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Prostaglandin D_2-induced catalepsy in rats: role of 5-hydroxytryptamine

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Prostaglandin D_2 (PGD_2), the major PG in the rat brain, induced a dose-related catalepsy in rats on intracerebroventricular (i.c.v.) administration. This cataleptic response was significantly attenuated following the i.c.v. administration of pharmacological agents that decrease rat brain 5-hydroxytryptamine (5-HT) activity. PGE_1 synergized but $PGF_{2\alpha}$ antagonized the catalepsy induced by PGD_2. PGD_2 and PGE_1 have previously been shown to augment rat brain 5-HT activity, whereas PGF_α inhibited it. It is therefore likely that the observed effects of these PGs on catalepsy involve a central 5-HT mechanism.

There is now considerable evidence that suggests a central neuronal modulator activity of prostaglandins (PGs) (Wolfe 1982). A wide variety of arachidonic acid metabolites are present in the mammalian central nervous system (CNS) and they have been shown to be synthesized afresh at neural and non-neural sites (Wolfe 1982). Until recently, PGs of the E and F series were thought to be the major eicosanoids in the CNS. However, it is now apparent that considerable species variation exists in the distribution of central PGs and that PGD_2 is by far the most abundant eicosanoid in the rat and mouse brain, the levels of PGE_2 and $PGF_{2\alpha}$ being much lower (Abdel-Halim et al 1977).

Most of the experimental data on the central actions of PGs pertain to the PGEs or PGFs (Wolfe 1982) while data on the central actions of PGD_2 are only recently available. One of the most pertinent features of the actions of PGD_2 on the CNS is the remarkable similarity with the reported actions of PGEs. Thus, both these PGs induce sedation, reduce spontaneous motility and produce hyperthermia (Laychock et al 1980). The electroencephalographic changes induced by PGD_2 and PGE_2 are indistinguishable, characterized by conversion from uniform low voltage fast pattern to high voltage slow waves (Laychock et al 1980). PGD_2, like PGE_1, potentiates hexobarbitone hypnosis (Bhattacharya et al 1976; Bhattacharya & Parmar 1985a), inhibits metrazol-induced convulsions (Bhattacharya & Sanyal 1978a; Bhattacharya 1987), potentiates anticonvulsant drug action (Bhattacharya & Sanyal 1978b; Bhattacharya & Parmar 1985b) and itself produces antinociceptive action on i.c.v. administration in rats (Sanyal et al 1979; Bhattacharya 1986). In addition, both PGD_2 and PGE_1 augment rat brain 5-HT turnover (Debnath et al 1978; Bhattacharya et al 1985) and depress sympathetic neurotransmission (Hedqvist 1977; Henker & Aiken 1980).

During an earlier study (Bhattacharya 1986), it was observed that higher doses of i.c.v. administered PGD_2 tended to induce catalepsy in rats, a phenomenon also observed in cats (Laychock et al 1980). PGEs (Coceani 1974) and PGI_2 are known to produce catalepsy in experimental animals (Brus et al 1985). The catalepsy induced by PGE_1 after i.c.v. administration in rats, has been shown to be, at least partly, a 5-HT-mediated behavioural response (Bhattacharya et al 1984). Several of the central actions of the PGEs and PGD_2 have been demonstrated to be 5-HT-mediated effects in rats, and have been reviewed recently (Bhattacharya 1985). The present study was designed to investigate the role of 5-HT in PGD_2-induced catalepsy in rats and to assess the effects of the other PGs on this behavioural phenomenon.

Materials and methods

Wistar strain albino rats (150–200 g), of either sex, were used. They were housed in individual Perspex cages with free access to standard pellet chow and water, at an ambient temperature of 22–25 °C and 45–55% relative humidity, with a 12 h light–dark illumination. The experiments were conducted at this ambient temperature between 0900 and 1400h, during the light phase.

Catalepsy was initially assessed by the staging system (Kuschinsky & Hornykiewicz 1972). Quantification of the intensity of the catalepsy was done by the 'ring test' (Pertwee 1972) in which the rat was placed on a steel ring, 12 cm in diameter, fixed to a steel stand at a height of 35 cm, at a predetermined time after the drug administration. The time during which the rat remained motionless, with complete cessation of snout and whisker movements, out of a total observation period of 5 min, was converted into 'percent immobility' (Pertwee 1972). Intracerebroventricular cannulation of the right lateral ventricle was performed in pentobarbitone sodium (40 mg kg^{-1} i.p.) anaesthetized rats (Feldberg & Lotti 1967). Experiments were conducted one week after the insertion of indwelling cannulae. All the drugs used were administered i.c.v., dissolved in 10 μL of artificial cerebrospinal fluid (Feldberg & Lotti 1967). Control animals received an equivalent volume of the vehicle via the same route.

All the PGs used were initially dissolved in 1% ethanol before dilution with artificial cerebrospinal fluid (csf). The control animals received 1% ethanol in artificial csf. The drugs used, apart from the PGs, with doses and pretreatment times mentioned in parentheses, were: 5,6-dihydroxytryptamine creatinine sulphate (75 μg/rat, 72 h), *p*-chlorophenylalanine methyl ester hydrochloride (100 μg/rat, once daily for 3 days),

* Correspondence.

metergoline (10 μg/rat, 15 min) and quipazine maleate (20 μg/rat, 30 min). The doses refer to the respective salts and the pretreatment time to the interval between the administration of these drugs and the administration of PGD_2, and are based on data available in this laboratory (Bhattacharya 1985). PGE_2 and $PGF_{2\alpha}$ were administered immediately before PGD_2 administration.

Earlier studies have indicated that the peak cataleptic effect of PGD_2 (Bhattacharya 1986) and PGE_1 (Bhattacharya et al 1984) is attained 15 min after their i.c.v. administration and that the effect wanes by 45–60 min. In the present study, the intensity of PGD_2-induced catalepsy was assessed 15 min after i.c.v. administration and no attempt was made to investigate drug effects on the duration of the catalepsy.

Statistical analysis of the data was initially done by using analysis of variance (ANOVA) and the data which proved to be statistically significant ($P < 0.05$) were then subjected to the Student's *t*-test.

Results and discussion

PGD_2 (10, 20 and 50 μg/rat, i.c.v.) produced a dose-related cataleptic effect, as assessed 15 min after its administration. Smaller doses (1, 2 and 5 μg/rat, i.c.v.) induced discernible sedation and reduced spontaneous motor activity only. PGD_2 (50 μg)-induced catalepsy was significantly attenuated following pretreatment of the rats with 5,6-dihydroxytryptamine (5,6-DHT), which induces selective degeneration of central 5-HT neurons, *p*-chlorophenylalanine (PCPA), a specific inhibitor of 5-HT biosynthesis, metergoline, a 5-HT postsynaptic receptor antagonist, and quipazine, a 5-HT presynaptic receptor agonist (Winter 1979). 5,6-DHT, PCPA, metergoline and quipazine did not produce any overt behavioural effect of their own in the doses used, nor did they show any effect themselves on the experimental parameter used. $PGF_{2\alpha}$ (20 μg/rat i.c.v.), had no effect of its own on the ring test but it significantly antagonized the cataleptic effect of PGD_2 (50 μg/rat). PGE_2 (10 and 20 μg/rat, i.c.v.) itself produced catalepsy, the effect being statistically significant with the higher dose, and appeared to have an additive synergistic effect with PGD_2 (10 and 20 μg/rat) (Table 1).

Catalepsy has been defined as a characteristic behavioural state in which the experimental animals retain the ability to sustain induced abnormal postures for considerable lengths of time, and it can be induced by both neuroleptic and non-neuroleptic agents (Pertwee 1972). Many methods are available to assess experimental catalepsy. However, the 'ring test' has been shown to be sensitive enough to be used for the bioassay of cannabinoids (Pertwee 1972).

PGs, including PGEs, PGD_2 and PGI_2, are known to induce experimental catalepsy in relatively large doses (Laychock et al 1980; Bhattacharya et al 1984; Brus et al 1985). Most of the catalepsy-inducing drugs, including morphine, neuroleptics and cannabis, produce this behavioural state in doses which are significantly higher than those inducing sedation alone (Bose & Bhattacharya 1979; Ghosh et al 1980; Bhattacharya & Bhattacharya 1982). The relatively large doses of PGD_2 required to induce catalepsy, as observed in the present study, may also be due to effective clearance of the PG from the brain by choroidal and extrachoroidal carrier-mediated transport processes (Bito et al 1976). Most of the PGs injected into the lateral ventricle can be recovered from the cisterna magna (Holmes 1970). The evidence currently available indicates that two mechanisms may account for the removal of the PGs from csf. One is the choroid plexus, which actively sequesters PGs from solutions, and the other an active transport in the pia mater (Davson 1976). Concerning the latter, it has been noted that radioactive PGs injected into the cisterna magna of dogs rapidly disappeared and only a minute fraction of the injected PG was detected in lumbar csf (Hagen et al 1977). Radioactivity detected in blood from the jugular vein, indicated that this PG rapidly egresses from csf to blood. Pretreatment of rats with probenecid, which inhibits the facilitated transport of PGs across the csf-blood barrier (Bito et al 1976), is an essential prerequisite to bring rat brain PGs within assayable limits by radioimmunoassay (Bhattacharya 1982a; Bhattacharya & Das 1984). Pretreatment with probenecid (120 mg kg^{-1} i.p.) has been observed to potentiate several pharmacological actions of centrally administered PGE_1 and PGD_2 (unpublished

Table 1. Effect of some drugs on PGD_2-induced catalepsy in rats.

Groups	n	Percent immobility Mean	s.e.m.	*P*
Control (artificial csf)	10	10·5	3·2	—
PGD_2 (10 μg)	6	21·7	6·6	NS[a]
PGD_2 (20 μg)	6	43·8	4·2	<0·001[a]
PGD_2 (50 μg)	10	76·4	2·4	<0·001[a]
PGE_2 (10 μg)	6	24·6	7·2	NS[a]
PGE_2 (20 μg)	8	48·2	3·7	<0·001[a]
$PGF_{2\alpha}$ (20 μg)	6	15·0	6·6	NS[a]
5-DHT	5	8·6	2·0	NS[a]
PCPA	5	8·0	2·6	NS[a]
Metergoline	5	12·4	2·8	NS[a]
Quipazine	5	14·8	3·2	NS[a]
PGE_2 (10 μg) + PGD_2 (10 μg)	6	51·9	4·0	<0·001[b]
PGE_2 (20 μg) + PGD_2 (20 μg)	6	88·6	1·6	<0·001[b]
$PGF_{2\alpha}$ (20 μg) + PGD_2 (50 μg)	6	64·6	2·9	<0·01[c]
5-DHT + PGD_2 (50 μg)	8	39·9	4·6	<0·001[c]
PCPA + PGD_2 (50 μg)	8	44·8	3·7	<0·001[c]
Metergoline + PGD_2 (50 μg)	6	52·1	4·3	<0·01[c]
Quipazine + PGD_2 (50 μg)	6	42·4	4·0	<0·001[c]

Statistically significant differences are indicated as follows: [a] in comparison with the control; [b] in comparison with the respective PGD_2 (10 or 20 μg) or PGE_2 (10 or 20 μg) groups; [c] in comparison with the PGD_2 (50 μg) group. NS indicates that the values are statistically non-significant.

data), presumably by enhancing their retention in the CNS.

PGs have been shown to be involved in a variety of drug-induced cataleptic states, and PG synthesis inhibitors are reported to inhibit morphine- (Bose & Bhattacharya 1979), cannabis- (Ghosh et al 1980) and haloperidol- (Bhattacharya & Bhattacharya 1982) induced catalepsy in rats. The role of PGs in tetrahydrocannabinol-induced catalepsy in mice has been demonstrated conclusively (Fairbairn & Pickens 1979, 1980).

PGD_2-induced catalepsy was significantly attenuated by pharmacological agents which reduce central 5-HT activity by different mechanisms. 5,6-DHT and PCPA have been shown to reduce rat brain 5-HT concentrations by approximately 50%, in the doses and pretreatment times used in this study (Amar et al 1982). The postsynaptic 5-HT receptor antagonist action of metergoline is well documented, while quipazine has been reported to decrease the neuronal release of 5-HT by acting as an agonist at the presynaptic 5-HT receptors and inducing intraneuronal accumulation of the amine consequent to reduced firing of the neurons (Winter 1979). Since none of these agents produced discernible behavioural effects of their own, in the doses used, and their effects on central 5-HT neurons is fairly selective and specific, it appears plausible that PGD_2-induced catalepsy in rats is a 5-HT-mediated response.

$PGF_{2\alpha}$, administered centrally, significantly inhibited the cataleptic action of PGD_2, while PGE_2 had a synergistic effect. $PGF_{2\alpha}$-induced antagonism of PGE_1-induced catalepsy is on record (Bhattacharya et al 1984). PGE_1 catalepsy, in rats, has also been shown to be a 5-HT-mediated response. Both PGE_1 (Debnath et al 1978) and PGD_2 (Bhattacharya et al 1985) facilitate rat brain 5-HT activity, which, however, is attenuated by $PGF_{2\alpha}$ (Bhattacharya 1982b). It is therefore likely that $PGF_{2\alpha}$-induced antagonism and PGE_2-induced synergism of PGD_2 catalepsy is consequent to their reported effects on rat brain 5-HT activity. $PGF_{2\alpha}$ has been shown to antagonize several 5-HT-mediated central actions of PGEs and PGD_2, as has been recently reviewed (Bhattacharya 1985).

The present study indicates that the reported similarity in the central actions of PGEs and PGD_2 can be extended to the induction of catalepsy in rats, after central administration. The mechanism of PGD_2-induced catalepsy in rats appears to be similar to that reported for PGE_1, since both these PGs facilitate 5-HT neurotransmission in the species. $PGF_{2\alpha}$, by virtue of its contrary effect on 5-HT activity, inhibits PGD_2 catalepsy. The data gathered from the present study lends credence to the postulate that PGs function as modulators of central 5-HT activity, with the PGEs and PGD_2 augmenting and $PGF_{2\alpha}$ reducing the activity of the transmitter in the rat brain (Bhattacharya 1985).

REFERENCES

Abdel-Halim, M. S., Hamberg, S., Sjoquist, B., Anggard, E. (1977) Prostaglandins 14: 633–643

Amar, A., Mandal, S., Sanyal, A. K. (1982) Acta Endocrinol. 101: 180–186

Bhattacharya, S. K. (1982a) Neurosci. Lett. 33: 165–168

Bhattacharya, S. K. (1982b) Res. Comm. Chem. Pathol. Pharmacol. 38: 149–152

Bhattacharya, S. K. (1985) Pharm. Res. 5: 195–198

Bhattacharya, S. K. (1986) Psychopharmacology 89: 121–124

Bhattacharya, S. K. (1987) Pharm. Res. in press

Bhattacharya, S. K., Bhattacharya, D. (1982) Ind. J. Pharmacol. 14: 263–267

Bhattacharya, S. K., Das, N. (1984) Neurochem. Pathol. 2: 163–169

Bhattacharya, S. K., Parmar, S. S. (1985a) J. Pharm. Pharmacol. 37: 915–916

Bhattacharya, S. K., Parmar, S. S. (1985b) Pharm. Res. 6: 313–315

Bhattacharya, S. K., Sanyal, A. K. (1978a) Psychopharmacology 56: 235–237

Bhattacharya, S. K., Sanyal, A. K. (1978b) Prostaglandins Med. 1: 159–164

Bhattacharya, S. K., Mukhopadhyay, S. N., Debnath, P. K., Sanyal, A. K. (1976) Experientia 32: 907–908

Bhattacharya, S. K., Rao Mohan, P. J. R., Bhattacharya, D. (1984) Pharm. Res. 5: 229–231

Bhattacharya, S. K., Goodall, W. M., Brumleve, S. J., Parmar, S. S. (1985) Proc. West. Pharmacol. Soc. 28: 217–220

Bito, L. Z., Davson, H., Hollingsworth, J. R. (1976) J. Physiol. (Lond.) 256: 273–285

Bose, R., Bhattacharya, S. K. (1979) Ind. J. Med. Res. 70: 281–288

Brus, R., Krzminski, T., Juraszczyk, Z., Kurocok, A. (1985) Biomed. Biochem. Acta 44: 637–644

Coceani, F. (1974) Arch. Intern. Med. 133: 119–129

Davson, H. (1976) J. Physiol. (Lond.) 255: 1–28

Debnath, P. K., Bhattacharya, S. K., Sanyal, A. K., Poddar, M. K., Ghosh, J. J. (1978) Biochem. Pharmacol. 27: 130–132

Fairbairn, J. W., Pickens, J. T. (1979) Br. J. Pharmacol. 67: 379–385

Fairbairn, J. W., Pickens, J. T. (1980) Ibid. 69: 491–493

Feldberg, W., Lotti, V. J. (1967) Br. J. Pharmacol. Chemother. 31: 152–161

Ghosh, P., Bose, R., Bhattacharya, S. K. (1980) Ind. J. Med. Res. 72: 605–609

Hagen, A. A., Gerber, J. N., Sweeley, C. C., White, R. P., Robertson, J. T. (1977) Stroke 8: 672–675

Hedqvist, P. (1977) Ann. Rev. Pharmacol. Toxicol. 17: 259–279

Henker, D. P., Aiken, J. W. (1980) Eur. J. Pharmacol. 67: 155–158

Holmes, S. W. (1970) Br. J. Pharmacol. 38: 653–658

Kuschinsky, K., Hornykiewicz, O. (1972) Eur. J. Pharmacol. 28: 167–173

Laychock, S. G., Johnson, D. N., Harris, L. S. (1980) Pharmacol. Biochem. Behav. 12: 747–754

Pertwee, R. G. (1972) Br. J. Pharmacol. 46: 753–763

Sanyal, A. K., Srivastava, D. N., Bhattacharya, S. K. (1979) Psychopharmacology 60: 159–163

Winter, J. C. (1979) Ibid. 60: 265–269

Wolfe, L. S. (1982) J. Neurochem. 38: 1–14