CHANGES IN RED CELL pH AND ION CONTENT DURING EXPOSURE OF SPINY DOGFISH (SQUALUS ACANTHIAS) TO ACUTE HYPOXIA

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Rainbow trout (Salmo gairdneri) exposed to acute hypoxia undergo a rapid acidification of the blood and a simultaneous increase in blood oxygen affinity. These two processes appear to be linked to the stimulation by catecholamines (released in response to respiratory stress) of the Na/H exchanger located in the red cell membrane. Activation of this exchanger results in the extrusion of protons from the red cell thereby contributing to acidification of the blood and alkalinization of the cell. The increase in blood oxygen affinity appears to be due at least in part to the Bohr effect following cell alkalinization (Nikinmaa, Mol. Physiol. 2: 287-297, 1982), and this is beneficial in aiding oxygen extraction from the water during respiratory stress. In a previous report, we demonstrated the presence of a Na/H exchanger in red cells of the dogfish shark, Squalus acanthias that was activated by phorbol esters (Payne and McManus, MDIBL Bulletin 28: 57-59, 1989); however, with in vitro proton efflux studies we were unable to show any sensitivity of this red cell Na/H exchanger to catecholamines. Therefore, in the present study, we examined the response of dogfish exposed to acute hypoxia to determine if the red cell Na/H exchanger is stimulated in vivo by catecholamines during a respiratory stress in a manner similar to the trout.

Male dogfish (2.09 ± 0.18 kg, mean ± S.D., n=6) were anesthetized (MS-222) and catheterized via the caudal artery. After a 24 h recovery period in a circulating sea water tank, dogfish were placed in a plexi-glass experimental chamber connected to a closed, seawater recirculation system (Claiborne and Evans, MDIBL Bulletin 25: 28-30, 1985). Water within the system was pumped at 7 l/min through a gas exchange column and recycled back to the fish chamber, and the system was flushed with fresh sea water every 4-6 h in order to prevent the build-up of metabolic wastes. Both the aeration column and fish chamber were partially immersed in circulating sea water to maintain experimental water temperature (15°-17°C). Air and air/N_2 mixtures were bubbled through the column while the PO_2 of the fish chamber was continuously monitored with a YSI Oxygen Monitor (Model 53) and electrode. Dogfish were allowed at least 12 h to adjust to the new environment and then a normoxic control blood sample (2.5 ml) was drawn from the caudal catheter. An air/N_2 mixture within the column was then adjusted such that the PO_2 of the chamber was maintained at ~20 torr (range 17-23 torr). Blood samples were obtained at 15, 30, 45, 60, and 90 min of hypoxia, after which the chamber was returned to normoxia and blood samples were taken at 1, 2, and 3 h during recovery. Blood pH (pH_o) and total CO_2 (TCO_2) were measured using a thermostated (16°C) pH microelectrode (Radiometer) and a TCO_2 detection system (Capni-Con II; Cameron Instruments Inc.). Plasma PCO_2 and [HCO_3] were calculated from pH_o and TCO_2 using values for alpha-CO_2 and pK at 16°C from Boutilier et al. (in Fish Physiology, eds. W.S. Hoar and D.J. Randall, Vol. XA, pp. 403-430, 1984). Red cell pH (pH_i) was measured by adding a trace amount (~0.1 μCi) of ^{14}C-DMO to 1 ml of whole blood and allowing 3-5 min equilibration at 16°C. Plasma and red cell samples were taken after centrifugation of blood (10,000g; 10 min). Ions (Na, K, and Cl) and activity of ^{14}C-DMO were measured after extraction of cell and plasma samples in 3.6% perchloric acid. pH_i was calculated from the transmembrane distribution of ^{14}C-DMO. Red cell and plasma water contents were determined from wet and dry (24 h at 80°C) weights. All red cell contents were corrected for 2% trapped space and plasma concentrations were corrected for dry content.

During the initial onset of hypoxia, there was a large and rapid decrease in PCO_2 resulting in a respiratory alkalosis as the pH_o increased in 15 min. Surprisingly, the ventilatory rate throughout hypoxia was significantly lower ($P< 0.01$; paired t-test) than the normoxic control value (49 min^{-1}); thus, the dogfish must be increasing the total volume flow across the gills in order to account for the dramatic decrease in PCO_2. After this initial respiratory alkalosis, the dogfish became acidotic. This acidosis was metabolic in origin with plasma [HCO_3] decreasing slowly and PCO_2 remaining fairly constant (~0.7 torr). There was no sign of recovery from the extracellular acidosis after 90 min of hypoxia. pH_i followed the pH_o with an initial alkalinization and was significantly less than the normoxic control value after 90 min of hypoxia. A linear plot of pH_i versus pH_o from hypoxic time points gave a slope of 0.68 (dpH_i/dpH_o) ($r= 0.95$) which is less than a similar plot of separate in vitro data where

dpH_i/dpH_o= 0.75 and r= 0.99. This small decrease in slope suggests a very weak, if any, pH regulation during hypoxia. After returning the dogfish to normoxia, blood and red cell pH returned to normoxic control values within 2 h. Plasma $[HCO_3]$ and PCO_2 were slow in recovering, and they were still significantly less ($P < 0.01$) than normoxic control values after the 3 h recovery time point.

Cell ion content measurements revealed that there was net uptake of Na and Cl during hypoxia. Additionally, mean cell K content increased throughout hypoxia and recovery periods which would be expected from an increased turnover rate of the Na/K ATPase in response to the higher cell Na content. The net uptake of Cl can be explained, in part, by the Cl shift following protonation of hemoglobin under acidotic and deoxygenated conditions. The mechanism of the net Na uptake is not known at this time; however, preliminary in vitro studies reveal that catecholamines do stimulate net Na uptake under deoxygenated conditions. We are currently investigating the mechanism of this catecholamine-stimulated net Na uptake.

Unlike the rainbow trout which shows rapid acidification of the blood and alkalinization of the red cell during exposure to acute hypoxia (Fievet et al., Am. J. Physiol. 252: R269-R275, 1987), the dogfish shark demonstrated very slow changes in blood and red cell pH in response to acute hypoxia. In trout, red cell pH is maintained significantly higher than normoxic values throughout hypoxia. However, in hypoxic dogfish the red cell pH is higher than the normoxic value only during the initial respiratory alkalosis and then begins to decrease with the ensuing extracellular metabolic acidosis. It appears from previous in vitro studies (Payne and McManus, 1989, ibid.) and the present in vivo study that although dogfish do possess a Na/H exchanger in the red cell membrane, it is not regulated by catecholamines in a manner similar to that in the trout. In comparing the physiological response of these two fish to hypoxic stress, one must consider the interactions of pH, oxygen, and the function of the hemoglobin molecule.

TABLE 1. HEMOGLOBIN AND RED CELL PROPERTIES OF DOGFISH AND TROUT

	SPINY DOGFISH Squalus acanthias	RAINBOW TROUT Salmo gairdneri
Bohr Effect ($\phi = d\log P_{50}/dpH$)	small[a,b] (-0.28)	large[b,c] (-0.57)
Haldane Effect	very small[a,b]	large[b]
Root Effect	none[a]	present[d]
Red Cell Buffering Power meq/(kg cell solid x pH)	large[b] 213[e]	small[b] —

Reference Code:
(a) Wells and Weber (J. exp. Biol. 103: 95-108, 1983); (b) Jensen (J. exp. Biol. 143: 225-234, 1989); (c) Tetens and Christensen (J. Comp. Physiol. 157: 667-675, 1987); (d) Boutilier et al. (J. exp. Biol. 123: 145-157, 1986); (e) Payne, unpublished.

It is evident from Table 1 that the two fish differ markedly in the characteristics of their hemoglobin and red cell buffering power. The large Haldane effect of trout hemoglobin will allow deoxyhemoglobin to remove a large number of protons during hypoxic conditions. This coupled with a small red cell buffering power and stimulation of Na/H exchange via catecholamines will contribute to maintaining or increasing cell pH even in the face of an extracellular acidosis. Overall, the goal during hypoxia in trout is to avoid the detrimental Root effect and possibly to increase hemoglobin-O_2 affinity via the Bohr effect, thus increasing O_2 loading at the gills. In direct contrast, Squalus hemoglobin does not show a Root shift and possesses a small Bohr coefficient. Additionally, we have measured the red cell buffering power to be extremely high in Squalus, therefore changes in cell pH would be expected to be small (as confirmed in this study) and may not affect hemoglobin-O_2 affinity to a great extent. This research was supported by a summer student fellowship from the American Heart Association, Maine Affiliate to JAP and NSF DCB-8801572 to DHE.