EFFECT OF ACETYLCHOLINE ON OXYGEN UPTAKE IN THE GILL OF S. ACANTHIAS

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Oxygen uptake in the gill is dependent on the oxygen gradient between the sea water and the blood perfusing the gill as well as the surface area in the gill available for gas exchange. Venous blood entering the branchial circulation is oxygenated during its course through the respiratory lamellae. The oxygen tension of blood measured beyond the lamellae in the dorsal aorta is often lower than that of the sea water indicating less than 100% efficiency in gas exchange i.e., the presence of venous admixture or shunting in the gill. The cholinergic system is known to participate in the control of blood flow in the gill. This study was designed to characterize the magnitude of the percentage of shunting in the shark gill and to determine the effect of shunting in the shark gill and to determine the effect of acetylcholine (Ach) on this shunt fraction.

Methods

Twenty-three female dogfish sharks ranging in weight from 2.5 - 7.0 kg were used. All were anesthetized with sodium pentobarbital (20mg/kg) and heparinized (1000μ/kg). Nine fish were autoperfused and in 14 blood flow in the ventral aorta was maintained constant by a total body perfusion circuit (Kent & Peirce, Comp. Biochem. Physiol. 60C, 37, 1978). Of the 9 intact fish, 6 were exposed to sea water equilibrated with air and 3 to sea water bubbled with 100% 0_2. Nine had gills superfused with sea water bubbled with 100% 0_2 and 5 by sea water equilibrated with air. Pre and post gill blood samples were taken from the ventral and dorsal aortas respectively for measurement of PO_2 and hematocrit; PO_2 determinations were also made on pre and post gill sea water samples. Ventral and dorsal aortic blood pressures were measured.

The oxygen content of arterial and venous blood was calculated using an oxygen dissociation curve for dogfish blood (Kent et al MDIBL (14) 15, 1974). The oxygen content of blood, if it were fully equilibrated with sea water, was calculated using the oxygen dissociation curve and the average PO_2 between sea water samples taken before and after irrigating the gill. The per cent shunt was then determined using the following formula $\dot{Q}S/\dot{Q}T = Ce'0_2-Ca0_2/Ce'0_2-C_{V0_2}$ x 100 of West (Resp. Physiol.-the essentials, 1977) where $\dot{Q}S/\dot{Q}T$ = % shunt; $Ce'0_2$ oxygen content of blood equilibrated with the average PO_2 of the sea water; $Ca0_2$ and C_{V0_2} oxygen content in dorsal aortic and ventral aortic blood. The resistance to the blood flow in the gill (RG) was calculated by dividing the pressure difference between the ventral and dorsal aortas by total ventral aortic blood flow ($\dot{Q}T$).

In each fish maintained on constant flow two control measurements of blood pressures and blood gases were made at 15 minute intervals. Ach (0.1mcg/kg/min in saline at 0.1mcg/ml) was infused into the perfusion circuit leading to the ventral aorta for 30 minutes while two more sets of measurements were made at 15 minute intervals. A paired t test was used to test for significant changes during Ach infusion. An unpaired t test was used to compare the data from the group given sea water bubbled with 100% 0_2 to the data from the group receiving sea water equilibrated with air.

Results

In 9 intact fish in plain sea water the calculated percentage of blood shunting across the gill without reaching equilibrium with sea water PO_2 is 13.8 $\pm$ 1.6%. The 3 intact fish with gills superfused by sea water bubbled with 100% 0_2 have a significantly lower per cent shunt, 3.3 $\pm$ 1.5%. The gill acts as a more efficient oxygen exchanger in a higher oxygen environment.

The results of Ach infusion on oxygen exchange and blood pressures are shown in Table 1. Ach infusion has

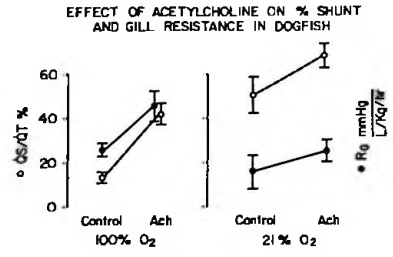
Table 1.--Effect of acetylcholine on oxygen exchange and blood pressure in perfused dogfish

	100%O_2 n = 9			Air n = 5		
	Control	Ach	%Δ	Control	Ach	%Δ
Ave PO_2 sea water mmHg	457 ±39	444 ±33	NS	151 ± 9	156 ±5	NS
Arterial PO_2 mmHg	309 ±37	181 ±45	-41	63 ±18	46 ±5	-27
Venous PO_2 mmHg	39 ±14	35 ±12	NS	31 ± 5	30 ±4	NS
$\dot{Q}T$ L/Kg/hr	0.7± 0.1	0.7± 0.1	NS	0.7± 0.1	0.7±0.1	NS
$\dot{V}O_2$ ml O_2/Kg/h	23 ± 4	17 ± 5	-24	9 ± 3	4 ±2	-53
Ca_{O_2} Vol. %	4.6± 0.2	3.2± 0.5	-30	2.8± 0.8	2.0±0.3	-29
cV_{O_2} Vol. %	1.3± 0.3	0.9± 0.3	-31	1.5± 0.4	1.3±0.4	-13
Ventral aortic press. mmHg	23.8± 1.8	34.4± 4.8	+45	21.3± 2.3	25.4±3.0	+20
Dorsal aortic press. mmHg	7.9± 1.3	6.5± 1.1	-18	10.9± 1	9.9±2	- 9

VO_2 = oxygen consumption. NS designates no significant difference at p 0.05 with paired t test between control and Ach Mean ± SEM.

no significant effect on the average PO_2 of the sea water superfusing the gill but the oxygen tension gradient between sea water and dorsal aortic blood widened from 148 to 263 mmHg in the high O_2 group and from 88 to 110 mmHg in the g roup on plain sea water. This is reflected by a 41% decrease in dorsal aortic PO_2 in the presence of Ach in the high O_2 group and a 27% decrease in the other group. Similar decreases are seen in arterial blood oxygen content. Since flow is maintained constant, oxygen delivery falls with Ach infusion as does $\dot{V}_{O2}$ in both groups. Ventral aortic pressure rises more in the high O_2 group in response to Ach infusion and dorsal aortic pressure falls slightly in both groups.

The effects of acetylcholine in % shunt and resistance to blood flow in the gill are shown in Figure 1. The



per cent shunt is significantly higher in both groups of perfused fish than in the intact fish and the fish in plain sea water demonstrate a considerably higher percentage of blood shunted through the gill than those in a high oxygen environment. In both groups acetylcholine acts to increase dramatically the percent shunt. In the high O_2 group, during Ach stimulation 42% of the blood passes through the gill without equilibrating with sea water PO_2 and in the other group almost 70% of the blood in the dorsal aorta represents venous admixture. Accompanying the large changes in % shunt are similar increases in the calculated gill resistance.

Discussion

Unanesthetized sharks normally move water from the mouth and spiracular openings in the buccal cavity by an outward movement of the gill arches. As the arches subsequently move in, water flows between the respiratory lamellae into water channels at the base of the filaments and is expelled. Normally, then, water flow is countercurrent to the flow of blood in the lamellae. In the countercurrent situation, if the gill were exchanging oxygen at 100% efficiency (zero % shunt), the blood leaving the lamellae will be completely equilibrated with the incoming sea water. Therefore the PO_2 used to calculate oxygen content of equilibrated blood for the % shunt should be the PO_2 of the incoming sea water. Anesthetized fish have no respiratory movements however, and must have irrigation of sea water across the gills performed externally. In our studies the fish were supinated and the gills were perfused with sea water through the spiracles. It is unlikely that the flow is completely countercurrent under these conditions and is more likely approximated by a concurrent flow model. The average of incoming and outgoing sea water oxygen tensions is used to estimate the oxygen content of fully equilibrated blood. Since the average drop in PO_2 across the gill is around 30 mmHg in these studies the use of average PO_2 could only underestimate the possible value by 15 mmHg. The % shunt reported may be slightly higher than actual, but this does not influence the relative differences between the groups.

An anatomically distinct blood shunt pathway around the respiratory lamellae in the gill of the dogfish has been shown (Olson & Kent, Cell Tissue Res. 209, 49-63, 1980), but its operation requires that central venous pressure exceed pressures in the post lamellar circulation. Possibly Ach acts on venous sphincters to increase pressure in the interlamellar vessels to such a degree that venous blood entering the afferent branchial circulation is shunted around lamellae by way of prelamellar AVAs to the interlamellar vessels and subsequently to the efferent branchial circulation by way of post lamellar AVAs. Ach causes firm muscle sphinter closing in excised hepatic veins in dogfish (Johansen & Hanson, J. Exp. Biol. 46, 195, 1967) and many studies indicate engorgement of the interlamellar vessels in teleost gills (Olson, SEM, 3,357, 1980; Holbert et al., J. Exp. Biol., 79, 135, 1979; Bergman et al., J. Comp. Physiol., 94, 267, 1974). It is quite likely that Ach causes a redistribution of blood flow in the gill to favor the interlamellar and collateral circulations at the expense of the lamellar, but it is questionable that this could account for the enormous venous admixture found in the efferent branchial circulation. A more likely explanation can be found by examining the compliance characteristics of the lamellar vasculature and the relationship between input pressure at the level of the lamellae and local lamellar distribution of blood flow. In lingcod the height of the intralamellar blood sheet is directly proportional to the transmural pressure in the lamella (Farrell et al., Am. J. Physiol., 239:R428, 1980) and when pressure decreases lamellar blood flow is redistributed away from the outer portions to the parts of the lamella embedded in the medial aspects of the filament. Such a redistribution tends to decrease oxygenation of blood flowing across the gill because the diffusion gradient (lamellar epithelium and pillar cell phlanges) is greater in the medial

than the outer lamella. If dogfish gills act similarly, a redistribution of lamellar flow may cause the apparent increase in the per cent of venous admixture found during Ach infusion.

The increase in resistance to blood flow with Ach infusion is to be expected since similar increases elicited by hypoxia or hypercapnia are shown to be abolished by vagotomy or atropinization in dogfish (Kent & Peirce, Comp. Biochem. Physiol., 60C, 37, 1978). The site of resistance increase is most likely prelamellar to be consistent with data from catfish showing preferential distribution of blood away from lamellar and into the interlamellar circulation with Ach stimulation. In many teleost species the collateral circulation subfuses areas such as water channels and filamental epithelium which are rich in chloride cells involved in osmoregulation. Chloride-like cells have been found in these areas in dogfish (Doyle, Bull. MDIBL 15, 27, 1975). These data suggest that the vagal reflexes such as seen in hypoxic vasoconstriction may play an important role regulating blood flow between portions of the gill involved primarily in respiration and those subserving osmoregulation. Certainly the cholinergic system in the dogfish has a profound effect on the oxygen uptake efficiency of the gill. This project was supported by funds (G7-002-01) from the Department of Surgery, Mt. Sinai School of Medicine, New York, New York. Michael Levy received an award from the Judy and Stan Fund.

AMOUNTS OF WATER, UREA, Na, AND K IN MAMMALIAN RENAL MEDULLA OF RATS AND HAMSTERS.

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The renal countercurrent system maintains a certain concentration of solutes in the renal medulla corresponding to the osmolality of the urine. When urine osmolality changes, the osmolality of the renal medulla changes. These changes are brought about by movement of solutes and water in or out of the tissue.

To elucidate the function of the countercurrent system, it is not enough to know the changes in solute concentrations that take place, but it is important to know the actual amounts of solute and water that move in and out of the various regions of the renal medulla during increases or decreases in papillary osmolality.

In these experiments these changes are compared in rats and hamsters using a new method which permits determination of water and solutes on the same piece of tissue. This approach reveals unexpected large changes in urea and water content.

A total of 33 Munich Wistar rats and 40 Syrian hamsters of both sexes were used in these experiments. (Since the results from the various groups were quite similar, the results from two groups only will be shown here.) The animals were maintained on a normal diet prior to sacrifice. Also prior to sacrifice the urine flow as manipulated as follows: some of the animals were made diuretic (lettuce feeding or water by stomach tube), some antidiuretic (water deprivation) and some intermediate (by giving water ad libitum).

The animals were killed in $C0_2$, the kidneys removed and the renal medulla divided into three parts: outer medulla (OM), inner medulla (IM) and (M_2) (see Fig. 3).

Water fraction (F_{H_20}) was determined by weighing the tissue after drying for 8 hours at 60°C. No urea and solutes are lost at this temperature (Schmidt-Nielsen et al., in preparation). Dried tissue was weighed and placed in 25 μl H_20 in plastic tubes which were then tightly capped and heated to 100°C. Analyses were made on supernatant 18-24 hours later. Calculating mg H_20 (or μl) per mg solute free dry tissue (s.f.d.t.):

$$\mu l\ H_2O/mg\ s.f.d.t. = \frac{F_{H_2O}}{1 - F_{H_2O} \times [(1 + osm \cdot 0.035)]}$$

(Schmidt-Nielsen, Am. J. Physiol., 230: 514-521, 1976).