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## Stimulation by uncouplers of the efflux of α-oxoglutarate from rat-liver mitochondria

The addition of 2,4-dinitrophenol or dicoumarol to rat-liver mitochondria oxidizing glutamate in the presence of phosphate and phosphate acceptor brings about an inhibition of the transamination pathway of glutamate oxidation[1–3], an increase in the oxidation level of NADP[3], and a stimulation of the oxidative deamination of glutamate[1–4]. Since the rate of glutamate deamination in rat-liver mitochondria is closely correlated with the extent of oxidation of NADP[5], this stimulation of the deamination is probably due to inhibition by uncouplers of the energy-linked transhydrogenase[6,7], and the consequent increase in the oxidation state of NADP.

HARRIS, VAN DAM AND PRESSMAN[8] and VAN DAM[9] have recently shown that uncouplers competitively inhibit the uptake of substrate anions (including glutamate) by mitochondria. In the present communication, another effect of uncouplers is reported, namely, a stimulation of the efflux of α-oxoglutarate from rat-liver mitochondria. DE HAAN AND TAGER[10] have shown that there is a permeability barrier for the entry or exit of α-oxoglutarate in rat-liver mitochondria. They found that α-oxoglutarate accumulates within the mitochondria during the oxidation of glutamate in the presence of arsenite (to prevent the oxidation of α-oxoglutarate), and that the rate of the oxidative deamination of glutamate is limited by the rate of efflux of α-oxoglutarate from the mitochondria. Malonate increases the rate of efflux, resulting in an increased rate of glutamate deamination[10].

Table I shows the effect of malonate and of dicoumarol on the formation of α-oxoglutarate and on the intra- and extramitochondrial concentration of this oxoacid during the oxidation of glutamate (*plus* arsenite) in rat-liver mitochondria. In the control, where glutamate was added to mitochondria preincubated with ADP, $P_i$ and arsenite, 0.84 μmole α-oxoglutarate was formed. The intramitochondrial concentration of α-oxoglutarate was 42 times higher than the extramitochondrial concentra-

TABLE I

EFFECT OF MALONATE AND DICOUMAROL ON THE FORMATION OF α-OXOGLUTARATE AND ITS EFFLUX FROM RAT-LIVER MITOCHONDRIA DURING THE OXIDATION OF GLUTAMATE (*plus* ARSENITE)

Rat-liver mitochondria[11] (24.8 mg protein) were preincubated at 25° for 2 min in a reaction mixture (final vol., 5 ml; final pH 7.5) containing 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris–HCl, 25 mM sucrose, 5 mM ADP, 10 mM $P_i$, 1 mM arsenite, 0.5 mg (440000 counts/min) [$^{14}$C]carboxydextran (mol. wt. 60000–90000) and (where indicated) 20 μM dicoumarol and 10 mM malonate. After preincubation, 10 mM glutamate were added and the incubation was continued at 25°. After 10 min incubation mitochondria were separated from the incubation medium by centrifugation filtration as described by PFAFF[12]. α-Oxoglutarate was determined enzymically in the mitochondrial extract and in the supernatant, using the Aminco-Chance double-beam spectrophotometer. Mitochondrial water was determined gravimetrically, correction being made for adherent supernatant; the latter was determined by [$^{14}$C]carboxydextran[12]. The mitochondrial water content ranged in this and other experiments from 2.5–2.9 μl/mg mitochondrial protein.

| *Additions* | *Δα-Oxoglutarate (μmoles/10 min)* | | | *[α-Oxoglutarate] (mM)* | |
|---|---|---|---|---|---|
| | *Inside* | *Outside* | *Total* | *Inside* | *Outside* |
| None | 0.320 | 0.520 | 0.840 | 4.52 | 0.107 |
| Malonate | 0.045 | 2.025 | 2.070 | 0.66 | 0.405 |
| Dicoumarol | 0.140 | 1.280 | 1.420 | 1.71 | 0.256 |

tion. When malonate was present, the intramitochondrial concentration of α-oxoglutarate was strongly diminished, the extramitochondrial concentration was increased and the net result was a stimulation of the formation of α-oxoglutarate. The ratio of the intra- to the extramitochondrial concentration of α-oxoglutarate was only 1.6 in the presence of malonate. Table I shows that dicoumarol, too, stimulated the efflux of α-oxoglutarate from the mitochondria, although much less effectively than malonate. With dicoumarol, the ratio of the concentration of the oxo-acid inside the mitochondria to that outside was 6.7.

A stimulation of the efflux of α-oxoglutarate is also brought about by other uncouplers, and the degree of stimulation is a function of the concentration of uncoupler. This is shown in Fig. 1. Increasing concentrations of uncoupler (up to approx. 25 μM in the case of dicoumarol and 100 μM in the case of 2,4-dinitrophenol) brought about a progressive decrease of the intramitochondrial and a progressive increase of the extramitochondrial concentration of α-oxoglutarate, so that the ratio between the intra- and extramitochondrial concentration of the oxo-acid declined. The net result was a progressive stimulation of the total amount of α-oxoglutarate formed.

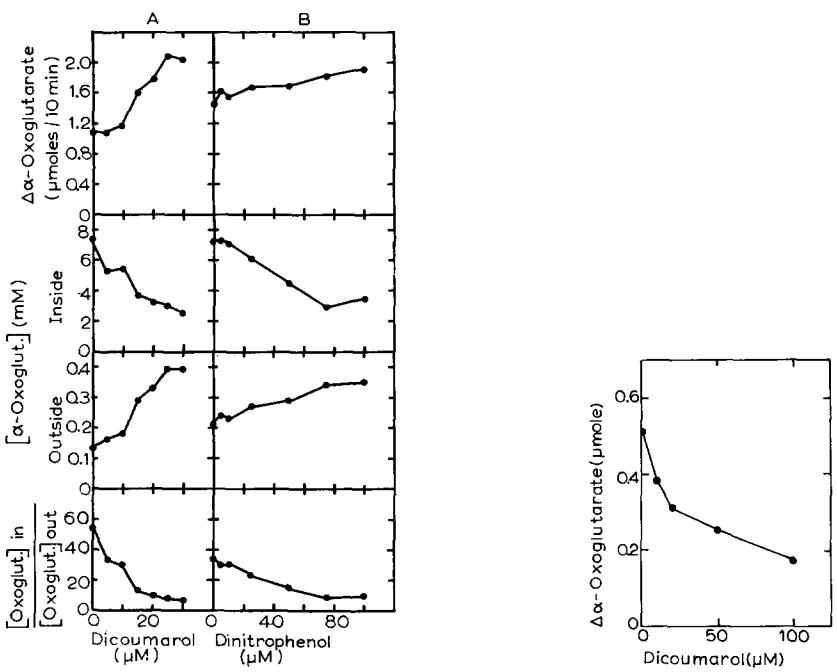


Fig. 1. Effect of different concentrations of dicoumarol and 2,4-dinitrophenol on the formation of α-oxoglutarate and its efflux from rat-liver mitochondria during the oxidation of glutamate (*plus* arsenite). Reaction mixture and experimental procedure as in Table I. Mitochondrial protein: 21 mg (A) or 22.5 mg (B). Δα-Oxoglutarate is the sum of the α-oxoglutarate found in the mitochondrial extract *plus* that found in the supernatant.

Fig. 2. Effect of dicoumarol on the formation of α-oxoglutarate during the oxidation of glutamate (*plus* arsenite) in rat-liver mitochondria in the presence of malonate. Reaction mixture (1 ml) contained 15 mM KCl, 5 mM $MgCl_2$, 50 mM Tris–HCl, 2 mM EDTA, 20 mM $P_i$, 0.5 mM ADP, hexokinase, 20 mM glucose, 25 mM sucrose, 10 mM glutamate and 2.0 mg mitochondrial protein. Final pH, 7.5. Reaction temperature, 25°. Reaction time, 20 min. Experimental procedure as in ref. 5.

Our results confirm the finding of DE HAAN AND TAGER[10] that the rate of oxidation of glutamate (*plus* arsenite) in rat-liver mitochondria is limited by the efflux of α-oxoglutarate from the mitochondria, and that malonate increases the permeability of the mitochondria for this oxo-acid. The mechanism by which malonate (or L-malate[10]) increases the permeability for α-oxoglutarate is unknown.

When malonate was present, so that α-oxoglutarate could move freely from the mitochondria (ref. 10 and Table I), high concentrations of uncouplers inhibited the net formation of α-oxoglutarate from glutamate (*plus* arsenite) as would be expected on the basis of the results of VAN DAM and coworkers[8,9]. This is shown for dicoumarol in Fig. 2. The degree of inhibition by uncoupler varied in different experiments and depended in part on the amount of mitochondrial protein present.

Thus on the one hand, uncouplers inhibit the uptake by mitochondria of substrate ions (including glutamate and α-oxoglutarate)[8,9]. On the other hand, they stimulate the exit of α-oxoglutarate from mitochondria; in this case, the uptake of the substrate is apparently not the rate-limiting factor. It is not known at present if these two effects are related to each other.

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