The efflux of Cl from the perfused pup head was 200 ± 45 uM, 100g-1.hr-1 (17), which is not significantly different from that described for the intact pup (219 μM.100g-1.hr-1, Evans et al., J. Exp. Biol., Op Cit., 1982). However, approximately 25% of the Cl efflux from the intact pup is via the rectal gland (Evans et al., ibid), so it appears that, while the Cl efflux from the perfused head approximates that from the intact fish, some 25% of this efflux is possibly from a mechanical leak. (We have recently monitored 14-C urea effluxes from perfused heads--the urea efflux from intact elasmobranchs is extremely low (Goldstein et al., Am. J. Physiol. 215:1493-1497, 1968)--and found that extremely small mechanical leaks (approximately 3 μl/min) are sometimes present and can account for approximately 25% of the Cl efflux.

Addition of ouabain (10^{-4} M) to the perfusate increased the efferent pressure slightly (4.7 ± 0.56 torr, N = 3), and stimulated the rate of Cl efflux by 222%, 329%, and 18% in three experiments. Addition of furosemide (10^{-4}M) to the perfusate reduced the afferent pressure slightly (3.7 ± 0.41 torr, N=3), but did not alter the rate of Cl efflux (control: 225 ± 57 μM.100g-1.hr-1, furosemide: 221 ± 122 μM.100g-1.hr-1, n=5). Unfortunately, we did not monitor urea fluxes in three experiments, so it is possible that slight changes in any structural leak (which even slight pressure changes could theoretically produce) could have masked any inhibition of Cl efflux produced by either ouabain or furosemide. Nevertheless these preliminary data are consistant with the proposition that Cl efflux from the perfused pup head is in the same range as that found in vivo, and is apparently not via a furosemide sensitive Na-Cl co-transport systems, which is secondarily sensitive to the inhibition of Na-K ATPase by ouabain (Fizzzell et al., Am. J. Physiol., 236:F1-F8, 1979). Further studies which will include measurement of the structural leak pathway by monitoring urea effluxes will enable us to separate passive, carrier-mediated, and structural leak pathways for Cl efflux across the gill epithelium of S. acanthias. This work was supported by NSF PCM 81-04046 to DHE.

PRELIMINARY INVESTIGATION OF THE ADRENERGIC CONTROL OF GILL HEMODYNAMICS IN THE SMOOTH DOGFISH, Mustelus canis

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Last year we examined the viability and hemodynamics of the perfused head of pups of Squalus acanthias (the spiny dogfish). We found thatthat preparation displayed long-term hemodynamic integrity and that addition of epinephrine (10^{-5}M) was followed by a significant reduction in gill resistance, which was inhibited by the concomitant addition of the beta-adrenergic receptor blocker propranolol. In some experiments this fall in gill resistance was preceded by a transient increase in gill resistance, which could be inhibited by the alpha-adrenergic blocker phentolamine (Evans and Claiborne, Bull. MDIBL 21:9-11, 1981). In addition, epinephrine stimulated the preferential flow of perfusate into the dorsal aorta, and this effect was blocked by propranolol. Thus, it appears that the branchial hemodynamics of the perfused S. acanthias pup head are similar to those described for a number of teleosts, with the exception that, in teleosts, the preferential flow into the dorsal aorta is mediated via alpha-adrenergic, rather than beta-adrenergic receptors (Claiborne and Evans, J. Comp. Physiol., 138:79-85, 1980, for relevant citations). A single pregnant female of the viviparous, smooth dogfish, Mustelus canis was caught this summer and we were able to perfuse the heads of the six pups (total body weights of from 103 to 122 g) which were removed from the sacrificed female. These experiments provided us with some comparative data to determine if the differences between the adrenergic control of gill hemodynamics in S. acanthias and teleosts was the result of species differences, or differences between teleosts and elasmobranchs. In addition, we wanted to determine if heads from pups from other available species could be routinely perfused and utilized for planned studies on elasmobranch gill solute transport (Evans et al., this volume).

The heads were perfused with elasmobranch Ringer's solution (Forster et al., Comp.Biochem. Physiol. 42A:3-13, 1972) using the perfusion and irrigation circuitry as described previously for the Squalus pups (Evans and Claiborne, 1981, op. cit.). The only major changes were the use of PE 90 for the entire cannula into the conus arteriosus, and the use of

a Gilson Duograph recorder for recording afferent pressures via a pressure transducer. Long-term viability was not tested routinely, because of the small number of pups available, but the first head utilized was perfused for nearly 7 hours (with intervening testing of pressure responses to flow changes and addition of epinephrine) and the afferent pressure was nearly identical at the end (12.7 torr at a perfusate inflow of 650 μl/min) to those measured at the beginning of the experiment (13.7 torr at 650 μl/min). In order to measure the apparent resistance (Δpressure/Δflow) of the branchial vasculature we reversibly increased the afferent perfusate inflow over the range of 125 μl/min to 1150 μl/min in some experiments and measured the change in afferent pressure. We found that the vascular resistance changed over this pressure range with a slope of approximately 1.0 torr per 100 μl/min flow change at flows between 125 and 500 μl/min and a slope of approximately 0.25 torr per 100 μl/min flow change over the range of 500 to 1150 μl/min. Since the apparent resistance of the S. acanthias pup head gill vasculature was also in the range of 1.0 torr per 100 μl/min flow change over the entire range of 400 to 1250 μl/min it appears that the vascular resistance of the gills of both species of pups are quite similar, but that apparent vascular resistance in the M. canis pups falls precipitously at flows above only 400 μl/min, presumably secondary to distension of the gill blood vessels. Opdyke et al (Comp. Biochem. Physiol. 62A:711-717, 1979), had previously found that the branchial (and systemic) resistance of intact, adult S. acanthias also fell with increasing afferent inflow, and concluded that the dogfish circulation responded passively to changes in perfusion. However, our recent data on S. acanthias pups (Evans and Claiborne, 1981, op. cit.) demonstrates that neural/hormone regulation is also present.

To determine if the branchial vasculature of M. canis pups is responsive to circulating or neural catecholamines we examined the effect on afferent pressure of the addition of epinephrine and propanolol. 10^{-5}M epinephrine prompted an initial increase in afferent pressure (6.7 ± 3.4 torr, N=3; mean ± s.e.) one to three minutes after adding the epinephrine to the perfusion line (time delay caused by dead space in the perfusion line). This initial pressure increase was transitory and by 10 minutes after the drug addition the pressure had fallen 7.4 ± 2.3 torr (N=3) below the initial, control afferent pressure. This final fall in afferent pressure was not transitory (at least over a time period of 10-20 minutes), but was reversed slowly (approximately 30 minutes) when epinephrine-free Ringer's was re-introduced. The addition of epinephrine also resulted in the preferential shunting of blood into the dorsal aorta. In two fish the ratio of dorsal artery to venous effluent (D/V) increased by 724% and 211%. Addition of the beta-adrenergic blocker propranolol (10^{-5}M) to the perfusate was followed by a fall in afferent pressure to 4.9 ± 2.5 torr (N = 3) below the initial control within 15 minutes. When propranolol (10^{-5}M) was added in conjunction with epinephrine (10^{-5}M) the afferent pressure increased rapidly (1-3 minutes) to 10 ± 0.6 torr above the control (N=3), but this increase was transient; the pressures fell to 2.2 ± 1.3 torr below the control within 10-15 minutes after adding the two drugs. A transient effect of propranolol (in conjunction with epinephrine) was also found in the perfused S. acanthias pup head; however, in this case the final pressures were still above those in the control period (Evans and Claiborne, unpublished). Addition of propranolol alone reduced the D/V flow slightly (30 ± 21%, N=3), but addition of the beta blocker with epinephrine was followed by a substantial increase in the D/V flow (45-, 29- and 4-fold).

Our results indicate that epinephrine produces an initial, and transitory increase in branchial vascular resistance, which is followed by a dominant fall in resistance, At the same time, perfusate is preferentially shunted into the dorsal aorta, at the expense of flow into other vessels. The fact that propanolol blocks (albeit transiently) the fall in vascular resistance (and actually results in a greater increase in branchial resistance than that seen with epinephrine alone), but does not affect the increase in perfusion of the dorsal aorta is consistent with the proposition that vascular resistance, but not differential perfusion of the dorsal aorta, is controlled by beta-adrenergic receptors. These preliminary data are similar to those found for both freshwater and marine teleosts (Claiborne

and Evans, 1980, op. cit.), but contrast with our recent findings with the perfused S. acanthias head where both afferent pressures and preferential shunting of perfusate into the dorsal aorta are controlled by beta receptors (Evans and Claiborne, 1981, op. cit.). The fact that epinephrine addition resulted in an initial increase in branchial vascular resistance (which is increased after beta-blockade) suggests that alpha-adrenergic receptors are involved in the pressure responses to epinephrine, but definitive statements await experiments testing the effects of pheontolamine on this response.

In summary our data indicate that the head of Mustelus canis pups can be readily perfused for relatively long periods and used to examine various factors in the control of elasmobranch branchial hemodynamics. Importantly, they, like the perfused S. acanthias pup head, also represent a potential resource for investigating mechanisms of solute transport across the elasmobranch branchial epithelium (Evans et al., this volume). This research was supported by NSF PCM 81-04046.

THE DISSOCIATION OF CO_2 HYDRATION AND RENAL ACID SECRETION IN THE DOGFISH, SQUALUS ACANTHIAS

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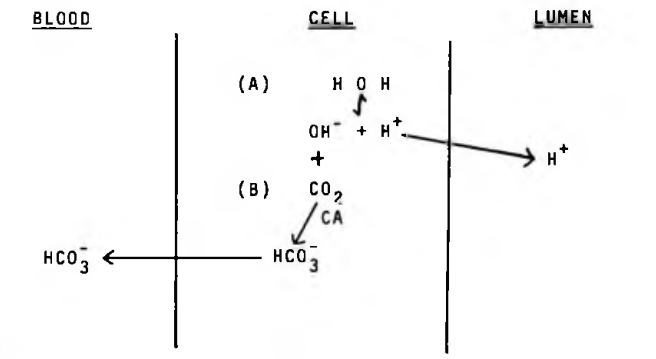
Since the discovery of renal hydrogen ion secretion and its reduction by carbonic anhydrase inhibition (Pitts and Alexander, Am. J. Physiol. 144:239, 1945), the enzyme, CO_2 and H^+ secretion have been inextricably linked. However, recent physiological work and reconsideration of the chemical reactions involved suggest that this linkage may be dissociated. The primary event in acid serretion is the formation of protons from the protolysis of water (an inexhaustible source) with translocation of H^+ toward one cell border and OH^- oppositely directed (Maren, Can. J. Physiol. Pharmacol. 52:1041, 1974). The proton is extruded into the tubular lumen and the hydroxyl ion moves into the blood (Figure 1, Step A). Our questions are: 1) how is this accomplished, and 2) is noncatalyzed or carbonic anhydrase catalyzed buffering of hydroxyl ion by CO_2 an absolute requirement for renal hydrogen ion secretion (Figure 1, Step B).

FIGURE 1

Since the discovery of renal H^+ secretion, (Pitts, 1945), the process has been linked with CO_2 hydration and carbonic anhydrase. In terms of what we now know of the chemistry of these processes, the scheme would be:



Marine fish are well suited chemically for such a study since they lack renal carbonic anhydrase, and their low body temperature and low pCO_2 result in a very slow uncatalyzed reaction. Marine fish are also well suited physiologically for such a study, since they reabsorb all filtered HCO_3^- and excrete acid at rates independent either of HCO_3^- loads or (since there is no renal enzyme) the administration of carbonic anhydrase inhibitors (Hodler et al., Am. J. Physiol. 183:155, 1955). Table 1 shows acid-base equilibria in plasma and urine of S. acanthias, both in the normal and alkalotic states.

In the present experiments we repeat the basic work of Pitts, but in S. acanthias, presenting a large buffer load to the kidney and measuring maximal rates of H^+ secretion. These rates will be compared to those of the uncatalyzed reactions of CO_2 with water, which yield H^+ ions. If the physiological rates are notably higher than the chemical, a process for H^+ secretion independent of CO_2 must be invoked.