since the inhibitions of J_{me}^{Cl} and J_{me}^{Na} elicited by Na- or Cl-free media or by furosemide are approximately equal. Humphreys (Am. J. Physiol. 230:1517, 1976) has reached similar conclusions with regard to the inhibition of NaCl absorption elicited by furosemide using in vivo rat ileum. Indeed, neutral NaCl transport processes have been suggested for gastrointestinal epithelia of a variety of species, ranging from arthropods through mammals, and direct evidence for a coupled NaCl influx process at the mucosal membrane has been obtained for rabbit gallbladder and small intestine (Frizzel et al., J. Gen. Physiol. 65:769, 1975 and Nellans et al., Am. J. Physiol. 225:467, 1973). Thus, as suggested for rabbit gallbladder, the energy required for active Cl absorption by flounder intestine may be derived from the Na gradient across the mucosal membrane. Information on the electrical potential profile and cellular Na and Cl activities is necessary to evaluate this possibility. In agreement with the findings reported here, cyclic nucleotides inhibit neutral NaCl influx mechanisms in gallbladder and small intestine; in flounder intestine, an increase in transepithelial Cl permeability also occurs.

Finally, these results lend further support to the notion (Field et al., J. Membrane Biol. in press) that a dissociation of neutral transcellular NaCl transport occurs at the level of the paracellular pathways leading to a preponderance of transepithelial Cl over Na absorption under short-circuit conditions. In this regard, it is of interest that a ouabain and furosemide-inhibitable active Cl pump has been postulated for the thick ascending limb of the loop of Henle which also displays a lumen-positive PD (Burg and Green, Am. J. Physiol. 224:659, 1973 and Burg, Kidney Internat. 9:189, 1976). The possibility of neutral NaCl transport modified by the paracellular pathway seems worthy of investigation in this nephron segment in view of the common physiologic and pharmacologic characteristics of these epithelia.

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FURTHER EVIDENCE THAT *Squalus acanthias* LACKS ANGIOTENSIN II ACTIVATED VASCULAR RECEPTORS

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Previous work from our laboratory reported that perfusion with perfusates containing angiotensin II (AII) did not result in an increase in blood flow resistance in the isolated dogfish gut preparation (Opdyke and Holcombe, MDIBL Bulletin 16:84-86, 1976). However, marked increase in flow resistance resulted when either epinephrine or norepinephrine was perfused in the same preparations. These results were interpreted as additional evidence that the pressor response to AII observed in the intact dogfish results solely from release of adrenergic catecholamines by AII (Opdyke and Holcombe Am. J. Physiol. 231:1750-1753, 1976). We have extended this study by observing the effect of AII, norepinephrine and isoproterenol on blood flow resistance in the gill and systemic circulations of dogfish whose hearts were replaced by a calibrated mechanical pump which perfused the circulation of the otherwise intact fish at known rates of flow.

Methods

Male and female dogfish averaging 3.2 Kg in weight were anesthetized with 20 mg/Kg pentobarbital sodium through an indwelling catheter introduced into the dorsal aorta via the caudal artery. The catheter served also for the recording of dorsal aortic pressure (DAP). The fish were placed belly up in a V-shaped trough and the gills bathed with cold running sea water through tubes inserted into both spiracles. The pericardial sac was carefully opened and, following heparinization (8 mg/Kg), a large polyethylene catheter (PE 280), which was filled with dogfish blood and connected to the perfusion pump, was tied into the conus arteriosus. Another large catheter (PE 320) was introduced into the atrium retrograde from the ventricle and tied

securely in place so that all systemic venous drainage exited from this catheter into a reservoir from which the re-oxygenated blood (95% O_2-5% CO_2) was recirculated via the pump. Additional blood for priming and drug testing (250-350 ml) was obtained from donor fish. Ventral aortic pressure (VAP) was recorded through an 18 gauge needle inserted into the conus arteriosus catheter near its terminal opening. The atrial catheter was also equipped with a similar needle for recording systemic venous outflow pressure (VOP). All recording was done by Statham P23AA transducers and an Electronics-for-Medicine IR 4 recorder. The unstressed manometer zero pressure of all transducers was carefully set at the level of the atria and each record was calibrated statically against a mercury manometer. For each fish an initial perfusion rate was selected which resulted in a mean dorsal aortic pressure which was well with in the normal range for an intact heart-perfused fish (20-30 mm Hg). The perfusion rates ranged from 0.92 to 1.92 ml/sec.

The drugs were added directly to 100 ml blood in the reservoir resulting in the concentrations indicated in Table 1. The actual concentration of a drug in the total volume of perfusate (reservoir volume plus blood volume of the fish) could not be easily determined accurately, but, based on calculations of dogfish blood

TABLE I

EFFECT OF ANGIOTENSIN II, NOREPINEPHRINE AND ISOPROTERENOL ON RESISTANCE TO BLOOD FLOW IN THE GILL, SYSTEMIC AND WHOLE BODY CIRCULATIONS OF THE DOGFISH

RESISTANCE DYNES SEC/CM 5		ANGIOTENSIN II 1 µG/ML * N = 7	NOREPINEPHRINE 1 µG/ML * N = 10	ISOPROTERENOL 1 µG/ML * N = 5
R_G (GILLS)	CONTROL	16033 ± 13554	181991 ± 14638	21407 ± 9218
	RESPONSE	16142 ± 13647	12476 ± 8887	16674 ± 6795
	Δ %	100.7 ± 5.3 %	65.7 ± 2.6 %	77.9 ± 12.6 %
		(N.S.)	P < 0.001	P < 0.05
R_{TPR-G} (SYSTEMIC)	CONTROL	22224 ± 7987	21466 ± 6481	42350 ± 13042
	RESPONSE	23450 ± 9098	32008 ± 10577	3338 ± 9678
	Δ %	105.1 ± 7.2 %	149.1 ± 15.4 %	78.8 ± 6.8 %
		(N.S.)	P < 0.001	P < 0.05
R_{TPR} (WHOLE BODY)	CONTROL	38298 ± 18978	40458 ± 18047	21370 ± 6107
	RESPONSE	39475 ± 19013	44602 ± 17820	15895 ± 4730
	Δ %	103.1 ± 5.8 %	112.8 ± 13.8 %	74.4 ± 4.6 %
		(N.S.)	(N.S.)	P < 0.001

* SEE TEXT

volume, approximated 0.3-0.4 µg/ml. The blood was allowed to recirculate for 5-10 minutes, or until any induced change in arterial pressures had returned to control level. The blood in the reservoir was then replaced with an aliquot of fresh blood before the next trial. The order in which drugs were tested was: AII first, then either norepinephrine or isoproterenol. If sufficient blood was available all three drugs were tested in the same preparation.

Resistance to blood flow was calculated in cm/gm/sec units by the usual equation: $R = \frac{\Delta P}{Q} \times 1332$ where R = resistance in dynes sec/cm^5; ΔP = the pressure gradient across the vascular bed; Q = the flow in ml/sec through the bed, and 1332 is the factor for converting the quotient to the metric system of units. Since ΔP could be determined for the gill circulation (VAP-DAP), the systemic circulation (DAP-VOP) and the whole body circulation (VAP-VOP), and, since the flow through each series-connected circuit was the same (and equal to pump output), the resistance to blood flow could be calculated for the gill circulation (R_G), the systemic circulation (R_{TPR-G}) and the whole body circulation (R_{TPR}).

Results

Table 1 summarizes the results of the investigation. Angiotensin II did not increase resistance to blood flow significantly in either the gill or systemic circulations . Norepinephrine, on the other hand, decreased gill resistance markedly but, at the same time, increased systemic blood flow resistance. Isoproterenol decreased resistance to blood flow in both gill and systemic circulations.

Discussion

These results confirm the conclusions drawn from the experiments on isolated dogfish gut. With a fixed vascular inflow (cardiac output) angiotensin II does not elicit a pressor response as it does in the intact fish. The pressor response to AII observed in intact fish must, therefore, be due to an increased cardiac output. Since, in the intact fish, heart rate does not increase significantly in response to AII, any increase in cardiac output must be due to an increase in ventricular stroke volume. Stroke volume could increase as a result of either increased venous return (venoconstriction, or recruitment of additional plasma volume) or increased myocardial contractility. These experiments do not shed light on that aspect of the problem, but experiments on isolated dogfish hearts show that AII does not increase myocardial contractility (Opdyke and Holcombe, unpublished observations). They do, however, offer additional evidence that AII is not a powerful vasoconstrictor in either the gill or systemic circulations of the dogfish. And, indeed, it now appears unlikely that the dogfish has evolved a vascular receptor for AII which mediates vasoconstriction.

The responses to norepinephrine and isoproterenol are in complete accord with the findings of other investigators (D. J. Randall. In Fish Physiology, Vol. 4, Chap. 4, page 166. Academic Press, New York, 1970). They do serve the purpose of showing that the pump-perfused preparations were capable of significant vasomotion.

Considerable variation in the calculated control resistances was observed in these experiments. This is explained partly by differences in fish size and initial hematocrits. The greater control resistances calculated for total peripheral resistance (R_{TPR}) are accounted for by the fact that gill and systemic resistances are arranged in series in the dogfish and, therefore, are summed algebraically. Hence, the change in R_{TPR} in response to norepinephrine is non-significant because gill resistance decreased to nearly the same extent as systemic resistance increased.

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