SHORT COMMUNICATIONS

BBA 73135

Lithium-stimulated sodium efflux in frog skeletal muscle

The fact that sodium efflux from skeletal muscle has a rate which can not be accounted for by the energy sources of the cell leads to the suggestion of an exchange–diffusion mechanism[1]. The experimental evidence came from the work of KEYNES AND SWAN[2], who showed that the efflux of sodium was notably reduced when this cation was replaced by lithium in the bathing solution. However, in muscles which spent some time in a K-free sodium medium, this effect disappeared or was even replaced by an increment in the rate of loss. In a detailed study on the matter BEAUGÉ AND SJODIN[3] showed that the reduction seen in fresh muscles also appeared in high sodium aged muscles, provided an inhibitor of the sodium pump, strophanthidin, was present in the solution. From this and from the fact that net sodium extrusion was induced against an electrochemical gradient by lithium ions, it was concluded that lithium ions have a direct stimulating action on the sodium pump in skeletal muscle. This view has been questioned very recently[4] on the basis that a similar increase in sodium efflux was obtained in fresh muscles when either lithium or magnesium was used as a substitute for sodium.

Considering the inhibitory effect of external sodium on the pump mechanism[5], if potassium is present in the bathing solution, it could be expected that the removal of sodium by itself, whatever the ion used to replace it, would result in an increment in the rate of sodium efflux provided the potassium concentration is below saturation. This may have been the case in the two papers mentioned[3,4]. However, this seems to be an insufficient test to rule out any lithium action on the sodium pump. The purpose of the present work is to add more experimental evidence to prove that, in fact, lithium ions do have a stimulating action on the sodium pump in skeletal muscle.

Sodium efflux was studied in aged sartorius muscles from the South American frog *Leptodactilus ocelatus*, which were kept 24 h at 3° in a K-free sodium Ringer's solution. They were loaded with ^{22}Na for 2 h at room temperature in the same solution (5 μC/ml of ^{22}Na). After this period sodium efflux was measured as described elsewhere[3] by attaching the muscles to a wire frame and collecting the radioactivity lost during 10 min in tubes with 5 ml of the testing solution; the tubes were then counted in a Nuclear-Chicago automatic gamma scintillation counter. At the end of the efflux the muscles were digested in a phosphonitric mixture and also counted; these counts were added to those lost into the efflux tubes. The results are given as the average fraction of ^{22}Na lost per min in each collection period. The temperature was 20°. The following solutions were used. (a) Potassium Ringer's solution: NaCl, 110 mM; KCl, 2.5 mM; $CaCl_2$, 2 mM; Tris–HCl, pH 7.4, 1 mM. (b) K-free Ringer's solution: NaCl, 110 mM; $CaCl_2$, 2 mM; Tris–HCl, pH 7.4, 1 mM. NaCl was in some cases substituted by LiCl (110 mM), $MgCl_2$ (73.5 mM) or $CaCl_2$ (73.5 mM).

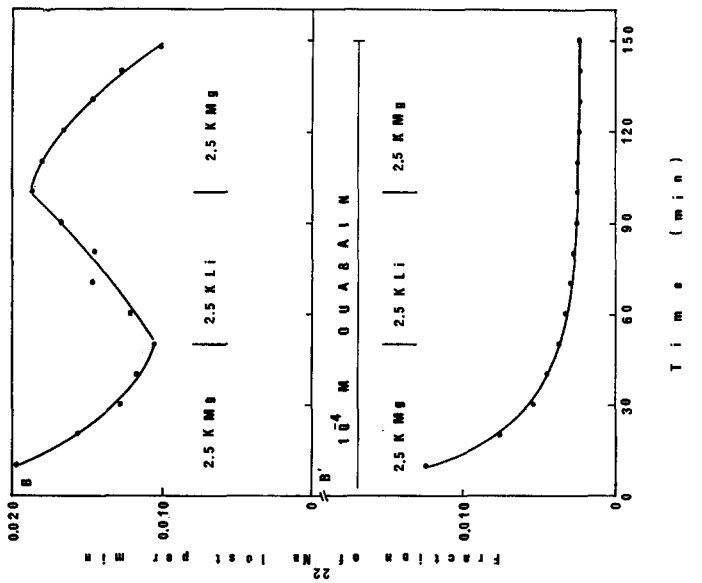

Fig. 2. Effect of the substitution of lithium or magnesium for external sodium on the sodium efflux from high sodium muscles into potassium-free solutions. Both muscles were dissected from the same frog.

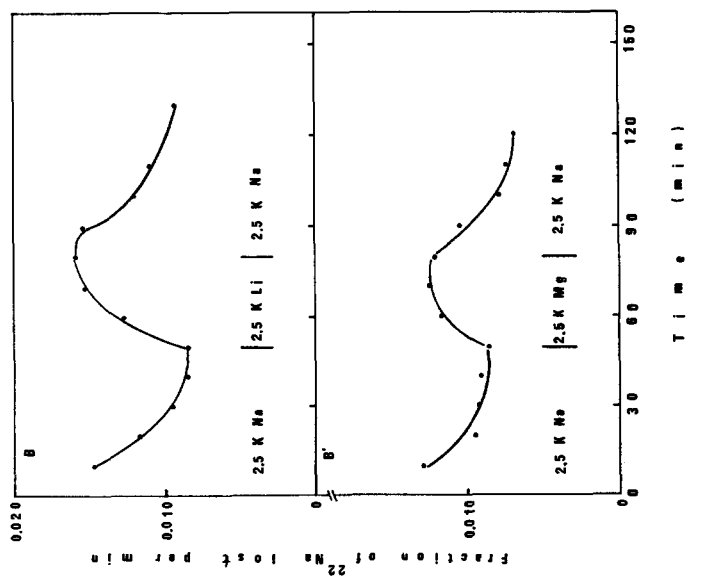

Fig. 1. Effect obtained from the substitution of lithium or magnesium for external sodium on the efflux of sodium from high sodium muscles when potassium was present in the media. Both muscles were dissected from the same frog.

A comparison of the effects obtained from the substitution of lithium for sodium on the sodium efflux with those from magnesium in the presence of potassium is shown in Fig. 1. It can be seen that when sodium was replaced by either cation there was an increase in the efflux. The extent of such increases varied from one experiment to another, but it was always larger in lithium; in five experiments the ratio Li increment/Mg increment averaged 3.2-fold (1.8–4.6). Despite the quantitative differences observed, qualitatively the behavior was the same; that is, when external sodium was removed, the efflux of sodium increased no matter what cation was used as a replacement.

However, as there was 2.5 mM potassium in the media, it might be that what happened was a liberation of the pump due to the removal of sodium. In such a case any direct effect of lithium would be obscured by the presence of potassium, and the fact that qualitatively the efflux of sodium behaves in the same way in the presence of either lithium or magnesium cannot be taken as an argument to rule out any stimulating effect of lithium which might not be shared by magnesium. To investigate this matter further, similar experiments, but without potassium in the outside solution, were performed. One of these is indicated in Fig. 2. It clearly shows that when there is no potassium outside, sodium efflux behaves in an opposite direction depending on which cation is used as a replacement for sodium. Thus, lithium produces

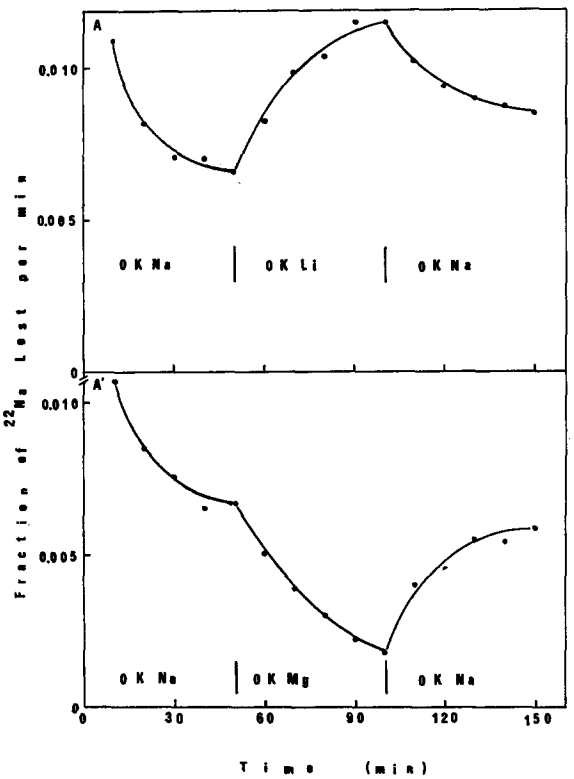

Fig. 3. The effect of changing from a magnesium-containing into a lithium-containing Ringer's solution on the sodium efflux from high sodium muscles when there is potassium in the solution in the absence and presence of 10^{-4} M ouabain. Both muscles were taken from the same frog.

a noticeable increase, whereas in the presence of magnesium there is a definite fall. To avoid the problem inherent to the sodium-dependent fraction of sodium efflux when this cation is removed and as a cross experiment of the previous ones, changes from magnesium-containing Ringer's into lithium-containing Ringer's solution and *vice versa*, with and without potassium and in the absence and presence of 10^{-4} M ouabain, were performed. Figs. 3 and 4 show two typical experiments. It can be seen that when going from a magnesium- into a lithium-containing Ringer's solution there was always an increase in sodium efflux, this being larger if potassium was taken away at the same time, while the changes from lithium into magnesium always brought a reduction. Also, all the increment due to lithium was abolished by 10^{-4} M ouabain. The same results were found when calcium was substituted for magnesium.

The experimental evidence which led to the conclusion that lithium ions have a potassium-like action on the sodium pump in skeletal muscle[3] is supported by the above results as well as by other considerations. Lithium really does have a stimulating effect on sodium efflux which is not shared by either magnesium or calcium, and this effect can be completely abolished by a poison of the sodium pump. On the other hand, all monovalent cations which stimulate ATPase *in vitro*[6] (K^+, Rb^+, NH_4^+, Cs^+, Li^+) have been able to stimulate sodium efflux and to produce net sodium extrusion against an electrochemical gradient in skeletal muscle[7]. This was found even

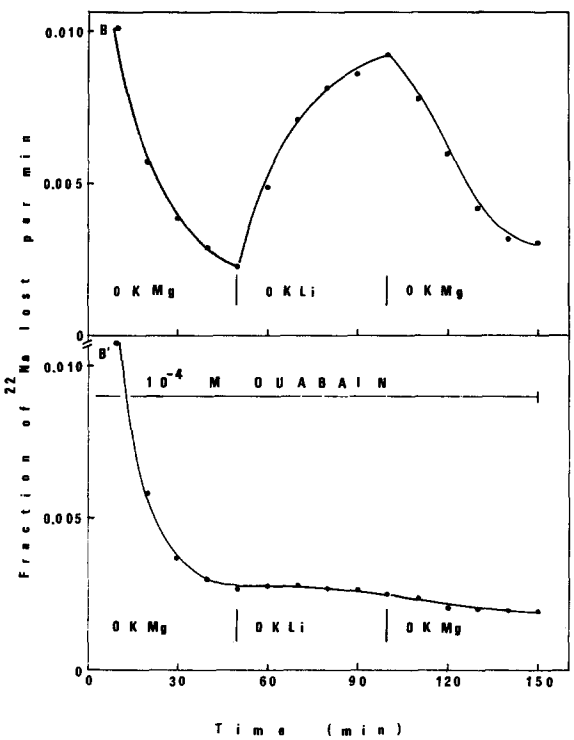

Fig. 4. The effect of changing from a magnesium-containing into a lithium-containing Ringer's solution on the efflux of sodium from high sodium muscles into potassium-free solutions in the presence and absence of 10^{-4} M ouabain. Both muscles were taken from the same frog.

when more than half of the external sodium was replaced by sucrose. This does not support the idea that active sodium transport could be inhibited by the presence of sucrose or by lowering the ionic strength[4].

L.A.B. is a member of the Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina. This work was supported by Grant 3118 from the Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina to L.A.B.

División de Biofísica,
Instituto de Investigación Médica Mercedes y Martín Ferreyra,
Casilla de Correo 389, Córdoba (Argentina)

L. A. BEAUGÉ
OLGA ORTIZ

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Received July 14th, 1970