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The Effects of Fluoxetine and Its Complexes with Glycerrhizic Acid on Behavior in Rats and Brain Monoamine Levels

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The effects of the serotonin reuptake inhibitor fluoxetine (FL) and its complexes with glycyrrhizic acid (GA) in molar ratios of 1:1 (FLG-1) and 4:1 (FLG-4) on the behavior of adult rats were studied in an elevated cross maze, with measurement of brain monoamine and monamine metabolite levels. Agents were given via the intragastric route using a cannula at a dose of 25 mg/kg 1 h before testing. FL increased anxiety in the rats and decreased their movement activity; FLG-1 and FLG-4 had no effect on behavior. None of the agents affected brain serotonin content, though all decreased the levels of its metabolite 5-hydroxyindoleacetic acid in the hypothalamus, FLG-4 also decreasing this in the cortex. Noradrenaline levels in the hypothalamus were increased after FLG-1 and FLG-4. In the striatum, FL increased the levels of dopamine and its metabolite dihydroxyphenylacetic acid but had no effect on the level of transmitter catabolism. Unlike FL, FLG-1 activated dopamine metabolism in the striatum. Overall, use of FL complexed with GA significantly modified its behavioral effects, which appears to be associated with the effects of FL and its complexes on the function of the monoaminergic systems involved in controlling behavior.

KEY WORDS: fluoxetine, glycyrrhizic acid, serotonin, dopamine, noradrenaline, 5-hydroxyindoleacetic acid, dihydroxyphenylacetic acid, anxiety, elevated cross maze.

Serotonin (5-hydroxytryptamine, 5-HT) is involved in the brain in controlling a multitude of physiological functions and mental processes, and alterations in serotonin levels or serotonin reception can lead to psychoemotional disorders [3, 5]. For example, decreases in 5-HT concentrations in the central nervous system are regarded as one of the causes of the development of depressive states and increases in anxiety. Agents prolonging the action of 5-HT on postsynaptic receptors therefore have anxiogenic and antidepressant actions. The most effective in this regard are 5-HT reuptake blockers [20]. Their mechanism of action consists of decreasing the activity and quantity of the serotonin transporter responsible for the reuptake into the cell of neurotransmitter released from nerve endings. The result of this inhibition is an increase in the serotonin concentration in the synaptic cleft and an increase in serotoninergic neurotransmission.

However, changes in psychoemotional state are regulated not only by the serotoninergic system, but also by the noradrenergic and dopaminergic systems of the brain. In addition, simultaneous changes in the functions of these neurochemical systems can in terms of behavioral consequences be significantly different from the effects of modulating the activity of either system alone. Changes in the activity of these monoaminergic systems in response to a variety of biologically relevant factors are known generally to occur simultaneously. This is a stimulus to the analysis of the actions of modified 5-HT reuptake inhibitors, which may have additional properties such as the ability to influence the functions of other neurotransmitters [20]. In this regard, molecular complexes protecting known agents from

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early metabolic degradation and facilitating their access to different parts of the brain have potential. Glycerrhizic acid has suitable complex-forming properties; interaction of this substance with a number of known antidepressants can increase their efficacy [1]. The present report describes comparative studies of the acute effects of the 5-HT reuptake inhibitor fluoxetine and two complexes of this agent with glycyrrhizic acid.

METHODS

Studies were performed on male Wistar rats. Animals were kept in the animal house of the Institute of Cytology and Genetics in natural illumination and with free access to water and feed. Study agents, i.e., fluoxetine and two complexes of fluoxetine with glycyrrhizic acid with different molecular ratios (fluoglycine-1, FLG-1, with GA:FL = 1:1; fluoglycine-4, FLG-4, with GA:FL = 4:1), were given as single doses of 25 mg/kg via an intragastric tube. Control animals received the corresponding volumes of physiological saline. Each experimental group consisted of 5–6 animals.

Animals were tested 1 h after dosage, tests lasting 5 min in an elevated cross maze. The number of entries into the open and closed arms were measured, along with the time spent in these arms, the time spent on the central platform, the number of downward glances from the open arms, the number of rearings onto the hindlimbs (vertical movement activity), and the number of defecations.

Immediately after tests, animals were decapitated and the brainstem, frontal cortex, hypothalamus, hippocampus, and striatum were rapidly extracted on ice; specimens were frozen in liquid nitrogen. Frozen materials were stored at –60°C until assays were performed.

High-performance liquid chromatography with electrochemical detection was used to measure the levels of 5-HT, noradrenaline (NA), and dopamine (DA) in the specimens, along with levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) and the DA metabolite dihydroxyphenylacetic acid (DHPA). Brain tissues were weighed and homogenized in 600 µl of 0.1 M KH_2PO_4. homogenates were then centrifuged for 15 min at 4°C at 15000 rpm and filtered; volumes of 20 µl were loaded onto a Waters C4 chromatography column (150 × 4.6 mm). The mobile phase contained 0.1 M KH_2PO_4, 10^{-4} M Na_2EDTA, 300 mg/liter octanesulfonic acid, and 4% methanol, and had a pH of 3.54; the working flow rate was 1 ml/min.

Data were analyzed by unifactorial dispersion analysis.

RESULTS

Behavior. Administration of FL led 1 h later to increases in anxiety in the animals as detected by the elevated cross maze test. This was indicated by a significant (compared with controls) decrease in the number of entries into the open arms (Fig. 1, *A*). Administration of FLG-1 and FLG-4 had no effect on the number of entries into the open arms, which was no different from the number in controls and was significantly greater than the number recorded after administration of FL.

The increase in anxiety in the rats after FL was also demonstrated by the significantly greater number of defecations (Fig. 1, *B*) than seen in animals given physiological saline or FLG-1. Male rats given FL also showed decreased investigative activity, indicated by a reduction in the number of downward glances from the open arms (Fig. 1, *C*). However, these differences were only significant in comparison with animals given FLG-1.

Apart from the increase in anxiety in male rats, FL also produced a decrease in total movement activity 1 h after administration (Fig. 1, *D*). The total number of entries into the open and closed arms was smaller in these animals than in those of other groups, though the difference was significant only in comparison with the FLG-1 group. It should be noted that these differences only applied to horizontal movement activity; vertical activity, i.e., the number of rearings on the hindlimbs, showed no significant differences (data not presented).

Monoamine and Metabolite Levels. Administration of FL and its two complexes with GA had no effect on 5-HT levels in the frontal cortex or hypothalamus at 1 h (Table 1). However, all agents produced a significant reduction in the level of the 5-HT metabolite 5-HIAA; FLG-4 also produced a decrease in 5-HIAA in the frontal cortex. Analysis of 5-HIAA levels in a further two brain areas, the brainstem and the hippocampus, revealed no changes in metabolite levels after administration of agents. Measurements of NA levels identified no effects for any of the three agents in the frontal cortex, brainstem, or hippocampus. However, FLG-1 and FLG-4 produced small increases in noradrenaline levels in the hypothalamus.

Concentrations of DA and its metabolite DHPA in the striatum were significantly increased after administration of FL. A significant increase in DHPA in the striatum was also seen after FLG-1. The DHPA:DA ratio, which reflects the level of DA catabolism, was no different after FL from controls, though there was a significant increase after FLG-1 compared with controls and animals of the other two experimental groups.

DISCUSSION

Increases in anxiety and suppression of overall movement activity in male rats 1 h after administration of FL is in accord with the acute effects of doses reported by other investigators. In these experiments, administration of FL at doses of 5.6–20 mg/kg also led to decreases in the numbers

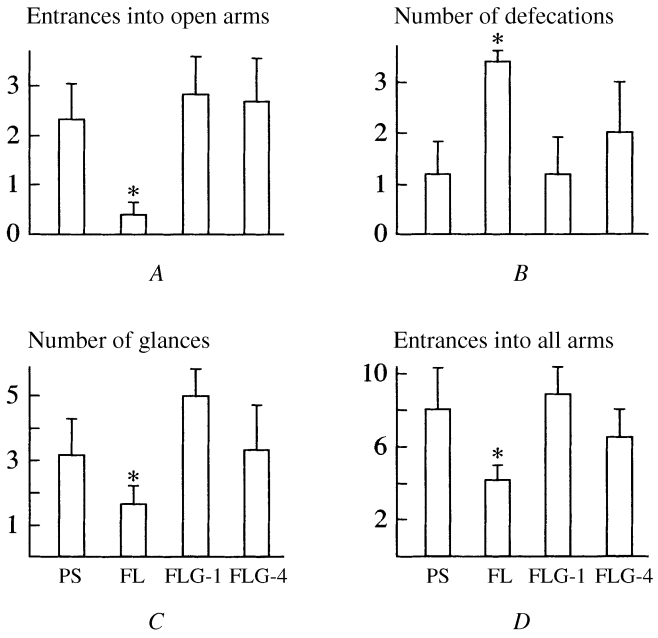

Fig. 1. Results of testing adult male rats in the elevated maze 1 h after administration of the serotonin reuptake inhibitor fluoxetine (FL) and its complexes with glycyrrhizic acid FLG-1 and FLG-4. *A*) Number of entrances into open arms, $*p < 0.05$ compared with all groups. *B*) Number of defecations, $*p < 0.05$ compared with controls (physiological saline) and FLG-1. *C*) Number of downward glances from the open arms, $*p < 0.05$ compared with FLG-1. *D*) Total number of entrances into all arms, $*p < 0.05$ compared with FLG-1. PS = physiological saline.

of entrances into the open arms, decreases in the time spent in these arms, decreases in the numbers of downward glances from the open arms, and decreases in the total numbers of entrances into the open and closed arms of an elevated cross maze [12, 17, 18]. The anxiolytic effect is seen only after 2–6 weeks of continuous use of this agent [20]. It should be noted that administration of complexes of FL with GA produced virtually no acute anxiogenic response in our experiments.

One reason for the increase in anxiety in animals after acute administration of FL may be the accumulation of neurotransmitter in synaptic clefts resulting from blockade of its reuptake, leading to activation of autoreceptors and, thus, a decrease in the discharge activity of serotoninergic neurons. The complete absence of the serotonin transporter in knockout mice is accompanied by increased anxiety in animals, seen in a variety of behavioral tests, including the elevated cross maze [8]. Increases in extracellular 5-HT levels have been seen in many parts of the brain, including the frontal cortex [7, 21] and hypothalamus [15] studied here, after acute administration of FL by different routes (i.p., s.c., via the esophagus) and over a wide range of doses (1–40 mg/kg). We found no changes in 5-HT levels in tissues 1 h after FL, though these changes, unlike those in extracellular neurotransmitter levels, were not seen 24 h after dosage. One point of evidence for a decrease in the reuptake of 5-HT into cells, the main site of its degradation, is the decrease in the hypothalamic levels of the 5-HT metabolite 5-HIAA after doses of FL. Decreases in 5-HIAA content have also been seen in cortex and hippocampus tissues 24 h after FL by other investigators [4]. Another explanation for the decrease in 5-HIAA levels may be direct suppression of the activity of the 5-HT-degrading enzyme monoamine oxidase (MAO). This pathway has been identified for FL: doses of 20 mg/kg produced a more-than-20% decrease in MAO A activity [14] and a 10–15% suppression of MAO B activity [13].

It should be noted that in rats given complexes of FL and GA, a decrease in 5-HIAA content was also seen in the hypothalamus after 1 h, though the level of anxiety assessed in the elevated cross maze was no different from that in controls. Differences in the acute behavioral effects of FL and its complexes with GA may be associated with the characteristics of the effects of these agents on the monoaminergic systems of the brain, which control the behavioral responses studied here. Thus, apart from changes in extracellular 5-HT levels, the effects of treatment with FL are known to include changes in NA and DA levels. Increases in extracellular catecholamine levels have been seen, for example, in the frontal cortex [7, 9] and hypothalamus [11, 15]. Complexes of FL with GA can, as has been demonstrated, protect this agent from early metabolic degradation [1] and,

TABLE 1. Levels ($M \pm m$, ng/g) of Monoamines and Their Metabolites in the Brains of Adult Male Rats 1 h after Administration of the 5-HT Reuptake Inhibitor Fluoxetine and Its Complexes with GA, FLG-1 and FLG-4

	Physiological saline (1)	FL (2)	FLG-1 (3)	FLG-4 (4)
Frontal cortex				
5-HT	363.7 ± 41.6 (6)	339.8 ± 25.0 (5)	312.0 ± 3.4 (5)	363.2 ± 34.8 (6)
5-HIAA	180.0 ± 7.5 (6)	164.4 ± 12.3 (5)	157.2 ± 9.4 (6)	144.8 ± 13.3 (5)[1]
NA	224.8 ± 20.7 (6)	205.2 ± 20.1 (5)	187.0 ± 14.3 (5)	232.2 ± 17.0 (6)
Hypothalamus				
5-HT	1594 ± 244 (5)	1150 ± 142 (6)	1277 ± 158 (6)	1192 ± 190 (5)
5-HIAA	561.0 ± 20.9 (5)	402.7 ± 34.8 (6)[1]	413.2 ± 47.5 (5)[1]	322.8 ± 34.6 (5)[1]
NA	1474 ± 180 (4)	1480 ± 112 (5)	1864 ± 63 (5)[2]	1937 ± 201 (6)
Hippocampus				
5-HIAA	234.2 ± 37.9 (5)	202.8 ± 9.7 (6)	227.2 ± 16.3 (6)	229.3 ± 10.0 (6)
NA	268.0 ± 17.0 (4)	269.8 ± 9.5 (6)	284.5 ± 21.5 (6)	316.5 ± 26.6 (6)
Stem				
5-HIAA	346.3 ± 16.9 (6)	367.7 ± 31.0 (6)	383.2 ± 11.4 (5)	366.2 ± 36.5 (6)
NA	415.0 ± 21.8 (6)	392.5 ± 23.5 (6)	344.7 ± 24.1 (6)	350.7 ± 34.2 (6)
Striatum				
DA	11949 ± 1320 (6)	15007 ± 1091 (6)[1]	12254 ± 523 (5)	13533 ± 835 (5)
DHPA	1585 ± 56 (5)	2101 ± 136 (5)[1, 4]	2088 ± 130 (5)[1, 4]	1696 ± 158 (5)
DHPA/DA	0.145 ± 0.016 (5)	0.150 ± 0.006 (5)	0.187 ± 0.006 (5)[1, 2, 4]	0.122 ± 0.015 (5)

Notes. Numbers in parentheses show numbers of animals. 1, 2, 4) Significant ($p < 0.05$) differences with the corresponding groups of animals.

judging from our data, can alter its access to other monoaminergic neurochemical systems. In fact, in our experiments, no changes in NA levels were ever seen in any of the brain areas studied 1 h after FL administration. At the same time, doses of FL-GA complexes were followed by increases in NA levels in the hypothalamus.

In addition, our observations showed that FL administration was followed by an increase in DA levels in the striatum. This could result from decreased release of neurotransmitter from nerve endings, which is in agreement with the decrease in extracellular DA levels in this part of the brain after doses of FL [10]. Support for the probable decrease in DA release into the synaptic cleft is also provided by data showing post-FL decreases in the electrical activity of dopaminergic neurons in the ventral tegmental area [16]. Brain dopamine may play an extremely important role in controlling both motor and psychoemotional responses [2]. It is entirely possible that the decrease in the extracellular neurotransmitter level could lead to a decrease in the motor activity of the animals in our experiments after FL. Unlike the situation with FL, administration of complex FLG-1 was, conversely, followed by a significant increase in DA catabolism in the striatum, and it is entirely possible that this is the reason for the increase in the movement activity of these animals as compared with animals given FL. Similarly, the increase in extracellular DA resulting from the absence of the dopamine transporter gene is accompanied by spontaneous hyperlocomotion in knockout mice [6, 19].

Overall, the use of FL complexed with GA significantly modifies its acute behavioral effects, which appears to be associated with the characteristics of the effects of FL alone and its complexes on the function of brain monoaminergic systems involved in controlling behavior.

CONCLUSIONS

The serotonin reuptake inhibitor fluoxetine increased anxiety in the elevated cross maze in rats 1 h after administration. Complexes of fluoxetine with glycyrrhizic acid had no significant effect on the animals' behavior in this test. Apart from the effects on the brain serotoninergic system, consisting of increases in 5-hydroxyindoleacetic acid levels, which were generally similar for FL alone and its complexes, complexes also had marked effects on the catecholaminergic systems of the brain, with increases in dopamine metabolism and noradrenaline levels in the hypothalamus. Overall, complexing of fluoxetine with glycyrrhizic acid significantly modifies its behavioral effects, which appears to be associated with the characteristics of the effects of FL alone and its complexes on the function of the monoaminergic systems controlling behavior.

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