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SHORT COMMUNICATIONS

On the Mg^{2+}— dependency of the mitochondrial ATPase activation by two azocarcinogens and a related cytotoxic styrylquinoline*

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FRESHLY-ISOLATED liver mitochondria possess a latent ATP-splitting activity which can be manifested by compounds such as DNP or by conditions which interfere with the structural intactness of the particles (e.g. ageing). The first type of ATPase, the DNP-activated ATPase, is independent of Mg^{2+}, whereas the second one, operative in structurally disorganized particles, is largely dependent on Mg^{2+} for its activity.[1] Since the mitochondrial ATPase assay is usually carried out in a medium containing Mg^{2+}, no distinction between the two possible kinds of effect is made. However, once a biologically-active compound has been found to induce ATPase activity in isolated mitochondria, it may be of interest for the study of its mode of action to know whether the compound acts by a DNP-like type of effect or, as may be inferred from the existence of the second type of ATPase, by interference with some component governing the structural organization of the particles.

Previous investigations in this laboratory on styrylquinolines and related compounds[2] and on azobenzene derivatives[3] have shown that some of the members of these series, which are biologically active either as cytotoxics or as hepatic carcinogens, are effective ATPase activators in mouse and rat liver mitochondria. The present note describes the difference in the mode of action, as formulated above, by which the anti-tumour drug DSQ and the hepatic carcinogen DAQ bring about the latter

TABLE 1. MITOCHONDRIAL ATPASE ACTIVATION BY DNP, DSQ, DAQ AND AAT IN THE PRESENCE AND ABSENCE OF Mg^{2+}

Addition	Phosphorus released (μg)					
	In the presence of Mg^{2+} after			In the absence of Mg^{2+} after		
	5 min	10 min	20 min	5 min	10 min.	20 min
None	0	0	7	0	0	0
DNP	39	62	113	49	79	129
DSQ	25	49	90	4	12	26
DAQ	30	52	93	35	58	98
AAT	31	61	105	0	3	7

Conditions described previously[2] (in Fig. 1 of this reference P_i should be read as phosphorus).

DNP: 10^{-4} M, all other compounds: $1{\cdot}3 \times 10^{-4}$ M.

effect (Table 1). ATPase stimulation in rat liver mitochondria by DSQ has been found to be largely dependent on the presence of Mg^{2+}, whereas that by DAQ was not. The molecular structure of the two compounds is similar except that in DSQ an ethylene and in DAQ an azo bridge link the benzene and quinoline rings. However, the presence of an azo bridge *per se* does not necessarily decide whether the ATPase stimulation by a given compound is independent of Mg^{2+} since the hepatic carcinogen AAT activated the ATPase only in the presence of Mg^{2+} (Table 1). The latter result also shows that different hepatic carcinogens, which activate the mitochondrial ATPase, may do so through a different

* Abbreviations used: ATP = adenosine triphosphate; DNP = 2 : 4-dinitrophenol; DSQ = 4-(4′-dimethylaminostyryl)quinoline; DAQ = 4-(4′-dimethylaminophenyl)azoquinoline· AAT = *o*-aminoazotoluene.

mechanism. The present results may thus allow the conclusion that DAQ exhibits a DPN-like type of effect on the mitochondrial ATPase, whereas DSQ and AAT appear to interact primarily with some component or reaction governing the structural integrity of the particles.

In view of the reverse relationship between water uptake by and structural integrity of isolated mitochondria, it is of interest that DNP has been shown[4, 5] to counteract the spontaneous and thyroxine-induced swelling of liver mitochondria incubated in the absence of oxidizable substrate and cofactors, as measured by (changes in) the optical density at 520 mμ of such suspensions. In similar experiments carried out with DAQ and DSQ a small increase, respectively a decrease, of the $o.d._{520}$ of the mitochondria, as compared with that of the controls, was observed. However, an interpretation of the results in terms of mitochondrial shrinkage or swelling was difficult, if not impossible, in view of the limited solubility of the compounds (approximately one-third of the added $1{\cdot}5 \times 10^{-4}$ was in real solution); part of the finely-dispersed compound may become attached to the particles and thus change the optical density of the suspension (measured at 660 mμ in the present case in view of the absorbency of DAQ at 520 mμ). However, in the case of AAT, which was in complete solution, a marked swelling (decrease of 30–50 per cent in $o.d._{520}$) has been noted previously.[3] It has also been reported[2] that DSQ causes a notable swelling of liver mitochondria when Mg^{2+} was present. This effect, i.e. fall in optical density which was interpreted as swelling, has now been found by phase contrast microscopical examination to be due to an agglutination of the particles. Addition of Mg^{2+} (as chloride, 5×10^{-3} M) to the control suspensions resulted in many very small aggregates of mitochondrial bodies without causing a change in the optical density of the suspension as compared with the controls. Addition of DSQ and Mg^{2+} gave rise to the formation of elaborate aggregates of particles and a marked fall in the optical density (60 per cent decrease). In the presence of DAQ and Mg^{2+}, however, no change in the optical density of the mitochondrial suspension was observed, the agglutination of the particles being less than in the former case but still more extensive than in the controls receiving Mg^{2+} only. Since DSQ or DAQ did not cause an agglutination of the mitochondria in the absence of Mg^{2+} these compounds, and DSQ especially so, appear to accelerate the agglutination of mitochondria incubated in the presence of Mg^{2+}.

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Variations in toxicity of some halogen derivatives of acetic acid in rats

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SOME halogen derivatives of acetic acid have been used extensively in biological systems and show a degree of toxicity. For example monoiodoacetate acts as an alkylating agent for sulphydryl groups[1] and monofluoroacetate is toxic because it is a precursor of fluorocitrate, which in turn is an inhibitor of aconitase.[2] Because of the lipotropic properties of ethyl trichloroacetate[3, 4] other halogen derivatives of acetate were examined for this property, and in this study their relative toxicity in young rats has been noted.