Effect of polyacetylene derivatives on mitochondrial swelling

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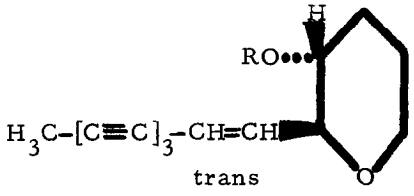
Preparations from the leaves of Clibadium sylvestre are used by the indigenous peoples in South America as a fish poison. The active principles have been extracted and shown to be a polyacetylenic alcohol (I) and its acetate ester (II) [Crook, Gorinsky & Quilliam, 1967].



R = H [I]
R = Ac [II]

As rotenone - another fish poison - has marked effects on oxidative phosphorylation it seemed important to ascertain whether these polyacetylenes (I, II) acted in an analagous fashion. Hence, the effects of these compounds (I, II) on mitochondrial swelling - a process known to be intimately associated with oxidative phosphorylation (Packer, 1961; Lehninger, 1962) - were studied. It is well established that orthophosphate will cause mitochondria suspended in a salt medium to swell and that this swelling may be reversed by the addition of ATP and Mg^{++} ions (Lehninger, 1959; Chappell & Greville, 1960; Greenbaum & Dicker, 1963). The experiments reported here show that whilst only the acetate (II) affected the rate of swelling in these conditions, both compounds inhibited the ATP, Mg^{++} induced contraction of phosphate swollen mitochondria.

Methods

Male Wistar rats were used of average weight 180 gms. Rat liver mitochondria were prepared by conventional means in 0.25 M Sucrose - 1mM EDTA (Clark, Greenbaum & Slater, 1965). Swelling was

followed at 25° and 520 mμ in a 0.125 M KCl - 0.02 M Tris pH 7.4 medium (see Lehninger, 1959) and was induced in all cases by 10 mM (f.c.) KH_2PO_4 pH 7.4. Both polyacetylenic compounds when used were suspended in the same KCl - Tris medium to avoid osmotic dilution. Reversal of swelling was brought about by adding a mixture giving the following final concentrations; ATP, 7.5 mM: $MgCl_2$, 5 mM: bovine plasma albumin 0.03 mg./ml. Swelling was initiated by the addition of 0.1 ml. (0.8 mg. protein) of a mitochondrial suspension in the KCl-Tris medium.

Results

Fig. 1 shows the effect that the polyacetylenic alcohol (I) had on the ATP induced contraction of phosphate swollen mitochondria. Complete inhibition of contraction was observed at a final concentration of 15.5×10^{-5} M alcohol (I).

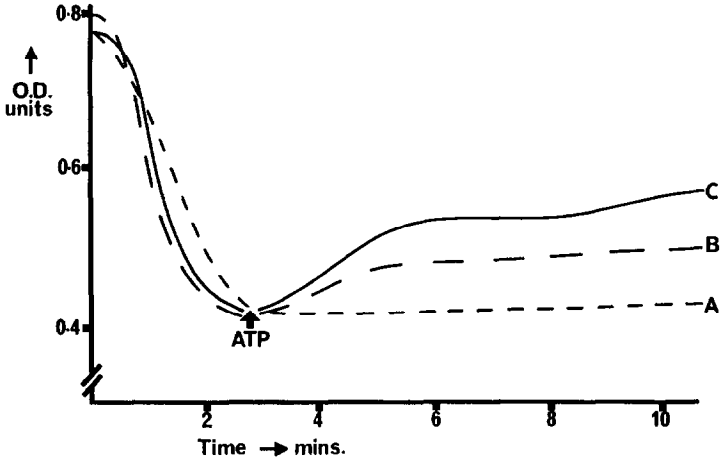


Fig. 1. Effect of the alcohol (I) on ATP induced contraction of phosphate swollen mitochondria

Conditions used were as in the methods section. Various amounts of the alcohol (I) were included in the cuvette prior to the addition of the mitochondria.
Curve A - in presence of 15.5×10^{-5} M alcohol (I).
Curve B - " " of 7.7×10^{-5} M alcohol (I).
Curve C - control in absence of alcohol (I).

Similar effects were observable in the case of the acetate (II) when complete inhibition of contraction was observed at a concentration of 12×10^{-5} M. If varying amounts of the alcohol (I) or the acetate (II) were added to the mitochondria, in both cases a linear relationship was found between the concentration present and the amount of inhibition of contraction up to a saturating level when 100 % inhibition of contraction occurred. (Fig. 2) Further additions of the compounds (I & II) caused no further effects on the mitochondrial swelling.

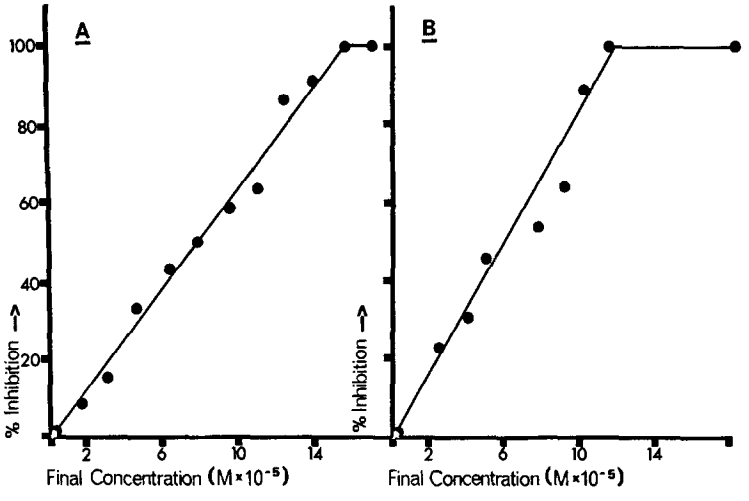


Fig. 2. Effect of varying the concentration of the alcohol (I) or acetate (II) on mitochondrial contraction
Experimental conditions are as described in the methods section and Fig. 1. The amount of contraction induced 4 mins. after the addition of the ATP mixture was noted and taken as a measure of the contraction rate.
A. The alcohol (I) concentration was varied. The average contraction of the control mitochondria was 0.16 optical density units.
B. The acetate (I) concentration was varied. The average contraction of the control mitochondria was 0.11 optical density units.

The alcohol (I) had no apparent effects on the rate or extent to which swelling occurred. However, the acetate (II) showed a marked stimulatory effect on the rate of swelling induced by phosphate (Fig. 3) whilst not affecting the extent to which swelling occurred. Again,

a linear relationship was apparent between the concentration of acetate (II) present and the increase in swelling rate up to a limiting level when further acetate (II) did not affect the swelling rate.

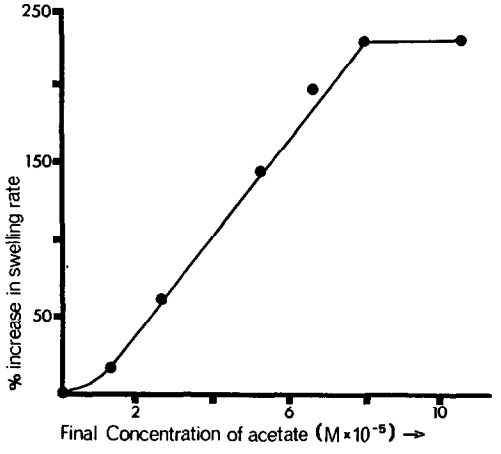


Fig. 3. Effect of varying acetate (II) concentration on mitochondrial swelling
Experimental conditions are as before. The time taken for the optical density to fall by 0.25 units was taken as a measure of the swelling rate.
The control mitochondria swelling rate was 0.04 optical density units/min.

Discussion

The effect of both these polyacetylene derivatives (I, II) on mitochondrial contraction is very similar to that reported by Neubert & Lehninger (1962) for oligomycin on mitochondria swollen by glutathione. Thus it may be suggested that these compounds (I & II) 'titrate' sensitive sites on the mitochondrial membrane to a saturation level. The acetate (II) appears to be able to penetrate the membrane before the mitochondria is fully swollen whereas the alcohol (I) can only act after swelling has occurred. As the acetate (II) is more lipophilic than the alcohol (I) it would seem that the sensitive sites are localised in the lipid regions of the membrane. Muscle relaxing factor has also been reported to inhibit mitochondrial contraction (Baltscheffsky & Baltscheffsky, 1964)

and has been implicated in Ca^{++} binding (Baltscheffsky, 1964). By analogy, it is possible that these polyacetylenic compounds act by virtue of their effects on membrane permeability to various ions which may explain the in vivo convulsive activity of the alcohol (I).

These polyacetylenic compounds and their derivatives may prove to be particularly useful tools in studying the membrane localisation of various metabolic activities. In these experiments the greater lipophilic character of the acetate (II) as compared with the alcohol (I) has manifested itself in an effect on swelling as well as contraction. By synthetically varying the lipophilic nature of these polyacetylene derivatives it may become possible to associate various activities with different parts of the membrane and build up a membrane activity 'map'.

References

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Acknowledgements

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