INVERSE ENZYMATIC CHANGES IN NEURONS AND GLIA DURING INCREASED FUNCTION AND HYPOXIA

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ABSTRACT

Following stimulation of the vestibular nerve in the rabbit, respiratory enzyme activities increased in Deiters' nerve cells. The anaerobic glycolysis, measured as 10^{-4} μl CO_2 per hour per cell, was found to decrease concomitantly by 25 to 40 per cent, suggesting a Pasteur effect. By contrast, in the surrounding glia the anaerobic glycolysis increased and the respiratory enzyme activity decreased, suggesting a Crabtree effect. The evidence is discussed for a regulatory metabolic mechanism operating between the neuron and its glia. Hypoxia of 8 per cent O_2 caused an increase of both oxygen consumption and CO_2 production in the nerve cells, but did not change the glia values.

It seems probable that a regulatory metabolic mechanism exists between the neuron and the surrounding glia. The base composition of the neuronal and glial RNA showed a complementarity with respect to guanine and cytosine, the ratios being 33.5 and 28.8 for the neuron, and 28.8 and 31.8 for the glia (1). When the vestibular nerve was physiologically stimulated, the amount of RNA and proteins increased in the Deiters' nerve cells as did the respiratory enzyme activities. The inverse changes were found in the surrounding glia (2). Under the same experimental conditions, a kinetic study of the succinate oxidizing enzyme system and its temperature dependence showed that there was a difference in behavior between the neuron and its glia as a function of stimulation (3). The neuron highly increased the capacity of its electron-transporting system. The glia did not change in this respect, nor did the calculated activation energy of the reaction reveal any temperature dependence within the range 37° to 27°C.

To gain more information on the linkage between the two cell types, the anaerobic glycolytic capacity of the Deiters' nerve cells and its glia was studied. The experimental conditions used were the same as those referred to above. In addition, some studies were carried out on animals subjected to decreased oxygen tension.

MATERIAL AND EXPERIMENTAL CONDITIONS

129 white rabbits weighing 1.6 to 1.8 kg were used. The vestibular nerve was stimulated by rotating the animal through 120° horizontally and 30° vertically with 30 turns per minute and for 25 minutes per day for 1 to 7 days, as described by Hydén and Pigon (2).

Moderate hypoxia was produced by subjecting the animals to 8 per cent O_2 and 92 per cent N_2 in a glass cage for about 15 hours. The flow rate of the gas was 3 liters per minute. The inlet and outlet of the gas were placed in opposite corners of the cage. The CO_2 content of the gas used was found not to exceed 0.35 per cent as analyzed by the micro method of Scholander (13). Therefore, no CO_2 absorber was used. The arterial oxygen tension of the animals and hence the tissue oxygen tension was calculated to be below 30 mm Hg (14). No pathological symptoms could be observed during or after the experiment, nor did a microscopic study of the brain reveal any pathological changes up to 1 month after the experiment.

The large nerve cells within the lateral vestibular nucleus, the so-called Deiters' nerve cells, and the glia surrounding each nerve cell were collected by free hand dissection according to a technique previously described (4, 2). The dry weight of the glia samples matched that of the nerve cells determined by x-ray microspectrography (5, 6) and was 20,000 $\mu\mu g$ for each sample.

Determinations of the cytochrome oxidase activity were carried out by means of a modified micro diver technique according to Zeuthen (7). The detailed use of this method in the study of single neurons and glial cells has been described by Hydén and Pigon (2). The following materials were used for the cytochrome oxidase assay: 0.0375 M phosphate buffer, pH 7.4; 10^{-4} M cytochrome *c* (beef heart, Sigma); 0.0125 M sodium ascorbate, prepared by neutralizing ascorbic acid with NaOH just before the experiment; 5×10^{-4} M $AlCl_3$; 1 mg/ml crystalline serum albumin.

For the measurements of anaerobic glycolysis, a slightly modified manometric method was used, according to Zajicek and Zeuthen (8), for determination of CO_2 production. The experimental work is more complicated than for respiration measurement because oxygen must be avoided, and all solutions used must be saturated with 5 per cent CO_2 and 95 per cent N_2. Glycolysis was determined in micro divers made from pyrex glass capillaries. The divers were preconditioned by 12 hours of internal exposure to CO_2-bicarbonate. Bicarbonate solution through which 5 per cent CO_2 and 95 per cent N_2 had previously been bubbled was sucked into the divers.

The nerve cells and glial samples were placed in a drop of reaction mixture saturated with 5 per cent CO_2 and 95 per cent N_2.

The vessels which contained bicarbonate solution were perfused with 5 per cent CO_2 and 95 per cent N_2 through a thin polyethylene tubing. The perfusion was continued for about 30 minutes after the divers had been introduced. Calculation of CO_2 production was made according to Zajicek and Zeuthen (8). CO_2 production was expressed in 10^{-4} μl CO_2 per hour per nerve cell or per corresponding weight of glia. The reaction mixture used was slightly modified after Stoesz and Le Page (9). The experiments were done in two series with a potassium concentration of 0.053 M and 0.026 M, respectively.

The reaction mixture for the first series consisted of sodium phosphate buffer, pH 7.6, 0.0024 M; $KHCO_3$ 0.025 M; nicotinamide, 0.04 M; K-ATP, 0.00033 M; K-DPN, 0.0002 M; sodium fructose 1.6 diphosphate, 0.002 M; glucose, 0.01 M; $MgCl_2$, 0.0067 M; sodium pyruvate, 0.005 M; KF, 0.01 M; KCl, 0.0154 M; sucrose, 0.1 M. For the reaction mixture for the second series, phosphate buffer and bicarbonate were changed to sodium salts. The remaining substances were the same as those in the first series. The concentrations given are those in the final mixture in the diver. The flotation medium was made up of 0.025 M $KHCO_3$ and $NaHCO_3$, respectively. Controls consisting of divers with complete substrate medium but no cells gave no measurable CO_2 production. All measurements were carried out at 37°C.

The determination of RNA in $\mu\mu g$ per nerve cell was carried out according to Edström (10, 11). RNA was extracted from the cells with a buffered solution of ribonuclease. The RNA in the extract was determined in micro drops by a photographic-photometric method using ultraviolet radiation at 2570 A. The coefficient of variation at the determination of 100 to 1000 $\mu\mu g$ of RNA averages 5 per cent.

TABLE I

Anaerobic Glycolysis of Deiters' Nerve Cells and Their Glia Following Vestibular Stimulation

Dry weight of the nerve cell and the glia samples, 20,000 $\mu\mu g$.
Rabbits stimulated for 25 minutes per day for 7 days.
Values expressed as 10^{-4} μl CO_2 per hour per sample $\pm$ S.E.M.

$10^{-4}\mu l$ CO_2	Controls	V*	Stimulated	V	Per cent changes
Nerve cell	9.1 ± 0.96	33.2	7.0 ± 0.77	34.9	−23%
Glia	7.4 ± 0.86	36.6	12.7 ± 1.41	38.4	+72%

Number of analyses, 42.
Number of animals, 14.

$* V = \frac{s \times 100}{\text{mean}}$.

RESULTS

Vestibular Stimulation

The intermittent physiological stimulation caused a marked decrease of the glycolytic

capacity of the vestibular nerve cells. In contrast, an increase of glycolytic capacity was observed in the glia constituting the immediate surroundings of the nerve cells. See Table I.

In the second series of experiments comprising 12 rabbits and 29 analyses, a potassium concentration of 0.026 M was used. Principally the same type of changes in the neurons and the glia was obtained.

Note that in both series of experiments the values of the CO_2 production are higher in the glia.

TABLE II

The Effect of Hypoxia for 15 Hours on the Amount of RNA of Deiters' Nerve Cells

Dry weight of the nerve cells, 20,000 $\mu\mu$g.
RNA expressed as $\mu\mu$g per cell ± S.E.M.

Controls*	V‡	Hypoxia	V
1550 ± 35	30	1919 ± 36	11

Number of analyses: controls, 180; hypoxia, 36.
Number of animals: controls, 50; hypoxia, 3.
* Control values taken from Hydén and Pigon (2) and Egyhazi and Hydén (1).

‡ $V = \frac{s \times 100}{\text{mean}}$

Hypoxia

To make possible a comparison between the effects of physiological stimulation and a medium grade hypoxia on the components of the lateral vestibular nucleus, the amount of RNA and of the cytochrome oxidase activities per cell sample were determined.

As is seen from Tables II and III, the hypoxia caused a significant increase of the RNA content and of cytochrome oxidase activity of the nerve cell. The enzyme activity of the glia, on the other hand, did not change.

The hypoxia effected a marked increase of the anaerobic glycolysis of the nerve cells and a slight increase of that of the glia (Table IV). Thus, in

TABLE III

The Effect of Hypoxia for 15 Hours on the Cytochrome Oxidase Activity of Deiters' Nerve Cells and Their Glia

Values expressed as 10^{-4} μl O_2 per hour per cell sample ± S.E.M.

$10^{-4}\mu$l O_2	Controls	V*	Hypoxia	V
Nerve cell	4.2 ± 0.61	58	14.2 ± 0.83	35
Glia	11.5 ± 0.84	30	11.3 ± 0.58	21

Number of analyses: controls, 68; hypoxia, 54.
Number of animals: controls, 17; hypoxia, 20.

* $V = \frac{s \times 100}{\text{mean}}$.

TABLE IV

Anaerobic Glycolysis of Deiters' Nerve Cells and Their Glia after Hypoxia, 8 per cent O_2, for 15 Hours

Dry weight of the nerve cell and the glia samples, 20,000 $\mu\mu$g.
Values expressed as 10^{-4} μl CO_2 per hour per sample ± S.E.M.
Concentration of K^+, 0.053 M.

$10^{-4}\mu$l CO_2	Controls	V*	Stimulated	V	Per cent changes
Nerve cell	9.1 ± 0.96	33.2	14.3 ± 3.22	67.5	+57%
Glia	7.4 ± 0.86	36.6	8.6 ± 1.23	42.8	+16%

Number of analyses, 38.
Number of animals, 11.

* $V = \frac{s \times 100}{\text{mean}}$.

neurons the changes were the reverse of those occurring during intermittent physiological stimulation.

DISCUSSION

Vestibular Stimulation

With the same type of vestibular stimulation experimental conditions, and material, it has previously been found that the amount of RNA and protein and the respiratory enzyme activities in *the nerve cells* increased significantly. *The glia* responded with inverse chemical changes under these conditions (2). The results were obtained on a dry weight basis and made likely an increased neural enzyme production as a function of stimulation. The kinetic studies (3) showed that the nerve cell has a greater capacity of the electron-transporting system, reflecting higher energy requirements of the neuron as compared with the glia. The decrease of the anaerobic glycolysis reported here in the nerve cells, when the respiratory enzyme activities increased, is most striking (Fig. 1). These changes have the character of a Pasteur effect. By the same type of considerations, the glia changes suggest a Crabtree effect.

From the point of view of a regulatory mechanism, the inverse qualitative differences and quantitative changes in the amount of RNA and protein and the enzyme activities of the neurons and their glia suggest strongly that such a mechanism operates between the two types of cells. The increase of the anaerobic glycolysis in the glia, when the respiratory enzyme activities of the neuron increased and the inverse changes occurred in the neuron, suggests that the neuron has priority in its high energy requirements when function so demands.

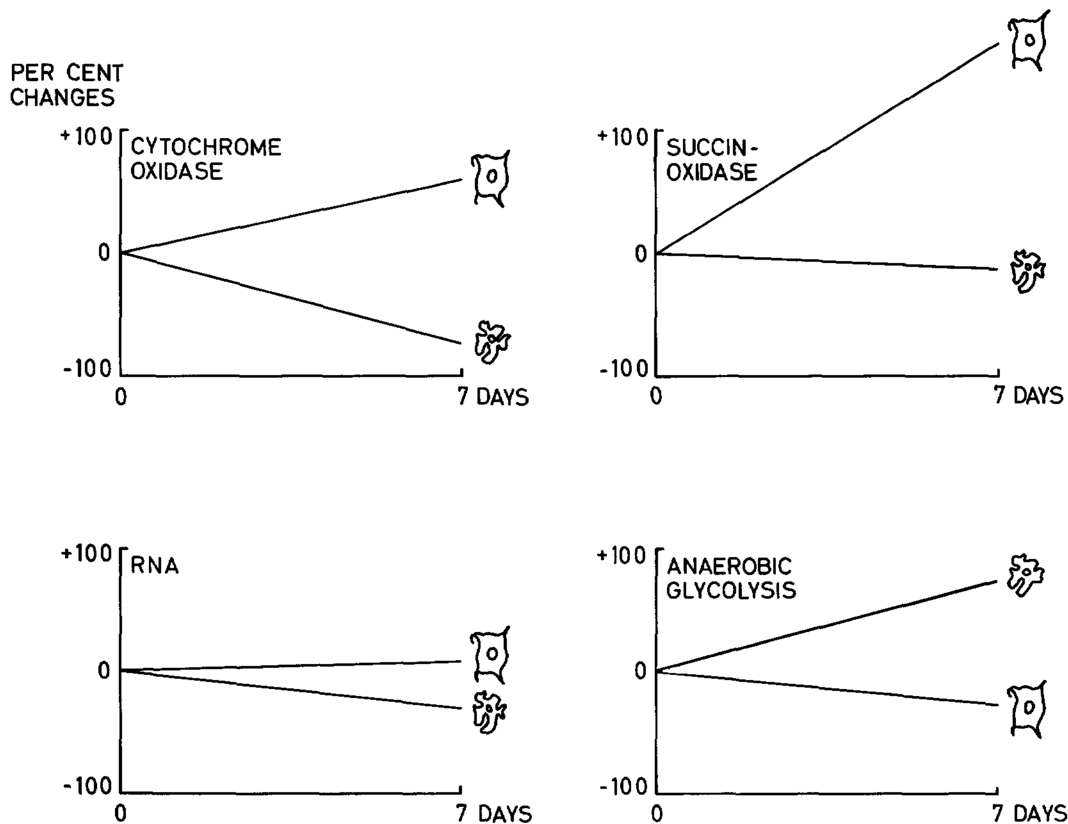

FIGURE 1

Survey of quantitative changes found in Deiters' nerve cells and glia after vestibular stimulation 25 minutes per day for 7 days.

The data discussed show that the neuron and its glia are linked in a coupled energetic system, the units of which can swing between two positions. Such a regulatory mechanism provides great stability from a cybernetic point of view.

Whether a regulatory mechanism between the neuron and its glia exists only at a metabolic level or also operates functionally is a problem for future studies. Hild and Tasaki (12) found that the resistance of the glial membranes was less than that of the neuronal membrane. Neuronal currents could, therefore, flow through the glia and possibly serve as an informative link in a feedback system glia to neuron.

Hypoxia

It has been inferred from results on muscle that increased function (stimulation) could induce cellular hypoxia. To judge from our results, the mechanism cannot be the same in the two instances in nerve tissue. It is to be noted that the decreased oxygen tension used here did not cause any acute or late functional or pathological changes. Rabbits can withstand well even more pronounced hypoxia.

In the neuron, the increase of RNA per nerve cell during hypoxia was considerable (20 per cent), and even more so the increase in the cytochrome oxidase activity (300 per cent). The anaerobic glycolysis increased by 57 per cent, contrasted with the decrease caused by stimulation. In the glia, the cytochrome oxidase activity during hypoxia did not change, nor did the anaerobic glycolysis. The neuronal changes during hypoxia evidently reflect an emergency situation. The inertia of the glia agrees well with its known capacity to withstand decreased oxygen tension.

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