# EFFECT OF ATRACTYLOSIDE ON MITOCHONDRIAL PROTEIN SYNTHESIS

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Bruni and Azzone (1964) concluded that atractyloside inhibits the mitochondrial formation of external ATP from phosphorylated compounds formed both at the level of the respiratory chain and at the substrate level. More recently Klingenberg and Pfaff (1966) and Vignais and Duee (1966) proposed that atractyloside acts on a catalytic carrier in the inner membrane which brings about the translocation of ADP and ATP in both directions. Winkler et al (1968) supported the hypothesis that the atractyloside sensitive binding site in rat liver mitochondria is an obligatory catalytic exchange diffusion carrier system specifically located in the inner membrane.

Stimulatory effects of high concentrations of atractyloside on two energy requiring processes in rat liver mitochondria: citrulline synthesis in the absence of added ADP and dinitrophenol insensitive fatty acid oxidation in the absence of added orthophosphate, have been reported by Charles and Van Den Bergh (1967). Allman et al (1966) presented evidence that atractyloside inhibits several of the component steps in fatty acid oxidation.

The present communication reports the effect of atractyloside on the incorporation of l-leucine $C^{14}$ into

protein by isolated muscle sarcosomes supported by either oxidative phosphorylation or external ATP in the presence of excess of phosphoenolpyruvate linked generating system.

## EXPERIMENTAL METHODS

Sarcosomes were prepared from pigeon breast muscle with 0.25 m sucrose, under aseptic conditions and washed 4 times with the isolation medium. The incubation medium was the same as described by Roodyn (1965) except for variations in the energy source. After one hour incubation at 30°C the reaction was stopped with 5 percent trichloroacetic acid (TCA). The TCA precipitates were washed, dissolved in NaOH and reprecipitated and rewashed as previously described (Hammel and Bessman, 1964). The washed precipitates were dissolved in formic acid and used for scintillation counting and protein determination (Lowry et. al., 1951).

The ATPase activity was determined in the presence of ATP (2 mM) with the generating system (Pullman et. al., 1960). The inorganic phosphate liberated after 15 min. incubation was determined by the method of Chen et. al., (1956).

## RESULTS

Table I shows the effects of atractyloside and oligomycin on leucine $C^{14}$ incorporation by mitochondria supported by α-ketoglutaric acid (αkg) + AMP, succinate + AMP, ATP + generating system and without any external energy source. Atractyloside causes a significant increase in the incorporation of leucine into mitochondrial protein. The stimulatory effect of atractyloside was diminished markedly by oligomycin in the system supported by oxidative phosphorylation and only slightly when ATP and generating system was added. The stimulatory effect of atractyloside was observed whether or

TABLE I

EFFECT OF ATRACTYLOSIDE ON LEUCINE $C^{14}$ INCORPORATION BY ISOLATED SKELETAL MUSCLE MITOCHONDRIA

| System | Addition | Per Cent Incorporation |
|---|---|---|
| 2 kg + AMP | | 100 |
| | Oligomycin 5 µgs | 93 |
| | Atractyloside 5 µM | 170 |
| | Atractyloside + Oligomycin | 129 |
| Succinate + AMP | | 100 |
| | Oligomycin 5 µgs | 26 |
| | Atractyloside 5 µM | 258 |
| | Atractyloside + Oligomycin | 43 |
| ATP + PEP + PK | | 100 |
| | Oligomycin 5 µgs | 50 |
| | Atractyloside 5 µM | 139 |
| | Atractyloside + Oligomycin | 129 |
| No Substrate or Nucleotide | | 100 |
| | Atractyloside 5 µM | 195 |

The incubation medium contained in 1 ml, 0.1 M sucrose, 0.04 M Kcl, 1.3 mM EDTA, 0.02 M nicotinamide, 0.016 M $KH_2PO_4$, 0.5 mM NAD, 5 mM $MgSO_4$, 50 µgs synthetic amino acid mix, minus leucine, 2-3 mgs mitochondrial protein, pH 7.4. The energy source was either α-ketoglutaric acid 10 mM or succinate 10 mM, and AMP 1 mM; or ATP 2 mM phosphoenolpyruvate (PEP) 5 mM, pyruvate kinase (PK) 100 µgs.

not the mitochondria were incubated with the energy source before the addition of atractyloside.

Table II shows the effect of different concentrations of atractyloside on leucine-$C^{14}$ incorporation supported either by αkg + AMP or ATP + generating system. It was noted that the stimulatory effect increased with increasing concentration of atractyloside up to 5 µm. Further increase did not seem to affect the incorporation significantly, in fact, decreased stimulation of incorporation was observed

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF ATRACTYLOSIDE ON LEUCINE $C^{14}$ INCORPORATION INTO PROTEINS BY SKELETAL MUSCLE MITOCHONDRIA

| Atractyloside Con. (μM) | System | |
|---|---|---|
| | α-kg + AMP (sp.act.) | ATP + PEP +PK ( sp. act.) |
| 0.0 | 704 | 618 |
| 1 | 732 | 661 |
| 2.5 | 1164 | 675 |
| 5 | 1816 | 1057 |
| 25 | 1855 | 704 |

The incubations were performed in the medium described under Table I.

TABLE III

EFFECTS OF VARIOUS CONCENTRATIONS OF ATRACTYLOSIDE ON LEUCINE $C^{14}$ INCORPORATION INTO PROTEIN AND ATPase ACTIVITY OF MITOCHONDRIA

| Atractyloside Concentration (μM) | (ATP + PEP + PK) Sp. Act. | ATPase Activity Pi μgs Liberated/15 min. |
|---|---|---|
| 0 | 887 | 71.3 |
| 1 | 883 | 67.5 |
| 2.5 | 1100 | 43.8 |
| 5 | 1387 | 40.1 |
| 25 | 914 | 34.5 |

Complete system as described in text.
ATPase activities and atractyloside effects were studied on the same batch of mitochondria.

at higher concentration of atractyloside (25 μM) when external ATP was used as the energy source but not when αkg + AMP was the energy source.

Table III shows the effect of various concentrations

of atractyloside on leucine $C^{14}$ incorporation and ATPase activity. No correlation was observed between the inhibition of ATPase activity and stimulation of leucine $C^{14}$ incorporation. At higher concentrations (25 μM) where maximum inhibition of ATPase occurred there was either no significant increase in stimulation or a decreased stimulation was observed.

## DISCUSSION

In intact mitochondria atractyloside inhibits the phosphate acceptor-stimulated respiration, the Pi-ATP, and ATP-ADP exchange reactions (Bruni and Azzone, 1964). This effect of atractyloside has been postulated to be due to interference with the step of the energy transfer sequence involving the reaction between the hypothetical phosphorylated high energy intermediate and ADP. Although the energy source for amino acid incorporation in mitochondrial protein has not been established either the internally generated energy of oxidative phosphorylation (Kroon, 1963) or external ATP (Wheeldon and Lehninger, 1966) has been shown to satisfy the energy requirement for incorporation. With the known action of atractyloside in preventing the respiration stimulated by phosphate acceptor, it is difficult to account for the substantial stimulation by atractyloside of amino acid incorporation by mitochondria, supported by energy from oxidative phosphorylation (αkg + AMP or succinate + AMP).

The reports by Winkler et al (1968) suggest that the uptake of the nucleotide by mitochondria occurs by an obligatory diffusion process resulting in a constant internal

pool size irrespective of its external concentration. This uptake system is specific for adenine nucleotides and the affinity being ADP>ATP>AMP. The action of atractyloside on the inner membrane of mitochondria, is at a site different from that used by adenine nucleotides on the "carrier" molecule and atractyloside by its binding prevents the binding or the release of the nucleotide from the membrane (Winkler and Lehninger, 1968).

The presence of 1 or 2 mM nucleotide in the incubation medium may partially reverse the inhibition by atractyloside depending upon its concentration and a part of the nucleotide may enter through the membrane. The stimulation thus observed may be adduced to the effect of atractyloside in preventing the loss of ATP in the outer medium thereby increasing its level inside the mitochondria. However Charles and Van Den Bergh (1967) have found that the intramitochondrial ATP level is not maintained in the presence of atractyloside (40$\mu$g); it continually falls although atractyloside strongly inhibits the appearance of ATP in the medium. Atractyloside has also been found to inhibit partially the phosphorylation of endogenous ADP at 30°C (Bruni, 1967, Heldt, 1967).

The stimulation of amino acid incorporation into mitochondrial protein in the presence of atractyloside may however suggest the possibility that protein synthesis in mitochondria can go on in the absence of ATP *per se* using intramitochondrially generated high energy phosphate intermediates which would accumulate in the presence of atractyloside. Alternatively atractyloside might block one or more of the ATP requiring processes in the mito-

chondria such as fatty acid oxidation which may otherwise compete for energy with the incorporating system. This, however, would postulate an increase in intramitochondrial ATP which has not been found (Charles and Van Den Bergh, 1967).

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