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Na^+ AND K^+ IN OUTER SEGMENTS OF RETINAL RODS

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SUMMARY

The amount of Na^+ and K^+ in isolated bovine retina outer segments and slices of outer segments obtained from frozen and freeze-dried bovine and frog retinas was established. It is shown that during the conventional procedure of isolation nearly 75 % of the Na^+ and K^+ present in native structures was lost.

The average amount of K^+ in bovine outer segments is 158 mmoles/kg dry wt.; Na^+, 136 mmoles/kg dry wt. In frog outer segments there is: K^+, 133 mmoles/kg dry wt.; Na^+, 91 mmoles/kg dry wt.

With the help of the electron microscopic technique Na^+ was shown to be located predominantly in the sacs of the outer segments. As for K^+, it is, in all probability, in the extrasaccular space which agrees with some experimental biochemical data obtained.

INTRODUCTION

Ion translocation in outer segments of retinal photoreceptors is one of the most possible processes of photoreception just after the decay of visual pigments caused by light[1–5]. Last year there were many discussions on the direction of ion translocation at different stages of the outer segment function[5]. But as yet there are no reliable data on the real content of Na^+ and K^+ in outer segments or on the distribution of these ions within a segment. Therefore, no conclusions can be drawn with respect to the ion concentration gradient in the systems: outer segment–extrasegmental medium and sac–extrasaccular space.

The aim of the present work was to establish the outer segment amount of Na^+ and K^+ as close as possible to that in native structures and to obtain some data on the localization of these ions within outer segment compartments.

RESULTS AND DISCUSSION

The first part of our experiments was carried out on the isolated preparations of bovine retina outer segments. It was shown that Na^+ and K^+ content in the isolated outer segments depends to a great extent on the amount of these ions in the preparative media. From the data in Fig. 1A it may be seen that the level of Na^+ and K^+ in the

isolated segments depends on the presence of these ions in the solutions used for washing excised retinas or outer segment preparations. The results of these model experiments show that while the retinas remained in the isolated eyes some changes of the outer segment amounts of Na^+ and K^+ may occur, owing to the differences between these ion contents in the outer segments and various eye media. This supposition was confirmed by the following experiments. It was observed that the outer segment preparations from cattle retinas excised (in the slaugtherhouse) just after the animals were killed contained more K^+ than did outer segments from isolated eyes kept intact for 2–3 h (Fig. 1B), *i.e.* under the conditions of common laboratory routine.

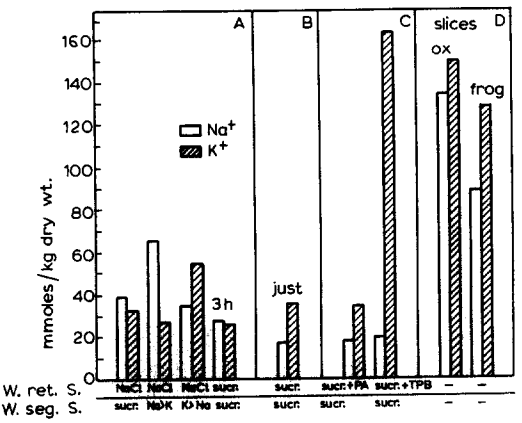

Fig. 1. The amount of Na^+ and K^+ in isolated outer segments of bovine retina (A, B, C) and in segment slices from frog and bovine retinas (D) (dark conditions). A, B and C. The preparation of the isolated fraction: first by the method of differential centrifugation[6] after which the fractions were purified by the method of centrifugation on a sucrose density gradient[7] with some modifications[8]. The purity of the fraction was checked by rhodopsin determination[9]. The rhodopsin content was on the average 4.2 mmoles/kg dry wt. Ion determination was accomplished by the method of flame spectrophotometry. Washing retina solution (W. ret. S.): 0.25 M sucrose (sucr.); 0.15 M NaCl. Washing segment solution (W. seg. S.): 0.25 M sucrose (sucr.); 0.10 M or 0.05 M NaCl; 0.10 M or 0.05 M KCl; 2% sodium tetrapenylboron (TPB); 2.5% potassium pyroantimonate (PA). D. The preparation of slices: the dark-adapted retinas of frog and ox were put out on filter paper, washed with an isotonic solution of sucrose or choline chloride and frozen in isopentane, which was cooled with liquid N_2. Then either the retinas were lyophilized and the segment layer scraped out under microscopic control or the preparations of segment were obtained by cutting the retinas (15 μ) with the help of a microtome. In Figs. 1 and 3 the mean values from several experiments are given and the difference in values is statistically significant.

All these data show that changes in outer segment ion content take place during the isolation procedure. Therefore, to prevent these changes it is necessary to fix ions in the outer segments immediately after the removal of the retina from the eye of the animal just killed. For this purpose we added to the medium, in which the retinas were placed immediately after their removal, such substances which form complexes with K^+ or Na^+ and thereby prevent the leakage of these ions. To fix K^+ we used sodium tetraphenylboron; to bind Na^+ we added potassium pyroantimonate. However, the latter did not produce the desirable effect under the available conditions, the content of Na^+ in outer segments being the same as in the samples without pyroantimonate (Fig. 1C); whereas segment preparations from the retinas placed in the medium with tetraphenylboron contained 4 times the K^+ as compared with control values. This

means that during the conventional procedure of isolation nearly 70 % of the K^+ may be lost.

In the following series of experiments we omitted outer segment isolation in general. Immediately after the removal of the retinas they were freeze–dried and slices containing outer segment layers were prepared by means of a modification of the technique of LOWRY *et al.*[10]. The analysis of these slices (Fig. 1D) showed that the amount of K^+ was 151 $\pm$ 4 mmoles/kg dry wt. for cattle segments and 133 $\pm$ 11 mmoles/kg dry wt. for the frog ones. It should be stressed that these data are identical with those obtained with outer segment preparations isolated in the presence of tetraphenylboron (165 mmoles/kg dry wt.). The amount of Na^+ in such slices was

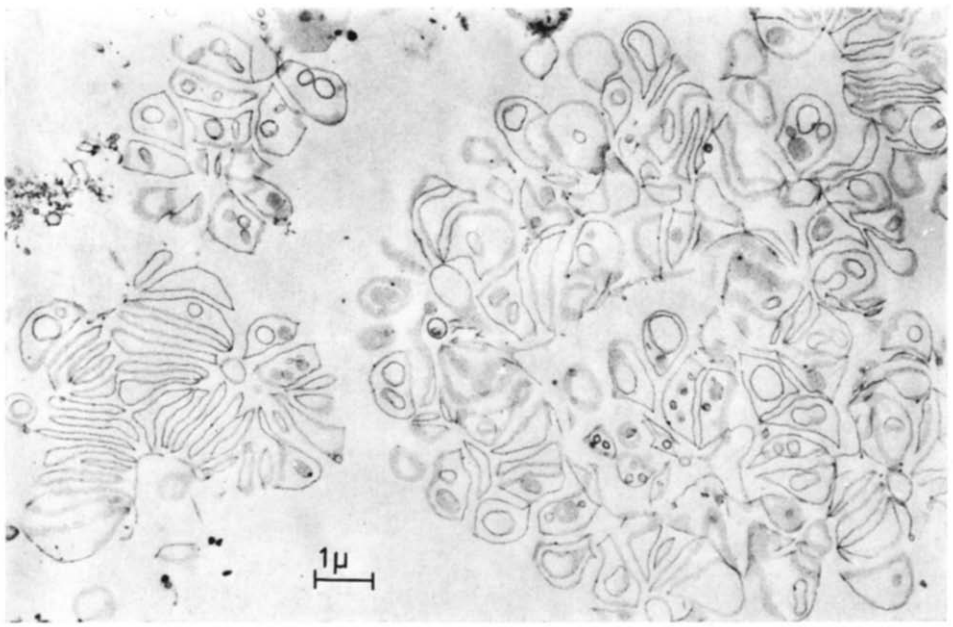

Fig. 2. Electron micrograph of the fraction of bovine retina outer segments after treatment with water (5 min; temp., 4°).

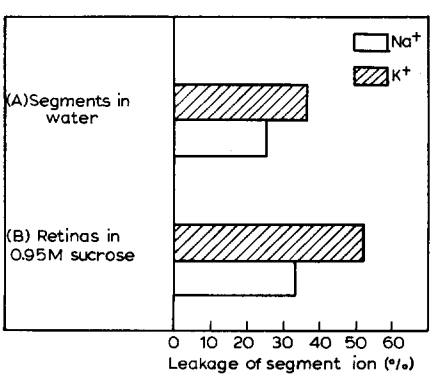

Fig. 3. The loss of bovine outer segment Na^+ and K^+ in removing the extrasaccular content. A. The isolated outer segments were washed 1 time with water. B. The retinas were washed in 0.95 M sucrose and then the segments were obtained by the procedures mentioned in Fig. 1.

136 ± 4 mmoles/kg dry wt. in cattle outer segments and 91 ± 8 mmoles kg/dry wt. in similar frog preparations. This means that similar to K^+ during the conventional isolation procedure we lose the main bulk of Na^+, too.

Thus, in concluding this part of our work, we may assert that the outer segments, compared to the entire retina tissue[11], contain a very large amount of Na^+ and a relatively high amount of K^+. During routine isolation procedures nearly 70–80% of these ions is lost. Therefore, data related to ion translocation in isolated outer segment preparations should be considered with care.

In the next part of our work we investigated Na^+ and K^+ localization within the outer segment. To begin with, we tried to remove the extrasaccular contents of the segments either by placing the retinas for washing in a highly hypertonic sucrose solution or by fragmentation of isolated outer segments by suspending them in hypotonic medium (water). The electron microscopic control of the fractions which were obtained after the treatment with water showed that as a result of this procedure the sacs were not destroyed, while most of the extrasaccular content was removed (Fig. 2). In the procedure in which hypertonic sucrose solutions were employed to wash out the retinas, the sacs were preserved well enough, too. Both of these procedures were

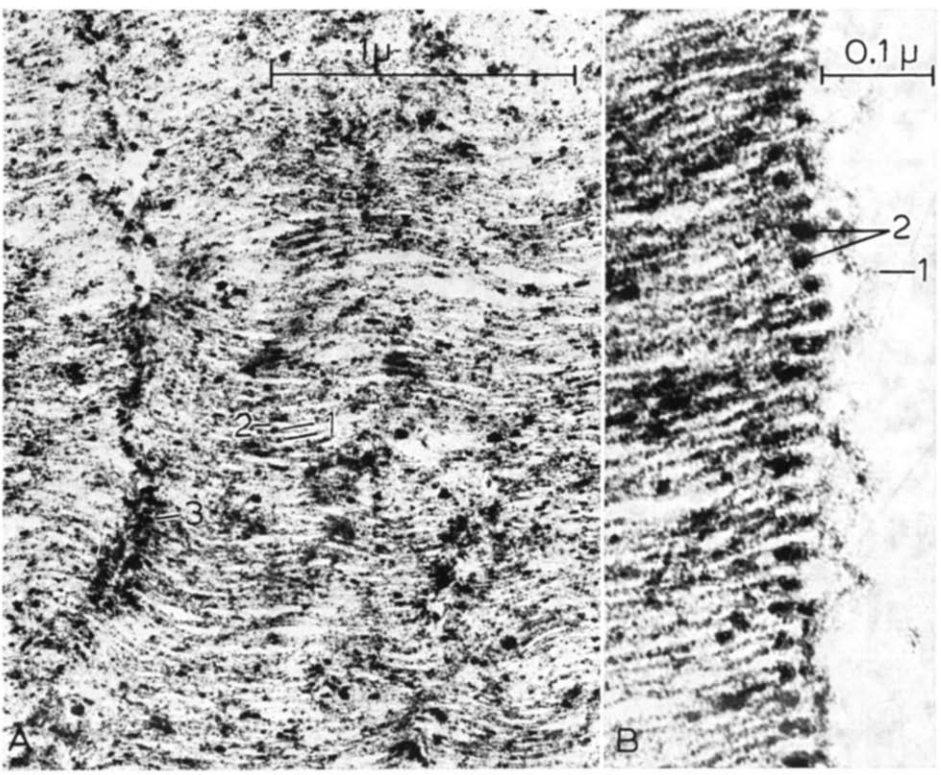

Fig. 4. Electron micrograph of Na^+ localization in different areas (A, B) of the outer segment of frog retina (dark conditions). The retina was frozen in isopentane at −160°, then the tissue water was substituted by −8° with a mixture of Komnick fixator[12] with 25% acetone; after this the preparations were dehydrated by the usual methods and embedding in Epon. A. 1, sacs; 2, extrasaccular space; 3, precipitate in loops on the incisures of the sacs. B. 1, outer membrane of rods; 2, sodium pyroantimonate in loops on the border of the sacs.

accompanied by a considerable loss of ions compared to the control; under these conditions the loss of K^+ was always greater than that of Na^+ (Fig. 3). Though the data obtained with isolated fractions should be referred to with care, we may suppose on these grounds that the main bulk of K^+ is localized in the extrasaccular space and Na^+ in the rod sacs, respectively. This suggestion remained to be proved, and this was done with Na^+ after we modified the electron microscopic technique to check the localization of this ion. From Fig. 4 it is obvious that Na^+ is located predominantly in the sacs. A large amount of pyroantimonate precipitate is clearly seen in the sacs incisures and loops and is not connected with the outer membrane of the rods (Fig. 4B).

Thus, the occurrence of an ion gradient within a segment was demonstrated by direct experiments, and contrary to the opinion of other investigators[3,4], it was shown that Na^+ is accumulated mainly in the sacs, whereas K^+ in all probability is located in the extrasaccular space. This conclusion is in accordance with the data on the structure of the outer segments and the origin of their sacs[13,14]. This ionic gradient, Na^+ predominantly in the sacs, K^+ predominantly in the extrasaccular space, must necessarily play a basic role in segment frunction.

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