# "EVALUATION OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION DURING Trypanosoma congolense INFECTION IN RABBITS: THE ROLE OF THYROXINE"



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# SUMMARY


## EVALUATION OF MITOCHONDRIAL OXIDATIVE PHOSPHORYLATION DURING Trypanosoma congolense INFECTION IN RABBITS: THE ROLE OF THYROXINE

Trypanosomiases is a protozoan disease commonly referred to as sleeping sickness in man and nagana in domestic animals. The disease is caused by various species of vector- transmitted trypanosomes.

The appetite of the trypanosome infected animals are not significantly affected yet chronic infection mimics conditions such as disease or starvation that lead to altered metabolic states. It was from this observation that a study was undertaken to evaluate possible changes in the mitochondria (the powerhouse) as well as assessing the pattern of changes in the concentrations of 3,5,3' -triiodothyronine ($T_3$) and thyroxine ($T_4$) during trypanosome infection. $T_3$ and $T_4$ are known to be involved in the regulation of basal metabolic rate and biogenesis of the mitochondria. The changes in packed cell volume was also studied as an index of trypanosome - induced anaemia.

In the first phase of experimentation sixteen sexually mature white New Zealand rabbits weighing between 2.8 and 3.0kg were used. The animals were divided into experimental (10 rabbits) and control (10 rabbits) groups. They were housed individually in cages (45cm x 45cm x 30cm) in a well lit room. The control were not infected whereas the experimental group were infected through the lateral

ear vein with about 2 x $10^4$ T. congolense (Strain IL - 3000: original stock from Transmara). Both the control and experimental animals were bled twice each week for the routine determination of packed cell volume and parasitaemia. The parasite titre was determined by hemocytometry and darkground/phase contrast buffy coat method (DG) where appropriate. The micro-hematocrit capillary tubes were used in the determination of packed cell volume.

The first wave of parasitaemia in the blood of the infected rabbits occurred within 3 to 5 days. The first peak parasitaemia (5 x $10^6$ to 5 x $10^7$ trypanosomes per ml of blood) occurred by day 8 of innoculation. This was followed by low transient parasitaemia levels (5 x $3^3$ to 5 x $10^4$ trypanosomes per ml) by the subsequent third day. The alternating low and peak parasitaemia occurred for eight weeks before the animals started to die.

A progressive decrease in PCV occurred over a period of eight weeks after infection by which time values of approximately 20% were reached. The fall in PCV correlated closely ($y = -0.376x + 15.189$, $r = 0.8185$, $P = 0.0011$) with the intensity and duration of parasitaemia. It was noted that the animals died at peak parasitaemia with a critical PCV percent of 15. Two animals had made self-recovery by week 10 post - infection with no detectable parasites in their blood. However, their PCV remained below normal values thus anaemia persisted in the animals despite the absence of living trypanosomes in their bloodstream.

In the second set of experiments, ten rabbits were infected with trypanosomes while another ten served as controls. The twenty animals were bled twice each week

for trypanosome count and plasma was kept at -20°C for subsequent thyroxine and triiodothyronine assay. The infected animals were sacrificed at peak parasitaemia with a PCV value of 16 to 15% and their liver mitochondria prepared for respiration studies. The mitochondria was prepared in a suitable medium by differential centrifugation technique and the protein content determined by the Lowry's method.

The respiratory activity of the mitochondrial suspension was measured as the oxygen consumption rate recorded polarographically by an oxygen electrode operated at room temp. (25°C) in a closed and magnetically stirred glass chamber. State 4 respiration was induced by addition of either $NAD^+$ - or FAD - linked substrate. Addition of ADP initiated state 3 respiration and after depletion of ADP, CCCP was added to initiate uncoupler stimulated respiration (state 3U). ADP/0 ratio was calculated on the basis of total oxygen consumption during state 3 respiration. Respiratory control ratio (RCR) was calculated as the ratio of oxygen consumption in state 3 and state 4. A marked decrease in the state 3 rate of oxygen consumption ($71.3 \pm 2.67$ vs $57.0 \pm 6.77$, $P < 0.02$) was observed in trypanosome infected rabbits whereas there was no significant difference in state 4 respiration (without ADP) relative to control. A lowered RCR ($5.03 \pm 0.21$ vs $3.10 \pm 0.20$; $P < 0.05$) and ADP/0 ratio ($2.85 \pm 0.05$ Vs $2.55 \pm 0.06$: $P < 0.05$) was observed in the infected animals suggesting an impairment of mitochondrial integrity.

The mitochondrial ATPase activity was measured in the direction of ATP hydrolysis by coupling to PK and LDH. A unit of ATPase activity was taken as the number of µmoles of ATP hydrolysed per min per mg protein. The latent ATPase

activity was 0.27 ± 0.01 and 0.47 ± 0.02 μmol/min/mg protein for infected and control animals respectively. The mitochondrial ATPase from experimental animals was found to be less sensitive to oligomycin inhibition (86.0 ± 1.7% vs 74.4 ± 2.2%; $P < 0.05$) as compared to the control. Incubation of the assay mixture with CCCP did not relieve the oligomycin inhibition in either control or experimental animals. No significant difference in sensitivity to CCCP stimulation was observed.

The effect of trypanosome infection on the plasma levels of $T_3$ and $T_4$ as well as the ratio between the two thyroid hormones were investigated. The concentration of $T_3$ and $T_4$ were measured in plasma by radioimmunoassay. The quality control parameters included intra-assay coefficient of variation which was 4.6% for $T_3$ at 2.16 nmol/l and 6.2% at 41.5 nmol/l for $T_4$ ($n = 20$). The inter assay CV for $T_3$ was 11.4% at 2.58 nmol/l and 7.5% at 75.1 nmol/l for $T_4$ ($n = 15$). The assay sensitivity taken as the value on the standard curve at two standard deviations below the maximum for specific binding was 0.11 nmol and 3.79 nmol for $T_3$ and $T_4$ respectively.

Although variations in the thyroid hormone levels within and between individual rabbits were noted they were not significantly different throughout the experimental period. In control animals, the mean ± SEM concentration of $T_3$ and $T_4$ was 2.6 ± 0.03 nmol/l and 78.3 ± 1.8 nmol/l respectively. However, in the experimental animals there was a rapid decline in the levels of both the thyroid hormones to minimum values of 1.38 ± 0.02 nmol/l for $T_3$ and 15.0 ± 1.35 nmol/l for $T_4$ by day 48 post - infection. It was thus proposed that trypanosome infection

probably causes the lowered RCR and ADP/0 ratio observed in the infected animals through depression of blood thyroid hormone levels since the mitochondria is known to be a target of thyroid hormone action.

The role of thyroid hormone in the impairment of mitochondrial integrity was investigated. Twenty male rabbits were infected with $2 \times 10^4$ T. congolense and then separated into two groups: ten experimental and ten controls. The pre-infection $T_3$ and $T_4$ levels had previously been determined to be $2.80 \pm 0.19$ n mol/l and $66.1 \pm 5.8$ nmol/l respectively. The experimental animals in this case were set to be given replacement doses of thyroxine each time the blood $T_4$ level went below the baseline. The intramuscular injection of sodium salt solution of thyroxine was not only able to bring the $T_4$ to normal levels but also did sustain physiological levels of the bioactive $T_3$ through ß - monodeiodinase activity. The 3, 3' - diiodothyronine ($T_2$) which is thought to have a direct effect on the mitochondria could be produced by further deiodination as shown below.

$$T_4 \overset{E_1}{\rightleftharpoons} I + T_3 \overset{E_1}{\rightleftharpoons} T_2 + I$$

$E_1$ is the type I iodothyronine monodeiodinase.

Besides the thyroid hormone levels, the PCV and parasitaemia levels were determined in both the infected - treated and infected (control) animals. The animals were sacrificed at peak parasitaemia with a critical level of anaemia. The state 3 rate of oxygen consumption was higher in the thyroxine treated infected

rabbits (57.0 ± 6.77 vs 96.3 ± 5.27 nmol 0 - atom/min/mg $P < 0.05$) as compared to the infected - untreated animals when malate and glutamate were used as substrates. State 3 respiration was also significantly higher in the infected - treated animals (60.20 ± 4.95 vs 108.7 ± 5.71 nmol 0 - atom/min/mg: $P < 0.05$) with succinate as a substrate. The infected - treated animals had an RCR and ADP/0 ratio of 4.89 ± 0.27 and 2.85 ± 0.05 respectively using malate plus glutamate as substrate whereas the infected animals had 3.10 ± 0.20 and 2.55 ± 0.06 (mean ± SEM, n = 5). A similar higher respective RCR and ADP/O ratio of 3.13 ± 0.21 and 1.88 ± 0.06 was observed in infected - treated animals with succinate as substrate when compared to the infected - untreated controls (2.23 ± 0.19 and 1.59 ± 0.03) . An inference drawn from the above observation is that trypanosomes or its toxins impairs mitochondrial integrity thus resulting to the lowered RCR and ADP/0 ratio observed. This condition can however be reversed to normal by thyroxine treatment.

The ATPase activity was 0.27 ± 0.01 and 0.61 ± 0.02 (mean ± SEM; n = 5) µmol/min/mg protein in infected and infected - treated animals respectively. The mitochondrial ATPase from infected animals was found to be less sensitive to oligomycin inhibition (74.4 ± 2.2%) as compared to 90.0 ± 0.9% (mean ± SEM; n = 5) in the infected - treated. A significantly lower CCCP stimulation was observed in the infected and treated (9.5 ± 0.8%) compared to their untreated counterparts (25.9 ± 5.5%; mean + SEM n = 5).

Two possible hypotheses may be put forward in an attempt to explain the mechanism by which the trypanosomes and, or its biologically active substances

lower the ATPase activity: (i) either the living parasites and, or its blood borne toxins promote the interaction of the $F_1$ ATPase with its naturally occurring inhibitor - protein or, (ii) they may interfere with ADP/ATP carrier or, alter the inner mitochondrial membrane properties especially in proton translocation thus dissipating the electrochemical potential required for the ATPase function. The latter possibility is supported by the previous observation of loose coupling of ATP synthesis to oxygen consumption or electron transport.

Maintaining the physiological levels of thyroid hormones by replacement therapy may increase the ATPase activity by:

(i) 'prevention' or slowing down combination of the ATPase and its inhibitor,

(ii) and, or favour the $H^+$ - translocation through $F_0$ coupled to the synthesis and hydrolysis of ATP.

The thyroid hormones apparently has no direct effect on the living trypanosomes but enhances the immunological competence of the host. Thyroxine when used as a complement in therapy would make the animals survive for a longer period while the trypanocidal drugs are being used.