence that some presynaptic $GABA_B$ receptors may be directly linked to K^+ channels[39]. Purtussis toxin is a bacterial toxin which ADP-ribosylates the α-subunit of Go and Gi[40]. Since $GABA_B$ receptor agonist, baclofen, potentiates haloperidol-induced catalepsy[41], it is suggested that there is an interaction between dopamine D_2 receptor and $GABA_B$ receptor activities. In this study, the stimulatory effects of intravenous injection of pertussis toxin on haloperidol-induced cataleptic responses (postsynaptic dopamine D_2 receptor inhibition) may be due to pertussis toxin-sensitive inhibition of adenyl cyclase via muscarinic M_2 receptor activation, or $GABA_B$ receptor activation, in striatum and/or nucleus accumbens.

That the cataleptic effects induced by SCH23390 and haloperidol can be suppressed by D_2 receptor agonists has been reported[42]. Furthermore, SCH23390 catalepsy mediated by dopamine D_1 receptor inhibition may be affected by altering dopamine D_2 receptor sensitivity (either super- or subsensitivity), whereas haloperidol catalepsy mediated by dopamine D_2 receptor inhibition may be modified by supersensitive, but not subsensitive, dopamine D_1 receptor changes[43]. Accordingly, SCH23390 catalepsy may be mediated by indirect blockade of dopamine D_2 receptor function through its D_1 receptor blocking action, whereas haloperidol catalepsy is mediated only by direct blockade of D_2 receptors, without being affected by dopamine D_1 receptor subsensitivity. The antagonism by pertussis toxin of the attenuated SCH23390 cataleptic response (D_1 receptor supersensitivity induced by chronic cocaine

treatment), may be due to an indirect inhibition of D_1 receptors (a synergistic effect) via blockade of postsynsptic dopamine D_2 receptors, and which may be mediated by an pertussis toxin-sensitive muscarinic M_2 receptor activation.

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