Effect of Sensory Input on the Levels of Enzymes Involved in Energy Metabolism

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Presentation of sensory stimuli of various modalities to rats immediately before their decapitation led to a significant increase in the level of succinate dehydrogenase in the hippocampus, the magnitude of the increase being dependent on the number of stimuli presented. In each individual case, this enzyme's activity was proportional to the amplitude of the population spike recorded in the hippocampus of the same rat. An inverse relationship was noted between the rate of plastic processes in the hippocampus upon rhythmic stimulation and succinate dehydrogenase activity.

Key Words: *sensory stimulation; hippocampus; succinate dehydrogenase; population spike; frequency facilitation*

The regulation of cell-cell interactions is inextricably bound up with assimilation-dissimilation processes. It has been shown that a major part of the glutamate secreted from nerve terminals becomes involved in energy metabolism [10-12,14,15]. Most of the glutamate is taken up by astrocytes [13]. The oxidation of exogenous glutamate provides an external energy source for astrocytes during neuronal excitation [5]. The remaining glutamate is taken up by neurons [13].

These findings have enabled a hypothesis to be formulated concerning astro-neuronal interactions during functional activity.

A convenient structure for verifying the validity of this hypothesis is the hippocampus. The main pathways in the latter are known to be glutamatergic [8]. Presentation of sensory stimuli should be accompanied by an elevation of glutamate secretion in the hippocampus and, according to the above hypothesis, will lead to increased uptake of glutamate by astrocytes followed by its entry into the Krebs cycle. This is bound to activate enzymes for the subsequent reactions. If so, then glutamate should be expected to influence primarily the level of succinate dehydrogenase (SDH). Indeed, as shown in several studies [1,3,5], glutamate has an activating effect on succinate oxidation, and such activation is usually explained by elimination of oxaloacetic acid, which inhibits SDH [1].

The purpose of this study was to elucidate the possible reasons for alterations in the level of SDH in response to sensory stimulation of different types.

MATERIALS AND METHODS

Four groups of Wistar rats were used for the experiments. Control rats were decapitated without being exposed to any stimulation. The second group were presented 15 light flashes immediately before decapitation, the third group was exposed to 15 electrocutaneous stimuli, and the fourth group,

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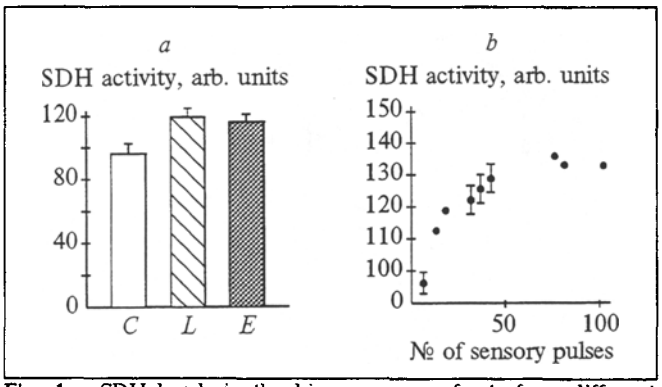

Fig. 1. SDH levels in the hippocampus of rats from different groups (*a*) and in relation to the number of stimuli presented before decapitation (*b*). *a*) *C*: control group ($n=18$); *L*: group of rats presented with 15 light flashes immediately before decapitation ($n=15$); *E*: group of rats presented with 15 electrocutaneous stimuli ($n=15$).

to various numbers (from 15 to 110) of photic and electrocutaneous stimuli in random order.

Electrophysiological studies were conducted on surviving hippocampal sections using the generally accepted procedure. Total electrical activity was recorded in the CA1 area with a glass microelectrode filled with potassium citrate. Stimulating electrodes were placed on Schaffer's collaterals. Stimulation parameters were selected 30 min after the preparation of a hippocampal section. The intensity of stimulation exceeded somewhat the threshold for eliciting a population spike (0.1 msec; 0.5-6 V). The main experiment was started 30 min after finding the threshold. Stimulation was carried out in series of 10 presentations each at a frequency of 0.2 Hz with 30-minute intervals between the series.

In the remaining part of the hippocampus, SDH activity was determined by a quantitative histochemical method [6,9]. Enzyme activity was expressed in arbitraty units (arb. units), namely in mmoles of formazan per mole of protein nitrogen per minute.

The significance of differences was evaluated by Student's test. Correlation coefficients were calculated to determine associations between the various parameters.

RESULTS

It was found that the presentation of sensory stimuli of various modalities immediately before

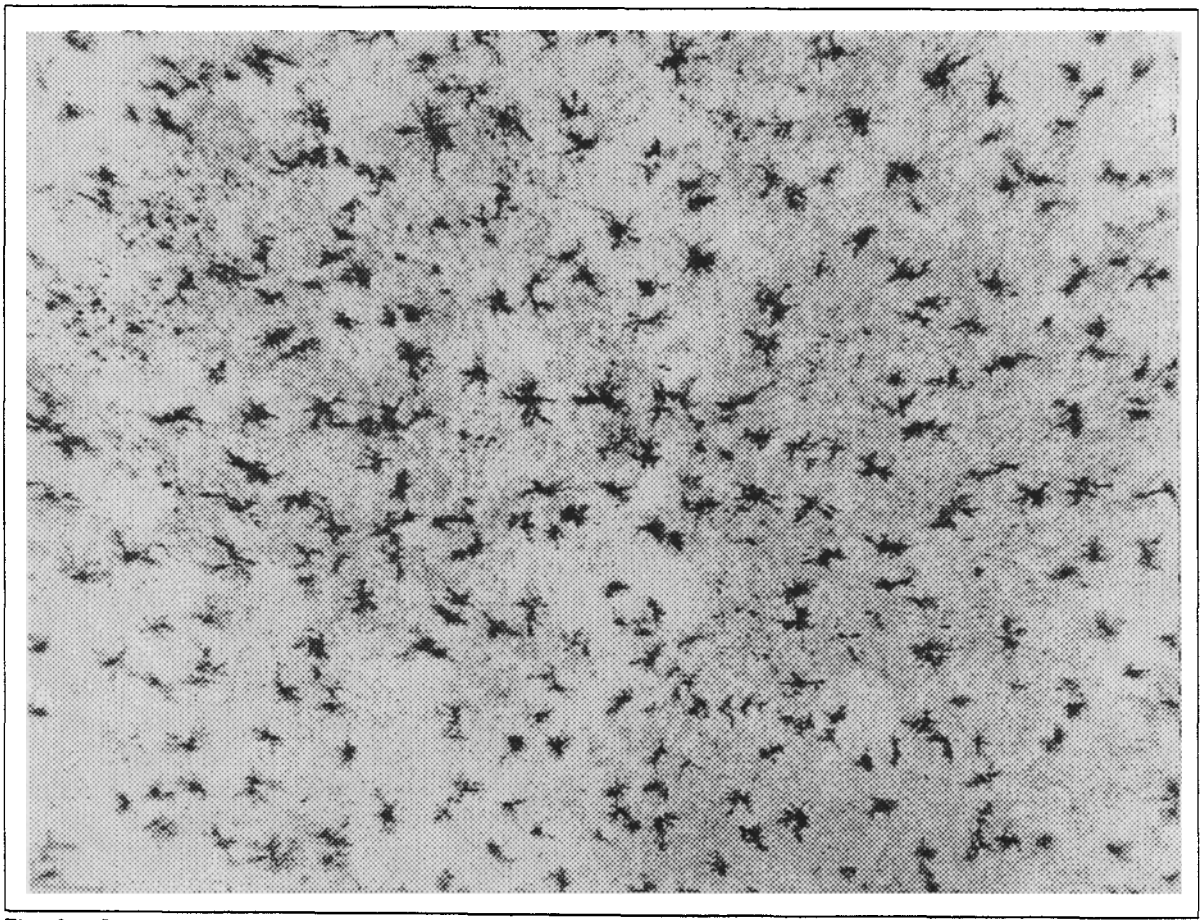

Fig. 2. Intensive reaction of SDH in cortical astrocytes of a rat in response to functional stimulation (spreading depression). ×200.

decapitation led to a significant rise of SDH in the hippocampus. Figure 1, *a* compares hippocampal SDH activity in the different groups of rats. In the control group, the mean SDH level was 96.6±0.8 arb. units, and the interindividual differences in this enzyme's activity were small (range, 90.4-102.1 arb. units). The presentation of any of the test stimuli resulted in a significant elevation of the hippocampal SDH level. The presentation of 15 light flashes just before decapitation brought about a significant elevation of enzyme activity ($p<0.01$) to a mean level of 119.6±0.6 arb. units (range, 116.4-123.5 arb. units). Similar effects were produced by 15 electrocutaneous stimulations prior to decapitation. In the latter group, the mean SDH level in the hippocampus was 116±0.4 arb. units, which also significantly exceeded ($p<0.01$) the control level. Even the lowest SDH activity recorded in this group (114.4 arb. units) was higher than the maximal activity in the control group, but the highest activity (119.5 arb. units) was somewhat lower than in the rats stimulated with light before decapitation. The mean SDH activity was significantly lower ($p<0.01$) than in the light-stimulated group.

Elevated SDH levels in the rat brain are usually thought to be associated either with individual characteristics of the animals [7] or with stress [2]. Our study indicates that the observed changes were most likely specific for sensory stimulation and suggests that any activation of hippocampal cells - not necessarily associated with motivational, emotiogenic, or reinforcing systems of the brain - should result in an elevated SDH level in these cells.

It appears, therefore, that the main reason for SDH elevation in the hippocampus was glutamate secretion as a result of sensory stimulation. We have shown using other models [4] that SDH elevation is primarily associated with activation of this enzyme in astrocyte-like cells (Fig. 2). These results support Hertz's hypothesis regarding astroglial interrelationships during sensory stimulation.

The hippocampal level of SDH depended on the number of stimuli presented before decapitation (Fig. 1, *b*). After the presentation of 15 sensory stimuli immediately before decapitation, the mean SDH level in the hippocampus was 23±0.4% higher than in the control group ($p<0.01$). When the number of stimuli was doubled, the control level was exceeded by 32±0.3%. In the range from 0 to 50 stimuli, this relation approached a linear one. Increasing the number of stimuli further had little or no effect. On the whole, the relationship of SDH activity to the number of sensory stimuli could best be described by a logarithmic function.

Thus, the effects from sequential presentation of stimuli were summated in time, though up to a certain limit. Continued stimulation led to a substantial reduction in the amount of secreted glutamate entering into the reaction that results in SDH production. The observed relationship is readily explainable in terms of Hertz's hypothesis.

Another argument in favor of Hertz's hypothesis is the dependence of SDH activity on parameters of synaptic transmission. One of the main parameters associated with the level of glutamate secretion in the hippocampus is the amplitude of the population spike. In our experiments, SDH activity recorded for each individual rat was found to be proportional to the amplitude of the population spike recorded in the section of the hippocampus from that animal (Fig. 3, *a*). The correlation between SDH activity and population spike amplitude in the hippocampus was high ($r=66$, $p<0.07$). The minimal amplitude (0.1 mV) recorded at the threshold current of stimulation corresponded to 92.5 arb. units of SDH. In the 0.2-1.1 mV range, SDH activity exceeded significantly ($p<0.05$) its minimal level. The maximal amplitude (1.3 mV) corresponded to the highest SDH activity (98.6 arb. units). It may be mentioned, though, that the scatter of data was greater than that found for the relationship between SDH activity and the number of behavioral stimuli presented prior to decapitation (data not shown). Such a relatively wide scatter appears to be due to the dependence of population spike amplitudes not only on the level of glutamate secretion, but also on other parameters not directly related to the SDH level.

On the other hand, an inverse relationship was noted between the rate of plastic processes

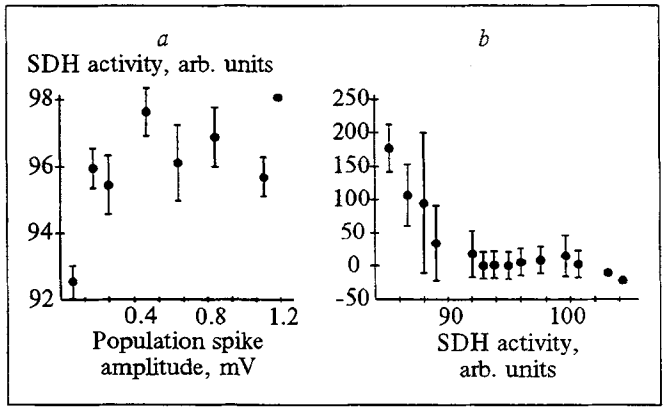

Fig. 3. SDH level as a funciton of the population spike amplitude (using near-threshold stimulation) recorded in the same hippocampus (*a*), and as a funciton of the rate of plastic processes using low-frequency (0.2 Hz) stimulation (*b*). Abscissa: increment of the amplitude of the hippocampal population spike during a stimulation series (10 pulses), expressed as % of the first response in the series.

upon rhythmic stimulation and SDH activity. When Schaffer's collaterals in the hippocampal CA1 area were stimulated at a low frequency (0.2 Hz), a progressive increase in the response to each subsequent stimulus was recorded in some hippocampal sections (frequency facilitation, FF). In other sections such stimulation resulted in a decrease in the population spike amplitude (low-frequency depression). Figure 3, *b* shows the relationship between the rate of change in responses to low-frequency stimulation and the SDH level recorded for the hippocampus from which the section was taken. The maximal level of FF (a 150% increase in the response per series of frequency stimulation) corresponded to 84 arb. units of SDH. In sections with a low SDH level (<90 arb. units), FF was recorded most often in the process of rhythmic stimulation. The hippocampi with intermediate SDH levels (90 to 103 arb. units) showed no plastic changes. In sections with high SDH levels (>103 arb. units), low-frequency depression predominated. From the viewpoint of Hertz's hypothesis this signifies that glutamate uptake by astrocytes is substantially lower in a hippocampus with a high level of FF. This is unlikely to be due to reduced secretion. Hence, enhanced glutamate reuptake by neuron terminals may be expected to occur in a rat hippocampus with increased FF. In fact, it may well be that such enhancement is one of the mechanisms of the FF phenomenon.

Taken together, our findings - SDH elevation as a result of sensory stimulation, specificity of this elevation for sensory stimulation, a direct relationship of the SDH level to the number of stimuli and the population spike amplitude, and its inverse relationship to the rate of plastic processes - all speak in favor of Hertz's hypothesis.

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