Inability of Tributyltin-Induced Chloride/Hydroxyl Exchange to Stimulate Calcium Transport in Mitochondria Isolated from Flight Muscle of the Sheep Blowfly *Lucilia cuprina*

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Tributyltin in the concentration range 1–4 μM failed to stimulate Ca^{2+} transport by *Lucilia* flight-muscle mitochondria in a medium containing KCl and respiratory substrate but devoid of P_i , despite its promotion of a rapid Cl^-/OH^- exchange. When 2 mM P_i was present, concentrations of tributyltin greater than 1 μM inhibited the initial rate of Ca^{2+} transport and induced efflux of the ion from the mitochondria in Cl^- - or NO_3^- -containing media. Lower concentrations had little effect. Oligomycin added at up to 10 $\mu\text{g}/\text{mg}$ of mitochondrial protein had no effect on Ca^{2+} transport. By contrast, approx. 0.3 μM -tributyltin completely inhibited respiration supported by α -glycerophosphate in either the presence or absence of added ADP. The data suggest that tributyltin can inhibit Ca^{2+} transport in *Lucilia* flight-muscle mitochondria other than by facilitating a Cl^-/OH^- exchange or producing an oligomycin-like effect.

The movement of Ca^{2+} between various cell compartments is fundamentally important for the way in which the ion influences a range of cellular metabolic activities (reviewed by Bygrave, 1978*a,b*). Since such movement is greatly facilitated by permeant anions (Lehninger *et al.*, 1967; Martonosi, 1972; Lehninger, 1974; Bygrave, 1977), it follows that an understanding of the mechanism of this facilitation is of wide biological interest.

We (Bygrave *et al.*, 1978*b*) have shown that trialkyltin compounds, known to promote Cl^-/OH^- exchange across the inner mitochondrial membrane (Selwyn *et al.*, 1970; Stockdale *et al.*, 1970; Skilliter, 1976), are potentially useful probes for elucidating further the interaction of the anion with Ca^{2+} movements in these organelles. Specifically, we observed that 1–2 μM -tributyltin markedly increased the initial rate of Ca^{2+} transport in a Cl^- -containing medium.

The clear dissimilarity between mammalian and insect flight-muscle mitochondria with regard to the effect of permeant anions, especially P_i , on Ca^{2+} transport (Carafoli *et al.*, 1971; Bygrave *et al.*, 1975, 1977; Smith & Bygrave, 1978) prompted the question as to whether this dissimilarity pertained also to the interaction of tributyltin with mitochondrial Ca^{2+} transport. Experiments in this report establish that this is so.

Experimental

Maintenance of flies, the isolation of flight-muscle mitochondria and measurement of mitochondrial respiration were carried out exactly as described previously (Bygrave *et al.*, 1975; Smith & Bygrave, 1978). Ca^{2+} transport was measured by the Ruthen-

ium Red-EGTA quench technique (Reed & Bygrave, 1974) or by using combination Ca and pH electrodes (Ramachandran & Bygrave, 1978). Each of the experiments described was carried out at least twice and usually three times, with agreement between experiments within approx. 10%. Tributyltin chloride (BDH, Poole, Dorset, U.K.) was prepared by diluting a portion of the stock solution (1.19 g/ml) to give a concentration of 4.5 mM in ethanol. This was further diluted periodically in water and portions added to the reaction mixtures as described in the Figure legends.

Results

The following points were established in initial experiments, the results of which are not presented.

In media containing KCl and α -glycerophosphate and devoid of added P_i , concentrations of tributyltin up to 4 μM failed to stimulate the initial rate of Ca^{2+} transport by *Lucilia* flight-muscle mitochondria isolated from flies ranging in age from 1 to 24 h. This situation contrasts with experiments using rat liver mitochondria in which 1–2 μM -tributyltin stimulated up to 100% the initial rate of Ca^{2+} transport (Bygrave *et al.*, 1978*b*).

The inability of tributyltin to stimulate Ca^{2+} transport by *Lucilia* mitochondria does not arise from an inability of the compound to promote a Cl^-/OH^- exchange. As shown in Fig. 1, addition of 3 μM -tributyltin, although it does not induce Ca^{2+} transport, does produce an immediate change in pH (increased alkalinity) in the medium bathing the mitochondria. Pyruvate plus malate replaced α -glycerophosphate in these experiments because of

Fig. 1. Effect of tributyltin on Ca^{2+} transport by *Lucilia* mitochondria in the absence of added P_i .

The incubation medium contained 100mM-KCl, 5mM-sodium pyruvate, 2mM-sodium malate, 2mM- KHCO_3 and 2mg of mitochondrial protein in a total volume of 4.0ml at 25°C . The pH was 7.4. Where indicated 50 μM - Ca^{2+} and 3 μM -tributyltin were added. Ca^{2+} transport and change in pH were measured by using a combination of Ca and pH electrodes (see the Experimental section).

the buffering experienced with the high concentrations of the latter substrate.

Only quite low concentrations of P_i are required to induce Ca^{2+} transport by *Lucilia* mitochondria (Bygrave & Smith, 1977; Smith & Bygrave, 1978). Fig. 2(a) shows the effect of tributyltin on the initial rate of Ca^{2+} transport by *Lucilia* mitochondria in the presence of 2mM- P_i . Stimulation does not occur at any concentration of added tributyltin. Indeed the compound has little effect until concentrations exceeding 1 μM are reached; at this point, inhibition of Ca^{2+} transport is observed, which becomes almost complete by 2 μM -tributyltin. A similar concentration-dependent effect is seen also on the steady-state concentration of accumulated Ca^{2+} . Identical experiments, carried out in a medium containing NO_3^- in place of Cl^- , produced similar results.

Fig. 2. Effect of tributyltin concentration on the initial rate of Ca^{2+} transport, on its efflux from mitochondria and on respiration supported by α -glycerophosphate in the absence and presence of ADP.

The incubation medium for (a) contained 100mM-KCl, 25mM- α -glycerophosphate and 2mM- K_2HPO_4 and the indicated concentrations of tributyltin in a total volume of 1.0ml at 25°C . The pH was 7.4. Mitochondria (1mg of protein), isolated from 6h-old flies, were added, and after 1 min 50 μM - Ca^{2+} (containing 0.3 μCi of $^{45}\text{Ca}^{2+}$) was added to initiate the reaction. In (b) the reaction system was similar, except that tributyltin at the indicated concentrations was added 1 min after the Ca^{2+} . At this point Ca^{2+} inside the mitochondria was approaching the steady-state concentration. The rate of Ca^{2+} efflux was then measured. In (c) respiration was measured in a medium identical with that in (a) in the absence (○) or presence (●) of 200 μM -ADP.

Fig. 2(b) shows the effect of adding various concentrations of tributyltin to *Lucilia* mitochondria after they had accumulated Ca^{2+} . Rather than promoting a further accumulation of Ca^{2+} (cf. Bygrave *et al.*, 1977, 1978b), tributyltin in these experiments induces an efflux of Ca^{2+} . The rate of efflux increases as the concentration of added tributyltin is increased; maximal rates of efflux were seen at approx. $4\text{ }\mu\text{M}$ -tributyltin. Again similar results were obtained when NO_3^- replaced Cl^- in the incubation medium.

Finally, data in Fig. 2(c) show the effect of tributyltin on the rates of respiration in the absence and presence of added ADP. Especially noteworthy is the finding that respiration is extremely sensitive to low concentrations of added tributyltin; the K_i is approx. $0.1\text{ }\mu\text{M}$ and near-maximal inhibition occurs at approx. $0.3\text{ }\mu\text{M}$ or at a concentration which has little effect on the initial rate of Ca^{2+} accumulation (cf. Fig. 2a). The concentrations of tributyltin that inhibit respiration of *Lucilia* mitochondria are similar to those that inhibit respiration in rat liver mitochondria (cf. Selwyn *et al.*, 1970; Stockdale *et al.*, 1970).

In other experiments, results of which are not presented, it was shown that addition of oligomycin to the incubation systems of Figs. 2(a) and 2(b) had no effect on mitochondrial Ca^{2+} transport, suggesting that any Cl^- permeation that may have been induced by oligomycin under the present experimental conditions (cf. Ariel & Avi-Dor, 1975) was unable to stimulate the initial rate of Ca^{2+} transport.

Discussion

The inhibitory effects of trialkyltin compounds on mitochondrial energy transduction (Aldridge, 1958, 1962) have been considered to result from either a Cl^-/OH^- exchange in a Cl^- -containing medium or from an oligomycin-like inhibition of phosphorylation which persists independently of the ionic composition of the medium (Selwyn *et al.*, 1970; Stockdale *et al.*, 1970; Skilliter, 1975, 1976; Ariel & Avi-Dor, 1973, 1975). Experiments in this report show that energy-dependent Ca^{2+} movement by *Lucilia* flight-muscle mitochondria is prevented by low concentrations of tributyltin through a mechanism that appears not to involve one or other of these mechanisms. Although the mechanism of inhibition cannot be gleaned from this work, it is pertinent that the inhibitory pattern is similar to that observed in the study of Ruthenium Red-insensitive Ca^{2+} transport by the microsomal fraction from rat liver (Bygrave, 1978c).

The second major conclusion to be drawn from this study is that α -glycerophosphate-supported respiration is sensitive to concentrations of tributyltin

that have no inhibitory effect on the initial rate of Ca^{2+} transport. This provides further evidence for the view that respiratory-chain activity is not the rate-limiting step in mitochondrial Ca^{2+} transport (Bygrave *et al.*, 1978a).

Finally the data indicate that something unique exists about P_i and its ability to facilitate Ca^{2+} transport by *Lucilia* flight-muscle mitochondria. Whereas in mammalian mitochondria tributyltin-facilitated Cl^-/OH^- exchange is as effective as P_i in promoting Ca^{2+} transport (Bygrave *et al.*, 1978b), only the latter is effective with *Lucilia* mitochondria. Yet, in most other respects, the properties of Ruthenium Red-sensitive Ca^{2+} transport is similar in mitochondria isolated from both species (Bygrave *et al.*, 1975).

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References

- Aldridge, W. N. (1958) *Biochem. J.* **69**, 367–376
- Aldridge, W. N. (1962) *Biochem. J.* **83**, 527–533
- Ariel, N. & Avi-Dor, Y. (1973) *Biochem. J.* **136**, 911–917
- Ariel, N. & Avi-Dor, Y. (1975) *Biochem. J.* **152**, 115–119
- Bygrave, F. L. (1977) *Curr. Top. Bioenerg.* **6**, 259–318
- Bygrave, F. L. (1978a) *Biol. Rev. Cambridge Philos. Soc.* **53**, 43–79
- Bygrave, F. L. (1978b) *Trends Biochem. Sci.* in the press
- Bygrave, F. L. (1978c) *Biochem. J.* **170**, 87–91
- Bygrave, F. L. & Smith, R. L. (1977) *Biochem. Biophys. Res. Commun.* **79**, 154–158
- Bygrave, F. L., Daday, A. A. & Doy, F. A. (1975) *Biochem. J.* **146**, 601–608
- Bygrave, F. L., Ramachandran, C. & Smith, R. L. (1977) *FEBS Lett.* **83**, 155–158
- Bygrave, F. L., Heaney, T. P. & Ramachandran, C. (1978a) *Biochem. J.* **174**, 1011–1019
- Bygrave, F. L., Ramachandran, C. & Robertson, R. N. (1978b) *Arch. Biochem. Biophys.* **188**, 301–307
- Carafoli, E., Hansford, R. G., Sacktor, B. & Lehninger, A. L. (1971) *J. Biol. Chem.* **246**, 964–972
- Lehninger, A. L. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1520–1524
- Lehninger, A. L., Carafoli, E. & Rossi, C. S. (1967) *Adv. Enzymol. Relat. Areas Mol. Biol.* **29**, 259–319
- Martonosi, A. (1972) *Curr. Top. Membr. Transp.* **3**, 83–197
- Ramachandran, C. & Bygrave, F. L. (1978) *Biochem. J.* **174**, 613–620
- Reed, K. C. & Bygrave, F. L. (1974) *Biochem. J.* **140**, 143–155
- Selwyn, M. J., Dawson, A. P., Stockdale, M. & Gains, N. (1970) *Eur. J. Biochem.* **14**, 120–126
- Skilliter, D. N. (1975) *Biochem. J.* **146**, 465–471
- Skilliter, D. N. (1976) *Biochem. J.* **154**, 271–276
- Smith, R. L. & Bygrave, F. L. (1978) *Biochem. J.* **170**, 81–85
- Stockdale, M., Dawson, A. P. & Selwyn, M. J. (1970) *Eur. J. Biochem.* **15**, 342–351