was able to displace an additional amount of [$^3$H]imipramine, an effect attributable to its interaction with low-affinity (or nonspecific) binding sites.

The benzofuran and morpholine derivatives befuraline and maclobamide, like the new derivatives of these compounds B1 and M1, according to the results of these experiments, virtually do not compete with [$^3$H]-imipramine for its receptors.

Comparison of the results obtained with data in the literature on the activity of compounds studied as monoamine oxidase inhibitors [1, 8] leads to the conclusion that relations between the parameters of these substances as revealed by the two tests used were reciprocal. For instance, these compounds occupy the following order of descending ability to inhibit monoamine oxidases: morpholine derivatives > pyrazidol > tricyclic antidepressants. Pyrazidol, incidentally, had comparable values of concentrations giving inhibition by 50% ($IC_{50}$), namely about $10^{-5}$ M.

On the whole it can be concluded from these results that, unlike tricyclic compounds, the atypical antidepressants have very weak affinity for imipramine receptors of mouse brain synaptic membranes. The therapeutic effect of the atypical antidepressants is evidently linked with their action on other receptor or enzyme systems of the brain.

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# EFFECT OF DIAZEPAM ON REACTIVITY OF HIPPOCAMPAL NEURONS DURING BLOCKADE OF THE GABA-ERGIC SYSTEM


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KEY WORDS: hippocampal slices; evoked potential; diazepam; bicuculline; picrotoxin.


The benzodiazepines (BD) are a group of compounds widely used in medical practice as tranquilizers and anticonvulsants. In recent years great attention has been paid to interaction between the BD and the GABA system. It has been shown, for instance, that BD potentiate the action of GABA, both liberated synaptically and extrinsically applied, on mammalian neuronal preparations [3, 6, 9].

The limbic system is evidently one of the principal structures of the CNS responsible for the clinical manifestation of the action of BD. We know that there is a high density of benzodiazepine receptors in the limbic system [11] and that GABA is the inhibitory mediator in this region of the brain [5]. Investigations conducted on formations of the mammalian limbic system have shown that BD inhibit both spontaneous and evoked unit activity [1, 4, 7]. Potentiation of GABA-inhibition is the presumed mechanism of the inhibitory effect of BD.


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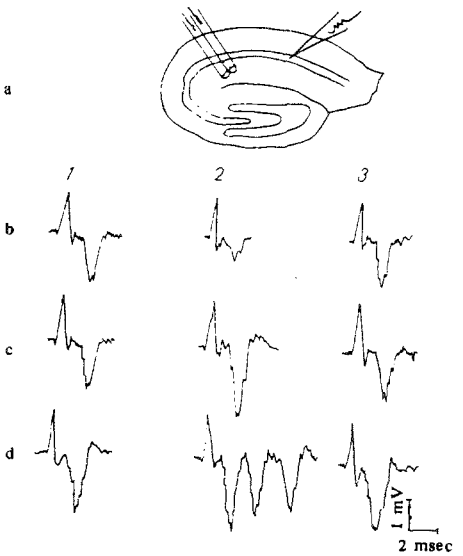


Fig. 1. Effects of diazepam, picrotoxin, and bicuculline on EP (one typical case): a) scheme of hippocampal slice, b) effect of diazepam ($10^{-6}$ M), c) effect of picrotoxin ($10^{-6}$ M), d) effect of bicuculline ($10^{-7}$ M). 1) Control EP, 2) EP during the action of the drugs, 3) EP after rinsing the preparation.

The aim of the present investigation was to test this hypothesis on a model of a hippocampal slice, by recording the evoked potential (EP) arising in area $CA_1$ in response to stimulation of Schaffer's collaterals (SC). For this purpose bicuculline, a competitive GABA-antagonist, and picrotoxin, a GABA antagonist at the chloride channel level [10] were used, for if diazepam interacts with the GABA system, these compounds should modify the effect of diazepam on EP.

## EXPERIMENTAL METHOD

Experiments were carried out on hippocampal slices by the method described previously [2] on C57BL/6 mice aged 14-20 days. After decapitation of the animals the brain was exposed and a slice of hippocampus 200-300 $\mu$ thick was cut and immediately transferred to a special constant-temperature chamber. The bathing fluid consisted of balanced Hanks' salt solution, saturated beforehand with carbogen (95% $O_2$ and 5% $CO_2$) and heated to 30-31°C. The slice was fixed to a grid by glass bipolar stimulating electrodes filled with Hanks' solution (diameter of tip 0.2-0.3 mm), which were located in the SC layer. A recording glass electrode filled with Hanks' solution (diameter of tip about 0.01 mm) was then led up to the pyramidal neurons of area $CA_1$ (Fig. 1a). Pulses of current 0.2 msec in duration with a frequency of 0.1 Hz, and voltage 30-60 V were used as stimulation. Recording to EP began 1 h after preparation of the slice and continued for 4-6 h. The conditions of stimulation were chosen so as to induce the appearance of a population spike (PS), consisting of the synchronized discharge of pyramidal neurons in area $CA_1$. The data were recorded on photographic film. The following solutions were used: 1) diazepam, from Hoffman-La Roche, $10^{-6}$ M in Hanks' solution; 2) picrotoxin, $10^{-6}$ M in Hanks' solution; 3) bicuculline (from Pierce), $10^{-7}$ M in Hanks' solution; 4) $10^{-6}$ M picrotoxin in $10^{-6}$ M diazepam solution; 5) $10^{-7}$ M bicuculline in $10^{-6}$ M diazepam solution. To apply these various substances the perfusion fluid was replaced by one of the above solutions.

## EXPERIMENTAL RESULTS

The action of diazepam ($10^{-6}$ M) was to produce a reversible decrease of 50-70% in the amplitude of the PS (Fig. 1b). The maximal effect developed 7-10 min after the beginning of application of diazepam, after which the drug was rinsed out with Hanks' solution. The amplitude of PS was restored to its initial level 10-15 min after the beginning of rinsing. Picrotoxin, in a concentration of $10^{-6}$ M, caused an increase in the amplitude of

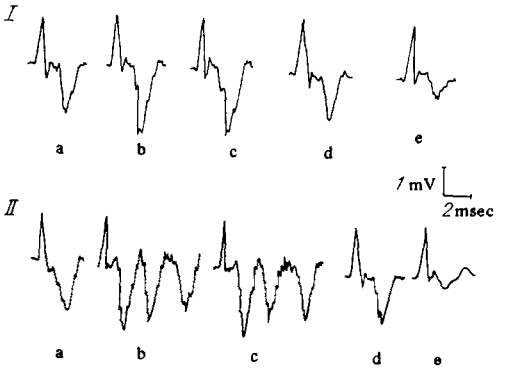


Fig. 2. Blocking of effect of diazepam on EP by GABA antagonists – bicuculline and picrotoxin (one typical case). I) Blocking by picrotoxin: a) control EP, b) EP after action of picrotoxin ($10^{-6}$ M) for 10 min, c) combined application of picrotoxin ($10^{-6}$ M) and diazepam ($10^{-6}$ M), d) EP after rinsing preparations for 20 min, e) restoration of effect of diazepam on EP. II) blocking by cuculline: a) control EP, b) after action of bicuculline ($10^{-7}$ M) for 10 min, c) combined application of bicuculline ($10^{-7}$ M) and diazepam ($10^{-6}$ M), d) EP after rinsing to remove drugs for 20 min, e) restoration of effect of diazepam on EP.

PS by 15-25% of the control level. The maximal effect was observed 8-10 min after the beginning of application. The amplitude of PS was restored 15-20 min after the beginning of rinsing (Fig. 1c). Bicuculline ($10^{-7}$ M) had a more marked effect on EP, expressed as an increase in the amplitude of PS by 30-50% and also the appearance of additional PS (seizure discharges), which were not observed in response to picrotoxin (Fig. 1d). The effect was reversible. Recovery occurred 15-20 min after the beginning of rinsing. Diazepam, applied in a concentration of $10^{-7}$ M after preliminary administration of $10^{-6}$ M picrotoxin or $10^{-6}$ M bicuculline, did not abolish the action of the latter drugs (Fig. 2, I, II), i.e., diazepam, if injected into the perfusion fluid simultaneously with GABA blockers, was ineffective as regards suppressing PS. After the preparation had been rinsed free from a combination of these drugs, diazepam injected into the perfusion fluid had an effective inhibitory effect on PS. In certain cases diazepam abolished seizure discharges evoked by bicuculline when these drugs were applied simultaneously.

A system of recurrent inhibition, the mediator for which is GABA [5], is known to participate in the formation of PS and, in particular, its polysynaptic components, in the hippocampus. An increase in the amplitude of PS under the influence of picrotoxin and bicuculline was demonstrated in the present investigation. Bicuculline also caused the appearance of several additional PS (seizure discharges). This change in PS can evidently be explained by abolition of the inhibitory effect of GABA.

The results of this investigation show that as a rule the inhibitory effect of diazepam on PS in the SC system in the hippocampus is not exhibited against the background of the action of bicuculline and picrotoxin, blockers of the GABA-ergic system. It can accordingly be concluded that the effect of diazepam is connected with GABA-ergic synapses. This conclusion is supported by data in the literature demonstrating a connection between effects of the BD and GABA-ergic processes in other parts of the mammalian CNS [7, 8].

The investigation showed that GABA antagonists with different binding sites on the postsynaptic membrane block the action of diazepam equally effectively. This suggests that diazepam potentiates the postsynaptic response of GABA. However, it is not yet clear where this potentiation takes place – at the level of the GABA receptor or of the ionic channel.

Suppression by diazepam of seizure discharges provoked by bicuculline, observed in some experiments, which can be explained from the standpoint of competition between effects evoked by these substances, also confirms the view that there is a common target for diazepam and for the GABA antagonist. Meanwhile the molecular mechanisms of competition between bicuculline and diazepam still await explanation, for we know that these compounds interact on the neuron membrane with different receptors [10].

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# EFFECT OF THYROTROPHIN RELEASING HORMONE ON SENSOMOTOR CORTICAL NEURONS


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KEY WORDS: thyrotrophin releasing hormone (TRH); sensomotor cortex; microiontophoresis.


Thyrotrophin releasing hormone (TRH), a hypothalamic neurohormone, has a stimulating action on the CNS. If administered by peripheral or central (intraventricular) routes it induces behavioral excitation and increases locomotor activity in different animals [4, 5, 9] and also induces electroencephalographic activation [1]. TRH has been shown to have analeptic properties, for it can reduce or block the central depressant effects of barbiturates, ethanol, neuroleptics, tranquilizers, and general anesthetics [15]. These effects of TRH are not associated with any influence on the endocrine system.

It has been suggested that TRH is an endogenous analeptic, regulating the levels of wakefulness and consciousness [6]. TRH can evidently perform these functions by virtue of its effect as a chemical transmitter (mediator) or a modulator of central nervous processes.

The object of the present investigation was to make an electrophysiological study of the effect of TRH on sensomotor cortical neurons in rabbits. TRH was injected intravenously and also applied to single cortical neurons by microiontophoresis.

## EXPERIMENTAL METHOD

Experiments were carried out on 14 unanesthetized rabbits weighing 3.0-4.0 kg, immobilized with diplacin* (5 mg/kg) and artificially ventilated. Action potentials (APs) of the neurons were derived extracellularly through one channel of a three-barreled glass microelectrode. The second barrel of the microelectrode was filled with an aqueous solution of TRH (0.03 M, pH 6.0). The third barrel, filled with 3 M NaCl, was used to pass the control microiontophoretic current and to compensate current artefacts. TRH for intravenous injection was dissolved in 1.5 ml 0.9 M NaCl and injected in doses of 0.1 to 7.0 mg/kg into the rabbit's auricular vein in the course of 1-1.5 min.

* 1,3-di ($\beta$-platynecinium-ethoxy) benzene hydrochloride.


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