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Increased plasma corticosterone, aggressiveness and brain monoamine changes induced by central injection of pertussis toxin

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Abstract

The effects of intracerebroventricular (i.c.v.) injection of pertussis toxin, a specific inhibitor of G_i/G_o proteins, on plasma corticosterone levels, aggressiveness, and hypothalamic and hippocampal monoamines and their metabolites levels were examined in mice. Plasma corticosterone level was markedly increased at 3 h after pertussis toxin injection (0.03 and 0.2 μg/mouse), peaked at 6 h and was still increased for up to 6 days after injection. Mice injected with pertussis toxin (0.2 μg/mouse) did not show weight gain between day 0 and day 6 after injection. In addition, pertussis toxin (0.2 μg/mouse) induced a progressive increase in aggressiveness, i.e. a decrease in attack latency and an increase in number of attacks, on day 1 and 6 after injection. Brain monoamines and their metabolites levels were changed on day 1 and 6 after pertussis toxin injection (0.2 μg/mouse): in the hypothalamus, levels of dopamine and 3,4-dihydroxyphenylacetic acid were increased, norepinephrine level decreased, and 5-hydroxyindole acetic acid (5-HIAA) level was markedly increased, with no changes in 5-hydroxytryptamine (5-HT) level, whereas in the hippocampus, 5-HT level was significantly decreased, with no changes in 5-HIAA and catecholamines. These results suggest that signal transduction through G_i/G_o proteins in the brain is involved in the modulation of hypothalamo-pituitary-adrenal axis, aggressiveness, and monoamine levels in vivo.

Keywords: Hypothalamo-pituitary-adrenal axis; Aggressive behavior; Monoamine; Pertussis toxin; (Mouse)

1. Introduction

A large group of neurotransmitter receptors are linked to their effectors via guanine nucleotide-binding proteins (G-proteins) (Gilman, 1987; Worley et al., 1987). Pertussis toxin inactivates G_i and G_o proteins by ADP-ribosylation of α_i subunit, and therefore uncouples the receptors from their effector mechanisms: i.e. inhibition of adenylate cyclase activity and calcium channels, and activation of K^+ conductance (Hildebrandt et al., 1983).

Central regulation of hypothalamo-pituitary-adrenal axis involves complex interactions among various neurotransmitters including catecholamines, serotonin, acetylcholine, gamma aminobutyric acid, glutamate, opioids and somatostatin (Herman and Cullinan, 1997). All of these neurotransmitters can activate receptor subtypes coupled to G_i/G_o proteins. However, the involvement of pertussis toxin-sensitive G_i/G_o proteins in the tonic regulation of hypothalamo-pituitary-adrenal axis in vivo has not been examined. Central administration of pertussis toxin is a useful means to assess the integrated role of various receptors coupled to G_i/G_o proteins in vivo for a given physiological parameter. Thus, we examined the effect of intracerebroventricular (i.c.v.) injection of pertussis toxin on the plasma corticosterone level in mice.

During the experiments, we unexpectedly observed increased aggressiveness, i.e. multiple biting sites on the body and fighting behavior, in mice pretreated i.c.v. with pertussis toxin. Thus, the aggressive behaviors induced by pertussis toxin injected i.c.v. were analyzed. Brain

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monoamine systems are intimately involved in the regulation of aggression (Eichelman, 1990) as well as hypothalamo-pituitary-adrenal axis (Johnson et al., 1992). Further, i.c.v. injection of pertussis toxin has been reported to induce a transient decrease in brain 5-hyroxytryptamine (5-HT) levels (Garzon et al., 1990). Thus, we examined the changes in norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT, and 5-hydroxyindole acetic acid (5-HIAA) levels in the hypothalamus and the hippocampus after pertussis toxin injection.

2. Materials and methods

2.1. Animals and drugs

Male ICR mice weighing 25–30 g were used for all the experiments. Animals were housed five per cage in a room maintained at 22 ± 1°C with an alternating 12 h light–dark cycle. Food and water were available ad libitum. All animal procedures were carried out as approved by the Animal Experimentation Committee at Hallym University. Pertussis toxin was purchased from Research Biomedicals International (Natick, MA, USA) and was dissolved in sterile saline (0.9% NaCl solution).

2.2. I.c.v. injection

The i.c.v. administration was performed following the procedure established by Laursen and Belknap (1986), which was modified from the method of Haley and McCormick (1957). Briefly, the animal was injected at bregma with a 50 μl Hamilton syringe fitted with a 26-gauge needle, which was adjusted to be inserted 2.4 mm deep. The i.c.v. injection volume was 5 μl.

2.3. Corticosterone assay

Blood was collected from the retro-orbital venous plexus 0, 3 h, 6 h, 1 day, and 6 days after an i.c.v. pertussis toxin injection (0.03 and 0.2 μg/mouse) at 8:30 a.m. Each mouse was bled once and sacrificed. Plasma was separated by centrifugation of the freshly drawn blood and stored at −70°C until assayed. The plasma corticosterone level was determined by the fluorometric determination method (Glick et al., 1964).

2.4. Assessment of aggressive behavior

Male ICR mice housed as a group were administered i.c.v with either saline or pertussis toxin (0.2 μg/mouse), and thereafter were also housed as a group. One or six days after the single injection of pertussis toxin or saline, an injected animal and an intact male (which had been also housed as a group) were put into a new cage. Attack latency and the number of attacks observed in the pertussis toxin-injected animal to the intact animal were measured during a 5-min session.

2.5. Assessment of spontaneous motor activity

On days 1 and 6 after pertussis toxin administration, the spontaneous motor activity of individual mouse was measured for 10 min with a motility meter (Rhema-Labortechnik, Hochheim, Germany).

2.6. Monoamine assays

Mice were killed by decapitation 0, 3 h, 6 h, 1 day and 6 days after an i.c.v. pertussis toxin (0.2 μg/mouse) or saline injection, and hypothalamus and hippocampus were dissected out, and stored at −70°C until they were assayed for norepinephrine, dopamine, DOPAC, 5-HT and 5-HIAA. Concentrations of monoamines and their metabolites were quantified using high-performance liquid chromatography with electrochemical detection. The hypothalamus and hippocampus were weighed and homogenized in 0.1 M perchloric acid containing 0.1 M sodium metabisulfite and 5-hydroxy-*N*-methyltryptamine as an internal standard. Following centrifugation at 12,000 × *g* for 2 min, the

Table 1
Effect of intracerebroventricular injection of pertussis toxin on plasma corticosterone levels in mice

	Time after an i.c.v. injection				
	0	3 h	6 h	1 day	6 days
Saline	80 ± 8	156 ± 21	160 ± 26	71 ± 11	83 ± 12
Pertussis toxin (0.03 μg)	83 ± 12	335 ± 51*	461 ± 49**	208 ± 31*	132 ± 29*
Pertussis toxin (0.2 μg)	76 ± 12	443 ± 33**	512 ± 38***	255 ± 19**	212 ± 29**

Plasma corticosterone levels (ng/ml) were measured 0, 3 h, 6 h, 1 day, and 6 days after the intracerebroventricular (i.c.v.) injection of pertussis toxin (0.03 and 0.2 μg/mouse). The data are means ± S.E.M. ($n = 8–10$).

*$P < 0.05$, significantly different from the i.c.v. saline-injected animals.

**$P < 0.01$, significantly different from the i.c.v. saline-injected animals.

***$P < 0.001$, significantly different from the i.c.v. saline-injected animals.

Table 2
Effect of an intracerebroventricular injection of pertussis toxin on aggressive behavior and spontaneous motor activity in mice

	Days after an intracerebroventricular injection	
	1	6
Attack latency (s)		
Saline	149 ± 16	145 ± 13*
Pertussis toxin	103 ± 15	85 ± 13**
Number of attacks		
Saline	4.6 ± 0.7	5.2 ± 1.0
Pertussis toxin	7.2 ± 1.4	14.6 ± 1.6***
Activity counts		
Saline	854 ± 175	1218 ± 103
Pertussis toxin	416 ± 93*	1218 ± 119

On days 1 and 6 after the intracerebroventricular (i.c.v.) injection of pertussis toxin (0.2 μg/mouse), aggressiveness was evaluated by measuring the attack latency and the number of attacks, and spontaneous motor activity of individual mouse was measured for 10 min using a motility meter (Rhema-Labortechnik, Hochheim, Germany). The data are means ± S.E.M. ($n = 8$).

*$P < 0.05$, significantly different from the i.c.v. saline-injected animals.

**$P < 0.01$, significantly different from the i.c.v. saline-injected animals.

***$P < 0.001$, significantly different from the i.c.v. saline-injected animals.

supernatant was filtered through a 0.45 μm Millipore HV-4 filter. Ten microliters of sample was injected onto a C18 μBondapak column (Waters, Milford, USA). As a mobile phase, 0.1 M KH_2PO_4 (adjusted to pH 3.8) containing sodium octanesulfonic acid (0.25 mM), disodium EDTA (0.1 mM) and acetonitrile (9% v/v) was used. The flow rate was 1 ml/min and the oxidation potential was 1 V.

2.7. Statistical analysis

Data were analyzed using two-way analysis of variance (ANOVA) test with the post hoc Bonferroni test. P values of less than 0.05 were considered to indicate statistical significance.

3. Results

As shown in Table 1, a single i.c.v. injection of pertussis toxin (0.03 and 0.2 μg/mouse) markedly increased plasma corticosterone levels in a dose-dependent manner. Plasma corticosterone levels peaked at 6 h, and were still increased for up to 6 days after pertussis toxin injection. An i.c.v. injection of saline induced a slight increase in plasma corticosterone levels at 3 h after injection due to the i.c.v. injection stress (Kim et al., 1998). Mice injected with pertussis toxin (0.2 μg/mouse) displayed no increase in body weight between days 0 (27.1 ± 0.4 g) and 6 (27.3 ± 0.6 g) after the pertussis toxin injection; whereas mice injected with saline showed increase in body weight from 27.0 ± 0.7 g on day 0 to 30.3 ± 0.7 g ($P < 0.05$) on day 6 after saline injection. The amount of food intake did not differ between mice injected with pertussis toxin (0.2 μg/mouse) and those treated with saline (data not shown).

The animals given pertussis toxin displayed an aggressive behavior on day 1 after pertussis toxin injection. Thus, the aggressive behavior was examined by measuring attack latency and the number of attacks observed in the pertussis toxin-injected animal to the intact animal during a 5-min session after each animal was put into a new cage. Mice which had been injected i.c.v. with pertussis toxin (0.2 μg/mouse) 1 day before the aggression test displayed a significant decrease in attack latency (Table 2). A separate

Table 3
Effect of intracerebroventricular injection of pertussis toxin on monoamines and their metabolites levels in the hypothalamus and hippocampus in mice

	Day	Norepinephrine	Dopamine	DOPAC	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT
Hypothalamus								
Saline	1	1256 ± 91	104 ± 10	16 ± 1	0.17 ± 0.02	1355 ± 55	359 ± 22	0.26 ± 0.02
Pertussis toxin	1	1148 ± 90	115 ± 7	28 ± 3***	0.26 ± 0.03*	1476 ± 118	686 ± 72***	0.48 ± 0.05***
Saline	6	1299 ± 51	113 ± 9	17 ± 3	0.19 ± 0.02	1341 ± 59	371 ± 32	0.28 ± 0.02
Pertussis toxin	6	988 ± 27*	151 ± 10*	48 ± 6***	0.33 ± 0.07***	1589 ± 109	641 ± 36***	0.41 ± 0.01***
Hippocampus								
Saline	1	436 ± 30	22 ± 3	n.d.	n.d.	784 ± 38	259 ± 16	0.33 ± 0.03
Pertussis toxin	1	355 ± 26*	21 ± 2	n.d.	n.d.	529 ± 42**	285 ± 19	0.56 ± 0.06***
Saline	6	426 ± 32	22 ± 3	n.d.	n.d.	774 ± 44	262 ± 14	0.34 ± 0.02
Pertussis toxin	6	378 ± 23	17 ± 2	n.d.	n.d.	503 ± 39**	290 ± 29	0.57 ± 0.04***

Norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) levels (ng/g tissue weight) were assayed 1 and 6 days after either pertussis toxin (0.2 μg/mouse) or saline injection. The data are means ± S.E.M. ($n = 8$).

*$P < 0.05$, significantly different from the i.c.v. saline-injected animals.

**$P < 0.01$, significantly different from the i.c.v. saline-injected animals.

***$P < 0.001$, significantly different from the i.c.v. saline-injected animals.

group of animals that had been injected with pertussis toxin 6 days before the test showed a further decrease in attack latency and a significant increase in the number of attacks (Table 2). When measured at 3 and 6 h after pertussis toxin injection, the animals did not display aggressive behavior (data not shown). The spontaneous motor activity of animals treated with pertussis toxin (0.2 μg/mouse) was significantly decreased on day 1 but it returned to control values on day 6 after the injection (Table 2). Higher counts on day 6 compared with on day 1 in control animals were due to the increased body weight of animals on day 6 in our motility-measuring device (Table 2).

Next, levels of norepinephrine, dopamine, DOPAC, 5-HT, and 5-HIAA in the hypothalamus and hippocampus were measured at 0, 3 h, 6 h, 1 day and 6 days after pertussis toxin (0.2 μg/mouse) injection. Norepinephrine level in the hypothalamus was decreased by 21% ($P < 0.05$) on day 6, while in the hippocampus it was reduced by 19% ($P < 0.05$) on day 1 and returned to the control value on day 6 after pertussis toxin injection (Table 3). Dopamine level in the hypothalamus, but not in the hippocampus, significantly increased on day 6 after the pertussis toxin injection (Table 3). DOPAC level in the hypothalamus was markedly increased by 131% on day 1 and 198% on day 6 after the pertussis toxin injection (Table 3). 5-HT level in the hippocampus, but not in the hypothalamus, was decreased by 33% on day 1, and 36% on day 6 after pertussis toxin injection (Table 3), while 5-HIAA levels in the hypothalamus, but not in the hippocampus, was markedly increased by 94% on day 1 and 79% on day 6 after the pertussis toxin injection (Table 3).

4. Discussion

We found in the present study that a single i.c.v. injection of pertussis toxin induces a marked increase in plasma corticosterone levels and aggressive behavior with changes in brain monoamine levels. The time-courses of these changes were different: the pertussis toxin-induced increase in plasma corticosterone peaked at 6 h whereas aggression and brain monoamines changes appeared on day 1 and progressively increased for up to 6 post-injection days.

Central pertussis toxin induced-increase in plasma corticosterone levels suggests that tonic activation of signal transduction through the G_i/G_o proteins in the brain maintains the basal levels of plasma corticosterone. Many of the neurotransmitters (listed in the Introduction) that are involved in the central regulation of hypothalamo-pituitary-adrenal axis are linked to pertussis toxin-sensitive G_i/G_o proteins. Therefore, pertussis toxin-induced blockade of tonic activation of at least one or more of these neurotransmitter systems may result in the increase in plasma corticosterone levels. Although markedly reduced on day 1, plasma corticosterone was still increased for up to 6 days after pertussis toxin injection. This chronic elevation of plasma corticosterone induced by central pertussis toxin may have resulted in the lack of increase in body weight. The attenuation of increased plasma corticosterone levels on days 1 and 6 may have resulted, at least in part, from the negative feedback effect of increased plasma corticosterone levels on hypothalamo-pituitary-adrenal axis. The increased plasma corticosterone levels do not appear to be caused by the aggressive behavior and the changes in brain monoamine systems, because of the different time-courses of these parameters. However, the possibility that chronically increased plasma corticosterone levels may somehow affect brain monoamine metabolism cannot be excluded. I.c.v. administration of pertussis toxin has been used as an experimental tool in various fields of neuropharmacology (Chung et al., 1994; Narvaez et al., 1992). The present finding that a single i.c.v. injection of pertussis toxin induces a long-lasting increase in plasma corticosterone indicates that one should consider the potential effect of increased plasma corticosterone on the parameters in one's study in which an i.c.v. injection of pertussis toxin is used, because corticosterone has a wide range of actions on virtually every cell in the body.

The pertussis toxin-induced brain monoamine changes generally coincided with the appearance of aggressive behavior. Hippocampal 5-HT levels were decreased in the pertussis toxin-treated mice (Table 3). Decrease in the brain 5-HT content has been shown to increase several types of aggressive behavior (Eichelman and Thoa, 1973; Goldstein, 1974; Gibbons et al., 1979). Limbic system structures (hippocampus, amygdala and hypothalamus) are implicated in aggressive behavior (Goldstein, 1974). Thus, it can be speculated that the decrease in 5-HT levels (33% and 36% on days 1 and 6, respectively) in hippocampus by pertussis toxin treatment may be, at least in part, related to the pertussis toxin-induced enhancement of aggressiveness. A mutant mouse strain lacking the 5-HT_{1B} receptor, a pertussis toxin-sensitive G_i/G_o protein-coupled receptor, was reported to show an increased aggressiveness in isolation-induced aggression (Saudou et al., 1994). However, when the mutants are housed as a group, they are not more aggressive than wild type mice (Saudou et al., 1994). Because the pertussis toxin-injected animals spontaneously became aggressive without an isolation period in this study, other pertussis toxin-sensitive G_i/G_o protein-coupled receptors in addition to 5-HT_{1B} receptor must be involved in the pertussis toxin-induced aggressiveness.

In contrast to hippocampus, pertussis toxin did not affect hypothalamic 5-HT levels, but markedly increased hypothalamic 5-HIAA levels, consequently increasing ratio of 5-HIAA to 5-HT levels. Increased ratio of 5-HIAA to 5-HT levels may suggest an increased 5-HT release from the hypothalamus. 5-HT_{1B} autoreceptors and pertussis toxin-sensitive G_i/G_o-coupled heteroreceptors located in the serotonergic terminals inhibit 5-HT release from the

serotonergic terminals (Göthert, 1990; Wolf and Kuhn, 1990). Thus, inhibition of signal transduction through these receptors by pertussis toxin may increase 5-HT release and subsequently lead to an increase of 5-HIAA levels in the hypothalamus. In the hippocampus, 5-HIAA content was not decreased in the presence of reduced 5-HT levels, with the consequent increase in ratio of 5-HIAA to 5-HT levels, suggesting an increased release of 5-HT by the same mechanism discussed above.

Although the reason for the pertussis toxin-induced decrease in hippocampal 5-HT levels is unclear at present, the reduction in hippocampal 5-HT content (33% and 36% on days 1 and 6, respectively) in this study is partially in line with a previous report showing that 5-HT content in the midbrain, thalamus and spinal cord decreased by 16–32% on day 2 after i.c.v. pertussis toxin (0.5 μg/rat) injection, which returned to the control levels on day 6 after injection (Garzon et al., 1990). The persistent decrease in 5-HT content for up to 6 days after a single injection in this study may result from the relatively higher dose of i.c.v. pertussis toxin used (0.2 μg/mouse) compared with that (0.5 μg/rat) used by Garzon et al. (1990). Intrathecal administration of a high dose of pertussis toxin (5 μg/mouse) also reduced 5-HT content without changes in 5-HIAA levels in the spinal cord on day 6 after injection (unpublished observation). The finding that the total amount of indoleamines (5-HT plus 5-HIAA) in the hippocampus was decreased on days 1 and 6 after the pertussis toxin injection may be due to the decrease in 5-HT synthesis. Because the hypothalamus did not show pertussis toxin-induced reduction of 5-HT levels, 5-HT neurons that innervate the hypothalamus may be different from those that innervate hippocampus and spinal cord in their response to pertussis toxin. The mechanism for this difference remains to be investigated.

In addition to 5-HIAA, DOPAC levels in the hypothalamus were markedly increased on day 1, and further increased on day 6. Dopamine levels in the hypothalamus significantly increased on day 6. The increase in DOPAC levels may have resulted from the increased dopamine release due to the inhibition of G_i/G_o-coupled presynaptic receptors (Goldstein et al., 1990; Wolf and Roth, 1990) by a similar mechanism as the case of hypothalamic 5-HIAA (Table 3). Microinjection of pertussis toxin to A10 dopamine region has been shown to increase dopamine synthesis and metabolism (Steketee et al., 1992). Increases in hypothalamic dopamine levels and brain dopamine utilization have been reported in the aggressive mice (Bernard et al., 1975; Barr et al., 1979). Furthermore, drugs that increase dopaminergic function induce aggressive behavior (Eichelman and Thoa, 1973; Avis, 1974). Thus, increases in hypothalamic dopamine level and turnover rate (or utilization) induced by pertussis toxin in the present study may be related, at least in part, to the enhanced aggressiveness. Additionally, the possibility that other neurotransmitter systems related with G_i/G_o proteins and other brain areas besides hypothalamus and hippocampus are involved in the pertussis toxin-induced aggressive behavior cannot be excluded.

I.c.v. injection of a high dose of pertussis toxin (5 μg/rat or 0.5–1 μg/mouse) has been shown to induce an elevation in locomotor activity (Nomura et al., 1987; Durcan et al., 1991). In contrast to these reports, a pertussis toxin dose of 0.2 μg/mouse i.c.v. caused a decrease in motor activity on day 1 but not on day 6 after injection (Table 2). A lower dose of pertussis toxin (0.03 μg/mouse i.c.v.) did not affect the locomotor activity on day 1, but decreased it on day 6 (data not presented). Thus, it is suggested that pertussis toxin injected i.c.v. may have a biphasic effect on locomotor activity; i.e. at low doses it may decrease locomotor activity (the present study), while at high doses it may increase locomotor activity (Nomura et al., 1987; Durcan et al., 1991). The progressive inhibition of G_i/G_o proteins by pertussis toxin as the post-injection time proceeds may account for both the significant decrease by 0.03 μg pertussis toxin on day 6, and the recovery to control values on day 6 after 0.2 μg pertussis toxin injection. Overall, it is suggested that the pertussis toxin-induced aggressiveness is not related to changes in locomotor activity.

To our knowledge, this is the first report on aggression induced by an agent interfering with a specific component of the intracellular signal transduction machinery. This simple animal model should be useful for aggression study and for development of anti-aggressive drugs with novel mechanism of action. Further localization studies in rats, wherein intracerebral microinjection is used, are needed to delineate the exact anatomical sites and neuronal pathways involved in the pertussis toxin-induced changes in these parameters.

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