ELECTROPHYSIOLOGICAL INVESTIGATION OF RELATION BETWEEN FUNCTIONAL STATE OF FROG SKIN RECEPTORS AND METABOLISM IN THE RECEPTOR FIELD

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We showed [6,7], that carbohydrate metabolism is one of the important factors in the provision of energy for maintaining the generation of nerve impulses in the receptors of frog skin. The action of glucose, or glucose and insulin on the skin of the foot causes an intensification of the generation of impulses in the receptors, and, as a result, the afferent impulsation is enhanced, and the time of the reflex by Turck's method is shortened. The same effect is produced by an additional supply of inorganic phosphates to the receptors. Insulin employed without glucose, on the other hand, causes a considerable reduction of the strength and frequency of the generated impulses and an increase in the time of reflex. The action of adrenalin leads to a brief enhancement of the impulsation and its subsequent weakening.

In this work, we studied how the generation of nervous impulses in the receptors was affected by the introduction of high-energy phosphates in the form of the sodium salt of adenosine triphosphoric acid (ATP), and also by the exclusion of phosphorylation and glycolysis, i.e., processes which lead to the formation of high-energy phosphates.

Method

The method consisted in a comparison of the frequency and amplitude of the afferent impulsation in the peripheral section of the frog sciatic nerve before and after the action of the investigated agents in the receptor field. The work was conducted on frogs with a destroyed central nervous system. For the registration we used platinum air electrodes with interelectrode distance 6-10 mm. The potentials were recorded on the loop oscillograph MPO-2 with an amplifier. The animals were screened. After determination of the initial background of impulses, one of the feet was immersed for 10-15 min in a solution of the test substance, and the other foot (control) was immersed for the same time in Ringer's solution. A second recording of the impulsation was made 5 min after removal of the feet from the solution. We studied both "spontaneous" impulsation, as well as impulsation due to stimulation of the receptors by sulfuric acid.

The action of a 1:1000-1:2000 ATP solution on the skin of the frog's foot leads to an increase in frequency and, in particular, amplitude of the spontaneous impulsation (Fig. 1a). Stimulation of the skin by sulfuric acid, in this case, as compared with the same stimulation of the skin of the control foot (not subjected to action of ATP), produces a greater increase in the frequency and amplitude of the impulses. These results are in good agreement with the fact established in experiments on the carotid gland receptors [3] and on the intestinal chemoreceptors [5] – the functionability of these receptors after their "exhaustion" can be restored by the addition of ATP. They are also confirmed by histochemical investigations [9] in which it was found that the receptors contain a specific adenosine triphosphatase, and that the enhancement of the receptor activity is associated with an increase in the activity of this adenosine triphosphatase and with the accumulation of orthophosphates [9].

The exclusion of oxidative phosphorylation in the skin of the frog's foot by immersing it in a 1:1000-1:5000 solution of 2,4-dinitrophenol leads to a pronounced and steadily increasing depression of the spontaneous impulsation (Fig. 1b), and, in some experiments, to its complete, or almost complete cessation. In these conditions, stimulation of the foot with sulfuric acid produces only a very weak

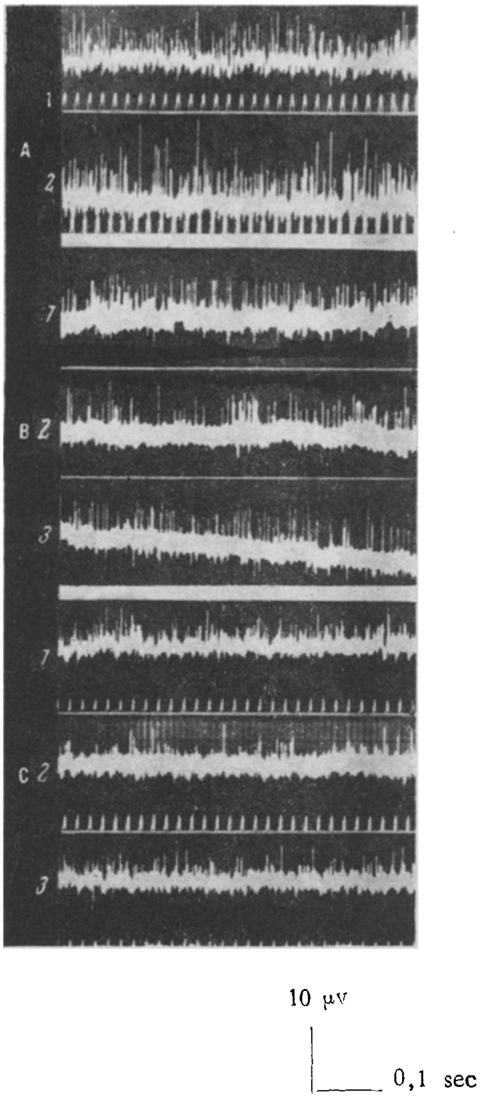

Fig. 1. Spontaneous impulsation of peripheral section of frog sciatic nerve. a) Before (1) and after (2) action of ATP on skin of foot; b) before (1), after (2) action of 2,4-dinitrophenol, and after subsequent action of ATP (3); c) before (1), after (2) action of monoidoacetic acid on skin of foot, and after subsequent action of ATP (3).

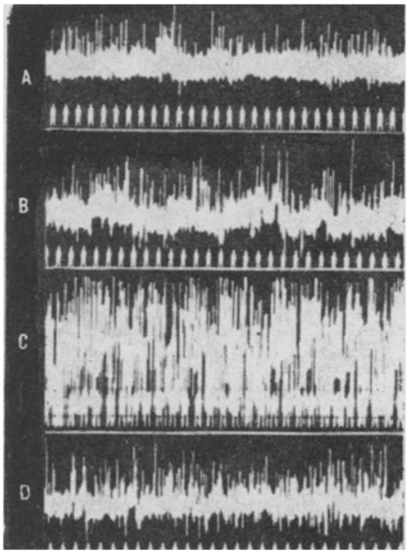

Fig. 2. Spontaneous impulsation of peripheral section of frog sciatic nerve. a) Before action; b) 5 sec after; c) 15 sec after action of sodium fluoride on skin of foot; d) after subsequent action of lactic acid.

impulsation – very much weaker than in the foot with intact metabolism. Immersion of a foot previously subjected to the action of 2,4-dinitrophenol in a solution of the sodium salt of ATP restores, through not completely, the frequency and amplitude of the spontaneous impulsation reduced by 2,4-dinitrophenol (Fig. 1b). Hence, oxidative phosphorylation, i.e., the process playing the most important role in the production of high-energy phosphates, according to the data of I. F. Seits and V. A. Engel'gardt [10], is also of great importance for maintaining the functionability of the receptors. In the absence of oxidative phosphorylation, the functionability of the receptors can be partially restored by the artificial introduction of high-energy phosphates to replace it.

The action on the skin of the frog's foot of a 1:2000-1:5000 solution of monoiodoacetic acid, a substance which blocks glycolysis at relatively early stages, leads to a gradual reduction in the frequency and amplitude of the spontaneous impulsation of the skin receptors (Fig. 1c). Stimulation by sulfuric acid in these conditions causes

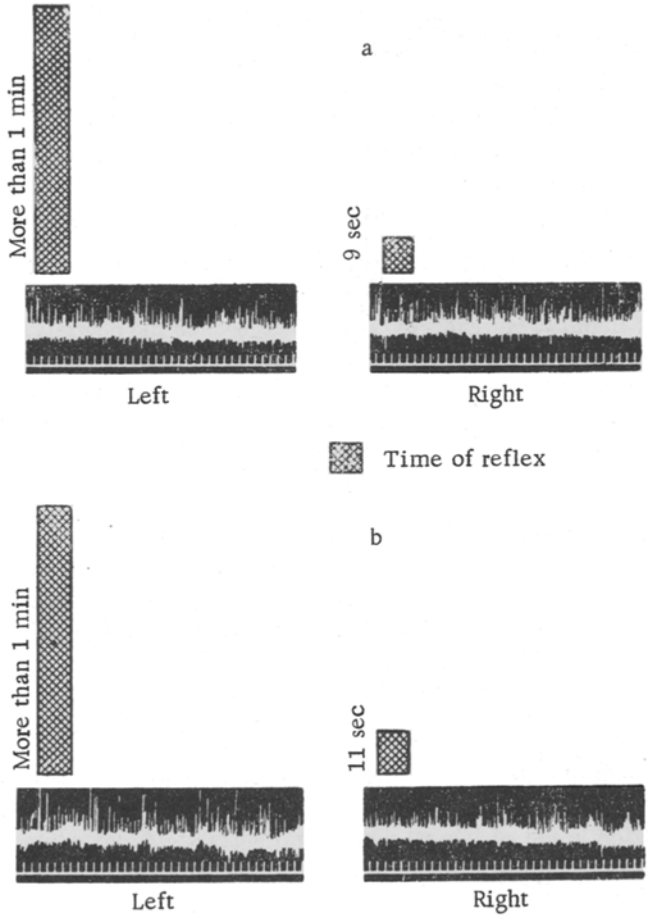

Fig. 3. Relation between time of the reflex by Türck's method and frequency of afferent impulses. Explanation in text.

a smaller increase in frequency and amplitude of the impulses than similar treatment of a control foot in which glycolysis in the skin is not blocked. These results agree with those obtained by other authors for the chemoreceptors of the carotid gland and the internal organs [1,2,4,8,11,12]. In our experiments, immersion of the foot in an ATP solution partially restored the frequency and amplitude of the impulsation which had been weakened by the effect of monoiodoacetic acid (see Fig. 1c), i.e., we observed the same effect as in experiments in which oxidative phosphorylation was inhibited by 2,4-dinitrophenol. We can conclude from this that, in the frog's skin chemoreceptors, the links of glycolysis which are blocked by monoiodoacetic acid, together with oxidative phosphorylation, play an important role in maintaining the quantity of high-energy phosphates at the level necessary to ensure the normal functioning of the receptors.

The action on the skin of the frog's foot of a 1:25-1:100 solution of sodium fluoride, i.e., a substance which blocks not the early, but the late, stages of glycolysis, produces an effect exactly the reverse of that produced by monoiodoacetic acid; the frequency and amplitude of the spontaneous impulsation are not reduced but, on the contrary, are considerably increased (Figs. 2a,b,c). At concentration 1:25, the flow of very large and frequent impulses continues for 20-30 min after removal of the sodium fluoride from the skin. Immersion of the foot in a 1:100 lactic acid solution completely removes this effect of sodium fluoride and restores the frequency and amplitude of the spontaneous impulsation to their initial values (Fig. 2d). In control experiments lactic acid had no effect on the spontaneous impulsation. It follows from these experiments that lactic acid during glycolysis has a suppressive effect on the generation of impulses in the receptors of the frog's foot. At present it is difficult to determine the cause of this effect. It is possibly due to part of the oxygen supplied by respiration being used up on oxidation of the lactic acid, thus limiting the rate of oxidative phosphorylation and, concomitantly, the supply of high-energy phosphates for impulse generation.

An investigation of the time of the reflex by Türck's method showed that the reflex flexure of a foot subjected to stimulation by sulfuric acid was considerably retarded and might even be absent altogether after the action, not only of 2,4-dinitrophenol or monoiodoacetic acid, but also of sodium fluoride [7], on the skin of this foot. Figure 3 shows the relation between the time of the reflex by Türck's method and the afferent impulsation in two frogs. The left foot of one of these had been subjected to the action of 2,4-dinitrophenol (a), and the left foot of the other to sodium fluoride (b). The right feet served as a control. In the first case (a), the long time of the reflex was accompanied by less intense impulsation than in the control foot, and in the second (b), the same long time of the reflex was accompanied by more intense impulsation than in the control.

Since 2,4-dinitrophenol and monoiodoacetic acid reduce the frequency and amplitude of the impulsation, the reason for the longer reflex time produced by them was obviously the weakening of the flow of impulses to the spinal column. Sodium fluoride, however, did not reduce but, on the contrary, greatly increased the frequency and amplitude of the impulsation arising in the skin receptors: the cause of the delay, and even complete cessation of the reflex in this case was probably the development of a pessimum in the spinal column owing to the excess intensity of impulses flowing to it. These experiments point out that the functional state of receptors cannot be determined from reflex effects alone, and that it is absolutely essential to employ the method of recording the afferent impulsation.

The results given here fit in well with the information which indicates that the functional state of frog skin receptors depends on the action of carbohydrate metabolism factors (glucose, insulin, adrenalin, sodium phosphate) on them. In correspondence with modern ideas on the biochemistry of nervous tissues, these results show that oxidative phosphorylation, glycolysis, and the high-energy phosphates formed in these processes play an important role in providing the energy for the functioning of these receptors.

SUMMARY

The functional conditions of cutaneous receptors in the frog's extremity is maintained at the expense of the energy provided by respiratory phosphorylation and glycolysis. As proved by experiments with the registration of the afferent impulsation in the peripheral portion of the sciatic nerve, submersion of the extremity in the ATP solution provokes an increase in the frequency and amplitude of spontaneous impulsation.

Exclusion of respiratory phosphorylation in the skin of extremity by means of 2,4-dinitrophenol, as well as exclusion of glycolysis by monoiodoacetic acid results in the decline of the ATP.

This impulsation is partially restored by amplitude and the frequency of the recorded impulses.

Conversely, exclusion of glycolysis by sodium fluoride, intensifies the impulsation, while lactic acid restores the impulsation to the initial level.

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