EFFECT OF THE GABA ANTAGONIST 4-ETHYLBICYCLOPHOSPHATE ON GLOBAL AND SYNAPTIC NEURONAL EVOKED RESPONSES IN HIPPOCAMPAL SLICES

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Systematic research into the biological activity and mechanism of action of 4-alkyl derivatives of bicyclophosphates (BCP) originated with the work of Bellet and Casida [3]. It was later shown [4] that the epileptogenic properties of these substances are connected with their ability to depress inhibitory influences of γ-aminobutyric acid (GABA). BCP inhibit specific binding of the labeled picrotoxin analog ^{3}H-dihydropicrotoxin [11]. The 4-ethyl- and 4-isopropyl-BCP depress presynaptic inhibition in n. cuneatum by a greater degree than standard GABA antagonists such as bicuculline and picrotoxin [6]. Meanwhile, there are no data in the literature on the effect of 4-alkyl derivatives of BCP on postsynaptic inhibition in the brain.

The aim of this investigation was to study the effect of the direct GABA antagonist 4-ethyl-BCP on global and synaptic activity of hippocampal pyramidal cells.

EXPERIMENTAL METHOD

Experiments were carried out on BALB mice aged from 1.5 to 2 months. Hippocampal slices were cut and incubated by the method described in [1]. The circulating fluid was a salt solution of the following composition (in mM): NaCl – 124, KCl – 3.5 – $CaCl_2$ – 2.0, $MgSO_4$ – 2.0, KH_2PO_4 – 1.24, $NaHCO_3$ – 26.0, glucose 10. The solution was saturated with a gas mixture of 95% O_2 + 5% CO_2. The slice was fixed in a thermostated cuvette (32-34°C) on a nylon grid. Bipolar glass stimulating electrodes were located in a radial layer through which pass Schaffer's collaterals (SC), which are axon collaterals of area CA3 neurons, terminating on dendrites of pyramidal cells in area CA1. The glass recording microelectrode was inserted into the layer of bodies of area CA1 pyramidal cells (Fig. 1b). The electrodes were filled with the fluid used for perfusion.

Stimulation consisted of square pulses 0.1 msec in duration, applied at a frequency of 0.1 Hz. The field potential began to be recorded 1 h after the slice was cut. The strength of the testing stimulus was usually 1.5 times greater than the threshold for evocation of a population spike (PS).

The FP were photographed from the screen of an S1-18 oscilloscope on film, after which the amplitude of the primary and secondary PS were measured (Fig. 1b). The results were subjected to statistical analysis on a Hewlett-Packard 98-35 A computer, as a result of which each point on the graphs reflects the amplitude of the primary and secondary PS, averaged for 5-12 experiments, for a period of 50 sec (interval between stimuli 10 sec).

Microelectrodes filled with 1 M potassium citrate solution and with a resistance of 50-80 MΩ were used for intracellular recording.

Solutions of the test substances were prepared before the experiment and added directly to the perfusion solution. The duration of exposure to the substances was 5 min. Picrotoxin was obtained from Sigma and 4-ethyl-BCP was synthesized by the method described in [12]. The structural formula of 4-ethyl-BCP is shown in Fig. 1a.

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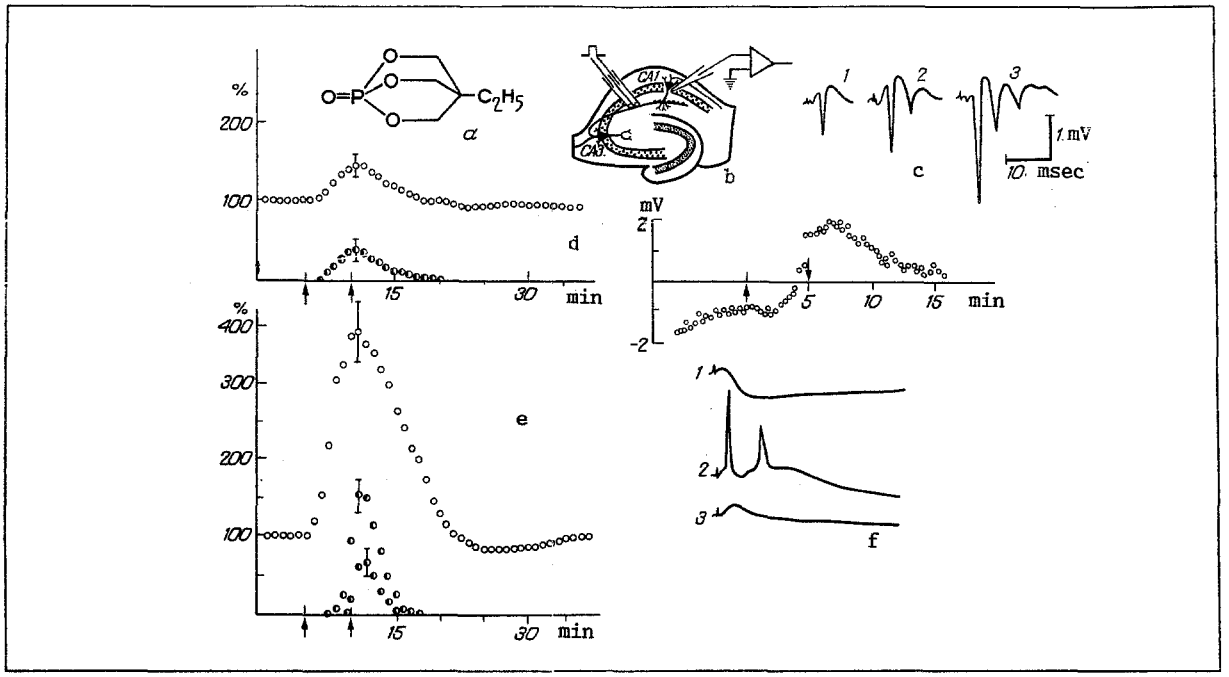

Fig. 1. Effect of 4-ethyl-BCP on inhibitory synaptic processes in hippocampal slices. a) Structural formula of 4-ethyl-BCP; b) diagram of transverse section through hippocampus; c) examples of field potentials. 1) In control, 2) on achievement of maximum of response to application of 4-ethyl-BCP (in concentration of 5 μM), 3) the same, after application of 4-ethyl-BCP in concentration of 10 μM; d) change in amplitude of primary and secondary PS in response to application of 4-ethyl-BCP in concentration of 5 μM; e) the same, application of 4-ethyl-BCP in concentration of 10 μM. d, e) Amplitudes of primary and secondary spikes shown as percentages of primary in control; empty circles indicate primary peak, half-shaded circles — first secondary peak, filled circles — second secondary peak, arrows indicate beginning and end of application, f) results of intracellular recording. Change in inhibitory postsynaptic potential of pyramidal nerve cell with time following application of 4-ethyl-BCP in concentration of 10 μM (period of application indicated by arrows) is shown. Resting potential of membrane at 55 mV taken as zero on ordinate. 1, 2, 3) Examples of postsynaptic potentials in control, with achievement of maximum of response to application of 4-ethyl-BCP in a concentration of 10 μM and after rinsing out for 10 min respectively.

EXPERIMENTAL RESULTS

FP recorded in the layer of bodies of hippocampal pyramidal neurons consists of a positive wave with a fast negative deviation superposed on it — a PS (Fig. 1b), reflecting the synchronized discharge of the pyramidal neurons. A change in amplitude of PS under the influence of physiologically active substances is often used as an indicator of their effect on reactivity of the pyramidal neurons [7, 8]. The magnitude and number of secondary PS in the response may also be regarded as the result of the epileptogenic action of the substance, for their presence is evidence of an increase in the number of pyramidal cell discharges in area CA1 in response to stimulation of SC. The number and magnitude of the secondary PS were therefore used in the work in addition to the change in amplitude of the primary PS with time, as a test of the epileptogenic activity of 4-ethyl-BCP. Effects induced by 4-ethyl-BCP were compared with effects of the well studied convulsant, picrotoxin [7].

Application of 4-ethyl-BCP in a concentration of 5 μM caused an increase of 45 ± 15% in the primary PS, and the maximal amplitude of the first secondary PS was 30 ± 10% of the amplitude of the primary PS in the control. The time taken to reach the maximum of the spikes was 3 and 5 min respectively (Fig. 1d).

Application of 4-ethyl-BCP in a concentration of 10 μM caused an increase in the primary PS by 300 ± 36% and the appearance of two secondary PS with amplitudes of 150 ± 18 and 60 ± 12% of the primary PS in the control (Fig. 1e).

Changes in the amplitude of PS in response to application of 4-ethyl-BCP considerably exceeded the changes induced by picrotoxin: to application in a concentration of 5 μM by half, to application in a concentration of 10 μM, tenfold. It must be noted, however, that 4-ethyl-BCP possesses a comparatively low index of specificity ($K_d \sim 10$ μM), which can be suggested for a substance with LD_{50} = 1.0 mg/kg [8]. For comparison, for picrotoxin $K_d \sim 1.0$ μM when LD_{50} = 1.5-3.0 mg/kg [5]. Our own results, together with data obtained by other workers, suggest partial disparity between populations of binding sites of 4-ethyl-BCP and for picrotoxin [2].

The aim of the next series of experiments was to study the effect of preliminary application of GABA on 4-ethyl-BCP activity and the effect of GABA on 4-ethyl-BCF activity when applied simultaneously to the slice. In both cases GABA was used in a concentration of 10 μM and 4-ethyl-BCP in a concentration of 5 μM. In the five experiments, 4-ethyl-BCP, applied after GABA to the hippocampal slice, caused a very small increase in amplitude of the underlying PS. No secondary PS were observed under these circumstances. Combined application of GABA and 4-ethyl-BCP induced mild inhibition of the primary PS in three cases. In two cases no effect was observed on the structure of FP.

The mechanism of depression of the effects of 4-ethyl-BCP under the influence of GABA is not clear and requires further study. However, the fact that GABA inhibited the effects of 4-ethyl-BCP in concentrations which had no appreciable effect on FP can be regarded as evidence of the existence of common points of application of the action of these two substances, and as evidence that 4-ethyl-BCP can influence GABA-ergic mechanisms.

Intracellular recording of evoked synaptic activity of hippocampal pyramidal neurons showed that application of 4-ethyl-BCP (5 μM) leads to a decrease in amplitude of evoked inhibitory postsynaptic potentials, which is accompanied initially by the appearance of short-latency action potentials in response to stimulation if they were absent in the control, and then by secondary action potentials (Fig. 1f). These findings confirm the view that the convulsant action of 4-ethyl-BCP is connected with blockade of inhibitory inputs to the neurons.

Thus 4-ethyl-BCP has a marked reversible dose-dependent facilitatory action on responses of the pyramidal cells in hippocampal area CA1 that is antagonistic to the action of GABA; the efficacy of 4-ethyl-BCP, moreover, considerably exceeds that of picrotoxin.

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