TETRODOTOXIN-SENSITIVE COMPONENT OF THE DEPOLARIZATION RESPONSE TO GABA APPLICATION TO PYRAMIDAL CELL DENDRITES IN HIPPOCAMPAL AREA CA1

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It is now firmly established that gamma-aminobutyric acid (GABA) is the inhibitory mediator in the hippocampus and other structures of the CNS [4]. During orthodromic stimulation of pyramidal neurons (PN) as a rule a biphasic hyperpolarization — inhibitory postsynaptic potential (IPSP), mediated by GABA, is recorded. The first phase of this potential is connected with an increase of permeability for Cl^-, which is blocked by picrotoxin (PTX) and mediated by $GABA_a$-receptors (blocked by bicuculline), whereas the second phase is connected with increased potassium conductance and is mediated through $GABA_b$-receptors [8, 9]. Relatively recently, by the use of various procedures, a third, depolarization component of the orthodromic IPSP, known to be generated on dendrites has been found [3, 11].

On local application of GABA to the soma of hippocampal PN as a rule membrane hyperpolarization is recorded, but depolarization in response to application to the dendrites [2, 6, 9, 12]. The pharmacological properties of depolarization responses (DR) are similar to the properties of hyperpolarization responses (HR) mediated by $GABA_a$-receptors: they are inhibited by bicuculline [10] and PTX [7]. The ionic nature of DR remains largely unexplained, but the fact that they are inhibited by PTX is evidence of increased membrane permeability for Cl^- [7].

Depolarization responses have an amplitude of 5-20 mV and are accompanied by a fall of membrane resistance by 30-50% [2, 9], and they perform an inhibitory function through intensive by-passing of the neuron membrane. However, despite intensive by-passing during the development of DR several workers have noted that it can induce action potentials [9]. On the basis of these data we suggested that the electrotonic spread of DR along the dendrite toward the soma can induce a local response in neighboring areas of the membrane not in contact with the applied GABA. In this case the DR recorded in the soma of pyramidal neurons to application of GABA to the dendrites ought to include a component linked with activation of voltage-sensitive Na-channels. The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices from C57BL/6 mice aged from 1 to 3 months. The method of preparing the slices and the conditions of incubation were described in [1]. Neuronal activity in area CA1 was recorded intracellularly by means of electrodes filled with 1 M potassium acetate solution, and field potentials were recorded to stimulation of Schaffer's collaterals.

GABA was applied iontophoretically (concentration 1 M, pH 7) to the region of the apical dendrites of PN at a distance of 150-250 μ from the pyramidal layer through an unsplit microelectrode. To ensure the local nature of the response, short (50-100 msec) pulses of current with amplitude from 8 to 20 nA were used. The membrane potential (MP) of the neuron was measured by passing hyper- and depolarizing direct currents. Current—voltage characteristic curves of the neurons and graphs of

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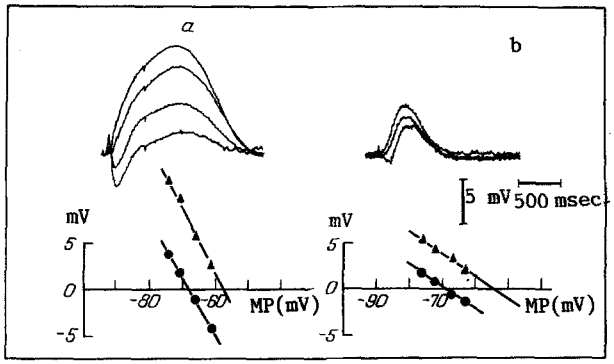

Fig. 1. Changes in reversal point of DR to iontophoretic application of GABA to dendrites of a neuron in hippocampal area CA1, using different injecting currents. Abscissa, MP (in mV); ordinate, amplitude of responses (in mV). a) Responses with injecting current of 40 nA and duration 400 msec, b) with current of 20 nA and duration of 100 msec. Here and in Fig. 2, above — separate responses of neuron to GABA at different MP levels. Graphs show dependence of DR (triangles) and HR (circles) to GABA on value of MP. Measurements of amplitude of responses made at their peak values. Each point of graph represents mean amplitude of eight responses. Resting potential of neuron 67 mV.

dependence of the amplitude of DR on MP were plotted. The reversal point of DR was determined by extrapolation of values obtained at different MP levels. Tetrodotoxin (TTX, 0.1-0.5 μM) and PTX (1-5 μM) were added to the perfusion solution.

The data were recorded on floppy disks and analyzed by PDP-9 computer (USA).

EXPERIMENTAL RESULTS

Iontophoretic application of GABA to the region of application of dendrites of PN by currents of 40-60 nA, and 0.4-1 sec in duration, induced a biphasic hyper- and depolarization response 2-4 sec in duration in the soma of PN (Fig. 1a). The development of DR was accompanied by lowering of the membrane resistance by 30-50%. The initial hyperpolarization component of the response (HR) was reversed at about —70 mV, whereas DR had a reversal point at about —60 mV (Fig. 1a, graph). These parameters of HR and DR agreed closely with those described in the literature [6, 9].

Limitation of the region of action of GABA to a local area of the dendrite by reducing the injecting current (see: Experimental Method) led to a decrease in the angle of slope of the dependence of the amplitude of DR on MP and displacement of the reversal point of DR in the direction toward depolarization by 5-15 mV (Fig. 1b, n = 3) or its disappearance (Fig. 2a, n = 11), whereas the reversal point of HR was unchanged (Fig. 1). The amplitude of DR with these parameters of GABA injection was 2-5 mV and its duration under 1 sec, with lowering of membrane resistance by 15-30%. Since with reduction of the injecting currents not only was the angle of slope of the curve of amplitude of DR as a function of MP reduced, but it was also shifted to the right (Fig. 1), we postulated that changes in the reversal point were connected with the presence of a voltage-dependent component of DR, with a relatively minor contribution when high concentrations of GABA were used. Later, therefore, GABA was injected using parameters described in "Experimental Method."

To determine whether this component was connected with activation of voltage-dependent sodium permeability, we used TTX, a blocker of voltage-dependent sodium channels, and which, in the concentrations used (0.1-1 μM), blocked action potentials of neurons to depolarizing pulses of current.

Perfusion of the slices with TTX solution led to reduction of the amplitude of DR to GABA by 20-80% (n = 12). Inhibition of DR, when TTX was used in higher concentrations (1 μM, n = 2) was considerable, and this made it difficult to determine the reversal points after administration of TTX. After application of TTX in lower concentrations (0.1-0.5 μM) an increase in the angle of slope of the dependence of amplitude of DR on MP was observed, and the reversal point of DR in this case was shifted toward hyperpolarization (Fig. 2a, c). This fact indicates that the sensitivity of DR to TTX depends on the MP level, in agreement with the voltage-sensitive character of the sodium current.

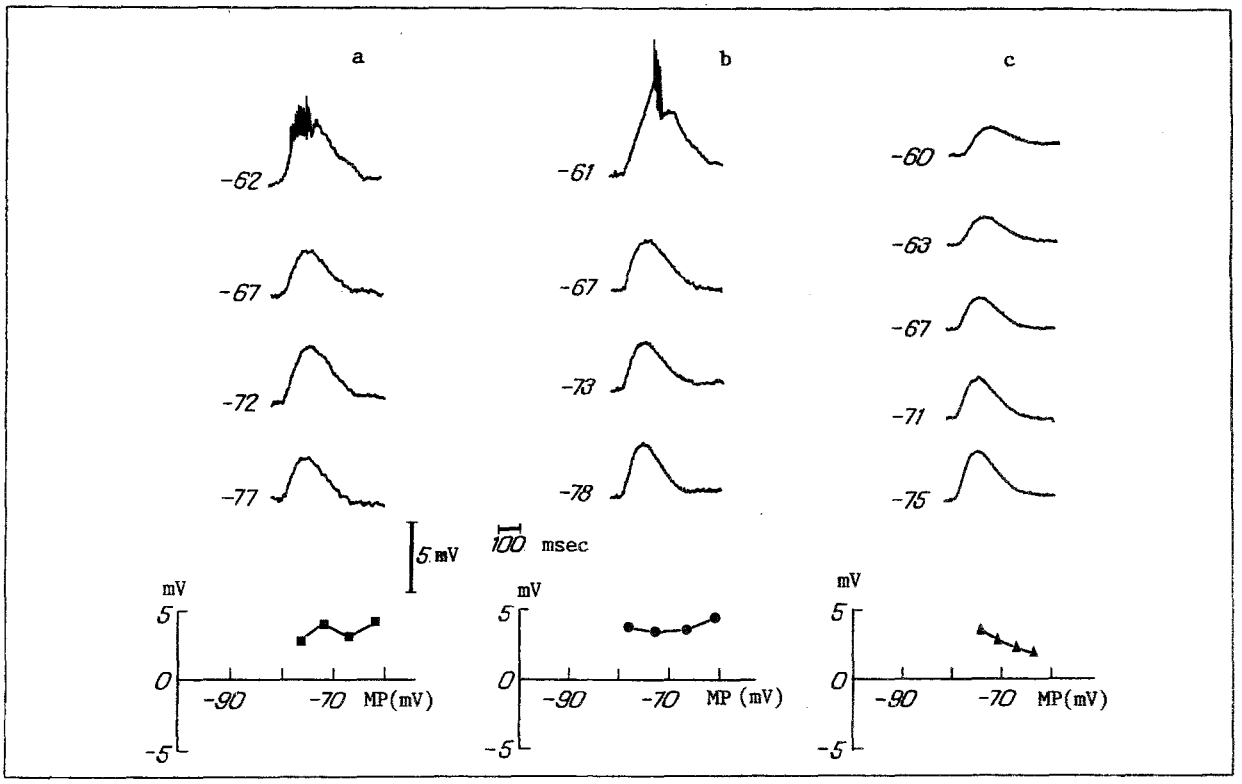

Fig. 2. DR of neuron to iontophoretic application of GABA (20 nA, 50 msec) in control (a), during blocking of synaptic transmission by solution containing low calcium concentration (0.5 mM) and high magnesium concentration (8 mM; b) and to application of TTX (0.5 μM) preceded by solution with low calcium concentration (c). Top left — value of MP (in mV). Below — graphs showing DR as a function of MP. Each point on graph represents mean amplitude of four responses. Resting potential of neuron 67 mV.

To rule out the possibility that the inhibitory action of TTX on DR is connected with the blocking of synaptic transmission caused by it, we studied the effect on DR of blocking synaptic transmission by the action of a solution with a low calcium (0.5 mM) and high magnesium concentration (8 mM). Perfusion of the slices with this solution led to inhibition of synaptic transmission but did not affect the reversal point of DR even during prolonged incubation (Fig. 2b), evidence against the participation of synaptic afferents in DR generation. Against the background of the blocking of synaptic transmission, TTX as before caused a shift of the reversal point of DR toward hyperpolarization (Fig. 2c, $n = 4$).

The DR described above were completely blocked by low concentrations of PTX (0.5-1.0 μM, $n = 3$), evidence of their chloride nature, and it rules out any possibility of direct activation of the dendrite by the currents used to inject the GABA.

Thus the present investigation showed that reducing the currents used to inject GABA shifted the reversal point of DR toward depolarization and toward the appearance of a component of DR which depends on MP.

Under the influence of small doses the reversal point of DR was shifted toward hyperpolarization; if it was absent in the control, moreover, it appeared and its amplitude lay between −30 and −50 mV. Since TTX is a blocker of voltage-sensitive sodium permeability, and since its action on the chloride channel or on the GABA receptor has not been described, we can postulate that the voltage-sensitive component of DR is due to sodium permeability, activated during electrotonic leaking of the GABA-induced depolarization to neighboring parts of the membrane. The existence of this component is partly responsible for the shift of the reversal point of DR toward depolarization or the absence of a reversal point of DR when determined by extrapolation, following local application of GABA.

In our view the increase in sodium conductance which we found in the responses to GABA is observed under natural conditions during activation of the inhibitory synapses on the dendrites and the spread of the GABA-induced depolarization to neighboring parts of the membranes, not exposed to the direct action of GABA. In this connection it can be tentatively suggested that activation of inhibitory inputs on certain regions of the dendrites may weaken the efficacy of some excitatory inputs and strengthen the efficacy of others.

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EFFECT OF ETHANOL ON BRAIN LEVELS OF DOPAMINE AND ITS METABOLITES IN RATS DIFFERING IN SENSITIVITY TO STRESS

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There is experimental evidence that addiction to ethanol and to other narcotics is realized through participation of the brain dopamine system. For instance, dopamine antagonists block voluntary ethanol consumption and destruction of dopamine neurons in the midbrain is accompanied by reduction of voluntary ethanol consumption [9, 11]. Ethanol administration is accompanied by activation of dopamine neurons, especially in those brain regions where their mesolimbic and mesocortical projections terminate [9, 10].

Studies of the effect of ethanol on dopamine metabolism have been undertaken many times but without taking account of differences in the organization of projections of dopaminergic neurons in the CNS or of initial differences between animals.

The aim of this investigation was to study the effect of ethanol on dopamine metabolism in rats differing in their sensitivity to stress. It was shown previously that this difference correlates with the level of ethanol consumption [4]. The animals were subjected to forced swimming and their sensitivity to stress was estimated as the duration of immobilization. Levels of dopamine and its metabolites were determined in the medial prefrontal cortex, nucleus accumbens, and striatum, so that dopamine metabolism could be characterized in mesocortical mesolimbic, and mesostriatal conducting systems [8].

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