STATE OF THE POTASSIUM AND SULFHYDRYL GROUPS IN THE SPINAL MOTOR NEURONS OF THE FROG DURING REFLEX EXCITATION

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Potassium ions play an important role in the process of excitation [1, 6, 8-12, 15-19]. There are two main views regarding the mechanism responsible for the movement of potassium in different functional states, and these are combined into the membrane [17, 18] and sorption [8, 9, 11, 12] theories of excitation. Information is given in the literature regarding the relationship between changes in the concentration of potassium ions and of sulfhydryl groups [8, 9, 11, 12], to which great importance is also attached in the mechanism of excitation [2, 3, 5, 7, 21].

The object of the present investigation was to study the potassium (in 30 experimental and 10 control animals) and SH-groups (in 55 experimental and 14 control animals) in the motor neurons of the spinal cord of the frog during reflex excitation.

EXPERIMENTAL METHOD

The experimental method was described in the preceding article. The motor neurons of the lumbar enlargement between the point of emergence of the eighth and ninth pairs of nerves were investigated for a period of between 15 sec and 6 min after reflex excitation. Against the background of electrical stimulation of the central end of the sciatic nerve, causing reflex excitation of the motor neurons, perfusion was carried out with a solution of sodium cobaltnitrite with acetic acid, followed by fixation with Carnoy's mixture, for detection of potassium, and with 10% neutral formalin, Carnoy's mixture, Brodskii's mixture, and a 1% solution of trichloroacetic acid in 80° alcohol for detection of SH-groups. Paraffin sections, 6 μ in thickness, were stained for potassium by Macallum's method and for sulfhydryl groups by the methods of Chèvremont and Frédéric and of Yakovlev and Nistratova.

EXPERIMENTAL RESULTS

During reflex excitation of the motor neurons, changes were found in the concentration and localization of the substances investigated. Breakdown of the protein-electrolyte complex took place in the excited motor neuron, with the loss of potassium from the neuron and liberation of SH-groups. The reaction used for potassium did not reveal the accurate localization of the ions, so that no definite conclusions could be drawn regarding the precise cell structures to which the potassium belonged. However, on the basis of the relative uniformity of the results obtained, it was assumed that the potassium was localized at the intracellular level. However, all these conclusions were made subject to the limitations of the method and could not be claimed to be absolute.

Investigation of the potassium in the motor neurons showed that some variability in the concentration of these ions was present also in the intact cells. As a rule the nucleus stained more intensively than the cytoplasm. The potassium activity took the form of dark granules, and at rest its intensity was slight. In individual neurons a more clearly defined localization of the granules was found at the periphery of the nucleus. During excitation the granules in the nucleus and cytoplasm became more distinct, i.e., the potassium concentration was increased, evidently as a result of the liberation of potassium from the protein-electrolyte complex, and the concentration of granules was highest at the periphery of the nucleus and cytoplasm. In addition, migration of granules from the nucleus into the cytoplasm was observed (Fig. 1).

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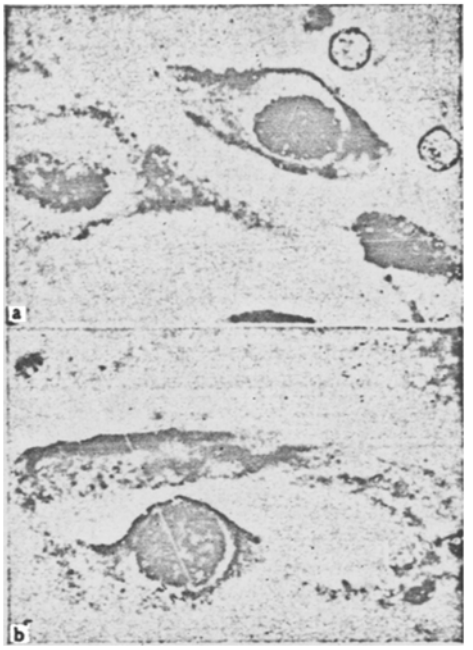

Fig. 1

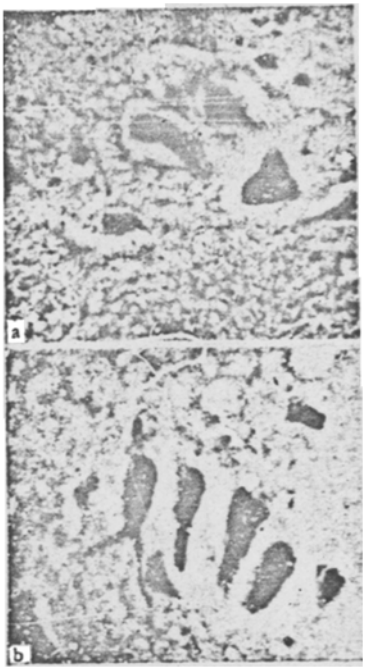

Fig. 2

Fig. 1. Motor neurons of the spinal cord of the frog: a) intact, b) after excitation for 2 min. Macallum's reaction for potassium. An increase in granularity during excitation can be seen, with migration of granules from the nucleus into the cytoplasm. Photomicrograph. Objective 100 ×, ocular 6.6 ×.

Fig. 2. Motor neurons of the spinal cord of the frog: a) intact, b) after excitation for 3 min. Reaction of Chèvremont and Frédéric. An increase in the concentration of SH-groups can be seen in the cytoplasm and nucleus, shown by an increase in the density of staining. Photomicrograph. Objective 20 ×, ocular 6.6 ×.

On the basis of the membrane theory it would be expected that during excitation the amount of potassium in the cell would fall because of its migration into the extracellular spaces. The results of the present experiments demonstrated an increase in the potassium ion concentration in the stimulated motor neurons, which was incompatible with the membrane theory but, on the other hand, which could be explained perfectly satisfactorily by the sorption theory, according to which the increase in the potassium concentration during excitation is associated with its liberation from the protein-electrolyte complex. As a result of this liberation, leading to an increase in the concentration of potassium ions inside the cell, the potassium attempts to leave the cell structures along the concentration gradient and migrates into the intercellular space.

Staining for SH-groups by the method of Chèvremont and Frédéric revealed an increase in their concentration in the nuclear structures and cytoplasm during excitation (Fig. 2). In the stimulated motor neurons the nucleus stained very intensively, while in the cytoplasm SH-groups were found diffusely and also in the granular structures. In the intact motor neurons the localization of SH-groups was fundamentally the same, although the granularity of the cytoplasm was less marked and the staining of the cell structures was weaker. The method of Chèvremont and Frédéric is not specific enough and cannot therefore determine the localization of the protein sulfhydryl groups perfectly accurately. However, staining the preparation by the method of Yakovlev and Nistratova, although revealing a somewhat different localization of SH-groups—the nucleolus and cytoplasmic structures, showed that the relationships between the concentration of SH-groups of the excited and intact motor neurons was of the same character as after staining by the method of Chèvremont and Frédéric, i.e., stimulated motor neurons gave a more intensive reaction than intact. This evidently indicated an increase in the concentration of sulfhydryl groups during excitation (Fig. 3).

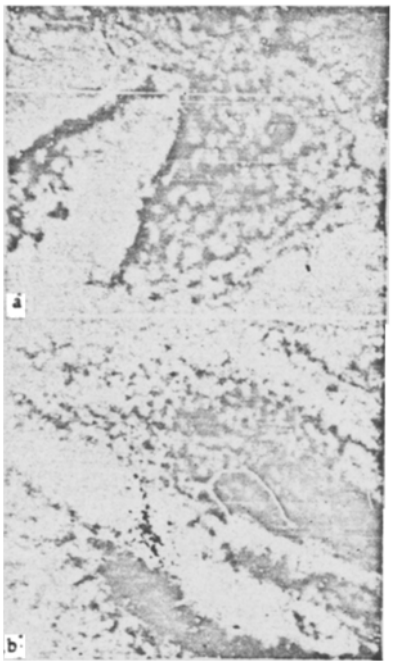

Fig. 3. Motor neurons of the spinal cord of the frog: a) intact, b) after excitation for 1 min. An increase in the granularity of the cytoplasm and an increase in the intensity of staining of the nucleus can be seen, indicating an increased concentration of SH-groups during excitation. Stained by the method of Yakovlev and Nistratova.

Hence, it may be concluded from these investigations that after comparatively brief excitation of the motor neurons of the spinal cord, breakdown of the protein-electrolyte complex takes place in these neurons, with liberation of potassium ions from the cell and the formation of free SH-groups.*

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