# EFFECT OF HYPEROSMOLARITY AND FUROSEMIDE ON RESTING MEMBRANE POTENTIAL AND VOLUME OF RAT SKELETAL MUSCLE FIBERS

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The ability of certian eukaryotes to restore their cell volume after changes in osmolarity of the external medium is known to be based on transmembrane movements of ions, notably of $Na^+$, $K^+$, and $Cl^-$ [10]. For instance, water-osmotic equilibrium in blood cells and neuroglial cells of mammals is maintained through the participation of the $Na^+$, $K^+$, $2Cl^-$-symport, whose activity depends on the osmotic pressure of the medium [6, 8, 9]. The work of this transfer leads to accumulation of osmotically active particles in the cell sarcoplasm, and in turn, this causes transmembrane movements of water and change in cell volume [1]. Sodium- and potassium-dependent mechanisms of active accumulation of chloride ions have also been found in the membrane of mammalian skeletal muscle fibers [7]. It can be tentatively suggested that this system of chloride transfer helps to maintain the volume of the muscle fibers by regulating their water-osmotic balance, just as takes place in other cells. To test this hypothesis, we studied the effect of blockade of active chloride transport on the resting membrane potential (RMP) and volume of skeletal muscle fibers in a medium with raised osmotic pressure.

## EXPERIMENTAL METHOD

Experiments were carried out on the diaphragm muscle of male laboratory albino rats weighing 150-200 g. A fragment 4-5 mm wide was excised from the left hemidiaphragm, parallel to the course of the muscle fibers, together with a long stump of the nerve supplying it in order to prevent an early postdenervation fall of RMP [5]. Control experiments confirmed that when the muscle was kept in ordinary medium, the RMP of the fibers did not fall. The preparation was placed in a chamber for electrophysiological investigations, containing continuously flowing Eagle's medium (USSR). The pH of the solution, saturated beforehand with a mixture of $O_2$ (95%) and $CO_2$ (5%), was maintained at pH 7.3-7.4, with the aid of Tris-maleate buffer ("Reanal," Hungary). The experiments were conducted at 36°C. The osmolarity of the solution was calculated by the equation $P = \Sigma C$, where P denotes the osmolarity of the solution in milliosmoles/liter: C the concentration of the particular type of ions, in mmoles/liter, in this case 292 milliosmoles/liter. In some cases sucrose ($2 \cdot 10^{-1}$ mole/liter, USSR) was added to the medium to increase the osmotic pressure of the solution [10], together with furosemide ("Lasix," $1 \cdot 10^{-3}$ M, "Hoechst," India) to block active chloride transfer through the membrane [1]. The RMP was measured in the extrasynaptic region of superficial

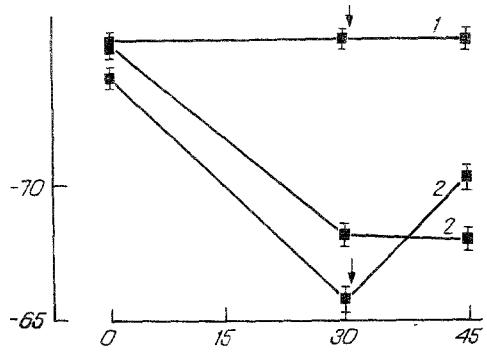


Fig. 1. Effect of furosemide on RMP of muscle fibers in solutions with normal (1) and increased (2) osmolarity. Abscissa, time (in min); ordinate, RMP of muscle fibers ± standard error (in mV). Arrows indicate addition of furosemide.

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TABLE 1. Effect of Furosemide on Area of Transverse Section of Fibers of Rat Diaphragm Muscle in Hyperosmolar Solution

| Solution | Red fibers | | | White fibers | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| A | 1,34±0,02 (96) | — | 1,32±0,03 (68) | 2,03±0,06 (29) | — | 2,10±0,07 (22) |
| B | 1,45±0,03 (94) | 1,04±0,03 (106) | 1,44±0,03 (102) | 2,21±0,09 (39) | 1,70±0,07 (35) | 2,08±0,08 (35) |
| C | 1,32±0,02 (127) | 1,18±0,02 (102) | 0,98±0,02 (150) | 2,13±0,03 (47) | 1,73±0,08 (30) | 1,29±0,05 (39) |

Legend. Mean values of area of cross section of muscle fibers ± standard errors (conventional units) are given. Number of fibers tested, taken from 3-5 animals, shown in parentheses. 1) Initial area of cross section in Eagle's medium; 2, 3) areas of cross section 5 and 45 min respectively, after addition of furosemide to medium, increasing osmolarity of medium, or increasing osmolarity and adding furosemide (solutions A, B, and C, respectively).

fibers, using a standard microelectrode technique. Succinate dehydrogenase activity was determined [2] in freshly frozen sections through the muscles by the method with nitro-blue tetrazolium, capable of identifying red and white fibers [3]. Areas of cross section were measured separately for the different types of muscle fibers by planimetry.

## EXPERIMENTAL RESULTS

Immediately after isolation of the muscle with a long stump of the nerve supplying it, the membrane potential measured in medium with normal osmotic pressure was 76.0 ± 0.3 mV (n = 55). After incubation of the preparation for 40 and 60 min the transmembrane potential difference was virtually unchanged at 75.9 ± 0.3 mV (n = 106) and 75.6 ± 0.2 mV (n = 82), respectively. Increasing the osmolarity of the solution to 500 millismoles/liter led in the course of 30 min to a decrease in RMP from 75.7 ± 0.3 mV (n = 76) to 68.3 ± 0.3 mV (n = 80, $p < 0.001$); during the next half hour, moreover, the transmembrane potential difference remained unchanged (Fig. 1). In another series of experiments, after the initial values of RMP had fallen in medium of increased osmolarity in the course of 30 min from 74.2 ± 0.4 mV (n = 68) to 65.8 ± 0.5 mV (n = 79, $p < 0.001$) addition of furosemide, a blocker of active chloride transport, increased RMP in the course of 15 min to 70.5 ± 0.4 mM (n = 102, $p < 0.001$). In iso-osmolar solution furosemide did not affect membrane polarization of fibers of the rat diaphragm muscle (Fig. 1), in agreement with results obtained previously [4]. Thus furosemide acted on RMP only in medium with raised osmotic pressure.

The fact that blockade of active transport of chloride ions by furosemide reduces the decrease in RMP of the muscle fibers arising under the influence of increased osmolarity suggests that depolarization may be due to a shift of equilibrium potential for $Cl^-$ in the positive direction because of activation of chloride tansport [4, 7].

As a result of the increase in flow of chloride ions inside the cells, osmotically active particles may accumulate in the sarcoplasm of the muscle fibers, and in turn, this may give rise to transmembrane movements of water [1]. In that case, intensification of the work of the chloride pump ought to be accompanied by changes in volume of the muscle fibers.

Immediately after the osmolarity of the external medium was raised to 500 milliosmoles/liter, the area of cross section of the red and white muscle fibers fell by 26% ($p < 0.001$). However, after only 45 min the areas of cross section of these fibers amounted to 94 and 99%, respectively, of their initial level (Table 1). In some experiments, simultaneously with an increase in osmolarity, furosemide was added to the solution. The results showed that after exposure for 5 min to medium containing furosemide and with increased osmotic activity, the areas of cross section of the red and white muscle fibers amounted to 89 and 81%, and after 45 min to 75 and 60%, respectively. In iso-osmolar solution, addition of furosemide caused virtually no change in the areas of cross section of the red and white muscle fibers of the rat diaphragm (Table 1).

Thus, during the first minutes after elevation of the osmotic pressure of the external solution, there was a decrease in volume of the muscle fibers due to loss of water. In the next 45 min of incubation of the muscle under these conditions the cell volume was restored

to its original values. Blockage of chloride transport by furosemide rendered the muscle fibers incapable of restoring their volume when exposed to the action of a medium with increased osmotic pressure. Consequently, chloride transfer can be regarded as an essential element of the mechanism controlling the volume of muscle fibers through regulation of the intracellular water content. Furosemide also reduces the depolarizing effect of the hyperosmolar medium. It can therefore be postulated that reduction of the transmembrane potential difference and restoration of the volume of the muscle fibers take place through an increase in the intracellular $Cl^-$ concentration, as a result of intensification of the work of furosemide-sensitive chloride transport. Changes in activity of this transport system may be of great importance for the maintenance of water and electrolyte homeostasis of skeletal muscle fibers in mammals.

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