SURAMIN: A POTENT INHIBITOR OF THE CALCIUM TRANSPORT IN SARCOPLASMIC RETICULUM

Derek LAYTON and Angelo AZZI

Istituto di Patologia Generale e Centro per lo Studio della Fisiologia dei Mitocondri, Università di Padova, Padova, Italy.

Received May 7,1974

SUMMARY

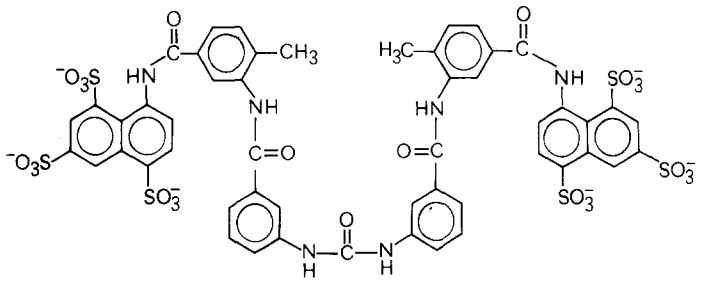
Suramin is shown to be a powerful inhibitor of the calcium uptake and the ATPase activity of sarcoplasmic reticulum. It would appear that suramin is a stoichiometric inhibitor of calcium transport.

INTRODUCTION

The trypanocidal drug suramin, an impermeant polyanion, has been shown to be a potent inhibitor of some hydrolytic and oxidative enzymes (1), but does not cross yeast (2) or red cell membranes (3). Suramin inhibits the Na^+ stimulated ATPase from guinea pig brain (4), and was recently reported to be a powerful inhibitor of the (Na^+ - K^+)-activated ATPase in erythrocyte ghosts (5). In this latter study, suramin was shown to inhibit the Na^+ - K^+ transport system through interaction with the intracellular part of the Na^+ pump located on the inside surface of the membrane. In view of this finding it was decided to investigate its effect on the calcium stimulated ATPase of sarcoplasmic reticulum, particularly with regard to its similarity to certain fluorescent probes in its constituent naphthalene trisulphonate groups and hence its potential usefulness as a site specific spectroscopically active ATPase inhibitor.

MATERIALS AND METHODS

Suramin, (antrypol), was a gift from ICI Pharmaceuticals Division, Alderly Park, Macclesfield, Ceshire, England. All other chemicals were of the highest purity commercially available. Sarcoplasmic reticulum was prepared from rabbit thigh muscle by the method of Hasselbach and Makinose (6). ATPase activity was measured by a standard phosphate assay technique and calcium



SURAMIN

movements were followed by measuring the light absorption changes undergone by murexide, a metallochromic indicator of calcium ion concentration (7).The optical changes were monitored by a dual wavelength (540-507 nm) spectrophotometer (8).

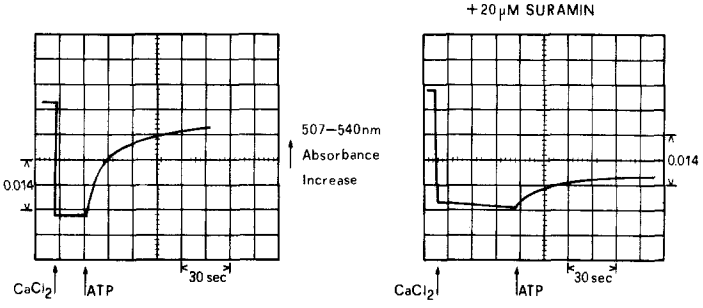


Figure 1. Ca^{2+} uptake by sarcoplasmic reticulum in presence and absence of suramin. The incubation medium contained 20 mM KCl, 10 mM HEPES pH 6.8, 5 mM $MgCl_2$, 200 μM murexide, 2.4 mg sarcoplasmic reticulum protein per ml. $CaCl_2$ was added at a concentration of 100 μM and ATP at a concentration of 1.5 mM. Changes in murexide absorbance were monitored at 507-540 nm in a dual wevelength spectrophotometer, and recorded in a storage oscilloscope (Tektronix 594).

RESULTS AND DISCUSSION

The effect of suramin on the ATP driven Ca^{2+} uptake as measured by the murexide method is shown in Fig. 1. Fig. 1a shows the ATP-induced Ca^{2+} uptake in the absence of suramin and indicates that virtually all of the external calcium was taken up by the sarcoplasmic reticulum

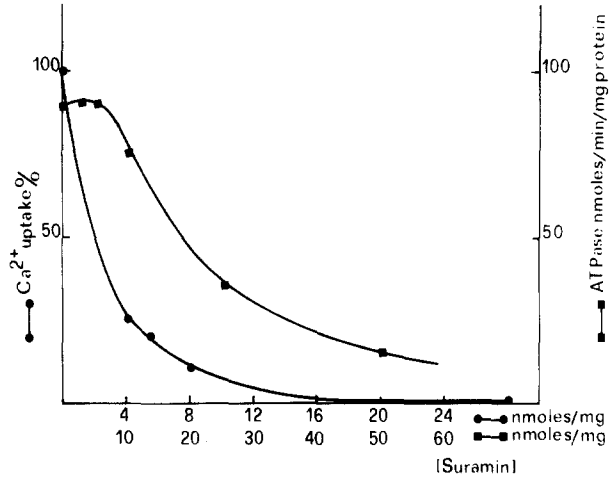


Figure 2. Ca^{2+} uptake and ATPase activity of sarcoplasmic reticulum at different suramin concentrations. Conditions as in Fig. 1 except that murexide was not present in the ATPase experiment, in which the protein concentration was 0.6 mg per ml and 2 mM $CaCl_2$-EGTA complex was also present. The ATPase reaction was stopped after 5 minutes incubation at 30°C by the addition of 10% trichloroacetic acid (final concentration), and Pi was measured by the method of Fiskie and Subbarow (9).

vesicles. In Fig. 1b, the presence of 20 μM suramin (5 nmoles per mg. protein) results in almost complete inhibition of Ca^{2+} uptake. Control experiments in the absence of sarcoplasmic reticulum have indicated that suramin alone does not affect the calcium monitoring system. In Fig. 2 the titration of the effect of suramin on the ATPase activity and the uptake of calcium is shown. It is apparent that suramin has a marked affect on these parameters, the uptake of calcium being most affected.

Calcium uptake shows 80-90% inhibition by 8 nmoles suramin per mg. protein. Assuming 1 mg. of sarcoplasmic reticulum protein contains approximately 0.7 mg. of pump protein of molecular weight 100,000, the concentration of pump would be 7 nmoles per mg. sarcoplasmic reticulum. Thus suramin appears to be a very potent inhibitor and possibly it inhibits with a stoichiometry of 1 to 1. The larger amount of inhibitor required for ATPase inhibition may mean that the inhibitor does not primarily interfere with the binding of ATP to the pump protein.

ACKNOWLEDGEMENTS

The authors are indebted to Mr. Mario Santato for excellent technical assistance. D.L. is in receipt of a long term EMBO fellowship.

REFERENCES

1. Wills, E.D. and Wormal, A. (1950) Biochem. J. 47, 158-170
2. Town, B.W., Wills, E.D., Wilson, E.J. and Wormal, A. (1950) Biochem. J. 47, 149-158
3. Wilson, E.J. and Wormal, A. (1949) Biochem. J. 45, 224-231
4. Schwartz, A., Bachelord, H.S. and McIllwain, H. (1962) Biochem. J. 84, 626-637
5. Fortes, P.A.G., Ellory, J.C. and Lew, V.L. (1973) Biochim. Biophys. Acta 318, 262-272
6. Hasselbach, W. and Makinose, M. (1963) Biochem. Z. 339, 94-103
7. Ohnishi, T. and Ebashi, S. (1963) J. Biochem. 54, 506-511
8. Chance, B. (1951) Rev. Sci. Instru. 22, 634-638
9. Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375-383