ELECTRICAL ACTIVITY OF HIPPOCAMPAL SLICES FROM MICE WITH METRAZOL KINDLING

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Repeated injections of metrazol in subthreshold doses causes the appearance of the kindling phenomenon, namely the appearance and progressive development of electrographic and behavioral seizures in response to a subconvulsant dose of an epileptogen, which initially does not evoke these effects. This state of enhanced predisposition to seizures persists in the animal for a long time after administration of the convulsant has ceased [6]. The pathogenetic mechanism of metrazol kindling have not been adequately studied. There was therefore good reason to investigate this phenomenon in a model of surviving slices of the hippocampus, a structure which has a special relationship with the development of epilepsy [1]. An advantage of experiments with hippocampal slices is that measured application of a substance directly to brain tissue is possible, and the stimulating and recording electrodes can be precisely located.

In the investigation described below evoked focal activity of surviving hippocampal slices from animals with developed metrazol kindling was studied.

EXPERIMENTAL METHOD

Inbred (CBA × C57BL/6)F_1 hybrid mice weighing 16-18 g were used. Kindling was induced by daily intraperitoneal injections of metrazol in a subconvulsant does (30 mg/kg, in a volume of 0.1 ml) for 1 month. The metrazol solution was made up immediately before injection. Physiological saline was injected in the same volume into animals of the control group. Investigations on hippocampal slices began not earlier than 1 week after the last injection of metrazol — against the background of developed kindling, manifested as behavioral seizures after injection of the same subthreshold dose of metrazol. After decapitation of the animals the brain was exposed and slices of hippocampus cut to a thickness of 200-300 μ, and immediately transferred into a special constant-temperature chamber. The slices were bathed in continuously flowing Yamomoto's salt solution [15], saturated beforehand with a gas mixture of 95% O_2 and 5% CO_2, and warmed to 34-35°C. The slice was fixed on nylon gauze by stimulating glass bipolar electrodes (diameter of tip 0.5-1 mm for the pair), filled with Yamomoto's solution. The electrodes were located in the radial layer through which pass Schaffer's collaterals, which are axons of neurons in area CA3 running to neurons in area CA1. The glass recording microelectrode (resistance 1-5 MΩ) also was filled with Yamomoto's solution and introduced into the layer of pyramidal neurons in area CA1. Fuller details of the technique were described previously [2]. Recording of evoked potentials (EPs) began 1 h after preparation of the slice and continued for 5-6 h. In the course of one experiment, hippocampal slices from mice of the experimental and control groups were studied consecutively.

The field potential in the hippocampus consists of a positive wave on which is superposed a negative wave or population spike (PS), reflecting the synchronized discharge of

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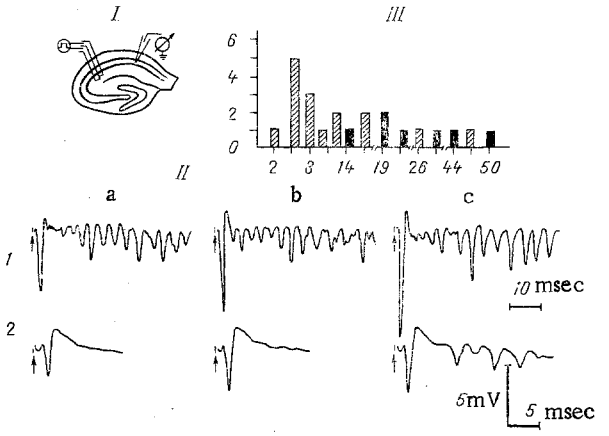

Fig. 1. EP in hippocampal slices from mice of control (2) and experimental (1) groups during stimulation by currents of different strengths. Abscissa, strength of stimulating current (in μA); ordinate, number of experiments. I) Scheme of hippocampal slice; II) EP during stimulation by current of 11 μA (a), 14 μA (b), and 60 μA (c). Arrows indicate time of application of stimulus; III) frequency of appearance of EPD depending on strength of stimulating current in control (obliquely shaded columns) and experimental (black columns) groups.

pyramidal neurons. The PS can serve as a measure of reactivity of the pyramidal neurons. The experiments began with investigation of the change in EP produced by a gradually increasing stimulating current (from 2 to 50 μA). Series of different stimuli (three stimuli per series, frequency 0.3 Hz, interval between series 10 sec) were used for this purpose. The parameters of stimulation were subsequently chosen so as to evoke the appearance of a single PS, whose amplitude remained unchanged in the course of several series of control tests (frequency of stimulation 0.1 Hz, 10 stimuli per series, interval between series 5 min). The second stage of the investigation was determination of the character of the change in focal responses to the action of epileptogens. For this purpose, solutions of metrazol (10^{-4} M), the sodium salt of benzylpenicillin (100 and 1000 U/ml), diazepam (10^{-5} M), metrazol (10^{-4} M) + diazepam (10^{-5} M), and the sodium salt of benzylpenicillin (1000 U/ml) + diazepam (10^{-5} M), made up in the incubation medium, were added to the surrounding solution.

EXPERIMENTAL RESULTS

During the study of changes in EP under the influence of a gradually increasing stimulating current, an evoked paroxysmal discharge (EPD), in the form of the additional PS in EP, was found to appear in animals of the experimental group during the action of a much weaker stimulating current (from 2 to 16 μA) (Fig. 1,II,III). Of the 15 hippocampal slices from animals of the experimental group studied there were two exceptions, when EPD appeared during testing with a strength of 26 and 50 μA (Fig. 1,III). In the control group EPD either did not appear at all even in response to a stimulating current of very high values (there was only an increase in amplitude of the first PS), or it appeared in response to a current of between 15 and 50 μA (Fig. 1,II, 2,III). An interval of 10-15 min was allowed after the end of investigation of the appearance of an EPD. After the interval the threshold of appearance of the EPD in the experimental group was even lower. In some cases, after investigation of the threshold of appearance of EPD in a hippocampal slice from a mouse of the experimental group depression of EP was observed, and it recovered after 20-30 min. In cases when a continuous recording of spontaneous activity was obtained, the appearance of spontaneous paroxysmal discharges was not observed. The next stage of the work was to investigate the appearance of EPD in response to the action of epileptogens, namely metrazol and the sodium salt of benzylpenicillin. EPD appears in hippocampal slices from mice in the experimental groups 20-110 sec after addition of 10^{-4} M metrazol (Fig. 2B, 2). In one experiment the latent period was 200 sec. The latent period of appearance of EPD varied from 50 to 130 sec. Differences between the experimental and control group were not significant, but just as with the action of metrazol, a tendency toward shortening of the reaction time of the animals of the experimental group to penicillin could be discerned.

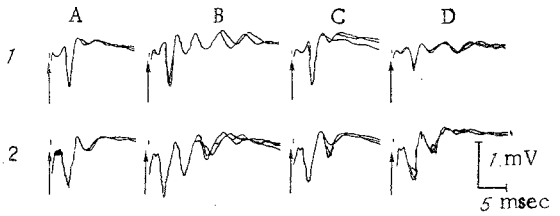

Fig. 2. Appearance of EPD in hippocampal slices from mice of experimental group in response to the action of penicillin (1) and metrazol (2). A) Control EP; B) EPD after addition of epileptogens to surrounding solution; C) EP after rinsing to remove epileptogens; D) EP after addition of epileptogens with diazepam to surrounding solution. Arrows indicate time of application of stimulus. Superposition of three responses.

When 100 U/ml of the sodium salt of benzylpenicillin was added the latent period of EPD in the experimental group varied from 50 to 190 sec (Fig. 2B,1), and in the control from 70 to 220 sec. Differences between the control and experimental groups are not significant, but just as in the case of metrazol, there was a tendency for the reaction time of animals of the experimental group to penicillin to become shorter.

When 1000 U/ml of the sodium salt of benzylpenicillin was added the latent period of EPD in a hippocampal slice from mice of the control group was 40-60 sec, compared with 70-80 sec in the experimental group.

To investigate inhibition of EPD, diazepam (10^{-5} M) was added to the external solution. Diazepam reversibly reduced the amplitude of PS by 50-70% in both control and experiment. When diazepam was added to the external solution simultaneously with penicillin, it also partially depressed the development of seizure activity (Fig. 2D,1). The previous level of the response was restored after rinsing to remove the added substances at a time which varied considerably in both the control and experimental groups, namely 5-60 min.

The investigations thus showed a marked fall in the threshold of EPD in the system of hippocampal pyramidal cells in area CA1 during stimulation of Schaffer's collaterals in mice with developed metrazol kindling. This was expressed at the appearance of EPD in response to a weaker stimulating current than in the control. The character of EPD in mice of the experimental group also is noteworthy: It continued after cessation of electrical stimulation and consisted of a large number of additional discharges (additional PS). This character of the response is evidence of the formation of a latent generator of pathologically enhanced excitation (GPEE), which is activated by trigger stimuli and then continues to work spontaneously for some time longer [4]. Some increase in predisposition to seizures also was observed in response to the action of metrazol and penicillin, reflected in shortening of the latent period of action of the convulsants compared with the control. The results are original for an *in vitro* model and agree with the results of *in vivo* studies [5], which showed that in developed metrazol kindling the sensitivity of the animals to the convulsant action of a number of GABA antagonists (picrotoxin, bicuculline, thiosemicarbazide, metrazol) is increased. These investigations, together with the results of *in vivo* experiments which demonstrated an increased predisposition to seizures in response to metrazol, picrotoxin, and lidocaine, in animals with kindling induced by electrical stimulation [9, 10, 11, 14], indicate a common pathogenesis for kindling evoked by electrical stimulation and by drugs. When the possible mechanisms of metrazol kindling are examined, it is important to note that, in view of data showing that metrazol is excreted comparatively quickly [8, 12, 13] and that disturbances of metrazol metabolism are not found if it is administered for long periods in subthreshold doses [9], the progressive increase in sensitivity to the action of convulsants cannot be attributed to accumulation of metrazol and disturbance of its biotransformation. This conclusion is also supported by the increased epileptogenicity of neurons in hippocampal slices for a long time after administration of metrazol has ceased, and the absence of seizures in the animals. It can be postulated on the basis of the results of investigation of electrical activity of various brain structures during the development of metrazol kindling, and also the results of the present investigation, that the hippocampus plays the role of determinant structure [4] in the development of chronic epileptization of the brain and the other behavioral changes associated with metrazol kindling. One possible mechanism of kind-

ling is a long-lasting change in efficiency of synaptic pathways in the hippocampus, which is probably similar in nature to the long-term potentiation which has been widely discussed in the literature [3]. The results now obtained are evidence that changes in reactivity of neurons accompanying kindling may be found in a separate hippocampal segment, so that this phenomenon can be studied at synaptic and molecular levels. This model can be used to study the effects of various antiepileptic agents.

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EFFECT OF THE SYMPATHETIC NERVOUS SYSTEM ON CARDIAC RHYTHM CONTROL DURING BURST STIMULATION OF THE VAGUS NERVE

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The cardiac rhythm can be controlled with a fair degree of accuracy by burst stimulation of the vagus nerve (VN) [2, 3, 6, 7]. The control phenomenon is manifested as close agreement between heart rate and the frequency of volleys of impulses used to stimulate VN [2, 3]. With a change in following frequency of the bursts within a certain range, the heart rate changes correspondingly. For each characteristic of the burst, determined by the number of impulses, there is a corresponding range of control of the cardiac rhythm. With the aid of a successive series of ranges, the cardiac rhythm can be controlled within wide limits [3]. No data could be found in the accessible literature on the effect of activation of the sympathetic nervous system on this phenomenon of heart rate control.

In this investigation the effect of the sympathetic nervous system on heart rate control was studied by means of burst stimulation of the vagus nerve.

EXPERIMENTAL METHOD

Experiments were carried out on 40 adult noninbred cats of both sexes. The animals were anesthetized intraperitoneally with a 1% solution of chloralose–pentobarbital mixture (75 and 15 mg/kg respectively). The right inferior cardiac nerve (ICN) and the right VN were identified and dissected. The peripheral ends of divided nerves were placed on platinum

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