CARBONIC ANHYDRASE IN THE UTERINE SEA WATER ACIDIFICATION PROCESS IN SQUALUS ACANTHIAS: LOCALIZATION AND THE EFFECT OF VARIOUS INHIBITORS

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Pups of the dogfish Squalus acanthias (L.) are maintained in utero during a gestation period that lasts nearly two years (Woodhead, Bull. MDIBL 16:103-106, 1976). During the latter months of the gestation period the solution in which the pups reside is apparently derived from sea water, but the pH is maintained at about 6, total CO_2 is about 0.2 mM and ammonia concentrations may approach 22 mM (Kormanik and Evans, J. Exp. Biol. 125:173-179, 1986). Thus CO_2 is extracted and the uterine sea water is acidified (Kormanik, Kremer and Patton, Bull. MDIBL 25:142-145, 1985). One possible role of the acidification is to protect the pups from the toxic effects of the accumulated ammonia (Kormanik, Kremer and Patton, Bull. MDIBL 25:140-141, 1985). In a previous investigation we reported that the acidification and reduction of total CO_2 in the uterine sea water was prevented by acetazolamide, an inhibitor of the enzyme carbonic anhydrase (CA) thus CA was implicated in the process (Kormanik and Kremer, Bull. MDIBL 26:142-144, 1986). On the one hand, the ubiquitous enzyme CA is involved in acidification processes and CO_2 movements in a variety of tissues (Maren, Physiol. Rev. 47:595-781, 1967), including the gill and red blood cells of Squalus (Swenson and Maren, Am. J. Physiol. 253:R450-R458, 1987). On the other hand, while Squalus produces an acid urine, no CA is found in the kidneys. Thus it is not required in this acidification process (Swenson and Maren, Am. J. Physiol. 250:F288-F293, 1986). We therefore extended our observations to include an assay for CA in the uterine endometrium. Since acetazolamide (ACZ) also had secondary effects in whole animal preparations, i.e. elevation of blood Tco_2 due to a reduction in CO_2 excretion as a result of inhibition of red blood cell CA, we induced an increase in blood TCO_2 by alkalosis to determine its effect on the uterine acidification process.

Late-term pregnant Squalus acanthias were collected from Frenchman Bay and maintained in live cars until they were used in experiments. For the CA assays, uterine tissue was removed immediately from sacrificed females and placed in ice-cold Elasmobranch Ringer's Solution (ERS, Forster et al., Comp. Bioch. Physiol. 42A:3-13, 1972). Endometrium lining the uterine horns was peeled from the underlying smooth muscle and rinsed again in ice-cold ERS. Accessible arterial vessels were perfused with ERS via syringe to rinse as much blood as possible out of the tissue. The tissue was homogenized using a Waring blendor and/or a Potter-Elvehjem tissue homogenizer, and stored on ice. The underlying smooth muscle samples were handled in the same manner. Tissues were assayed immediately for CA at 0° C. with 100% CO_2 using the methods of Maren (J. Pharmacol. Exp. Ther. 130:26-29, 1960), thus the enzyme units presented below are comparable to Swensen and Maren (1987). Since it was impossible to rinse this highly vascular tissue completely free of blood, a correction factor was applied to eliminate the contribution of CA from the red blood cells. The absorbance (O.D.) of an aliquot of the tissue extracts was measured at 545nm, the absorbance peak for hemoglobin. The aliquot was then bleached with 5% sodium hypochlorite (1:20) and the O.D. was again determined.

The same procedure was used on pup blood samples of known CA activity. This procedure yielded a correction factor, in enzyme units/O.D. Hb, which was applied to the tissue extracts to yield a RBC correction factor (Table 1). In the experiments where we determined the effects of alkalosis on the uterine sea water acidification process in whole animals, the fish were prepared as previously described (Kormanik and Kremer, op. cit.).

Table 1. Carbonic anhydrase activity (in EU) in tissues of pregnant mothers and pups, reported per gram wet weight of tissues. Blood was diluted 25:1 and uterine tissues, 16:1 for the assay.

	maternal	fetal
Red blood cells (n = 9)	2300 ± 100	2800 ± 100*

Uterine tissues

	total activity	− RBC activity	= tissue activity
endometrium (n = 6)	57 ± 14	17 ± 8	40 ± 7
smooth muscle (n = 3)	0	0	0

* − $p < 0.01$

CA activities of the various tissues are reported in Table 1. The activity we detected in the homogenates was inhibited by methazolamide (10^{-4} M. in the reaction mixture). Activities for blood we determined are about 50% lower than those previously reported for comparable tissues using this assay (Swenson and Maren, op. cit.). Nevertheless, the results provide some relative comparisons. CA activity in pup blood is 20% higher than that of the mothers. However, since CA is usually found in excess in the tissues where it has a physiological role, the difference is probably not functionally important. Activity of CA in the uterine endometrium is detectable, but very low, on the order of 2% of that found in RBCs, and is therefore among the lowest values reported for this method (Maren, op. cit.). Nevertheless, our observations of CA in the endometrium have been recently confirmed by histochemical localization of CA on the basal and lateral membranes of the epithelial cells (Drs. C. Flügel and E. Lütjen-Drecoll, Anatomisches Institut der Universitat Erlangen-Nürnberg, FRG, personal communication). We found no activity in uterine smooth muscle. These preliminary observations suggest that C.A. activity is present in the epithelium of the uterine endometrium.

The effects of acetazolamide and alkalosis are shown in Table 2. As previously reported, ACZ inhibits uterine SW acidification and the reduction

Table 2. The effect of CA inhibitors and alkalosis on uterine sea water acidification and CO_2 removal in S. acanthias. ACZ (acetazolamide, 20 mg kg^{-1}) data and Cntrl (control) are averages for 4-12 animals from Kormanik and Kremer, op. cit. Alk (alkalosis, 10 mmol HCO_3^- kg^{-1}, over 8 hrs) blood data are from one animal, uterine values are the average of 2 horns. Tco_2 is in mM, Pco_2 is in mm Hg.

	-0.5 hr (control)			4 hr			8 hr		
	pH	Tco_2	Pco_2	pH	Tco_2	Pco_2	pH	Tco_2	Pco_2
Blood									
ACZ	7.80	7.3	2.6	7.67	15.5	6.8	7.72	15.4	6.7
Alk	7.91	7.9	2.2	8.42	37.7	3.3	8.34	38.1	3.9
Uterine SW									
ACZ	8.2	2.1	0.2	7.20	2.7	2.6	7.10	2.1	3.4
Alk	"	"	"	7.65	2.8	1.0	7.85	5.3	1.2
Cntrl	"	"	"	6.75	1.2	3.6	6.30	0.3	2.2

of Tco_2 seen in the controls (Kormanik and Kremer, op. cit.). ACZ also causes an increase in blood Pco_2 and Tco_2, both of which more than double, from reduced CO_2 excretion (Swensen and Maren, op. cit.). To see if these secondary effects might be responsible for the inhibition of uterine SW acidification, we induced a metabolic alkalosis by continual infusion of HCO_3^- (Table 2). Blood pH, Tco_2 and Pco_2 all were elevated, and uterine sea water acidification was inhibited. In fact, uterine Tco_2 increased over that of the controls. In previous experiments, acetazolamide inhibited the uterine acidification process and the reduction of Tco_2 typically seen in controls by 8 hours (Kormanik and Kremer, op. cit.). Acetazolamide can be expected to inhibit not only the epithelial CA, but also the CA of the red blood cells (Swenson and Maren, op. cit.). The net result is a change in the blood to uterine sea water gradients, especially for Tco_2. When the gradients were modified by induction of severe metabolic alkalosis, uterine acidification and the reduction of Tco_2 was eliminated. It is therefore difficult to ascribe the inhibition of uterine sea water acidification and reduction in Tco_2 to an inhibition of uterine CA by ACZ alone. Nevertheless, combined with the data demonstrating activity of CA in our crude extracts of uterine endometrium, and the more sensitive method of histochemical localization (Flügel and Lütjen-Drecoll, op. cit.) it appears that CA is of catalytic importance in the process of uterine acidification and removal of Tco_2. Further work with potent and localizable inhibitors of epithelial CA (e.g. Benzolamide, see: Swenson and Maren, 1987) should help to define its role. (Supported by NSF DCB-8502251 to G.A.K.).