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EFFECT OF DIAZEPAM ON EVOKED UNITARY RESPONSES OF HIPPOCAMPAL SLICES

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KEY WORDS: diazepam; neurons; brain; hippocampal slices.

Compounds of the benzodiazepine series (BDZ) have become widely used in recent years in medical practice as tranquilizers and anticonvulsants [1]. Despite many investigations, the mechanism of the effect of BDZ has not yet been completely studied [4]. With the discovery of brain formations sensitive to BDZ, notably the limbic system [9], the necessity has arisen for a study of the effects of BDZ at the synaptic level, i.e., of the possible effect of BDZ on neurotransmitter or other cellular and subcellular systems through which BDZ may exert their influence on the neuron.

The object of the present investigation was to examine the effect of diazepam (a member of the BDZ) on evoked potentials (EP) arising in hippocampal area CA_1 in response to stimulation of Schaffer's collaterals (SC).

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices from C57BL/6 mice by the method described previously [2, 10]. The animals were decapitated, the bones of the upper part of the skull removed, and a transverse slice of the hippocampus was excised and placed in an experimental chamber through which balanced Hanks' salt solution at 25°C flowed continuously; the solution was saturated with a gas mixture consisting of 95% O_2 and 5% CO_2. Bipolar glass stimulating electrodes filled with Hanks' solution were introduced into the radial layer, where SC running from neurons of area CA_3 to neurons of area CA_1 are located, and the recording glass microelectrode, also filled with Hanks' solution, was inserted into area CA_1 (Fig. 1A).

Recording of the response began 1 h after preparation of the slice, when its electrophysiological parameters were stabilized. Pulses 0.2 msec in duration with a frequency of 0.1 Hz and a voltage of 30-60 V were used for electrical stimulation. The conditions of stimulation were chosen so as to evoke a population spike (PS), which is the synchronous discharge of pyramidal neurons in area CA_1 [5], and whose amplitude reflects the reactivity of this hippocampal synaptic system.

The diazepam used was synthesized by the method described previously [8]. The resulting preparation was purified by liquid chromatography on Sephadex LH-20 (from "Pharmacia," Sweden) in 70% methanol. The chemical structure was confirmed mass-spectrometrically. After preparation of a solution of diazepam in phosphate buffer, saturated at 4°C, its concentration (3.96×10^{-5} M) was determined from the coefficient of molar extinction (ε = 31,800 at a wavelength of 233 nm) measured in preliminary experiments, and from this solution a standard Hanks' solution was prepared. A proprietary preparation of diazepam (Seduxen, Hungary) also was used in the form of a solution in ampuls for injection. The results were recorded on photographic film and on disks of the PDP-8 computer, and were processed manually and by a program composed by A. G. Gusev [3].

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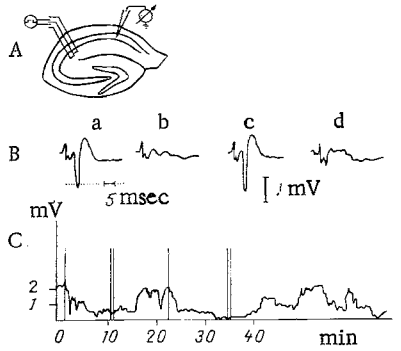

Fig. 1

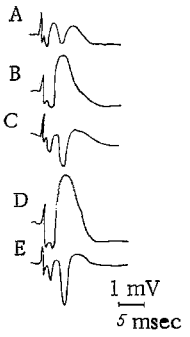

Fig. 2

Fig. 1. Effect of diazepam in a concentration of 3.96×10^{-6} M. A) Diagram of hippocampal slice; B) summation of 30 EPs: a) control, b) during administration of drug, c) after rinsing out drug, d) during administration of a second dose of drug; C) graph of current amplitude of PS. Single vertical line denotes time of application of drug, double vertical line — beginning of rinsing. Abscissa, time (in min); ordinate, amplitude of PS (in mV).

Fig. 2. Effect of diazepam in concentration of 3.96×10^{-5} M. A) Control response; B) response after diazepam; C) response after rinsing out diazepam; D) response after a second dose of diazepam; E) response after rinsing out diazepam again. All traces obtained by summation of 10 EPs on PDP-8 computer.

EXPERIMENTAL RESULTS

The action of diazepam in a concentration of 3.96×10^{-6} M was expressed as a reversible decrease in the amplitude of PS (Fig. 1B, a-d). This effect was obtained initially by the use of diazepam in solution in ampuls (Seduxen). In view of misgivings about the action of the solvent used to make up the preparation, a series of experiments was carried out with diazepam dissolved in Hanks' solution. The effect was the same in principle. From a graph of the current amplitude of PS (Fig. 1C) it was possible to judge the sequence of changes taking place in the amplitude of PS during the action of the drug and its rinsing out with control Hanks' solution.

Addition of diazepam in a concentration of 3.96×10^{-5} M led to more complex effects (Fig. 2A-E) connected with a change in amplitude of the population EPSP. Against the background of a gradual fall in amplitude of PS (or even its complete disappearance), the amplitude of the population EPSP increased, but this was followed by gradual disappearance of the response as a whole. A typical case of an increase in amplitude of the population EPSP is shown in Fig. 2B. After a second exposure to diazepam in a concentration of 3.96×10^{-5} M, its effect appeared to be stronger (Fig. 2D) but was reversible. In a concentration of 3.96×10^{-6} M, diazepam evidently acts on postsynaptic structures, for in all the experiments examined above, the amplitude of the presynaptic potential was unchanged. When diazepam was added in a concentration of 3.96×10^{-5} M, the amplitude of the presynaptic component of the response decreased.

Interpretation of the data was clearer in the case when the concentration of diazepam was 3.96×10^{-6} M and the changes were confined to a decrease in amplitude of PS, which evidently reflects a decrease in reactivity in the hippocampal pyramidal system. Since the synaptic inflow, to judge from the amplitude of the presynaptic component of the response, was unchanged, the decrease in reactivity may have been the result of increased efficiency of action of inhibitory interneurons. According to modern views of mutual interaction between γ-aminobutyric acid (GABA) and BDZ [6, 7], this effect can be presumed to take place because diazepam strengthens the affinity of GABA for its own receptors. The action of higher concentrations of the drug evidently leads to strong hyperpolarization of the pyramidal cells, accompanied by an increase in amplitude of evoked EPSPs, although they do not reach the level of spike generation. This is expressed as depression of PS and an increase in amplitude of the population EPSP. However, interpretation of the effects of these concentrations is made difficult because of their pos-

sible action not only on the receptor apparatus, but also on the excitable presynaptic membrane, i.e., on the conduction of impulses along fibers. An indication that this is so is given by the change in amplitude of the presynaptic component. A more detailed analysis of the mechanism of action of the drug may be obtained by studying single unit activity.

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HEMODYNAMIC RESPONSE OF NORMOTENSIVE AND HYPERTENSIVE RATS TO PROSTAGLANDINS AND INDOMETHACIN

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High arterial pressure in spontaneously hypertensive rats (SHR; Okamoto-Aoki line) is associated with increased tone of the sympathetic nervous system [6, 8, 9], whereas in rats with renovascular hypertension it is associated with increased activity of the renin-angiotensin system [5]. It has recently been shown [4], however, that intravenous injection of depressor prostaglandins (PG) is accompanied by more severe hypotension in spontaneously hypertensive than in normotensive animals. Meanwhile, in rats with renovascular hypertension, definite inhibition of PG biosynthesis has been found in the kidney and other tissues. These and other investigations suggest that depressor PG play a role in the development of hypertension [1].

The aim of this investigation was to compare the effects of PGI_2, PGE_1, and $PGF_{2\alpha}$ and of indomethacin, which inhibits PG synthesis, on the hemodynamics in rats of the above groups.

EXPERIMENTAL METHOD

Male rats weighing 250-300 g were divided into three groups: noninbred normotensive rats — NR (control), SHR (Okamoto-Aoki line), and rats with renovascular hypertension (RVHR). A coil with internal diameter of 0.35 mm was wound around the left renal artery of the last group 28-30 days before the experiment, and their right kidney was completely removed.

The rats were anesthetized with urethane (600 mg/kg) and chloralose (40 mg/kg). The arterial pressure (BP) was measured in the carotid artery by an EMT-34 electromanometer. Momentary values of the heart rate (HR) were determined with a digital pulsotachometer, triggered

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