Br. J. Pharmac. (1979), **66**, 185–190

5-HYDROXYTRYPTAMINE UPTAKE INHIBITORS BLOCK *para*-METHOXYAMPHETAMINE-INDUCED 5-HT RELEASE

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1 Activation of myoclonic twitch activity (MTA) of suprahyoideal muscle after *p*-methoxyamphetamine (PMA) administration in rats anaesthetized with urethane has previously been reported to be due to brain 5-hydroxytryptamine (5-HT) release. Increased MTA caused by PMA was blocked by chlorimipramine (0.1 to 1 mg/kg) and fluoxetine (0.3 to 3 mg/kg) but not by desipramine (3 mg/kg).

2 The 5-hydroxytryptophan-induced increase of MTA of suprahyoideal muscle in rats pretreated with pargyline was not blocked by chlorimipramine but was blocked by methysergide.

3 [^{3}H]-5-HT was injected intraventricularly 30 min before the beginning of ventricular perfusion with artificial cerebrospinal fluid in urethane anaesthetized rats. PMA (2 mg/kg i.v.) increased the release of [^{3}H]-5-HT in the perfusate after injection. Chlorimipramine (0.1 to 1 mg/kg) and fluoxetine (0.1 to 1 mg/kg), injected 10 min before the PMA injection, caused a dose-related blockade of the increased release of [^{3}H]-5-HT induced by PMA. Desipramine at 3 mg/kg slightly inhibited the increased release of [^{3}H]-5-HT caused by PMA but was inactive at 1 mg/kg.

4 It is proposed that these 5-HT uptake inhibitors block the increased MTA caused by PMA by preventing the PMA-induced release of 5-HT in the central nervous system.

Introduction

Previous studies have revealed that the hallucinogen, *p*-methoxyamphetamine (PMA) releases [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) from rat brain preloaded with [^{3}H]-5-HT. The release of [^{3}H]-5-HT induced by PMA was blocked by chlorimipramine, a 5-HT uptake inhibitor (Tseng, Menon & Loh, 1976; Tseng, Harris & Loh, 1978). In behavioural studies, chlorimipramine blocked PMA-induced 5-hydroxytryptaminergic responses (Tseng *et al.*, 1978). To test the specificity of action of chlorimipramine on the effect of PMA, further studies using other 5-HT uptake inhibitors seemed warranted. The present studies show that another 5-HT uptake inhibitor, fluoxetine (Wong, Horng, Bymaster, Hauser & Molloy, 1974; Wong, Bymaster, Horng & Molloy, 1975), which is similar to chlorimipramine, also antagonizes PMA-induced pharmacological actions.

Methods

Animals and reagents

Male Sprague–Dawley rats (Simonsen Laboratories, Gilroy, CA) weighing between 250 g and 420 g were used.

The hydrochloride salt of (±)-*p*-methoxyamphetamine ((±)-PMA), was obtained from the National Institute on Drug Abuse, Rockville, MD. Other drugs used in this study were fluoxetine (Lilly 110140, Eli Lilly and Co., Indianapolis, IN), desipramine (USV Pharmaceutical Corp., Tuckahoe, NY), chlorimipramine (Anafranil, Ciba-Geigy Pharmaceutical Co., Summit, NJ) and pargyline. [1, 2-^{3}H]-5-hydroxytryptamine binoxalate (sp. act. 27.4 Ci/mmol) was obtained from New England Nuclear Corp., Boston, MA. 5-Hydroxytryptamine creatinine sulphate complex and 5-hydroxytryptophan were purchased from Sigma Chemical Co., St. Louis, MO.

Measurement of myoclonic twitch activity (MTA) of suprahyoideal muscle in rats

The experimental procedure employed was essentially the same as that of Bieger, Larochelle & Hornykiewicz (1972) with minor modifications. In the present study, instead of placing needle electrodes in the exposed suprahyoideal muscle, the force of contraction of the muscle was monitored by a Grass force-displacement transducer FT 03C and recorded

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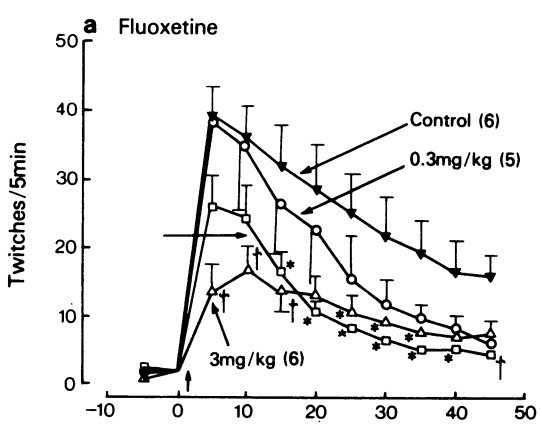
0007-1188/79/060185-06 $01.00

on a Grass Polygraph (Model 7). Urethane (1.2 g/kg) was administered intraperitoneally, and the external jugular vein was cannulated for drug administration. Drugs dissolved in 0.9% w/v NaCl solutions (saline) were administered intravenously in a volume of 0.1 ml/100 g body wt. The frequency of twitch before and after injection of drugs was then counted and the data are presented as number of twitches per 5 min.

Ventricular perfusion and analysis of perfusate

The method of ventricular perfusion and subsequent analysis of the perfusate for [^{3}H]-5-HT and [^{3}H]-5-hydroxyindoleacetic acid ([^{3}H]-5HIAA) has been described in a previous publication (Tseng *et al.* 1978). The technique used was similar to that of Myers & Brophy (1972) and Hirvonen, Karlsson & Salorinne (1969). The tip of the inflow cannula (20 gauge) for ventricular perfusion was stereotaxically inserted into the lateral ventricle under urethane (1.2 g/kg) anaesthesia. The inflow cannula and connecting tubing were filling with artificial cerebrospinal fluid (CSF) before insertion. To obtain outflow from the cisterna magna, a 20 gauge needle was inserted into the exposed atlanto-occipital membrane and connected to a 20 cm length of polyethylene tubing. The external end of the outflow tube was set in position at a level 6 cm below the cisterna. The ventricular system was then perfused with a peristaltic pump (minipals, II, Gilson) at a rate of 35 µl/min. The outflow of artifical CSF was received in a tube which contained 10 µg of 5-HT and 5-HIAA and 200 µg of ascorbic acid in 10 µl. A fraction collector was used to collect each 5 min sample. Thirty min before the start of ventricular perfusion, [^{3}H]-5-HT (0.06 µg, 10 µCi) was injected into the lateral ventricle at the place where the inflow cannula was to be inserted. The perfusate



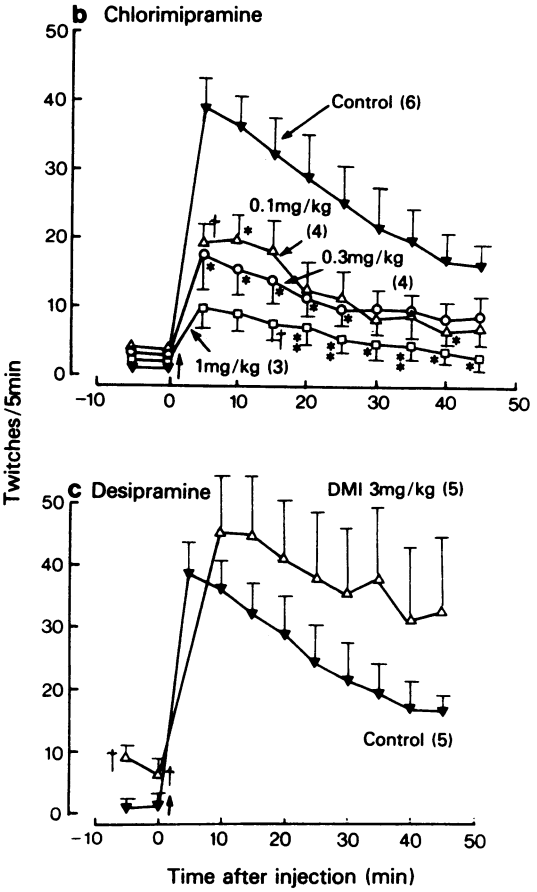


Figure 1 Effect of (a) fluoxetine, (b) chlorimipramine and (c) desipramine on the *p*-methoxyamphetamine (PMA)-induced increase of myoclonic twitch activity of the suprahyoideal muscle of urethane-anaesthetized rats. Fluoxetine, chlorimipramine and desipramine at various doses were injected 10 min before the injection of PMA, 2 mg/kg. Controls were injected with saline followed by PMA, 2 mg/kg. The vertical bars represents s.e. mean of frequency of MTA at 5 min intervals. The numbers in parentheses indicate the numbers of rats used. $*P < 0.05$; $**P < 0.02$; $\dagger P < 0.01$ compared with the control (Student's *t* test).

was at ambient temperature. The CSF-substitute perfusion solution was based on an artificial CSF solution of Myers (1971), derived from electrolyte values of Reed, Withrow & Woodbury (1967), and Chutkow (1968), and contained the following concentrations of ions (mM): Na 127.6, K 2.5, Ca 1.3, Mg 1.0 and Cl 134.5.

Ten µl of perfusate obtained before and after injection of drugs was spotted on glass thin-layer chromatography (t.l.c.) plates precoated with silical gel G

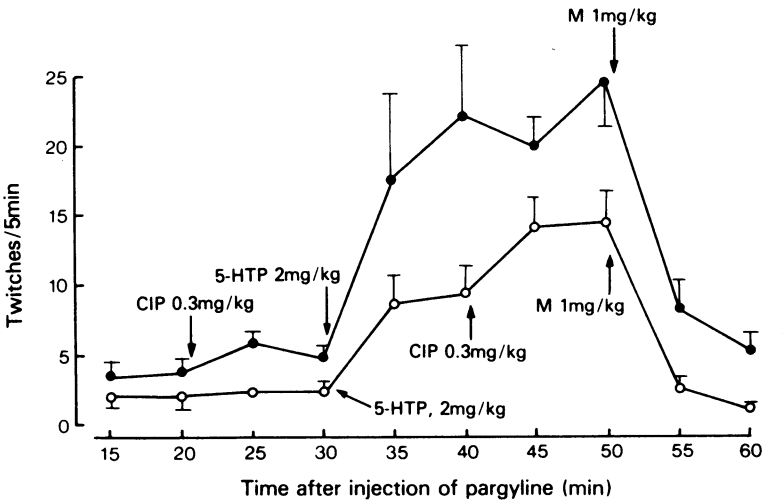


Figure 2 Effect of chlorimipramine and methysergide on 5-hydroxytryptophan (5-HTP)-induced myoclonic twitch activity (MTA) in rats pre-injected with pargyline. Two groups of rats, 6 in each, were injected with pargyline, 10 mg/kg at zero time. Chlorimipramine injected either before or after the 5-HTP injection failed to antagonize increased MTA caused by 5-HT. The vertical bars represent the s.e. mean. CIP, chlorimipramine; M, methysergide.

(E. M. Laboratories, Inc., Elmsford, NY). Replicate t.l.c. plates containing standard 5-HT and 5-HIAA were developed concurrently in a solvent system containing ethyl acetate, *n*-propanol and ammonia (45:35:20) in an ascending chromatography tank at room temperature. The standard was visualized by iodine. The silica gel powder from each spot containing [^{3}H]-5-HT or [^{3}H]-5-HIAA from the t.l.c. plates was then scraped separately into test tubes, extracted with 1 ml of distilled water, and the radioactivity of the aqueous extract determined by liquid scintillation spectrometry.

Results

Effect of 5-hydroxytryptamine uptake inhibitors on p-methoxyamphetamine-induced activation of myoclonic twitch activity (MTA) of suprahyoideal muscle in rats

A dose of 2 mg/kg of PMA was used since previous studies indicated that this dose produced about 90% of the maximum activation in MTA frequency. PMA (2 mg/kg) activated MTA immediately after injection and reached maximum frequency 5 min after injection. The frequency of MTA gradually decreased but 40 min after injection, it was still higher than the preinjection level. Fluoxetine (0.3 to 3 mg/kg) and chlorimipramine (0.1 to 1 mg/kg) caused a dose-related inhibition of increased MTA induced by PMA. Chlorimipramine was more potent than fluoxetine (Figure 1a, b). On the other hand, desipramine (3 mg/kg) appeared to increase MTA and did not prevent the increased MTA caused by PMA (Figure 1c).

Effects of chlorimipramine and methysergide on 5-hydroxytryptophan (5-HTP)-induced activation of myoclonic twitch activity of suprahyoideal muscles in rats

Bieger *et al.* (1972) demonstrated that 5-HTP activates MTA in monoamine oxidase-inhibited rats via 5-hydroxytryptaminergic mechanisms since it is blocked by methysergide, a 5-HT-receptor blocker. To compare the mode of action of 5-HTP with that of PMA, the effect of chlorimipramine on the action of each was studied. 5-HTP (2 mg/kg) activated MTA in rats injected with pargyline (10 mg/kg) 10 min before 5-HTP injection. Chlorimipramine at a dose (0.3 mg/kg) which markedly antagonized the PMA-induced increase of MTA did not antagonize but slightly potentiated the 5-HTP-induced MTA. The increased MTA induced by 5-HTP was blocked by 1 mg/kg of methysergide (Figure 2).

Effects of 5-hydroxytryptamine uptake inhibitors on p-methoxyamphetamine-induced increased release of [^{3}H]-5-HT

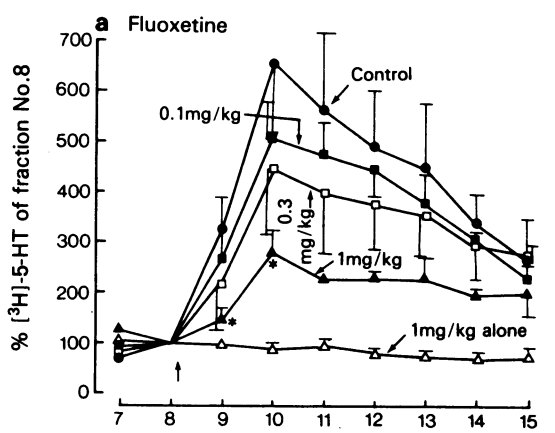
After intraventricular injection of [^{3}H]-5-HT, there was a high level of radioactivity in initial samples of perfusate and a rapid decline of radioactivity in

succeeding samples. The decline of radioactivity gradually slowed down at about the fourth or fifth sample. In view of this time course, saline or (±)-PMA was injected intravenously immediately after sample number 8. Various doses of 5-HT uptake inhibitors were injected 10 min before (±)-PMA which was given in a dose of 2 mg/kg since this dose greatly increased the frequency of MTA.

The content of [^{3}H]-5-HT and [^{3}H]-5-HIAA in the perfusate before PMA injection (sample No. 8) was identical to that of similar samples taken during control sessions, with about 75% of the radioactivity as [^{3}H]-5-HIAA and 4% as [^{3}H]-5-HT. After injection of 2 mg/kg PMA, there were marked increases of [^{3}H]-5-HT without any significant change in the [^{3}H]-5HIAA level in the perfusate. The increase of [^{3}H]-5-HT induced by PMA (2 mg/kg) lasted more than 35 min. At 35 min, [^{3}H]-5-HT activity was still higher than control. [^{3}H]-5-HIAA activity declined slowly as it did in samples obtained from rats injected with saline. Fluoxetine (0.1 to 1 mg/kg) and chlorimipramine (0.1 to 1 mg/kg) injected 10 min before PMA (2 mg/kg) caused a dose-related reduction of the increased release of [^{3}H]-5-HT induced by PMA (Figure 3a, b). Chlorimipramine was more potent than fluoxetine. Injection of chlorimipramine (1 mg/kg) or fluoxetine (1 mg/kg) alone did not affect the spontaneous efflux of [^{3}H]-5-HIAA and [^{3}H]-5-HT. Desipramine (1 mg/kg) did not block but slightly potentiated the PMA-induced release of [^{3}H]-5-HT. However, 3 mg/kg of desipramine caused a slight reduction of [^{3}H]-5-HT release induced by PMA (Figure 3c).

Discussion

It has been shown previously that PMA activates the MTA of rat suprahyoideal muscle by increasing the release of brain 5-HT. This is supported by the finding that increased MTA induced by PMA is blocked by methysergide and *p*-chlorophenylalanine (Menon, Tseng & Loh, 1976). The results agree well with the biochemical finding that PMA greatly increases the release of [^{3}H]-5-HT in brain preloaded with that



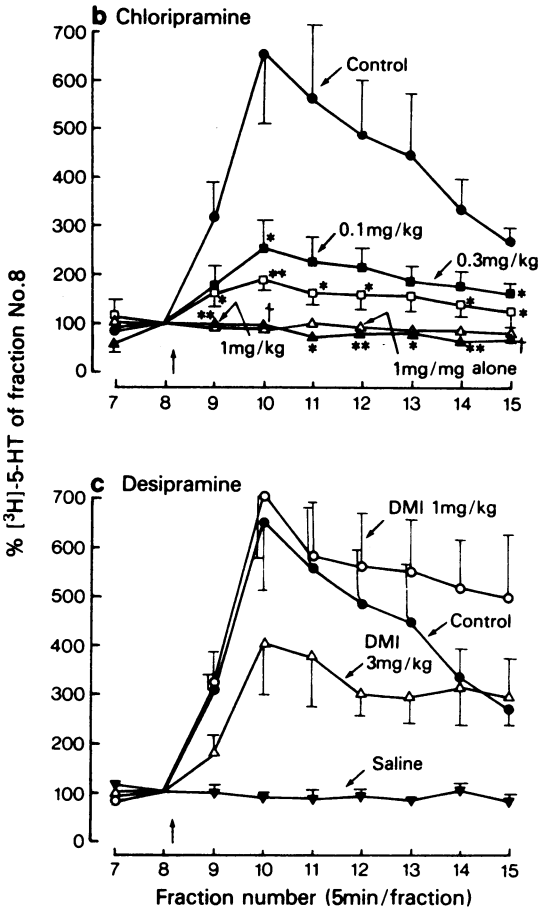


Figure 3 Effects of (a) fluoxetine, (b) chlorimipramine and (c) desipramine on the increased release of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) into the perfusate induced by *p*-methoxyamphetamine (PMA) in perfused cerebral ventricle of urethane-anaesthetized rats preloaded intraventricularly with [^{3}H]-5-HT. For details of the method, see text. Radioactivity is expressed as a percentage of the respective pre-PMA radioactivity level (sample No. 8 = 100%). The vertical bars represent s.e. mean (n = 3 to 5). Control: injected with saline at No. 7 and PMA at No. 9. The mean total amount of radioactivity recovered in sample No. 8 is $1.23 \pm 0.95 \times 10^5$ d/min per fraction (n = 8) in a control group which contains $3.88\% \pm 0.93\%$ of [^{3}H]-5-HT and $74.59 \pm 3.66\%$ of [^{3}H]-5-HIAA. $^*P < 0.05$; $^{**}P < 0.02$ and $\dagger P < 0.01$ compared with control (Student's t test).

compound (Tseng *et al.*, 1976). Further evidence leading to the conclusion that PMA activates the central 5-hydroxytryptaminergic system by an indirect mechanism (via 5-HT release rather than acting directly on the 5-HT receptor) was obtained from the experiment with chlorimipramine (Tseng *et al.*, 1978), a potent 5-HT uptake inhibitor (Carlsson, 1970; Lidbrink, Jonsson & Fuxe, 1971). Chlorimipramine blocked PMA-induced MTA and other PMA-linked 5-hydroxytryptaminergic behaviour patterns, such as increased locomotor activity, 5-HT-like stereotyped behaviour and disruption of fixed-ratio responding; it also blocked the increased release of [^{3}H]-5-HT into the ventricle in brain preloaded with [^{3}H]-5-HT (Tseng *et al.*, 1978). In the present studies another specific 5-HT uptake inhibitor, fluoxetine (Wong *et al.* 1974; 1975; Fuller, Perry & Molloy, 1975) was found to block the increased MTA induced by PMA although chlorimipramine is more potent than fluoxetine in this respect. These results parallel the biochemical findings that chlorimipramine and fluoxetine block PMA-induced increased release of [^{3}H]-5-HT into the ventricle in rat brain preloaded with [^{3}H]-5-HT. Chlorimipramine again was found to be more potent than fluoxetine. On the other hand, desipramine, a selective noradrenaline uptake inhibitor (Carlsson, 1970; Lidbrink *et al.* 1971) was unable to antagonize the PMA-induced increase of MTA and was also much less effective than chlorimipramine and fluoxetine in blocking the increased release of [^{3}H]-5-HT induced by PMA. The present studies support the conclusion that PMA produces its pharmacological actions by releasing brain 5-HT.

The activation of MTA induced by 5-HTP appears to be different from that observed after PMA treatment. Although activation of MTA by both 5-HTP and PMA was blocked by methysergide (Bieger *et al.*, 1972; Menon *et al.*, 1976), indicating that the 5-hydroxytryptaminergic system is involved, the increase of MTA induced by 5-HTP was not blocked but slightly potentiated by chlorimipramine. Thus 5-HTP activates MTA by increasing the availability of 5-HT and its effect is potentiated by chlorimipramine which blocks the reuptake of 5-HT.

The blockade of PMA-induced 5-HT release by 5-HT uptake inhibitors may be explained in a variety of ways. The most likely explanation is that PMA enters the neurone by the 5-HT uptake process in brain 5-HT neurones and that this transport is blocked by 5-HT uptake inhibitors, denying PMA access to intraneuronal releasing sites. The action of PMA on 5-HT neurones appears to be analogous to that of (+)-amphetamine on catecholaminergic neurones. The potent noradrenaline uptake inhibitors, desipramine and cocaine, have been shown to block the release of [^{3}H]-noradrenaline by (+)-amphetamine (Lundborg & Waldeck, 1971; Azzaro, Ziance & Rutledge, 1974).

This work was supported by a NIDA research grant DA-01314. The author would like to thank Miss Maureen Ann O'Rourke and Mrs Barbara Hitzemann for their technical assistance and Ms Cheryl Beyer for her typing assistance.

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(Received March 28, 1978.
Revised November 9, 1978.)