HEMODYNAMICS OF THE PERFUSED *SQUALUS ACANTHIAS* PUP HEAD: SENSITIVITY TO IN VIVO LEVELS OF EPINEPHRINE

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We have recently initiated a series of studies of the hemodynamics and transport characteristics of the perfused head of *Squalus acanthias* pups (Evans and Claiborne, J. Exp. Biol. 105:363-371, 1983; Evans et al. Bull. MDIBL 22:46-47, 1982) and have found that this preparation is hemodynamically stable for periods of hours and displays Cl and ammonia effluxes near to those found in the intact fish. Like other perfused head preparations (eg. Claiborne and Evans, J. Comp. Physiol. 138:79-85, 1980), the pup head reacts to the addition of 10^{-5}M epinephrine with a transient, alpha-adrenergic mediated increase in vascular resistance (not always present), followed by a longer lasting, beta-adrenergic mediated fall in vascular resistance (Evans and Claiborne, 1983, Op. Cit.). However, 10^{-5}M epinephrine is substantially greater than the resting levels of 3.2×10^{-8}M found in *Scyliorhinus canicula* (Butler et al., J. Comp. Physiol. 127:325-330, 1978). It is even substantially above the concentrations of 4.5×10^{-7}M found in *S. canicula* under hypoxic conditions. Shuttleworth (J. Exp. Biol. 103:193-204, 1983) has recently shown that the secretory rate of the perfused rectal gland of *S. canicula* is sensitive to catecholamine concentrations of this magnitude (ie. 5×10^{-8}M to 10^{-6}M), so it is appropriate to examine the sensitivity of the branchial vasculature of the *S. acanthias* pup head to levels of epinephrine which more nearly approximate the in vivo condition.

Pup heads were prepared as described previously (Evans and Claiborne, 1983, Op. Cit.) and were perfused for 15 to 30 minutes to clear the branchial vasculature of blood, and to reach stable afferent pressures. (Temperature of the preparation was controlled by running the perfusate through a coiled tubing in a bath maintained at approximately 10° C by a cooling plate. In addition, the irrigation fluid was maintained at approximately 10° C with another cooling plate. In this way the temperature of the perfusate and irrigate were approximately 12°-14° C as they entered the head). After stable afferent pressures were obtained, the perfusate was changed to contain epinephrine in concentrations ranging from 10^{-8}M to 10^{-6}M and the change in afferent pressures was noted. In many cases, the effects of the epinephrine were transient, ie., an initial fall followed by a relatively slow (few minutes) increase in pressure. In this case the fall in pressure calculated for a subsequent increase in epinephrine concentration was taken as the difference between the final pressure at the first concentration and the initial drop in pressure at the next highest epinephrine concentration.

Table 1 presents the results and it is quite clear that epinephrine is effective at reducing the resistance of the

Table 1.--Effects of low concentrations of epinephrine on the afferent pressure of the perfused pup head

	10^{-8}M	5×10^{-8}M	10^{-7}M	10^{-6}M
Pressure Change (torr)	-6.3 ± 3.1*	-5.1 ± 0.6	-3.8 ± 1.3	-8.0 ± 0.7

*$\bar{X}$ ± S.E. (N=4).

branchial vasculature at extremely low concentrations, certainly in the range of in vivo levels. Thus, it appears that the resistance of the elasmobranch branchial vasculature is tonically maintained by circulating catecholamines. Importantly, these data also indicate that the hemodynamic effects of epinephrine on the isolated, perfused pup head demonstrated at higher concentrations (Evans & Claiborne, 1983, Op.Cit.) cannot be ascribed to pharmacological levels of the hormone. Supported by NSF PCM 81-04046 to DHE.

UREA EFFLUXES FROM THE *SQUALUS ACANTHIAS* PUP: THE EFFECT OF STRESS

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The extremely high concentration of urea found in elasmobranch blood is secondary to nearly complete re-

absorption of urea in the renal tubules (90-95%) and a branchial urea permeability which is approximately 1% of that of the toad bladder. Measured urea effluxes from various species of elasmobranchs range from 19 to 59 $\mu M.100g^{-1}.hr^{-1}$ (Evans in "Comparative Physiology of Osmoregulation in Animals", ed. by G.M.O. Maloiy, Academic Press, 1979). As part of our studies on the osmoregulatory physiology of the S. acanthias pup, it is appropriate to measure the rate of urea efflux from intact pups, especially since we found that pups displayed a significantly reduced (18%) blood urea concentration after 6 days in sea water (Evans et al., J. Exp. Biol. 101:295-305, 1982). Moreover, recent studies have shown that stress can increase circulating levels of catecholamines in elasmobranchs (eg. Butler et al., J. Comp. Physiol. 127:325-330, 1978), and epinephrine is capable of lowering branchial resistance in perfused S. acanthias pup heads (Evans and Claiborne, J. Exp. Biol. 105:363-371, 1983), presumably via lamellar recruitment, as has been described for teleosts (Booth, J. Exp. Biol. 83:31-39, 1979). Since lamellar recruitment would increase functional branchial surface area, and thereby the efflux of permeant blood solutes, it is of interest to examine the effect of stress on the efflux of urea from intact pups. Stress was produced by the experimentally-common procedure of anesthesia, followed in some experiments by ligation and removal of the yolk sac, in order to examine the role of this structure in urea efflux.

Pups were removed from sacrificed females and used in experiments within 24 hours. Individual pups were placed into 200 mls of sea water in a plastic container, maintained at approximately 15°C in a running seawater table. At hourly intervals for 3-5 hours thereafter, 5 ml samples of the efflux baths were removed and frozen for subsequent analysis for urea using the diacetyl monoxime reaction analysis of Sigma Kit #531. At the end of the control efflux period the pups were anesthetized by pipetting appropriate volumes of MS 222 (to reach a concentration of 0.01%) into the bath. Anesthetized pups were then placed into new efflux baths, in some cases after the yolk sac had been ligated and removed. Animals were weighed during the period of anesthesia, and effluxes were calculated from the change in bath urea concentration with time, using the initial weights of the pups (including yolk sac), even in the experiments where the yolk sac was removed, to avoid a weight bias of the calculated efflux.

Tables 1 and 2 present the results of the experiments. It is clear that in both cases the simple transfer of un-

Table 1.--The effect of stress and yolk-sac removal on the efflux of urea from intact S. acanthias pups

	Hours after initial transfer		Hours after anesthesia (minus yolk sac)			
	0-1	4-5	0-1	1-2	2-3	3-4
Urea efflux* (n=4)	14.5 ± 4.1	7.4 ± 1.2	29.6 ± 2.6	15.7 ± 2.3	9.7 ± 3.0	15.4 ± 1.2
Significance (P)**	< 0.05		<0.01	<0.02	> 0.05	>0.05

*Efflux in $\mu M \cdot 100g^{-1} \cdot hr^{-1}$, $\bar{x} \pm$ S.E. **Significance between respective effluxes, paired t-test, one-tail.

Table 2.--The effect of stress on the efflux of urea from intact S. acanthias pups

	Hours after initial transfer		Hours after anesthesia		
	0-1	2-3	0-1	1-2	3-4
Urea efflux (n = 12)	26.3 ± 10.3	13.5 ± 6.1	40.3 ± 8.8	22.5 ± 6.4	25.8 ± 8.2
Significance (P)	<0.01		< 0.01	< 0.02	> 0.25

anesthetized pups into the initial bath resulted in a significant stimulation of urea efflux, which was reduced to levels below that described for other (in this case adult) elasmobranchs (Evans, 1979, Op.Cit.) by 2-5 hours after the initial transfer. One might argue that our baseline urea effluxes are low because the pups are physiologically different from

the adults, but the counter argument is that previous studies utilized stressed experimental animals. Further experiments on adults that are known to be unstressed are needed. In the present study, anesthesia and weighing was followed by a significant increase in urea efflux, which was not dependent upon the presence of the yolk sac. In fact, there was a 3-fold stimulation in animals with an intact yolk sac, and a 4-fold stimulation in animals without a yolk sac. Importantly, the stress-induced increase in urea efflux declined with time so that by two hours after transfer the efflux had fallen to pre-stress levels, at least in the case of pups without yolk sacs. The fact that the urea efflux from pups with intact yolk sacs did decline significantly in the second hour, but continued thereafter at levels above the control rates may indicate that the yolk sac does play a role in urea efflux, at least over the long term. This interesting possibility should be explored. However, it is clear that rather simple stress is followed by a significant, but transient increase in the efflux of urea, secondary presumably to epinephrine-induced lamellar recruitment and therefore increased functional branchial surface area. Data on the extent of lamellar recruitment by epinephrine in S. acanthias pups is lacking (although concomitant studies have shown that the branchial resistance of the perfused pup head is reduced significantly by as little as 10^{-8}M epinephrine (Evans and Hooks, this volume), but studies on teleosts indicate that there is only an approximately 50% increase in the number of perfused lamellae (Booth, 1979, Op.Cit.). If similar levels of lamellar recruitment are found in elasmobranchs, then one must propose that the stress also induces an increase in branchial permeability to urea, since Table 1 indicates a 300-400% increase in urea fluxes after stress. Supported by NSF PCM 81-04046 to DHE.

SODIUM FLUXES ACROSS THE HAGFISH, MYXINE GLUTINOSA

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The different classes of aquatic vertebrates display quite different, and characteristic, ionic efflux rates (Evans, in "Comparative Physiology of Osmoregulation in Animals", G.M.O. Maloiy, ed., Academic Press, 1979). Generally speaking, the teleosts have Na and Cl effluxes which are similar and in the range of 1000 to 4000 μM. 100g.hr for marine species, and less than 50 μM.100g.hr for freshwater species. The elasmobranchs, on the other hand, have decidedly different efflux rates for Na and Cl, with effluxes of Na in the range of 50-100 μM.100g.hr, and effluxes of Cl 5-10 times higher. While differences in functional branchial surface area may account for some of the discrepancies between marine teleosts and elasmobranchs, it is generally considered that elasmobranchs, like freshwater teleosts, maintain a very low ionic permeability. This low ionic permeability is adaptive since elasmobranchs maintain blood Na and Cl concentrations distinctly below sea water levels. In fact, the question of why marine teleosts maintain relatively high ionic permeabilities remains unanswered.

Like elasmobranchs, hagfishes are approximately iso-osmotic to sea water and therefore are relatively freely permeable to water (Evans, ibid). However, hagfish do not retain urea and TMAO, and their iso-osmolarity with sea water is the result of relatively high blood concentrations of both Na and Cl. In fact hagfish blood Na and Cl levels are only slightly below sea water concentrations. Because of this near iso-ionic condition, and their evolutionary position near to the origin of the vertebrates (Hardisty, 1979), it is of interest to determine the relative ionic permeabilities of this group in order to address the questions of whether low ionic permeability is a primitive condition, or related to the needs for ionic regulation.

Hagfish were collected in the Bay of Fundy and supplied by the Huntsman Marine Laboratory, St. Andrews, N.B. They were maintained in running sea water at ambient temperatures (approximately 15°C). Experimental fish were anesthetized with MS 222 (.02% solution), injected with 2μCi of carrier-free 22 Na solution (10 μl) and returned to 200 ml of aerated sea water in a plastic container maintained at approximately 15°C on a running sea water table. A time zero sample (2ml) of the efflux bath was taken 15-30 minutes later and mixed with 10 mls Aquasol 2 for liquid scintillation counting. Subsequent samples were taken at timed intervals for periods up to 48 hours after time zero.