MECHANISMS OF HYPOTHALAMO-RETICULAR INFLUENCES ON THE CONTENT OF SULFHYDRYL GROUPS IN THE CEREBRAL CORTEX

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Data are given to show the effect of electrical stimulation of the hypothalamus and mesencephalic reticular formation on the content of tissue sulfhydryl groups in different parts of the brain and on the EEG. Posterior-hypothalamic influences on the EEG and the content of sulfhydryl groups in the cortex were shown to occur only in the case of morphological integrity of the hypothalamo-cortical and hypothalamo-reticular connections and they have a common cholinergic component at the cortical level.

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Stimulation of nonspecific components of the mesencephalic reticular formation and hypothalamus under certain conditions gives EEG responses which are accompanied by cerebral metabolic and circulatory changes [14, 18] and, in particular, by changes in neuro-humoral and energy-producing processes in the cortex [1, 5, 7]. An important role in the mechanism of peripheral nervous influences is played by the sulfhydryl groups of protein complexes as a result of their high physiological activity [3]. More recently, findings have been published indicating changes in the content of sulfhydryl groups in nervous structures during stimulation of their function [4, 10, 15, 20].

The object of the present investigation was to study the mechanisms of ascending influences of the hypothalamus and mesencephalic reticular formation on the content of tissue sulfhydryl groups in the cerebral cortex and on its electrical activity.

EXPERIMENTAL METHOD

Acute experiments were carried out on dogs (50) and cats (15) treated with muscle relaxants. In some experiments the animals were anesthetized with intranarcon or transection of the brain was performed at different levels. Subcortical structures were stimulated by monopolar electrodes 75μ in diameter, introduced into the brain relative to stereotaxic coordinates in conjunction with x-ray control [6].

Structures of the posterior hypothalamus and mesencephalic reticular formation were stimulated with the cathode through a radiofrequency output by square pulses (up to 300/sec, 0.5-1 V, 0.5-0.8 msec). The duration of each series of stimuli was 60 sec. Low frequencies (2-5/sec) were used for stimulation of the anterior hypothalamus. Up to seven series of stimuli were applied. Cortical potentials were recorded through contact electrodes. The EEG from different parts of the brain, respiration, and the heart rate were recorded on a multichannel electroencephalograph.

Quantitative determination of the tissue sulfhydryl groups was carried out in homogenates of various parts of the brain by the method of mercurimetric titration described by Kolthoff and co-workers [19] as modified by Nistratova [8]. The homogenate was prepared in 0.85% NaCl solution. In some experiments the homogenate was dialyzed through a semipermeable membrane for 24 h against 25 volumes physiological saline, with rotation of the dialyzer, and at a temperature of 2-4°. Sulfhydryl groups were determined both in the whole homogenate and in the residue not passing through the semipermeable membrane. Their content was calculated per 100 mg fresh tissue and expressed in μmoles.

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TABLE 1. Effect of Stimulation of Posterior Hypothalamus and Mesencephalic Reticular Formation on Content of Sulfhydryl Groups (M ± t) in Tissue Homogenates of Different Parts of Dog's Brain (in μmoles/100 mg fresh tissue)

Part of brain	Stimulation of hypothalamus			Stimulation of reticular formation			Stimulation of hypothalamus of "cerveau isolé" preparation		
	control	stimulation	P	control	stimulation	P	control	stimulation	P
Frontal cortex	1,20±0,07	1,60±0,09	<0,01	1,20±0,07	1,44±0,07	<0,05	1,06±0,04	1,15±0,05	=0,2
Occipital cortex	1,13±0,08	1,55±0,10	<0,01	1,13±0,08	1,36±0,06	<0,05	1,03±0,03	1,14±0,04	=0,2
Limbic cortex	1,17±0,07	1,58±0,08	<0,01	1,17±0,07	1,41±0,06	<0,05	1,02±0,06	1,16±0,05	<0,05
Hypothalamus	1,23±0,09	1,61±0,07	<0,01	1,23±0,09	1,46±0,05	<0,05	1,04±0,09	1,18±0,06	=0,2
Mesencephalic reticular formation	1,05±0,03	1,44±0,07	<0,01	1,05±0,03	1,45±0,01	<0,001			
Bulbopontine reticular formation	1,06±0,05	1,36±0,01	<0,001	1,06±0,05	1,15±0,03	>0,05			

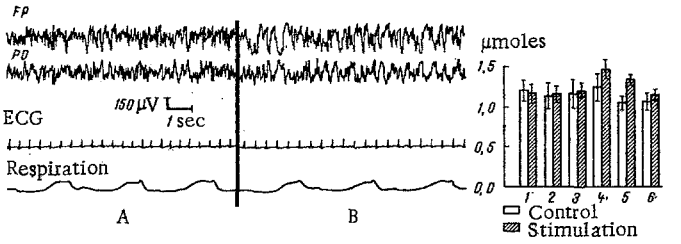

Fig. 1. Effect of stimulation of anterior hypothalamus on bioelectrical activity and content of tissue sulfhydryl groups in various parts of the dog's brain. A) Initial background EEG; B) stimulation of anterior hypothalamus; FP) fronto-parietal lead; PO) parieto-occipital lead. Content of sulfhydryl groups: 1) frontal cortex; 2) occipital cortex; 3) limbic cortex; 4) hypothalamus; 5) mesencephalic reticular formation; 6) bulbopontine reticular formation.

EXPERIMENTAL RESULTS AND DISCUSSION

The investigations showed that the content of sulfhydryl groups was practically identical in different parts of the brain (hypothalamus, mesencephalic and bulbar reticular formation, frontal, occipital, and limbic cortex), in agreement with data in the literature [11]. Some quantitative differences in the content of sulfhydryl groups were observed between investigated parts of the brain of dogs and cats (Tables 1 and 2).

In most cases stimulation of the posteromedial hypothalamus led to the development of an ECoG activation response in all areas of the cortex, and an increase in the respiration and heart rates. These effects of the posterior hypothalamus on the ECoG and indices of autonomic responses could be observed for 15-20 min after the end of stimulation. In some experiments, however, the ECoG response to stimulation of the posterior hypothalamus was ill-defined and sometimes absent, but at other times it was characterized by the appearance of spindle-like activity. The intensity of the changes in frequency and amplitude characteristics of the EEG was assessed by Faure's method of histographic analysis. In all experiments, regardless of the character of the ECoG responses, during prolonged stimulation there was a statistically significant increase in the total content of tissue sulfhydryl groups in all parts of the brain investigated (Table 1). As Table 2 shows, the increase in content of sulfhydryl groups affected both the high-molecular-weight fraction of thiol compounds, evidently bound with proteins and the low-molecular-weight fraction. The influx of low-molecular-weight thiol compounds into the cortical cells increased, presumably on account of the increase in velocity of the cortical blood flow previously demonstrated under these conditions [7]. The experimental results indicate the possibility of a parallel activation of hypothalamic mechanims responsible for this particular trophic change and regulating cortical electrical activity.

Since the total content of sulfhydryl groups in a tissue correlates with the level of its energy metabolism [9, 11, 12], the observed increase in content of sulfhydryl groups in the cortex can be regarded as a factor raising its elements to a higher level of energy metabolism as the ascending influences of the posterior hypothalamus are put into effect.

Electrical stimulation in the region of the anterior hypothalamus increased the intensity of autonomic responses of the parasympathetic

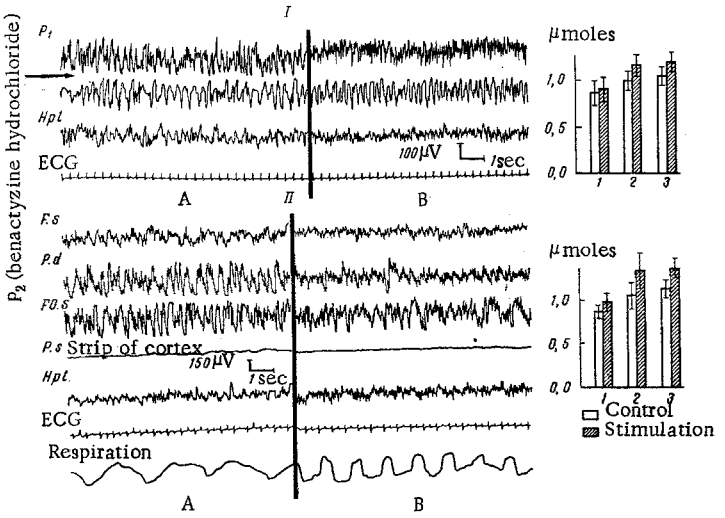

Fig. 2. Effect of posterior hypothalamic stimulation on bioelectrical activity and content of sulfhydryl groups in cerebral cortex of dogs following application of benactyzine hydrochloride (I) and undercutting (II). A) Initial background EEG; B) stimulation of posterior hypothalamus; P_1) EEG of intact parietal cortex; P_2) EEG of area of symmetrical cortex of opposite hemisphere treated with benactyzine; Hpt) hypothalamus. Content of SH-groups: 1) in area of cortex treated with benactyzine; 2) in surrounding cortex; 3) at symmetrical point of cortex of opposite hemisphere. P.s) EEG of isolated strip of cortex of left hemisphere; P.d) EEG of symmetrical point of cortex of opposite hemisphere; F.s and FO.s) EEGs of intact areas of cortex surrounding strip. Content of SH-groups: 1) in strip of cortex; 2) in surrounding cortex; 3) at symmetrical point of cortex of opposite hemisphere.

type, and a generalized hypersynchronization response appeared in the ECoG. The content of tissue sulfhydryl groups remained unchanged in the various parts of the cortex and in the bulbar reticular formation (Fig. 1). Meanwhile, in the hypothalamus and mesencephalic reticular formation, a significant increase occurred in the content of thiol compounds, reflecting to some extent an increase in functional activity of the reticulo-hypothalamus systems.

To study the mechanism whereby hypothalamic influences are exerted on the dynamics of sulfhydryl groups in the cortex, experiments were carried out on dogs in which the cortex was undercut by circumferential and horizontal incision. The strip of cortex thus formed remained connected to the rest of the body through the meningeal vessels and vasomotor nerves. Control experiments showed that undercutting the cortex itself leads to a slight decrease in the content of thiol compounds in the strip. Spontaneous electrical activity of the strip was sharply reduced. Stimulation of the posterior hypothalamus under these conditions caused no changes in its electrical activity, and the content of sulfhydryl groups in the strip of cortex likewise remained substantially unchanged, in contrast to intact zones of the cortex, where a statistically significant shift occurred (Fig. 2, II). These experiments thus showed that structural integrity of hypothalamocortical connections is essential for the exertion of posterior hypothalamic influences on changes in the content of sulfhydryl groups in the cortex in the early stages after stimulation.

Since high-frequency electrical stimulation of the mesencephalic reticular formation causes changes in the content of sulfhydryl groups in the cerebral cortex and other parts of the brain, similar in direction to the effect observed during stimulation of the posterior hypothalamus, the next step was to study the role of the mesencephalic reticular formation in the mechanism of this effect. Experiments showed that the

TABLE 2. Content of Sulfhydryl Groups (M ± t) in Fractions of Tissue Homogenates of the Frontal Cortex and Hypothalamus of Control Cats and after Stimulation of the Posterior Hypothalamus (in μmoles/100 mg fresh tissue)

Part of brain	Type of SH-groups	Control	Stimulation	P
Cortex	Total	0,78±0,018	1,00±0,036	<0,001
	Nondialyzable	0,4±0,03	0,54±0,038	<0,02
	Dialyzable	0,38±0,012	0,46±0,025	=0,01
Hypothalamus	Total	0,79±0,04	0,96±0,04	<0,01
	Nondialyzable	0,44±0,007	0,55±0,026	<0,01
	Dialyzable	0,35±0,015	0,41±0,021	<0,05

Note. Dialyzable SH-groups were determined by substracting values for the nondialyzable fraction from the total content of sulfhydryl groups.

content of sulfhydryl groups in different parts of the brain fell slightly after total transection of the brain stem at the precollicular level. Stimulation of the posterior hypothalamus in these animals no longer caused significant changes in the content of sulfhydryl groups in the cortical and subcortical tissues (only a slight tendency toward an increase could be detected). The response of the ECoG under these conditions to stimulation of the posterior hypothalamus was reduced and phasic in character. Evidently, therefore, influences of the posterior hypothalamus on changes in the level of sulfhydryl groups in the cortex can be exerted only if functional links are intact between the posterior hypothalamic systems and the mesencephalic reticular formation.

In a series of experiments in which a 0.75% solution of benactyzine hydrochloride (muscarine-like cholinolytic of central action) was applied to the surface of the cortex it was observed (Fig. 2, I) that the ECoG of this area showed slow high-voltage activity which did not increase in response to stimulation of the posterior hypothalamus, although in the surrounding cortex and in the cortex of the opposite hemisphere a distinct response of ECoG activation was observed. No increase in the level of the tissue sulfhydryl groups likewise took place in this area, in contrast to other areas of the cortex, although application of benactyzine itself slightly lowered the content of thiol compounds in this area compared with the cortex of the opposite hemisphere.

On the basis of data in the literature [2, 16, 17] concerning the link between the cortical response of EEG activation and cholinergic processes, and also of data showing that the protein receptor of actylcholine contains sulfhydryl groups [13], it can be postulated that blocking of muscarine-like cholinergic receptors in the cortex disturbs the integrity of the acetylcholine–cholinergic receptor protein enzymochemical cycle essential for realization of hypothalamic influences on the genesis of cortical electrical activity and the level of cortical sulfhydryl groups. The results of the present series of experiments suggest that the hypothalamic projections responsible for activation of the ECoG and for the increase in content of tissue sulfhydryl groups have a common cholinergic component at the cerebral cortical level.

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