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Prostaglandins: effect of prostaglandin E_1 on brain, stomach and intestinal serotonin in rat

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Prostaglandins (PGs) have been found in brain, gastro-intestinal tract and other mammalian organs, together with complete systems capable of synthesizing and metabolizing distinct PG types [1–5]. PGs of E series have been shown to produce sedation, stupor, catatonia and inhibit electrical and chemically-induced convulsions [6, 7]. Recent studies from this laboratory have shown that PGE_1-induced potentiation of morphine analgesia, antinociceptive effect of PGE_1, *per se*, and PGE_1-induced potentiation of hexobarbitone hypnosis in rat are probably serotonin mediated. PGE_1-induced inhibition of gastric secretion was also shown to be a serotonin mediated response. In addition, PGE_1 potentiated the inhibitory effect of serotonin on gastric secretion and the smooth muscle contractility of serotonin [8–12]. Haubrich *et al.* [13] have demonstrated that PGE_1-induced sedation in rat was associated with increased brain serotonin turnover. It thus seemed possible that an inter-relationship exists between PGs, more specifically PGE_1, and serotonin in the brain and the gastro-intestinal tract. It was therefore thought worthwhile to study the effect of PGE_1 on the level, rate of synthesis (accumulation) and decline of serotonin in brain, stomach and proximal intestine.

MATERIAL AND METHODS

The study was conducted on male Wistar albino rats (100–120 g). The rats were maintained on standard Hind Lever diet and were housed in an air cooled room (25°) in colony cages. Animals were fasted overnight but water was allowed *ad lib.* before experimentation. PGE_1 (0.4 mg/kg s.c.) was administered and the rats were killed by decapitation at different time intervals (30, 60, 120 and 240 min) after drug administration. The time of the experiments was kept constant between 9 and 11 a.m. Tissue serotonin were done by the method of Snyder *et al.* [14]. The rate of accumulation and the rate of decline of serotonin, after administration of pargyline (75 mg/kg i.p.) and *p*-chlorophenylalanine (PCPA, 316 mg/kg i.p.), respectively, were done with the help of the methods adopted by Neff *et al.* [15] and Neff and Tozer [16]. For studying the rate of accumulation, PGE_1 was administered along with pargyline and the animals were sacrificed after 30, 60 and 90 min whereas for the study of rate of decline, PGE_1 was administered 6 hr after PCPA and animals were sacrificed at 6, 7, 8, 9 and 10 hr after PCPA treatment. Statistical significance was done by Student's *t* test.

The choice of the dose and route of administration of PGE_1 was based on earlier reports from this laboratory [8, 9, 11].

RESULTS

The results are summarised in Tables 1 and 2. After administration of PGE_1, the brain serotonin level was maximally increased within 60 min and tended to normalise within 240 min. However, serotonin levels of both stomach and intestine were not significantly affected. PGE_1 increased only the rate of accumulation of brain serotonin but enhanced both rates of accumulation and decline of stomach serotonin. There was no significant effect on either the rate of accumulation or rate of decline of intestinal serotonin.

DISCUSSION

The results indicate that serotonin metabolism is strongly affected by PGE_1 in both brain and stomach of rat but not in the intestine. There was a marked increase in brain serotonin level after PGE_1 administration, an effect not noted in either stomach or intestine. However, when the results are analysed after pargyline and PCPA treatments, it is apparent that PGE_1 markedly accelerated both the rate of accumulation (4-fold) and rate of decline (1.5-fold) of serotonin in the stomach whereas only the rate of accumulation was increased in the brain (1-fold) with no appreciable change in rate of decline. There was no significant effect on either rates in the intestine.

Our results support the observations of Haubrich *et al.* [13], who have shown that the sedative effect of PGE_1,

Table 1. Effect of PGE_1 (0.4 mg/kg s.c) on serotonin levels in brain, stomach and intestine of Wistar albino rat

Time (min)	Serotonin μg/g wet tissue		
	Brain	Stomach	Intestine
0	0.41 ± 0.07	3.65 ± 0.36	2.07 ± 0.18
30	0.57 ± 0.06	4.01 ± 0.42	1.84 ± 0.22
60	1.20 ± 0.15†	3.36 + 0.31	2.42 ± 0.24
120	0.73 ± 0.08*	3.76 ± 0.19	2.53 ± 0.31
240	0.36 ± 0.08	3.34 ± 0.21	2.90 ± 0.36

Results expressed as Mean ± S.E.M. of five different determinations.
$n = 5$ in each group.
* and † indicate statistical significance in comparison to 0 min level as $P < 0.05$ and $P < 0.01$ respectively.

Table 2. Effect of PGE_1 (0.4 mg/kg s.c.) on the rates of accumulation and decline of serotonin in different tissues of rats

	Rate of accumulation*		Rate of decline*	
Tissue	Control	PGE_1	Control	PGE_1
Brain	0.43 ± 0.07	0.84 ± 0.13†	0.382 ± 0.07	0.327 ± 0.08
Stomach	3.24 ± 0.48	12.40 ± 1.34‡	0.115 ± 0.06	0.275 ± 0.07
Intestine	0.51 ± 0.08	0.41 ± 0.07	0.107 ± 0.05	0.161 ± 0.07

Results expressed as Mean ± S.E.M. of three different determinations $n = 3$ in each group.

* Rates were expressed as μg serotonin/g wet tissue/hr.

† and ‡ indicate statistical significance in comparison to control as $P < 0.05$ and $P < 0.001$ respectively.

To measure the rates of accumulation and decline the rats were treated (i.p.) with pargyline (75 mg/kg) and PCPA (316 mg/kg) respectively and animals were sacrificed at different time intervals thereafter as stated in detail in Methods. The rate constant was calculated by plotting the levels of 5HT at different time on semilogarithmic graph paper. The rate constant was multiplied by the zero hour concentration of the serotonin to measure the rate of decline [15, 16]. Accumulation rate was calculated simply by observing the rate of accumulation of serotonin at different time intervals [16].

after parenteral administration, was linked to an increase in brain serotonin turnover. They observed that single doses of PGE_1, varying from 0.5 to 2 mg/kg increased brain concentration of 5-hydroxyindole acetic acid (5HIAA) by 30–60 per cent. The turnover of brain serotonin almost doubled after administration of two doses of PGE_1, 1 mg each given 45 min apart. They also reported that PGE_1 markedly increases synthesis of [^{14}C]5HT from [^{14}C] tryptophan. Thus our own results are in harmony with these findings.

The results lend support to our earlier contention, based on indirect observations, that PGE_1-induced potentiation of morphine analgesia [8] and hexobarbitone hypnosis [9] and the antinociceptive effect of PGE_1, *per se* [10] in rats are mediated through an increase in brain serotonin turnover.

Although both serotonin and PGE_1 and other PGs, have been implicated in the gastric secretory process [18–26], the precise relationship between the two is not clear. Earlier observations from this laboratory suggest that PGE_1-induced inhibition of gastric secretion may be serotonin mediated [11]. Our results also indicate that the turnover and not the absolute levels of serotonin may be a critical factor in the physiological regulation of gastric secretion. Thompson and Angulo [17], have concluded that PGs of the E series have no effect on stomach and intestinal serotonin levels and hence their gastric secretion inhibitory effect was not serotonin mediated. However, they studied only absolute levels and not the turnover rate of serotonin in coming to their conclusion. The present findings are thus in conformation to their observations as regards the effect of PGE_1 on serotonin levels of stomach and intestine.

The present observations linked with the earlier data emanating from this laboratory, suggest that, at least in rat, some of the physiological effects of PGE_1, on brain and stomach, are mediated through alterations in the serotonin turnover of the target organs.

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The acute effects of amphetamine, chlorpromazine, amitriptyline and lithium on adenosine 5-triphosphatase activity in the cortex of the rat brain

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A reuptake process appears to be the principle mechanism for the inactivation of noradrenaline, dopamine and serotonin following their physiological release from neurons. It has been demonstrated that the uptake of noradrenaline into both nervous and non nervous tissue is antagonized by ouabain [1, 2], a drug which has long been known to block the ATPase mediated flux of Na^+ and K^+ across cell membranes [3]. Bogdanski and Brodie [4] have proposed a model for the amine carrier mechanism. They propose that the affinity of the carrier for noradrenaline is dependent on the concentration of Na^+ and is a non energy requiring process. Once within the nerve ending, noradrenaline is mainly stored in specific vesicles and the Na^+ extruded from the cell by the energy dependent ATPase system. Thus any situation leading to a change in the cation flux across a nerve membrane would affect the affinity of the carrier for the neuro-transmitter. However, other invistigators [5] have shown that in cardiac tissue the onset of the ouabain inhibition of noradrenaline accumulation was uncorrelated with the changes in the intracellular Na^+ and K^+ concentrations. From such studies it would appear that although there is evidence which implicates Na^+, K^+ ATPase in the amine transport process, it is uncertain whether these processes are causally related. Furthermore most studies of the effect of drugs on ATPase activity and amine transport have been conducted *in vitro*. It was with a view to finding if there was a causal or coincidental involvement of Na^+, K^+ and Mg^+ dependent ATPases with amine transport into partially purified synaptosomes and vesicles that we decided to study the actions of some psychotropic durgs known to affect the uptake and metabolism of biogenic amines in the rat brain *in vivo*. Studies were made of the major tranquillizing drug chlorpromazine, of lithium chloride (a drug used most frequently in the treatment of manic depression), of the tricyclic antidepressant amitriptyline and of the stimulant drug D-amphetamine.

MATERIALS AND METHODS

Male Wistar rats (approximate weight 300 g) were used. The control and experimental groups consisted of 6 animals. The control group was injected with physiological saline and the experimental groups with D-amphetamine (5 mg/kg i.p.), chlorpromazine (10 mg/kg i.p.), amitriptyline (20 mg/kg i.p.) or lithium chloride (50 mg/kg i.p.). One hr after drug administration, the animals were decapitated; at this time all the drugs had clearly observable effects on the gross behaviour of the animals. The brains were rapidly removed, washed in cold saline, the excess saline removed and placed on ice. The cerebral cortex was removed from each brain. The cortices were homogenized for 5 min, in 10 volumes of 0.32 M sucrose containing 1 mM EDTA pH 7.4 at 0° using a Thomas teflon pestle tissue grinder (clearance 0.15 mm rotating at 1200 rev/min). The partially purified synaptosomes and vesicular fractions were separated by density gradient centrifugation using the method of Gray and Whittaker [6]. The vesicular fraction occurred at the junction of the 0.32 and 0.80 M sucrose band while the synaptosomal fraction occurred at the interface of the 1.0 and 1.2 M sucrose layer. The molarity of the sucrose containing the synaptosomal fraction was adjusted to 0.40 M and both the vesicular and the synaptosomal fractions centrifuged at 100,000 *g* for 30 mins. The pellets were resuspended in distilled water and retained at 4° for estimation of the ATPase activity. The incubation medium contained (final concentrations) 5 mM-$MgCl_2$, 15 mM-Kcl, 90 mM-NaCl and 5 uM ATP (sodium free); ouabain (1 mM) was added in some cases to inhibit Na^+, K^+ ATPase activity. Total ATPase activity was taken to be the activity occurring in the absence of ouabain while Mg^{2+} dependent ATPase activity was assumed to be that remaining after ouabain inhibition. ATPase activity was determined by measuring the release of inorganic phosphate from ATP after 5 min incubation at 37°. The enzyme activity is expressed as μmoles of inorganic phosphate formed per hr per mg protein. All estimations were carried out in duplicate. The statistical significance of the results was assessed using Student's *t*-test.

RESULTS AND DISCUSSION

The acute effects of amphetamine, chlorpromazine, amitriptyline and lithium chloride on vesicular and synaptosomal ATPase activity are summarized in Table 1.