ROLE OF THE CHOLINERGIC STRUCTURES OF THE HYPOTHALAMUS IN THE REGULATION OF THE BLOOD CLOTTING SYSTEM

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It has recently been shown [7, 14] that local stimulation of the hypothalamus with rectangular pulses of a duration of 2 msec and voltage 2-3 V causes changes in the prothrombin time and in the concentration of factor V and fibrinogen. Morphological, physiological, and clinical findings suggest that the hypothalamus contains adrenergic, cholinergic, and serotoninergic systems [1-3, 8, 10, 11, 13, 15]. On these grounds it may be assumed that each of these systems has a specific influence on homeostasis and, in particular, on the blood clotting system.

In the present investigation the role of the cholinergic structures of the hypothalamus in the regulation of the blood cutting system was studied. For this purpose the method of microinjection of acetylcholine into the anterior and posterior zones of the hypothalamus was used. This method of microinjection of physiologically active substances has recently been widely used by many investigators [4, 9, 12]. At the same time as the components of the blood clotting system were studied, the bioelectrical activity of the hypothalamus and cerebral cortex was recorded.

EXPERIMENTAL METHOD

Chronic experiments were carried out on 17 rabbits. A cannula-electrode — an injection needle with a point 60-65 μ in diameter, coated with acetone varnish — was inserted into the region of the anterior and posterior hypothalamus. The cannula-electrode was used both for local recording of the electrical activity of the hypothalamus and for injection of the pharmacological agent. Acetylcholine (0.01 ml) was injected by means of a microsyringe attached to a Kovacs stereotaxic apparatus. The electrodes were introduced into the corresponding zones of the hypothalamus with the aid of Sawyer's bone coordinates [16] with the corrections made by M. M. Kozlovskaya and A. V. Val'dman [5]. Biopotentials were detected from the sensorimotor, occipital, and parietal regions of the cortex by means of epidural nicrome electrodes. The indifferent electrode was inserted into bone posteriorly to the occipital tuberosity. The respiration was recorded as an index of the functional state of the hypothalamus.

Of all the various indices of the functional state of the blood cutting system, the following were studied: Quick's test, the recalcification time by the method of Bergerhof and Roka, the free heparin concentration by Sirmai's method, the concentration of factor VII by the Adams – Andreenko method and of factor VIII by Bunamo's method. The fibrinogen concentration and the fibrinolytic activity of the blood were determined by the method of Gorshkova and Lomazova. To obtain a general picture of the blood clotting process, the thromboelastrogram (TEG) was recorded. The study of the blood cutting indices and recording of the electrical activity of the brain were carried out before the microinjection of acetylcholine and on the 1st, 5th, 20th, etc., minute thereafter until the initial level was restored. The postoperative period continued for 10-12 days after implantation of the cannula-electrode into the hypothalamus. At the end of the investigation the location of the cannula-electrode was confirmed histologically in material stained by Nissl's method. The results obtained by the investigation of the blood clotting indices were subjected to statistical analysis by the small sample method.

EXPERIMENTAL RESULTS

The microinjection of acetylcholine (1 μg) into the region of the nuclei of the anterior zone of the hypothalamus was accompanied by hypocoagulation of the blood (Fig. 1A). The study of the component of the blood clotting system revealed a decrease in the concentration of prothrombin ($t = 7.8$, $P < 0.001$) and of factors VI and VIII ($t_1 = 4.9$, $t_2 = 6.3$, $P < 0.001$). The fibrinogen concentration fell from 344 to 309 mg% at the 20th minute ($t = 2.6$, $P < 0.02$), and the fibrinolytic activity rose very slightly ($t = 2.1$, $P < 0.05$). The heparin concentration in the blood rose by 20-25% over its initial value ($t = 2.8$, $P < 0.01$). The hypocoagulation began 1 min after microinjection of acetylcholine, and this was confirmed by the significance of the values obtained. The indices of

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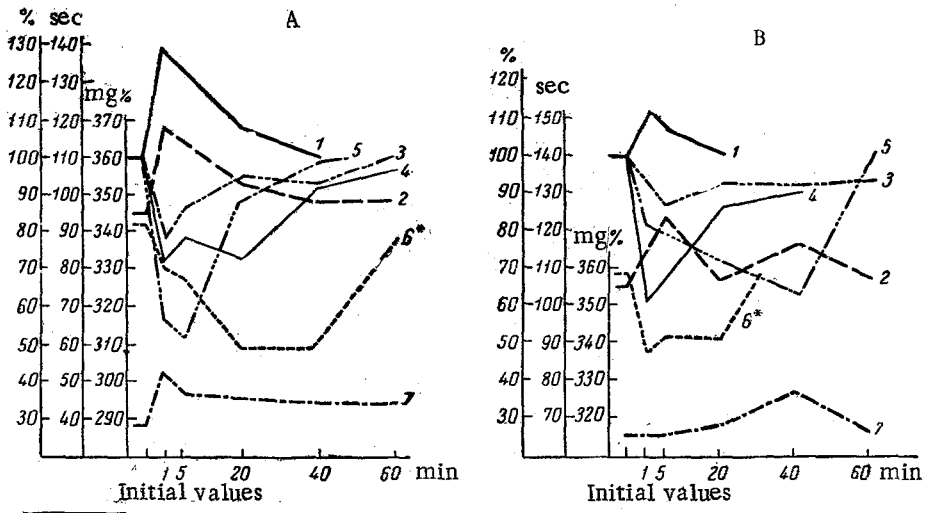

*The notation 1, 2, 3, 4, 5, 6 probably refers to the parameters r, K, T, S, Ma, Ci mentioned in the text. Their exact correspondence is not given in the Russian original—Publisher's note.

Fig. 1. Coagulogram of the blood after microinjections of acetylcholine into the anterior (A) and posterior (B) hypothalamus. Recalcification time in sec; factor VI, VIII, heparin, prothrombin (Quick's test) and fibrinolysis in percent; fibrinogen in mg %.

blood clotting selected for study returned almost to their initial levels 35-40 min after the injection. The maximal changes in blood clotting, accompanied by hypocoagulation, developed 1 min after the microinjection of acetylcholine. The possible explanation of this rapid but transient effect may be that acetylcholine is quickly destroyed by cholinesterase. The TEG showed an increase in the reaction time (r) the coagulation (K) the total clotting time (T) and of syneresis (S). The maximal amplitude (Ma) and index of coaguloability (Ci) were reduced. The changes in the indices of the TEG after local injection of acetylcholine into the anterior hypothalamus were statistically significant.

The electrical activity of the hypothalamus and of the sensorimotor, parietal, and occipital regions of the cortex was characterized by the appearance of fast low-amplitude activity from the first minute after microinjection of acetylcholine. Against the background of this fast low-amplitude activity of the hypothalamus and cortex, a paroxysmal activity appeared, in the form of fast, sporadic spikes and pointed waves (Fig. 2I). The activation reaction of the EEG after the microinjection of acetylcholine was most marked in the hypothalamus. Determination of the integrated electrical activity of the hypothalamus and cortex by means of a digital integrator also showed that the structures of the anterior hypothalamus react to the microinjection of acetylcholine into that region by a sharp increase in the integrated activity affecting the waves of all frequencies. The activation reaction of the brain persisted for 20-25 min from the beginning of the microinjection of acetylcholine, when it was replaced by the background electrical activity of the brain. The period of more intensive bioelelectrical activity of the brain was accompanied by an abrupt slowing and decrease in the amplitude of respiration.

Microinjections of acetylcholine in the same dose into the posterior nuclei of the hypothalamus were also accompanied by hypocoagulation (Fig. 1B). However, in contrast to the effect of microinjections of acetylcholine into the anterior hypothalamus, the decrease in the concentration of procoagulants and the increase in the concentration of anticoagulants only began to develop 5 min after the microinjections in the posterior hypothalamus.

From the quantitative point of view the changes in the blood clotting indices were less marked than after microinjections of acetylcholine in the same dose into the anterior hypothalamus. For instance, when acetylcholine was injected into the anterior hypothalamus the recalcification time was prolonged by 23 sec ($t = 3.7$, $P < 0.001$), but when injected into the posterior hypothalamus the recalcification time was prolonged by 15 sec ($t = 2.6$, $P < 0.01$). The differences after microinjections of acetylcholine into the anterior and posterior hypothalamus were particularly clear in the concentrations of factor VIII, prothrombin, and heparin. Microinjection of acetylcholine into the anterior hypothalamus lowered the blood concentration of prothrombin by 22% ($P < 0.01$), and of factor VIII by 47% ($P < 0.001$), and increased the heparin concentration by 27% ($P < 0.01$), whereas injections of this substance into the posterior hypothalamus lowered the prothrombin concentration by 14% ($t = 2.05$, $P < 0.05$), and the factor VIII concentration by 34% ($t = 2.9$, $P < 0.01$), and increased the heparin concentration by only 13% ($t = 2.35$, $P < 0.02$).

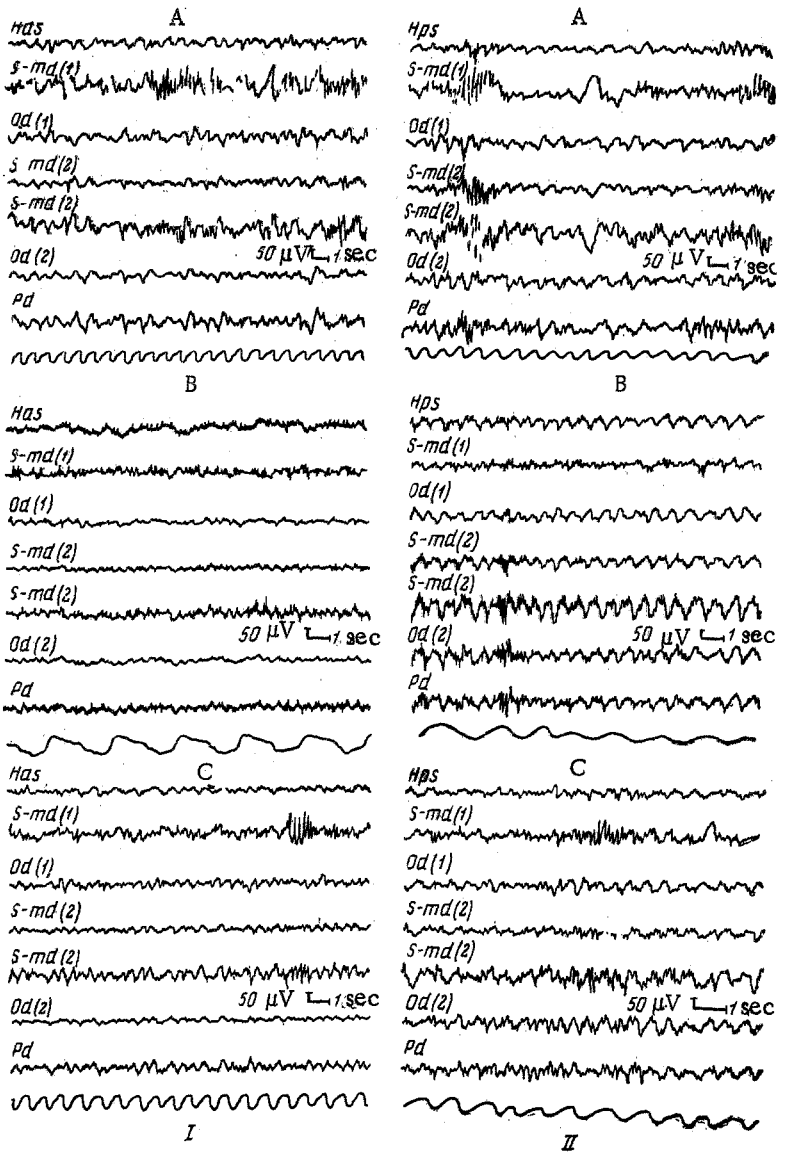

Fig. 2. EEG of the hypothalamus and cortex during microinjection of acetylcholine (1 μg) into the hypothalamus. I) Anterior hypothalamus; a) background; b) 1 min after microinjection; c) 40 min after microinjection. II) Posterior hypothalamus; a) background; b) 5 min after microinjection; c) 20 min after microinjection. Has – anterior hypothalamus; Hps – posterior hypothalamus; S-md – sensorimeter cortex; Od – occipital (right) cortex; Pd – parietal (right) cortex.

As is clear from Fig. 1B, the blood clotting indices were restored almost to their initial levels 20 min after the microinjections of acetylcholine into the posterior hypothalamus. The TEG indices were very slightly changed in the direction of hypocoagulation.

The electrical activity of the hypothalamus and cortex after microinjections of acetylcholine into the posterior nuclei of the hypothalamus was accompanied by a temporary (15-20 min) activation dominated by fast, low-amplitude activity (Fig. 2II). However, in contrast to the EEG during microinjection of acetylcholine into the anterior hypothalamus, only very few sporadic epileptiform spikes were observed in the phase of activation of the EEG following microinjection into the posterior hypothalamus.

The results obtained show that the hypothalamus is one of the principal links in the neuro-humoral regulation of blood clotting at the level of the subcortical brain structures. The results of the study of individual blood clotting indices and the indices of the thromboelastogram (r, K, Ma, T, S, Ci) showed that activation of the cholinergic

elements of the hypothalamus was reflected in all three phases of blood clotting. However, the most marked changes in blood clotting in the direction of hypocoagulation took place in the first and second phases of blood clotting. In the present investigations, following microinjections of acetylcholine into the anterior and posterior regions of the hypothalamus, well defined changes were found in the components of the blood clotting system, typical of hypocoagulation. It was observed, moreover, that the maximal changes in the blood clotting indices occurred in the first 5 min. The existing evidence concerning the effect of acetylcholine on blood clotting when injected intravenously is contradictory [6]. These contradictions are evidently due to the fact that the investigators used different doses of acetylcholine, and in addition, to the fact that acetylcholine, when injected intravenously, is quickly inactivated by cholinesterase.

The investigations described above show that cholinergic elements are present in both the anterior and the posterior regions of the hypothalamus. Nevertheless, the nuclei of the anterior hypothalamus evidently contain more cholinergic elements than the posterior nuclei of the hypothalamus. This may account for the fact that microinjections of acetylcholine into the anterior nuclei of the hypothalamus caused a more marked hypocoagulatory effect and activation reaction of the EEG than microinjections of acetylcholine in the same dose into the posterior hypothalamus. Undoubtedly the injection of any pharmacological agent into the hypothalamus must be accompanied by mechanical injury to its structures. However, the results of experiments in which microinjections of catecholamines and serotonin were given into the hypothalamus, and also of control experiments in which 0.01 ml of physiological saline was injected into the same structures of the hypothalamus, demonstrate excitation of the specific chemosensitive structures of the hypothalamus by specific mediators.

The hypothalamus is in two-way communication with the adenohypophysis and the higher levels of the central nervous system. The influence of the cholinergic structures of the anterior and posterior hypothalamus on blood clotting and on the bioelectrical activity of the brain must undoubtedly depend on the functional state of the pituitary and of the higher levels of the central nervous system.

The hypothalamus thus plays the role of a direct regulator of the blood clotting process. A special feature of this regulation is that, depending on the functional state of the various chemosensitive systems of the hypothalamus, hypercoagulation or hypocoagulation is observed. Activation of the cholinergic structures of the anterior and posterior nuclei of the hypothalamus causes distinct changes in blood clotting in the direction of hypocoagulation. Its action on the blood clotting process evidently takes place both directly and through the endocrine system.

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