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Effect of Electrical Cutaneous Stimulation on the Level of Succinate Dehydrogenase Activation and Changes in Glutamatergic Synaptic Transmission in Response to Sensory Stimulation

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It is shown that the rise of succinate dehydrogenase activity in the hippocampus depends on the number of sensory stimuli presented before decapitation, which correlates with changes in the efficiency of glutamatergic synaptic transmission in hippocampal sections from the same animal. Electrocutaneous stimulation potentiates the activation of succinate dehydrogenase induced by sensory stimulation probably due to enhanced glutamate release.

Key Words: *succinate dehydrogenase; population spike of the hippocampus; frequency-dependent facilitation; frequency-dependent depression*

The major afferent pathways of the hippocampus are glutamatergic [3,10]. Hence, long-term rearrangements caused by different types of sensory stimulation are probably mediated through modulations of glutamate metabolism [4,8,9,12,14].

Glutamate is taken up from the synaptic gap by nervous endings or glial cells [7,11]. It has been shown that part of glutamate released during excitation replenishes the pool of the transmitter in the synaptic gap, while the other part becomes involved into energy metabolism in astrocytes at the level of α-ketoglutarate and succinate [5,6,13]. Thus, in any case glutamate should be expected to influence the level of succinate dehydrogenase (SDH) activity.

A correlation was previously found between SDH activity and the parameters of synaptic transmission in the hippocampus (population spike amplitude, PSA, and the direction of plastic processes in rhythmic stimulation) [2].

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The aim of the present study was to evaluate the effect of electrocutaneous stimulation (ECS) on changes in SDH activity and parameters of synaptic transmission in hippocampus sections of the same animals in response to sensory stimulation.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats. Control animals (group 1) were sacrificed without being exposed to any stimulation, various numbers of light flashes were presented to group 2 immediately before decapitation, group 3 was exposed to various numbers of electrocutaneous stimuli, and various number of light stimuli and ECS were presented to group 4.

Electrophysiological studies were carried out on surviving hippocampal sections using the generally accepted procedure. The total electrical activity was recorded in the CA1 area using a glass microelectrode filled with potassium citrate. Stimulating electrodes were placed on Schaffer's collaterals. Stimulation parameters were selected 30 min after preparation of a hippocampal section. The intensity of stimulation slightly exceeded the threshold for eliciting a population spike (0.1 msec; 1-20 V). The main experiment was started 30 min after establishing the threshold. Stimulation was carried out as series of 10 presentations at a frequency of 0.2 Hz at 30-min intervals.

In the remaining part of the hippocampus, SDH activity was measured using a quantitative histochemical method [1] and expressed in arbitrary units (arb. units, mmol formazan/mol protein nitrogen/min).

The asymptotic function of the rise of SDH activity in response to increasing number of light flashes presented before decapitation was aligned using the regression equation: $Y=A-D\times10^{-kx}$, where A is the magnitude of asymptote, D is the difference between the asymptote and the experimental value, and k is the growth coefficient.

RESULTS

The relation between the rise of SDH activity and changes in the parameters of synaptic transmission varied in different modes of sensory stimulation.

It was found that the hippocampal level of SDH depended on the number of stimuli presented before decapitation. Figure 1, *a* shows that SDH activity was significantly lower in rats presented only ECS at any number of stimuli. At first glance, motivational or stressory component of the sensory stimulus inhibits some factors underlying the rise of SDH activity in response to sensory stimulation. However, it cannot be excluded that glutamatergic endings of

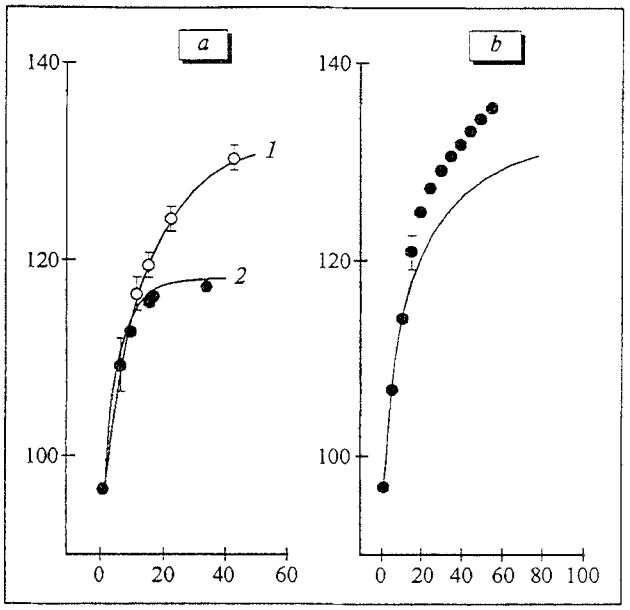

Fig. 1. Activity of succinate dehydrogenase (SDH) as a function of the number of sensory stimuli. Abscissa: number of stimuli presented before decapitation; ordinate: SDH activity, arb. units in rats exposed to photic (*a*, *1*) or electrocutaneous stimulation (*a*, *2*), or to both stimuli (*b*). Solid lines: calculated regression functions.

the ECS projections in the hippocampus are less abundant.

For verification of this assumption the rats of group 4 were presented different number of sensory stimuli of various modalities (light and electric current).

Figure 1 shows that as the number of stimuli increases, SDH activity rises more slowly. The rise of SDH activity can be best described by an asymptotic function (Fig. 1, *a*).

From the given parameters of these two functions the rate of elevation of SDH activity in response to successive presentation of light and electrocutaneous stimuli (Fig. 1, *b*) can be calculated. It is evident that in the absence of negative effect of the stimulus on SDH activity, the experimental curve should coincide with the calculated one. It was found that hippocampal SDH activity in rats presented various numbers of different stimuli significantly differs from the calculated values (Fig. 1, *b*). Consequently, ECS enhances some processes underlying SDH activation in response to sensory stimulation. This suggests that the rise of hippocampal SDH activity in response to specific sensory stimulation is presumably modulated by a constant factor such as a motivational component or stress.

Published data [5-7,11,13] and our experimental findings [2] suggest that the rise of SDH activity in the hippocampus in response to sensory stimulation is associated with the uptake of glutamate released

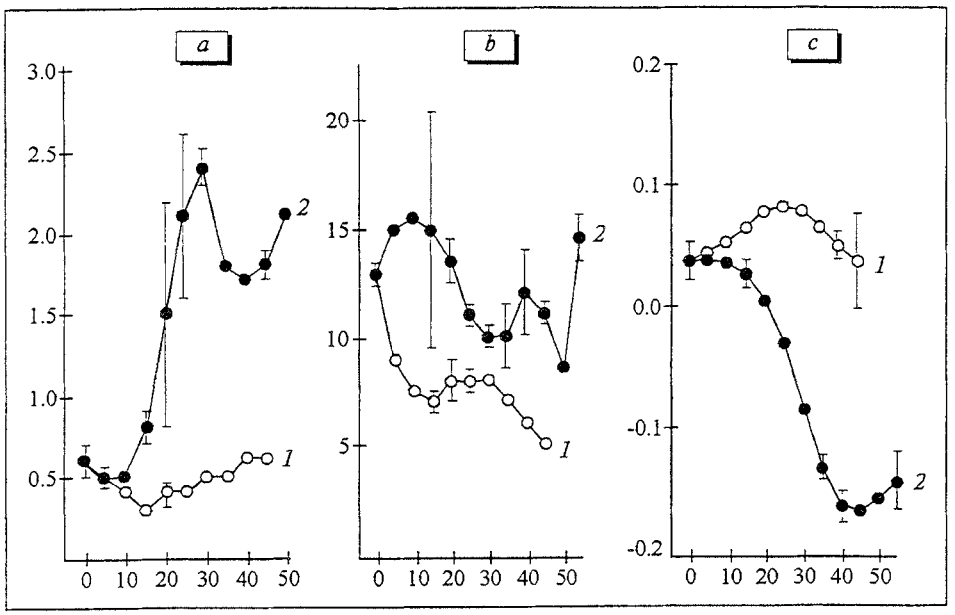

Fig. 2. Parameters of synaptic transmission in hippocampal sections as a function of the number of sensory stimuli. Abscissa: number of stimuli presented before decapitation; ordinate: *a*) amplitude of population spike, mV; *b*) population spike threshold, V; *c*) change in the amplitude $\bar{x}$-x_1, mV, during rhythmic stimulation of Schaffer's collaterals (10 stimulus, 0.2 Hz). *1*) light flashes; *2*) stimulation of both modalities (light flashes and electrocutaneous stimuli).

from the nerve endings by astrocytes and its subsequent transformation in the Krebs cycle. From the viewpoint of this hypothesis, presentation of ECS, both isolated or in combination with light flashes, enhances glutamate incorporation into the Krebs cycle. Thus, the observed rise of SDH activity caused by sensory stimulation is probably associated with the effect of defense motivation on both glutamate release in response to the sensory stimulus and the proportion between glutamate uptake by astrocytes and nerve endings.

The amplitude of the population spike is an important parameter reflecting the level of glutamate release in the hippocampus. If the hypothesis on the modulating effect of aversive stimulus on the efficiency of glutamatergic transmission is valid, PSA in hippocampal sections from animals presented ECS and those presented light flashes will be different.

The rate of plastic processes and PSA also depended on the number of sensory stimuli presented before decapitation. Figure 2, *a* shows the dynamics of PSA values recorded in hippocampal sections of animals presented various numbers of light or mixed stimuli prior to decapitation. The dynamics was different in different experimental groups (Fig. 2, *a*). We observed an initial decrease of PSA in rats of groups 2 and 4, which can be attributed to utilization of glutamate as a substrate for energy metabolism leading to progressive depletion of its content in nerve endings [13].

Further photostimulation caused a gradual increase in PSA (Fig. 2, *a*). Using dispersion analysis, we previously showed that changes in PSA in the hippocampus in response to photostimulation are largely determined by a lower rise of SDH activity, which may result in a reduced utilization of glutamate in energy metabolism of astrocytes. In this case, the rise of PSA starting from the 20th stimulus is probably to some extent associated with enhanced glutamate reuptake during photostimulation. This also manifests itself in enhanced frequency-dependent facilitation during a low-frequency rhythmic stimulation (Fig. 2, *c*). Dispersion analysis showed that the frequency-dependent facilitation in the hippocampal sections from rats decapitated after photostimulation resulted from the rise of SDH activity.

Thus, on the basis of the hypothesis on astroneuronal interactions during excitation it can be expected that changes in PSA caused by photostimulation are determined predominantly by altered proportion between glutamate uptake by astrocyte and nerve terminals.

The addition of ECS to photostimulation has a considerable effect on this process, which manifests itself as a progressive rise of PSA in response to increasing numbers of mixed stimuli (Fig. 2, *a*). Starting from the 20th stimulus, PSA significantly exceeded the values observed in the group presented only light stimuli; this difference between experimental groups increases during further stimulation. PSA in hippocampal sections from rats decapitated after a sufficient number of ECS attained 2.4±0.1 mV.

The fact that against the background of ECS the increase in both SDH activity and PSA is more pronounced than after isolated photostimulation suggests that ECS enhances glutamate release in response to stimulation. This is also confirmed by higher PSA threshold in animals exposed to ECS (Fig. 2, *b*). Consequently, the rise of PSA cannot be attributed to elevated neuron excitability.

Moreover, the direction of plastic processes during rhythmic stimulation was different in groups 2 and 4 (Fig. 2, *c*). For instance, unlike isolated photostimulation, the long-term presentation of ECS caused progressive frequency depression (Fig. 2, *c*). The different level of glutamate reuptake should have an immediate impact on plastic changes during rhythmic stimulation. Hence, the inhibition of glutamate reuptake caused by ECS in comparison with isolated photostimulation can be expected.

Thus, the aversive stimulation results in inhibition of glutamate uptake by nerve endings.

These findings suggest that neuron activity in the hippocampus in different experimental situations, in particular those related to negative reinforcement, is to a great extent determined by modulation of glutamate metabolism.

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