

**RNA SYNTHESIS AND ANOXIC SURVIVAL
IN CRUCIAN CARP (*CARASSIUS CARASSIUS*)**

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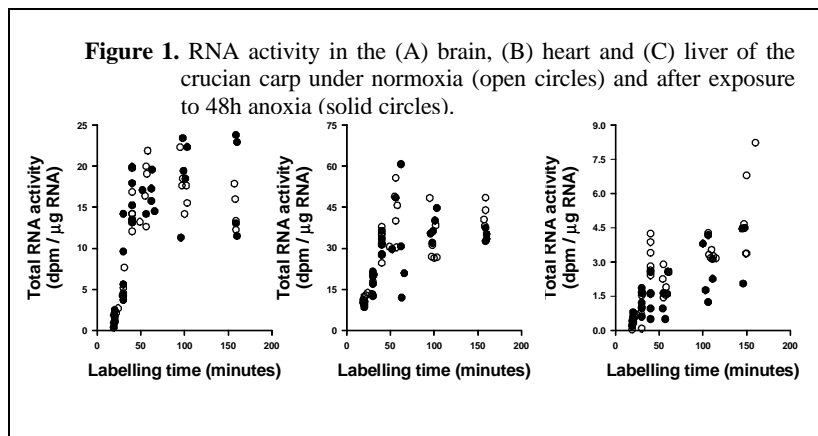
EXTENDED ABSTRACT ONLY - DO NOT CITE

During anoxia the crucian carp downregulates protein synthesis in the liver, heart and muscle, yet maintains synthesis in the brain. This results in a 40% reduction overall and ensures neuronal survival (Smith *et al*, 1996). Tissue specific downregulation is associated with reductions in RNA to protein ratio (heart) or RNA translational efficiency, *i.e.* mg protein synthesised μg^{-1} RNA day⁻¹ (liver, and muscle), whereas an increase in brain RNA translational efficiency counters the reduction in RNA to protein ratio (Smith *et al*, 1996). Since we know little about how events upstream of RNA translation affect anoxic protein synthesis, here we describe how changes in RNA synthesis contribute to anoxic survival.

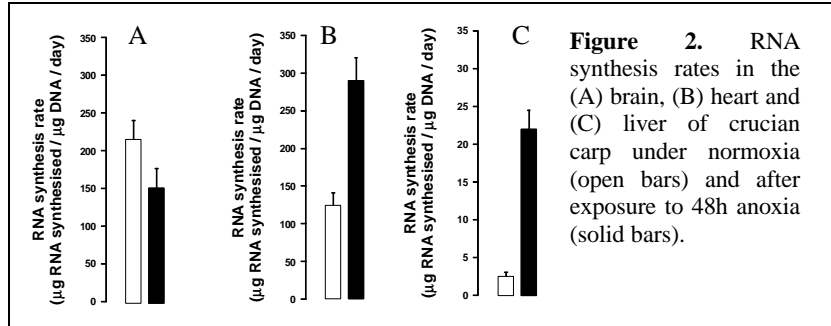
After normoxia or 48 h anoxia (described by Smith *et al*, 1996), each fish was injected with 10 μl g⁻¹ body weight of 100 mM uridine @ 3.7 MBq (100 μCi ml⁻¹) ³H-uridine and returned to the appropriate conditions. Individuals were then killed and the brain, heart ventricle and liver removed and immediately frozen. Free intracellular nucleotides were liberated by homogenisation in 0.5M perchloric acid (PCA) and were analysed by HPLC, with the eluted UMP, UDP and UTP fractions being collected (Smith *et al*, 1999). PCA insoluble material was dissolved in NaOH and RNA extracted by precipitation of DNA and protein with 20% PCA. RNA (and also DNA, following further PCA extraction) was

determined by dual absorbance spectrophotometry. Radioactivity levels were determined by scintillation counting.

UDP was not detected in any tissue and UTP was not detected in the heart. Stabilised normoxic UMP specific activities (dpm nmol^{-1}) were ranked: liver (490.2 ± 63.2) > heart (99.6 ± 12.5) > brain (43.1 ± 6.3). Under anoxia liver UMP specific activity declined whereas the brain and heart were unaffected (201.3 ± 25.6 , 36.7 ± 3.2 and 81.7 ± 8.6 dpm nmol^{-1} , respectively). Stabilised normoxic brain and liver UTP specific activities were similar (5.2 ± 1.8 and 7.5 ± 1.6 dpm nmol^{-1} , respectively). Both were increased by anoxia (11.9 ± 3.1 and 13.3 ± 2.8 dpm nmol^{-1} , respectively). RNA radioactivities ($\text{dpm } \mu\text{g}^{-1}$) increased linearly, with labelling time, for 40 mins. Afterwards there were no further changes (Fig. 1).



From the linear RNA labelling phase and total nucleotide specific activities we have developed a novel RNA synthesis rate calculation (Smith *et al*, 1999). This allows for the differential uridine salvage, with synthesis rates expressed relative to DNA (Fig. 2). This defines tissue specific RNA synthesis rates, brain > heart > liver and show that, after 48 h anoxia, RNA synthesis is reduced in the brain yet is increased in the heart and the liver (Fig. 2).



RNA synthesis is not downregulated during anoxia. However these changes in synthesis rate do correspond to known anoxic survival mechanisms. In the brain these data confirm an earlier investigation where neuron survival is increased following RNA synthesis inhibition; RNA synthesis being required for neuron apoptosis (Rosenbaum *et al*, 1994). In the liver, a reduction in newly synthesised proteins reduces rRNA survival yet enhanced recovery depends on the replacement of pre-existing ribosomes plus the synthesis of specific mRNA's, as shown in anoxia tolerant turtles (Douglas *et al*, 1994). Thus, the large reduction in liver protein synthesis rate (>95%; Smith *et al*, 1996) corresponded to the greatest increase in RNA synthesis rate (>800%; Smith *et al*, 1999).

These data provide evidence that these survival strategies are possible because they conform to the combined energetics of RNA and protein synthesis; *i.e.* contains both a variable and fixed component, the latter being RNA synthesis (refer to Smith and Houlihan, 1995). Nucleotide supply constitutes the greatest energy demand of RNA synthesis, but exogenous salvage avoids the higher costs of intracellular synthesis (Rudolph, 1994). Although the extent of nucleotide salvage (Smith *et al*, 1999) corresponded to normoxic protein synthesis rates (Smith *et al*, 1996) there was no increase of exogenous salvage (as could be expected) under anoxia. However in the liver an increase in RNA species, with an increased turnover, could, via nucleotide recycling, reduce even the necessity for exogenous salvage. It would therefore be of interest to know exactly which nucleotide sequences are being transcribed. In the brain, a reduction in RNA synthesis would incur an energetic commitment to intracellular synthesis in order to maintain a fixed RNA synthesis cost.

RNA synthesis may therefore represent an area where energy demand is not so much downregulated as restructured. Therefore maintaining the fixed cost of

RNA synthesis may be as vital to anoxic survival as exploiting the variable cost of protein synthesis (Smith *et al*, 1999).

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