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Differential Properties of Behavioral Responses to Bromocriptine and Apomorphine in Rats

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Abstract The study was carried out in an attempt to understand the contribution of dopamine D-1 and D-2 receptor activities to yawning responses induced by bromocriptine (BRC) in rats. Intraperitoneal (i. p.) injection of BRC ranging from 1 to 20mg/kg produced yawning behavior. The yawning responses were increased by reserpine (2mg/kg) and α-methyl-p-tyrosine (α-MPT ; 200mg/kg) but decreased by α-MPT (400mg/kg). Reserpine (2mg/kg) plus α-MPT (200mg/kg) completely inhibited the yawning induced by BRC. The yawning responses were stimulated by a low dose of apomorphine (0.1mg/kg). a dopamine receptor agonist, but were inhibited by a high dose (1mg/kg). Furthermore, BRC alone did not produce stereotypy but potentiated, in a dose-dependent manner, apomorphine (1mg/kg)-induced stereotypy, which was inhibited by sulpiride (20mg/kg), a dopamine D-2 receptor antagonist. The result suggest that the important factors in the occurrence of yawning and stereotyped behaviors, include not only dopamine D-2 receptor stimulation, but also endogenous dopamine or D-1 receptor activation, and seem to depent upon the ratio and potency of D-1 receptor versus D-2 receptor activity

Key Words : bromocriptine, dopamine D-2 agonist, yawning, stereotypy, endogenous dopamine, apomorphine

Introduction

Bromocriptine (BRC) has been used to treat patients with Parkinson's disease[1]. This drug is dopamine D-2 receptor agonist, which inhibits dopamine-stimulated synthesis of cyclic adenosine 3', 5'-monophosphate (cyclic AMP)[2]. BRC produces long-lasting locomotor stimulation in mice, which is preceded by locomotor depression. These effects are considered to be the result of post- and pre-synaptic dopamine receptor stimulation, respectively[3-5]. However, there is evidence suggesting that BRC acts differently from other dopamine receptor agonists such as apomorphine. Thus, its locomotor stimulant effect in mice and stereotypy in rats[3], and its ability to produce

contralateral rotation in rats[3,6] could be reduced or antagonized by the dopamine synthesis inhibitor α-MPT or with the granule depletor reserpine. Recently we found that administration of BRC produces yawning behavior, which may be due to : (a) stimulation of presynsptic dopamine autoreceptors and subsequent cholinergic stimulation, or (b) dopamine D-2 receptor stimulation and cholinergic stimulation. Reserpine also elicited yawning which was antagonised by sulpiride and by α-MPT, suggesting that dopamine synthesis and release are needed to produce the yawning response[7]. The present studies were designed to understand the effect of endogenous dopamine and apomorphine (dopamine D-1 and D-2 agonist) on yawning behavior induced by BRC, as well as the effect of BRC on apomorphine-induced stereotypy.

Materials and Methods

Animals. Healthy male Wistar rats (200-250 g) were obtained from Kyudo Animal Laboratory (Kumamoto, Japan). They were permitted food (MF, Oriental Yeast Ltd.) and water *ad libitum* except during trials. All trials and breeding were carried out at an environmental temperature of 23±1℃, with a 12 h light-dark cycle (7 : 00 a. m. -7 : 00 p. m.)

Measurement. Pairs of rats were placed in transparent plastic boxes (35x30x17cm) containing wood shavings. Yawns were counted for 180/min after i. p. injection of BRC and 3% Tween 80 solution as control. Stereotypy was rated for 180/min after saline or apomorphine according to the scale of Costall and Naylor (1973)[8], i. e., 0 : normal behavior, 1 : exploratory activity, discontinuous sniffing, 2 : continuous sniffing, 3 : continuous sniffing, discontinuous licking or biting, 4 : continuous licking or biting.

Administration of drugs. To induce yawning responses, rats received intraperitoneal (IP) injections of BRC (1-20 mg/kg). The time interval between pretreatment with the following drugs and BRC was 24 h for reserpine (2 mg/kg), 6 h for α-MPT (200 and 400 mg/kg), and 10 min for apomorphine (0.25 and 1 mg/kg). To examine the effects of reserpine plus α-MPT, BRC (2.5mg/kg) was administered after 24 h of reserpine (2 mg/kg) and 6 h of α-MPT (200 mg/kg). To observe stereotyped behavior, apomorphine or saline was injected 15 min after BRC. In the control experiments, 3% tween 80 solution instead of BRC was injected at the same time interval.

Drugs. The following drugs were used. Bromocriptine mesylate (BRC ; Sandoz A. G.), Reserpine (Sigma, St. Louis), α-methyl-p-tyrosine methylester (α-MPT ; Sigma, St. Louis) and apomorphine hydrochloride (Sigma, St. Louis). Bromocriptine was suspended in 3% Tween 80 solution. The other drugs were dissolved in saline. These drugs were injected intraperitoneally (IP) using 1 ml/kg. Doses are expressed in term of the salt.

Statistical analysis. Yawning and stereotyped behaviors were expressed as mean values, and statistical analysis was calculated using ANOVA and subsequently two-tailed Mann-Whitney U-test[9]. The level of significance chosen was $P<0.05$.

Results

Yawning responses induced by BRC. Intraperitoneal injection of BRC at doses ranging from 1-20 mg/kg elicited yawning responses, with protrusion of the tongue and with the head moving mainly downward. A dose of 2.5 mg/kg of BRC produced maximal yawning behavior. Each yawn was preceded by penile erection, chewing, head twitches or, occasionally, by sudden stretching of the forelimbs. Yawning began about 20-30 min of injection of BRC (2.5 mg/kg), and was pronounced after 30-60 min. BRC, at all of doses, did not produce stereotypy and hyperlocomotion during 120 min (data not shown).

Effects of α-MPT, reserpine or reserpine plus α-MPT on BRC-induced yawning responses. As shown in Table 1, α-MPT (200 mg/kg) and reserpine (2 mg/kg) markedly potentiated the yawning induced by BRC (2.5 mg/kg), but a high dose of α-MPT (400

mg/kg) and reserpine (2.0 mg/kg) plus α-MPT (200 mg/kg) completely inhibited the yawning behavior.

Table 1 Effects of Reserpine, α-MPT or Reserpine Plus α-MPT on BRC-induced Yawning.

Pretreatments (mg/kg)		Yawns in 120 min	
		Vehicle	BRC (2.5mg/kg)
Saline		0.0	24.9 ++
Reserpine	(2)	2.5	41.0**++
α-MPT	(200)	0.0	40.7* +
α-MPT	(400)	0.0	13.7**++
Reserpine	(2)		
+α-MPT	(200)	0.0	9.3**+
Apomorphine	(0.1)	7.8**	52.5* ++
Apomorphine	(1)	0.3	12.0**+

The respective drugs were injected as described in the text. *, +; P<0.05, **, ++; P<0.002; significant difference from the saline-injected group (*, **), and from vehicle-injected group (+, ++), determined by analysis of variance and subsequent Mann-Whitney U-test

Table 2 Potentiation of Yawning and Stereotyped Behaviors by Combined Treatment with BRC and Apomorphine.

Dose of BRC (mg/kg)	Dose of apomorphine (mg/kg) 0	0.1	1.0
	Yawns in 180 min		
0.0	0	7.8**	0.3
1.0	12.0++	37.4**++	1.2*
2.5	29.0++	52.5**++	12.0*+
5.0	6.8++	34.3**++	8.2+
10.0	5.4+	21.3**++	3.5+
20.0	2.3+	11.2**	0.3
	Stereotypy in 180 min		
0.0	0.0	0.0	5.4*
1.0	0.0	0.0	12.4**+
2.5	0.0	0.4	15.3**++
5.0	0.0	1.2	20.6**++
10.0	0.0	2.1	22.0**++
20.0	0.0	3.0*+	25.4**++

Apomorphine, at all doses, wsa injected 15 min after BRC. *P<0.05, **P<0.002; significant difference from the vehicle (absent of apomorphine) group and +P<0.05, ++P<0.002; from the vehicle (absent of BRC) group, determined by analysis of variance and subsequent Mann Whitney U-test

Potentiation of yawning and stereotyped behaviors by the combined treatment with BRC and apomorphine. Apomorphine, at 0.1 mg/kg increased yawning behavior induced by BRC (1-20 mg/kg) and decreased this behavior at 1.0 mg/kg. BRC (1-20 mg/kg) alone did not produse stereotyped behavior, but when BRC was administered in combination with apomorphine, the behavior occurred more prominently than with apomorphine alone, and however, increased at 1.0 -2.5 mg/kg of BRC and decreased at 2.5-20 mg/kg, in dose dependent manner. The stereotypy was proportional to the dose of BRC (Table 2). The stereotypy potentiated by apomorphine (1.0 mg/kg) in combination with BRC (20 mg/kg) was inhibited by sulpiride (20mg/kg) (date not shown).

Discussion

BRC, a selective dopamine D 2 agonist, induced yawning without producing typical symptoms such as stereotypy and hyperlocomotion at all doses. Apomorphine exerts biphasic effects on behavior ; that is, yawning and hypomotility at low doses, and stereotypy and hyperlocomotion at higher doses[10,11]. Low doses of apomorphine preferentially activate presynaptic dopamine autoreceptors, which results in an inhibition of dopamine release and consequent decrease of its synthesis, while higher doses stimulate postsynaptic receptors[12-14]. Since scopolamine, an anticholinergic agent, as well as low doses of haloperidol inhibit apomorphine-induced yawning, it appears that this behavior may be mediated by cholinergic activation secondary to the inhibition of dopamine transmission[10]. Recently, We found that BRC-induced yawning was inhibited by sulpilide, a selective dopamine D-2 antagonist, and by a low dose of haloperidol which preferentially inhibits presynaptic dopamine autoreceptors[15]. BRC-induced yawning is characterized by the head moving downward, which is similar to the effects produced by apomorphine[16]. Accordingly, the neuronal mechanism

involved in BRC-induced yawning seems to be similar to that of low doses of apomorphine. Most recently, SCH 23390, a selective D-1 receptor blocker[17] prevented apomorphine-induced yawning. suggesting that dopamine receptors mediating yawning may be both D-1 and D-2 receptors[18]. Increased apomorphine-induced stereotypy following subacute treatment with neuroleptics such as haloperidol and sulpiride, correlates with changes in D-2 receptor number, but not with changes in D-1 receptors[19]. In this study, BRC alone did not induce the stereotypy, but markedly potentiated the stereotypy induced by apomorphine. Therefore, BRC but not apomorphine appears to require another dopamine receptor agonist for the induction of stereotypy. This might suggest a dissimilarity in possible mode of action between BRC and apomorphine.

The BRC-induced yawning was potentiated by reserpine and by a low dose of α-MPT (200 mg/kg) but was inhibited by a high dose of α-MPT (400 mg/kg) or reserpine plus α-MPT (200 mg/kg). The apomorphine-induced yawning is markedly potentiated by combined treatment with these drugs (unpublished observation). The effects of these drugs on BRC-induced yawning are different from those of apomorphine. Reserpine treatment facilitates catecholamine release in the inactivated form, however, tyrosine hydroxylase acitvity may also be potentiated. Serra et al. (1986)[7] reported that it is likely that the newly synthetized dopamine, released in the synaptic cleft, is sufficient to induce yawning 24 h following reserpine treatment, partly because supersensitivity has developed in postsynaptic receptors. On the other hand, reserpine pretreatment potentiates the yawning and stereotypy indused by low and high doses of apomorphine, respectively, suggesting that both pre-and postsynaptic dopamine receptors may be supersensitive (Kishimoto et al. unpublished observations). The inhibitory effect of combined treatment with reserpine and α-MPT on BRC-induced yawning may be due to the complete depletion of endogenous dopamine.

Accordingly, in addition to supersensitivity of dopamine receptors, the important factors in the occurrence of yawning include not only dopamine D-2 receptor stimulation but also stimulation of another dopamine receptor probably D-1 receptor. Since apomorphine possesses the ability to stimulate both dopamine D-1 and D-2 receptors, it appears that apomorphine-induced yawning may be potentiated by combined treatment with reserpine and α-MPT. Combined treatment with BRC (1.0-20 mg/kg) and low dose of apomorphine (0.1 mg/kg) increased yawning responses at 1.0-2.5 mg/kg of BRC and decreased at 2.5-20 mg/kg, proportionaly to the dose of BRC, whereas stereotypy induced by apomorphine (1.0 mg/kg) was potentiated in dose dependent fashion. from these results, the ability of dopamine agonists to induce yawning and stereotyped behaviors seems to depend on the ratio and potency of D-1 receptor versus D-2 receptor activities.

BRC has relatively long half-life and crosses the blood brain barrier with difficulty[20] so that an optimal and stable brain concentration could be obtained for the induction of yawns during the whole period of observation[21]. Furthermore, the locomotor stimulation in mice and stereotypy in rats observed later[3] may be due to the combined action of long-lasting BRC and endogenous dopamine progressively released.

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