

Effect of parachlorophenylalanine, a specific 5-HT depleter on fluoxetine and d-fenfluramine induced penile erections in rats

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Abstract

Objective: To determine whether fluoxetine (FLU) induces penile erection (PE) in rats either indirectly via its 5-HT neuronal reuptake inhibiting activity or directly by stimulating the 5-HT_{2C} receptors in the brain. **Method:** The effect of pre-treatment with para-Chlorophenylalanine (PCPA) on fluoxetine and d-fenfluramine (d-FEN) induced PEs (Penile erections) was studied in albino rats. **Results:** FLU (10mg/kg) and d-FEN (2.5mg/kg) treated groups exhibited a significant increase in number of PEs as compared to their respective control (DW treated) groups. Pre-treatment with PCPA (100mg/kg/day) had no significant effect on the PEs induced by FLU (10mg/kg) but did significantly decrease the PEs induced by d-FEN (2.5 mg/kg). **Conclusion:** Our results indicate that pre-treatment with selective 5-HT depleter PCPA (100mg/kg/day) significantly decreased PEs induced by d-FEN (2.5mg/kg) but had no significant effect on the number of PEs induced by FLU (10 mg/kg). This indicates that the PE inducing effect of FLU is probably due to the stimulation of 5HT_{2C} receptors.

Key words: Fluoxetine (FLU), d-Fenfluramine (d-FEN), Parachlorophenylalanine (PCPA), Penile Erections (PEs), Distilled water (DW), Intraperitoneal injection (ip), Metachlorophenylpiperazine (mCPP), 5-Hydroxytryptamine (5-HT).

Introduction

Penile erections (PEs) induced by meta-Chlorophenylpiperazine (mCPP) and the indirectly acting agonists (E.g. fluoxetine and fenfluramine) were suggested to be 5HT_{1B} receptor mediated. However following discovery of 5HT_{1C} receptor, mCPP a drug considered to be 5HT_{1B} receptor selective agonist was found to bind more strongly to 5HT_{1C} receptors [1]. Later the 5HT_{1C} receptor was included in 5HT₂ receptor family and redesigned as 5HT_{2C} receptors (2). 5HT_{2C} receptor activation is involved in induction of PEs [2, 3, 4].

Penile erections (PEs) can be induced in rats by serotonergic (5-HT) releasing compounds like fenfluramine and SSRIs (Selective Serotonin Reuptake Inhibitors) like fluoxetine. Direct or indirect activation

of central 5HT_{2C} receptors induces penile erections in rats [5, 1, 2]. Fluoxetine induces penile erections in rats either indirectly via its 5-HT reuptake inhibiting activity or directly by stimulating the 5HT_{2C} receptors [5, 6]. Further in the present study we have determined that fluoxetine induces penile erections directly by stimulating central 5HT_{2C} receptors.

Materials and Methods

Animals: Albino male wistar rats weighing 100-180 grams, bred in Central Animal House of the Institute, were used. The animals were housed under standard conditions, maintained on a 12 hour light/dark cycle and had free access to food and water up to the time of experimentation. The animals were brought to the department and kept in a noiseless diffusely illuminated laboratory, at least 1 hr before the experiments for acclimatization to the laboratory environment. Each group consisted of 10 animals. Each animal was used only once. All observations were made between 10:00

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and 17:00 Hrs at 27°-30°C. Observations were made blind with respect to the treatments used. The experimental protocols were approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs: Drugs used were fluoxetine hydrochloride (Sun Pharmaceuticals), d-fenfluramine hydrochloride (Wockhardt) and d-parachlorophenylalanine methyl ester hydrochloride (Sigma). All drug solutions were prepared immediately before use and were injected intraperitoneally. The volume of injection was 5 ml/kg body weight for PCPA and 2 ml/kg body weight for the remaining drugs. Doses refer to the forms mentioned and were selected on the basis of previous studies conducted in our laboratory and those reported in literature.

PCPA on fluoxetine and d-fenfluramine (d-FEN) induced penile erections (PEs) in male rats: The methodology followed was similar to that of Berendsen

Results

In preliminary experiments, it was observed that 2.5 to 20 mg/kg FLU produced neither gross behavioural changes nor induced stereotyped behaviour (SB) of oral movement variety (OMV) or any feature of the 5-HT_{1A} and 5-HT_{2A} receptor mediated behavioural syndrome in the rats. However, male rats given 5, 10 and 20 mg/kg FLU exhibited the 5-HT_{2C} receptor mediated PEs. As FLU at 40 mg/kg dose had produced ataxia, motor incoordination and muscular hypotonia, for subsequent studies it was therefore used in the dose range of 2.5 to 20 mg/kg.

Effect of PCPA pre-treatment on FLU and d-FEN induced PEs in male rats: The results are given in Table 1. FLU (10 mg/kg) and d- FEN (2.5 mg/kg) treated groups pre-treated with DW (5 ml/kg ip daily for 4 days) exhibited a significant increase in the number of PEs as compared to their respective control DW (2 ml/kg ip) treated group pre-treated with DW (5 ml/kg ip daily for 4 days).

Pre-treatment with PCPA (100 mg/kg/day for 4 days) had no significant effect on the number of PEs induced by 10 mg/kg FLU. However, pre-treatment with PCPA (100 mg/kg/day for 4 days) did significantly decrease the number of PEs induced by 2.5 mg/kg d- FEN.

Table 1: Effect of PCPA pre-treatment on FLU and d- FEN induced PEs in male rats.

Treatment dose mg/kg	Number of PEs Mean ± S.E.M.
1. DW + DW	0.3 ± 0.15
2. DW + FLU 10	3.6 ± 0.16 ^a
3. PCPA 400 + FLU 10	3.7 ± 0.15
1. DW + DW	0.4 ± 0.16
2. DW + d- FEN 2.5	3.2 ± 0.13 ^a
3. PCPA 400 + d- FEN 2.5	0.7 ± 0.15 ^b

and Broekkamp [5]. For observation of distilled water (DW, 2 ml/kg ip, control group), FLU (10 mg/kg) and d- FEN (2.5 mg/kg) induced PEs the rats were placed in individual Perspex cages (30×20×20cm) immediately after the injection of DW (2 ml/kg), FLU (10 mg/kg) and d- FEN (2.5 mg/kg). The number of PEs induced by DW (2 ml/kg), FLU (10 mg/kg) and d- FEN (2.5 mg/kg) was counted between 5 and 60 min observation period. The total number of PEs scored by each rat in the group was taken to compute the mean value of the group.

DW (5 ml/kg ip) was administered daily for 4 days, the last dose was given 18 hr before DW (2 ml/kg ip, control group), FLU (10 mg/kg) and d- FEN (2.5 mg/kg). PCPA (100 mg/kg/day) was administered daily for 4 days, the last dose was given 18 hr before FLU (10 mg/kg) and d- FEN (2.5 mg/kg).

Statistics: The results were statistically analysed by the Student's unpaired t-test with differences considered significant at P < 0.05.

^a P < 0.001 as compared by the Student's unpaired t-test to the respective distilled water (DW, 2 ml/kg ip) treated control group pre-treated with DW (5 ml/kg ip daily for 4 days).

^b P < 0.001 as compared to the d- FEN (2.5 mg/kg ip) treated group pre-treated with DW (5 ml/kg ip daily for 4 days).

Discussion

The effect of pre-treatment with the selective 5-HT depletor PCPA was investigated on FLU induced PEs and d-FEN induced PEs. The study was conducted to determine whether FLU induces the PEs by stimulating the 5-HT_{2C} receptors either directly or indirectly via the accumulated 5-HT in the synaptic cleft resulting due to blockade of 5-HT reuptake.

Penile erections (PEs) result from activation of 5-HT_{2C} receptors [6, 1, 3]. FLU and d- FEN are reported to induce PEs [5]. The mechanism whereby FLU, fenfluramine and d- FEN induce PEs is explained as follows.

FLU at 5, 10 and 20 mg/kg doses, by blocking the neuronal reuptake of 5-HT, causes accumulation of 5-HT in the synaptic cleft with resultant stimulation of the 5-HT_{2C} receptors and occurrence of PEs. Alternatively, since FLU has moderate affinity for the 5-HT_{2C} receptors [6], it might be inducing the PEs by directly stimulating the 5-HT_{2C} receptors.

Fenfluramine and its d-isomer, d-FEN, like 5-HT and para-Chloroamphetamine (PCA), are taken up into the 5-HTergic (serotonergic) neurons by the same uptake carrier protein SERT [7]. After entering into the 5-HTergic neurons fenfluramine and d-FEN cause a brisk release of 5-HT from the 5-HTergic neurons with resultant stimulation of all the 5-HT receptor subtypes by the released 5-HT [7]. PEs induced by 5-HT_{2C} receptor activation are functionally inhibited by the activation of 5-HT_{1A} and 5-HT_{2A} receptors. They are evident only when the 5-HT_{1A} and 5-HT_{2A} receptor mediated behaviors are blocked by the 5-HT_{1A} and 5-HT_{2A} receptor antagonists respectively [8,5,1]. PEs are therefore induced only by smaller doses of fenfluramine (2.2 and 4.6 mg/kg) (5) which do not induce the 5-HT_{1A} and 5-HT_{2A} receptor mediated behaviours that occur following administration of higher doses of fenfluramine [9,10].

In preliminary studies it was observed that treatment with 1.25 mg/kg d-FEN did not induce PEs. However, treatment with 2.5 mg/kg d-FEN induced only PEs whereas treatment with 5, 10 and 15 mg/kg d-FEN

induced only 5-HT_{1A} and 5-HT_{2A} receptor mediated behaviors. For further study d-FEN was therefore used in the dose of 2.5 mg/kg.

The anti-immobility effect of FLU in the rat forced swimming test (FST), which is dependent on FLU's 5-HT neuronal reuptake blocking activity and consequent accumulation of 5-HT in the synaptic cleft, was reported to be antagonized by PCPA pre-treatment [11]. The antagonism of the anti-immobility effect of FLU in the rat FST by PCPA pre-treatment was explained by Page et al [11] as follows. PCPA, by selectively inhibiting tryptophan hydroxylase, the rate limiting enzyme in 5-HT biosynthesis, decreases the intraneuronal stores of 5-HT [12]. Consequently less amount of 5-HT is released from the 5-HTergic neurons resulting in a decrease in the concentration of 5-HT in the synaptic cleft and less amount of 5-HT being available for FLU to block the reuptake. Less amount of 5-HT will therefore accumulate in the synaptic cleft due to FLU's 5-HT neuronal reuptake blocking activity. Consequently the various 5-HT receptor subtypes involved in the anti-immobility effect of FLU will be stimulated to a lesser extent with resultant antagonism of FLU's anti-immobility effect. We studied the effect of pre-treatment with PCPA on FLU (10 mg/kg) induced PEs to determine whether FLU elicits the PEs indirectly via its 5-HT neuronal reuptake blocking activity and subsequent stimulation of the 5-HT_{2C} receptors by the accumulated 5-HT in the synaptic cleft or by direct stimulation of the 5-HT_{2C} receptors.

Pre-treatment with PCPA, by depleting brain 5-HT [12], makes less amount of 5-HT available for release by fenfluramine and thus antagonizes the fenfluramine-induced 5-HT_{1A} and 5-HT_{2A} receptor mediated behavioral syndrome [9,10]. We studied the effect of pre-treatment with PCPA on d-FEN (2.5 mg/kg) induced PEs to determine whether PCPA pre-treatment decreases the number of PEs induced by 2.5 mg/kg d-FEN.

In the present study pre-treatment with PCPA (100 mg/kg/day for 4 days) had no significant effect on the number of PEs induced by 10 mg/kg FLU but did

significantly decrease the number of PEs induced by 2.5 mg/kg d-FEN. Since PCPA pre-treatment had antagonized the effect of d-FEN it indicates that the dosing schedule of PCPA had caused significant depletion of brain 5-HT. PCPA pre-treatment, despite causing significant depletion of brain 5-HT, however, failed to decrease the number of PEs induced by 10 mg/kg FLU. This indicates that the PE inducing effect of FLU is not dependent on the availability of brain 5-HT.

The anti-immobility effect of FLU in the rat FST is dependent on the availability of brain 5-HT and is antagonized following depletion of brain 5-HT by PCPA pre-treatment [11]. However, in our study the PE inducing effect of 10 mg/kg FLU was not antagonized following depletion of brain 5-HT by PCPA pre-treatment. Our finding thus indicates that the PE inducing effect of 10 mg/kg FLU is not dependent on the availability of brain 5-HT and that FLU induces the PEs by a direct agonistic action on the 5-HT_{2C} receptors.

Our finding and the conclusion derived thereof that FLU induces the PEs by directly stimulating the 5-HT_{2C} receptors is in agreement with the reports of Zhang et al [13] and Chen et al [14]. These workers have reported that FLU, like nanomolar concentrations of 5-HT, induces glycogenolysis and an increase in free cytosolic Ca²⁺ concentration in cultured astrocytes and glial cells by directly stimulating the 5-HT_{2C} receptors.

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