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The Effect of Neurotransmitters on Cataleptic Behavior Induced by PG D_2 in Rats

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SAITO, R , M FUJIWARA, H KAMIYA AND N ONO *The effect of neurotransmitters on cataleptic behavior induced by PG D_2 in rats* PHARMACOL BIOCHEM BEHAV 26(3) 543–546, 1987 —The effects of several neurotransmitters on prostaglandin (PG) D_2-induced cataleptic behavior in rats were investigated by the high bar test Intracerebroventricular administration of PG D_2 elicited cataleptic behavior in a dose-dependent manner without producing a marked change in spontaneous motor activity The incidences of cataleptic behavior were 20% and 100% at doses of 2 nmol and 50 nmol of PG D_2, respectively Intraperitoneal pretreatment with L-DOPA (100 mg/kg), apomorphine (1 mg/kg), amantadine (0 2 mg/kg), atropine (0 5 mg/kg) or p-chlorophenylalanine (300 mg/kg) significantly decreased the cataleptic behavior induced by 50 nmol of PG D_2 Conversely, simultaneous treatment with 5-hydroxy-L-tryptophan (30 mg/kg), 5-methoxy-N,N-dimethyltryptamine (5 mg/kg), imipramine (20 mg/kg) or clomipramine (10 mg/kg) markedly increased the cataleptic behavior induced by 2 nmol of PG D_2 Propranolol (10 mg/kg) and phenoxybenzamine (10 mg/kg) did not affect the induction of cataleptic behavior by either 2 nmol or 50 nmol of PG D_2 These results suggest that PG D_2 might be involved in inducing cataleptic behavior by modulating serotonergic, cholinergic and dopaminergic systems

Prostaglandin D_2 Cataleptic behavior Dopaminergic system Serotonergic system Cholinergic system

RECENTLY prostaglandins (PGs) have been proposed to modulate catecholaminergic [2, 4, 17], serotonergic [3,4] and cholinergic [21] neurons in the central nervous system. Until recently, the function of only the E and F series of PGs in mammalian brain have been investigated. But it is now evident that there is considerable species variation in the distributions of central PGs and that PG D_2 is a major PG in the central nervous systems of various mammals, including human [1, 14, 15, 22, 26], the levels of PG E_2 and PG $F_{2\alpha}$ being considerably lower. Recently, the following studies on PG D_2 were reported (1) The localization of ^{3}H-PG D_2 binding sites was demonstrated [33]. (2) PG D_2 synthetase [22] and 15-hydroxy-PG D_2 dehydrogenase [27,32], which are responsible for the biosynthesis and degradation of PG D_2, were purified from the brain (3) PG D_2 was shown to have a sedative action and to increase the pentobarbitone-induced sleeping time in rodents [3, 9, 12]. (4) PG D_2 was found to exert several neurophysiological effects, such as sleep induction [29,30] and hypothermia [31] when administered intracerebroventricularly or intracerebrally. However, the participation of central monoaminergic systems to PG D_2-induced cataleptic behavior is still unclear.

In this study, we found that central administration of PG D_2 induced marked cataleptic behavior in rats, and we investigated the roles of classical neurotransmitters in the cataleptic effect of PG D_2

METHOD

Animals

Male Wistar strain rats weighing 220–250 g were obtained from Kyudo Co (Saga, Japan). The animals were kept under a 12 hr-12 hr light-dark cycle (7·00 a.m.–7·00 p.m.) in a room at 25±1°C and 50±10% humidity, and had free access to commercial diet (CE-2, Clea Co , Japan) and tap water, except during measurement of catalepsy

Intracerebroventricular (ICV) Administration

For ICV administration of PG D_2, a 22-gauge guide cannula of stainless steel was implanted in the right lateral ventricle of rats under pentobarbital anesthesia according to the stereotaxic coordinates of Paxinos and Watson [18]. One week after surgery, ICV injections of volumes of 10 μl of drugs were given over a period of 1 min through a 27 gauge injection tube connected to the implanted cannula. After the experiment, the site of the injection was determined by injecting malachite green dye and then examining the distribution of dye in the ventricle.

Behavioral Measurement

Cataleptic behavior was measured by the high bar test between 9 00 a.m and 4·00 p.m in a dark, sound-proof room.

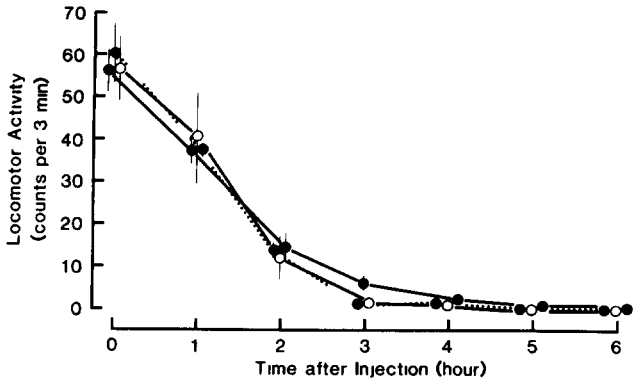

FIG 1 Effect of ICV administration of PG D_2 on locomotor activity observed by the open-field method in rats. Each value at the zero time represents the mean±S E M before the injection of PG D_2 or vehicle ○—○, vehicle (n=4), ● ●, PG D_2 50 nmol (n=4), ●—●, PG D_2 100 nmol (n=6)

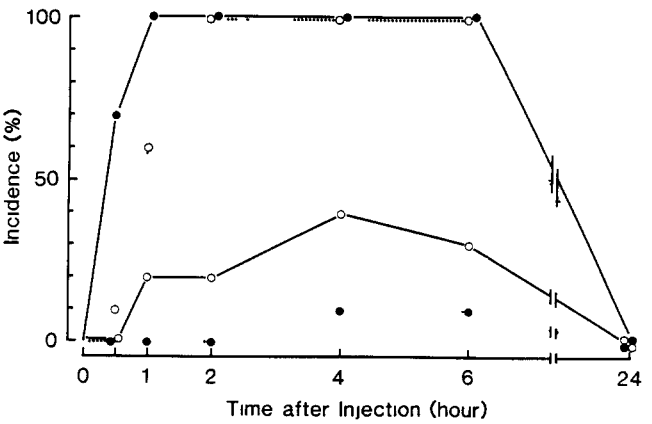

FIG 2 Cataleptic behavior induced by ICV administration of PG D_2 in rats Each point represents the incidence of the behavior ● ●, vehicle (n=10), ○—○, PG D_2 2 nmol (n=10), ○ ○, PG D_2 10 nmol (n=10), ●—●, PG D_2 50 nmol (n=10)

TABLE 1

EFFECTS OF DOPAMINERGIC AND CHOLINERGIC DRUGS ON PG D_2-INDUCED CATALEPTIC BEHAVIOR

Drug	Dose (mg/kg)	PG D_2 (Incidence of Catalepsy) 2 nmol	50 nmol
Saline		2/10	10/10
L-DOPA	50	—	5/8
	100	—	1/8†
Apomorphine	0 5	—	6/8
	1	—	3/8†
Amantadine	0 1	—	7/8
	0 2	—	3/8*
	0 4	—	2/8†
Atropine	0 2	—	6/8
	0 5	—	2/8†

Incidences are shown as numbers of cataleptic animals/numbers of animals tested *†Significant differences from control at $p<0$ 05 and $p<0$ 01, respectively

TABLE 2

EFFECT OF SEROTONERGIC AND NORADRENERGIC DRUGS ON PG D_2-INDUCED CATALEPTIC BEHAVIOR

Drug	Dose (mg/kg)	PG D_2 (Incidence of Catalepsy) 2 nmol	50 nmol
Saline		2/10	10/10
PCPA	300	—	0/10†
5-MDMT	3	4/8	—
	5	8/8†	7/8
5-HTP	10	4/7	—
	30	6/7*	—
	100	—	8/8
Imipramine	10	4/6	—
	20	8/9†	8/8
Clomipramine	10	5/7*	—
	20	7/7†	8/8
Phenoxybenzamine	10	3/8	8/8
Propranolol	10	4/8	7/8

Incidences are shown as numbers of cataleptic animals/numbers of animals tested *†Significant differences from control at $p<0$ 05 and $p<0$ 01, respectively

This test was carried out by placing the front paws of the rat on a horizontal metal bar suspended 12 cm above a platform In the present experiment, the catalepsy test was considered to be positive when the animal did not remove its paws within 30 sec. The degree of cataleptic behavior was expressed as the number of rats showing a positive response When the effects of drug pretreatment were investigated, the incidence of cataleptic behavior was determined only once at one hour after administration of PG D_2 to avoid the complication of the effect of the testing procedure on that of the drug [25].

The spontaneous motor activity was measured by open-field test. The open-field chamber was 60 cm in diameter and 50 cm in height, the floor was divided into 19 blocks A rat was placed on the center of the floor and observed for 3 min Locomotor activity, ambulation, was expressed in terms of the number of blocks traversed.

All drugs except PG D_2 were administered intraperitoneally (IP) and in a volume of 1–2 ml/kg.

Statistical analyses were carried out by the two tailed Fisher's exact probability test.

Drugs

PG D_2 (Funakoshi), p-chlorophenylalanine (PCPA) (Sigma), 5-methoxy-N,N-dimethyltryptamine (5-MDMT) (Sigma), L-dihydroxyphenylalanine (L-DOPA) (Daiichi Chemicals), amantadine hydrochloride (Fujisawa) and phenoxybenzamine hydrochloride (Tokyo Chemicals) were suspended in 0.5% carboxymethylcellulose sodium solution. Imipramine hydrochloride (Ciba-Geigy), apomorphine hydrochloride (Fujisawa), propranolol hydrochloride (Sigma), atropine sulfate (Merck) and clomipramine (Ciba-Geigy) were dissolved in 0 9% physiological saline

RESULTS

Effect of PG D_2 on Spontaneous Motor Activity

Spontaneous motor activity, ambulation, was measured at several time intervals after a single ICV administration of PG D_2 or vehicle. As Fig. 1 shows, spontaneous locomotor activity after PG D_2 was not significantly different from that after vehicle. Furthermore, stereotypy, aggressive behavior or rigidity were also not found, but marked hyperirritability characterized by squealing and startling responses to stimuli such as touch or a puff of air in home cage was observed.

PG D_2-Induced Cataleptic Behavior

As shown in Fig 2, ICV injections of PG D_2 induced dose-related increase of cataleptic behavior The incidences of this behavior at doses of 0, 2, 10 and 50 nmol/animal of PG D_2 one hour after administration were 0, 20, 60 and 100%, respectively, and this effect persisted for more than 6 hours.

Influences of Various Drugs on PG D_2-Induced Cataleptic Behavior

For observation of the influences of various drugs on PG D_2-induced cataleptic behavior, cataleptic behavior was measured 1 hour after ICV administration of PG D_2. As shown in Table 1, the incidences of cataleptic behavior with doses of 2 nmol and 50 nmol of PG D_2 were 20% and 100%, respectively Simultaneous treatments with L-DOPA (50–100 mg/kg), apomorphine (0.5–1 mg/kg) and amantadine (0 1–0 4 mg/kg) reduced the incidence of cataleptic behavior induced by 50 nmol of PG D_2 dose-dependently. It is noteworthy that the effective dose range of amantadine was very low Atropine (0 5 mg/kg) also significantly reduced the incidence of cataleptic behavior No abnormal behavior in home cage was observed after these treatments by these drugs Apomorphine produced a stereotyped behavior such as discontinuous sniffing

Treatment with PCPA (300 mg/kg) 72 hours before treatment with 50 nmol of PG D_2 significantly reduced the incidence of catalepsy, but simultaneous treatment with 5-MDMT (5 mg/kg), 5-HTP (100 mg/kg), imipramine (20 mg/kg) or clomipramine (20 mg/kg) had little effect (Table 2) On the other hand, the simultaneous treatment with 5-MDMT (3–5 mg/kg), 5-HTP (10–30 mg/kg), imipramine (10–20 mg/kg) or clomipramine (10–20 mg/kg) dose-dependently increased the incidence of cataleptic behavior induced by 2 nmol of PG D_2 Simultaneous treatment with phenoxybenzamine (10 mg/kg) or propranolol (10 mg/kg) rarely affected the incidence of cataleptic behavior induced by either 2 nmol or 50 nmol of PG D_2

DISCUSSION

In this work, we injected PG D_2 ICV, because PGs do not readily penetrate into the central nervous system when they are injected systemically [8] and they are rapidly inactivated in the pulmonary circulation [6,20].

ICV administration of PG D_2 had dose-dependently exhibited cataleptic behavior In the meanwhile, any sedative effect of PG D_2 was not detected as judged by measuring the effects of PG D_2 on spontaneous locomotion in open-field tests. This observation in the motor activity was not in accordance with several reports [7,12]. Forstermann *et al* [7] reported that ICV administration of PG D_2 reduced spontaneous motor activity measured continuously using an Animex electronic mobility meter set at maximum sensitivity. This difference might be due to the use of different methods of testing. In fact, PG D_2 induced hyperactive responses of external stimuli such as noise and touch in home cage. When cataleptic behavior had once been induced, however, the animals maintained an unnatural posture as long as stimuli were not applied.

Catalepsy is known to be induced in several species by neuroleptics. From the view point of brain dopamine system, this catalepsy is chiefly due to blockade of dopamine receptors in the nigrostriatal system In the present studies, L-DOPA and apomorphine significantly suppressed the cataleptic behavior. Furthermore, amantadine, a dopamine releaser and dopamine-receptor agonist, was significantly inhibitory at a dose as low as 0 2 mg/kg Accordingly, PG D_2 might be involved in producing cataleptic behavior mainly by lowering the activity of dopamine neurons and suppressing dopamine release from neuronal terminals

Cholinergic functions are also known to be involved in catalepsy induced by neuroleptics [13,35]. Klemm [10] postulated that catalepsy is due to modifying an interaction between cholinergic and dopaminergic systems. Since dopamine is generally thought to act as an inhibitory neurotransmitter in the striatum [24] that might be a main part in promotion of cataleptic behavior, the dopaminergic system may prevent the cataleptic behavior by inhibiting the cholinergic system in the striatum. In this work, atropine inhibited PG D_2-induced cataleptic behavior. Moreover, other types of PG E and F series administered ICV as well as pilocarpine IP produced weak cataleptic behavior in rats compared with haloperidol The pilocarpine-induced behavior was potentiated by PG $F_{2\alpha}$ ICV [16]. Therefore, PGs including PG D_2 seem to participate in dopaminergic-cholinergic interaction and the development of cataleptic behavior.

Recently, a specific PG D_2-binding protein was found in the synaptic membrane fraction of rat brain [23,28]. Furthermore, Yamashita *et al* [34] examined the localization of [^{3}H]PG D_2-binding protein and observed no significant binding in the striatum. Thus, PG D_2-induced cataleptic behavior may be manifested via some neuronal system to the striatum.

The central serotonergic system acts as an inhibitory control to the nigro-striatal dopaminergic pathway [19]. Neuroleptics-induced cataleptic behavior also apparently depends on the balance between dopaminergic and serotonergic systems [5]. In the present studies, PG D_2-induced cataleptic behavior, at a dose of 50 nmol, was significantly inhibited by PCPA, an inhibitor of serotonin synthesis In addition, a dose and treating schedule of PCPA hereby used elicited 90% depletion of serotonin concentration in rat brain with no influence in catecholamine [11], while PG D_2 (2 nmol)-induced cataleptic behavior was potentiated by serotonergic drugs such as 5-HTP, 5-MDMT, imipramine and clomipramine Thus, unlike catalepsy induced by neuroleptics, changes in the activity of serotonin neurons are apparently important in the manifestation of PG D_2-induced cataleptic behavior Bhattacharya and Parmar [3] had shown that PG D_2 potentiated hexobarbitone hypnosis by enhancing serotonin turnover in rat brain.

PG D_2 probably exerts its cataleptic effect by modulating dopaminergic, cholinergic and serotonergic neurotransmissions This PG D_2-induced cataleptic behavior may be associated with increased cholinergic and serotonergic activities, and reduced dopaminergic activity in rat brain. Imipramine and clomipramine inhibit norepinephrine uptake as well as

serotonin uptake However, central noradrenergic functions may not be involved in PG D_2-induced catalepsy, because this cataleptic behavior was not affected by phenoxybenzamine or propranolol.

In conclusion, these results suggest a possible involvement of PG D_2 to serotonergic system with central dopaminergic and cholinergic systems in the induction of cataleptic behavior.

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