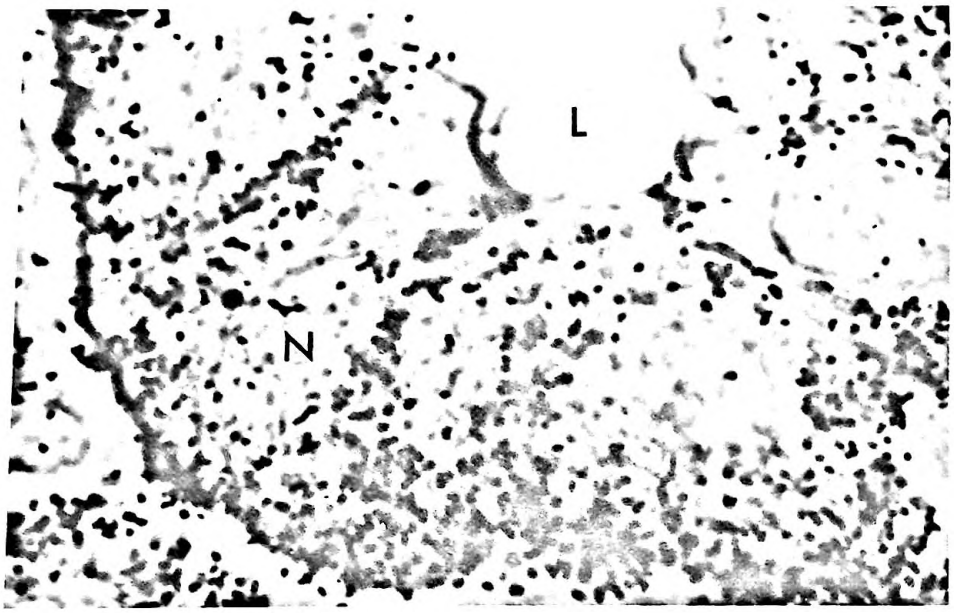

Figure 2. ^{3}H-ouabain autoradiograph of dogfish rectal gland (incubated 2 h in medium containing 5 μM radiolabeled ouabain, 60 μCi/ml) showing good morphological preservation of a secretory tubule in cross-section. Many silver grains (black dots) are located over the regions occupied by basal infolds or lateral plasma membranes and essentially no grains over the membrane bounding the tubule lumen (L), the cell nuclei (N), or the mitochondria (hazy gray dots) packed between basal folds. X 2000.

EFFECT OF PARASYMPATHECTOMY ON THE RESPONSE TO HYPOXIA IN *Squalus acanthias*

Barbara Kent, Jean-Francois Eid, Michael Levy and E. Converse Peirce II, Department of Surgery, Veterans Administration Hospital, Bronx, New York; and Mt. Sinai School of Medicine, New York, N.Y.

Neural control of the cardiovascular system in the dogfish *S. acanthias* is almost completely by way of the vagus nerve (Satchell, Circulation in Fishes, Cambridge Univ. Press, 1971; Johansen K, Ann. Rev. Physiol., 1971). The vagus is the efferent pathway of a reflex bradycardia and increased resistance to blood flow across the gills induced by an increase in CO_2 in the sea water perfusing the gills (Bull. MDIBL 9:13, 1969). When O_2 tension in sea water is lowered there is also a decrease in heart rate and cardiac output accompanied by an increase in gill resistance (Bull. MDIBL, Vol. 15; Kent et al., Cardiovascular responses to hypoxia in *S. acanthias*, 1975). In the present study the role of the parasympathetic nervous system in the response to hypoxia was assessed.

Thirteen fish of either sex ranging in weight from 1.7 to 7.0 kg were used. They were surgically prepared for measurement of cardiac output ($\dot{Q}_B$) by an electromagnetic flow probe (18-25 mm circumference, Carolina Medical) placed on the conus arteriosus (Bull. MDIBL 8:20, 1968). Catheters were placed in the ventral aorta and dorsal aorta for pressure recording (VAP and DAP) and for drawing venous and arterial blood samples. Pressures and flow were recorded simultaneously on a

2-Channel Beckman dynograph recorder. Heart rate (H.R.) was counted from the pressure pulse. Resistance to blood flow across the gills (R_G) was calculated as $R_G=\overline{VAP}-\overline{DAP}/\dot{Q}_B$ and systemic resistance as $Rs=\overline{DAP}/\dot{Q}_R$. R_G and Rs are expressed in peripheral resistance units (PRU=mmHg/L/Kg/hr). Duplicate arterial and venous blood samples were taken at ten-minute intervals in heparinized syringes for measurement of oxygen content (Lexington Instrument oxygen analyzer, Lex-O_2-con), pO_2 (Clark type electrode and Radiometer gas monitor), pH (micro-pH electrode and Radiometer pH meter) and hematocrit (micro-hematocrit centrifuge). All fish were exposed to the "control" protocol in which gills were flushed with fresh 15°C sea water (3L/min) equilibrated through a bubble oxygenator (Seal Corp.) with 100% O_2 for ten minutes. This was followed by a ten-minute period in which sea water was equilibrated with 100% N_2. Finally the sea water was again equilibrated with 100% O_2. Blood samples were taken at the end of each 10 minute period and hemodynamic responses to hypoxia were recorded.

The fish were then divided into two groups. Surgical vagotomy was done on 6 fish via the oro-pharyngeal approach to the ventral aspect of the brain stem. The vagi were sectioned at the medullary junction. In another group of 7 fish a pharmacological vagotomy was accomplished by injection into the dorsal aorta of 12 mg/Kg atropine. Both groups of fish were then again exposed to 10 minutes flushing with sea water equilibrated with 100% N_2, bracketed by 10 minutes of exposure to sea water and 100% O_2. Paired data were compared for statistical differences by the paired t-test.

The effects of vagotomy and atropine can be seen in Figure 1. With both the heart rate is almost doubled over control values. There is no significant change in the pressure drop across the gills ($\Delta P=\overline{VAP}-\overline{DAP}$) and only in the atropinized fish was there a significant decrease in cardiac output.

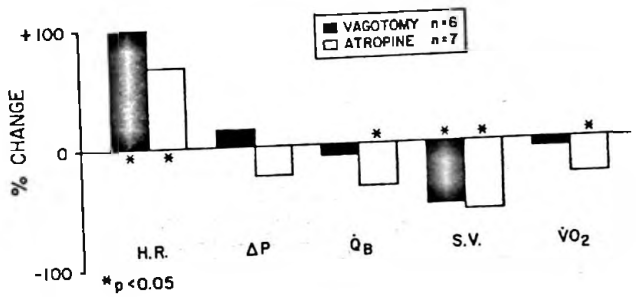

Figure 1. Percent change from control of variables after atropine and vagotomy.
*indicates $p < 0.05$.

Stroke volume in both groups decreased significantly and the oxygen consumption in the fish receiving atropine decreased by 30%. Although there were changes in gill and systemic resistance in individual fish after vagotomy and atropine there was no consistent directional change and hence no statistically significant change. Arterial and venous pH and pO_2 were unchanged by the parasympathectomy.

The effects of 100% N_2 in the sea water on hemodynamic variables and blood gases in the control group were similar to those described in Bull. MDIBL, Vol. 15, 1976; Kent et al., Cardiovascular Responses to Hypoxia in *S. acanthias*. In Table 1 it can be seen that although there

TABLE 1

Blood gases during sea water equilibration with 100% O_2 and 100% N_2

	Control 13		Vagotomy 6		Atropine 7	
	100% O_2	100% N_2	100% O_2	100% N_2	100% O_2	100% N_2
Pa_{O_2}	359	28	280	34	347	24
mmHg	±42	±11	±30	±7	±40	±5
$P\bar{v}_{O_2}$	23	9	20	9	20	11
mmHg	±7	±1	±7	±3	±2	±3
$\dot{V}$ O_2	0.40	0.06	0.41	0.13	0.20	0.04
ml/Kg/min	±0.20	±0.02	±0.20	±0.04	±0.09	±0.02
pH (arterial)	7.41	7.41	7.39	7.43	7.36	7.40
	±0.08	±0.10	±0.07	±0.13	±0.11	±0.12

All values are given as mean ± standard deviation.

is a precipitous fall in blood oxygen tension and oxygen consumption when 100% N_2 equilibration begins, there is essentially no difference in oxygen tension or pH in the three groups. The pH does not change with the development of hypoxia. There is, however, a marked difference in the hemodynamic response to hypoxia before and after parasympathectomy. In the intact control group hypoxia elicited a greater than 100% increase in gill resistance (Figure 2) and a 40-70% decrease in heart rate. There was a significant increase in the pressure drop across the gills, while cardiac output tended to decrease. After both atropine and vagotomy there was no longer the dramatic change in resistance across the gills during hypoxia and the heart rate response was abolished.

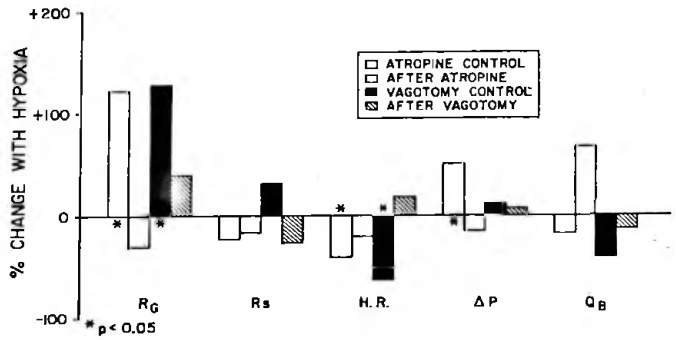

Figure 2. Percent change between 100% O_2 and 100% N_2 before and after vagotomy or atropinization.
* indicates $p < 0.05$.

In intact fish the response to hypoxia is almost identical to the response to hypercapnia. The response to hypercapnia can be maintained for several hours, but prolonged periods of hypoxia

will, of course, lead to irreversible deterioration of the fish. In the first ten minutes, the responses are remarkably similar. Since response to both hypoxia and hypercapnia is abolished by vagotomy or atropinization, the efferent pathway is the same. That the same central chemoreceptors are stimulated is only speculation. Hypoxemia may create local pH imbalance which in turn could stimulate receptors sensitive to high CO_2 tension. The lack of increase in resistance with hypoxia after parasympathectomy suggests there is no local constrictor effect of hypoxemia in the fish gill as there is in the vasculature of the mammalian lung.

From studies on the isolated perfused teleost gill it is known that acetylcholine increases the pressure drop across the gill (Comp. Biochem. Physiol., 12:127-142, 1964). Presumably blood is shunted away from respiratory lamellae through non-respiratory shunts to raise the pressure. Anatomical shunts have not been demonstrated in *S. acanthias*, but vagal innervation has been described for both basilar and distal arteries which deliver blood to and from the lamellae. If the increase in gill resistance in the spiny dogfish in response to hypoxia and hypercapnia is accompanied by a decrease in lamellar perfusion, then a reflex sensitive to post-gill blood gas changes might have great survival potential. Instead of losing oxygen as well as other solubles in the blood across the gills in a low pO_2 environment, the fish may simply decrease perfusion of the gill and the functional surface area until a more favorable environment is reached and pO_2 in the arterial blood increases. Studies quantitating the functional surface area of the gill during changes in gill resistance will be interesting.

This work was supported by the Veterans Administration Hospital, Bronx, New York. Project # 7240-03.

PREPARATION AND ENZYMATIC PROPERTIES OF BRUSH BORDER AND BASAL-LATERAL MEMBRANES FROM FLOUNDER KIDNEY TUBULES

Rolf Kinne, Rudolf Maier, Jill Eveloff, William Kinter, Max Planck Institute for Biophysics, Frankfort, West Germany and Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

Isolated flounder kidney tubules, first described by Forster in 1948 (Science, 108:65-67), have proved valuable for studying tubular transport, particularly active secretion of organic acids. Using rat kidney, recent studies with purified luminal and contraluminal plasma membranes have extended our understanding of the cellular and molecular mechanisms underlying transepithelial transport (Kinne, In: MTP International Review of Science, Kidney and Urinary Tract Physiology, Vol. II, edited by K. Thurau, Baltimore University Press, in press). Corresponding experiments with flounder kidney membranes are lacking and the present study was undertaken to evaluate procedures for purifying brush border (luminal) and basal-lateral (contraluminal) membranes from flounder tubules. Membranes separated by free-flow electrophoresis were compared with brush border membranes prepared by differential centrifugation. These membrane preparations were characterized by the presence of marker enzymes.

For the initial homogenate, kidneys of 6-8 flounder (*Pseudopleuronectes americanus*) were excised, chilled in ice-cold Forster's medium, and cut into small pieces with scissors. The tubules were released from the haematopoetic tissue by suction of the tissue suspension through a syringe and separated by low speed centrifugation. Freed tubules were homogenized to a 10 gm% homogenate in sucrose-Tris buffer (250 mM sucrose - 10 mM triethanolamine-HCl, pH 7.6 at 20°C). In accordance with the free-flow electrophoresis technique of Heidrich et al. (J. Cell Biol., 54:232-245, 1972)