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Report

Prostaglandin D_2 Inhibits Pentylenetetrazole-Induced Convulsions in Rats by a Serotonin-Mediated Mechanism

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Prostaglandins (PGs) of the E series are known to exert anticonvulsant action in experimental animals. Earlier studies from this laboratory have indicated that PGE_1 inhibits pentylenetetrazole (PTZ)-induced convulsions in rats through a serotonin-mediated mechanism. PGD_2, the major PG in the rodent brain, shares a number of central pharmacological actions of the PGEs, and like the latter it potentiates the anticonvulsant action of phenobarbitone and phenytoin in rats. The present study was undertaken to investigate the putative anticonvulsant action of PGD_2 against PTZ-induced convulsions in rats and to evaluate the role of serotonin in the anticonvulsant action of PGD_2. PGD_2 (5, 10, and 20 μg, icv) produced a dose-related inhibition of PTZ-induced clonic convulsions in rats. The anticonvulsant action of PGD_2 (20 μg, icv) was significantly attenuated following pretreatment of the rats with pharmacologic agents known to reduce central serotonergic activity, including 5,6-dihydroxytryptamine, a selective neurotoxin for serotonergic neurons, *p*-chlorophenylalanine, a specific inhibitor of serotonin biosynthesis, metergoline, a serotonin postsynaptic receptor antagonist, and quipazine, which is known to inhibit neuronal release of serotonin. These findings, in conjunction with an earlier study from this laboratory indicating that PGD_2 augments rat brain serotonergic activity, suggest that the anticonvulsant activity of PGD_2 against PTZ-induced convulsions in rats is mediated through a serotonergic mechanism.

KEY WORDS: prostaglandin D_2; pentylenetetrazole convulsions; serotonin.

INTRODUCTION

Considerable evidence now exists that makes it possible to assign a physiological role for prostaglandins (PGs) in the mammalian central nervous system (CNS) (1). PGs are suggested to exert a modulatory influence on central neuronal activity (1). Catecholamines and serotonin have been reported to stimulate PG biosynthesis and release (1,2). Likewise, PGs are reported to affect central catecholaminergic and serotonergic activity (3–5). Until recently, mostly PGs of the E and F series were investigated in mammalian brain functions. However, it is now evident that there is considerable species variation in the distribution of the central PGs and that PGD_2 is by far the most dominant PG in the rat and mouse CNS (6). Recent studies have indicated that PGD_2 shares several of the central actions of the PGEs. Thus, like PGE_1 (7), PGD_2 potentiates the hypnotic action of barbiturates by a serotonin-mediated mechanism (8). In addition, PGD_2 has been shown to potentiate the anticonvulsant action of phenobarbitone (9), as was reported earlier for PGE_1 (10), the effect in either case being shown to be serotonin mediated. Furthermore, PGD_2 (11) has been shown to exhibit significant antinociceptive activity on central administration in rats, as was previously reported with PGE_1 (12).

We have earlier reported that PGE_1 inhibits pentylenetetrazol (PTZ)-induced clonic convulsions in rats, effected through a serotonin-mediated inhibitory mechanism (13). In this communication we report the effect of centrally administered PGD_2 on PTZ-induced convulsions in rats and demonstrate the role of serotonin in the PGD_2-PTZ interaction.

MATERIALS AND METHODS

Wistar-strain albino rats (150–200 g) of both sexes were used. The animals were caged individually with free access to standard pellet chow and water, at an ambient temperature of 22 ± 2°C and 45–55% relative humidity, with a 12-hr light–dark cycle. All the experiments were conducted at this ambient temperature between 9:00 AM and 2:00 PM. PTZ (60 mg/kg, sc) was administered and the rats were observed for 60 min for the appearance of clonic convulsions. No attempt was made to study either the duration or the intensity of the convulsions. The end point was either the presence or the absence of an episode of clonic seizure lasting for at least 10 sec. The choice of the dose of PTZ was based on pilot experiments.

Intracerebroventricular (icv) cannulation of the right lateral ventricle was performed in pentobarbital sodium (40 mg/kg, ip)-anesthetized rats and indwelling cannulae were inserted stereotaxically (14). The rats were used 1 week after the cannulation. All the drugs, except PTZ, were administered icv dissolved in 10 μl of artificial cerebrospinal fluid (CSF) (14). PGD_2 was suspended in 1% ethanol prior to

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dilution with artificial CSF and administered in a fixed volume of 10 μl. Control rats received an equivalent volume of 1% ethanol in artificial CSF. Initially a pilot time-course study was done to determine the onset, peak effect, and duration of action of PGD_2. Groups of five rats were administered PGD_2 (20 μg, icv) and were then challenged with PTZ (60 mg/kg, sc) after 5, 15, 30, 45, and 60 min in the different groups. Since the peak anticonvulsant effect of PGD_2 was noted at 15 min, graded doses of PGD_2 (5, 10, and 20 μg, icv) were administered 15 min before the PTZ challenge.

The drugs which were used to investigate PGD_2–PTZ interaction, with doses and pretreatment times given in parentheses, were as follows: 5,6-dihydroxytryptamine creatinine sulfate (DHT; 75 μg, 72 hr), *p*-chlorophenylalanine methyl ester hydrochloride (PCPA; 100 μg, once daily for 3 days), metergoline (10 μg, 15 min), quipazine maleate (20 μg, 30 min), and $PGF_2\alpha$ (20 μg, 15 min). The doses refer to the respective salts, and the pretreatment time to the time interval between the administration of these drugs and the administration of PGD_2.

Statistical analysis of the data was done by the chi-square test.

RESULTS

PGD_2 (5, 10, and 20 μg, icv) produced a dose-related inhibition of PTZ-induced clonic convulsions in rats; the percentage inhibition was 32.3, 55.6, and 88.9%, respectively, the effect of the last two doses being statistically significant (Table I). A time-course study on the anticonvulsant effect of PGD_2 (20 μg, icv) done earlier had indicated that the peak effect is achieved 15 min after the administration of PGD_2. Hence these and the subsequent data refer to this protocol. PGD_2-treated rats showed signs of sedation and reduced spontaneous motility but no motor incoordination. Pretreatment with DHT, which induces selective degeneration of central serotonergic neurones on icv administration, and PCPA, a specific inhibitor of serotonin biosynthesis, significantly attenuated PGD_2 (20 μg)-induced inhibition of PTZ convulsions. In an earlier study from this laboratory (15), DHT and PCPA have been shown to reduce rat brain serotonin concentrations by nearly 50%, at the doses and pretreatment times used in this study. Metergoline, a well-documented antagonist of postsynaptic serotonin receptors, also attenuated the anticonvulsant action of PGD_2, as did quipazine, which has been reported to decrease the neuronal release of serotonin by acting as an agonist at the central presynaptic serotonergic receptors, resulting in inhibition of neuronal firing and intraneuronal accumulation of serotonin (16). $PGF_2\alpha$ had a statistically insignificant inhibitory effect on the anticonvulsant action of PGD_2 (Table I).

Table I. The Effect of PGD_2 on PTZ-Induced Clonic Convulsions in Rats and the Effect of Selected Drugs on PGD_2–PTZ Interaction

Groups	*N*	Incidence of convulsions (%)	*P*
Vehicle + PTZ	20	90	—
PGD_2 (5 μg) + PTZ	10	70	NS[a,*]
PGD_2 (10 μg) + PTZ	10	40	<0.05[a]
PGD_2 (20 μg) + PTZ	10	10	<0.01[a]
DHT + PGD_2 (20 μg) + PTZ	10	80	<0.01[b]
PCPA + PGD_2 (20 μg) + PTZ	10	70	<0.01[b]
Metergoline + PGD_2 (20 μg) + PTZ	10	60	<0.05[b]
Quipazine + PGD_2 (20 μg) + PTZ	10	60	<0.05[b]
$PGF_2\alpha$ + PGD_2 (20 μg) + PTZ	10	50	NS[b]

* a and b denote statistical significance in comparison to the vehicle + PTZ and the PGD_2 (20 μg) + PTZ groups, respectively. NS signifies that the data are statistically insignificant.

DISCUSSION

The findings of the present study are contrary to an earlier report that PGD_2 has no inhibitory effect on PTZ convulsions in rats (17). It is possible that this discrepancy is due to the difference in the route of administration of PGD_2. These workers administered the PG peripherally; however, PGs are rapidly metabolized on the first pass through the lungs (1). The discrepancy is not likely to be related to a lack of accessibility of PGD_2 through the blood–brain barrier since the PG has been reported to augment pentobarbital sleeping time and to reduce locomotor activity in rodents when administered either intraventricularly or intravenously (18).

The role of PGs in experimental convulsions has been reviewed (1). PGs of the E series have been shown to inhibit convulsions induced by PTZ, picrotoxin, strychnine, or isoniazid in rodents. Since the convulsive effects of these agents appear to be related to a direct antagonism of γ-aminobutyric acid (GABA)-mediated postsynaptic inhibition and PGE_2 has been shown to enhance mouse brain GABA levels (19), it has been postulated that this PGE_2 action could contribute to the anticonvulsant effect (1). Some reports indicate that the anticonvulsant action of PGEs may be linked to their effect on central cyclic nucleotides. Thus, PGE_2 has been shown to increase the levels of cyclic 3′,5′,-adenosine monophosphate (cAMP) and prevent the increase in the levels of cyclic 3′,5′-guanosine monophosphate (cGMP). An increase in central cGMP levels is likely to be the triggering factor for induction of convulsions, whereas cAMP is thought to function as a convulsion suppressant (1,20). PTZ has been found to enhance rat brain cGMP, which is prevented by PGE_2 (20). Unlike the PGEs, $PGF_2\alpha$ does not alter the concentration of central cyclic nucleotides and also lacks anticonvulsant activity (20). There are, however, reports that indicate that $PGF_2\alpha$ is proconvulsant, since it decreases the threshold of electroconvulsive seizures and potentiates PTZ convulsions in rats (21). The anticonvulsant effect of PGE_1 against PTZ-induced clonic convulsions in rats has been shown to be serotonin mediated (13). The clinical relevance of the role of PGs in convulsive episodes is still unclear, although inhibitors of PG synthesis such as corticosteroids, which inhibit phospholipase A_2, and indomethacin, a cyclooxygenase inhibitor, have been reported to aggravate epileptic seizures (22,23).

Pharmacological agents such as DHT, PCPA, metergoline, and quipazine, which selectively attenuate central serotonergic activity by diverse mechanisms, antagonized the anticonvulsant activity of PGD_2. Recent studies from this laboratory have shown that PGD_2 augments rat brain seroto-

nergic activity (24), a property it shares with PGEs (4). A number of central actions of PGD_2 have been reported to be serotonin mediated, including potentiation of hexobarbitone hypnosis (8), potentiation of anticonvulsant actions of phenobarbitone and phenytoin (9), and per se antinociceptive activity (11), in rats. The role of serotonin in experimental seizures is equivocal (25), although there is sufficient evidence to suggest that the amine may have an inhibitory role in PTZ convulsions (25). Experimentally induced increases in rat brain serotonergic activity have been shown to attenuate PTZ convulsions, whereas an induced decrease in the amine activity had the opposite effect (26).

The present study, thus, indicates that the anticonvulsant action of centrally administered PGD_2, against PTZ-induced convulsions in rats, is an indirect one mediated through the inhibitory serotonergic system. Apart from serotonin, a number of other central neurotransmitter systems have been implicated in convulsive seizures, including catecholamines, acetylcholine, GABA, and endogenous opioid peptides (25). PGs are known to affect these neurotransmitters (1–3,27). However, their putative role in PG-induced changes in seizure threshold remains unknown (25).

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