BBA 71 034

Effects of ethacrynic acid on sodium fluxes in frog sartorius muscle

HOROWICZ[1] and more recently KEYNES[2,3] found that the Na^+ efflux from frog sartorius muscle has two independent and additive components, one is strophanthidin- and K^+-sensitive, while the second is not blocked by strophanthidin and requires Na^+ in the external solution. The strophanthidin-sensitive component is presumably the "Na^+ pump" proper; the Na^+-dependent component may result from exchange diffusion[3]. Recently HOFFMAN AND KREGENOW[4] concluded that Na^+ extrusion from erythrocytes occurs through two independent mechanisms. Pump I is strophanthidin-sensitive and dependent on external K^+. Pump II requires external Na^+ and is inhibited by ethacrynic acid. In the present experiments we have tested the effects of ethacrynic acid on the Na^+ efflux of frog skeletal muscle. We also used this substance to determine whether or not the efflux through the external Na^+-dependent component is coupled to an influx of Na^+.

The techniques used were the same as those employed by KEYNES[5].

In the experiment of Fig. 1 the effect of a Na^+-free solution* on the ^{22}Na efflux from a strophanthidin($3 \cdot 10^{-5}$ M)-treated frog sartorius muscle was first determined. This solution reduced Na^+ efflux to 0.21 time the resting level. Afterwards the muscle was reimmersed in Na^+ Ringer *plus* strophanthidin, and the efflux increased to a value close to that reached at the end of the initial period in this solution. Then ethacrynic acid (2 mM) was added to the Na^+ Ringer *plus* strophanthidin solution. The compound caused a reduction of Na^+ efflux to a level close to that observed in the Na^+-free solution. On the average, ethacrynic acid reduced the efflux from strophanthidin-treated muscles to 0.18 ± 0.02 ($n = 8$) times the resting efflux, while during immersion in Na^+-free solutions *plus* strophanthidin, the level was equal to 0.14 ± 0.03 times the resting efflux. Once the effect of ethacrynic acid had been determined, the muscle was transferred to a Na^+-free Ringer *plus* strophanthidin and ethacrynic acid. An increase of Na^+ efflux was observed in 6 out of 7 experiments. These results suggest that in strophanthidin-treated muscles, as in strophanthidin-treated erythrocytes, ethacrynic acid partially blocks the Na^+-dependent component of Na^+ efflux.

The last part of Fig. 1 shows that when a muscle was finally transferred from Na^+-free Ringer *plus* strophanthidin and ethacrynic acid into Na^+ Ringer *plus* strophanthidin and ethacrynic acid, there was always ($n = 8$) a marked rise in Na^+ efflux. Although we have no explanation for this Na^+-induced increase in Na^+ efflux, observed after treating the muscles with Na^+-free Ringer *plus* ethacrynic acid and strophanthidin, we believe that it is the result of a drastic change of the muscle cells because once the new level of efflux is reached, Na^+ efflux is not affected by eliminating the external Na^+ or by concentrations of strophanthidin larger than those necessary to have a full effect in control muscle.

Fig. 2 illustrates another experiment with ethacrynic acid on a pair of sartorius

* Na^+ Ringer's composition (in mM) was: NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; Tris–maleate buffer, 4 mM. This concentration of buffer was sufficient to bring the pH of the solution to 7.2 after the addition of ethacrynic acid. In most experiments, Na^+-free Ringer was prepared by substituting NaCl by equivalent amounts of LiCl. In a few experiments, choline chloride was used as a substitute.

muscles. One muscle was used as a control (open circles) and the other was treated with ethacrynic acid (black circles). When ethacrynic acid was added, the Na^+ efflux increased; after 100 min it was equivalent to 1.7 times the resting value. This rise was always observed. It leveled off 90–120 min after the addition of ethacrynic acid at a value equivalent to 2.0 ± 0.2 ($n = 12$) times the resting level. On several occasions it was preceded by a transient decrease of Na^+ efflux to about 0.8 times the

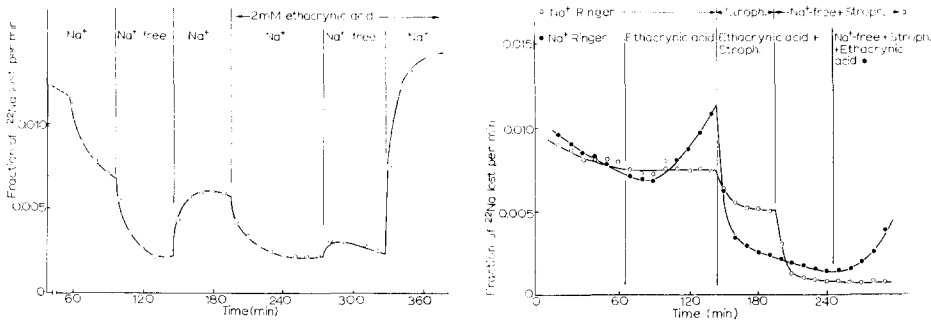

Fig. 1. The effects of ethacrynic acid (2 mM) on the ^{22}Na efflux from a strophanthidin-treated frog sartorius muscle. The broken line shows the level of efflux reached during an initial period in Na^+ Ringer. The starting point of the continuous graph is the moment when the muscle was immersed in Na^+ Ringer *plus* strophanthidin. The effects of Na^+-free Ringer (Li^+ substituted for Na^+) were then determined. After reimmersing the muscle in Na^+ Ringer *plus* strophanthidin, ethacrynic acid was added, and the effects of Na^+-free Ringer *plus* strophanthidin and ethacrynic acid were tested. Finally the muscle was immersed again in Na^+ Ringer *plus* strophanthidin and ethacrynic acid.

Fig. 2. Comparison of the effects of strophanthidin and Na^+-free solutions on a control and an ethacrynic acid-treated muscle. Initially the efflux of ^{22}Na from two sartorii (dissected from a single frog) into Na^+ Ringer was determined; afterwards one muscle was treated with 2 mM ethacrynic acid (●) while the other remained as a control (○). During the third period, strophanthidin ($3 \cdot 10^{-5}$ M) was added to the Ringer solution bathing both muscles. Then the control muscle was transferred to Na^+-free Ringer *plus* strophanthidin, and finally the test muscle was immersed in Na^+-free Ringer *plus* strophanthidin and ethacrynic acid. In this experiment Li^+ was substituted for Na^+ in the Na^+-free Ringer.

resting level. In the next part of the experiment, the effects of strophanthidin on both the control and the ethacrynic acid-treated muscles were investigated. The effect of the aglycone on the ethacrynic acid-treated muscles was larger than that observed in control muscles. In 27 muscles immersed in Na^+ Ringer, strophanthidin reduced the Na^+ efflux to a value of 0.59 ± 0.04 times the resting level, while in 4 muscles immersed in Na^+ Ringer *plus* ethacrynic acid, strophanthidin reduced the efflux to a value of 0.20 ± 0.3 times the resting value. After testing the effects of strophanthidin on both sartorii, the control muscle was immersed in Na^+-free Ringer *plus* strophanthidin. Its efflux dropped, reaching a value (0.16 ± 0.02, $n = 4$) similar to that observed in muscles immersed in Na^+ Ringer *plus* strophanthidin and ethacrynic acid. Finally, when the muscle treated with strophanthidin and ethacrynic acid was transferred to Na^+-free Ringer *plus* strophanthidin and ethacrynic acid, an increase in Na^+ efflux was again observed as in Fig. 1. These observations can be explained if ethacrynic acid, in addition to an inhibition of the Na^+-dependent component of the Na^+ efflux, stimulates the Na^+ pump, so that when strophanthidin is not present, the net effect is an increase in the Na^+ efflux. If this explanation is correct, it should

be possible to show that strophanthidin is not indispensable to the inhibition of the Na^+-dependent component; that is, in muscles treated only with ethacrynic acid, no decrease in efflux follows the elimination of external Na^+. When muscles treated only with ethacrynic acid in Na^+ Ringer were transferred to Na^+-free Ringer *plus* ethacrynic acid, a large stimulation of the efflux, lasting for at least 60 min, was observed.

In other experiments using the technique of KEYNES AND STEINHARDT[3], we measured the effects of ethacrynic acid on the Na^+ influx into strophanthidin-treated muscles. Ethacrynic acid reduced influx to 0.62 $\pm$ 0.6 ($n = 6$) times the resting level. In the same muscles the efflux was reduced from 0.59 to 0.18 $\pm$ 0.05 times the resting level.

In summary, we found that ethacrynic acid affects the two components of Na^+ efflux from frog skeletal muscle differently, stimulating the strophanthidin-sensitive, external K^+-dependent component, and inhibiting the external Na^+-dependent, strophanthidin-insensitive component. More interesting, perhaps, are the results of the uptake experiments indicating that in contrast to the erythrocyte—where the inhibition of efflux caused by ethacrynic acid is not associated with a depression of Na^+ influx[4]—the Na^+-dependent component of Na^+ efflux in skeletal muscle may involve an exchange of Na^+ for Na^+.

This work was supported by grants from the National Institutes of Health (U.S.A.) and the Life Insurance Research Fund. We thank Merck, Sharp and Dohme of México for the ethacrynic acid used in these experiments.

Departamento de Fisiología, GERARD LEBLANC
Centro de Investigación y de Estudios Avanzados del I.P.N., DAVID ERLIJ
Apartado Postal 14-740, México 14, D.F. (México)

1 P. HOROWICZ, *Acta Physiol. Acad. Sci. Hung.*, 26, Suppl. 14 (1965).
2 R. D. KEYNES, *J. Physiol. London*, 184 (1966) 31P.
3 R. D. KEYNES AND R. A. STEINHARDT, *J. Physiol. London*, 198 (1968) in the press.
4 J. F. HOFFMAN AND F. M. KREGENOW, *Ann. N.Y. Acad. Sci.*, 137 (1966) 566.
5 R. D. KEYNES, *J. Physiol. London*, 178 (1965) 305.

Received October 28th, 1968

Biochim. Biophys. Acta, 173 (1969) 149–151

BBA 71032

Effects of ATP and Ca^{2+} on a K^+-activated phosphatase from red blood cell membranes

In a previous communication[1] we showed that ATP at low concentrations increases the hydrolysis rate of *p*-nitrophenyl phosphate by a K^+-activated phosphatase present in fragmented red blood cell membranes. Since this observation is at variance with the inhibitory action of ATP on K^+-sensitive phosphatases from other tissues[2–4], a more detailed study of the phenomenon seemed worthwhile. In this communication we wish to report our findings on the role that Ca^{2+} plays in the interaction of the enzyme with the nucleotide.

As previously reported[1], fragmented membranes were prepared by freezing and thawing "hemoglobin-free" human red blood cell ghosts prepared by successive washes in hypotonic Tris–HCl solutions.