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Isolation Increases a Behavioral Response to the Selective 5-HT_{1B} Agonist CGS 120 66B

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FRANCES, H. AND C. MONIER. *Isolation increases a behavioral response to the selective 5-HT_{1B} agonist CGS 120 66B.* PHARMACOL BIOCHEM BEHAV **40**(2) 279–281, 1991.—The effect of two serotonergic drugs, CGS 120 66B acting specifically and TFMPP acting preferentially onto 5-HT_{1B} receptors, was compared in preisolated and in pregrouped mice. Two mice put under an inverted beaker attempt to escape. The number of escape attempts of mice preisolated for 7 days was half that of pregrouped mice. In preisolated mice, TFMPP and CGS 120 66B increased the number of escape attempts up to, respectively, 200% and 300% of that of preisolated control mice. In pregrouped mice, CGS 120 66B was nearly inactive and TFMPP exerts a smaller effect. These results suggest that isolation increases the apparent responsiveness to 5-HT_{1B} stimulants.

Isolation Behavior Mice 5-HT_{1B} agonists

A brief period of isolation (7 days) modified the behavior of mice. Two mice (one preisolated mouse + one pregrouped mouse) observed together under a reversed beaker attempted to escape. However, for the first two minutes of observation, the number of escape attempts of preisolated mice was half that of pregrouped mice: this difference was named the isolation-induced social behavioral deficit; this deficit was reversed by some serotonergic agonists (2). It was later found that the behavioral deficit was reversed by drugs acting through stimulation of 5-HT_{1B} receptors such as TFMPP, m-CPP, RU 24969 but not 5-HT_{1A} receptors (8-OH DPAT), and that the effect of TFMPP was unchanged by serotonergic antagonists acting through 5-HT_2 (ritanserine), 5-HT_3 (ICS 205-930) or 5-HT_{1C} receptors (cyproheptadine, mianserine) but completely impaired by penbutolol, a beta-blocking drug binding also to 5-HT_1 receptors (4).

It has been shown that a prolonged isolation reduced the number of 5-HT receptors in the brain of aggressive mice (1) and that the number and affinity of 5-HT receptors was decreased in rats isolated for 3 months (8). These data prompted us to undertake the following experiments to specify whether the increase in escape attempts induced by serotonergic drugs in preisolated mice was also present in pregrouped mice and, if so, to what extent. In other words, the influence of isolation on the sensitivity of a response mediated through 5-HT_{1B} receptors was investigated.

METHOD

Animals

Male Swiss NMRI mice (20–24 g), from CERJ, Genest St Isle, 53940, France, were used in all experiments. Mice were housed in groups of six in home cages of $30 \times 20 \times 10$ cm (pregrouped mice) or were isolated during 7 days in home cages of $24 \times 10 \times 8$ cm. Mice were 4–5 weeks old when they were housed in isolation. The room was thermostatically maintained at 21 ± 1°C with a 12-h light/dark schedule. Food and water were freely available. Experiments were performed between 9 a.m. and 4 p.m. A partner mouse was a pregrouped mouse which did not receive any treatment (neither drug nor vehicle).

Experimental Procedure

Escape attempts. Mice were observed in pairs (one preisolated + one partner mouse) or (one pregrouped + one partner mouse) under a transparent beaker (height 14 cm, diameter 10 cm) inverted on a rough surface glass plate. The number of escape attempts was defined as any one of the following: 1) the two forepaws were placed against the beaker wall, 2) the mouse sniffed at the rim of the beaker, 3) the mouse scratched the glass floor. Escape attempts were counted for 2 minutes. There was no minimal duration for one escape attempt. For a long-lasting attempt, a new attempt was counted for each period of 3 seconds. All mice were tested only once, except in Experiment 2. Behavioral observations were made by an observer blind to the treatments received by the mice. Control mice were, according to the protocols, preisolated or pregrouped mice receiving demineralized water.

In Experiment 2, the same mice were their own controls. Each mouse, preisolated or not, received on the 7th day demineralized water and was observed in pair with a partner mouse 30 minutes later. Then, the preisolated mice were reisolated for an additional day, and on the 8th day, all mice were given TFMPP 2 mg/kg and observed with the same pool of but not the same individual partners.

Open-field. The wooden, white painted open field (50×50 cm) was separated in 25 squares of 10×10 cm. The walls were 22 cm high. The number of squares crossed and of rearings were counted for 2 minutes of observation, 30 min after drug administration.

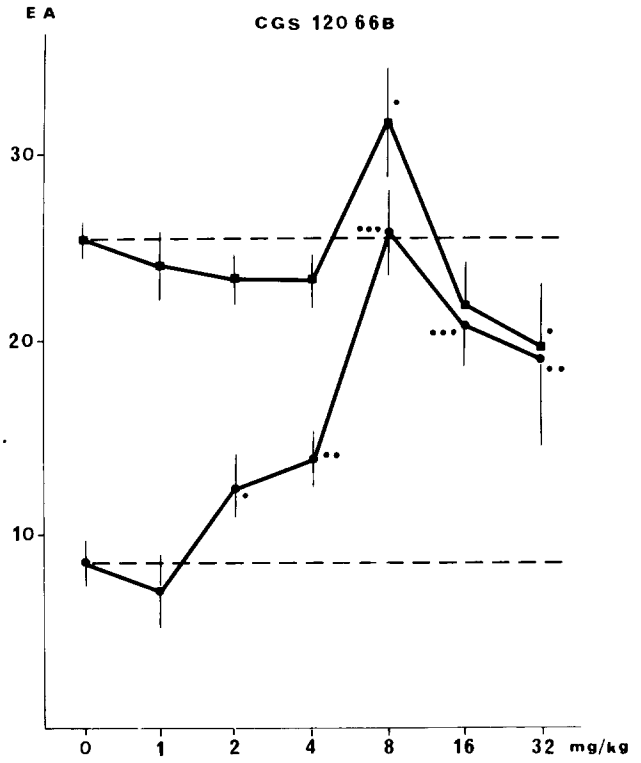

FIG. 1. Effect of CGS 120 66B on the number of escape attempts in pregrouped mice (■) and in preisolated mice (●). Ten to 40 mice per dose. Significant values refer to the respective controls receiving water. ●$p<0.05$, ●●$p<0.01$, ●●●$p<0.001$.

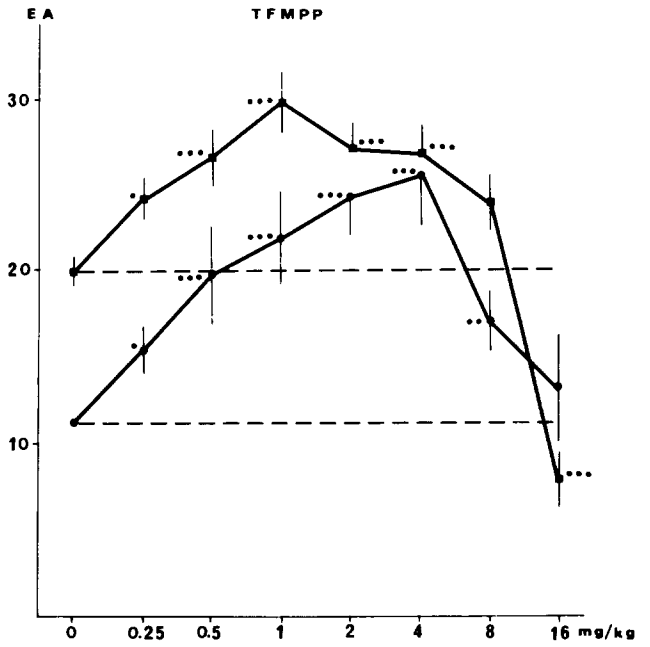

FIG. 2. Effect of TFMPP on the number of escape attempts in pregrouped mice (■) and in preisolated mice (●). Ten to 40 mice per dose. Significant values refer to the respective controls receiving water. ●$p<0.05$, ●●$p<0.01$, ●●●$p<0.001$.

Drugs

The drugs used were: 1-(-m-trifluoromethylphenyl)piperazine (TFMPP) (Aldrich Chemical Co., Strasbourg, France) and 7-trifluoromethyl-4(4-methyl-1-piperazinyl)-pyrolol (1,2-a) quinoxaline 1:2 maleate (CGS 120 66B) (R.B.I., Natick, MA).

TFMPP was dissolved in demineralized water; CGS 120 66B was suspended in gum arabic. Drugs were administered in a volume of 0.2 ml/20 g body weight by the IP route 30 min before the test. During the delay between the drug administration and the test, preisolated as well as pregrouped mice were maintained in individual cages.

Statistics

Results were analyzed using the one-way analysis of variance followed by the Dunnett test, or using the Student *t*-test.

RESULTS

Experiment 1

Effect of CGS 120 66B (Fig. 1). In preisolated mice, CGS 120 66B increased the number of escape attempts in a dose-dependent way up to 8 mg/kg. For higher doses, the effect decreased. With 8 mg/kg, the most effective dose, the escape attempts reached 300% of the score of control preisolated mice receiving water. In pregrouped mice, only the dose of 8 mg/kg increased significantly the number of escape attempts.

Effect of TFMPP. In preisolated mice, TFMPP increased the number of escape attempts in a dose-dependent way up to 4 mg/kg (Fig. 2). For higher doses, the effect decreased and disappeared. With 2 and 4 mg/kg, the more effective doses, the effect reached 200% of control preisolated mice receiving water.

In pregrouped mice, TFMPP increased significantly the number of escape attempts (Fig. 2); the effect was dose-dependent up to 1 mg/kg and then decreased, disappeared and a significant reduction in escape attempts was observed with 16 mg/kg. The first effective dose was the same (0.25 mg/kg) in both preisolated and pregrouped mice; however, with 1 mg/kg, the more effective dose, the escape attempts reached 150% of the score of control pregrouped mice receiving water.

Experiment 2

The number of escape attempts of mice preisolated for 7 days and of pregrouped mice was counted as usually 30 min after water administration. Each mouse was individually stamped. Preisolated mice were reisolated for an additional day. The 8th day, the same preisolated and pregrouped mice received TFMPP 2 mg/kg and were tested 30 min thereafter. So, each drugged mouse preisolated or pregrouped was its own control the day before. For each mouse, the difference between the score with drug and with water was calculated. The effect of TFMPP 2 mg/kg appeared significantly higher in preisolated than in pregrouped mice (Table 1).

Experiment 3

Pregrouped mice received TFMPP 2 mg/kg or water and were individually tested in the open-field. TFMPP (Table 2) increased, but in a nonsignificant way, the number of squares crossed for 2 minutes; the number of rearings was unchanged.

DISCUSSION

In preisolated mice, the selective 5-HT_{1B} agonist CGS 120 66B increased highly significantly the number of escape at-

TABLE 1

COMPARISON BETWEEN PREGROUPED AND PRE-ISOLATED MICE IN RESPONSE TO TFMPP

	Escape Attempts m ± S.E.M.		
	Water	TFMPP 2 mg/kg	Individual Differences
Pregrouped Mice (n = 11)	28.7 ± 1.6	31.3 ± 3.0	2.6 ± 2.7
	n.s		$p<0.01$
Preisolated Mice (n = 14)	10.4 ± 1.2	28.1 ± 3.9	17.7 ± 3.9
	$p<0.001$		

The same mice were observed for 2 minutes, 30 min after water on the first day (7 days of isolation) and 30 min after TFMPP administration on the day after (8 days of isolation).
Student *t*-test.

TABLE 2

EFFECT OF TFMPP IN PREGROUPED MICE OBSERVED IN AN OPEN-FIELD

	Water (n = 15)	TFMPP 2 mg/kg (n = 15)
Crossed Squares mean ± S.E.M.	47.7 ± 5.5	65.9 ± 8.2
	n.s.	
Rearings mean ± S.E.M.	6.3 ± 1.1	5.4 ± 0.9
	n.s.	

Mice were observed for 2 minutes, 30 min after treatment.
Student *t*-test.

tempts. On the contrary, in pregrouped mice, the same drug was nearly inactive: this result suggests that preisolated mice are more responsive than pregrouped mice to 5-HT$_{1B}$ agonists. TFMPP, another serotonin agonist binding preferentially but not specifically to 5-HT$_{1B}$ receptors, also increases the number of escape attempts of pregrouped mice but in a smaller way. The difference between CGS 120 66B and TFMPP in their activity in pregrouped mice may be accounted for by the different specificity of these drugs. CGS 120 66B has been reported as highly specific for 5-HT$_{1B}$ receptors (7), whereas TFMPP binding preferentially to 5-HT$_{1B}$ receptors may also stimulate 5-HT$_{1C}$ receptors (5). TFMPP has been shown to reduce locomotor activity in rats (6) and in mice (3) and this effect appears to be mediated in rats through the stimulation of 5-HT$_{1C}$ receptors. So, the increase in escape attempts provoked by TFMPP in pregrouped mice cannot have been augmented through an effect of this drug on locomotor activity. However, in the open field, where the activity of mice has been measured for two minutes, TFMPP did not change the number of rearings but slightly increased the number of crossings; this increase, although nonsignificant, may have been sufficient to augment the number of escape attempts.

A direct comparison of TFMPP effect in pregrouped and in preisolated mice is given in Table 1 where the same mice are controls the first day and treated the second day: the increase in the number of escape attempts was significant in preisolated mice and not in pregrouped mice; the effect of TFMPP was highly greater in preisolated mice. This result strengthens the suggestion of a greater sensitivity of preisolated mice to 5-HT$_{1B}$ agonists.

The isolation-induced apparent increase in the responsiveness to 5-HT$_{1B}$ agonists may result from an increase in the number or affinity of some of these receptors. However, such an increase seems questionable since Essman and Valzelli (1) observed a reduction in the number of serotonin binding sites in three brain regions of mice isolated for 35 days and since Popova and Pletkov (8) obtained a decrease in the number and affinity of brain 5-HT$_1$ receptors in rats isolated for 3 months. Possibly, the receptor's modification vary with time: the duration of our isolation (one week) was brief regarding to 35 days or 3 months. Another possibility is that all 5-HT receptors may not vary in the same direction with isolation. We tested specifically a behavior mediated through 5-HT$_{1B}$ receptors, whereas Essman and Valzelli (1) measured all sorts of 5-HT receptors and Popova and Petkov (8) all the 5-HT$_1$ receptors together. Biochemical experiments are required to assert the hypothesis of an increased sensitivity of 5-HT$_{1B}$ receptors.

These results suggest an isolation-induced increase in the sensitivity of 5-HT$_{1B}$ receptors and also that preisolated mice represent a better substrate than pregrouped mice to study the effect of drugs acting through 5-HT$_{1B}$ receptors in this model.

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