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VANADATE SELECTIVELY INHIBITS THE K_0^+-ACTIVATED NA^+ EFFLUX IN SQUID AXONS

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Summary

The effects of internally applied 1 mM vanadate on the Na^+ efflux in dialysed squid axons were found to depend on the presence of external K^+. In K^+-free artificial sea water, vanadate did not produce any change in the rate of Na^+ efflux, whereas in the presence of 10 mM K^+ the Na^+ efflux was reduced to values even lower than those observed in the absence of K^+ (inversion of the K^+-free effect). In vanadate-poisoned axons, K^+ and NH_4^+ at low concentrations activated Na^+ efflux, but at high concentrations both cations were inhibitory. However, NH_4^+ was always a better activator and a poorer inhibitor than K^+.

Orthovanadate, a contaminant found in Sigma "Sigma Grade" ATP, is a powerful inhibitor of the $(Na^+ + K^+)$-ATPase [1—3]. This inhibitory effect is enhanced by increasing the K^+ concentration in the media [1,2]. Very recently it has been shown in experiments on red cells that vanadate acts from the inside of the cell [4], whereas the effects of K^+ are exerted from the external surface [5]. With vanadate concentrations of a few micromolar inhibition of the $(Na^+ + K^+)$-ATPase was not detected with K^+ concentrations below 1 mM [1] (Beaugé, L., unpublished). In addition, the vanadate concentration for half maximal inhibition for Na^+-ATPase activity was about 1500 times higher than for $(Na^+ + K^+)$-ATPase activity (Beaugé, L., unpublished). When squid axons are dialysed with solutions containing ATP and phosphoarginine and in the absence of ADP and internal calcium, practically all Na^+ efflux goes

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through the Na^+ pump, either coupled to K^+ influx or in an uncoupled mode [6,7]. This makes the dialysed axon an ideal preparation to study further the effects of vanadate. The present results show that at 1 mM concentration, vanadate inhibited the Na^+ efflux stimulated by external K^+ without any effect on the Na^+ efflux in the absence of K^+. In addition, the differential inhibition seen with K^+ and NH_4^+ resembled the effect of those cations on the Na^+ efflux in axons dialysed with low ATP.

In the experiment described in the legend to Fig. 1, when the axon was dialysed with 3 mM ATP, 5 mM phosphoarginine and no vanadate, removal of K_0^+ reduced Na^+ efflux by about 60%. The effects of the addition of 1 mM vanadate into the dialysate depended on the presence or absence of K^+ in the external solution. In the absence of K^+ no inhibition was seen. In the presence of 10 mM K^+ a large inhibition developed, with the levels of flux being even lower than in K^+-free conditions. With 10 mM Rb^+ the efflux of Na^+ from the vanadate-poisoned axon was the same as (or even a little smaller than) with K^+; on the other hand with 10 mM NH_4^+ the levels of flux from the vanadate-poisoned axon were the same as in K^+-free solutions.

Fig. 2 shows the effects of varying the K^+ and NH_4^+ concentrations in a vanadate-poisoned axon. With both cations an increase in Na^+ efflux at low

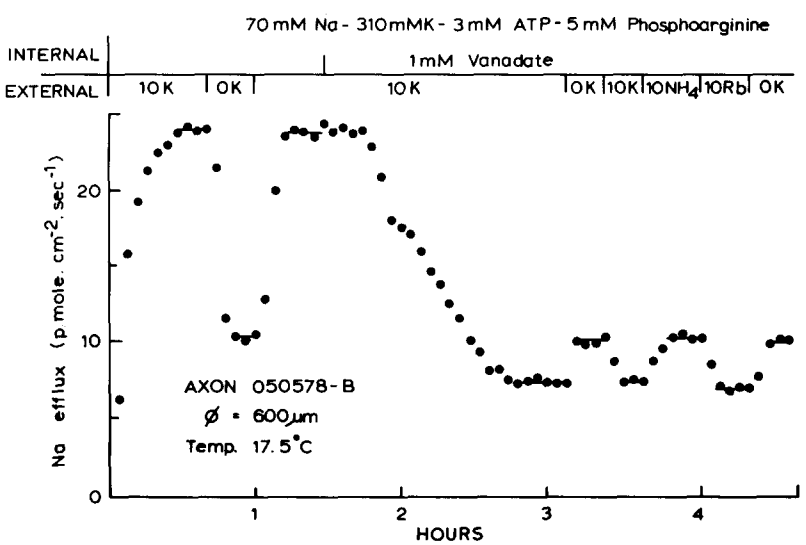

Fig. 1. The effects of 1 mM vanadate applied internally on the efflux of Na^+ in dialysed squid axon. The dialysis solution had the following composition: 70 mM Na^+, 310 mM K^+, 4 mM Mg^{2+} in excess of ATP concentration, 5 mM $Tris^+$, 84 mM Cl^-, 310 mM aspartate , 1 mM ethyleneglycol-bis-(β-aminoethyl-ether)-*N,N'*-tetraacetate ($EGTA^{4-}$), and 330 mM glycine. The osmolarity was 980 mosM and pH 7.1 (at 20°C). ATP was obtained from Boehringer and phosphoarginine from Calbiochem as sodium salts; they were neutralized with Tris (ATP) and HCl (phosphoarginine) to pH 7.1 (at 20°C) and stored at 250 mM solutions at −90°C. The ATP solution contained in addition 250 mM $MgCl_2$. The composition of the standard artificial sea water was: 440 mM Na^+, 10 mM K^+, 50 mM Mg^{2+}, 10 mM Ca^{2+}, 10 mM $Tris^+$, 580 mM Cl^-, 0.1 mM $EDTA^{4-}$. The osmolarity was 1050 mosM and pH 7.6 (at 20°C). The removal or replacement of external K^+ was made without changing the other constituents. At zero time the dialysis was started with the indicated solutions and radioactive Na^+. The apparent rise in Na^+ efflux between 0 and 20 min does not represent a real flux increase but the time taken for the isotope to reach steady-state distribution. The external solutions flow was about 1 ml/min. More details on the dialysis technique can be found in refs. 6 and 7.

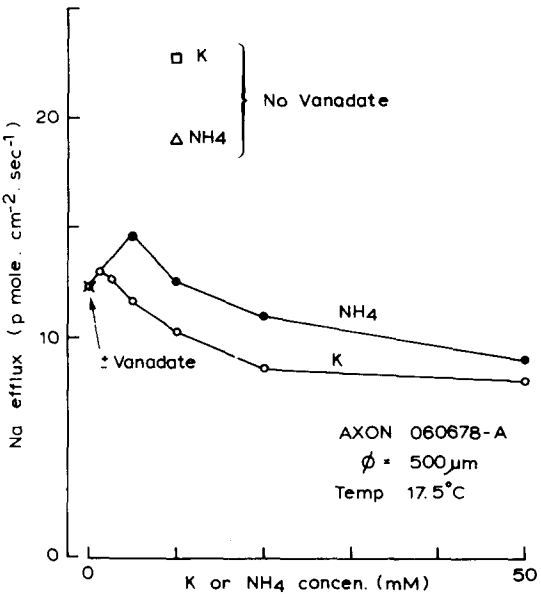

Fig. 2. The effects of different external K^+ and NH_4^+ concentrations on the Na^+ efflux in vanadate-poisoned axon. The dialysis began with no vanadate in the dialysate, and once a steady flux was obtained the effect of 10 mM K^+ (open square) and 10 mM NH_4^+ (open triangle) were investigated. Immediately thereafter 1 mM vanadate was added to the dialysate with the axon immersed in 10 mM K^+ sea water. Once inhibition was fully developed (about 1.5 h after vanadate was introduced) the effects of different K^+ (open circles) and NH_4^+ (filled circles) concentrations were investigated. The values of Na^+ efflux into K^+-free sea water were the same in the presence and absence of vanadate (± vanadate in the figure), and are represented by the half-filled circle and the cross. The general dialysis technique was the same as that described in the legend to Fig. 1.

concentrations was followed by an inhibition as their concentration increased, with the absolute values of flux eventually falling below those in K^+-free conditions. At any given concentration the levels of Na^+ efflux were higher with NH_4^+ than with K^+; i.e. ammonium was a better activator and a poorer inhibitor than potassium. This contrasts with K^+ being a better activator of Na^+ efflux in the absence of vanadate (same Fig. 2); it is in line with the observation that the relative effectiveness of K^+ congeners in promoting vanadate inhibition of renal $(Na^+ + K^+)$-ATPase is $Rb^+ > K^+ > Cs^+ \simeq NH_4^+ > Li^+$ (Grantham, J.J. and Glynn, I.M., unpublished).

In axons with low ATP, external NH_4^+ was also found to be a better activator of the Na^+ efflux than external K^+ [7]; this followed the same pattern as has been described for their effects on the levels of phosphoenzyme [8], and it is consistent with the idea that by binding to a low affinity site, ATP reduces the stability of an enzyme-potassium (E_2K) complex formed after dephosphorylation. The data on vanadate inhibition shows some of the features of low ATP axons, and the hypothesis that vanadate increases the stability of the E_2K complex, even in the presence of high ATP concentrations, seems attractive. The inversion of the K^+-free effect due to vanadate is unlikely to be the consequence of a shift from an uninhibited Na-Na exchange to an inhibited Na-K exchange, because the dialysates were free of ADP and P_i and contained large amounts of phosphoarginine. On the other hand, if, as is

likely, the Na^+ efflux in the absence of K^+ was largely due to uncoupled pumping (driven by the Na^+-ATPase reaction) its reduction by K_0^+ could be the result of a reduction in the availability of pump units because they are stuck in the E_2K conformation. This is consistent with the inhibitory effect of external K^+ on the Na^+-ATPase activity in the presence of low ATP concentrations seen in resealed red cell ghosts [9] and inside-out red cell vesicles [10]. But this mechanism of K^+-vanadate interaction, where every pump unit dephosphorylated by K^+ rephosphorylates more slowly than those spontaneously dephosphorylated, demands that the addition of external K^+ should always result in the inhibition of Na^+ efflux. However, as is seen in Fig. 2, this was not the case, and inhibition of Na^+ efflux at high concentrations of K^+ or NH_4^+ was preceded by activation at low cation concentrations. In order to explain these biphasic curves a secondary effect of external K^+, perhaps stimulating the vanadate binding, could be postulated; alternatively we may suppose that the behaviour of the dephosphoenzyme varies depending whether one or two K^+ ions were used in catalyzing the dephosphorylation. A simple positive cooperative effect between K^+ and vanadate in promoting inhibition of the Na^+ pump could also account for the data; in this case the similarities between the effects of vanadate poisoning and ATP depletion would just be coincidence.

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