EFFECTS OF GILL CARBONIC ANHYDRASE (CA) INHIBITION ON COMPENSATION TO RESPIRATORY ACIDOSIS IN THE SHARK, SQUALUS ACANTHIAS

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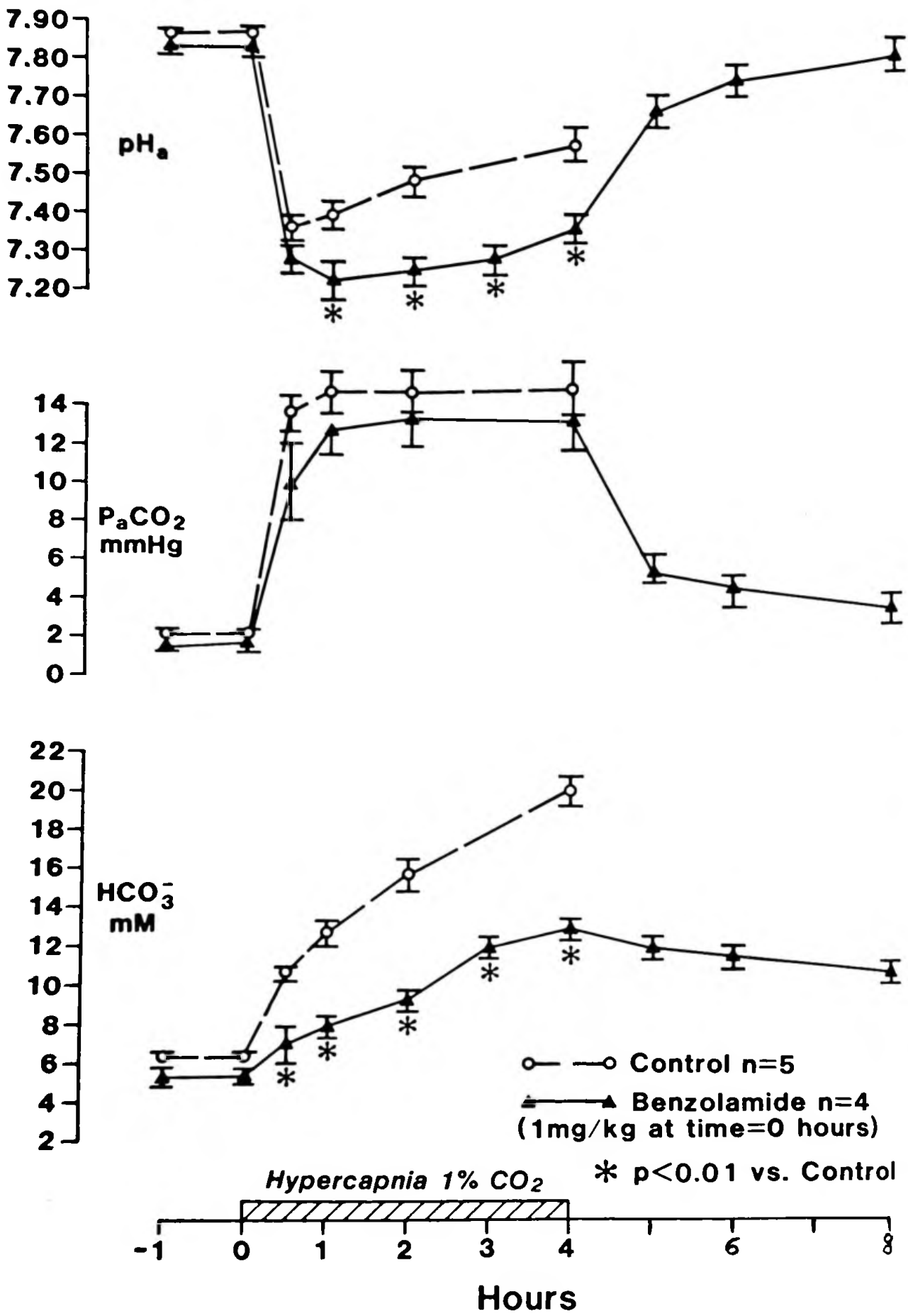
Introduction: Compensation to respiratory acidosis in marine elasmobranchs occurs by branchial uptake of bicarbonate or excretion of protons. (Heisler et al, Bull Europ Physiopath Resp 12: 77; 1976). While Swenson et al (Bull MDIBL 24:72: 1984) have demonstrated gill CA dependent branchial excretion of HCO_3 in metabolic alkalosis, it is unknown whether HCO_3 uptake in respiratory acidosis also requires gill enzyme. Therefore we studied the effects of gill CA inhibition on HCO_3 uptake during hypercapnia and HCO_3 excretion following the return to normocapnia in the dogfish shark, Squalus acanthias.

Methods: Male dogfish (1.8-2.2 kg) were used 1-3 days following capture. A catheter was introduced into the caudal artery for withdrawal of arterial blood and drug injections. Following catheterization, the fish were placed in a closed recirculation system (~25 liters) consisting of a plastic box slightly larger than the fish, a circulating pump and an aerator to which CO_2 could be added. (Claiborne and Evans, Bull MDIBL: this volume). Following a 16-20 hour recovery period, several control arterial blood and seawater samples were taken for pH, PO_2, PCO_2, HCO_3, and NH_4 and then the system was closed. Hypercapnia was imposed by the addition of ~ 1% CO_2 into the aerator and arterial blood was sampled at 1/2, 1, 2, 3, and 4 hours and seawater at 0 and 4 hours. Blood and seawater pH and pO_2 were measured on a blood gas analyzer maintained at 14-15 °C. and calibrated with known standards. Total CO_2 was measured either manometrically (Kopp-Natelson microgasometer) or by a conductometric method (Capnicon III, Cameron Instruments Inc.). Seawater ammonia concentrations were determined by the phenolhypochlorite method. In a separate series of fish, gill carbonic anhydrase was inhibited by benzolamide (1mg/kg) given at the onset of hypercapnia. This dose was chosen because it selectively inhibits gill carbonic anhydrase and causes no acidosis and only a 0.6 mM rise in arterial HCO_3 after four hours. (Swenson and Maren, Bull MDIBL, this volume). These fish were switched back to normally aerated seawater at 4 hours and blood samples were taken at 5, 6 and 8 hours.

Results: The figure shows the results of four hours of respiratory acidosis on the blood acid base parameters in control and treated fish and their return toward normal in the treated fish. Our results in control fish are similar to those of Heisler et al (Bull Europ Physiopath Resp 12: 77; 1976). In both control and drug treated fish the arterial PCO_2 rose to 13-14 mmHg in one hour and remained at this level over the subsequent three hours. The arterial pH fell to 7.22 in the drug treated fish and 7.39 in the controls after one hour. Thereafter the arterial pH rose as the plasma HCO_3 rose. The rate of rise in arterial pH and HCO_3 was markedly slowed by inhibition of gill CA such that by four hours the control animals had corrected their pH to 7.58 with a rise in plasma HCO_3 to 20.0 mM while the inhibited animals had only reached a pH of 7.36 and HCO_3 of 13.0 mM. Analysis of seawater showed that the controls exhibited a net uptake of 2.64 mmol/kg of HCO_3 from the seawater while inhibited fish had


7.90
7.80
7.70
7.60
7.50
7.40
7.30
7.20
pHa
14
12
10
8
6
4
2
0
PaCO2
mmHg
22
20
18
16
14
12
10
8
6
4
2
HCO3-
mM
Control n=5
Benzolamide n=4
(1mg/kg at time=0 hours)
* p<0.01 vs. Control
Hypercapnia 1% CO2
-1
0
1
2
3
4
6
8
Hours

only taken up 0.84 mmol/kg during the 4 hour period. Ammonia excretion appeared to increase in the inhibited fish when compared to the control fish (1.06 and 0.40 mmol/kg respectively), thus the net base taken up was 3.04 mmol/kg in the control animals and 1.90 mmol/kg in the CA inhibited sharks. In the post hypercapnic period the inhibited animals showed only a 2 mM decline in plasma HCO_3 in four hours. This can be contrasted with the very rapid fall in plasma HCO_3 seen in control animals in the first 4 hours following a 24 hour period of hypercapnia, when HCO_3 fell from 26 to 7.2 mM. (Claiborne and Evans, Bull MDIBL: this volume).

Discussion: We have shown that a powerful branchial HCO_3 uptake mechanism in elasmobranchs induced by respiratory acidosis is dependent on gill carbonic anhydrase. A previous study by Maren (Am J. Physiol 222: 885, 1972) did not show an effect of acetazolamide on the compensation to 5% CO_2, however the combination of mild metabolic acidosis (induced by surgery and restraint) superimposed on severe respiratory acidosis may have compromised normal gill function in these experiments. We did not test the effects of either methazolamide or acetazolamide, drugs which inhibit both gill and red cell enzyme, because generalized systemic CA inhibition in itself causes a respiratory acidosis. It is evident from these studies that mechanisms of rapid HCO_3 transfer across the gills require carbonic anhydrase for normal acid base regulation, both in respiratory acidosis and metabolic alkalosis.

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