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COMPARATIVE STUDY OF THE EFFECT OF 3-CARBOXY-β-CARBOLINE METHYLAMIDE AND DIAZEPAM ON RAT BEHAVIOR

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KEY WORDS: aggression; fear; diazepam; FG 7142

Discovery of the molecular mechanisms of the onset and development of anxiety states is essential for the development of concrete methods of prevention and treatment of mental disorders. In this respect a very promising line of research is the study of the effect of anxiogenic compounds on behavioral responses of animals, which could provide a new approach to the stimulation of processes characteristic of mental pathology at the biochemical level.

It has recently been shown that several compounds, which are derivatives of β-carboline, possess anxiogenic properties [12, 15]. They are behavioral antagonists of the benzodiazepines [5, 13] and compete with the latter for highly specific binding sites with the protein components of the brain (benzodiazepine receptors) [5, 12, 13, 15]. The most active representative of this class of compounds is 3-carboxy-β-carboline methylamide (FG 7142), administration of which, as experiments on animals [7, 9] and tests on human volunteers [6] have shown, induces somatic, behavioral, and endocrine syndromes characteristic of a state of fear and anxiety.

The aim of this investigation was to compare the effects of administration of diazepam and FG 7142 on a group of behavioral responses of rats, reflecting a broad spectrum of emotional states.

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TABLE 1. Effect of Drugs on Behavior of Rats in Open Field Test (in % of control, taken as 100%)

Strain of rats	Drug	Dose, mg/kg	Number of animals	Ambulation	Rears	Time spent in center of arena	Grooming	Defecation, number of boli	Urination
Wistar	F67142	10	15 (15)	31**	22**	0**	33**	139*	200*
	Diazepam	1	12 (19)	138**	134**	250*	40*	105	110
August	The same	1	15 (15)	157**	372**	510**	37*	102	118

Legend. *$p \leqslant 0.05$, **$p \leqslant 0.01$ compared with control. Number of animals in control given in parentheses. Duration of exposure 4 min.

EXPERIMENTAL METHOD

The investigation was conducted in the fall and winter on adult male Wistar (weighing 250-300 g) and August (weighing 200-250 g) rats.

3-Carboxy-β-carboline methylamide was synthesized by the method developed previously [2]. FG 7142 was injected intraperitoneally in the form of an 0.4% solution in a mixture of physiological saline and dimethylformamide (2:1). Control animals were injected with a corresponding amount of a mixture of physiological saline and dimethylformamide (2:1). Diazepam was injected intraperitoneally in the form of a 0.015% solution in physiological saline. Control animals received an intraperitoneal injection of physiological saline.

A battery of tests was used to evaluate behavioral changes. The level of investigative activity and of emotional reactivity in a new situation was assessed by the open field method [10]. The rat was placed for 4 min in the center of a brightly lit arena 90 cm in diameter, divided into squares. The level of investigative activity and of emotional reactivity was determined by the ratio of the number of squares crossed (ambulation), the number of times the rat stood up on its hind limbs (rears), time spent in the middle of the arena, and the number of fecal boli deposited and the number of urinations.

Intraspecific aggression was studied on the following models. Aggressive-defensive behavior, induced by electrical stimulation of the limbs [3, 4], was provoked in a pair of rats placed beneath the hood on a grid floor. The voltage applied to the grid was increased by 1 V every 15 sec until the appearance of boxing postures and fighting. The thresholds of these responses and the frequency of flashes before and after stimulation were determined. Aggression evoked by social isolation [8] was produced by keeping the rats (residents) in a standard cage for 5 weeks, after which the intensity of their aggressive syndrome was determined. For this purpose, a partner (intruder) was put into a resident's cage for 10 min and a complex program of interaction between the two animals was set up. The latent period of the first attack by the resident, the number and duration of attacks and of acts of aggressive grooming were recorded: types of the intruder's defensive behavior were noted. Interspecific aggression was investigated on a model of stereotyped behavior of mouse-killing rats [11]. All observations were made at mid-day (11 a.m. to 3 p.m.). The effect of the compounds used on behavior began to be analyzed 10 min after injection.

EXPERIMENTAL RESULTS

Diazepam in small doses (0.25, 5, and 1 mg/kg) induced facilitation of orienting-investigative activity of the Wistar rats in the open field test; not only the parameters of horizontal ambulation and vertical activity were significantly increased, but so also was the frequency of visits to the center of the arena, evidence of lowering of the fear level. Meanwhile, with an increase in dose a tendency was found for autonomic responses (urination and defecation) to be intensified, possibly as a result of the nonspecific activating effect of diazepam on various physiological functions of the experimental rats (Table 1).

The experiments with August rats showed that the ratio between parameters of the level of investigative activity and emotional reactivity (control group) indicated a higher level of "anxiety" in these animals than in Wistar rats.

After injection of diazepam in a dose of 1 mg/kg the behavior of the August rats changed sharply toward strengthening of all components of investigative activity. Whereas the control group of rats virtually never spent time in the center of the arena, under the influence of diazepam, they spent longer in the center than Wistar rats. Facilitation of investigative

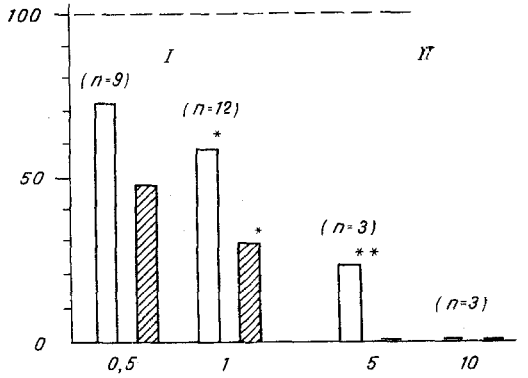

Fig. 1

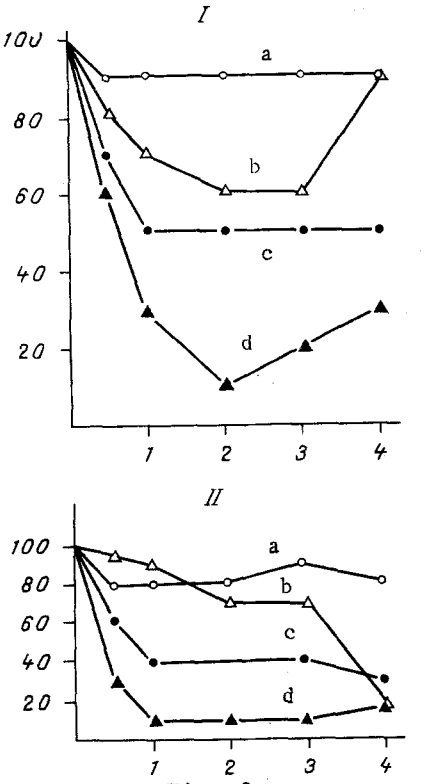

Fig. 2

Fig. 1. Effect of diazepam (I) and of FG 7142 (II) on intraspecific aggressive behavior of rats induced by prolonged social isolation. Abscissa, dose of drug (in mg/kg); ordinate, value of parameter (in % of control). Unshaded columns — number of acts of aggressive grooming; shaded columns — number of cycles of attacks. Horizontal line indicates control (n = 17). Duration of social contact 10 min. *p < 0.05; **p < 0.025 compared with control.

Fig. 2. Effect of various doses of FG 7142 on muricidal activity of mouse-killing rats (I) and on duration of attack on intruder (II). Abscissa, time (in h); ordinate, value of parameter (in % of initial). a) Control (n = 10); b) 0.5 mg/kg (n = 10); c) 1 mg/kg (n = 10); d) 10 mg/kg (n = 10).

activity accompanied by a minor degree of strengthening of the corresponding autonomic responses could indicate abolition by diazepam of the inhibitory effect of hyperemotionality (anxiety), genetically determined in August rats.

The opposite effect was observed after injection of FG 7142 in a dose of 10 mg/kg; all components of the orienting-investigative activity were inhibited whereas responses of urination and defecation were intensified. Under the influence of the β-carboline the rats spent absolutely no time in the center of the arena.

To discover the nature of the inhibitory effect of FG 7142 on components of orienting-investigative behavior, additional experiments were carried out. In a modified open field test (2 min with weak and 2 min with bright lighting) the effect of β-carboline on the response to presentation of a bright light and the ringing of a bell simultaneously was evaluated [1]. Facilitation of the start response was found, and was manifested not as starting, which was characteristic of animals of the control group (this response was found in seven of 10 rats), but as a sudden jump (in nine of 10 rats), followed by panic running. Thus activity provoked by additional stimuli revealed a high state of readiness of the animals for motor responses, and at the same time, showed significant facilitation of responses typical of anxiogenic behavior in response to provocation. Weakening of investigative activity induced by injection of FG 7142 was thus not the result of a disturbance of locomotion. In addition a significant ($p \leqslant 0.05$) increase in the latent period of departure of the rats from the start compartment for the illuminated area was found, further evidence of a lowered level of investigative activity, on the one hand, and of a raised level of fear, on the other hand.

TABLE 2. Effect of Drugs on Threshold of Aggression Provoked by Painful Electrical Stimulation

Drug	Dose, mg/kg	Threshold of aggression, V	Number of pairs
Control (physiological saline)	—	35—40	10
FG 7142	10	60—85	5
Diazepam	0,5	40—45	7
	1	40—55	8

FG 7142 inhibited all types of intraspecific aggressive interaction studied. Whereas diazepam in doses of 0.25 and 0.5 mg/kg, which did not disturb locomotion, preserved the basic patterns of aggressive behavior induced by prolonged social isolation, and in a dose of 1 mg/kg inhibited only the highest ranks of this type of aggression (sudden attacks with holding the intruder in a posture of submissiveness, chasing and biting), FG 7142 in a dose of 5 mg/kg inhibited all the main components of aggressive behavior, while preserving only social grooming in a reduced form. In a dose of 10 mg/kg FG 7142 also inhibited these elements of aggressive behavior (Fig. 1). FG 7142 almost doubled the threshold of aggression provoked by painful electrical stimulation. Although the activity of diazepam in this test system was the same in direction, it was much weaker (Table 2). FG 7142 also had a marked inhibitory action on interspecific aggression in rats. For instance, its injection caused a marked decrease in muricidal activity of mouse-killing rats. The effect was dose-dependent: maximal inhibition of muricidal activity was observed 2-3 h after injection. The duration of attack on a dead mouse presented to it also was reduced (Fig. 2).

Thus distinct antagonism was found in the effect of diazepam and of FG 7142 on the behavior of rats in the open field test, evidence that the action of the latter is anxiogenic in direction.

Meanwhile FG 7142 was found to have an inhibitory action on various types of intraspecific and interspecific aggression in rats. An unexpected discovery was that in models of intraspecific aggression diazepam and FG 7142 acted in the same direction, although in the open field test and with respect to certain other features FG 7142 is a behavioral antagonist of the benzodiazepines [6, 7, 9]. Since the biological activity of these compounds is considered to be mediated through the same molecular target, namely the benzodiazepine receptors of the brain, it can be postulated that there are at least two mechanisms of regulation of the aggressive behavior of animals, mediated through the GABA-ergic system. For instance, the lowering of the level of interspecific aggression observed under the influence of diazepam is evidently determined by its anxiolytic activity, whereas inhibition of aggression induced by injection of FG 7142 probably takes place because of a sharp rise of the level of fear. This suggestion is partly confirmed by data in the literature indicating that the ethyl ester of β-carboline-3-carboxylate, possessing anxiogenic activity, and diazepam inhibited aggression evoked in mice by social isolation; combined administration of these drugs moreover, led to preservation of aggressiveness at the control level [14]. Inhibition of stereotyped behavior of muricidal rats also was probably due to the anxiogenic action of FG 7142.

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EFFECT OF DELTA SLEEP PEPTIDE IN CORTICAL EPILEPTIC ACTIVITY IN RATS AND CATS

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KEY WORDS: epileptic activity; delta sleep-inducing peptide (DSIP); penicillin; strychnine; epileptic focus; epileptic complex

Experimental and clinical investigations have shown the therapeutic effects of delta sleep-inducing peptide (DSIP) in several pathological states: insomnia, the withdrawal syndrome, and stress [7, 8, 10]. The next step was to study the effects of DSIP on epileptic activity (EPA).

In the investigation described below the effect of DSIP was studied on foci of EPA and on their complexes, created in the cerebral cortex of rats and cats.

EXPERIMENTAL METHOD

Experiments were carried out on 47 male Wistar rats weighing 200-220 g and on 20 cats weighing 2.5-3.5 kg. The preparatory operation on the animals was carried out in accordance with the method described previously [5]. Under hexobarbital anesthesia (150 mg/kg, intraperitoneally) a burr-hole (2 × 4 mm) was drilled in the region of the sensomotor cortex of the rats, the dura was divided, and recording electrodes were secured in the sensomotor and visual cortex. To prevent the exposed surface of the cerebral cortex from drying it was moistened with physiological saline and the burr-hole was covered with waterproof film. Next day, to create foci of EPA, the film was removed and a piece of filter paper soaked in a solution of the sodium salt of benzylpenicillin in a concentration of 12,000 or 20,000 IU/ml was applied to the surface of the cortex. In the experiments on cats, under ether anesthesia tracheotomy was performed and a burr-hole drilled in the skull to provide access to the frontal regions of the cortex. Tubocurarine (0.12-0.28 mg/kg) was injected into the animals 2.5-3 h after the administration of ether ceased and the cats were artificially ventilated. Single foci of EPA were created in different parts of the sigmoid, coronary, and orbital gyri by application of a piece of filter paper (2 × 2 mm) soaked in a 0.1% solution of strychnine nitrate. The determinant focus of the epileptic complexes was formed by application of a 3% solution of strychnine to the middle sigmoid gyrus. Electrical activity of the brain was recorded by a monopolar technique and the reference electrode was fixed in the nasal bones. Potentials were recorded on a polygraph (Nihon Kohden, Japan) and 4-ÉÉG-3 electroencephalograph. In experiments on unrestrained rats the ECoG of two animals, one of which was given DSIP intraperitoneally, the other physiological saline, was recorded simultaneously. In experiments on cats, control determinations of the effect of intravenous injection of physiological saline (1 ml) on activity of the foci and of the multifocal complexes were undertaken initially. EPS in the foci was then restored by repeated applications of solutions of the convulsants and DSIP was injected intravenously. The DSIP was diluted in physiological saline immediately before injection and was used in a dose of 100 µg/kg. The experimental results were subjected to statistical analysis by variance and parametric methods [11].

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