EFFECT OF A TEN-DAY COURSE OF FENIBUT AND DIAZEPAM ON GABA AND BENZODIAZEPINE RECEPTORS IN MOUSE BRAIN

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The clinical spectrum of action of fenibut (β-phenyl-GABA), which is used in clinical practice as a tranquilizer [2], differs in many respects from that of tranquilizers of the benzodiazepine series [3]. The writers showed previously that bicuculline-insensitive receptors take part in the mechanism of action of fenibut, whereas bicuculline-sensitive GABA receptors take part in the mechanism of action of diazepam [1]. Evidence has now been obtained to suggest that the tranquilizing effect of the benzodiazepines is closely linked with their effect on benzodiazepine receptors [7]. However, the role of benzodiazepine receptors in the mechanism of action of fenibut still remains unexplained.

Since fenibut and diazepam are usually used repeatedly in clinical practice over a long period of time, the effect of a 10-day course of fenibut and diazepam on bicuculline-sensitive and bicuculline-insensitive GABA receptors ($GABA_A$ and $GABA_B$ receptors respectively [5]) and also on benzodiazepine receptors was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male mice weighing 22-28 g. Fenibut (100 mg/kg) and diazepam (5 mg/kg) were injected intraperitoneally twice a day for 10 days. The animals were decapitated 24 and 48 h after stopping administration of the drugs. The brain was removed in the cold and the forebrain detached for analysis. To prepare a suspension of membranes the pooled forebrains of the mice were homogenized in 10 volumes of 0.32 M sucrose by means of a glass-Teflon homogenizer (500 rpm). The suspension thus obtained was centrifuged at 1000g for 10 min. The supernatant was made up to the initial volume with 0.32 M sucrose and centrifuged at 20,000g for 20 min. The residue was then rehomogenized in Tris-HCl buffer (pH 7.3) and centrifuged at 50,000g for 20 min. The membranes (fraction P_2) were washed by rehomogenization in a blender (8000 rpm) and centrifuged at 50,000g twice for the experiments to study binding with benzodiazepine receptors and a further seven times for experiments to study binding with GABA receptors. After the last centrifugation the residue was resuspended in Tris-HCl and protein was determined [8]. Binding of [^{3}H]-GABA was determined in medium with a volume of 1 ml containing 0.4-0.6 mg/ml protein and 8 nM [^{3}H]-GABA (50 Ci/mmole, from Amersham Corporation, England). Binding with bicuculline-insensitive GABA receptors was determined in the presence of 2.5 mM $CaCl_2$ and 50 μM (+) bicuculline at 20°C for 10 min, whereas binding with bicuculline-sensitive GABA receptors was determined without the addition of the ions at 0°C for 10 min. Binding of [^{3}H]flunitrazepam was determined in medium in a volume of 1 ml containing 1 nM [^{3}H]flunitrazepam (80 Ci/mmole, from Amersham Corporation) and 0.20-0.25 mg/ml protein for 60 min at 0°C. Specific binding of the label with GABA or benzodiazepine receptors was determined as the difference between binding of the label in the presence and absence of 100 μM GABA or 100 nM diazepam in the reaction mixture respectively. The reaction was stopped by addition of 4 ml of cold Tris-HCl buffer and rapid filtration through glass fiber filters of the CF/B type (Whatman, England). The filters were washed three times, with 4 ml of the same buffer each time, and transferred to flasks with scintillator. Dioxan scintillator was used, with an average efficiency of 30%. Radioactivity was measured on an UltroBeta 1210 counter (from LKB, Sweden).

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TABLE 1. Effect of a 10-Day Course of Fenibut (100 mg/kg twice a day) and Diazepam (5 mg/kg, twice a day) on $GABA_A$, $GABA_B$, and Benzodiazepine Receptors in Mouse Forebrain (determinations carried out 24 and 48 h after discontinuation of these drugs)

Substance	Time of determination, h	Binding, fmoles/mg protein		
		$GABA_A$ receptors	$GABA_B$ receptors	benzodiazepine receptors
Control (physiological saline)	—	284±21	167±25	640±42
Fenibut	24	388±31*	143±13	942±64†
	48	274±32	96±12*	1209±108†
Diazepam	24	440±38†	244±19*	408±36†
	48	269±29	183±28	1037±94†

Legend. *$P < 0.05$; †$P < 0.01$. Each experiment was carried out separately with three groups of animals (six animals in each group). Mean values and standard errors of means are shown.

EXPERIMENTAL RESULTS

A 10-day course of fenibut lowered the specific binding of [^{3}H]-GABA with bicuculline-insensitive GABA ($GABA_B$) receptors. Although fenibut did not affect bicuculline-sensitive ($GABA_A$) receptors *in vivo* [6], specific binding of [^{3}H]-GABA with $GABA_A$ receptors was increased 24 h after stopping the fenibut. Administration of fenibut also increased binding of [^{3}H]flunitrazepam with mouse brain cell membranes (Table 1).

A reduction in the number of specific binding sites for benzodiazepines has recently been found after administration of clonazepam and chlordiazepam for several days [4]. These findings agree with the results of the present experiments, according to which the specific binding of [^{3}H]flunitrazepam was reduced 24 h after stopping the course of diazepam injections. However, 48 h after stopping diazepam an increase was observed in specific binding of [^{3}H]flunitrazepam. It must be pointed out that 24 h after stopping the 10-day course, diazepam inhibited binding of [^{3}H]-GABA with $GABA_B$ receptors. Thus not only $GABA_A$ receptors, but also $GABA_B$ receptors, the existence of which has been conclusively proved [5], are evidently closely linked with benzodiazepine receptors. Prolonged administration of benzodiazepines is known to lead to increased binding of [^{3}H]muscimol with $GABA_A$ receptors [9]. In the present experiments 24 h after stopping diazepam, an increase in binding with $GABA_A$ receptors also was observed. It may be that enlargement of binding sites of $GABA_A$ receptors after prolonged administration of diazepam reflects the direct participation of GABA receptors in the mechanism of action of benzodiazepines.

It can be postulated on the basis of these data that after a 10 day course of fenibut marked hypersensitivity of benzodiazepine receptors develops. To some degree this can explain the mechanism of action of fenibut as a unique kind of tranquilizer, when used in clinical practice for this purpose. At the same time, prolonged administration of fenibut may be useful for patients whc have developed resistance to benzodiazepines, i.e., a course of fenibut may restore the effectiveness of the benzodiazepine tranquilizers by inducing hypersensitivity of benzodiazepine receptors.

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ACTION OF NALOXONE IN TRAUMATIC SHOCK

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According to recent reports naloxone, a specific antagonist of opiates and opioids, is effective in animals against various types of shock: hemorrhagic, endotoxic, spinal, and painful electric shock [2, 6-8]. It has accordingly been postulated that endogenous opioid peptides (enkephalins, endorphins, etc.) play a part in the genesis of these shock processes. However, the most typical form of shock and that most frequently found in life is traumatic shock [1]. No data have yet been published on the action of naloxone in traumatic shock.

It was accordingly decided to study the effect of naloxone on the course of traumatic shock under experimental conditions.

TABLE 1. Effect of Naloxone on BP, HR, RR, and TV in Torpid Phase of Traumatic Shock (M ± m)

Experimental conditions	BP, mm Hg	HR, beats/min	RR, cycles/min	TV, % in 1 min
Control	133±3 / 111±1	267,1±8,5	66,2±4,7	100
Immediately after trauma	73±1* / 58±2	283,8±15,4	88,3±5,1*	92±12
Before injection of naloxone	73±2 / 58±2	262,1±8,2	84,3±5,3	94±12
Naloxone	77±4 / 61±4	258,7±7,4	100,1±7,7	204±33*

Legend. TV before trauma taken as 100. Number of experiments 10.
*$P < 0.01$ compared with control.

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