ROLE OF GABA-ERGIC BRAIN SYSTEMS IN THE ACTIVATING EFFECT OF DIAZEPAM

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Experiments on rats showed that bicuculline (2 mg/kg), which selectively blocks GABA-receptors, does not reduce the activating effect of diazepam (1 mg/kg) on the self-stimulation test. The GABA-mimetic muscimol (0.5 and 1 mg/kg) did not affect the frequency of self stimulation, but in a dose of 2 mg/kg, causing disturbance of behavior, it sharply reduced the frequency (by 93.3%). During combined administration of diazepam (1 mg/kg) and muscimol (0.5 mg/kg) no potentiation of the diazepam effect was observed. It is concluded that facilitation of the self stimulation test by diazepam is not connected with changes in activity of GABA-ergic processes.

KEY WORDS: tranquilizers; GABA-ergic mechanisms; self-stimulation.

Tranquilizers of the benzodiazepine series, within a definite dose range, have an activating action: They increase spontaneous motor activity of mice and rats in an "open field," facilitate operant behavior, increase the number of "punishable" responses, and activate the response to self-stimulation [1, 10, 12]. Since the activating effect can be regarded as the equivalent or the result of the tranquilizing action, the neurochemical mechanism of this effect is an interesting question. It has been shown [3] that bicuculline, which blocks GABA-receptors, reduces the disinhibiting action of diazepam in a conflict situation. The tranquilizing effect of the benzodiazepines could thus be linked with activation of GABA-ergic processes [2, 3]. Meanwhile elevation of the brain GABA level is not accompanied by a diminution of anxiety in a situation of zoosocial interaction [6]. GABA has been shown to be an inhibitory mediator in the substantia nigra [13], one of the regions which participates in the organization of projections of the positive reinforcement system [5]. Meanwhile the question of the role of GABA in the "award" system has not been studied.

EXPERIMENTAL METHOD

Experiments were carried out on 15 noninbred albino rats with monopolar electrodes implanted into the region of the lateral hypothalamus and medial forebrain bundle (the coordinates taken from [8]). Pedal self-stimulation activity was evaluated in a Skinner's box with constant conditions of reinforcement: a volley (0.25 sec) of square pulses (1 msec) of negative polarity with a frequency of 100 pulses/sec. The strength of current used was such that the number of presses on the pedal was 400-700 in the course of a 10-min self-stimulation session. The spontaneous motor activity of the rats was evaluated by the number of squares of the "open field" crossed in the course of 2 min. An ampul solution of diazepam (Seduxen, from Hungary) was used. Muscimol was dissolved in distilled water and bicuculline in 1N HCl solution (with subsequent titration with 1N NaOH). Diazepam and muscimol were injected 30 min and bicuculline 5 min before the beginning of experiments; when bicuculline was given in combination with diazepam, it was injected 25 min after the diazepam. The effects of the drugs were calculated as percentages relative to the control (results obtained on the same group of animals but receiving physiological saline), taken as 100%. The significance of differences was determined by Student's t-test (the bicuculline and muscimol were generously provided by Dr. W. Haefely, Basel, Switzerland).

EXPERIMENTAL RESULTS

The experimental results are given in Tables 1 and 2. Diazepam (1 mg/kg) increased the frequency of brain self-stimulation by 23.4% compared with the control. Bicuculline (2 and 4 mg/kg) caused no significant

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TABLE 1. Effect of Diazepam, Bicuculline, Muscimol, and Their Combinations of the Self-Stimulation Response (M ± m)

Drug	Dose, mg/kg	Number of experiments	Number of presses on pedal		Change in number of presses on pedal, % of control
			control	after administration of drugs	
Diazepam	1	8	512,1±29,6	632,5±30,6	+23,5±5,9*
Bicuculline	2	5	525,2±47,4	536,6±32,8	+2,2±6,2
"	4	7	533,3±39,3	559,7±30,8	+4,9±5,8
Diazepam + bicuculline	1+2	8	535,7±56,4	680,4±61,6	+27,0±11,5*
Muscimol	0,5	5	499,6±30,1	481,4±71,8	−3,6±14,4
"	1	9	494,7±29,8	460,6±52,6	−6,9±10,6
"	2	8	515,8±36,9	34,4±26,0	−93,3±5,0*
Diazepam + muscimol	1+0,5	8	672,0±34,2	879,6±70,7	+30,9±10,5*

* $P < 0.05$.

TABLE 2. Effect of Bicuculline and Muscimol on Spontaneous Motor Activity of Rats in an "Open Field" (M ± m)

Drug	Dose, mg/kg	Number of experiments	Motor activity (number of squares crossed in 2 min)	
			control	after administration of drugs
Bicuculline	2	9	12,0±2,2	13,8±2,4
"	4	7	12,6±2,7	15,0±3,0
Muscimol	0,5	5	27,8±7,1	26,4±3,8
"	1	9	29,8±6,0	26,9±4,3
"	2	10	19,7±2,2	21,9±2,7

change in the frequency of self-stimulation or in the motor activity of the rats. No changes in general behavior were observed in most of the animals. In two of nine animals, after injection of bicuculline in a dose of 4 mg/kg, clonico-tonic convulsions were observed and lasted 4-6 min. Subsequently these animals were withdrawn from the experiment. With combined administration of diazepam (1 mg/kg) and bicuculline (2 mg/kg) the activating effect of diazepam on the self-stimulation response showed no significant change.

Muscimol (0.5 and 1 mg/kg) caused no appreciable change in the general behavior of the animals and did not affect their motor activity in an "open field." The frequency of self-stimulation showed no significant change under these circumstances, although a tendency was observed for the number of presses on the pedal to diminish. Simultaneous injection of muscimol (0.5 mg/kg) and diazepam (1 mg/kg) did not lead to potentiation of the activating action of diazepam on self-stimulation. In a dose of 2 mg/kg, muscimol caused a disturbance of the adequate response of the animals to external stimulation and the appearance of abnormal forms of behavior. In some rats stereotyped movements in a circle were observed in the "open field." Signs of catalepsy and marked muscle relaxation were observed in all the animals. The frequency of self stimulation fell sharply in this case (by 93.3%).

It has been shown [4, 9] that diazepam binds specifically with high affinity with the receptor protein of the synaptosomal membranes of the brain. No neuromediator yet known or suggested, including GABA, has affinity for the benzodiazepine receptor [11]. However, an important factor in the action of benzodiazepines is the specific interaction of these compounds with GABA-ergic transmission [2, 7].

The results of the present investigation indicate that changes in activity of central GABA-ergic mechanisms are not essential for realization of the activating effect of diazepam. Unlike diazepam, the GABA-mimetic muscimol had no facilitating action on the self-stimulation response, it did not activate motor activity, and did not potentiate the activating effect of the tranquilizer on the self-stimulation response. Bicuculline, a specific blocker of GABA-receptors, did not significantly change the activating effect of diazepam on the self-stimulation response. Hence, although GABA-ergic systems are involved in the mechanism of some of the pharmacological effects of the benzodiazepine tranquilizers (relieving anxiety, anticonvulsive) [2], the activating manifestations (increased motor activity in an "open field," activation of brain self-stimulation) are unchanged by modulation (blocking or activation) of the GABA-ergic system of bicuculline and muscimol. Characteristically, neither bicuculline nor muscimol replaces diazepam for binding with the hypothetical "benzodiazepine receptors" [11].

The fact that the GABA-mimetic muscimol and the GABA receptor blocker bicuculline, in doses not causing any sharp changes in behavior, had no effect on the frequency of self-stimulation indicates that GABA-ergic systems do not play a decisive role in the mechanism of the "award" effect during central stimulation.

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