EFFECT OF DIAZEPAM ON THE STATE OF Na,K-ATPASE IN A CORTICAL FOCUS OF HYPERACTIVITY INDUCED BY PENICILLIN

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Intramuscular injection of diazepam into rats in doses of 0.01 and 2 mg/kg 25-30 min after application of penicillin to the cerebral cortex was shown to lead to a disturbance of the periodic occurrence of epileptic fits (EF), to a change in their character, and to the appearance of periods of increased frequency of epileptiform discharges (ED). Injection of diazepam in a dose of 2 mg/kg 20 min before application of penicillin led to shortening of the latent period of ED in the epileptic focus and to an increase in their frequency in the period before the fit compared with the control without diazepam. EF appeared irregularly, their number was sharply reduced, and their duration increased. Injection of diazepam led to disappearance of the animal's motor response during ED and EF. Diazepam (2 mg/kg) in experiments in vivo did not affect Na,K-ATPase activity of unpurified cerebral cortical synaptosomes, but after creation of an epileptic focus it led to an increase in Na,K-ATPase activity in the primary focus and secondary foci dependent on it. It is suggested that the anticonvulsant action of diazepam may be based on its activating effect on Na,K-ATPase in neuron membranes in the epileptic focus.

KEY WORDS: focal epileptic focus; penicillin; diazepam; synaptosomes; Na,K-ATPase, increase in activity.

An important role in the mechanisms of formation of foci of epileptic activity may be played by changes in the activity of Na,K-ATPase, an enzyme which participates directly in the maintenance and restoration of ionic gradients relative to neuronal membranes [14, 15].

It was shown in the writers' laboratory that electrical stimulation of the brain [2], evoking convulsions, and application of penicillin to the cerebral cortex of rats [3] are accompanied by a decrease in Na,K-ATPase activity of unpurified synaptosomes isolated from the cortex; this effect, moreover, is observed actually during the latent period of formation of paroxysmal activity. It has been suggested that a decrease in Na,K-ATPase activity in the membranes of nerve endings is one of the mechanisms of epileptogenesis [1].

These conditions explain interest in a combined study of the state of Na,K-ATPase and electrophysiological characteristics in a focus of epileptiform activity and their changes under the influence of anticonvulsants. In the investigation described below the action of diazepam was studied on Na,K-ATPase and brain electrical activity in the zone of an epileptic focus created by local application of penicillin to the cerebral cortex in rats.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male albino rats weighing 180-200 g. Global electrical activity was recorded in the sensomotor cortex of both hemispheres and in the visual cortex of the right hemisphere by means of silver cortical electrodes. A focus of epileptic activity was created by application of a piece of filter paper measuring 1.5 × 3.5 mm, soaked in penicillin solution (sodium salt, 20,000 i.u./ml physiological saline) to the surface of the brain in the left sensomotor area. Pieces of filter paper soaked in physiological saline were applied to the surface of the cortex of the right hemisphere. The dura was removed from

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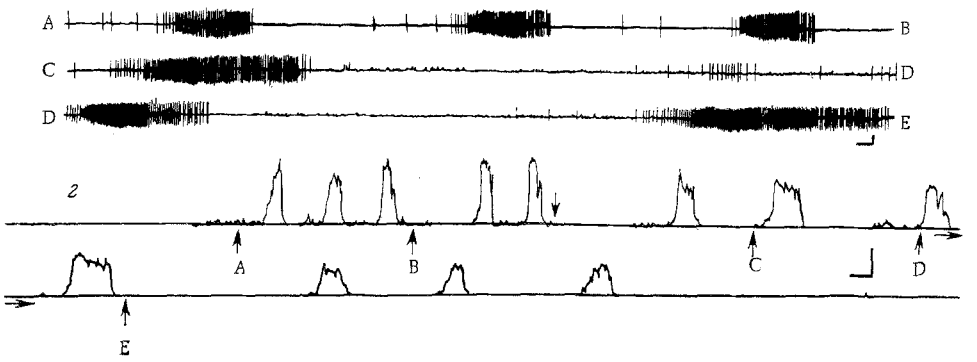

Fig. 1. Development of ED and EF in motor cortex of rats after application of penicillin solution to it followed by injection of diazepam. Letters at beginning and end of electrocorticograms (ECoG) (1) reflect ECoG recording periods designated by corresponding letters with arrow on histogram of momentary frequency of ED and EF (2). For ECoG, calibration of amplitude 400 μV, time 10 sec. For histogram: vertical calibration 10 spikes in 3 sec, horizontal, 1 min. Intramuscular injection of diazepam, 2 mg/kg, indicated by arrow (↓) on histogram of momentary frequency of ED and EF.

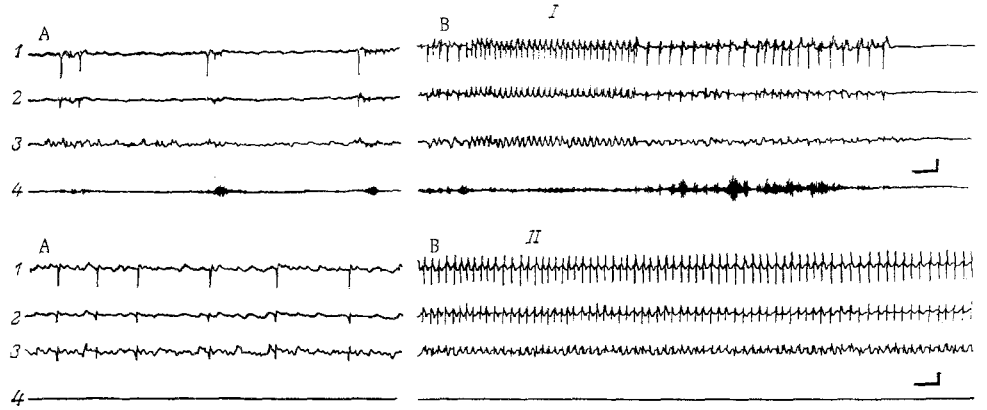

Fig. 2. Electrical activity in left (1) and right (2) sensomotor cortex and in visual cortex (3) in preconvulsive period (A) and during marked EF (B) in animals after application of penicillin to surface of left sensomotor cortex; seismogram (4). I) ECoG after application of penicillin; II) ECoG after application of penicillin preceded by injection of diazepam, 2 mg/kg, 20 min beforehand. Calibration: amplitude 400 μV, time 1 sec.

the brain surface in the regions for study the day before the experiment [3]. Diazepam (Gedeon Richter, Hungary) in doses of 0.01 and 2 mg/kg was injected intramuscularly. Samples for determination of Na,K-ATPase activity in unpurified cortical synaptosomes from rats receiving an injection of diazepam in a dose of 2 mg/kg 20 min before penicillin application were obtained with a special cooled spoon with sharpened edges from all regions to be studied 7-11 min after application. The micromodification of determination of Na,K-ATPase activity by a fluorometric method was described previously [6].

EXPERIMENTAL RESULTS

Changes in the Electrophysiological Characteristics in a Focus of Hyperactivity under the Influence of Diazepam. Epileptiform discharges (ED) in the zone of the focus, accompanied by contractions of muscles of the animal's contralateral forelimb, appeared 4-6 min after application of penicillin to the rats' cortex. The mean frequency of ED in the period before convulsions was 8.3/min. Epileptic fits (EF) with a mean duration of 24 ± 1.5 sec developed 15-30 min after application of penicillin. The frequency of EF averaged 0.5/min during the first 60 min. The life of the focus was 100-140 min.

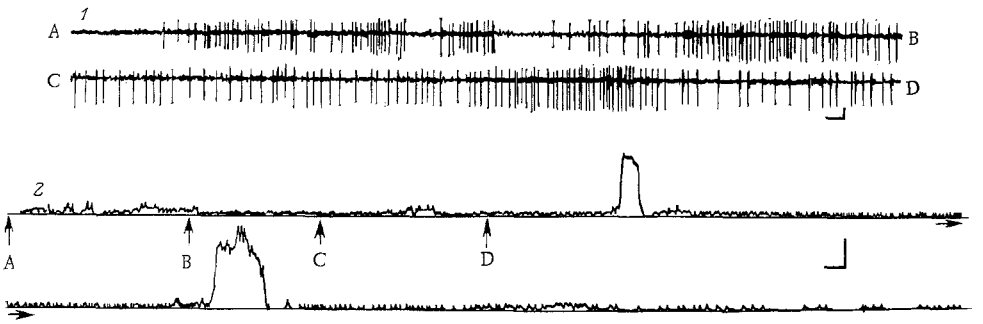

Fig. 3. Changes in ECoG in zone of focus of hyperactivity formed 20 min after intramuscular injection of diazepam (2 mg/kg). Legend as to Fig. 1.

Injection of diazepam in a dose of 0.01 mg/kg 25-30 min after application of penicillin led to an increase in the intervals between EF and to a decrease in the motor response during EF and ED. The effect lasted 25 min, after which the frequency of EF returned to the control level. Diazepam, injected in a dose of 2 mg/kg 25-30 min after application of penicillin, led to a reduction in the frequency of EF and an increase in their duration. The EF alternated with periods of increased frequency of ED. The number of EF fell sharply and the mean life of the focus was shortened to 90 min (Fig. 1). The decrease in the motor response, sometimes amounting to complete disappearance, under the influence of diazepam agreed with data showing the stabilizing effect of diazepam on the excitability of the brain-stem centers [7], and the muscle-relaxing action of diazepam can also evidently be connected with an increase in the intensity of presynaptic inhibition at the spinal level [13]. Diazepam, however, mainly altered the character of EF without abolishing ED, as was observed previously also [5, 17]. Disturbance of the appearance of EF was evidently connected with the fact that diazepam activates inhibitory polysynaptic processes at all levels in the CNS [10].

Injection of physiological saline and the solvent of diazepam (a mixture of propylene glycol and alcohol in the ratio of 7:3) did not cause any of the changes mentioned above.

Injection of diazepam in a dose of 2 mg/kg 20 min before application of penicillin shortened the latent period of onset of ED in the epileptic focus from 5.2 ± 0.8 to 2.8 ± 0.5 min ($P < 0.05$). The frequency of ED in the preconvulsive period increased on average to 17.3/min, but the ED were not accompanied by motor activity of the animal (Fig. 2). Either EF did not develop and only periods of an increase in the frequency of ED were observed, or they did develop, but the discharge frequency during EF was reduced and irregular, and the mean duration of these "modified" EF was 60 ± 7.6 sec (Fig. 3). EF were observed only during the first 50 min of existence of the focus, and later only ED were recorded. The total life of the focus was shortened to 80 min.

State of Na,K-ATPase in Focus of Hyperactivity during the Action of Diazepam. Na,K-ATPase and Mg-ATPase activity of unpurified rat cortical synaptosomes were unchanged under the influence of diazepam in all regions tested. However, in the presence of a focus of hyperactivity, diazepam led to an increase in Na,K-ATPase activity in both the sensomotor and the visual cortex of the rat. No significant change took place in Mg-ATPase activity under these circumstances (Table 1).

In experiments in vitro diazepam is known to inhibit Na,K-ATPase activity in membrane preparations [12], but the results obtained in experiments in vivo are contradictory: both moderate inhibition of enzyme activity [2] and no effect have been reported [4]. These disagreements can evidently be explained by differences in the methods used to isolate the membrane preparations.

The present experiments showed that Na,K-ATPase activity in the "penicillin" focus was increased by preliminary injection of diazepam. A similar effect was observed when a paroxysmal focus was created by electrical stimulation of the rat brain [2]. The experiments showed that an increase in Na,K-ATPase activity is observed not only in the primary focus, but also in the contralateral sensomotor and visual areas, and its mean level is 60% of the corresponding control. It was shown previously [3] that Na,K-ATPase activity in the sensomotor and visual areas contralateral to the focus 9-11 min after application of penicillin, in the absence of diazepam, is indistinguishable from the control, but in the primary focus it is depressed by about 40%. Preliminary administration of diazepam thus increases Na,K-ATPase activity in the primary epileptic focus on the whole by 100%, but in other regions of the cortex the effect is reduced by half. These results confirm the hypothesis that diazepam acts differently on Na,K-ATPase activity in the intact and "epileptic" neuron.

TABLE 1. Changes in Na,K-ATPase Activity of Unpurified Synaptosomes Isolated from the Cerebral Cortex of Rats Receiving Diazepam and in Which an Epileptic Focus Was Formed by Application of Penicillin to the Left Sensomotor Cortex

Object	Intact rats		Experimental rats receiving diazepam (2 mg/kg)		Experimental rats receiving diazepam (2 mg/kg) + penicillin	
	Na, K-ATPase	Mg-ATPase	Na, K-ATPase	Mg-ATPase	Na, K-ATPase	Mg-ATPase
Left sensomotor cortex	7,3±0,4 $n = 10$	10,3±0,5 $n = 10$	6,4±0,7 $n = 9$	9,4±0,8 $n = 9$	12,0±1,6* $n = 6$	12,4±1,5 $n = 8$
Right sensomotor cortex	7,5±0,4 $n = 8$	9,0±1,0 $n = 8$	7,2±0,6 $n = 9$	9,0±1,0 $n = 9$	10,9±0,9* $n = 8$	11,8±1,3 $n = 8$
Right visual cortex	6,8±0,6 $n = 10$	7,1±0,7 $n = 10$	6,9±0,7 $n = 9$	8,0±0,7 $n = 9$	10,4±1,0* $n = 8$	9,9±1,2 $n = 8$

Legend. 1) Na,K- and Mg-ATPase activity given in micromoles ADP/mg protein/h. 2) n – Number of experiments. 3) *$P < 0.05$.

Inhibition of Na,K-ATPase activity by 45% under the influence of ouabain is known to be accompanied by a twofold increase in the K^+ concentration in conger eel gill homogenate [18]. The increase in the K^+ concentration in the extracellular medium, as a result of inhibition of Na,K-ATPase activity, during convulsions may perhaps play an essential role in the mechanism of the different action of diazepam on Na,K-ATPase in intact and "epileptic" neurons, for it has been shown that K^+ ions increase binding of diazepam-^{3}H with rat cerebral cortical membranes by 20% [8]. Diazepam-^{3}H binds specifically with brain membranes, and the combining sites incorporate protein and phospholipid components [9]. Disturbance of the hydrophobic environment in the protein–lipid system as a result of diazepam binding may be a factor in the modification of Na,K-ATPase activity under normal conditions and during excitation of nerve endings.

A high K^+ concentration in the extracellular medium in a focus of hyperactivity is an important factor synchronizing the activity of "epileptic" neurons and the development of EF [16]. The increase in Na,K-ATPase activity found in the cortex in the "penicillin" focus after preliminary injection of diazepam may perhaps play an important role in the mechanism of "removal" of the surplus K^+ from the surrounding medium, and in turn, this may explain the disturbance of organization of ED into EF.

The paradoxical initial electrographic intensification of ED after preliminary injection of diazepam in a dose of 2 mg/kg deserves attention. Diazepam, increasing Na,K-ATPase activity, evidently facilitates hyperpolarization of the neurons of the epileptic focus. Under these conditions the effectiveness of the IPSP is reduced [11]. In an epileptic focus hyperpolarization potentials are one of the factors limiting the frequency and synchronization of ED. The decrease in the effectiveness of inhibitory interaction between neurons may be expressed as strengthening of asynchronous paroxysmal activity, and under these circumstances the latent period of origin of ED will be shortened and their frequency and variability of amplitude increased.

The results now obtained thus show that one of the molecular mechanisms of the anticonvulsant action of diazepam may be its activating effect on Na,K-ATPase of the neuronal membranes in a focus of epileptic activity.

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ALLEVIATION OF STRESS AND GASTRIC ULCERATION BY GAMMA-HYDROXYBUTYRIC ACID

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The effect of preliminary administration of sodium hydroxybutyrate (GHBA) on activation of the adrenergic and pituitary-adrenal systems during emotional-pain stress and on the severity of gastric ulcers after the end of such stress was studied in experiments on rats. Preliminary administration of GHBA was shown to restrict excitation of the systems responsible for stress and to prevent the development of ulceration of the gastric mucosa. It can be suggested that activation of the GABA-ergic inhibitory system arising during stress is the sole mechanism of limitation of the stress reaction and of prevention of stress-induced injuries.

KEY WORDS: emotional-pain stress; noradrenalin; corticosterone; sodium γ-hydroxybutyrate; gastric ulcers.

Recent investigations have shown that in emotional-pain stress (EPS) marked activation of the inhibitory GABA-ergic system of the brain is regularly observed [3]. This activation is brought about in such a way that it must lead to an increase in the formation of a terminal metabolite of the GABA system in the brain – sodium hydroxybutyrate (GHBA), a substance which has now been shown to have a strong and direct inhibitory action [6]. It has accordingly been postulated that activation of the GABA-ergic system through the action of GHBA limits excitation of the adrenergic and pituitary-adrenal system in stress and thereby prevents stress-induced injuries to the internal organs.

To test this hypothesis, in the present investigation the effect of preliminary injection of GHBA on activation of the adrenergic and pituitary-adrenal systems during EPS and on the severity of gastric ulceration, which are usually observed after exposure to EPS, was studied.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. EPS was evoked in the form of an anxiety neurosis by Desiderato's method [4] and it lasted 6 h. GHBA was injected subcutaneously in a dose of 100 mg/kg 30 min before EPS, and also 2 and 4 h after the beginning of EPS. Physiological saline was injected into the control animals at these same times.

The concentration of corticosterone in the adrenals, blood plasma, and heart was determined by chromatography on silica-gel columns [1]. The noradrenalin concentration in the adrenals and heat was determined by a fluorometric method [2]. The animals were decapitated for determination of corticosterone and noradrenalin 1 and 6 h after the beginning of EPS, and also 2 h after the end of EPS.

Ulcers observed in the gastric mucosa after EPS are the result of digestion of areas of the mucosa in which foci of ischemic necrosis occurred previously [5]. They were assessed quantitatively by measurement of the total length of the ulcers present in the stomach of rats killed at the end of a period of rest of 2 h after termination of EPS.

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