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STIMULATION OF THE Na^+ PUMP BY HYPOTONIC SOLUTIONS IN SKELETAL MUSCLE

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Summary

The fractional loss of $^{22}Na^+$ from frog sartorius muscle is increased when the tonicity of the external solution is reduced. The effect, which is larger the lower the osmolarity, exhibits the following characteristics: (1) quick onset and reversibility, (2) is not reduced in the absence of external Na^+, (3) is completely abolished by strophanthidin ($3 \cdot 10^{-5}$ M), (4) is neither the result of membrane depolarization nor K^+ accumulation in the extracellular space.

It has been shown that within a wide range of osmotic strengths, frog skeletal muscle fibers practically behave as perfect osmometers [1—7]. Since the osmotic water permeability of the plasma membrane is much higher than that of any of the solutes present in the external and the internal media, alterations of the tonicity of the Ringer's solution are quickly followed by the corresponding changes in fiber volume [8], internal concentrations and resting membrane potential (V_m). The changes in V_m are in fair agreement with those predicted by the Goldman-Hodgkin-Katz equation [9] assuming that no major changes in relative ionic permeabilities ($P_{Na}/P_K = 0.01$) occur at least in the range of 0.80—1.26 times the normal osmolarity (Tables 7 and 8 of ref. 3).

Little is known, however, about the effect of osmotic changes on ionic fluxes in this preparation. Keynes [10] observed that an increase in the external tonicity stimulates appreciably the active Na^+ efflux in low Na^+ muscles, while in Na^+-loaded muscles Mullins and Awad [11] found that raising the tonicity of the external medium has no significant effect on Na^+ efflux. It might be expected, therefore, that a decrease in the relative osmotic pressure (π) of the external medium, if anything, would reduce the active Na^+ extrusion. The purpose of the experiments reported here was to study the movements of

Na^+ across the plasma membrane of frog skeletal muscle fibers in the presence of hypotonic solutions. As is shown below, it was seen that hypotonicity produces a marked stimulation of the Na^+ pump in this preparation. To the author's knowledge, this effect has not been described before.

Na^+ and K^+ fluxes ($^{22}Na^+$ and $^{42}K^+$ as tracers) were measured using techniques previously described [12, 13]. Standard microelectrode procedures were used to determine V_m. The normal Ringer's solution had the following composition (mM): NaCl, 115/KCl, 2.5/$CaCl_2$, 1.8/Na_2HPO_4, 2.15/NaH_2PO_4, 0.85 (pH: 7.15). In order to change the tonicity π without altering the ionic strength the NaCl content in most of the experimental solutions was reduced to 55 mM ($[Na^+]_o$ = 60 mM) and sucrose was used in adequate amounts to obtain media of different tonicities from π = 0.5 (0 mM sucrose) to π = 1.0 (94 mM sucrose). The rest of the ionic composition was similar to that of the normal Ringer's solution. In some experiments where π was lowered to 0.33 the NaCl concentration was 41.5 mM and in this instance the control π = 1.0 solution contained 116 mM sucrose. In replacing NaCl by sucrose 1 mol of NaCl was taken as osmotically equivalent to 1.57 mol sucrose [14]. This equivalence was tested in control experiments where the weight of eight sar-

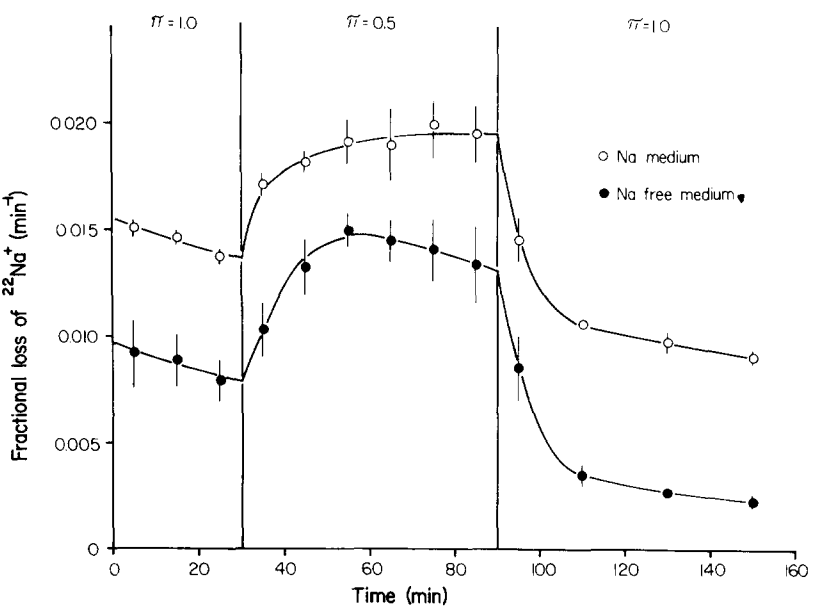

Fig. 1. Effect of halving the tonicity (π) of the external solution on the fractional loss of $^{22}Na^+$ (see below) in the presence of Na^+ (60 mM, upper curve) and its absence (Na^+ substituted by $Tris^+$, lower curve). The open circles represent the mean (±1 S.E.) from three sartorius muscles and the filled circles the mean (±1 S.E.) from their paired companions. All muscles were first exposed to normal Ringer's solution labelled with $^{22}Na^+$ for 2.5 h. They were pre-washed from t = —30 min to t = 0 min in unlabelled π = 1.0 solution similar to that used during the experiment from t = 0 min to t = 30 min and from t = 90 min to t = 150 min. Clearly, the fractional loss of $^{22}Na^+$ in the presence of Na^+-containing medium is considerably higher than that in Na^+-free medium throughout the run. This difference most likely represents a Na^+ for Na^+ exchange component of the Na^+ efflux. It is apparent that the increase in Na^+ efflux promoted by hypotonic solutions (π = 0.5) does not require the presence of Na^+ in the external medium, indicating that the effect is not due to an increased exchange of internal for external Na^+. The fractional loss of $^{22}Na^+$ represents the mean amount of radioactivity released per unit time (min) during a given collecting period divided by the total radioactivity present in the muscle midway during the collecting period [12].

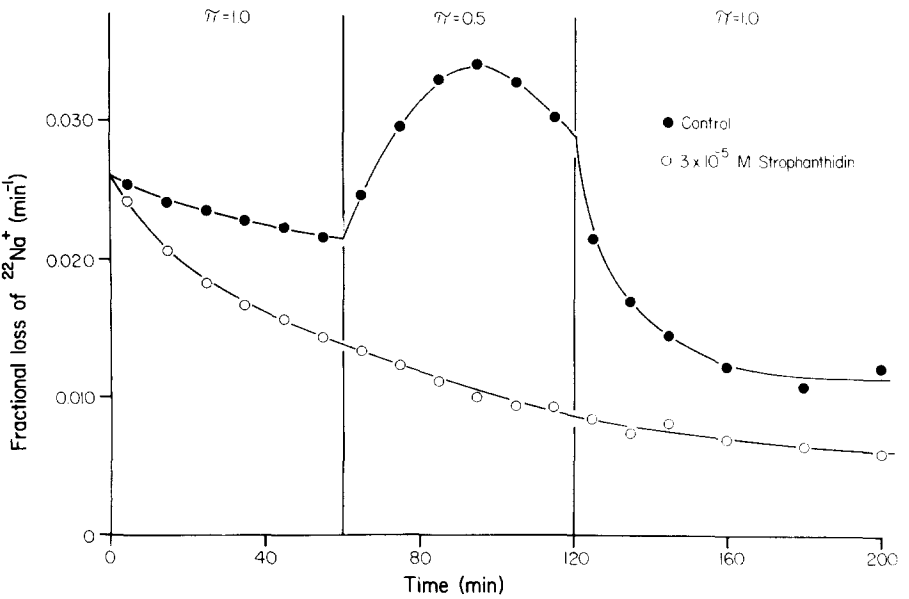

Fig. 2. Fractional loss of $^{22}Na^+$ from a pair of muscles loaded as in Fig. 1. Both sartorii were pre-washed in $\pi = 1.0$ Na^+-containing solution for 60 min. All the experimental solutions had $[Na^+]_o = 60$ mM. One member of the pair (○) was exposed to $3 \cdot 10^{-5}$ M strophanthidin from $t = 0$ to the end of the experiment while the other muscle (●) remained in strophanthidin-free media throughout the run. The inhibitory effect of the drug in the initial period in $\pi = 1$ is apparent. At the end of the first hour of the $^{22}Na^+$ washout the tonicity was halved ($\pi = 0.5$). The hypotonic response seen in the presence of strophanthidin-free medium is completely absent in the muscle exposed to the inhibitor. This type of experiment together with that of Fig. 1 shows that the increment in Na^+ efflux promoted by hypotonicity is exclusively due to an increase in the active extrusion of Na^+.

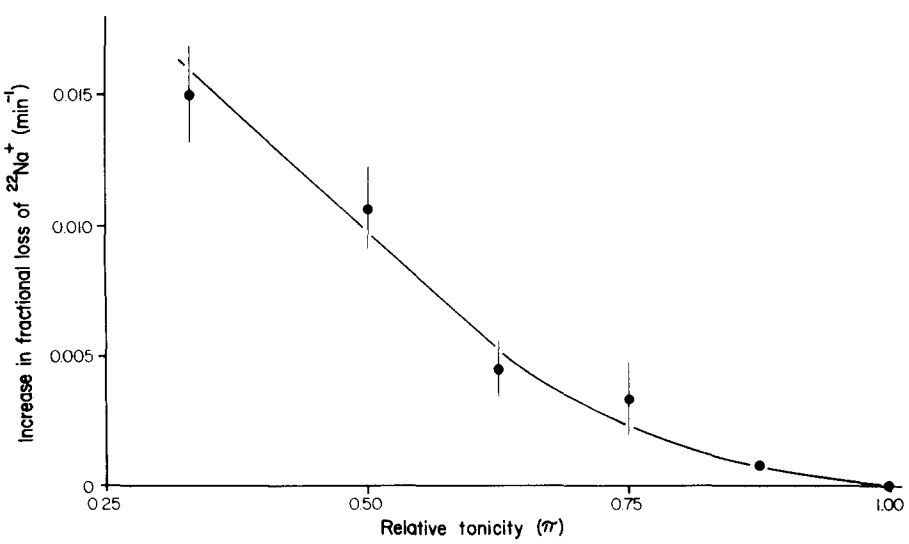

Fig. 3. Increase of the fractional loss of $^{22}Na^+$ as a function of the relative external tonicity in the range $\pi = 1.0$—0.33. Each point is the mean (±1 S.E.) of 3—8 experiments. The increase at $\pi = 0.33$ is equivalent to a fractional loss of $^{22}Na^+$ about 2.2 times larger than that at $\pi = 1.0$. The hypotonic increase is all strophanthidin sensitive. Since at $\pi = 1$ about half of the fractional loss of $^{22}Na^+$ is strophanthidin sensitive, it means that a reduction of π from 1.0 to 0.33 increases the active component of the fractional loss by a factor of about 4. The mean fractional loss of $^{22}Na^+$ at $\pi = 1.0$ from 26 control muscles was 0.0123 ± 0.0005 min^{-1} (S.E.).

torii did not change to any appreciable extent when transferred from normal Ringer's to a solution containing either 55 mM NaCl and 94 mM sucrose or 41.5 mM NaCl and 116 mM sucrose. In some experiments Na^+ was replaced by $Tris^+$ on a one to one basis. All the experiments were carried out at 20—22°C.

Fig. 1 shows the effect of halving the normal tonicity of the external medium on the $^{22}Na^+$ efflux from paired sartorii. It is apparent that the increase in the fractional loss of $^{22}Na^+$ produced by hypotonicity is completely reversible and is present in the absence of the external Na^+. The time course of the response is similar to that of the volume changes observed under identical conditions in control experiments. These data suggest that this effect is not the result of an increase in the Na^+ for Na^+ exchange component of the Na^+ efflux. In the presence of Na^+-containing media ($[Na^+]_o$ = 60 mM), on the other hand, the Na^+ influx at π = 0.5 was 79 ± 5% (S.E.; n=4) of that under isotonic conditions (π = 1.0). In π = 1.0 about half of the total Na^+ influx is passive and half represents an Na^+ for Na^+ exchange [13]. The observed reduction in Na^+ influx in π = 0.5 probably represents a decrease in P_{Na} since a reduction in the Na^+ for Na^+ exchange component can be ruled out due to the fact that in the presence of strophanthidin Na^+ efflux is not reduced by π = 0.5 (Fig. 2). Under those conditions the passive component of the efflux that should be affected by a drop in P_{Na} is usually too small (1—2% of the total Na^+ efflux) for any change in it to be detected. It seems clear, therefore, that Na^+ exchange diffusion is not involved either in the increase of the Na^+ efflux or in the reduction of Na^+ influx promoted by hypotonicity.

Fig. 2 illustrates another typical experiment where the Na^+ efflux was studied also in paired sartorii. Clearly, the hypotonic effect is completely abolished by the presence in the external medium of strophanthidin ($3 \cdot 10^{-5}$ M), a well known inhibitor of the active Na^+ transport in muscle [15, 16]. That is, hypotonicity stimulates the Na^+ pump in frog sartorius. In agreement with this finding it has recently been reported that the K^+ stimulation of the strophanthidin-sensitive fraction of the Na^+ efflux is enhanced by hypotonicity [17].

As is shown in Fig. 3, the hypotonic effect is larger the lower the osmolarity of the external solution in the range π = 0.33—1.0.

At present, the mechanism by which hypotonicity increases the pumping rate is not known and several factors might be involved in its production. Two possibilities, however, have been discarded in control experiments. One of them is that depolarization by itself could be the direct cause of the stimulation of the pump. This does not seem to be the case because when $[K^+]_o$ was reduced to 1 mM and π to 0.5 simultaneously, the effect was still present despite the fact that under those conditions, the fibers do not depolarize. Thus, in a control experiment the mean V_m measured in the presence of $[K^+]_o$ = 2.5 mM and π = 1.0 was −88.4 ± 0.9 mV (S.E.; n=27) and −95.5 ± 1.0 mV (S.E.; n=27) after 50 min in the presence of $[K^+]_o$ = 1.0 mM and π = 0.5.

The other possibility, namely that the observed effect was mediated by an accumulation of K^+ in the extracellular space produced by an increased leak of this ion from the cells, is also unlikely. In four control experiments

the $^{42}K^+$ efflux rate coefficient in $\pi = 0.5$ was actually reduced to 43.8 ± 1.7% (S.E.) of its value in $\pi = 1.0$. This probably reflects a decrease in K^+ permeability since changes in tonicity should not alter the driving force on K^+.

If it is assumed that the rate of Na^+ pumping is mostly dependent on the intracellular Na^+ activity ($^{Na}a_i$), then an increase in $^{Na}a_i$ might be expected under hypotonic conditions. Some investigators have suggested that a substantial fraction (up to 60—70%) of the intracellular Na^+ is not in free solution but sequestered within or bound to organelles or macromolecules [18]. If this were the case, it is conceivable that, under hypotonic conditions, bound Na^+ might be released into free solution in sufficient amount to produce an increase of $^{Na}a_i$ and thereby a stimulation of the Na^+ pump. Thus, an extreme situation can be imagined in which all the bound Na^+, say 66% of the total Na^+ content, is released into free solution when, for instance, the tonicity of the external solution is halved (and the intracellular solvent water roughly doubled). In such a hypothetical case $^{Na}a_i$ would increase by about 50%. However, following this line of reasoning, when π is further reduced to 0.33 (and the intracellular solvent water roughly tripled), $^{Na}a_i$ should be similar, if not lower, than it is at $\pi = 1.0$. Therefore, if the level of $^{Na}a_i$ were the determinant factor in the activation of the Na^+ pump, as is assumed in the model above, one would expect a larger stimulation of the Na^+ extrusion at $\pi = 0.5$ than at $\pi = 0.33$. As shown in Fig. 3, the experimental data indicate just the opposite: at $\pi = 0.33$ the fractional loss of $^{22}Na^+$ is significantly higher than at $\pi = 0.5$ ($P<0.001$). These considerations seem to rule out the possibility that the hypotonic effect were the result of an increase in $^{Na}a_i$.

A factor which might play a relevant role in the effect described here is the decrease in $^{K}a_i$. If this is so, one must assume that the intracellular K^+ exerts an inhibitory effect on the Na^+ pump. Moreover, such an inhibition should mainly depend on the absolute value of $^{K}a_i$ rather than on the $^{K}a_i/^{Na}a_i$ ratio. An inhibitory effect of $[K^+]_i$ on the active extrusion of Na^+ in frog sartorius has already been suggested [19,20]. In this preparation the internal K^+ activity coefficient ($^{K}\gamma_i$) is apparently similar to that in the external solution [18]. Furthermore, measurements carried out with K^+-sensitive microelectrodes in rabbit cardiac muscle cells have indicated that in the hypotonic region changes in π and $^{K}a_i$ are linearly related, suggesting a constant $^{K}\gamma_i$ [21]. Probably the situation is much the same in frog sartorius muscle where the changes in V_m produced by altering π are not far from those predicted by assuming a linear relationship between $[K^+]_i$ and π, constant $^{K}\gamma_i$ and no major change in the P_{Na}/P_K permeability ratio [3].

In summary, hypotonicity promotes a quick and reversible stimulation of the active Na^+ extrusion in frog skeletal muscle. Such stimulation is not the result of fiber depolarization or of K^+ accumulation in the extracellular space and it occurs whether or not Na^+ is present in the external solution. Although other factors may be involved in the production of the hypotonic effect, the data at hand are consistent with the notion that in this preparation the intracellular K^+ might have an inhibitory effect on the Na^+ pump. Thus, if that were the case, the hypotonic stimulation of the strophanthidin-sensitive Na^+ efflux would reflect a reduction of such inhibition subsequent to the fall in $^{K}a_i$.

A preliminary account of these experiments has been presented elsewhere [22].

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References

1 Hill, A.V. (1930) Proc. Roy. Soc. B 106, 477—505
2 Boyle, P.J. and Conway, E.J. (1941) J. Physiol. 100, 1—63
3 Adrian, R. (1956) J. Physiol. 133, 631—658
4 Dydynska, M. and Wilkie, D.R. (1963) J. Physiol. 169, 312—329
5 Parisi, M.N., Montoreano, R. and Lew, V. (1965) Acta Physiol. Latinoam. 15, 38—43
6 Blinks, J.R. (1965) J. Physiol. 117, 42—57
7 Reuben, J.P., López, E., Brandt, P.W. and Grundfest, H. (1963) Science 142, 246—248
8 Caputo, C. (1968) J. Gen. Physiol. 52, 793—809
9 Hodgkin, A.L. and Katz, B. (1949) J. Physiol. 108, 37—77
10 Keynes, R.D. (1965) J. Physiol. 178, 305—325
11 Mullins, L.J. and Awad, M.Z. (1965) J. Gen. Physiol. 48, 761—775
12 Venosa, R.A. and Horowicz, P. (1973) J. Membrane Biol. 14, 33—56
13 Venosa, R.A. (1974) J. Physiol. 241, 155—173
14 Gage, P.W. and Quastel, C.M.S. (1966) J. Physiol. 185, 95—123
15 Johnson, J.A. (1956) Am. J. Physiol. 187, 328—332
16 Horowicz, P. (1965) Acta Physiol. Acad. Hung. (Suppl.) 26, 14—15
17 Szabó, B. and Kovács, J. (1977) Proc. XXVII Int. Congr. Physiol. Sci. 13, 731
18 Lev, A.A. and Armstrong, W. McD. (1975) Current Topics in Membranes and Transport, (Bronner, F. and Kleinzeller, A., eds.), Vol. 6, pp. 59—123, Academic Press, New York
19 Chaplain, R.A. (1973) Experientia 29, 794—795
20 Beaugé, L. (1975) J. Physiol. 246, 397—420
21 Fozzard, H.A. and Lee, C.O. (1976) J. Physiol. 256, 663—689
22 Venosa, R.A. (1977) Proc. 27th Int. Congr. Physiol. Sci. 13, 783