THE EFFECT OF CYPROHEPTADINE ON OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

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The ability of cyproheptadine to modify appetite (Oomura etal 1973) would appear to be secondary to its known action as a potent 5-hydroxytryptamine antagonist or anti-histamine agent (Pawlowski, 1975) and therefore the mechanism by which this compound stimulates food intake during the treatment of anorexia remains unknown. Recent invivo experiments have shown appetite modifying agents to be capable of changing intracellular energy production (McNamee etal 1985). The present study investigates the invitro effects of appetite-modifying agents on mitochondrial adenosine triphosphate formation.

Tightly coupled rat hepatic mitochondria were prepared from male Wistar rats by the method of Chappell and Hansford (1969). Oxygen consumption was measured polargraphically using a Clark-type oxygen electrode (Rank Bros. Bottisham U.K.) according to the method of Sweetman and Weetman (1972). ATP hydrolysis was measured by the method of Beechey (1966), with phosphate release being determined by the method of Fiske and Subbarow (1925). Protein was determined by the method of Gornall etal (1949).

Cyproheptadine 3.33-100μM was found to produce a concentration-dependent stimulation of State 4 respiration (substrate and oxygen in excess, ADP absent) during the NAD^+-linked oxidation of 5mM glutamate plus 5mM malate by tightly coupled rat hepatic mitochondria, with the rate of respiration increasing from 22.6 ± 1.0 to 47.4 ± 4.0 ng atoms O_2 consumed min^{-1}mg of $protein^{-1}$ (n=4), resulting in an EC_{50} value of 28.2μM. State 3 respiration (ADP present with substrate and oxygen in excess) was inhibited between 30 and 70 per cent by the presence of cyproheptadine (150-333μM), concentrations below 100μM had no significant effect ($p>0.05$). When 5mM succinate replaced glutamate plus malate, low concentrations of cyproheptadine (3.33-100μM) had no significant effect ($p>0.05$) on either State 3 or State 4 respiration. The addition of cyproheptadine (18.3μM) in place of ADP (166.7μM) produced a stimulation of State 4 respiration similar to that obtained with the uncoupling agent 2,4-dinitrophenol (DNP, 10μM). Cyproheptadine (3.33 - 100μM), like DNP was found to release oligomycin (2μg) inhibited State 3 respiration. These results indicate that the antagonist is acting as an uncoupling agent.

Cyproheptadine was shown to cause a concentration-dependent stimulation of mitochondrial ATPase (E.C.3.6.1.4) activity over the same concentration range that produced a stimulation of State 4 respiration; with the rate of activity increasing from 4.87 ± 0.39 to 10.84 ± 1.34 nmoles inorganic PO_4 released min^{-1}mg of $protein^{-1}$ (n=4). A similar but significantly ($p<0.05$) more potent effect was observed with DNP (1-100μM) with the rate increasing from 4.90 ± 0.53 to 16.05 ± 0.87 nmoles inorganic PO_4 released min^{-1}mg of $protein^{-1}$ (n=4).

Data presented indicate that under invitro conditions cyproheptadine can modify mitochondrial ATP synthesis by a specific action on NAD^+-linked oxidations via the NADH dehydrogenase enzyme complex, or by its ability to act as weak uncoupling agent.

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