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Effects of the Chronic Ingestion of Therapeutic Doses of Chlorimipramine on the Behavioral Action of Agonists and Antagonists of Serotonin in Male Rats[1]

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RODRIGUEZ ECHANDIA, E. L., S. T. BROITMAN AND M. R. FOSCOLO. *Effects of the chronic ingestion of therapeutic doses of chlorimipramine on the behavioral action of agonists and antagonists of serotonin in male rats.* PHARMACOL BIOCHEM BEHAV **19**(2) 193–197, 1983.—Locomotor activity and hole-board exploration (frequency and time spent head-dipping) were impaired in male rats by injecting IP the 5-HT agonists, fluoxetine and 5-HTP. This treatment produced also myoclonus and increased the time spent resting during trials. The chronic ingestion of chlorimipramine (CIM) or the injection of the 5-HT receptor blocker, methysergide (15 mg/kg) prevented the action of the 5-HT agonists on locomotion and resting and blocked the appearance of myoclonus. Both, CIM and methysergide prevented to a minor degree the fluoxetine-5-HTP-induced decrease of exploration. The chronic ingestion of CIM clearly potentiated the effects of methysergide on hole-board exploration. Results suggest that the chronic treatment with therapeutic doses of CIM reduces the functional activity of some 5-HT systems in the brain of the rat, probably by blockade of post-synaptic 5-HT receptors. This does not preclude, however, that CIM may also alter some NA systems.

Chlorimipramine Fluoxetine 5-HTP Locomotion Exploration Rat

BRAIN serotonin (5-HT) participates in the regulation of behavior [1, 2, 3, 22] as well as in some sensory [17,23] and motor mechanisms [18]. Injection of 5-hydroxytryptophan (5-HTP) or MAOI and tryptophan was found to produce stereotyped hyperactivity [10,15] and myoclonus [5,14]. It was shown recently that myoclonus is mediated by 5-HT neurons and would not involve a catecholamine link [16,20]. On the contrary, the stereotyped hyperactivity would be mediated by catecholamines; this alteration is antagonized by α-MPT but not by the 5-HT blockers [7,8]. Aprison and Hingtgen [3] showed that the effect of injecting 5-HTP into rats is to cause a type of hypoactivity. Such effect would be mediated also by 5-HT neurons; it is blocked by methysergide, a postsynaptic blocker of 5-HT and is potentiated by fluoxetine, a specific 5-HT agonist [24]. Recently, it was found that the acute treatment with the antidepressant drugs mianserin, amitryptiline, imipramine and iprindole resulted in blockade of the 5-HTP-induced behavioral depression [24]. It was postulated that these drugs can act as antagonists of 5-HT at the postsynaptic 5-HT receptors [13,24]. Growing evidence suggests that 5-HT neurons are involved in human depression as well as in the mechanism of action of some antidepressant drugs [2, 4, 21, 22, 26]. Inasmuch as clinical usage of antidepressants involves chronic treatment, an analysis of the effects of chronic administration of chlorimipramine (CIM) might be of interest. It is known that chlordesipramine, the major metabolite of CIM, is a potent noradrenaline (NA) uptake inhibitor [29]. In the rat, however, CIM would not be demethylated to its secondary amine metabolite [25]. The chronic ingestion of therapeutic doses of CIM causes hyperactivity and blocks the behavioral inhibition produced by a mild stress in male rats [27]. These findings were tentatively associated to an inhibitory action of CIM on some central 5-HT systems. However, experiments combining chronic CIM with 5-HT agonists and antagonists were not performed. In the present report IP injections of

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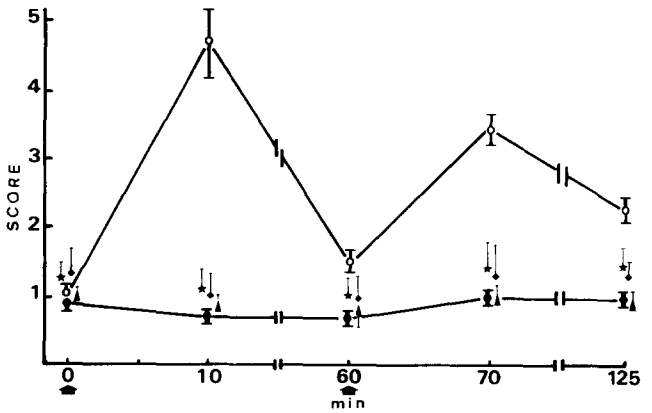

FIG. 1. Myoclonic syndrome test. Each value is the mean±S.E.M. of 15 observations for ○, ● and ★ and 10 observations for ◆ and ▲. ○: fluoxetine and 5-HTP injected rats. ●: saline injected controls. ★: CIM-fluoxetine-5-HTP rats. ◆: Methysergide-fluoxetine-5-HTP rats. ▲: CIM-methysergide-fluoxetine-5-HTP injected animals. Arrows: at min 0 and 60: first and second injection.

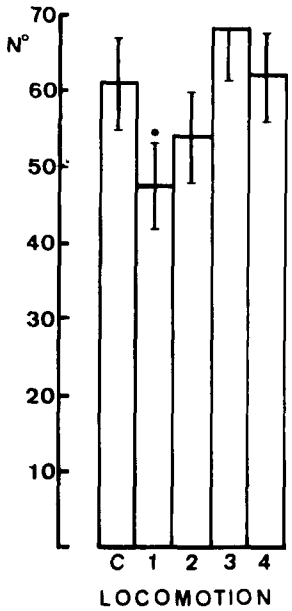

FIG. 2. Hole-board test. Scores of locomotion (means±S.E.M. of the number of animals within columns). C: saline-injected controls. 1: Fluoxetine and 5-HTP injected rats. 2: same treatment in CIM ingesting rats. 3: Methysergide (15 mg/kg)-fluoxetine and 5-HTP injections. 4: the same treatment in CIM ingesting rats. $^{*}p<0.05$.

fluoxetine and 5-HTP were used to analyze the effect of acute stimulation of the 5-HT neurons on hole-board locomotion and exploration in normal rats (Experiment 1) and in rats chronically treated with therapeutic doses of CIM (Experiment 2). Also, to obtain further clarification, groups of controls and CIM-treated rats were injected with methysergide and fluoxetine prior to a 5-HTP injection (Experiment 3).

METHOD

Male rats (Holtzman strain) weighing 200–230 g were used. These were housed in groups of 5 in stainless steel cages (40×27×20 cm) and maintained at 23±1°C with 14-hr lights 10-hr dark cycle, with lights on at 6.00 hr. Food and water were available ad lib. All injections were performed between 10.00 and 12.00 hr and the animals were tested between 12.30 and 14.00 hr.

Experiment 1

Rats were allocated in 2 groups: saline controls (15 animals) and fluoxetine-5-HTP experimentals (15 animals). Fluoxetine (10 mg/kg) was injected IP 60 min before injecting IP 30 mg/kg of 5-HTP according to Clemens *et al.* [6]. Both drugs were dissolved in 0.9% saline and the volumes injected were 0.4 ml/100 g. Control animals received equal volume IP injections of 0.9% saline.

Experiment 2

Fifteen rats were provided with tap water containing 25 mg/kg of CIM. The amount of drug ingestion was about 3 mg/kg/24 hr. As reported previously, [28] this was calculated from the mean amount of water intake/24 hr. After 30 days of CIM ingestion the rats were submitted to fluoxetine and 5-HTP injections as described in Experiment 1.

Experiment 3

A group of 20 CIM-treated animals and 10 controls were injected IP with a mixture of methysergide (15 and 30 mg/kg) and fluoxetine (10 mg/kg); 60 min later they were injected IP with 5-HTP (30 mg/kg).

Apparatus and Tests

(a) Myoclonic syndrome test. Myoclonus was evaluated in control and experimental rats as described by Trulson *et al.* [31]. Immediately before and after each injection, the spontaneous behavior of the individual rats was observed during 10 min in a cage similar to the home cage. Each animal was evaluated on a scale of 1 to 5 for the presence of head-twitches, hind limb adduction, forepaw treading, straub tail and rigidity. Results in Fig. 1 represent the summed scores for each 10 min period of observation. Myoclonus was evaluated also during the hole-board test.

(b) Hole-board test. The arena was a square surface (1.0×1.0 m) with 35 cm high walls painted black. The floor was of black plastic marked off in 20×20 cm squares. A total of 16 holes, each 2 cm in diameter, were regularly spaced 20 cm apart from one another. The field was placed 10 cm above the floor of the room. A single 40 W white lamp was suspended over the center of the field 2.8 m from the floor. All rats were tested 60 min after the second IP injection. They were placed singly in the center of the hole-board for a 5 min trial. The number of squares entered completely by each rat was scored as locomotor activity. The frequency of head-dips and the time spent head-dipping were scored as exploration. Resting, i.e., the time spent without performing any activity, was also scored. At the end of the trial the rat was removed from the hole-board and the field wiped clean.

Statistics

All variables were parametrics according to the Kolmogorov and Smirnov's test of goodness of fit. The signifi-

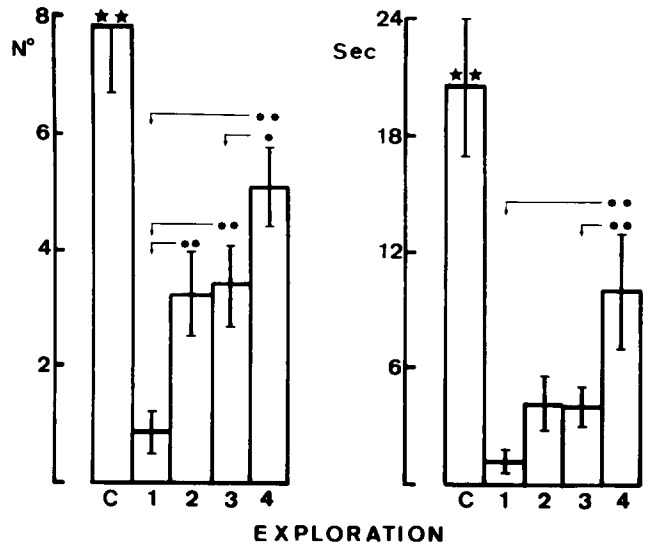

FIG. 3. Hole-board test. Frequency (N°) and time-spent (sec) head-dipping (means±S.E.M.). For explanation of columns and number of animals: see Fig. 2. Stars on column C: $p<0.01$ compared to groups 1 to 4. *$p<0.05$ and **$p<0.01$ compared to the column pointed by arrows.

cance of the difference of the mean was obtained by the ANOVA one way test and Duncan's multiple range test. A level of probability of less than 0.05 was considered statistically significant.

RESULTS

Experiment 1. Effect of Fluoxetine and 5-HTP Treatment

(a) Myoclonic syndrome test. As soon as 3.6±0.5 min after injection of fluoxetine the myoclonic syndrome scores were significantly higher than controls (Fig. 1). Immediately before the 5-HTP injection the syndrome scores approached those of the control animals but scores rised significantly at min 4.2±0.69 after 5-HTP injection (Fig. 1). The IP injection of 5-HTP induced rubescence of the ears, paws and tail from min 4.0±0.5 after injection up to the end of the experiment (65 min).

(b) Hole-board test. In the hole-board trials performed 60 min after that fluoxetine-5-HTP treatment was completed, the treated animals and the control rats showed similar myoclonic syndrome scores (results are not shown). Figure 2 shows that fluoxetine-5-HTP injections resulted in a significant decrease in the scores of locomotion and exploration. The treated animals approached and walked between and over the holes, as did the controls, but their head-dips were scarce. Figure 3 shows that fluoxetine-5-HTP rats displayed less frequency of head-dips ($p<0.01$) and less time spent head dipping ($p<0.01$) than controls. The time spent resting increased significantly in the treated rats (C: 34.5±11.65; fluoxetine-5-HTP: 106.1±19.21, $p<0.01$).

Experiment 2. Effect of Fluoxetine-5-HTP Injections in Rats Submitted to Chronic Ingestion of CIM

(a) Myoclonic syndrome test. The chronic ingestion of CIM prevented the increase of the myoclonic syndrome scores induced by the injection of the 5-HT agonists. Figure 1 shows that the syndrome scores in these animals were similar to controls. Rubescence of ears, paws and tail was prevented as well.

(b) Hole-board test. Figure 2 illustrates that CIM prevented the inhibitory action of fluoxetine- and 5-HTP injections on hole-board locomotion. The CIM treatment prevented to a minor degree the fluoxetine-5-HTP-induced blockade of exploration (Fig. 3). Scores of exploration of CIM animals were significantly lower than controls ($p<0.01$). However, if exploration scores of CIM-fluoxetine-5-HTP rats and fluoxetine-5-HTP rats are compared, it can be observed that chronic CIM enhanced significantly the frequency of head-dips ($p<0.01$) but not the time spent head-dipping. CIM fully prevented the effect of fluoxetine-5-HTP injection on the time spent resting (C: 34.5±11.65; CIM-fluoxetine-5-HTP: 34.4±13.58).

Experiment 3. Effect of Methysergide on the Behavioral Response to Fluoxetine and 5-HTP Injections in Control and CIM Treated Rats

(a) Myoclonic syndrome test. The injection of either 15 and 30 mg/kg methysergide prevented the increases of the myoclonic syndrome scores produced by fluoxetine and 5-HTP (see Fig. 1) in both normal and CIM treated rats. Rubescence was also prevented by methysergide.

(b) Hole-board test. Both locomotion and exploration were normal in the animals injected with 30 mg/kg methysergide in addition to fluoxetine and 5-HTP (Locomotion: C: 60.7±6.34; methysergide: 63.6±5.53. N° of head-dips: C: 7.8±1.2; methysergide: 6.9±1.93. Time spent head-dipping: C: 20.7±4.66; methysergide: 21.6±5.84). The time spent resting substantially decreased if compared to the animals treated with fluoxetine-5-HTP only (Fluoxetine-5-HTP: 106.1±19.21; methysergide-fluoxetine-5-HTP: 53.2±12.27, $p<0.01$).

The CIM treatment did not modify the protective action of 30 mg/kg methysergide against the effect of the 5-HT agonists on hole-board locomotion and exploration. Methysergide, in the lower dose of 15 mg/kg fully prevented the effect of the 5-HT agonists on locomotion (Fig. 2). In these rats, however, the scores of exploration (Fig. 3) remained lower than controls ($p<0.01$), though the frequency of head-dips was significantly higher than in the animals injected with fluoxetine-5-HTP only ($p<0.01$). Methysergide in doses of 15 mg/kg prevented also the effect of fluoxetine-5-HTP on the time spent resting (C: 34.5±11.65; methysergide: 56.7±11.78). The chronic treatment with CIM clearly potentiated the effect of 15 mg/kg methysergide on hole-board exploration. Figure 3 shows that for CIM treated rats both frequency and time spent head-dipping were significantly higher ($p<0.05$ and $p<0.01$ respectively) but did not reach control levels. The time spent resting in CIM treated rats was similar to controls (C: 34.5±11.65; CIM-methysergide: 31.5±12.62).

DISCUSSION

Fluoxetine is a selective blocker of 5-HT uptake into 5-HT neurons [11] but produces a reduction in tryptophan hydroxylase activity which results in an inhibition of 5-HT synthesis [12] and decreases neuronal impulse flow [6]. When 5-HTP is given to rats pretreated with fluoxetine the tryptophan hydroxylase step in 5-HT synthesis is circumvected and more 5-HT would be available at serotonergic synapses. Consistently, the behavioral depression produced

by 5-HTP injection is potentiated by fluoxetine [24,26].

In experiment 1, the rats submitted to fluoxetine-5-HTP injection displayed the typical myoclonus described by other authors [5, 14, 18]. This syndrome was induced by both 5-HT agonists and appeared shortly after injections. It was prevented by methysergide in doses of 15 and 30 mg/kg. These results thus support previous evidence on the selective responsibility of the 5-HT neurons in the mechanism of the myoclonic syndrome [8,20]. The fact that CIM was also effective to prevent the myoclonic syndrome induced by the 5-HT agonists supports the speculation that a chronic treatment with therapeutic doses of CIM induces also a postsynaptic blockade of 5-HT in rats. This result agrees with previous observations on the action of acute doses of some tricyclic and tetracyclic antidepressants [13, 24, 26].

It has been reported that the injection of 5-HTP and fluoxetine causes hypoactivity in rats [3, 20, 24]. Such hypoactivity has been temporally correlated with an increase of 5-HT in specific brain areas but not with changes in catecholamine levels [3]. In the present study the fluoxetine and 5-HTP treatment impaired locomotion, substantially reduced hole-board exploration and increased the time spent resting. This behavioral inhibition would be a consequence of enhancement of the 5-HT neuron activity since, it was fully prevented by methysergide in doses of 30 mg/kg. The effect of the 5-HT agonists on locomotor activity was blocked also by methysergide at the lower dose of 15 mg/kg and to a minor extent by the chronic administration of CIM. However, exploratory activity in these animals remained lower than controls. Therefore, a stronger blockade of serotonergic stimulation appears necessary for a full restoration of exploration. Distinction between exploration and locomotion is widely accepted; it has been suggested that alterations of exploration may have different pharmacological bases from alterations of locomotion [9]. Without minimizing the involvement of 5-HT in locomotion, it is possible that serotonergic neurons might play a more significant role in modulation of exploration than in the regulation of locomotion. This speculation correlates with the finding that lesions of the median raphe 5-HT cells induced by 5,6-DHT did not affect locomotor activity in rats [19], but requires further analysis.

Despite the different quantitative effects of the chronic CIM treatment on locomotion and exploration it is clear that CIM treated rats were less responsive to the behavioral inhibitory action of the 5-HT agonists. Moreover, the CIM treatment clearly potentiated the effect of the 5-HT postsynaptic blocker, methysergide when used in the dose of 15 mg/kg. These findings support the speculation that the chronic treatment with therapeutic doses of CIM reduces the functional activity of the 5-HT systems in the brain of the rat.

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