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Prostaglandin E_1 causes sedation and increases 5-hydroxytryptamine turnover in rat brain

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Summary

1. Administration of prostaglandin E_1 (1 mg/kg, i.p.) to rats induced sedation and a decrease in muscular tone. Prostaglandin E_1-induced sedation was accompanied by the low voltage–high frequency E.E.G. pattern characteristic of the waking animal.

2. Administration of prostaglandin E_1 also increased the turnover rate of 5-hydroxytryptamine and raised the concentration of acetylcholine in brain.

3. The behavioural effects of prostaglandin were blocked by prior administration of *p*-chlorophenylalanine or pargyline, drugs which lowered the brain concentration of 5-hydroxyindoleacetic acid (5-HIAA), and was potentiated by pretreatment with probenecid, which elevated the 5-HIAA concentration. Pretreatment with atropine sulphate failed to alter prostaglandin E_1-induced sedation.

4. The results are compatible with the possibility that prostaglandin E_1 induces a state resembling paradoxical sleep through an action on 5-hydroxytryptamine metabolism in brain.

Introduction

Prostaglandins are present in brain and other organs of mammals and are extremely active in a number of biological systems (Eliasson, 1959; von Euler & Eliasson, 1967; Samuelsson, 1964). Prostaglandins of the E series have been shown to cause profound sedation, stupor and catatonia when administered intraventricularly to cats or intravenously to chickens (Horton, 1964; Horton & Main, 1965; 1967a). The present study shows that intraperitoneal administration of prostaglandin E_1 to rats induces sedation accompanied by diminished muscular tone, and increases the turnover rate of brain 5-hydroxytryptamine.

Methods

Male Sprague-Dawley rats (180–200 g) were housed individually and electroencephalograms were recorded from two stainless steel electrodes which had been permanently implanted in the parieto-occipital cortices two weeks beforehand. Electromyograms (E.M.G.) were recorded from coiled insulated electrodes implanted in the dorsal neck muscles. After normal baseline recordings had been obtained

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for 1 h, prostaglandin E_1 (1 mg/kg, i.p.) was administered and the tracings were continued for another 2 hours. Control animals received only the appropriate amount of 4% ethanol in 0·9% w/v NaCl solution (2·5 ml/kg) used to dissolve the prostaglandin E_1.

The effects of drugs on prostaglandin E_1-induced sedation were measured in groups of 4–5 rats housed in plastic cages (43 × 17 × 16 cm). The number of animals sedated (i.e., lying flat on their abdomen with their eyes closed) was determined at ten five-min intervals beginning 15 min after administration of prostaglandin E_1 (1 mg/kg) or of an equivalent amount of 4% ethanol in 0·9% w/v NaCl solution (saline).

For biochemical studies, rats were killed at various times after administration of prostaglandin E_1 (two doses of 1 mg/kg, 45 min apart) and the brains were rapidly removed and either assayed immediately for acetylcholine (Reid, Haubrich & Krishna, 1971) or frozen on dry ice and later assayed for 5-hydroxytryptamine (Bogdanski, Pletscher, Brodie & Udenfriend, 1956) and the deaminated metabolite of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid (5-HIAA ; Udenfriend, Weissbach & Brodie, 1958). The rate constant of 5-HIAA efflux was determined from the rate at which 5-HIAA disappeared from the brain after administration of the monoamine oxidase inhibitor, pargyline hydrochloride (75 mg/kg). The turnover rate of brain 5-hydroxytryptamine was calculated as previously described (Tozer, Neff & Brodie, 1966). All drugs were administered intraperitoneally. Statistical comparisons were made with Student's *t* test.

Results

After treatment of rats with prostaglandin E_1 (1 mg/kg) sedation was evident within 15 min and persisted for about 1 hour. Spontaneous movements almost

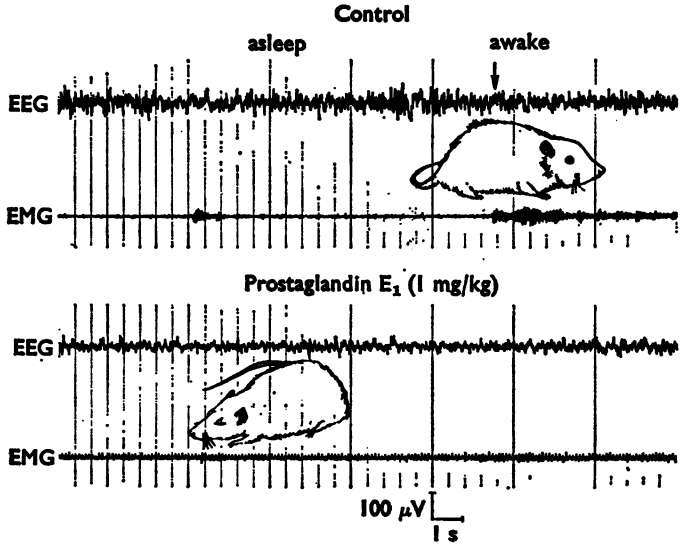

FIG. 1. Electroencephalogram (E.E.G.) and electromyogram (E.M.G.) recordings from a single rat before (upper trace) and 30 min after (lower) prostaglandin E_1 administration (1 mg/kg). Figures are artist's reproductions of photographs of control and prostaglandin E_1-treated rats. The results are typical of 6 animals.

ceased, and the animals lay flat on their abdomens and kept their eyes closed. The prostaglandin E_1-treated animals could be easily aroused by gentle handling, but would quickly return to their original quiescent posture when left undisturbed. Sedation was accompanied by a variable decline of rectal temperature (0·5 to 2·5° C) and by diminished muscular tone, as shown by decreased voltage of the E.M.G. (Fig. 1). During prostaglandin E_1-induced sedation, the predominant E.E.G. pattern was one of low voltage and high frequency (Fig. 1, lower tracing) similar to that of normal waking animals (Fig. 1, upper right tracing). The waking E.E.G. pattern was present for more than 80% of the period during which the prostaglandin E_1-treated animals were sedated, and lasted for intervals of 15–20 min interrupted by relatively brief intervals (2–3 min) of slow wave sleep.

In addition to producing sedation, administration of prostaglandin E_1 also altered the metabolism of brain 5-hydroxytryptamine. Single doses of prostaglandin E_1 ranging from 0·5–2·0 mg/kg increased the concentration of 5-HIAA in the brain by 30–60%, and administration of two doses (1 mg/kg) 45 min apart produced more consistent and prolonged elevations in the acid concentration as shown in Figure 2. The rate constant of 5-HIAA efflux from the brain was increased slightly by prostaglandin E_1 treatment, and the turnover rate of 5-hydroxytryptamine was almost doubled (Table 1).

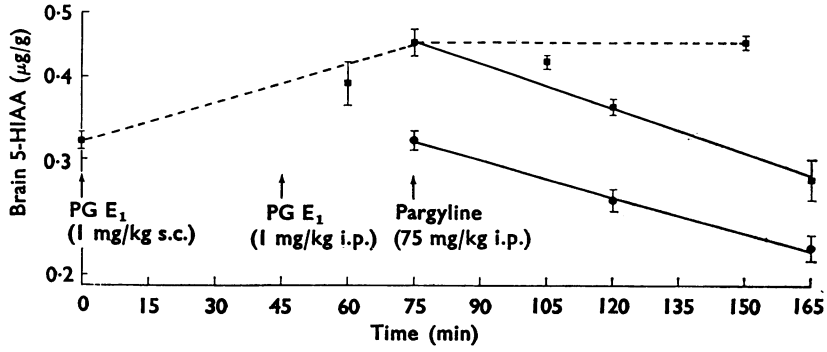

FIG. 2. Effect of prostaglandin E_1 on brain 5-hydroxyindoleacetic acid (5-HIAA) levels and on the disappearance rate of brain 5-HIAA after pargyline administration (75 mg/kg). Broken line shows the increase in brain 5-HIAA levels after administration of 2 doses of prostaglandin E_1 (1 mg/kg). Solid lines were drawn by the method of least squares and indicate the rate of 5-HIAA disappearance in control rats (circles) and in prostaglandin E_1-treated animals (squares) following pargyline administration. Each point is an average of 5–6 animals and bars indicate standard error.

TABLE 1. *Effects of prostaglandin E_1 (PGE_1) on 5-hydroxytryptamine (5-HT) turnover rate and on the concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and acetylcholine in rat brain*

Treatment	Acetylcholine concentration (μg/g±S.E.)	5-HIAA concentration (μg/g±S.E.)	Rate constant (k) of 5-HIAA efflux* (h^{-1})	5-HT turnover rate† ((μg/g)/h)
Control‡	3·07±0·11 (10)	0·32±0·01 (6)	0·26±0·04 (17)	0·077
PGE_1‡	3·94±0·11 (10) ($P<0·01$)	0·45±0·02 (6) ($P<0·01$)	0·35±0·03 (18) ($P>0·05$)	0·146

Numbers in parentheses are the number of animals in each group. * Calculated from the data in Figure 2. † Calculated from the product of the steady-state concentration of 5-HIAA, the rate of constant of 5-HIAA efflux and 0·92 (correction factor for the difference in molecular weight between 5-hydroxytryptamine and 5-HIAA). ‡ Two doses of prostaglandin E_1 (1 mg/kg) were given 45 min apart; control animals received two 0·5 ml injections of 4% ethanol/saline.

Prior administration of probenecid (150 mg/kg, 2·5 h before prostaglandin E_1), a drug which raises the 5-HIAA concentration by blocking the transport of organic acids from the brain (Neff, Tozer & Brodie, 1967), potentiated the sedative effect of a low dose of prostaglandin E_1 (0·25 mg/kg) (Table 2). In contrast, reduction of the 5-HIAA concentration beforehand either by blockade of 5-hydroxytryptamine synthesis with *p*-chlorophenylalanine (300 mg/kg, 48 and 24 h before prostaglandin E_1) or by inhibition of monoamine oxidase with pargyline (75 mg/kg, 2·5 h before prostaglandin E_1), completely blocked the induction of sedation by prostaglandin E_1 (Table 2). As expected, both pargyline and *p*-chlorophenylalanine lowered the concentration of 5-HIAA but had opposite effects upon the concentration of 5-hydroxytryptamine in brain, whereas probenecid treatment increased brain 5-HIAA without appreciably changing the 5-hydroxytryptamine concentration (Table 3). Thus the effects of pargyline, probenecid and *p*-chlorophenylalanine on prostaglandin E_1-induced sedation correlated with the drugs' effects on 5-HIAA but not on 5-hydroxytryptamine concentrations.

TABLE 2. *Effects of drug on sedation induced by administration of prostaglandin E_1 to rats*

Drug pretreatment	Sedation index ‖		
	Control	Prostaglandin E_1 (0·25 mg/kg)	Prostaglandin E_1 (1·0 mg/kg)
Saline	0·24±0·06	0·50±0·06†	0·72±0·06*
Probenecid	0·37±0·11‡	0·92±0·04§	—
p-Chlorophenylalanine	0·15±0·07	—	0·18±0·06‡
Pargyline	0·03±0·03†	—	0·22±0·04‡
Atropine	0·18±0·08	—	0·62±0·05¶

* $P<0·01$ compared to saline-pretreated controls. † P 0·05 compared to saline-pretreated controls. ‡ $P>0·1$ compared to saline-pretreated controls. § $P<0·01$ compared to saline-pretreated animals receiving 0·25 mg/kg prostaglandin E_1. ¶ $P>0·1$ compared to saline-pretreated animals receiving 1·0 mg/kg prostaglandin E_1. ‖ Starting 15 min after prostaglandin E_1 (1 mg/kg) administration, rats were observed every 5 min and the number of immobile rats were divided by the total number in each group (4 or 5). The values shown are the average of 10 observations ±S.E. Probenecid (150 mg/kg) or pargyline (75 mg/kg) were administered 2·5 h before prostaglandin E_1. *p*-Chlorophenylalanine was administered 48 and 24 h before prostaglandin E_1 in doses of 300 mg/kg. Atropine sulphate (10 mg/kg) was administered 15 min before and 15 min after prostaglandin E_1. Control animals received 4% ethanol/saline (2·5 ml/kg).

TABLE 3. *Effects of drugs on the concentration of 5-hydroxytryptamine (5-HT) or 5-hydroxyindoleacetic acid (5-HIAA) in brain*

Treatment	5-HT concentration (μg/g±S.E.)	5-HIAA concentration (μg/g±S.E.)
Saline	0·34±0·02	0·35±0·03
Probenecid	0·40±0·01*	0·90±0·08*
p-Chlorophenylalanine	0·08±0·01†	0·10±0·01†
Pargyline	1·27±0·05†	0·14±0·01†

Values are the means of 4 animals ±S.E. Animals were killed 2 h after drug treatment. Doses are given in legend of Table 2. * $P<0·05$ compared with saline control. † $P<0·01$ compared with saline control.

Administration of prostaglandin E_1 (two doses of 1 mg/kg, 45 min apart) also increased the brain concentration of acetylcholine by 30% (Table 1). To determine whether cholinergic neurones might play a role in prostaglandin E_1-induced sedation, rats were given atropine sulphate (10 mg/kg) 15 min before and 15 min after prostaglandin E_1. Atropine administration failed to alter the sedative effect of prostaglandin E_1 (Table 2).

None of the behavioural, physiological or biochemical changes recorded in prostaglandin E_1-treated rats were observed in animals which received only 4% ethanol in saline.

Discussion

These studies show that parenteral administration of prostaglandin E_1 to rats induces sedation accompanied by a reduction in skeletal muscle tone. The behavioural effect of prostaglandin E_1 in rats superficially resembles normal sleep. However, the E.E.G. pattern in prostaglandin E_1-treated animals is similar to that seen in the normal waking state. This behavioural state, characterized by sedation, diminished muscular tone, and an activated E.E.G. pattern closely resembles paradoxical or activated sleep which occurs periodically in normal sleeping rats (Swisher, 1967) and cats (Dement, 1958 ; Jouvet, 1969). A major difference between paradoxical sleep in normal rats compared to those treated with prostaglandin E_1 is that both the duration (2–3 min) and proportion (10–15%) of the time asleep are shorter in untreated rats than in animals given prostaglandin E_1. Thus, prostaglandin E_1 administration appears to induce a state of persistent paradoxical sleep, although more extensive physiological measurements are necessary to validate this impression.

The induction of paradoxical sleep is believed to be regulated in part by tryptaminergic neurones (see Jouvet, 1969). The parenteral administration of prostaglandin E_1 to rats increases the turnover rate of 5-hydroxytryptamine (Table 1), a finding which we recently confirmed (unpublished data) using the isotopic method of Neff, Spano, Groppetti, Wang & Costa (1971). Furthermore, our data (Tables 2 and 3) suggest that sleep induced by prostaglandin E_1 may be related to the brain concentration of deaminated metabolites of 5-hydroxytryptamine, but not to the concentration of the parent amine. This finding is consistent with other reports which indicate that paradoxical sleep may be suppressed either by administration of drugs which lower the brain 5-HIAA concentration (Matsumoto & Jouvet, 1964 ; Delorme, Jeannerod & Jouvet, 1965 ; Mouret, Bobillier & Jouvet, 1968) or by the production of lesions in the 5-hydroxytryptamine containing raphé nuclei (Kostowski, Giacalone, Garattini & Valzelli, 1968). Moreover, administration of other 5-hydroxytryptamine metabolites to mice also induces sleep (Feldstein, Chang & Kucharski, 1970). These findings are all compatible with the view that prostaglandin E_1 may elicit its sedative effect by increasing the rate of formation of a deaminated metabolite of 5-hydroxytryptamine. They do not, of course, exclude the possibility that changes in the metabolism of brain amines represent a response by which the brain attempts to counteract the physiological effects of prostaglandin E_1.

Our findings do not rule out the possibility that the central effects of prostaglandin E_1 may be mediated through neurohormones other than 5-hydroxytryptamine. Reports demonstrating that paradoxical sleep may be induced by intraventricular injection of oxotremorine or carbachol and blocked by atropine sulphate suggest that acetylcholine may be involved in paradoxical sleep (George, Haslett & Jenden, 1964 ; Khazan & Sawyer, 1964 ; Khazan, Bar & Sulman, 1967). Moreover, the rate of acetylcholine liberation from the cortical surface of cats is greater during paradoxical sleep than in slow wave sleep (Jasper & Tessier, 1971). Our findings that prostaglandin E_1 administration increases the acetylcholine concentration is

consistent with the possibility that prostaglandin E_1 may interact with cholinergic neurones to induce paradoxical sleep. However, administration of prostaglandin E_1 elicited no overt signs of muscarinic stimulation (such as diarrhoea, tremors, chromodacryorrhoea or salivation), and prior administration of atropine failed to block the sedation elicited by prostaglandin E_1. We conclude therefore that prostaglandin E_1 probably does not induce its overt behavioural effects by increasing the concentration of acetylcholine at muscarinic receptors in the brain. It is perhaps more likely that the increased acetylcholine concentrations are secondary to prostaglandin E_1-induced sedation, since other central nervous system depressants have been shown to induce similar increments (Crossland & Merrick, 1954). Other studies in this laboratory indicate that the synthesis of ^{14}C-dopamine from ^{14}C-tyrosine in the caudate nucleus of rats is increased by only about 20% after prostaglandin E_1 administration, compared with an 80% increase in the amount of ^{14}C-5-hydroxytryptamine synthesized from ^{14}C-tryptophan (unpublished data). Thus, although prostaglandin E_1 clearly can affect other neurohormones, its effects are most dramatic on 5-hydroxytryptamine.

Endogenous prostaglandins have been identified in brain (Horton & Main, 1967b) and it has been reported that they are associated with nerve endings of the cerebral cortex (Kataoka, Ramwell & Jessup, 1967). They may be released into superfusates of the brain either spontaneously or by electrical stimulation (Coceani & Wolfe, 1965 ; Ramwell & Shaw, 1967 ; Bradley, Samuels & Shaw, 1969). It has been proposed that prostaglandins may be neurotransmitters or may modulate the activity of other neurohormones (Avanzino, Bradley & Wolstencroft, 1966a, b ; Horton & Main, 1967a ; Bradley *et al.*, 1969). Our findings are compatible with the possibility that endogenous prostaglandins may play a role in the induction of paradoxical sleep. A major criticism of this hypothesis is that sedation is produced by administration of relatively large doses of prostaglandin E_1, which could lead to unphysiological concentrations within the brain. However, 80% of injected prostaglandin E_1 is reportedly removed by the liver and 95% by the lungs (Ferreira & Vane, 1967) so that the concentration actually reaching the brain after an intraperitoneal injection might well be extremely small.

Prostaglandins have strong actions on vascular and intestinal smooth muscle, and we have not eliminated the possibility that the effects reported here result from the peripheral actions of prostaglandin E_1. However, intraventricular injection of prostaglandin E_1 to cats causes central nervous system depression (Horton, 1964 ; Horton & Main, 1965 ; 1967a), indicating that this behavioural effect may occur in the absence of peripheral changes induced by prostaglandin E_1. Prostaglandin E_1 also causes hyperthermia when injected into the third ventricle of cats (Milton & Wendlandt, 1970), and an increase in body temperature could elicit changes in 5-hydroxytryptamine turnover similar to those observed in our animals treated with prostaglandin E_1 (Reid, Volicer, Smookler, Beaven & Brodie, 1968). However, intraperitoneal injection of prostaglandin E_1 in rats produced a variable decrease in body temperature. The mechanism of the prostaglandin E_1-induced decrease in rectal temperature is uncertain, but could be due either to the decreased muscle tone or to a reduced mobilization of free fatty acids (Steinberg, Vaughan, Nestel, Strand & Bergstrom, 1964 ; Moskowitz, Harwood, Forn, Krishna, Rodgers & Morrow, 1971).

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