ACID-BASE BALANCE IN THE SPINY DOGFISH (SQUALUS ACANTHIAS) DURING HYPERCAPNIA

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Fish rapidly develop a respiratory acidosis when exposed to elevated levels of ambient CO_2. In most species studied to date, this obligatory plasma pH depression is slowly compensated by an increase in extracellular $[HCO_3^-]$ (reviewed by Heisler, in "Fish Physiology", eds. W.S. Hoar and D.J. Randall, Vol Xa, pp. 315-401, 1984). Several reports have now indicated that the increase in plasma $[HCO_3^-]$ is due to an uptake of HCO_3^- from (or an excretion of H^+ to) the surrounding water. Transbranchial Cl^-/HCO_3^-, Na^+/NH_4^+, and Na^+/H^+ exchanges have been postulated as the mechanisms responsible for the pH compensation (Cameron, J. Exp. Biol. 64:711-725, 1976; Claiborne & Heisler, J. Exp. Biol. 108:25-43, 1984). A detailed study of acid-base balance during hypercapnia in an elasmobranch, has only been accomplished in one species to date (the spotted dogfish, Scyliorhinus stellaris; Heisler et. al., Bull. Europ. Physiopathol. Respir. 12:77-85, 1976). When exposed to an external Pco_2 of ~8 torr, this species was capable of adjusting serosal pH to values approaching pre-hypercapnic levels (by taking up HCO_3^- from the surrounding water), within 24 hours. In the present study, the ability of the spiny dogfish (Squalus acanthias) to compensate for hypercapnic acidosis, and the possible underlying mechanisms responsible for this compensation, will be investigated.

Male dogfish (1.81 $\pm$ 0.08 kg, mean $\pm$ S.E., n=5) were briefly anesthetized (MS-222; 1:10,000), catheterized via the caudal artery, and placed in a darkened, plexi-glas experimental chamber connected to a closed, seawater recirculation system (total volume ~29 l). Water within the system was pumped at 4-6 liters/ min (16-19 °C) through an aeration column (within which, air or air/CO_2 mixtures were bubbled at ~4 l/min) and then recycled back to the fish chamber (Heisler et. al., 1976, ibid.). Animals were allowed to recover from anesthesia and adjust to the new environment for at least 24 hours before experiments were begun. Prior to the start of hypercapnia, duplicate control blood samples (1-2 ml) were drawn from the caudal catheter. The air bubbled through the aeration column was then adjusted to a ~1% CO_2/air mixture (Pco_2: 8-10 torr), and subsequent blood samples were obtained at hours 1, 4, 8, and 24. The animal was then returned to normocapnia, and additional samples were drawn 1, 4, and 8 hours after this change. Blood pH and Tco_2 were measured utilizing a thermostated pH electrode/pH meter combination (Orion 601A) and a Tco_2 detection system (Capni-Con II; Cameron Instruments Inc.). Plasma Pco_2 and $[HCO_3^-]$ were calculated from pH and Tco_2 using values for CO_2 and pK' at 17 C from Boutilier et. al. (in "Fish Physiology", eds. W.S. Hoar and D.J. Randall, Vol Xa, pp. 401-426, 1984). During a 4-14 hour control period prior to the start of hypercapnia, and throughout the hypercapnic and post-hypercapnic periods, water samples (22 ml) were collected and analyzed for $[HCO_3^-]$ (by volumetric titration of a portion of the sample to a pH of 3.80 with 0.1 N HCl), and total ammonia (T_{amm}; by the phenolhypochlorite method). The total amount of H^+ transferred between the shark and the water (ΔH^+, in mMole/kg) could then be calculated for each time period by subtracting the rates of HCO_3^- movement, from T_{amm} loss (effectively NH_4^+ at normal and hypercapnic seawater pH since the pK' of the NH_3/NH_4^+ equilibrium is about 9.6; see Cameron and Heisler, J. Exp. Biol. 105:107–125, 1983), and adjusting for volume changes due to sampling, and the mass of the animal. During each experiment, the water within the experimental system was periodically flushed with fresh seawater to prevent the build up of T_{amm}.

The changes in blood acid-base status during the hypercapnic and post-hypercapnic periods are shown in Table 1. As expected, plasma P_{CO_2} increased by more than 12 torr within one hour after the onset of hypercapnia and then remained 10-12 torr above control levels for the remainder of the period. Extracellular pH was rapidly depressed by the elevation in P_{CO_2} (by 0.5 units after 1 hour), but then slowly increased throughout the remainder of the period. This secondary pH compensation was due to a ~4.5x augmentation of plasma $[HCO_3^-]$ (by ~20 mM over 24 hours). One hour after the return to normocapnia, P_{CO_2} approached control values once again while plasma $[HCO_3^-]$ was still ~10 mM higher than pre-hypercapnic measurements. This induced an 'over-shoot' in pH (by about 0.2 units) which disappeared as extracellular $[HCO_3^-]$ continued to decrease over the next few hours.

Table 1. The effect of hypercapnia on selected acid-base parameters in Squalus acanthias.

Period	P_{CO_2} (torr)	pH	$[HCO_3^-]$ (mM)	ΔHCO_3^- ($mMole \cdot kg^{-1} \cdot h^{-1}$)	ΔNH_4^+ ($mMole \cdot kg^{-1} \cdot h^{-1}$)
control:	2.07 ± 0.08	7.87 ± 0.02	6.19 ± 0.52	−0.05 ± 0.02	0.06 ± 0.01
hypercapnia: (hours)					
1	14.71 ± 1.32	7.39 ± 0.04	12.65 ± 0.61		
2	14.66 ± 1.79	7.48 ± 0.04	15.61 ± 8.84	−0.59 ± 0.16	0.17 ± 0.03
4	14.81 ± 1.88	7.58 ± 0.06	20.00 ± 0.73	−0.83 ± 0.13	0.15 ± 0.04
8	12.86 ± 0.99	7.70 ± 0.04	24.40 ± 1.02	−0.41 ± 0.07	0.20 ± 0.02
24	12.40 ± 1.41	7.75 ± 0.05	26.75 ± 2.41	0.04 ± 0.07*	0.18 ± 0.05*
post-hypercapnia: (hours)					
1	3.00 ± 0.15	8.11 ± 0.03	16.55 ± 1.43		
4	2.12 ± 0.14*	7.93 ± 0.02*	7.24 ± 0.51*	1.38 ± 0.16	0.07 ± 0.03*
8	2.38 ± 0.15	7.84 ± 0.02*	6.46 ± 0.41*	0.27 ± 0.05	−0.01 ± 0.02*

Mean ± S.E. (n=5), test of significance by paired t-test, $p < 0.05$, two tailed. Values marked with '*' are not significantly different from controls. P_{CO_2} and $[HCO_3^-]$ are calculated from measured pH, T_{CO_2} and the appropriate solubility and pK' derived from Boutilier et al., 1984, op. cit.). Negative ΔHCO_3^- values represent a net uptake of HCO_3^- from the water.

S. acanthias is capable of regulating internal pH via the modification of plasma $[HCO_3^-]$. Indeed, had the $[HCO_3^-]$ remained constant, a plasma P_{CO_2} of 14 torr would have driven pH down to a calculated value of 7.09. By evaluating the rates of HCO_3^- and NH_4^+ transferred between the fish and the external milieu (Table 1), it becomes clear that the observed compensatory pH adjustment was due to an increase in both the net uptake of HCO_3^- from the water, and the excretion of T_{amm} to the water. In the first 8 hours of hypercapnia, the rate of HCO_3^- uptake and NH_4^+ loss averaged 11x and 3x that of control rates, respectively. Reinstatement of normocapnia induced a reversal in HCO_3^- transfer, as this ion was rapidly excreted to the water. The cumulative effect of these transfers is shown in Figure 1 as ΔH^+. In both experimental periods, the major alteration in H^+ movements occurred during the first 8 hours of the respective period. The net ΔH^+ (the difference between experimental and control rates of transfer) during hypercapnia was +5.3 mMole/kg. If the extracellular space in these animals is on the order of 20% (Heisler, J. Exp. Biol. 99:9–28, 1982), than this net loss of H^+ could have easily accounted for the observed 20 mM increase in plasma $[HCO_3^-]$. Indeed, it is likely that some compensatory intracellular HCO_3^- uptake also occurred (Cameron, J. Exp. Biol. 86:171–184, 1980). The return

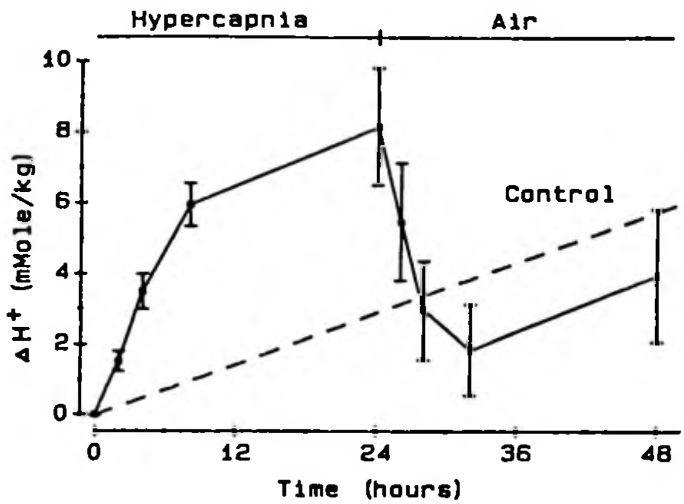

Figure 1. Changes in cumulative H^+ transfer during and after hypercapnia (mean $\pm$ S.E., n=5). The control line represents the measured control rate of efflux extended as a reference over the subsequent experimental periods.

to normocapnia and the concomitant excretion of extra- and intracellular HCO_3^- was observed as a net ΔH^+ of -7.1 mMole/kg. In contrast to the spotted dogfish (Heisler et. al., 1976, op. cit.), it is interesting that T_{amm} excretion in S. acanthias does increase during hypercapnia, perhaps indicating the utilization of NH_4^+ (or NH_3 together with H^+) excretion for acid-base regulation in this ureotelic species. It remains to be seen whether the HCO_3^- and NH_4^+ transfers observed in spiny dogfish are due to Cl^-/HCO_3^-, Na^+/NH_4^+, or Na^+/H^+ exchanges, as have been proposed for other fish. Likewise, the carbonic anhydrase mediated hydration of CO_2, may also play a role in the regulation of HCO_3^- transfers between the fish and the water (Swenson and Claiborne, this volume). (Funded by a Faculty Research Grant from GSC to JBC and NSF PCM 83-02621 to DHE)