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ANTIWITHDRAWAL ACTION OF FENIBUT AND BACLOFEN ON EXPERIMENTAL WITHDRAWAL INDUCED BY CGS 8216, A BENZODIAZEPINE RECEPTOR ANTAGONIST, IN RATS RECEIVING DIAZEPAM

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KEY WORDS: GABA receptor agonists; CGS 8216; diazepam; withdrawal.

Fenibut (FB, a phenyl derivative of GABA) was originally introduced into clinical practice as a tranquilizer and sedative [3, 7]. However, animal experiments have shown that FB is virtually without properties characteristic of the benzodiazepine tranquilizers [8]. In clinical practice FB, unlike diazepam, had little effect on fear, phobias, and anxiety, but was effective against symptoms of asthenic neurosis [4]. This particular feature of FB can most probably be explained by the presence of a nootropic component of its action. The question accordingly arose of whether it is justifiable to regard FB as a tranquilizer [2, 4]. The absence of activity of FB in the conflict situation test or in the antimetrazol effect is most probably evidence that FB differs from the tranquilizers of the benzodiazepine series, but it does not disprove that it has tranquilizing properties.

This paper gives data to show that FB and its chlorophenyl analog baclofen are highly effective in abolishing withdrawal symptoms induced by CGS 8216, a benzodiazepine receptor antagonist, in rats chronically receiving diazepam.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 220-250 g. For 20-30 days the animals were given diazepam by intraperitoneal injection in a dose of 10 mg/kg, after which it was withdrawn and the benzodiazepine receptor antagonist CGS 8216 (generously provided by the firm of Ciba Geigy, Switzerland) was given after 24-72 h in a dose of 2.5 mg/kg which induced well-defined features of withdrawal for 1-1.5 h after its administration in 90-95% of the animals. The behavioral signs characterizing this syndrome were recorded for 1 h. The test substances – diazepam (5-20 mg/kg), FB (10-100 mg/kg), baclofen (1.25-10 mg/kg), and TGIP, an agonist of $GABA_A$-receptors (5-20 mg/kg, from Lundbeck Denmark) were injected 5 min before the CGS 8216.

The experimental results were subjected to statistical analysis by the Mann–Whitney U-test and by methods of variance analysis.

EXPERIMENTAL RESULTS

Injection of CGS 8216 caused a behavioral syndrome in 95% of the animals chronically receiving diazepam, which included shaking of the head, attacks of myoclonus of the forelimbs, turning of the body, intensive chewing movements and sniffing, increased emotional reactivity to external tactile or acoustic stimuli, and tension of the tail muscles (Table 1). The high in-

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TABLE 1. Effect of Test Substances on Signs of Withdrawal Induced by Intraperitoneal Injection of CGS 8216 (2.5 mg/kg) into Rats Receiving Diazepam (10 mg/kg daily, intraperitoneally) for 20 Days

Substance	Dose, mg/kg	Number of animals	Sign of withdrawal	
			number of head shakings	number of attacks of myoclonus of the forelimbs
Control	Physiological saline	10	6,1±2,2	8,0±1,3
Diazepam	5	8	4,4±1,2	8,3±1,9
	10	8	1,4±0,7*	8,2±1,9
	20	8	1,6±0,4**	4,2±0,4*
Control	Physiological saline	12	6,9±1,0	11,1±1,5
FB	10	8	4,5±1,7	3,7±1,1*
	50	8	0,8±0,5*	0,3±0,1**
	100	8	0**	1,0±0,6**
Control	Physiological saline	12	28,8±7,7	13,8±3,3
Baclofen	1,25	6	27,6±6,7	4,5±2,8*
	2,5	8	5,3±2,3**	4,0±1,4**
	5	6	6,7±2,7**	1,0±0,8**
Control	Physiological saline	19	6,0±0,9	6,0±0,6
TGIP	10	6	10,6±1,5*	11,2±2,7*
	20	19	12,1±2,4*	9,0±1,7

Legend. *p < 0.05, **p < 0.01 (Mann–Whitney U-test).

tensity of exhibition of these features in the animals chronically receiving diazepam suggests that they reflect withdrawal after administration of diazepam. A detailed account of the withdrawal syndrome induced by CGS 8216 will be given in a separate publication.

Starting with a dose of 10 mg/kg diazepam abolished the signs of withdrawal. Like diazepam, FB and baclofen abolished all the features of withdrawal, and FB selectively suppressed the myoclonus of the forelimbs in a dose of 10 mg/kg, which had virtually no effect on the animals' behavior. Conversely, TGIP not only did not inhibit the withdrawal syndrome, but significantly potentiated it.

Thus FB and its analog baclofen, even in small doses, abolished the withdrawal syndrome induced by CGS 8216 in rats chronically receiving diazepam. They were actually more effective on this model than diazepam itself, which abolished the syndrome only in doses causing a marked sedative and muscle-relaxing effect. Both experimentally and clincally, other workers also have demonstrated the high effectiveness of FB in abolishing the alcohol withdrawal syndrome [1, 5].

In our opinion, the ability of FB and baclofen to abolish benzodiazepine withdrawal may be important evidence that these substances have a tranquilizing action, although it differs from that of the benzodiazepines. Consequently, the original suggestion [3, 7, 8] that FB has tranquilizing activity is reinforced by data obtained in the present investigation on a model of diazepam withdrawal. The mechanism of the antiwithdrawal action of FB and baclofen is not clear. There is as yet no clear evidence that it is realized through the GABA-benzodiazepine-barbiturate complex, for activation of $GABA_A$-receptors by TGIP, with which the benzodiazepine receptor is closely bound, actually intensifies the features of withdrawal. In addition, $GABA_B$ receptors, on which, in the modern view, baclofen [9] and FB [6] act, are not directly connected with benzodiazepine receptors. It can be tentatively suggested that the action of FB and baclofen is realized through other neurotransmitter systems (serotoninergic, endopioid), which may perhaps be involved in the development of benzodiazepine withdrawal.

It can be postulated that the withdrawal syndrome described above provides an important model with which to study the tranquilizing and antiwithdrawal action of drugs.

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INDEPENDENT BENZODIAZEPINE AND BETA-CARBOLINE BINDING SITES IN THE BRAIN OF AGGRESSIVE AND TIMID-DEFENSIVE MICE

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The view has now been formulated that specific ligands of benzodiazepine binding sites constitute a continuum: from complete agonists through antagonists to complete inverting agonists, the beta-carbolines [4]. However, binding sites with increased affinity for beta-carbolines only may perhaps exist.

The aim of this investigation was to study the distribution of specific binding sites of labeled benzodiazepine and beta-carboline derivatives in parts of the brain of intact aggressive and timid-defensive mice, and also of animals subjected to subchronic administration of diazepam.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male albino mice weighing 28-30 g — aggressive (AG) and timid-defensive (TD) animals, kept in isolation for 6 weeks. The TD mice were kept in the same cage as an AG partner, which was changed daily, and was subjected to painful electrical stimulation of threshold strength (5 min).

Intact animals and also mice receiving subchronic treatment with diazepam (14 days, 5 mg/kg intraperitoneally), were decapitated 24 h after the last injection, the brain was removed and the cerebral hemispheres, diencephalon, and brain stem were separated in the cold [8] and frozen in liquid nitrogen. The tissue was then thawed and homogenized in isolation medium (0.32 M sucrose, 0.05 M phosphate buffer, pH 7.4, at 25°C) in a glass homogenizer with Teflon pestle and for 10 sec on a RT-2 mechanical tissue microblender. The homogenate (10%) was centrifuged for 10 min at 1000g and the supernatant for 20 min at 12,000g. The residue of the fraction of unpurified synaptosomes thus obtained was washed 5 times in 0.05 M phosphate buffer (pH 7.4), poured into polyethylene flasks and kept at −20°C for not more than 2 weeks. The incubation mixture for binding of ^{3}H-flunitrazepam (^{3}H-flu) and ^{3}H-beta-carboline-3-carboxylate ethyl ester (^{3}H-BCCE; both substances were obtained from Amersham Inrernational, England) contained ^{3}H-flu in concentrations of 0.5, 1.1, 1.8, 2.4, 3.1, 3.7, 4.4, and 5 nM or ^{3}H-BCCE in concentrations of 0.6, 1.4, 2.1, 2.9, 3.7, 4.4, 5.2, and 6 nM, nonradioactive diazepam (10^{-7} M), phosphate buffer, and 0.4 ml of homogenate of synaptic membranes. Incubation continued for 60 min at 0°C when the reaction was stopped by addition of 4 ml of cold buffer to each sample, followed by filtration of the material in vacuo through glass fiber

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