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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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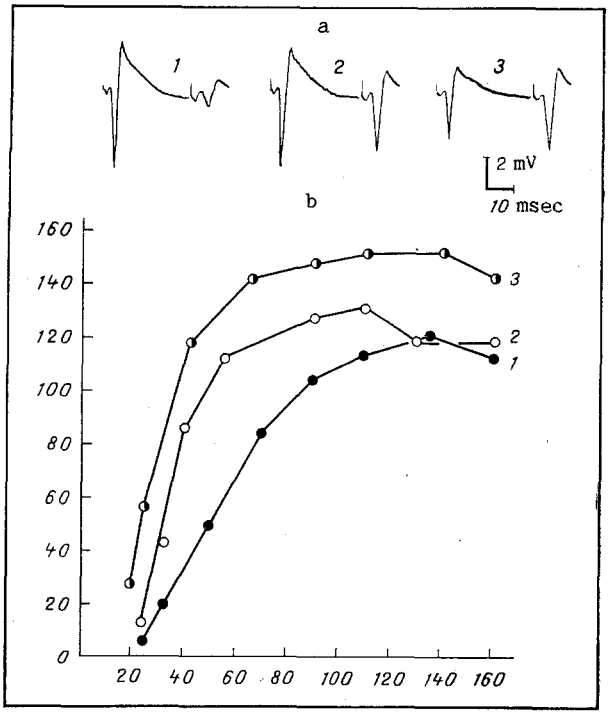

Fig. 1. Action of Ro 15-1788 on EP during paired stimulation: a) traces of EP; b) graph showing dependence of Ats/Acs ratio on interspike interval. 1) Control EP, 2) EP after application of Ro 15-1788 (5 μM, 10 min), 3) EP after rinsing for 50 min. Here and in Figs 2 and 3: abscissa, interspike interval (in msec); ordinate, Ats/Acs × 100%.

in the control in these experiments was 20-71%, and the corresponding CS-TS interval was 20-30 msec. After application of the compound in a concentration of 5 μM for 3-6 min the amplitude of TS was increased by 150-270%, so that the Ats/Acs ratio was increased, but the amplitude of CS under these circumstances was unchanged. Inhibition of CS was then observed, to a value of 32-84%. In four of nine experiments no inhibition of CS was observed during administration of Ro 15-1788. If an inhibitory effect of Ro 15-1788 on CS was not exhibited during application of the drug, it could be observed on rinsing. After rinsing for 60 min, the original parameters of the evoked potential (EP) were not fully restored. The results of one experiment to study the action of Ro 15-1788 are given in Fig. 1.

The action of Ro 15-1788 on the effect of diazepam was studied in 10 experiments. Application of diazepam (2 μM, 10 min) caused depression to TS by 52-100% accompanied by inhibition of CS by 5-40%. Administration of Ro 15-1788 (5 μM) against the background of the developing effect of diazepam, and when the drug was present in the system, led to abolition of its depressant effect on TS. During combined application of the drugs, additional depression of CS by 0-25% was observed. The results of a typical experiment to study the effect of Ro 15-1788 on the effect of diazepam are given in Fig. 2.

The action of GABA during paired stimulation was studied in six experiments. In the presence of GABA (40 μM) TS was reduced by 50-95% and CS by 21-50%. Ro 15-1788 (5 μM), when applied against the background of GABA, reversed its effect.

Blocking of the effect of hexobarbital during application of Ro 15-1788 was discovered in seven experiments. Application of hexobarbital (10 μM, 25 min) led to a decrease in the value of the Ats/Acs ratio with all values of interspike intervals. Depression of the amplitudes of CS and TS amounted to 7-18 and 33-62%, respectively. Application of Ro 15-1788 (5 μM) against the background of hexobarbital for 4-7 min caused complete reduction of the effect of the latter.

Application of Ro 5-4864 (10 μM, 15 min) during stimulation by single pulses with a frequency of 0.1 Hz led to depression of PS by 13-25% (10 experiments). In two experiments an additional PS appeared, while the main PS was inhibited simultaneously. With a frequency of stimulation of 1 Hz application of the compounds caused an increase in amplitude of the additional PS if it appeared in the control (three experiments) or its appearance de novo is absent in the control (seven experiments). Under these circumstances the amplitude of the principal PS was reduced by 10-25%. During paired stimulation, application of Ro 5-4864 increased the amplitude of TS by 53-142% with an interval of 20-30 msec, and in this case the amplitude of CS was reduced by 2-30%. It was difficult to rinse out the preparation, and recovery of EP to its original value did not occur even after 60 min of rinsing. The results of a separate experiment to study the action of Ro 5-4864 on EP during paired stimulation are given in Fig. 3.

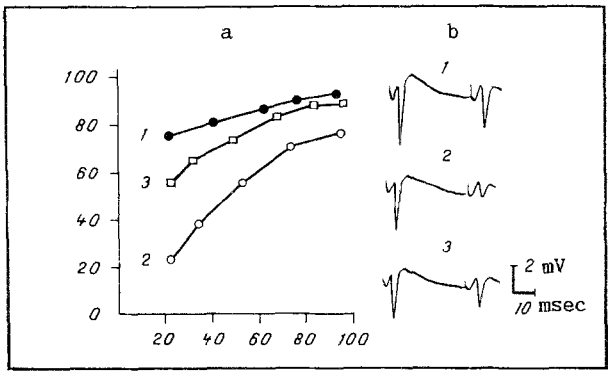

Fig. 2

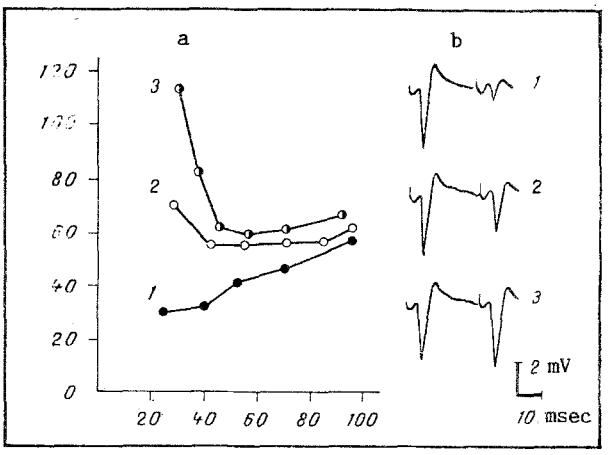

Fig. 3

Fig. 2. Action of Ro 15-1788 on effect of diazepam: a) graph showing dependence of Ats/Acs × 100% on interspike interval; b) traces of EP. 1) Control, 2) after application of diazepam (2 μM, 10 min), 3) after application of Ro 15-1788 (5 μM) for 5 min against the background of diazepam.

Fig. 3. Action of Ro 5-4864 on EP during paired stimulation. a) Graph showing dependence of Ats/Acs × 100% on interspike interval; b) traces of EP from the same experiment. 1) Control, 2) after application of Ro 5-4864 (10 μM, 15 min), 3) after rinsing for 10 min.

An important experimental discovery in this investigation was facilitation of TS by Ro 15-1788. The probable explanation of this effect is depression of reciprocal inhibition due to interaction of the antagonist with an endogenous BDR ligand, possibly present in the tissue of the slice, and possessing activity of an agonist. Displacement of the endogenous ligand in this case would abolish its potentiating action on inhibition. The observed effect of Ro 15-1788 cannot be explained by intrinsic activity of a reverse agonist, for in experiments on neurons in a spinal cord culture, in which the presence of an endogenous ligand is unlikely, this compound exhibited the properties of a partial BD agonist [4].

Application of Ro 15-1788 against the background of the developing effect of diazepam led to abolition of the potentiating action of this BD on inhibition. These results are in agreement with biochemical data relating to displacement of BD from binding sites by the action of Ro 15-1788 and they suggest that the effect of diazepam on a model of hippocampal slices is realized through BDR. The abolition of the effect of GABA and hexobarbital by Ro 15-1788 was unexpected. We know that the site of action of barbiturates, as exemplified by hexabarbital, and the GABA receptor are located in the region of the BDR – GABA-receptor – chloride channel complex. It has been shown that allosteric interaction takes place between the above-mentioned components of the complex, but competition for the binding site between barbiturates, GABA, and BDR ligands has not been found [7]. The effect discovered may be the result of displacement of an endogenous ligand, possessing agonist activity, through the action of Ro 15-1788. Displacement of this ligand would lead to abolition of potentiation of the action of both endogenous and exogenous GABA, and this would be expressed as facilitation of evoked neuronal activity. In the case of hexobarbital, the facilitatory action of Ro 15-1788 evidently concealed its inhibitory effect.

The discovery of so-called peripheral BD binding sites in the CNS is evidence of a probable role of these receptors in the regulation of its functions. PBDR are located on glial cells of nerve tissue, unlike CBDR, which are located on bodies of neurons and their processes [3]. The physiological role and the possible pharmacologic significance of PBDR are not clear. At the behavioral level, sedative and anxiogenic effects of Ro 5-4864, a ligand of these receptors, have been found [3, 6]. The action of Ro 5-4864 on hippocampal unit activity, as the present investigation has shown, likewise was not consistently identical. Against the background of inhibition of the response, additional PS appeared or those already present were increased. Facilitation of TS during paired stimulation suggests that GABA-ergic reciprocal inhibition is depressed by Ro 5-4864. Inhibition of CS may be explained by potentiation of adenosine inhibition, for Ro 5-4864 binds with the reuptake sites of this nucleotide [3].

It can be postulated on the basis of these results that a common property of BDR ligands with an anxiogenic component in the spectrum of their behavioral effects is depression of reciprocal inhibition in the hippocampus.

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N-METHYLCYTISINE: A SELECTIVE LIGAND OF NICOTINIC ACETYLCHOLINE RECEPTORS IN THE CENTRAL NERVOUS SYSTEM

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The presence of two types of nicotinic acetylcholine receptors (NACR) in the animal brain has now been established. Receptors of the first type interact with the α-neurotoxins of snakes and, with respect to some pharmacologic, chemical, and biochemical properties, they closely resemble neuromuscular NACR [10, 11, 14]. However, there are various circumstances which compel a more critical consideration of the view that the α-neurotoxin-binding components of the brain are NACR. One of the main arguments in support of this approach is the fact that according to electrophysiological data α-neurotoxins do not block cholinergic functions of certain neuronal systems [7, 8]. Accordingly, to study NACR in the CNS, radioactive derivatives of traditional low-molecular-weight ligands have been used more extensively in recent years: acetylcholine, nicotine, and tubocurarine [9, 12, 15]. With their aid nicotine receptors of a second type, insensitive to snake α-neurotoxins and differing in various pharmacologic properties from neuromuscular NACR, have been found in the brain. The authors cited also have described NACR in the optic ganglia of the squid, similar in their pharmacologic properties (in particular, high affinity for cytisine and insensitivity to α-neurotoxins), to the type II nicotinic receptors of the CNS [1, 2, 5]. For the reasons described above, it is interesting to look for new low-molecular-weight ligands capable of interacting selectively with type II NACR.

This paper describes a comparative study of the pharmacologic activity of cytisine and its N-methyl derivatives with respect to neuromuscular NACR of the electric organ of the skate Torpedo marmorata and the nicotinic receptor of the optic ganglia of the squid.

EXPERIMENTAL METHOD

The following substances were used in the experiments: ^{14}C-tubocurarine (^{14}C-TC, 94 Ci/mole) and ^{3}H-methyl iodide (13 Ci/mmole; from Amersham International, England). Cytisine was obtained from the Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek

Brain Research Institute, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow.

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