SHORT COMMUNICATIONS

BBA 43158

Stimulation of mitochondrial reactions by high concentrations of atractyloside

Early work on the mechanism of the inhibition of mitochondrial reactions by atractyloside suggested that the drug interferes with the energy-transfer reactions of oxidative phosphorylation, in the same way as does oligomycin[1,2]. Later work by the group of BRUNI[3,4] provided evidence that the two inhibitors act on different reactions of the energy-transfer system and that atractyloside inhibits the interaction of the phosphorylated intramitochondrial compounds with the external adenine nucleotides. BRUNI[5] conceived of an action of atractyloside in oxidative phosphorylation before the formation of ATP. More recently, however, it has been demonstrated[6–8] that it prevents the reaction between internal ATP and external ADP and thus acts after ATP is synthesized inside the mitochondria. Concentrations of atractyloside as low as 1 mμmole per mg mitochondrial protein, sufficient for the complete inhibition of the phosphorylation of added ADP, are without effect on the phosphorylation of endogenous ADP.

In this communication evidence is presented for a second effect of atractyloside that appears to be unrelated to the one described above. At concentrations 100–200

TABLE I

EFFECT OF ATRACTYLOSIDE ON CITRULLINE SYNTHESIS AND FATTY ACID OXIDATION BY RAT-LIVER MITOCHONDRIA

Both reactions were carried out for 30 min at 25° in 1 ml of a medium containing 15 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 50 mM Tris–HCl buffer (pH 7.5), 25 mM sucrose and 5.6 mg mitochondrial protein. For fatty acid oxidation, which was followed by measuring oxygen uptake in differential manometers, 3 mM octanoate, 0.1 mM dinitrophenol and 30 mM glucose were added. The values given are means of duplicate determinations. For citrulline synthesis the mitochondria were incubated in round-bottomed tubes in a Dubnoff metabolic shaker, and 10 mM L-ornithine, 10 mM NH_4Cl, 10 mM L-glutamate, 5 mM potassium phosphate buffer (pH 7.5) and 16.6 mM $KHCO_3$ were added to the medium; the gas phase was 95% O_2 and 5% CO_2. After 30 min the reaction was stopped by addition of $HClO_4$ and citrulline was determined as described by CHARLES, TAGER AND SLATER[9].

Atractyloside (μg)	*Citrulline synthesis* *Δ Citrulline* (μmoles)	*Fatty acid oxidation* $-\Delta O_2$ (μmoles)
0	2.6	3.0
200	2.7	3.6
400	3.0	4.1
600	3.3	4.3
800	3.5	4.5
1000	3.0	4.4
1200	3.8	4.4
1400	3.8	4.4

times higher than those required for the inhibition of phosphorylation of exogenous ADP, atractyloside has a stimulatory effect on two energy-requiring processes in rat-liver mitochondria: citrulline synthesis in the absence of added ATP, described by CHARLES, TAGER AND SLATER[9], and the dinitrophenol-insensitive fatty acid oxidation in the absence of added orthophosphate, described by VAN DEN BERGH[10,11]. The former process utilizes intramitochondrially generated ATP, and the latter probably GTP.

The effect of increasing concentrations of atractyloside on the two processes is shown in Table I. In both cases a maximal stimulation of about 50 % was obtained. Plots of percentage stimulation against concentration of atractyloside show a typical saturation curve, concentrations of atractyloside above 1 mg/ml having no further stimulatory effect.

Fig. 1 shows the effect of atractyloside on citrulline synthesis, in the absence of ATP, as a function of time. The lag-period in the production of citrulline is always observed[9]. In the absence of added ATP the rate of citrulline synthesis decreases with time. In the presence of 3 mM ATP (not shown in the figure) the amount of citrulline synthesized is much greater than in its absence, the rate of the reaction is constant after the initial lag phase and addition of atractyloside has no effect.

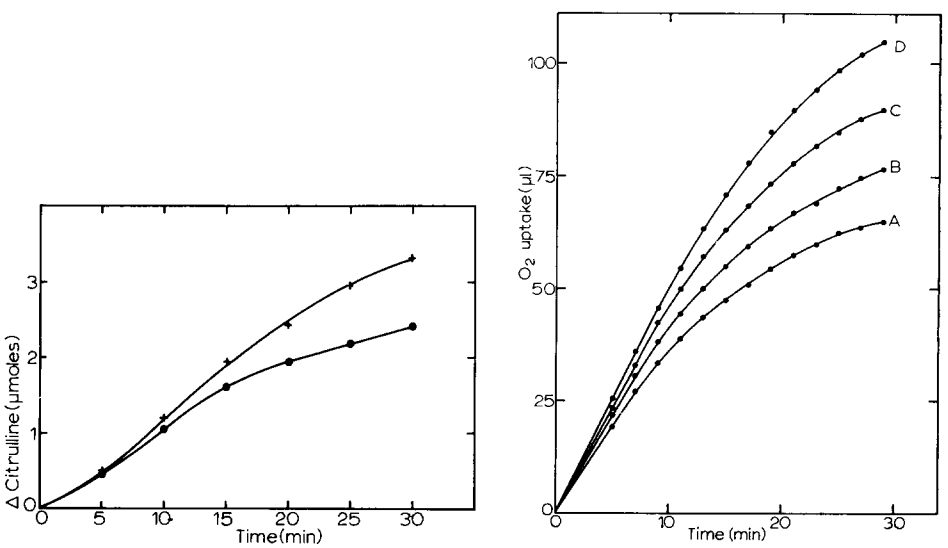

Fig. 1. Time course of citrulline synthesis by rat-liver mitochondria. Experimental conditions as in Table I. 4.7mg mitochondrial protein were present. ●—●, no atractyloside; +—+, with 1 mg atractyloside.

Fig. 2. Stimulation by increasing concentrations of atractyloside of octanoate oxidation in rat-liver mitochondria. Experimental conditions as in Table I. 5.6 mg mitochondrial protein were present. Curve A, no atractyloside; Curves B, C and D, with 200, 400 and 800 μg atractyloside, respectively.

In Fig. 2 the time course of fatty acid oxidation in the presence of different concentrations of atractyloside is given. A similar set of curves is found when the uncoupling of oxidative phosphorylation is brought about by high concentrations of fatty acid instead of by dinitrophenol. When fatty acid oxidation takes place in the presence of added malate, the oxygen uptake is more rapid, the rate of oxidation

does not decline and atractyloside is without effect. The stimulatory effect of atractyloside is not observed in the normal, dinitrophenol-sensitive fatty acid oxidation system.

From the experiment depicted in Fig. 3 it can be seen that the high concentrations of atractyloside affect the level of intramitochondrial ATP. In this experiment the amounts of intra- and extramitochondrial ATP were measured during citrulline synthesis. In the absence of atractyloside a rapid and extensive decrease of intramitochondrial ATP was observed, which was unaffected by low concentrations of atractyloside. In the presence of high concentrations of atractyloside both the rate and the extent of this decrease were smaller.

BRUNI AND AZZONE[3] have described a stimulatory effect of atractyloside on succinate oxidation by rat-liver mitochondria in the absence of phosphate and phosphate acceptor, and in the presence of 2.5 mM arsenate. Since this effect of atractyloside is abolished by EDTA[5], it must be of a different nature from the effects described in this paper.

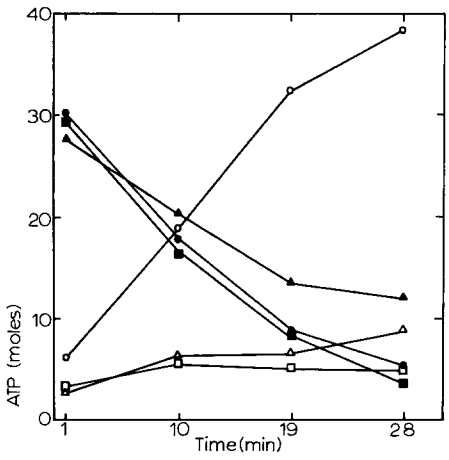

Fig. 3. Effect of atractyloside on the amounts of intra- and extramitochondrial ATP during citrulline synthesis by rat-liver mitochondria. Reactions were carried out at 25° in 6 ml of the reaction medium (see Table I) containing 16.5 mg of mitochondrial protein, with magnetic stirring in 15-ml beakers. At the times indicated a sample of 1 ml was taken from the incubation medium and the mitochondria were rapidly separated from the reaction medium with the help of a Millipore filter. The mitochondria on the filter were washed twice with 1 ml of ice-cold reaction medium from which ornithine, NH_4Cl and atractyloside were omitted, and extracted with 1.65 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.5) to which 3.2% $HClO_4$ had been added. ATP was determined in the filtrate and in the neutralized extract with hexokinase and glucose-6-phosphate dehydrogenase, using an Aminco dual-wavelength spectrophotometer. The open symbols refer to the extramitochondrial and the closed symbols to the intramitochondrial ATP. ○—○, without atractyloside; □—□, with 40 μg atractyloside; △—△, with 1 mg atractyloside.

Several possible explanations for this second effect of atractyloside may be considered. PFAFF[12] originally suggested that, at these high concentrations, atractyloside may occupy binding sites on the inside of the inner membrane and thereby set free bound nucleotides, thus increasing the effective internal nucleotide concentration. The experiment given in Fig. 3, however, shows that the high atractyloside concentrations affect the concentration of the total intramitochondrial ATP.

From Figs. 1 and 2 it can be seen that the high atractyloside concentrations seem to delay the decline in rate of the two processes rather than stimulate their initial rates. Therefore, the protective effect of atractyloside on the intramitochondrial ATP level may well explain its effect on citrulline synthesis and fatty acid oxidation.

From Fig. 3 it is clear that atractyloside strongly inhibits the appearance of ATP in the medium. (It had no effect on the extramitochondrial ADP or AMP.) Surprisingly, however, it does not stop the fall in intramitochondrial ATP. Since this discrepancy is also observed at low concentrations of atractyloside, it is irrelevant to the point under discussion.

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