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The actions of disulfiram and 2,2′-dithiopyridine on oxidative phosphorylation and ion transport by rat liver mitochondria

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In previous papers[1-3] we have reported that 5,5′-dithio-bis-(2-nitrobenzoic acid; Ellman's reagent or DTNB) inhibits oxidative phosphorylation and uptake of phosphate and calcium ions by rat liver and heart mitochondria. DTNB is a disulfide and acts presumably by forming mixed disulfides with sulfhydryl groups present on the mitochondrial membranes. This view is supported by the observation that the action of DTNB on mitochondrial metabolism can be reversed by the sulfhydryl compound, dithiothreitol (DTT). The present report concerns the actions on liver mitochondria of two other disulfides, tetraethylthiuram disulfide (disulfiram) and 2,2′-dithiopyridine. In contrast to DTNB, these substances are not strongly charged and can be expected to penetrate biological membranes.

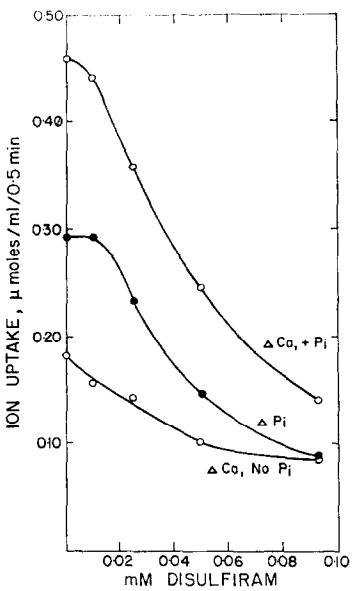

Fig. 1. Effect of disulfiram on the uptake of calcium and phosphate ions by rat liver mitochondria. Mitochondria were incubated at 25° in the presence of different concentrations of disulfiram. Inhibitor was added at zero time, potassium phosphate (pH 7·4 to give 0·57 mM) at 50 sec, $CaCl_2$ (to give 0·54 mM) at 1 min; millipore filtration after 1·5 min. Components of reaction mixtures: 24 mM sucrose, 33 mM HEPES (pH 7·4), 8·1 mM potassium succinate, 8·1 mM $MgCl_2$, KCl to 250 mOsmolar; total volume, 2·65 ml. Mitochondrial protein, 2·39 mg/ml.

Freshly prepared rat liver mitochondria were used in all experiments. The methods used for incubation and analytical determinations were identical to those described in earlier publications from this laboratory.[2,3] Experimental conditions are reported in the legends to the figures.

The effect of disulfiram on the uptake of calcium and phosphate ions is shown in Fig. 1. In the absence of inhibitor, the mitochondria removed calcium from the incubation medium both in the presence and absence of added inorganic phosphate. The addition of phosphate, however, produced a large increase in calcium uptake. The ratio of calcium to phosphate uptake was 1·6, close to the

figure reported by Rossi and Lehninger.[4] As the concentration of disulfiram was increased, there was a decrease in both phosphate and calcium uptake. At each concentration of inhibitor, the extra calcium uptake in the presence of phosphate was about equal to the amount of phosphate ions entering the mitochondria. In identical experiments with 2,2′-dithiopyridine, similar results were obtained. It appears then that these two disulfides act like DTNB in that they inhibit the process by which one calcium and one phosphate ion enter the mitochondrion.[5] Calcium uptake in the absence of phosphate is also depressed, but to a smaller extent.

Studies in which respiration of liver mitochondria was measured showed that disulfiram and dithiopyridine, like DTNB, inhibit oxidative phosphorylation. The experiments showed also that the disulfides had an additional effect on mitochondrial metabolism in that they inhibited stimulation of respiration by 2,4-dinitrophenol (DNP).

The results of experiments with mitochondria incubated in the presence of glutamate are reported in Fig. 2. Curve A shows the normal response to the addition of ADP and phosphate, the cut-off of

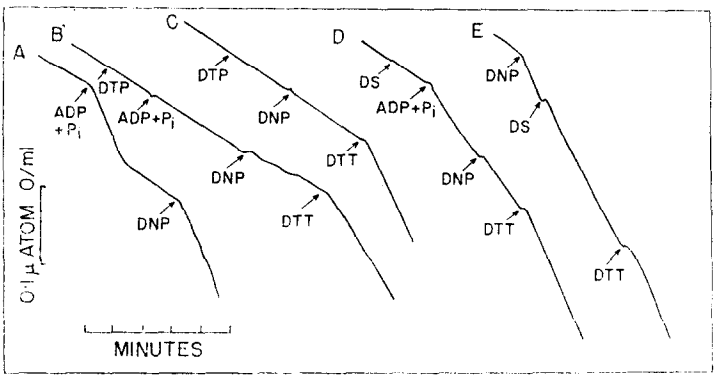

FIG. 2. Effects of dithiopyridine and disulfiram on respiration of rat liver mitochondria. Mitochondria were incubated at 26° in a Gilson Oxygraph with Clark oxygen electrode. Components of reaction mixture: 25 mM sucrose, 35 mM HEPES (pH 7·4), 8·8 mM potassium glutamate, 8·8 mM $MgCl_2$, KCl to 250 mOsmolar; total volume, 1·48 ml. When added: ADP, 0·135 mM; potassium phosphate (Pi), 2·70 mM; 2,4-dinitrophenol (DPN), 0·010 mM; 2,2′-dithiopyridine (DTP), 0·10 mM; dithiothreitol (DTT), 0·67 mM; disulfiram (DS), 0·10 mM. Mitochondrial protein, 1·11 mg/ml.

the respiratory response and the subsequent increase in respiration produced by DNP. In experiment B, the addition of dithiopyridine (DTP) caused no change in the rate of respiration, but the responses to ADP and phosphate and to DNP were abolished. There was a partial reversal of the inhibition after addition of the sulfhydryl reagent, dithiothreitol (DTT). When added before DNP (not shown here), DTT also caused a partial restoration of the inhibited respiration. The experiment depicted in curve C shows that DTP prevents the stimulatory effect of DNP on respiration in the absence of ADP and phosphate and that the inhibition is overcome by DTT. Disulfiram (curve D) in the same concentration as dithiopyridine inhibited the response of the mitochondria to ADP plus phosphate and blocked the effect of DNP. The addition of dithiothreitol resulted in some relief of the inhibition. In experiments not reported here it was found that a small increase in the concentration of disulfiram led to a complete inhibition of the respiratory response to ADP and phosphate. It is interesting that disulfiram had only a slight effect when respiration had been stimulated by the prior addition of dinitrophenol. This indicates that the two compounds may combine with the same active site.

The present experiments provide additional evidence for the importance of sulfhydryl groups in oxidative phosphorylation and ion transport.[1–3,5–7] The action of disulfiram shown here is consistent with its demonstrated ability to combine with sulfhydryl groups[8] in a manner similar to that of DTNB.[9] The effect of disulfiram on DNP-stimulated respiration is in agreement with the observation

of Hassinen[10] that this compound strongly inhibits dinitrophenol-induced ATPase of liver mitochondria.

It is clear from these experiments that dithiopyridine and disulfiram are less specific than DTNB in their effect on mitochondrial metabolism. While the action of DTNB[1-3] appears to be concerned almost entirely with the effect of inorganic phosphate on mitochondria (probably because it inhibits phosphate transport), the less charged disulfides, dithiopyridine and disulfiram, have additional effects such as inhibition of DNP-stimulated respiration.

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Release of norepinephrine and normetanephrine from cat brain by central nervous system stimulants*

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IT HAS been suggested[1-3] that the central actions of a number of psychoactive drugs are related to their ability to alter the concentration of norepinephrine at specific postsynaptic receptor sites within

* Supported by U. S. Public Health Service MH Grant 13174. A preliminary report covering some aspects of this work was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, August, 1969 (*Pharmacologist* **11**, 258, 1969). The word "release" is used here to denote the release of amines from the brain and does not imply any specific releasing mechanism at the neuronal or molecular level.