METABOLISM OF ISOLATED GILL ARCHES OF EMBRYOS OF THE SPINY DOGFISH, SQUALUS ACANTHIAS

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Embryos of the dogfish Squalus acanthias must osmoregulate in a uterine solution resembling sea water during the second half of a nearly two year gestation period (Evans et al., J. Exp. Biol. 101:295-305, 1982). These embryos possess mitochondria-rich cells in the gills (Kormanik et al., Bull. MDIBL 30:4-7, 1991) thus implicating them in ion regulation. We assessed the metabolic activity of isolated gill arches by measuring oxygen consumption, in order to better understand their potential role in osmoregulation as well as the way by which metabolic activity of gill tissue is controlled.

Late-term embryos of Squalus acanthias (25-55g.) were collected and prepared as previously described (Kormanik and Totten, Bull. Mt. Desert Isl. Biol. Lab. 32:107-109, 1993). Gill tissue was minced and placed into the oxygen chamber with Elasmobranch Ringer's Solution (ERS: in mM; 280 Na, 6 K, 3 Mg, 5 Ca, 350 urea, 70 trimethylamine N-oxide; 300 Cl, 2 PO_4, 8 HCO_3, 5 glucose, pH = 7.9) and oxygen consumption was measured as previously described (Kormanik and Totten, ibid.). Tissue and chamber were allowed to come to steady state (5 min) after set-up or addition of drugs. A control rate (Period 1) was measured over the next ten minutes, followed by either another control or an experimental period (Period 2). Drugs and metabolites (Sigma Chem. Co.) were prepared as concentrated stock and injected into the O_2 chamber to yield the following final concentrations: ouabain (0.3 mM); methacholine (0.5mM); α-methyl tyrosine (3mM); ±arterenol (0.1mM); ±isoproterenol (0.1mM). All data are presented as mean ± SEM, and compared using Student's t-test for paired or unpaired data.

In one series of experiments we added to the ERS the metabolic fuels typically favored by the gill, alanine (2mM) or lactate (3mM; see Perry and Walsh. J. Exp. Biol. 144:507-520, 1989). Oxygen consumption (umol g^{-1} h^{-1}, n=5) in the presence of glucose + alanine (4.34 ± 0.31) or glucose + lactate (4.50 ± 0.50) was not significantly different from a comparable control period where only glucose was provided ($p > 0.1$, unpaired). Cyanide reduced gill oxygen consumption to zero. These data show that the glucose supplied plus endogenous metabolites were sufficient to maintain oxygen consumption during the duration of the experiments, which lasted about 45 min.

In order to determine how gill metabolism is controlled, we examined the effects of several drugs on gill oxygen consumption. The results are shown in Table 1. The α-adrenergic agonist arterenol, which inhibits chloride secretion in the isolated opercular preparation (Zadunaisky, in Fish Physiology, eds. Hoar and Randall, Vol. XB pp. 129-176, 1984) reduced gill oxygen consumption slightly but not significantly. α-methyl tyrosine, which has been shown to reduce oxygen uptake in resting dogfish (Metcalfe and Butler, J. Exp. Biol. 141:21-32, 1989) and isolated hepatocytes, had no significant effect on gill cells. Nor was any effect observed with the ß-adrenergic isoproterenol, which has been shown to stimulate short-circuit current in the opercular preparation (Degnan and Zadunaisky, J. Physiol Lond. 294:484-495, 1979) and gluconeogenesis and glycogenolysis during acidotic states (Wright et al., J. Exp. Biol. 147:169-188, 1989). However, catecholamines had no effect on erythrocyte metabolism at normal blood pH (Wood et al., J. Exp. Biol. 154:475-489, 1990). We did not measure the effect of catecholamines in the presence of lactate.

The only compound we found thus far that significantly stimulated gill metabolism was the parasympathomimetic methacholine (Table 1). However, oxygen consumption was still slightly enhanced in the presence of methacholine + ouabain, compared to experiments where ouabain alone was added. These data suggest that methacholine-stimulated oxygen consumption is not transport related. Cholinergic agonists are strong inhibitors of salt transport in isolated opercular epithelia (see Zadunaisky, Op. cit.).

Table 1. Oxygen consumption of isolated gills of Squalus acanthias embryos. Data are expressed in umol O_2 g wet wt.$^{-1}$ h^{-1}, ± SEM (n).

Period 1	Period 2			
Control 1	Control 2	Arterenol	α-methyl tyr	isoproterenol
5.22 ± 0.36 (26)	4.48 ± 0.23 (7)	4.01 ± 0.58^{ns} (7)	4.22 ± 0.54^{ns} (6)	4.58 ± 0.24^{ns} (6)
		methacholine	methacholine + ouabain	ouabain
	4.50 ± 0.28 (14)	5.40 ± 0.44 (14)	4.59 ± 0.62 (8)	3.40 ± 0.10* (6)
		\-- p < 0.001 --\-- p < 0.005 --\ paired data		

ns - p > 0.1, unpaired data versus Control 2

* - p < 0.01, paired data versus Control 2

The O_2 consumption of gills we measured is comparable to that of isolated chloride cells from seawater-acclimated teleosts (ca. 5-15 umol g^{-1} h^{-1}, Perry and Walsh, ibid.), and higher than that which we measured for elasmobranch liver tissue (2.2 ± 0.4 umol g^{-1} h^{-1}, n=4). In previous experiments we were unable to significantly stimulate O_2 consumption with theophylline and c-AMP (Kormanik and Totten, ibid.), known stimulators of salt transport in teleost chloride cells. Oxygen consumption, however, was reduced by 24% by ouabain, which inhibits secretion by its inhibition of Na-K-ATPase. Gill tissue is involved in numerous activities, including acid-base balance, ion and nitrogenous waste excretion and gas transfer. These data indicate that a relatively small portion of metabolism (ca. 24%) is related to ion transport by elasmobranch gill tissue, and its control may therefore be difficult to dissect out, at least in whole gill preparations. We plan to isolate mitochondria-rich cells from the elasmobranch gill and directly assess their metabolism. (Supported by NSF DCB8904429 to GAK and a Hearst Foundation Scholar award to AW)