ANTIPYRESIS AND THE INHIBITION OF MITOCHONDRIAL FUNCTION

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Introduction

The antipyretic actions of paracetamol and the NSAIDs has traditionally been attributed to their ability to inhibit the cyclooxygenase (COX) enzymes particularly COX-2[1]. However it has widely been accepted that paracetamol in particular is a poor inhibitor of COX-2 activity, suggesting other mechanisms may be involved. Pyresis is thought to result from an increase in metabolic activity, including and ultimately increased mitochondrial activity. There are numerous targets for compounds which affect the mitochondria such as disruption of the supply of substrates to complex I and II, the integrity of the mitochondrial membranes and direct inhibition of the electron transport chain (Figure 1). In this study, the ability of paracetamol and other compounds, known to possess antipyretic properties were assessed for their ability to inhibit complexes of the mitochondrial electron transport .

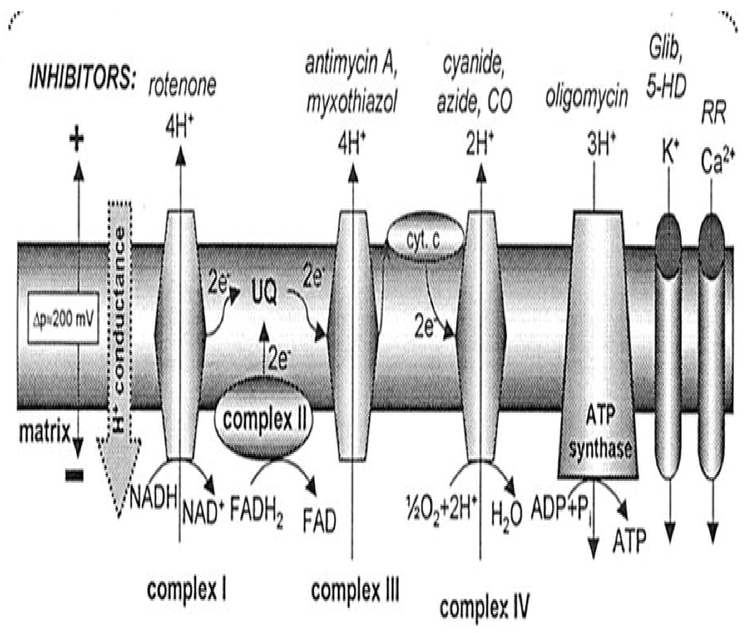

Figure 1 Schematic representation of an electron transport chain showing known inhibitors. Pictured adopted from Szewczyk and Wojtczak, 2002)

Objective

- To investigate the extent to which a range of antipyretic agents could inhibit complexes (I and II) of the mitochondrial transport chain.

Methods

ISOLATION OF RAT LIVER MITOCHONDRIA

Rat liver mitochondria were isolated by differential centrifugation in a sucrose buffer (tris HCL 25mM, pH 8.2, 0.25M sucrose and 2mM EDTA) then either used fresh or stored at -20ºC until when required[3]. The protein concentration was determined using the Bradford protein assay.

DETERMINATION OF NADH-UBIQUINONE OXIDOREDUCTASE ACTIVITY

The activity of NADH-ubiquinone oxidoreductase (complex 1) was determined by measuring the reduction of 0.05mM of the electron acceptor 2,6-dinitrophenolindophenol (DCPIP) and NADH (0.05mM) and either paracetamol, or a range of antipyretic agents at 600nm. Complex 1 activity was confirmed using rotenone (0.1mM). The reaction mixture consisted of potassium phosphate buffer (35mM, pH 7.4) in the presence and absence of KCN and antimycin A. The reaction was started by adding the mitochondria homogenate (6.22mg/ml) and incubated for 10 minutes at room temperature (37ºC).

Methods

DETERMINATION OF SUCCINATE –UBIQUINONE OXIDOREDUCTASE ACTIVITY

The activity of Succinate–Ubiquinone Oxidoreductase activity (Complex II) activity was determined by measuring the reduction of DCPIP (0.05mM) and succinate (5-100mM) and either paracetamol, or a range of antipyretic agents at 600nm. The reaction mixture consisted of potassium phosphate buffer (35mM, pH 7.4) in the presence and absence of KCN (0.5mM) and antimycin A (0.5mM) over 5-100mM of succinate. Complex II activity was confirmed using malonate (0.1mM).The reaction was started by adding the mitochondria homogenate (6.22mg/ml) and incubated for 10 minutes at room temperature (37ºC).

Results

Paracetamol and a range of antipyretic agents, at concentrations up to 10mM were found to inhibit in a concentration dependent manner complex I activity by decreasing the rate of rotenone sensitive NADH oxidation (Figure 2 and 3). Similarly complex II activity was inhibited by the malonate sensitive of DCPIP reduction (Figure 4 and 5). The inhibition appears to be more pronounced at higher succinate concentrations.

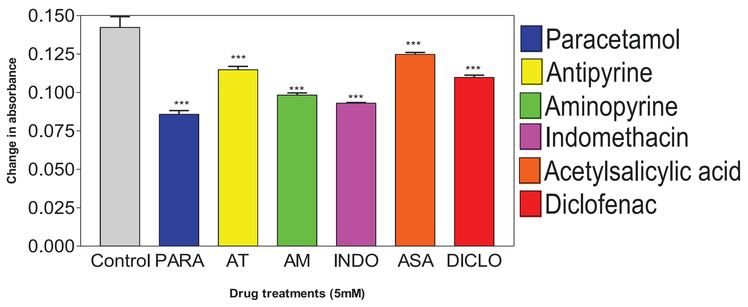

Figure 2 Effects of Antipyretic agents (5mM) on isolated rat liver mitochondria complex I activity. Values are expressed as mean ± SEM (N= 7). ***p< 0.001 was statistically significant compared to the control (NADH with no treatment).

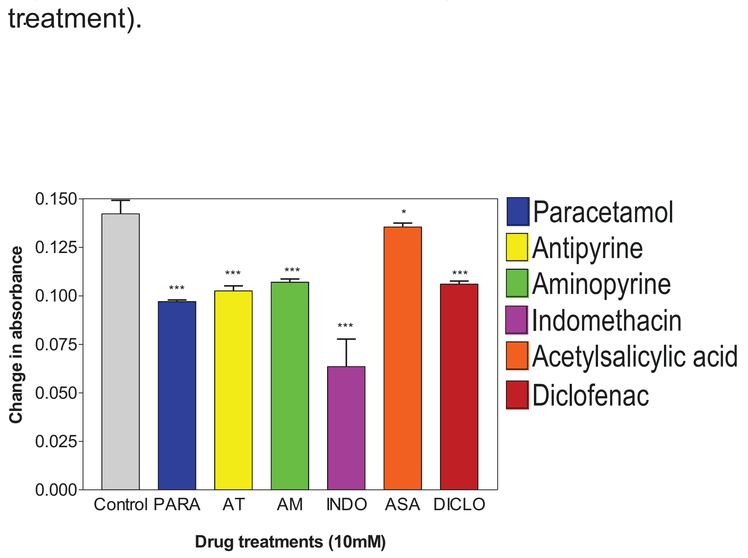

Figure 3 Effects of antipyretic agents (10mM) on isolated rat liver mitochondria complex I activity. Values are expressed as mean ± SEM (N=7). *p<0.05 and ***p<0.001 statistically significant to compared to control (no treatment/inhibitor).

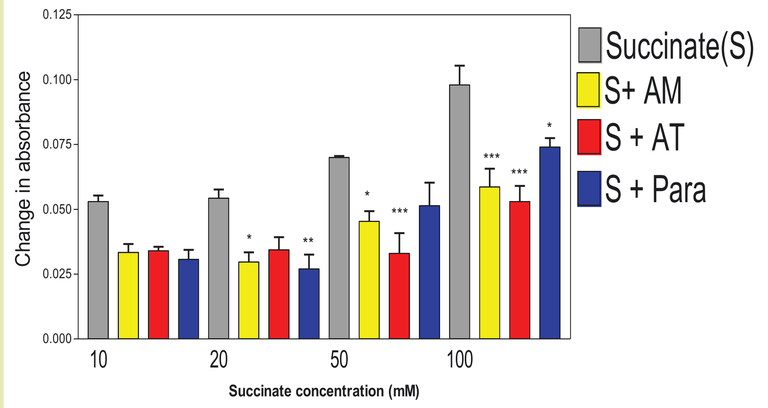

Figure 4 Effects of Paracetamol, aminopyrine and antipyrine (5mM) on isolated rat liver mitochondria complex II activity. Values expressed as mean ± SEM (N=20). *p<0.05 and ***p<0.001

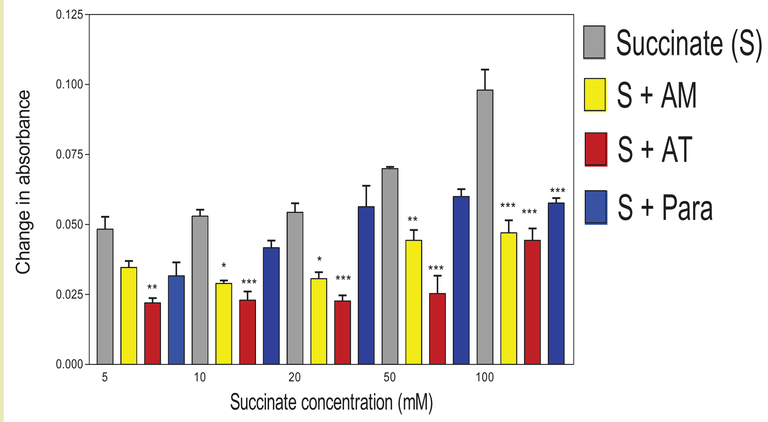

Figure 5 Effect of varying concentration of paracetamol and related compounds (10mM) on succinate. Values are expressed as the mean ± SEM (N=20). **p< 0.01 ***p< 0.001 statistically significant relative to succinate alone.

Conclusions

For some time it has been accepted that different classes of antipyretic agents inhibit COX-2 to different extents, particularly in the CNS. However, following oral administration paracetamol and other antipyretic agents accumulate in the liver at concentrations that were found to inhibit mitochondrial function in this study[2]. These results suggest that the inhibition of mitochondrial electron transport chain by paracetamol and the NSAIDs may disrupt energy metabolism enough to impact on pyresis and even cause hypothermia in certain animals. The limited inhibition observed at these concentrations also suggest these effects may be reversible and could explain the lack of toxicity of these compounds . If replicated *in vivo,* these observations may in part be responsible for the antipyretic actions of these compounds regardless of their impact on the COX enzymes. Further, these observation could also provide a new direction for the design of novel antipyretic agents which have a limited impact on the immune system.

References

1. Szewczyk, A. and Wojtczak, L. (2002) Mitochondria as a Pharmacological Target', Pharmacological Reviews vol. 54 (1) pp.101-127
2. Simmons, D. L., et al (2000), *Clinical Infectious Disease*, 31(5), s211-8.
3. C. Sandoval-Acuña,et al ,(2012), *Chemico-biological Interactions*, 199(1), 18-28.