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BRIEF COMMUNICATION

Anxiogenic β-Carboline FG 7142 Produces Activation of Noradrenergic Neurons in Specific Brain Regions of Rats

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IDA, Y., J. D. ELSWORTH AND R. H. ROTH. *Anxiogenic β-carboline FG 7142 produces activation of noradrenergic neurons in specific brain regions of rats.* PHARMACOL BIOCHEM BEHAV **39**(3) 791–793, 1991.—By measuring the levels of two major metabolites of rat brain noradrenaline (NA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3,4-dihydroxyphenylglycol (DHPG), we investigated the effects of anxiogenic β-carboline FG 7142, an inverse agonist of benzodiazepine (BZD) receptors, on brain noradrenergic activity of rats. Thirty min after treatment with FG 7142 (15 mg/kg, IP), levels of both MHPG and DHPG in the hypothalamus, amygdala and thalamus, but not in the hippocampus and cerebral cortex, significantly increased. These increases were significantly antagonized by pretreatment with BZD receptor antagonist Ro 15-1788 (15 mg/kg, IP). Sixty min after treatment with FG 7142 at the same dose, significant increases in both metabolite levels occurred in the hypothalamus, amygdala, thalamus and cerebral cortex, and increases in MHPG levels only were observed in the hippocampus. These increases were significantly blocked by pretreatment with α_2-adrenoceptor agonist clonidine (100 μg/kg, IP). The present findings suggest that FG 7142 can produce increases in brain noradrenergic activity in specific brain regions by interacting with BZD receptors, and may support the hypothesis that hyperactivity of brain noradrenergic systems may be one neural mechanism in provocation of aversive emotional changes (anxiety, fear or panic).

Anxiogenic β-carboline FG 7142 MHPG DHPG Benzodiazepine receptors Ro 15-1788 Clonidine

SEVERAL lines of human and primate studies have raised a hypothesis that hyperactivity of brain noradrenergic systems may be one neural mechanism in provocation of anxiety (1,12). This hypothesis may be supported by the previous data that various stressful stimuli cause hyperactivity of brain noradrenergic systems in rodents (6, 7, 13, 14), which could be attenuated by the anxiolytic benzodiazepine (BZD) receptor agonist, diazepam (6).

β-Carbolines such as FG 7142 and β-CCE, which are classified as inverse agonists of BZD receptors, have been reported to have anxiogenic actions in humans (3), primates (2), cats (10) and rats (11). It has been reported that a behavioral, physiological and biochemical syndrome of anxiety-related responses induced by β-CCE in primates was blocked by the α_2-adrenoceptor agonist, clonidine, as well as diazepam (2). This raises the possibility that the anxiogenic actions of β-carbolines might be linked to hyperactivity of brain noradrenergic systems, resulting in their anxiogenic actions. However, there have been few reports about the effect of β-carbolines on brain noradrenergic activity.

In the present study, by measuring two major metabolites of rat brain noradrenaline (NA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and 3,4-dihydroxyphenylglycol (DHPG), we investigated whether FG 7142 could elicit increases in brain noradrenergic activity of rats, and whether the effect of FG 7142 on brain noradrenergic activity could be reversed by the BZD receptor antagonist Ro 15-1788 or by clonidine.

METHOD

Subjects

Male Sprague-Dawley rats weighing 280–320 g were housed 4 per cage at constant room temperature (24 ± 1°C) and humidity (50 ± 10%) and were allowed free access to food and water. The animal colony was maintained on a 12-h alternating light-dark cycle with light on at 0700 h. All experiments were carried out between 1000 and 1400 h.

Drugs

FG 7142 (Ferrosan) and Ro 15-1788 (Hoffmann-La Roche) were prepared as microsuspensions with vehicle (one drop of

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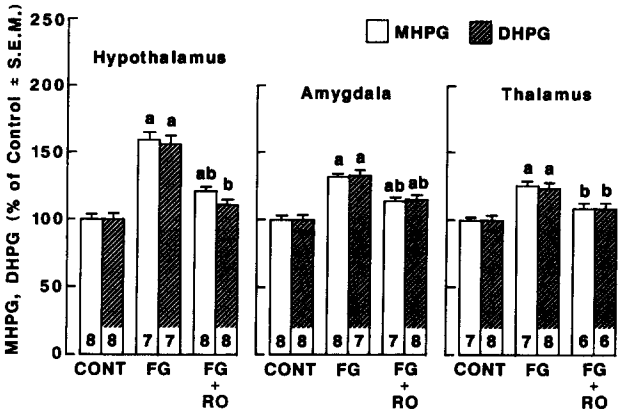

FIG. 1. Effects of FG 7142 (FG) and an FG 7142-Ro 15-1788 (FG + RO) combination on MHPG and DHPG levels in the hypothalamus, amygdala and thalamus of rats. FG 7142 (15 mg/kg) and Ro 15-1788 (15 mg/kg) were given IP 30 min and 45 min before decapitation, respectively. Each value indicates the mean ± S.E.M. of the number of rats shown in the column and is expressed as percentage of control (CONT). Respective control values (ng/g) of MHPG and DHPG are: hypothalamus, 113 ± 5 and 396 ± 18; amygdala, 145 ± 4 and 197 ± 7; and thalamus, 183 ± 2 and 243 ± 9. [a]Significantly different ($p<0.05$) from control group. [b]Significantly different ($p<0.05$) from FG 7142 group.

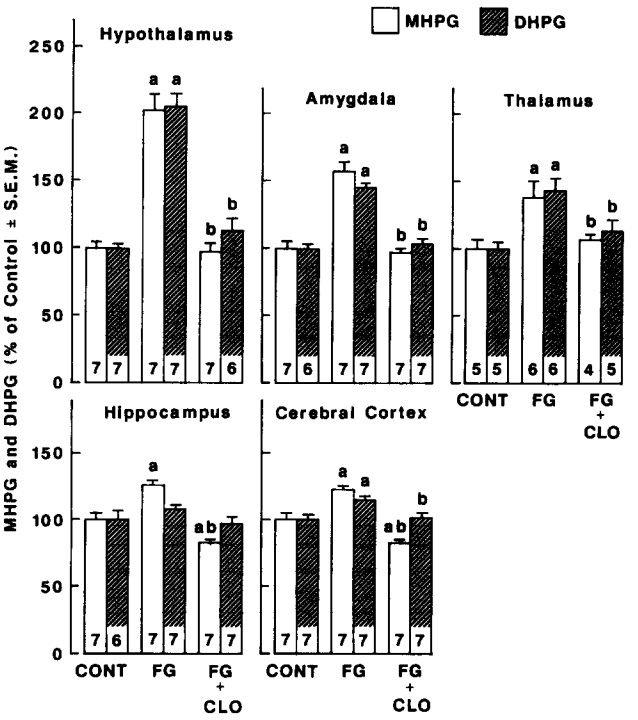

FIG. 2. Effects of FG 7142 (FG) and an FG 7142-clonidine (FG + CLO) combination on MHPG and DHPG levels in the hypothalamus, amygdala, thalamus, hippocampus and cerebral cortex of rats. FG 7142 (15 mg/kg) and clonidine (100 μg/kg) were given IP 60 min and 75 min before decapitation, respectively. Each value indicates the mean ± S.E.M. of the number of rats shown in the column and is expressed as percentage of control (CONT). Respective control values (ng/g) of MHPG and DHPG are: hypothalamus, 104 ± 4 and 229 ± 6; amygdala, 97 ± 4 and 131 ± 4; thalamus, 128 ± 8 and 234 ± 11; hippocampus, 79 ± 4 and 115 ± 7; and cerebral cortex, 70 ± 4 and 88 ± 3. [a]Significantly different ($p<0.05$) from control group. [b]Significantly different ($p<0.05$) from FG 7142 group.

Tween 80 per 3.5 ml of distilled water). Clonidine (Sigma) was dissolved in saline.

Experimental Procedure

Twenty-four rats were randomly assigned to one of three groups of 8 rats for each experiment. Two rooms were used for drug treatments and sacrifice, respectively. In the first experiment, Ro 15-1788 (15 mg/kg) or vehicle and FG 7142 (15 mg/kg) or vehicle were administered IP 45 min or 30 min before sacrifice, respectively. In the second experiment, clonidine (100 μg/kg) or saline and FG 7142 (15 mg/kg) or vehicle were administered IP 75 min and 60 min before sacrifice, respectively.

Tissue Preparation and Biochemical Assay

Immediately after each experimental procedure, rats were sacrificed by decapitation. The brains were rapidly removed and were dissected into five regions: the hypothalamus, amygdala, thalamus, hippocampus and cerebral cortex by the method of Gispen et al. (5). Total levels of MHPG and DHPG were measured by GC-MS (4).

Statistical Analysis

Statistical analyses for the data of MHPG and DHPG in each brain region involved one factorial analysis of variance (ANOVA) and post hoc Newman-Keul's tests for multiple comparisons. They were considered statistically significant when p value was equal or less than 0.05.

RESULTS

Figure 1 shows the mean (± S.E.M.) of MHPG and DHPG levels in the first experiment. ANOVA revealed a significant drug treatment effect on both MHPG and DHPG levels in the hypothalamus, $F(2,20)=38.5$, $p<0.01$; $F(2,20)=20.0$, $p<0.01$, amygdala, $F(2,20)=43.7$, $p<0.01$; $F(2,20)=20.0$ $p<0.01$ and thalamus, $F(2,17)=21.1$, $p<0.01$; $F(2,19)=2.1$, $p<0.01$, but did not in the hippocampus, $F(2,19)=2.7$; $F(2,19)=2.1$ and cerebral cortex, $F(2,20)=1.8$; $F(2,20)=2.5$. FG 7142 caused significant increases in levels of both MHPG and DHPG in the hypothalamus (159 and 156% of control, respectively), amygdala (132 and 133%) and thalamus (126 and 124%) 30 min after treatment. However, both metabolite levels were unchanged in the hippocampus and cerebral cortex (data not shown). These FG 7142-induced increases in the hypothalamus, amygdala and thalamus were significantly antagonized by pretreatment with Ro 15-1788.

Figure 2 shows the mean (± S.E.M.) of MHPG and DHPG levels in the second experiment. ANOVA revealed a significant drug treatment effect on both MHPG and DHPG in the hypothalamus, $F(2,17)=47.4$, $p<0.01$; $F(2,18)=52.3$, $p<0.01$, amygdala, $F(2,18)=39.8$, $p<0.01$; $F(2,17)=35.2$, $p<0.01$, thalamus, $F(2,12)=4.9$, $p<0.05$; $F(2,13)=7.2$, $p<0.05$ and cerebral cortex, $F(2,18)=32.5$, $p<0.01$; $F(2,18)=6.0$, $p<0.05$. In the hippocampus, ANOVA showed a significant drug treatment effect on MHPG, $F(2,18)=32.5$, $p<0.01$, but did not on DHPG, $F(2,18)=1.5$. FG 7142 produced significant increases in MHPG and DHPG levels in the hypothalamus (202 and 205%), amygdala (158 and 145%), thalamus (138 and 143%) and cerebral cortex (123 and 116%), and in MHPG levels in the hippocampus (126%). These FG 7142-induced increases in NA metabolite concentrations in five brain regions were significantly blocked by pretreatment with clonidine.

DISCUSSION

In the first experiment, FG 7142 treatment raised MHPG and DHPG levels in the hypothalamus, amygdala and thalamus, and these increases were antagonized by BZD receptor antagonist Ro 15-1788. These data suggest that, by interacting with BZD receptors, FG 7142 elicits increases in brain noradrenergic activity in the hypothalamus, amygdala and thalamus. The findings of increased metabolite levels in the second experiment also suggested that FG 7142-induced elevations in noradrenergic activity extended to the hippocampus and cerebral cortex 60 min after treatment, although the magnitude of the increases were lower than those shown in the hypothalamus, amygdala and thalamus. The relatively low DHPG/MHPG ratio in hippocampus may at least partly explain the lack of FG 7142 effect on DHPG levels in this region (4).

There are several treatments, e.g., electrical stimulation of the nucleus locus coeruleus (LC) (12), yohimbine administration (1), and stress exposure (6), that have anxiogenic effects in humans and animals by increasing brain noradrenergic activity. LC stimulation could elicit larger activation of noradrenergic neurons in the cerebral cortex than in the hypothalamus (8). This is interpreted by the neuroanatomical evidence that noradrenergic axon terminals in the cerebral cortex (as well as the hippocampus) are projected from the LC, and noradrenergic innervation of the hypothalamus (as well as the amygdala and thalamus) is shared by the LC and the nuclei referred to as the lateral tegmental group (9). Yohimbine has a pharmacological action to cause a similar degree of noradrenergic activation in the hypothalamus, hippocampus and cerebral cortex (15). It has been reported that an intensive stress, immobilization stress, remarkably increases noradrenergic activity in eight brain regions, and that noradrenergic neurons in different regions display characteristic responses to stressful stimuli, a greater effect in the hypothalamus, amygdala and thalamus than in the hippocampus and cerebral cortex (13). It has also been reported that a low degree of activation in noradrenergic neurons induced by mild stressful stimuli such as conditioned fear and a psychological stress model is observed only in the hypothalamus and amygdala (14). Since the pattern of response induced in noradrenergic neurons by stress rather than by nonstress treatments is similar to that after FG 7142 administration, it can be concluded that FG 7142 appears to have stress-like effects on the metabolic activity of central noradrenergic systems.

It has been reported that oral treatment of healthy volunteers with FG 7142 produced severe recurrent attacks of anxiety accompanied by increased blood pressure and pulse rate, and elevated plasma cortisol, prolactin and growth hormone levels (3). Another anxiogenic β-carboline, β-CCE, has been reported to elicit anxiety-related responses in monkeys such as behavioral agitation, increases in heart rate and blood pressure, and elevations in plasma catecholamines, cortisol and adrenocorticotropic hormone levels; all changes in these parameters could be blocked by either clonidine or diazepam (2). The present study biochemically demonstrated that pretreatment with clonidine could block FG 7142-induced hyperactivity of brain noradrenergic neurons. Taken together, the previous data and the present study suggest that β-carbolines such as FG 7142 and β-CCE may elicit anxiogenic actions, at least in part, by increasing the activity of brain noradrenergic neurons following interacting with BZD receptors.

In conclusion, the anxiogenic β-carboline, FG 7142, produces increases in rat brain noradrenergic activity, mainly in the hypothalamus, amygdala and thalamus, as a result of a specific interaction with BZD receptors. The present data support the hypothesis that brain noradrenergic systems, in addition to other neurotransmitter systems, may be related to provocation of aversive emotional changes (anxiety, fear or panic).

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