GABA-ERGIC MECHANISMS OF CEREBROVASCULAR EFFECTS OF PHENAZEPAM AND DIAZEPAM

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The writer showed previously that benzodiazepine tranquilizers (phenazepam and diazepam) have a depriming effect on central regulation of the cerebral circulation: They inhibit changes in the cerebral blood flow and in the tone of the cerebral vessels due to stimulation of afferent fibers of somatic nerves and they also potentiate central inhibition of tonic sympathetic activity and somatosympathetic reflex responses [5]. Meanwhile phenazepam and, to an even greater degree, diazepam lower the arterial blood pressure (BP) in animals under general anesthesia, and this reduces the circulation in the brain.

In view of these observations it was important to study the mechanism of action of phenazepam and diazepam on nervous regulation of the cerebral circulation. The depriming effect of diazepam on cortical neurons and also its tranquilizing and anticonvulsant effects are based on GABA-ergic mechanisms [2, 5, 12, 13]. However, there is information in the literature on antagonistic relations between benzodiazepines and GABA at the level of vestibular and cerebellar neurons [11]. It has also been suggested that diazepam can act on cholinergic [6] and monoaminergic [7, 10] transmission of impulses in the CNS. Finally, the effects of benzodiazepine tranquilizers have been linked with their interaction with specific receptors in brain tissue [8, 9].

The object of this investigation was to analyze the cerebrovascular effects of phenazepam and diazepam, by using a specific blocker of GABA-receptors, bicuculline.

EXPERIMENTAL METHOD

Experiments were carried out on 36 cats weighing 3-4 kg under general anesthesia (urethane, chloralose) with artificial ventilation of the lungs. The inflow of blood into the cats' brains through the internal maxillary artery was determined by means of an electromagnetic flowmeter. The EEG in the parietal region, ECG in lead II, and BP in the femoral artery were recorded simultaneously. Tonic activity and reflex responses in the sympathetic nerves of the kidney also were recorded [1]. The vascular component of the action of the drugs on the cerebral hemodynamics was differentiated by separate perfusion of the carotid and vertebral arteries on the two sides [3]. In all experiments the partial pressure of carbon dioxide (pCO_2) in arterial blood samples was determined by the ABC-1 apparatus and maintained within the control limits (35-40 mm Hg). The drugs for testing were injected intravenously: phenazepam 0.05 mg/kg, diazepam 0.5 mg/kg, and bicuculline 0.15-0.2 mg/kg. The animals were anesthetized with a mixture of urethane and chloralose.

EXPERIMENTAL RESULTS

The experiments showed that during blockade of GABA-receptors by bicuculline the effect of phenazepam and diazepam on the cerebral circulation and BP was considerably weakened (Fig. 1). Under these conditions phenazepam reduced the cerebral blood flow on average by 8 ± 2.2%, compared with by 20 ± 4.8% in control experiments ($P < 0.05$). Meanwhile BP was reduced by 9±0.9% (control 14 ± 3.3%). Diazepam, given after bicuculline, reduced the cerebral blood flow by 11 ± 1.8%, compared with 39 ± 4.7% in the control ($P < 0.002$). The hypotensive effect

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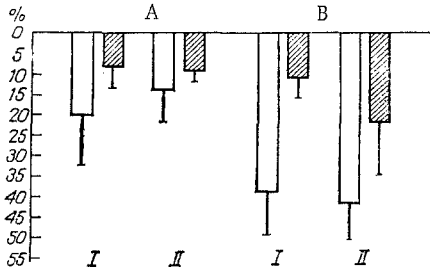

Fig. 1. Changes (in % of initial level) in cerebral blood flow (I) and BP (II) under the influence of phenazepam (A) and diazepam (B) in control experiments (unshaded columns) and after administration of 0.2 mg/kg bicuculline (shaded columns).

of diazepam also was weakened (22 ± 5.2%, compared with 42 ± 4.2% in the control; $P < 0.001$). The weakening of the reduction in the inflow of blood into the brain observed under the influence of phenazepam and diazepam after blockade of GABA-receptors was evidently due to the weaker hypotensive effect of the drugs under these conditions. This suggestion was confirmed by experiments using the technique of resistography: benzodiazepine tranquilizers, administered after bicuculline, lowered the tone of the cerebral vessels by a lesser degree.

In a separate series of experiments the effect of phenazepam and diazepam on nervous regulation of the cerebral circulation was investigated after preliminary injection of bicuculline. The results showed that after blockade of the GABA receptors the effect of phenazepam (+14 ± 11.7%) on the change in the cerebral blood flowduring formation of the vasomotor reflex was not statistically significant. In control experiments, however, phenazepam depressed changes in the cerebral blood flow in all experiments on average by 70 ± 11% (Fig. 2). Bicuculline also blocked the depriming effect of phenazepam on reflex constrictor responses of the cerebral vessels in the systems of the carotid and vertebral arteries. After blockade of the GABA-receptors, the effect of phenazepam on reflex responses of BP was prevented or significantly weakened (+9 ± 12.7%, compared with −40 ± 8.5% in the control).

The study of the effect of diazepam on changes in the cerebral blood flow during formation of the vasomotor reflex also revealed considerable weakening of the depriming effect of the drug after blockade of the GABA receptors. The depriming effect of diazepam on pressor reflex responses of BP also was reduced.

After blockade of the GABA receptors, in most experiments phenazepam and diazepam caused no significant changes in tonic and reflex activity in sympathetic nerves (Fig. 2). Consequently, against the background of bicuculline, potentiation of central inhibition of spontaneous activity and of somatosympathetic responses, a characteristic feature of the benzodiazepine tranquilizers, was not manifested.

The effect of bicuculline on changes in the cerebral circulation and cerebrovascular reflexes after preliminary administration of phenazepam or diazepam was investigated in another series of experiments. These experiments showed that bicuculline, preceded by administration of benzodiazepine tranquilizers, raised BP on average by 18 ± 5%. The changes in the cerebral circulation were varied: In some experiments the cerebral blood flow was reduced, whereas in others the inflow of blood into the brain was increased. Under these conditions bicuculline potentiated reflex vasoconstrictor responses in the carotid and vertebrobasilar systems and the responses of BP. Under the influence of bicuculline an increase in amplitude and discharge frequency of tonic and reflex activity in sympathetic nerves was observed (Fig. 2).

This investigation thus showed that when GABA-receptors are blocked by bicuculline, the effects of phenazepam and diazepam on the cerebral circulation and on BP are weakened. Bicuculline prevents or considerably weakens the effects of benzodiazepine tranquilizers on nervous regulationof the cerebral circulation. In most experiments the depriming effect of these tranquilizers on changes in the cerebral blood flow during formation of the vasomotor reflex and on the constrictor responses of the cerebral vessels is not manifested. Under these conditions the inhibitory effect of phenazepam and diazepam on tonic activity and reflex responses in sympathetic nerves is considerably weakened. Antagonism is found between phenazepam and diazepam, on the one hand, and bicuculline on the other hand. The results confirm data on

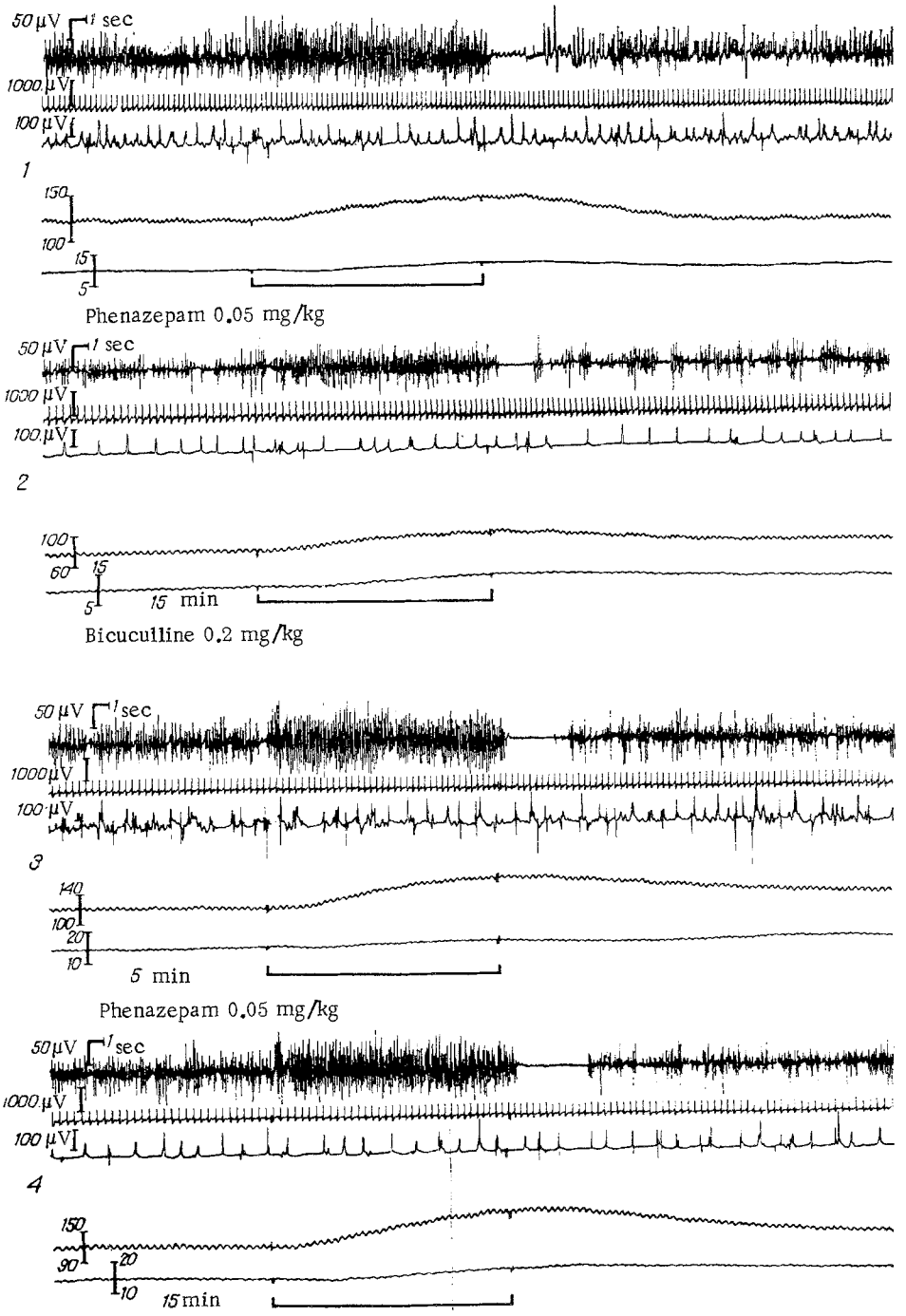

Fig. 2. Effect of phenazepam on changes in cerebral blood flow due to electrical stimulation of afferent fibers of tibial nerve before and after injection of bicuculline (0.2 mg/kg). 1) Control response, 2) 15 min after injection of phenazepam, 3) 5 min after injection of bicuculline, 4) 15 min after injection of phenazepam preceded by bicuculline. From top to bottom: tonic and reflex activity in sympathetic nerve of kidney, ECG in lead II, EEG in parietal region, BP in femoral artery, blood flow in internal maxillary artery, marker of stimulation (20 V, 20 stimuli/sec, 1 msec).

the central nature of the depriming effect of benzodiazepine tranquilizers on nervous regulation of the cerebral circulation. They indicate that GABA-ergic mechanisms participate in the mechanism of the cerebrovascular effects of phenazepam and diazepam, in agreement with data in the literature on the role of the GABA-ergic component in the central effects of diazepam. These results also are evidence of the importance of GABA in the central regulation of the cerebral circulation.

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EFFECT OF LITHIUM HYDROXYBUTYRATE ON THE CORTICAL AND SUBCORTICAL CATECHOLAMINE LEVEL IN THE RABBIT BRAIN

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Lithium hydroxybutyrate, synthesized and studied experimentally at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, successfully combines the antimaniacal properties of the lithium ion and the tranquilizing effect of γ-hydroxybutyric acid (GHBA), a natural metabolite of brain tissue [1, 3]. Investigation of the electroencephalogram (EEG) revealed a predominantly depriming effect of lithium hydroxybutyrate on spontaneous bioelectricalactivity and electrical excitability of the cortex and of some deep brain formations in rabbits [9]. As a result of synergism between Li^+ and the anionic component, the compound is superior to lithium chloride in its activity, as reflected in several EEG indices [7].

Considering the possible role of central monoamines in the mechanism of the psychotropic action of lithium salts [4, 15, 16], the effect of lithium hydroxybutyrate was investigated and compared with that of sodium hydroxybutyrate and lithium chloride on the catecholamine content in individual structures of the rabbit brain.

EXPERIMENTAL METHOD

Experiments were carried out on 35 rabbits of both sexes weighing 1.5-2 kg. Aqueous solutions of the compounds were injected intravenously: lithium hydroxybutyrate 10 mg/kg, lithium chloride 10 mg/kg (the isoeffective dose according to EEG indices), and sodium hydroxybutyrate 10 mg/kg (the equimolar dose). Control animals received injections of the same volume of physiological saline. The animals were decapitated 1 h after injection of the compound. A weighed sample of the corresponding part of the brain (200 mg for the hypothalamus and caudate nucleus, 400-600 mg for other structures) was frozen without delay. The catechol-

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