FUNCTIONAL ADJUSTMENTS OF THE BRUSH RECEPTOR DURING INHIBITION OF ANAEROBIC GLYCOLYSIS

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We provisionally explained our data relating to the high resistance of tissue brush receptors to anoxia by the ability of these structures to switch their energy metabolism within a short time from oxidative phosphorylation to glycolysis [4]. The role of glycolysis in the energy supply for nerve cells during ischemia has been studied in fair detail [6, 7, 9, 10]. Meanwhile the role of this mechanism during functioning of brush receptors has virtually not been studied.

This paper describes an investigation of brush receptor function when anaerobic glycolysis is inhibited.

EXPERIMENTAL METHOD

Living brush receptors in the wall of the urinary bladder of Rana temporaria served as the test object. The bladder was isolated from the spinal frog, and a piece of its wall was excised, with an area of about 1.5-2 cm^2, including the bladder neck, which is rich in receptors. Spike activity of the receptors was recorded from the vesical nerves by means of a suction electrode. The bladder preparation was stretched in a cuvette with a thin transparent bottom (170 μ), which enabled receptors to be examined by intravital microscopy, on the MBI-14 inverted microscope. The receptors were stained with methylene blue ($6.5 \cdot 10^{-5}$ M).

Decolorization of single receptor plaques in the course of time was recorded by the FMÉL-1A cytophotometer. Afferent spike activity was recorded by FOR-2 camera synchronously with intravital microscopy and cytophotometry. Oxygen was expelled from the solutions with nitrogen. There were three series of experiments. In all series the receptors were initially stained with methylene blue in a normoxic medium. The dye solution was then replaced by a colorless solution (without the dye): in series I – by anoxic medium with glucose (1 g/liter); in series II – by anoxic medium without glucose; in series III – by anoxic medium with glucose and monoiodoacetate (1 mM); the pH of the solutions was maintained at 6.7. There were 30 experiments altogether. The results were obtained by Student's criteria and the nonparametric criteria of Wilcoxon–Mann–Whitney.

EXPERIMENTAL RESULTS

The visual microscopic control (without using the cytophotometer) gave an estimate of the general pattern of decolorization of the receptor brushes and revealed a difference in the rate of this process depending on exposure to different modifying solutions. If glucose was present in the anoxic solution, the time of complete decolorization was 5.7 ± 0.4 min, if the anoxic solution was without glucose, this time was considerably increased, to 14.1 ± 0.4 min. Exposure to an anoxic solution with glucose and monoiodoacetate, however, shortened the decolorization process to 2.2 ± 0.2 min ($p < 0.05$).

With the aid of the cytophotometer the time course of this process could be evaluated for the individual structural elements of the receptor and, in particular, of the single terminal plaques. The latter are flat formations of irregular shape, measuring (on the longer diameter) 6.5 ± 0.6 μ.

The diameter of the optical probe of the cytophotometer, with the objective (×60) which we used, is about 2 μ, so that not only large, but also quite small plaques (2.5-3 μ) can be studied photometrically. The results showed that although the time course of decolorization

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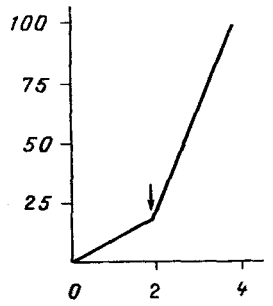

Fig. 1

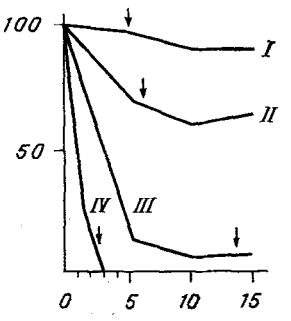

Fig. 2

Fig. 1. Decolorization of receptor plaques in anoxic solution after addition of monoiodoacetate. Abscissa, time (in min); ordinate, degree of decolorization (in %). Arrow indicates time of addition of monoiodoacetate.

Fig. 2. Reduction of firing rate of receptors stained by methylene blue. Abscissa, time (in min); ordinate, frequency (in %). Frequency at time of replacement of staining solution by solutions not containing methylene blue taken as 100%: I) normoxic; II) anoxic with glucose; III) anoxic without glucose; IV) anoxic with glucose and monoiodoacetate. Arrows indicate time of complete decolorization of receptors.

of each individual plaque may differ, the tendency is close to the general pattern of decolorization of the receptors described above. In an anoxic solution with glucose complete decolorization took place after 5.0 ± 0.4 min, compared with 10.0 ± 0.5 min in anoxic solution without glucose. The rate of decolorization of single plaques after addition of monoiodoacetate to the external solution rose sharply. It will be clear from Fig. 1 that the slope of the curve changed substantially after addition of the inhibitor and complete decolorization took place after 2.0 ± 0.3 min ($p < 0.05$).

The frequency characteristics of the spontaneous discharge of the receptors showed appreciable changes. The general tendency was for impulsation to be inhibited; for each external agent, moreover, the dynamics showed particular features (Fig. 2). In anoxic solutions with or without glucose, slowing of the discharge began immediately after the beginning of exposure to the solution, and the effect was nonlinear in character: initially its rate was quite rapid, but toward the time of complete decolorization of the solutions it decreased. After decolorization a very small increase was observed in the frequency, and under anoxic conditions and in the absence of glucose, the rate and degree of reduction of frequency were more marked than in the presence of glucose. The time course of the change in frequency under the influence of anoxia and in the presence of monoiodoacetate showed characteristic differences. It will be clear from Fig. 2 that in this case inhibition of the spike discharge took place rapidly, and there was no tendency toward an increase of firing rate at the time of complete decolorization.

Thus deprivation of glucose and inhibition of glycolysis modify, although differently, electrogenesis and the staining properties of receptors exposed to the effects of anoxia. This is shown by the more rapid inhibition of spike activity and by changes in the time of decolorization of the structures stained by the vital dye.

We know that monoiodoacetate blocks sulfhydryl groups of enzyme molecules and depresses the activity of the latter or completely suppresses it [3, 5, 8]. It is considered that monoiodoacetate is a specific inhibitor for enzymes of glycolysis [8, 12]. Admittedly, as has been stated [12], in a concentration of more than 1 mmole this inhibitor interacts with sulfhydryl groups of other enzymes and its specificity is reduced. The fact that we used a low concentration of the inhibitor suggests that its blocking action is expressed mainly on the group of enzymes responsible for glycolysis. The rapid depression of spike activity is therefore evidence in this case of inhibition of the sodium-potassium pump as a result of the energy deficiency which arises under anaerobic conditions when glycolysis as a source of energy, alternative to tissue respiration, is blocked [1, 11].

The gradual (and not instantaneous) depression of spike activity (Fig. 2) is evidently connected not only with special features governing interaction between inhibitor and enzymes, but also with the presence of tissue diffusion barriers: initially receptor units located subepithelially, i.e., those to which the inhibitor penetrates sooner, are blocked initially, whereas those in the deeper layers of the wall of the preparation are blocked later.

A change in the time of decolorization of the receptors under the influence of modifying solutions reflects the time course of the change in level of reducing equivalents which, in turn, is an indicator of the intensity of energy metabolism [2]. Thus a more than twofold increase in the time of decolorization in series II (absence of glucose) compared with series I (presence of glucose) reflects lowering of the energy level of the receptor. However, as the synchronous electrophysiological control showed, under these conditions complete energy hunger does not arise, for the reserves are still intact, in the form of tissue deposits of glucose and glycogen, so that metabolic processes can be maintained, although at a lower level [4].

A completely different situation arises under the influence of monoiodoacetate. We know that the sensitivity of enzymes of glycolysis to this inhibitor differs: SH-groups of some are alkylated almost instantaneously, inhibition of the dehydrogenases participating in the glycolytic cycle therefore takes place at different times. As a result, the proton transport chains are interrupted and protons accumulate in the substrate. The methylene blue present in it, which is an active proton acceptor, takes up their excess and is rapidly reduced, i.e., it is converted from the colored to the colorless form.

Thus the data confirm the view that under anaerobic conditions glycolysis plays a very important role in the maintenance of the energy metabolism of tissue receptors. It is evidently responsible for the high resistance of the brush receptor to anoxia.

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