SIMILAR REGULATION OF CENTRAL AND PERIPHERAL BENZODIAZEPINE BINDING SITES BY GABA RECEPTOR AGONISTS IN RATS

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Specific binding sites of benzodiazepines (BD) can be divided into binding sits of central and peripheral types. Binding sites of the central type are specific and are not found outside the brain. Peripheral BD binding sites are found in many organs: kidneys, lungs, heart, liver, etc., and also on platelets and monocytes, but they differ sharply from central BD binding sites in a number of pharmacologic and biochemical properties. The affinity of peripheral BD binding sites for clonazepam is about 10,000 times lower, whereas their affinity for Ro 5-4864 is 10,000 times higher than that of central BD binding sites [1].

According to the results of experiments in vitro, peripheral BD binding sites, unlike central, are not linked with $GABA_A$-receptors [5]. Our previous experiments showed that injection of $GABA_A$-receptor agonists (muscimol) and of $GABA_B$-receptor agonists (fenibut) significantly increases binding of ^{3}H-flunitrazepam (^{3}H-FNZ) with mouse kidney membranes in experiments in vivo, i.e., under conditions when peripheral binding sites were labeled in vivo by a radioligand [6]. However, the technique of binding ^{3}H-FNZ in vivo which we used did not permit specific binding sites to be characterized more precisely: it was impossible to determine the dissociation constant (K_d) and the maximal number of binding sites (B_{max}) or to avoid possible pharmacologic interaction between the test substances and the radioligand.

For the reasons given above it was decided to study the effect of injection of muscimol and stereoisomers of baclofen in vivo and in vitro on binding of ^{3}H-FNZ with central and peripheral benzodiazepine binding sites in rats.

EXPERIMENTAL METHOD

Experiments were carried out from October through January on Wistar albino rats weighing 200-250 g, kept on a schedule of 12 h daylight (8 a.m. to 8 p.m.) and 12 h darkness (from 8 p.m. to 8 a.m.), with food and water ad lib. Muscimol (Fluka, Switzerland) was injected intraperitoneally 65 min, and (−) and (+) baclofen (Ciba Geigy, Switzerland) 50 min before the the experiment. The animals were decapitated in the cold and the cerebral cortex and kidneys removed. These tissues were homogenized on a Potter S glass-Teflon homogenizer in 10 volumes of cold 50 mM Tris-HCl buffer, pH 7.4. Before the experiment the homogenates were diluted with buffer to a tissue protein concentration of 2-3 mg/ml. Protein was determined by Lowry's method [3]. Binding of ^{3}H-FNZ (79 Ci/mmole, Amersham International, England) was determined at 0°C over a period of 60 min. After incubation the samples were filtered through GF/B filters (Whatman, England), which were then washed 3 times with 3 ml of buffer. Radioactivity of the filters was determined in modified Bray's scintillator on an LS-6800 counter (Beckman, USA). For the experiments to study binding with cerebral cortical membranes ^{3}H-FNZ was used in concentrations of 0.125-8 nM, and for those with kidney membranes, 0.5-38 nM; specific binding was determined after addition of 1 μM of diazepam or Ro 5-4864 (Hoffman La Roche, Switzerland) respectively.

EXPERIMENTAL RESULTS

The comparative study of ^{3}H-FNZ binding with rat cerebral cortical and kidney homogenates revealed specific binding sites for this ligand in both these structures. The number

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TABLE 1. Effect of Muscimol and (−) and (+) Baclofen on ^{3}H-FNZ Binding Sites in Rat Cerebral Cortical and Kidney Homogenates (M ± m; n = 4)

Substance	Dose, mg/kg	Cerebral cortex		Kidneys	
		K_d	B_{max}	K_d	B_{max}
Control (physiological saline)		1,81±0,09	1240±60	24,5±1,5	2420±120
Muscimol	1,5	1,07±0,10*	1180±120	17,2±1,7*	1995±210
(−) Baclofen	5	1,12±0,08*	918±85*	12,8±2,8*	1490±150*
(+) Baclofen	5	1,29±0,18	1110±90	15,9±2,1*	2030±180

Legend. Each separate Scatchard plot related to 7 points, each point to three repetitions. B_{max} in fmoles/mg protein. *$p < 0.05$.

of peripheral binding sites in the kidneys was found to be about 2 times greater, but their affinity about 20 times less, than for central binding sites in the brain (Table 1). These findings agree with those of previous workers [7]. Preliminary injection of muscimol into the animals (1.5 mg/kg) significantly increased affinity for the ligands not only in the cerebral cortex, but also in the kidneys; however, the number of binding sites was not significantly changed. This type of effect of muscimol on central BD binding sites in vitro and in vivo is well known, but it is completely absent in peripheral tissues in experiments in vitro. Injection of (−) baclofen (5 mg/kg) not only increased the affinity of central and peripheral BD binding sites for the ligand, but also significantly reduced the number of binding sites in both structures tested. Although changes in ^{3}H-FNZ binding in the brain and kidneys after injection of (+) baclofen (5 mg/kg) resembled changes brought about by the action of (−) baclofen (5 mg/kg), a significant fall in K_d was observed only in the kidneys. Since at the present time the existence of a link between $GABA_B$ and benzodiazepine receptors is denied [4], on the basis of experiments in vitro, it can be postulated that in vivo $GABA_B$ receptors participate in the regulation of function of the GABA-BD-receptor molecular complex. Since the effect of (−) baclofen resembles, on the one hand, the effect of muscimol (affinity is increased), and on the other hand, that of bicuculline (the number of binding sites is reduced), it can be postulated that in vivo baclofen will behave as a mixed agonist-antagonist of $GABA_A$ receptors. This hypothesis is confirmed indirectly by data in the literature [8], according to which both isomers, in high concentrations (IC_{50} = 38 μM) bind stereochemically unselectively with low-affinity $GABA_A$ receptors. This may also explain why the two stereoisomers of baclofen have the same effect on BD binding sites. Our experiments showed that after injection in vivo, agonists of $GABA_A$ and $GABA_B$ receptors modulate the function of peripheral BD binding sites equally. Since the presence of GABA receptors in the kidneys has not been established, it can be tentatively suggested that GABA agonists may have a protective action in stress, as a result of which the content of the hypothetical endogenous ligand of BD-receptors is changed.

According to the available data, it is impossible to decapitate an animal in an acute experiment without exposure to stress [2]. Preliminary injection of GABA agonists may perhaps exert a stress-protective action and thereby counteract the development of stress-induced changes in BD-receptors. This direct interaction betwen GABA receptors and peripheral binding sites is not obligatory and it indicates the possibility of further investigation of the regulatory mechanisms of functioning of peripheral BD binding sites. Our experiments also provide a basis for studying the possibility of using peripheral BD binding sites (for example, on blood cells) as sensitive markers of central BD-receptor function.

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ACTION OF Ro 15-1788 AND Ro 5-4864 ON EVOKED NEURONAL ACTIVITY IN HIPPOCAMPAL SLICES

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The imidazobenzodiazepine derivative Ro 15-1788 is a widely used benzodiazepine (BD) antagonist. This compound blocks binding of labeled BD with BD receptors (BDR), and the behavioral and electrophysiological effects of BD [8]. The structural analog of diazepam, Ro 5-4864 (4'-chlorodiazepam), is a high-affinity ligand of BDR of the peripheral type (PBDR). These receptors, which differ from receptors of the central type (CBDR), were found originally in kidney, liver, and lung tissues. Later their presence was demonstrated in the CNS also [3]. Testing in behavioral experiments showed that Ro 5-4864 and Ro 15-1788 are anxiogenic [5, 6]. The writer demonstrated previously that reciprocal inhibition in hippocampal slices is depressed by the anxiogenic BDR ligand FG 7142 [2].

The aim of this investigation was to study the action of Ro 15-1788 and Ro 5-4864 on electrophysiological parameters of global neuronal activity in hippocampal slices in order to test the hypothesis that depression of reciprocal inhibition in the hippocampus is a common property of anxiogenic BDR ligands.

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices from Wistar rats weighing 70-100 g, by the method described previously [1]. Activity was recorded in area CA1.

To assess reciprocal inhibition, Schaffer collaterals were subjected to paired stimulation by equal monopolar square pulses (5-20 V, 100-200 μsec, 0.1 Hz). Two population spikes (PS) in response to these stimuli were recorded extracellularly. The quantitative parameter of inhibition was the ratio Ats/Acs × 100%, where Ats is the amplitude of the test spike (PS in response to the 2nd stimulus) and Acs the amplitude of the conditioning spike (PS in response to the 1st stimulus). The interspike interval varied in each experiment from 20 to 160 msec. Stimulation by single pulses with a frequency of 0.1 and 1 Hz also was used.

All substances were added to the external solution. Working concentrations were made up by diluting concentrated (20 mM) solutions of GABA and hexobarbital in distilled water, and of Ro 5-4864 in ethanol, in physiological saline. Diazepam and Ro 15-1788 were disolved in physiological saline to a concentration of 10 μM, followed by the required dilution. Addition of the corresponding quantities of ethanol to the external solution caused no appreciable changes in unit activity.

When the curves were plotted, each point corresponded to the average of 5 to 7 evoked responses; the standard error of the mean did not exceed the dimensions of the circles. The significance of effects of the compounds was estimated by the signs test. Effects of the drugs were significant at the $p < 0.01$ level.

EXPERIMENTAL RESULTS

The action of Ro 15-1788 was studied on slices with marked inhibition of the test spike (TS) relative to the conditioning spike (CS). The minimal value of the Ats/Acs ratio observed

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