A KINETIC STUDY OF THE NEURON-GLIA RELATIONSHIP

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ABSTRACT

Kinetic studies have been performed on the activity of the succinate oxidizing enzyme complex in living nerve cells and glial cells after increasing duration of stimulation. The nerve cells and glia of the lateral vestibular nucleus were used. The results show a clear difference between the neuron and its surrounding glia. The nerve cell reacted by highly increasing the capacity of the electron transporting system, reflecting an increased consumption of energy as a function of the stimulation. The glia, in contrast, did not change in this respect.

Qualitative as well as quantitative biochemical differences have been shown to exist between the neuron and the glia immediately surrounding the neuron (1, 24). Differences were found even down to the base composition of RNA in the two types of cells (3). Physiological stimulation of the vestibular nerve was found to be followed by a significant increase of the amount of RNA, protein, and certain enzyme activities in the neuron. Inverse changes were found in the glia.

The following investigation is a kinetic study of the behaviour of an enzyme reaction and its temperature dependence in the neuron and neighbouring glia, as a function of increased physiological stimulation.

The comparison between the reactions in the neuron and in the glia could be made on a dry weight basis. Therefore, it was possible to study differences in energy requirement as the functional demand on the vestibular organ was changed. The activity of the succinate oxidizing enzyme complex (here called succinoxidase) was chosen because of the unique position of succinate in the Krebs cycle with direct transfer of electrons to the cytochrome system of the respiratory chain.

MATERIAL AND EXPERIMENTAL CONDITIONS

87 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. The animal was placed in a tight-fitting box with the head away from the centre. Vestibular tests were used to check that the peripheral organ had not been damaged.

The animals were killed by bleeding and the brain rapidly removed. The large nerve cells within the lateral vestibular nucleus, the so called Deiters' nerve cells, and the neuroglia immediately surrounding each nerve cell were used for the study. The brain material was immersed in 0.25 M sucrose solution. The cells were rapidly removed by free-hand dissection under a stereomicroscope at × 100 magnification as described earlier (2, 5). The nerve cells were transferred to a clean solution of sucrose and cleaned from glia. The glia cells surrounding each nerve cell were collected in a dense cluster and trimmed down to a spheroid having a volume approximately equal to that of the nerve cell. This was checked as described earlier (2). The dry weight per unit volume of the nerve cell and the glia was determined by quantitative x-ray micro-

radiography at 10 to 12 A using for the evaluation of the x-radiograms a scanning and computing analyzer (6, 7). Each nerve cell and glia sample had the dry weight of 20,000 to 21,000 $\mu\mu$g. Most of the glia is composed of oligodendroglial cells. We would like to stress that the enzyme activities thus are measured on a dry weight basis, and also that these cells, isolated as described, can be considered to be living, because they can be cultivated if they are transferred to a fortified Eagle medium. At this laboratory such nerve cells plus glia have been cultivated for periods up to 5 months (8).

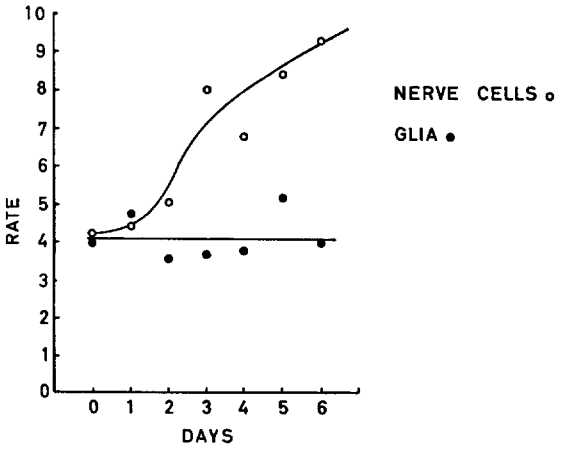

FIGURE 1

The activity of the succinate oxidizing enzyme complex expressed as $\mu l O_2 \cdot 10^{-4}$ per nerve cell and glia sample per hour at 37°C, as a function of increased duration of vestibular stimulation.

METHOD OF ANALYSIS

Determinations of the activity of the succinoxidase were carried out using a modified micro-diver technique according to Zeuthen (9). For the succinoxidase assay we used: 0.0375 M phosphate buffer, pH 7.4; 10^{-4} N cytochrome *c* (beef heart, Sigma Chemical Company, St. Louis); 0.025 M Na-succinate; 5×10^{-4} M $AlCl_3$; 5×10^{-4} M $MgCl_2$; 1 mg/ml crystalline serum albumin.

The weight of the divers was 0.3 to 0.2 mg, corresponding to a gas volume of 0.15 to 0.10 μl. Not more than one nerve cell or glia sample was placed in each diver. Four samples could be analyzed at the same time. Before recordings were taken, the cells were allowed to stay in the substrate and the diver was immersed in the temperature-controlled water bath for 1 hour. The calculations were carried out using Zeuthen's formula (9). The results are expressed in μl $O_2 \cdot 10^{-4}$ per nerve cell or glia sample per hour.

RESULTS

Since all enzymatic reactions occur at 37°C, the rate of the reaction (measured as succinoxidase activity) at this temperature was studied. Fig. 1 demonstrates the results obtained.

For both nerve cells and glia the reaction rate during the first 2 days was practically the same. The increased duration of the stimulation, however, caused a significant difference in the reaction rate between the two types of cells. After 6 days of intermittent stimulation (total stimulation for 150 minutes) the reaction rate of the nerve cells was

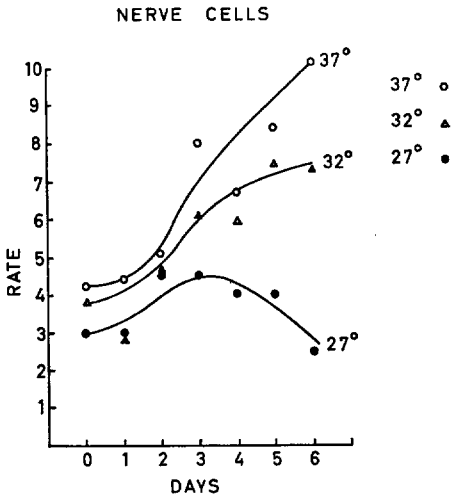

FIGURE 2

The reaction rate of succinoxidase of living nerve cells at 37°, 32° and 27°C, as a function of increased duration of vestibular stimulation.

more than twice that of the neuroglia. In earlier experiments (2) it was found that the total amount of proteins per nerve cell had increased by 15 per cent after 6 days of vestibular stimulation. The enzyme activity is expressed in terms of dry weight. Thus, the change in reaction rate is only slightly less pronounced after 6 days if results are expressed in terms of $\mu\mu$g of dry weight instead of per cell. The nerve cell thus must have access to a more abundant supply of energy than before.

In striking contrast to the nerve cell, the glia cells do not respond to the stimulation by increasing the activity of the electron-transporting system, *i.e.* reflecting energy requirements surpassing those during control vestibular conditions.

As a next step, the temperature dependence of

the succinoxidase activity (measured at 27°, 32°, and 37°C) was studied as a function of the stimulation of the nerve cells. Fig. 2 and Table I give the results. After about 3 days of stimulation (a total of 50 minutes) the reaction rate at 27°C begins to show a trend different from that at 32° and 37°C, as can be seen from the curves in Fig. 2. From the 3rd to the 7th day the reaction rate at 27°C steadily decreases.

plicated systems as living cells, the greatest caution must be taken.

The results are most easily discussed with the aid of the curves in Fig. 4, showing the log rate as a function of the inverse value of the absolute temperature, T. As is seen, the activation energy for the control nerve cells reveals practically no temperature dependence within the temperature range studied. It is interesting to note that the

TABLE I

Succinoxidase activities of individual Deiters' nerve cells, and of the same weight and volume of their neuroglia, expressed as $\mu l\ O_2 \cdot 10^{-4}$ per sample per hour.

Control material and material after vestibular stimulation of the rabbits for 25 min per day and for 1 to 6 days were analyzed at 37°, 32°, and 27°C. Number of analyses: 410.

Nerve cells

Controls		Stimulated animals					
Days	0	1	2	3	4	5	6
Total stimulation in minutes	—	25	50	75	100	125	150
Enzyme activity at							
37°C	4.2	4.4	5.1	8.0	6.7	8.4	9.8
32°C	3.8	2.8	4.7	6.1	5.9	7.4	7.3
27°C	3.0	3.0	4.6	4.5	4.0	4.0	2.5

Neuroglia

Controls		Stimulated animals					
Days	0	1	2	3	4	5	6
Total stimulation in minutes	—	25	50	75	100	125	150
Enzyme activity at							
37°C	4.0	4.7	3.6	3.7	3.8	5.2	4.0
32°C	3.3	4.0	4.4	3.5	2.9	4.7	4.0
27°C	2.5	3.5	4.0	3.0	3.0	3.0	2.9

In contrast to the reaction in the nerve cells, the reaction in the glia bears no relation to the duration of the stimulation. The values at the three different temperatures show no systematic variation as a function of the stimulation. Therefore, all values obtained have been averaged for each temperature and are represented by the three straight lines in Fig. 3.

A further possibility for studying the differences between the glia and the nerve cell is offered by calculating the apparent activation energy of the reaction. The authors are well aware that, in a kinetic study of enzymic reactions in such com-

glia cells, in both control material and stimulated material, show the same behaviour as do the control nerve cells. The numerical value of the activation energy for these cases is calculated to be between 4,000 and 8,000 cal. These approximate values have been obtained by using the values of the smooth curves for 32° and 37°C in Fig 2. With increasing duration of the stimulation the apparent energy of activation for the enzyme reaction in the nerve cells shows an increasing temperature dependence.

It seems that within the temperature range of 27° to 32°C the decreasing rate of the reaction

reflects a change of the enzyme reactions as a result of the stimulation. Another step in the reaction chain may have become the limiting factor. In other words, the stimulation has diminished the capacity of a regulating mechanism.

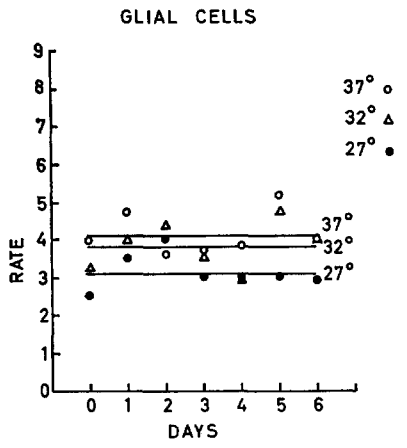

FIGURE 3

The reaction rate of succinoxidase of living glial cells at 37°, 32°, and 27°C, as a function of increased duration of vestibular stimulation.

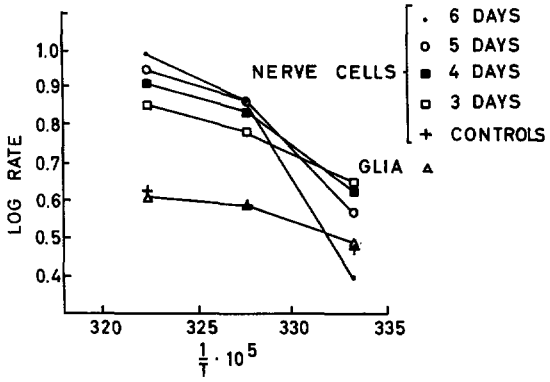

FIGURE 4

Log rate of the activity of succinate oxidizing enzyme complex of living nerve cells and glial cells after increased duration of vestibular stimulation, as a function of the inverse value of the absolute temperature.

We would like to stress, however, that the systematic deviation of this straight-line relationship between log rate and $\frac{1}{T}$, as a function of the duration of the stimulation, supports the significance of the experimental data obtained in this work.

The question may be raised as to whether the substrate has equal access to neurons and glial cells, or whether these cells differ in their ability to transport the divalent ion, succinate, across the cell membrane. A substrate-enzyme curve has, therefore, been made (16). Lowering of the substrate concentration by 50 per cent did not produce a decrease of the enzyme activity. Therefore, under the experimental conditions used, a membrane effect does not seem to have constituted an error.

DISCUSSION

The results of the kinetic studies reported here have shown that there is a clear difference in behaviour between the neuron and its surrounding glia as a function of physiological stimulation.

These results should be viewed against the background of other relevant data. With the same type of stimulation, experimental conditions, and material, it was found (2) that the amount of RNA and protein in *the nerve cells* increased significantly, as did the cytochrome oxidase activity. Furthermore, since the enzyme activities were expressed on a dry weight basis, the results indicated an increased enzyme production and a greater activity of the electron-transporting system. Even though the results do not prove that new neuronal enzyme was produced, it seems likely that it was. Since the amount of RNA and protein increased as a function of the stimulation, as did the respiratory enzyme activities, it would seem probable that the proteins produced included also enzyme proteins. Whether the increased enzyme activities may depend on an activation reaction or release from inhibition is a problem for future studies.

The neuroglia, on the other hand, responded with inverse quantitative changes. The amount of RNA (matching that of the nerve cell) decreased as did the cytochrome oxidase activity.

The kinetic findings reported in this study permit a discussion of the neuron-glia difference from the point of view of energy relationships. The cytochrome oxidase results in previous studies can be included. After 6 days of intermittent stimulation (150 minutes) the reaction rate (both succinoxidase and cytochrome oxidase activities) of the nerve cell was about twice that of the glia (1). This shows a greater capacity of the electron transporting system of the nerve cell. The data presented here reflect not only the higher energy requirements of the nerve cell as compared with the glia, but also might reflect the difference in speed of the electri-

cal activities in the two types of cells. In contrast to the action potentials in the neuron, the action potentials recorded from glial cells are slow (11). Wilkie (12) made the analogy between a chemical reaction in a cell and an electrical unit having a certain voltage and producing a current. In both systems power is dissipated in order to drive a process along at a finite rate.

How can the "inertia" of the glia be explained? Under experimental conditions the same as those in the present study, the glia cells surrounding the Deiters' nerve cells were found to increase their capacity for anaerobic glycolysis (10), but did not increase their respiratory enzyme activities. On the other hand, the nerve cell did. It, therefore, seems that with increasing functional demands the neuron has priority in its high energy requirements. As is seen from the present data, the reaction of the glia cells differs fundamentally from that of the nerve cells. The rate of enzyme reaction in the glial cells bears no relation to the duration of the stimulation. The glia cells have been shown to resort partly to anaerobic glycolysis with increasing stimulation (10). It is appropriate to point out that no increased modulated frequencies (13) have been observed as a result of stimulation of the glia.

The observed decrease in the reaction rate of the nerve cell with increasing stimulation, as measured at 27°C, cannot at present be commented upon. The changes which may have taken place in the enzyme reactions and/or in the enzyme substrate complex, as a function of the increasing amount of stimulation, are problems for future work. It may be mentioned that Ungar *et al.* (14, 15) found that after stimulation there was indication of a reversible change in the configuration of protein toward denaturation.

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