10. S. Ögren, S. Ross, H. Hall, et al., Acta Physiol. Scand., 63, Suppl. 290, 127 (1982).
11. A. Sherman, G. Allers, F. Petty, et al., Neuropharmacology, 18, 891 (1979).
12. A. Sherman, and F. Petty, Behav. Neural. Biol., 30, 119 (1980).

EFFECT OF THE SPECIFIC BENZODIAZEPINE ANTAGONIST RO15-1788 ON INHIBITION OF HIPPOCAMPAL UNIT ACTIVITY EVOKED BY PHENAZEPAM

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High-affinity specific binding sites for benzodiazepines (BD), which function as receptors and mediate the pharmacologic activity of these compounds, have been found comparatively recently in the mammalian CNS [5, 12].

The writers previously described the effect of BD on the evoked potential (EP) of hippocampal neurons [1]. However, the question whether this effect is mediated through interaction of BD with a specific receptor remained unanswered.

The aim of this investigation was to test the hypothesis that the effect of BD on EP arising in hippocampal area CA1 in response to stimulation of Schaffer's collaterals (SC) is mediated through the benzodiazepine receptor (BDR). For this purpose the action of the specific BD antagonist, RO15-1788, on inhibition induced by phenazepam in the hippocampus was studied. The effect of RO15-1788 on EP also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices from Wistar rats aged 2-3 weeks by the method described previously [1]. The animals were decapitated, the upper cranial bones were removed, and a transverse slice of the hippocampus about 400 μ thick was removed, and transferred to a thermostatically controlled experimental chamber through which flowed balanced Hanks' solution. The solution was previously saturated with a gas mixture containing 95% O_2 and 5% CO_2 (pH 7.2-7.4), and for the next 30-40 min the temperature in the chamber was gradually adjusted to 25-30°C. Bipolar glass stimulating microelectrodes filled with Hanks' solution were introduced into the stratum radiatum, in which SC are located. Recording microelectrodes filled with Hanks' solution were led up to neurons in area CA1.

EP began to be recorded 1-1.5 h after preparation of the slice. Pulses of direct current 0.2 msec in duration, with a frequency of 0.1 Hz and a voltage of 10-20 V, were used for stimulation. The parameters of stimulation were chosen in order to evoke a population spike (PS), which is a synchronous discharge of the pyramidal neurons in this area. In the present experiments the amplitude of PS varied from 2 to 5 mV in different experiments. The results were recorded on photographic paper from an oscilloscope.

Solutions containing 2 μM phenazepam, 4 μM RO15-1788, and 2 μM hexobarbital were used. Phenazepam and RO15-1788 were dissolved beforehand in ethanol to a concentration of 10^{-2} M. From these solutions, working solutions to the required concentration in Hanks' solution were prepared. Appropriate quantities of ethanol were added to the flow of liquid in the control.

The experiments were conducted by the following scheme: phenazepam was added to the system and applied for 10-15 min; during application of phenazepam, the antagonist RO15-1788 was

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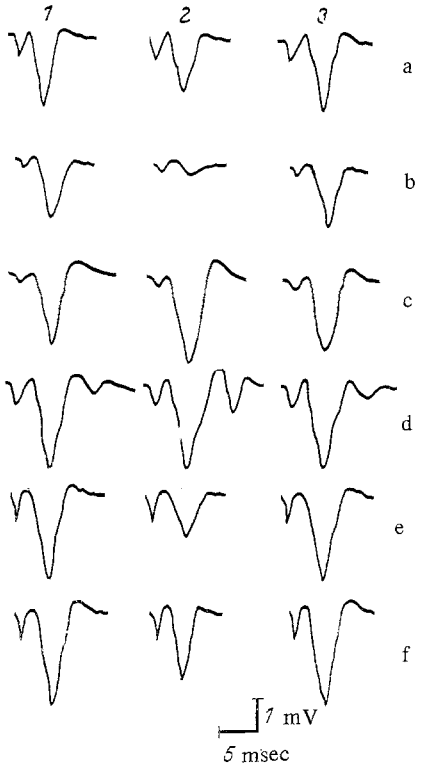

Fig. 1

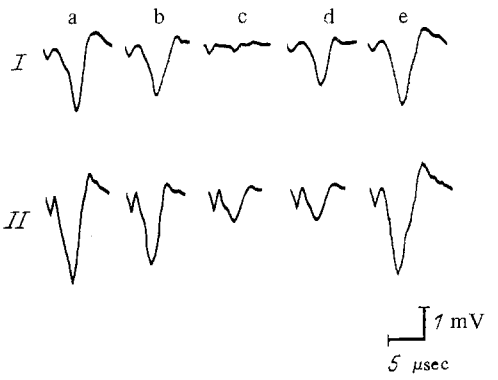

Fig. 2

Fig. 1. Effects of test substances on EP (separate typical case): a) BD-like effect of R015-1788 (4 μM), b) strong BD-like action of R015-1788 (1 μM), c) increase in PS under the influence of R015-1788 (4 μM), d) increase in second PS under the influence of R015-1788 (4 μM), e) effect of phenazepam (2 μM), f) effect of hexobarbital (2 μM). 1) Control EP, 2) EP under influence of substances, 3) EP after rinsing out substances.

Fig. 2. Effect of the specific BDR antagonist R015-1788 on reduction of PS by phenazepam and hexobarbital. I) Effect of R015-1788 on inhibition by phenazepam: a) control EP, b) after action of R015-1788 (4 μM) for 10 min; c) EP after action of phenazepam (2 μM) for 10 min, previous preparation rinsed out; d) EP 5 min after addition of R015-1788 to 4 μM to perfusion solution containing phenazepam (2 μM), preliminary application of phenazepam for 10 min; e) EP after rinsing out the substances. II) No effect of R015-1788 on reduction of PS by hexobarbital: a) control EP, b) EP after action of R015-1788 (4 μM) for 10 min, c) EP after application of hexobarbital (2 μM) for 10 min, preceding substance washed out; d) EP 10 min after addition of R015-1788 to 4 μM to perfusion solution containing hexobarbital (preliminary application of 2 μM hexobarbital for 10 min); e) EP after rinsing out substances.

added to the perfusion fluid up to the required concentration. Similar procedures were carried out in the case when hexobarbital was applied.

EXPERIMENTAL RESULTS

The action of the specific BDR antagonist R015-1788 in a concentration of 4 μM on EP in most cases (seven slices) was manifested as a decrease of 10-20% in PS, i.e., its action was BD-like (Fig. 1a). In one experiment R015-1788 had a stronger inhibitory action on EP: PS was reduced by 80% after application of the antagonist in a concentration of 10^{-6} M (Fig. 1b). In two cases, however, during application of R015-1788 in a concentration of 4 μM an effect opposite to that of BD was observed: in one case the amplitude of PS was increased by 13% (Fig. 1c), in another case an accessory PS appeared whereas the first PS remained visibly unchanged (Fig. 1d). The effects described above developed 5-10 min after the beginning of addition of the drug and disappeared after rinsing of the preparation for 15 min.

The effect of BD on EP of hippocampal CA1 neurons is manifested as a reversible decrease in PS [1]. To verify the specificity (i.e., mediation through BDR) of this effect, against

the background of the action of phenazepam, RO15-1788 was applied to the system. This was done in cases when the action of the antagonist itself was BD-like and was weaker than the effect of phenazepam (five slices).

Phenazepam (2 μM) reduced the amplitude of PS to 15-70% of the control level (Fig. 1e). Against the background of application of RO15-1788 (4 μM), in these cases the PS reduced by phenazepam rose in the course of 4-6 min to 66-95% of the control level (Fig. 2, I).

Hexobarbital had an effect on PS outwardly similar to that of phenazepam. Application of hexobarbital (22 μM) caused a reduction of PS to 30-50% of the control value (Fig. 1f). RO15-1788 (4 μM) applied against the background of hexobarbital did not change the amplitude of the response (five slices, Fig. 2, II). All the effects described are reversible. PS was restored to its initial value after rinsing out the drugs for 10-20 min.

Much evidence obtained by various methods in support of the existence of several types of BDR in the mammalian CNS has recently been published [6, 10]. For example, the presence of proteins or protein complexes with four BD-binding sites located immediately adjacent to one another, so that conformational changes due to interaction of one of them with a ligand affect the functioning of the others, in the synaptic membrane has been postulated. In addition, receptors independent of this group including, in particular, what are considered [6] to be receptors for BD antagonists, also exist. The physiological role of BDR is not clear, for no endogenous ligand of this receptor has been isolated. The list of claimants for the role of endogenous ligand includes hypoxanthine, inosine [11], carboline-3-carboxylates [3], prostaglandins of group A [2], and also many unidentified agents. It is also probable that several physiologically active BDR ligands may function in the body. This hypothesis is confirmed by the heterogeneity of BDR in mammals. Among the substances suggested as endogenous ligands there are some with opposite pharmacologic effects. For example, harman has activity opposite to the effect of BD, i.e., when injected intravenously it behaves as a convulsant, only four times less active than picrotoxin [9]. Meanwhile inosine and hypoxanthine exhibit BD-like activity in the CNS [11].

In the present experiments the synthetic BD antagonist RO15-1788 exhibited BD-like activity in most cases, but in two experiments it increased the amplitude of PS and behaved as an "antibenzodiazepine." Accordingly it seems likely that the opposite nature of the effects of RO15-1788 is the result of interaction of the antagonist on BDR with endogenous ligands differing in the character of their pharmacologic action. These ligands may probably be present in different proportions in the tissue of the slice depending on its functional state. Another possible explanation of the results is interaction of RO15-1788 with different subpopulations of BDR, represented in individual slices in different proportions [8].

The present investigation shows that the specific BDR antagonist RO15-1788 increases the amplitude of PS when depressed by phenazepam. Inhibition of PS by hexobarbital developed whether RO15-1788 was applied or not. It can accordingly be postulated that the effect of phenazepam on this particular model is linked with its interaction with BDR.

Most of the effects of BD observed clinically are evidently mediated through interaction with BDR. It has been shown, for instance, that the pharmacologic efficacy of the various BD correlates closely with their ability to bind in biochemical experiments with BDR [12]. However, the action of BD may also be exhibited on experimental models [4] in which no BDR can be found biochemically [7]. The results of the present investigation suggest that hippocampal slices can be used as a model with which to study the pharmacology of BD at the receptor level.

LITERATURE CITED

1. P. G. Glushankov, S. L. Buldakova, V. G. Skrebitskii, et al., Byull. Éksp. Biol. Med., No. 10, 44 (1982).
2. T. Asano and N. Ogasawara, Eur. J. Pharmacol., 80, 271 (1982).
3. C. Braestrup, M. Nielsen, and C. Olsen, Proc. Natl. Acad. Sci. USA, 77, 2288 (1980).
4. S. Faugier-Grimaud, Neuropharmacology, 17, 905 (1978).
5. H. Mohler and T. Okada, Science, 198, 849 (1977).
6. H. Mohler, Eur. J. Pharmacol., 80, 435 (1982).
7. M. Nielsen, C. Braestrup, and R. F. Squires, Brain Res., 141, 342 (1978).
8. D. J. Nutt and P. J. Cowen, Nature, 295, 436 (1982).
9. H. Rommelspacher, C. Nanz, H. O. Barbe, et al., Eur. J. Pharmacol., 70, 409 (1981).

10. W. Sieghart and M. Karobath, Nature, 286, 285 (1980).
11. P. Skolnick, P. J. Marangos, F. K. Goodwin, et al., Life Sci., 23, 1473 (1978).
12. R. F. Squires and C. Braestrup, Nature, 266, 732 (1977).

ENDOGENOUS ETHANOL LEVEL AND ALCOHOL MOTIVATION

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Predisposition to the development of experimental alcoholism in animals has been shown to depend on the rate of ethanol metabolism [1]. Meanwhile differences in the rate of elimination of ethanol by patients with chronic alcoholism and nonalcoholics have been reported [3-5]. However, the possibility of studying the kinetics of ethanol as a diagnostic and prognostic criterion reflecting the risk of development of an addiction for alcohol is limited by the fact that such investigations require administration of alcohol to the subjects and repeated taking of blood samples. The study of endogenous ethanol (EE), a natural substrate of ethanol-oxidizing systems, indirectly reflecting the level of their activity, could be of great practical value in this respect.

The object of this investigation was to study correlation between the intensity of alcohol motivation and the blood EE level in animals and man.

EXPERIMENTAL METHOD

The EE level was determined in 57 men aged 28-42 years suffering from chronic alcoholism in stage II, on the 20th-25th day of their stay in the clinic after relief of withdrawal symptoms. They had received no drugs during the 4-5 days before the investigation. The control group consisted of 47 healthy male volunteers aged 24-42 years who had consumed no alcoholic drinks during the 7-10 days before the investigation. Blood (2-3 ml) was taken from the cubital vein always at the same time of day — 9:30-11:00 a.m., 1.5-2 h after breakfast. Each subject was tested once only.

Experiments were carried out on 25 noninbred male rats weighing 180-200 g, 10 C57BL mice weighing 30 g, and genetically predisposed toward alcohol consumption, and on 10 CBA mice weighing 30 g, with genetically determined aversion for alcohol. The intensity of the initial alcohol motivation of the rats was measured in a situation of minimal stress, namely being kept in groups of four in a plastic cage measuring 32 × 47 × 16 cm, equipped with a feeding bowl and two graduated receptacles — one with water, the other with 15% ethanol solution. Four groups of animals, each containing four rats, were selected: two groups were formed by rats with an EE level below 4 μg/ml (mean 3.75 μg/ml), the other two consisted of animals with EE above 7 μg/ml (mean 7.53 μg/ml). The testing period was 10 days. The intensity of alcohol motivation of the rats was determined by the standard method, by keeping them for 10 days in individual cages measuring 5 × 9 × 5 cm, equipped with two graduated receptacles — one containing water, the other a 10% solution of ethanol.

The EE level was determined by gas—liquid chromatography. Samples of 0.5 ml blood, taken from the subjects, were treated with 0.5 ml of 50% TCA and 0.5 ml of 1% isopropyl alcohol, which served as internal standard. Flasks were sealed and 0.25 ml of 30% $NaNO_2$ was injected into them from a syringe. The contents of the flask were shaken and the gaseous phase withdrawn by means of a syringe and introduced into the chromatograph.

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