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Serotonin-Mimetic and Antidepressant Drugs on Passive Avoidance Learning by Olfactory Bulbectomised Rats

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BROEKKAMP, C. L., D. GARRIGOU AND K. G. LLOYD. *Serotonin-mimetic and antidepressant drugs on passive avoidance learning by olfactory bulbectomised rats.* PHARMAC. BIOCHEM. BEHAV. 13(5) 643–646, 1980.—Olfactory bulbectomised rats were treated with drugs and their rate of acquisition of a passive avoidance task was measured. The acquisition-rate, which is disturbed by the bilateral ablations, was completely restored by acute administration of fenfluramine or fluoxetine. Partial restoration was found with quipazine. Clonidine was without effect. Repeated treatments with imipramine and mianserine improved passive avoidance of bulbectomised rats. Metergoline blocked these effects of imipramine and mianserin. These results indicate a serotonergic mechanism in the effect of antidepressants on olfactory bulbectomised rats.

Antidepressant drugs Mianserin Olfactory bulb removal Passive avoidance learning Serotonin-like drugs

THE removal of both olfactory bulbs in rats induces a behavioural syndrome characterized by an augmentation in irritability, muricide, activity in an open field and fluid intake, and a decrease in the capacity to learn a passive avoidance task [10, 20, 23]. At the physiological level it has been found that the blood levels of corticosteroids are increased [9]. From pharmacological studies, it is known that a series of antidepressants are able to restore the deficit in passive avoidance learning and to normalize the corticosteroid levels [9,25]. Furthermore, chlorpromazine, amphetamine, diazepam and chlordiazepoxide were ineffective in this respect. Thus, the olfactory-bulbectomised rat is proposed to be useful as a screening test for potential antidepressant compounds [7,19]. However, the predictability and specificity of this model is still uncertain. Thus, tranylcypromine, an antidepressant-monoamine oxidase inhibitor, is reported to be inactive. Additionally, a theoretical relation between the behaviour shown by the olfactory bulbectomised rat and depression in man is absent. However this model seems worth pursuing as mianserin, a new antidepressant drug which lacks activity in the majority of test models for antidepressant drugs [5], is active in this model.

The following experiments were performed in order to increase our pharmacological understanding of the olfactory bulbectomised rat as a model for antidepressant drug screening. Olfactory-bulbectomised rats were treated with the post- and presynaptic α-receptor stimulant clonidine [4,20] or with putative serotonin-mimetic compounds such as fluoxetine (a 5-HT-reuptake blocker, [28]), fenfluramine (a 5-HT-releasing agent, [14]) and quipazine (a potent 5-HT-like agent with both direct and indirect receptor stimulating properties, [17,26]). The serotonin antagonist metergoline [2,11] was used to further evaluate serotonin involvement in this test system.

METHOD

Male albino rats (180–200 g, CS-COBS strain, Charles River, France) were anaesthetized with 80 mg/kg sc Brietal®. The skull was exposed and two 2 mm holes were trepanized through the skull 7 mm anterior to bregma. The olfactory peduncles were cut according to Cairncross *et al.* [7] and the bulbs were removed by suction. The resultant cavity was filled with hemostatic resorbable gauze (Surgicel®). After the operation the rats were housed individually and a period of 6 days without manipulations was respected for recovery from surgery.

The environment in which the passive avoidance training occurred consisted of an experimental cage (30×30×28 cm) in which a platform of 7.5×30 cm was mounted 4.5 cm above the floor at one side. The rat was placed in the experimental cage and allowed one minute of exploration. For a learning trial the animal was placed on the platform. When it had left the platform with all four paws, it received for two seconds a shock of 0.6 mA via the grid floor of the cage. The animal was immediately removed from the experimental cage and replaced in its home cage. After 1 minute the next trial on the same rat was initiated. The training of a rat was stopped if the rat learned not to leave the platform before the passage of three minutes or if ten trials were given.

The measurements of passive avoidance acquisition were made at the 7th day postoperatively except if a drug treatment was given repeatedly. In the latter situation the acquisition test was done on the 11th postoperative day for imipramine treatments and on the 18th day for mianserin administration.

Single treatments with fluoxetine HCl, fenfluramine HCl, quipazine maleate, and clonidine HCl were given 30 min before the start of conditioning. Repeated treatments of imipramine HCl were given daily between 9–10 a.m. The first

TABLE 1

DRUG TREATMENT EFFECT ON ACQUISITION OF A PASSIVE AVOIDANCE TASK BY BULBECTOMISED RATS

Treatment (dose)	Number of animals	No. of trials to criterion Mean ± SEM	p, in two-tailed *t*-test
Sham operated controls	9	2.8 ± 0.4	
bulbectomised controls	51	6.6 ± 0.3	<0.01*
+ clonidine (0.1)	8	6.9 ± 0.8	NS
+ clonidine (0.5)	8	5.2 ± 0.7	NS
+ fluoxetine (10)	8	3.4 ± 0.4	<0.01†
+ fenfluramine (3)	7	2.9 ± 0.3	<0.01†
+ quipazine (5)	16	4.0 ± 0.3	<0.01†
+ metergoline (10)	8	4.9 ± 0.7	NS†
+ metergoline (10) + fenfluramine (3)	7	5.6 ± 0.6	<0.01‡
+ metergoline (10) + quipazine (5)	8	5.6 ± 0.8	<0.05‡

*Significance in comparison with sham operated controls.
†Significance in comparison with control treated bulbectomised rats.
‡Significance in comparison with corresponding drug treated group.

injection was given on the 7th post-operative day and the last injection was given on the 11th day after the operation, half an hour before conditioning. Mianserin HCl was injected twice daily between 9 and 10 a.m. and 4 and 5 p.m. The first injection was given on the 7th post-operative day and the last injection was given between 4 and 5 p.m. on the day before the passive avoidance test. The antagonist metergoline was given 1 hour before conditioning. Drugs were dissolved in distilled water and administered intraperitoneally in a volume of 5 ml/kg except metergoline which was suspended in distilled water with a drop of Tween 80 and administered orally. Controls were handled similarly and injected with distilled water.

After the experiments the rats were sacrificed and the brain was examined for verification of the olfactory bulb lesions. Data from rats with incomplete olfactory bulb ablations or additional damage to the frontal cortex were discarded.

RESULTS

As shown in Table 1, unoperated rats learned the passive avoidance situation in 2.8±0.4 trials. Rats with bilateral olfactory bulb ablations needed an average of 6.6±0.3 trials to reach criterion. Among the drugs that restored the deficit, fenfluramine was the most potent. Doses of 3 mg/kg IP reduced the number of trials to 2.9±0.3. Fluoxetine in a dose of 10 mg/kg IP also restored the acquisition capacity to the level of control operated rats. Partial restoration was observed with quipazine (5 mg/kg). Clonidine, up to 0.5 mg/kg was ineffective. The effects of fenfluramine and quipazine were prevented by pretreatment with metergoline (10 mg/kg). Metergoline by itself did not increase the number of trials for acquisition. Rather, it had a tendency to improve the acquisition.

Beneficial effects with the antidepressants imipramine and mianserin were obtained with repeated but not acute treatments. Thus, after imipramine was given daily for 5 days, the rats learned the task in 4.5±0.6 trials ($p<0.05$; Fig. 1). After a single injection of imipramine, OLB rats needed 5.1±0.5 trials. A single injection of mianserin before conditioning seemed to worsen rather than ameliorate the acquisition rate (7.9±0.8 trials). Repeated injections twice daily during 10 days improved the learning rate significantly (4.9±0.7; $p<0.05$; Fig. 1). Metergoline pretreatment prevented completely the beneficial effects of repeated treatments of imipramine or mianserin (Fig. 1).

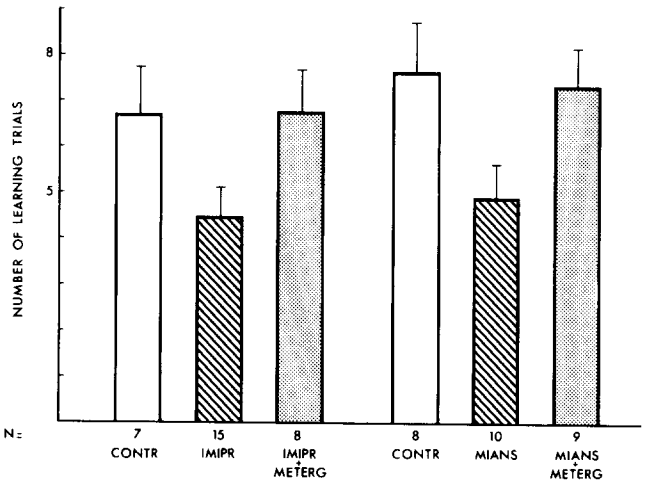

FIG. 1. Effect of repeated treatment with imipramine (10 mg/kg, 5 days) or mianserin (10 mg/kg twice daily, 10 days) on acquisition of a passive avoidance task and antagonism of the drug effects by metergoline (10 mg/kg PO). Imipr vs imipr+meterg. $p<0.05$ (*t*-test; two-tailed) Mians vs mians+meterg. $p<0.05$ (*t*-test; two-tailed)

DISCUSSION

Fluoxetine, fenfluramine and quipazine all potentiate 5-HT transmission (see Introduction); all these compounds at least partially restore the acquisition deficit in bulbectomised rats. This suggests that some of the disturbances in the brain caused by the removal of the olfactory bulbs can be compensated for by stimulating directly or indirectly certain 5-HT receptors. The antagonism by metergoline of the effect of fenfluramine and quipazine supports this interpretation.

The importance of a serotonin mechanism in restoring another bulbectomy induced behavioral change was demon-

strated before by DiChiara *et al.* [10]. Thus, the OLB-enhancement of mouse-killing behavior can be antagonized by potentiating serotonin transmission in the brain [10]. Perhaps, these behavioral changes are based on the same disequilibrium in a specific part of the brain affected by the lesion induced denervation.

One possible explanation for the restoring effect of a serotonergic activation is that the bulb ablations might have caused a reduced activity in a serotonin-system by indirect mechanisms and, as a consequence of this reduced activation, impair the acquisition of the passive avoidance response. This is in line with the observations that the serotonin depletor parachlorophenylalanine results in an impairment of the acquisition or maintenance of conditioned inhibition [15, 22, 24] and facilitates mouse-killing [16]. Another possibility is that increased serotonin activity compensates functionally for another, non-serotonergic pathway disturbed by the lesions.

The drugs ameliorating passive avoidance learning as described above have behaviorally inhibitory effects as shown by a potentiation of haloperidol or morphine catalepsy [1,18] or antagonism and prolongation of amphetamine and pentobarbital respectively [3]. In contrast, clonidine, in doses that are inhibitory on overt behavior and that potentiate haloperidol catalepsy [6], does not improve passive avoidance learning of bulbectomised rats. Thus, the drug effects on OLB rats and behavioral inhibition therefore are not necessarily related. This is in agreement with results by Cairncross and coworkers [7] who showed that sedative drugs, like benzodiazepines, and the neuroleptic chlorpromazine (3 mg/kg) are ineffective on the OLB model.

The finding that fenfluramine restores the passive avoidance deficit is not in accordance with the hypothesis that the bulbectomised rat can be used as a test model for antidepressant drugs. Fenfluramine is devoid of antidepressant properties in man [27]. Other parameters of the proposed test model such as the corticosteroid level and the acquisition rate of sham operated controls should be measured before it can be concluded that fenfluramine is a false positive in this test. However, from our results the possibility occurs that it is the serotonergic component in the actions of a number of antidepressants that might be responsible for the effect of the antidepressants in this model. In agreement with this suggestion is the antagonism by the serotonin-receptor blocker metergoline of the effect of repeated imipramine or mianserin treatment. Imipramine is known to inhibit serotonin uptake and can, by this mechanism, potentiate serotonin transmission. The blockade by metergoline of the effect of a repeated treatment with mianserin indicates that such a treatment has, directly or indirectly, an activating effect on a group of serotonin receptors. This seems to conflict with available data showing that mianserin by itself is a serotonin receptor blocker [5]. It is important to emphasize that the positive effect was obtained after repeated treatments. A single treatment with mianserin had no effect on the passive avoidance acquisition of OLB rats. The effects of repeated injections can be different in view of compensatory and sensitisation mechanisms [12,13]. In view of our finding, a potentiating action or indirect stimulation of a serotonin system in the brain should be taken into consideration in the search for an explanation of the antidepressant activity of mianserin in man.

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