EFFECT OF THE HYPOTHALAMUS ON SUCCINATE DEHYDROGENASE ACTIVITY IN THE CILIARY EPITHELIUM OF THE RABBIT'S EYE

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In the structures of the ciliary processes succinate dehyrogenase (SDH) was detected selectively and in high concentration in the ciliary epithelium, especially in its apical parts. After electrical stimulation of the anterior hypothalamus SDH activity in the epithelium of the ciliary body was reduced. After stimulation of the middle part of the hypothalamus there was a very slight increase in activity of the enzyme. Comparison of the cytophotometric results with the ophthalmotonus under these conditions shows that hypothalamic effects on intra-ocular pressure may be excreted through the respiratory chain of the mitochondria and, in particular, through the SDH activity of cells secreting the aqueous humor.

Constancy of the intra-ocular pressure is an essential condition for the specific function of the visual system. This is shown, in particular, by the fact that the commonest cause of incurable blindness is glaucoma, the principal manifestation of which is a permanent increase in intra-ocular pressure. Many clinicians at the present time [3-5, 8, 10, 13, 17, 18] link the pathogeneis of glaucoma with disturbances in the hypothalamo-hypophyseal system. The mechanism of the effect of the hypothalamus on ophthalmotonus has not been studied. It has merely been suggested that the effect is exerted through a change in the secretion of aqueous humor. The action of neurohumoral factors on function is known to be connected with metabolic changes in the structures responsible for their function. However, the available information on the biochemical basis of secretion of the aqueous humor is inadequate. Virtually nothing is known of the enzymic processes which provide the energy for this process. It was accordingly interesting to study experimentally how individual parts of the hypothalamus influence the tropic component in the system stabilizing the intra-ocular pressure and, in particular, how they affect the state of oxidation-reduction processes in the cells secreting the aqueous humor.

This paper describes a histochemical investigation of the activity and character of distribution of succinate dehydrogenase (SDH) in the ciliary epithelial cells of the rabbit's eye under normal conditions and after stimulation of the anterior and middle parts of the hypothalamus. No information of this type could be found in the literature.

EXPERIMENTAL METHOD

Experiments were carried out on albino rabbits. The hypothalamus was stimulated with square pulses (0.05-0.1 msec, 60-80 Hz, 0.5-4 V, 3 min) applied through unipolar electrodes implanted by a stereotaxic apparatus. The stimulation was given repeatedly at intervals of 3-4 days for 1 month. The animals were unanesthetized during the experiments. The position of the electrodes was verified histologically. After the last stimulation the animals were killed by air embolism. Immediately after embolism the eye was investigated on the side of the electrode. In seven animals the anterior, and in four the middle

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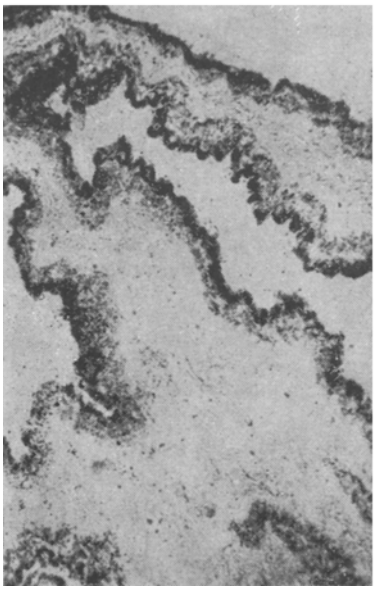

Fig. 1

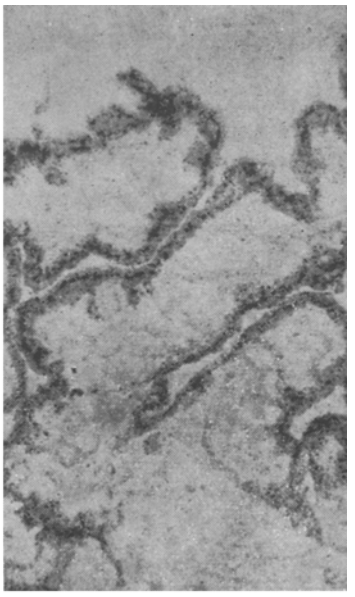

Fig. 2

Fig. 1. Distribution of SDH in structures of processes of ciliary body in the normal rabbit's eye. Highest enzyme activity visible in ciliary epithelium, especially in its apical parts (200 ×).

Fig. 2. Decrease in SDH activity in ciliary epithelium of a rabbit's eye after stimulation of the anterior hypothalamus (200 ×).

part of the hypothalamus was stimulated. The eyes of 11 intact rabbits were used as the control. Pieces of tissue were frozen and sections cut in a cryostat. To obtain a more precise assessment of the SDH activity pieces of tissues from the control and experimental animals were mounted in the same block, so that they were cut simultaneously and the sections were of equal thickness (8 μ), and subsequent incubation also took place at the same time. The sections were incubated for 40 min by the method of Shelton and Schneider [15], using nitro-BT. The SDH activity in the ciliary epithelial cells was assessed by microscopic examination of the specimens, followed by their cytophotometry by the scanning method [2, 7] on the MUF-5 instrument in visible light at a wavelength of 546 nm, with a 50 × objective. The work of Anders [1] showed that cytochemical analysis of dehydrogenase activity by the use of nitro-BT can be carried out quantitatively. During cytophotometry the optical density of the diformazan grains in the different areas (at least 60 in each group of experiments) of the ciliary epithelium was recorded. Altogether 297 measurements were made. The cytophotometric results were subjected to statistical analysis. The mean values and variants curves of optical density in the control and experimental groups were compared.

EXPERIMENTAL RESULTS

In the section the sites of SDH activity were revealed by deposits of dark blue diformazan granules. A marked zonal distribution of SDH was observed. The diformazan granules were deposited mainly and in large numbers in the epithelial cells. Only solitary granules were found in the stroma, while in the blood vessels of different caliber and the capillaries they were present mainly in the endothelium. As a result, on a general inspection of the sections the ciliary epithelium appeared as a clearly demarcated band consisting of diformazan granules (Fig. 1). Some granules were small and round, others large and round, and others were irregular in shape. In some cells the diformazan granules were regularly distributed in the cytoplasm. In other cells (the majority) the granules were concentrated close under the nuclear membrane as a thick dotted line or they appeared as conglomerations of tiny diformazan deposits. The abundance of diformazan granules in the ciliary epithelium is in agreement with observations on the large numbers of mitochondria found in these cells by means of the light microscope [14], and more recently

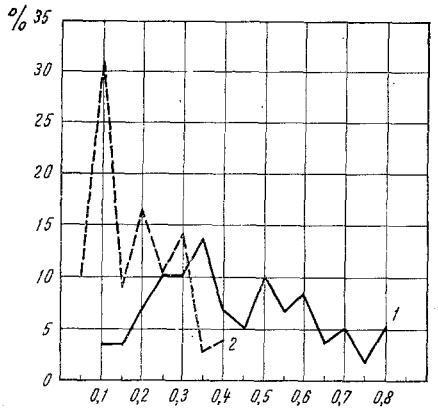

Fig. 3. Variance curves of optical density of diformazan deposits in ciliary epithelium of rabbit's eye under normal conditions and after stimulation of anterior hypothalamus: 1) control; 2) experiment. Abscissa, optical density; ordinate, frequency (in percent).

also by the electron microscope [11, 16]. The formation of the aqueous humor is accompanied by considerable expenditure of energy [9, 12], and this also agrees with the high SDH activity detected in the cells of the ciliary epithelium. The energy is evidently produced by oxidation of substrates brought by the blood-stream, in agreement with the rich blood supply of the processes of the ciliary body. The high level of SDH activity in the perinuclear zone of the cells is evidence of the high intensity of the biochemical processes, probably connected with the secretion of the components of the aqueous humor, taking place in that zone.

After stimulation of the anterior portion of the hypothalamus (the region of the supraoptic nucleus) the localization of diformazan granules remained unchanged, but the total activity of the enzyme was appreciably reduced. This was shown by a decrease in the number of granules, so that in some places only very tiny specks of diformazan could be seen. The intensity of staining of the granules was reduced, and in some places the granules looked like "ghosts" or were completely absent. The density of their deposits in the cells was reduced, especially in the basal portions of the epithelium. As a result, the width of the band corresponding to the epithelium covering the processes, which was abundantly filled with granules in the control sections, was appreciably narrower after stimulation of the hypothalamus (Fig. 2). Hardly any of the clusters of diformazan granules in the apical parts of the cells, characteristic of the normal appearance, were found in the sections through the ciliary body of the experimental animals. Against this background of a general decrease in SDH activity, however, some areas still retained a fairly high diformazan content, and in those places their distribution was still of the the same type: concentration of granules in the outer epithelial cells, in their apical portions and in the perinuclear zone.

The results of this visual study of the sections concurred with the cytophotometric results. For example, the optical density of the diformazan granules and of their deposits in the ciliary epithelium of the control sections averaged 0.428 ± 0.024 (in optical density units), and after electrical stimulation of the anterior zone of the hypothalamus this figure was reduced to 0.118 ± 0.015. In other words, SDH activity in the ciliary epithelium was reduced by 2.2 times below the control level ($P < 0.001$). The decrease in the mean optical density in the experimental group was accompanied by a shift to the left of the variance curve (Fig. 3). The results thus show that the state of the SDH activity in the cells secreting the aqueous humor depends on influences arising from the anterior hypothalamic region. It was accordingly interesting to compare these results with the opthalmotonus under the same conditions. The writers' earlier investigations [6] and also the study of the ophthalmotonus in the present group of animals, revealed a relatively long wave of decrease of ophthalmotonus (up to 3.4 h) approximately 20-30 min after electrical stimulation of the anterior hypothalamus (the region of the supraoptic nucleus). These relatively prolonged changes in ophthalmotonus arising sometimes after hypothalamic stimulation are evidently due to the influence of hormonal factors. At the time of stimulation and immediately after it, a very small increase in intra-ocular pressure was observed, or in some cases the pressure was reduced. The rapid appearance (short latent period) and the short duration of these changes are evidence of their neurogenic origin. It can accordingly be concluded that in these experiments SDH activity in the ciliary epithelium accompanied the decrease in intra-ocular pressure. The influence of the anterior hypothalamus on ophthalmotonus may therefore be exerted through a trophic component in the system responsible for stabilizing the intra-ocular pressure or, more specifically, through its influence on SDH activity in the cells secreting the aqueous humor.

In the experiments in which the middle portion of the hypothalamus (region of the ventromedial nucleus) was stimulated no clear and regular changes in the deposition and character of the diformazan granules in the ciliary epithelium could be seen on visual inspection. Only here and there could slight signs of increased SDH activity be detected. The mean density of the diformazan deposits in the epithelial cells of the ciliary body after stimulation of the middle hypothalamus was 0.263 ± 0.017, while in the parallel control sections it was a little lower, namely 0.210 ± 0.012. In these experiments a very slight increase

in optical density of SDH compared with the control was thus found ($P < 0.05$). Analysis of the variance curves for the comparable groups of experiments showed no definite difference between them. Only a slight shift of the curve for the experimental group to the right relative to the control curve could be noted. In these experiments there was no decrease in the opthalmotonus, but the intra-ocular pressure, on the other hand, increased.

The results described are important in connection with the role of the hypothalamo-hypophyseal neurosecretory system in the homeostatic regulation of the intra-ocular pressure.

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