Potassium excretion was variable. Although each fish that received chlorthiazide demonstrated an increase in potassium excretion, indicating some physiologic response to the drug, the same was not the case with those diuretics that induced natriuresis. With the latter group of drugs potassium excretion usually decreased if potassium excretion was high during the control periods but increased if potassium excretion was less than 10 μEq/Kg/Hr during the control periods.

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1970 #31

EFFECT OF SPINAL CORD STIMULATION AND SYMPATHOMIMETIC DRUGS ON DORSAL AORTIC PRESSURE IN Squalus acanthias

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Descriptions of a neuronal system in elasmobranchs corresponding to the sympathetic division of the mammalian autonomic system are nebulous. Squalus acanthias exhibits vascular responses to sympathomimetic drugs and possesses abundant chromaffin tissue. This suggests that some degree of sympathetic control or regulation of peripheral circulation may occur. Experiments were conducted in an attempt to establish whether peripheral vascular responses could be elicited by stimuli which produce sympathetically mediated reflex vascular responses in mammals.

The spinal cord was stimulated in thirteen spino-medullary transected dogfish using faradic stimuli of 5-15 volts at 2-50 Hz. The gills of the fish were perfused with 2-4 L/min cold sea water via the spiracles. The stimulating electrode was introduced into the spinal canal and advanced from the tail region rostrally in 2 cm steps to the point of spinal transection (or beyond in some cases). No significant changes in dorsal aortic pressure or gill movement rate could be elicited by cord stimulation. Inhibition of the heart rate and gill movements could be elicited by stimulation just above the site of transection (in the fourth ventricle). Failure to elicit vascular or respiratory responses by direct stimulation of the spinal cord is presumptive evidence against the existence of a sympathetic autonomic control of the peripheral vascular system.

Shark Ringer's saline was rapidly infused into the dorsal aorta of six fish in an attempt to actuate vascular baroreceptor reflexes by volume loading since the efferent limb of such reflexes involve autonomic nervous system activity. The rapid injection of 30 ml saline in 5 ml increments resulted in an increase in dorsal aortic blood pressure in 3 fish but no decrease in heart rate or gill movement rate. No increase in blood pressure was observed in the other fish, nor did heart rate change. The decrease in hematocrit was consistent with the volume of saline infused. These experiments gave no evidence of sympathetic autonomic control.

If the dogfish possesses a sympathetic neuronal control of its peripheral circulation then it should respond to neurotransmitters, their blocking agents, ganglionic stimulators and blockers in a fashion similar to that observed in mammals. Table 1 presents the effects of several sympathetically acting drugs on dorsal aortic blood pressure, heart and gill movement rates in

Table 1

EFFECT OF SYMPATHOMIMETIC DRUGS ON DORSAL AORTIC PRESSURE, HEART RATE AND GILL MOVEMENT RATE OF 1 - 1-1/2 KGM DOGFISH (*Squalus acanthias*). ALL INJECTIONS WERE MADE INTRA-ARTERIALLY.

Test No.	Drug treatment and comment on the most significant response	N	Drug(s), sequence and dose(s)	B.P./mmHg systolic/diastolic†		H.R./min.*		G.M.R./min.*	
				Control	Max. response (t) after inj.	Control	Max. response (t) after inj.	Control	Max. response (t) after inj.
1	Norepinephrine[1] gives a pressor response	9	200 ugm	28 ± 1.4 22 ± 0.9	39 ± 2.0 ** 30 ± 1.1 ** (2')	25 ± 1.5	28 ± 2.5 X (2')	40 ± 1.8	40 ± 2.7 X (2')
2	Phentolamine[2] gives a depressor response	20	2 mgm	28 ± 1.3 22 ± 1.0	20 ± 1.0 ** 17 ± 0.7 ** (2-1/2')	30 ± 1.6	30 ± 2.1 X (2-1/2')	50 ± 1.4	50 ± 1.6 X (2-1/2')
3	Phentolamine blocks the pressor effect of norepinephrine	5	As above	17 ± 1.6 15 ± 1.2	17 ± 1.8 X 15 ± 1.6 X (2')	32 ± 2.1	47 ± 5.6 *	49 ± 2.7	53 ± 4.2 X
4	Propanolol[3] does not block the pressor effect of norepinephrine	5	Pro. 2 mgm norepi. 200 ugm	28 x 2.5 21 x 1.7	35 ± 3.6 * 26 x 2.6 * (2-1/2')	23 ± 0.8	20 ± 1.2 X (2-1/2')	42 ± 2.6	43 ± 2.7 X (2-1/2')
5	Isoproterenol[4] gives a depressor effect	5	200 ugm	24 ± 0.7 20 ± 0.5	18 ± 0.9 ** 16 ± 0.7 ** (4')	26 ± 2.4	28 ± 3.0 X (4')	49 ± 5.0	52 ± 3.4 X (4')
6	Propanolol alone exerts little effect	5	2 mgm	20 ± 2.1 16 ± 1.2	21 ± 2.0 X 18 ± 1.6 X	25 ± 3.6	23 ± 3.3 X	52 ± 4.3	49 ± 4.5 X
7	Isoproterenol 3' after propanolol may give a pressor response	5	Pro. 2 mgm isopro. 200 ugm	21 ± 2.0 18 ± 1.6	28 ± 4.3 X 22 ± 2.7 X (4')	23 ± 3.3	27 ± 3.3 X (4')	49 ± 4.5	50 ± 4.6 X (4')
8	Hexamethonium[5] does not produce an effect	10	10 mgm	28 ± 2.1 22 ± 1.4	28 x 2.1 X 22 ± 1.1 X (4')	32 ± 2.8	34 ± 1.9 X	45 ± 1.5	44 ± 1.6 X
9	DMPP[6] results in a pressor response with marked cardiac slowing and respiratory depression	3	1.5 mgm	26 ± 2.6 20 ± 0.7	37 ± 3.6 X 25 ± 2.4 X (2')	27 ± 7.6	17 ± 3.6 X (2')	47 ± 1.4	Depressed (2') Stopped (10')
10	Atropine[7] increased heart rate with only slight effect on blood pressure and GMR	16	2 mgm	27 ± 1.4 21 ± 1.0	29 ± 1.7 X 23 ± 1.1 X (1'-5')	24 ± 1.9	38 ± 2.4 ** (1'-5')	43 ± 3.0	42 ± 2.8 X (1'-5')
11	DMPP 5' after atropine gives only a pressor effect	5	DMPP 1.5 mgm Atrop. 2 mgm	30 ± 1.4 26 ± 1.2	41 ± 2.4 ** 32 ± 1.6 * (2')	37 ± 1.9	34 ± 2.4 X (2')	47 ± 2.5	45 ± 3.9 X (2')
12	DMPP 4' after hexamethonium is ineffective in producing a pressor response	10	DMPP 1.5 mgm Hexa. 10 mgm	27 ± 1.6 22 ± 1.1	29 ± 1.4 X 23 ± 1.1 X (4' after hexa)	33 ± 1.8	34 ± 1.8 X (4')	45 ± 1.4	50 ± 1.9 X (4')
13	Phentolamine, with or without atropine, prevents the pressor response to DMPP (1.5 mgm)	9	Phentol. 2 mgm Atrop. 2 mgm (in 4 fish)	17 ± 0.4 15 ± 0.4	16 ± 0.0 X 14 ± 0.8 X (4' after phent)	31 ± 1.4	29 ± 0.5 X	52 ± 2.3	Depressed
14	Atropine and propanolol together do not prevent the pressor response to DMPP	5	Atrop. 2 mgm Prop. 2 mgm DMPP 1.5 mgm	25 ± 1.5 20 ± 1.3	31 ± 1.8 * 34 ± 1.6 X (4' after DMPP)	34 ± 1.5	32 ± 2.7 (4')	41 ± 13.8	44 ± 14.6 X (4')

1, norepinephrine - an alpha receptor stimulator; 2, phentolamine - an alpha receptor blocker; 3, propanolol - a beta receptor blocker; 4, isoproterenol - a beta receptor stimulator; 5, hexamethonium - an autonomic ganglia blocker; 6, DMPP - (1, 1-Di-methyl-4-phenylpiperazinum) - an autonomic ganglia stimulator; 7, atropine - cholinergic effector junction blocker.

*$p < 0.05$.

**$p < 0.01$. X = non-significant response.

†± one standard error.

unanesthetized, unrestrained fish. The order in which the drug responses are arranged in Table 1 shows that:

1. Norepinephrine produces a pressor response which is blocked by phentolamine, but not by propanolo. (Indicating the presence of sympathetic alpha receptors.)

2. Isoproterenol gives a depressor effect which tends to be reversed by propanolol. (Indicating presence of beta receptors.)
3. Hexamethonium (a ganglionic blocker) has no effect on blood pressure (presumptive evidence of absence of sympathetic vasoconstrictor tone), but DMPP (1-1 dimethyl-4-phenylpiperzinium), a ganglionic stimulator, caused a pressor effect which was blocked both by hexamethonium (ganglionic blockade) and by phentolamine (neuroeffector junction blockade).
4. DMPP stimulated parasympathetic ganglia resulting in depression of heart and gill movement rate. This effect was abolished by atropine. Otherwise, there was no evidence of autonomically mediated reflexes affecting heart or gill movement rates with the exception of an increase in heart rate caused by norepinephrine after phentolamine.

The drug experiments demonstrate that the dogfish has the capacity to respond to sympathetically active drugs in a fashion similar to mammals. The sympathetic ganglion-post-ganglionic, sympathetic neuron-neuro-effector structure appears to be developed. However, no evidence of operational sympathetic reflexes affecting the peripheral circulation (dorsal aortic system) could be observed. It is tentatively concluded that such reflexes have not yet developed in this primitive species.

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1970 #32

EFFECTS OF CATECHOLAMINES, SEROTONIN AND OTHER DRUGS ON GILL AND SYSTEMIC VASCULATURE OF S. acanthias

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The actions of drugs in S. acanthias are of interest both from the standpoint of comparative pharmacology and of studies of the hypothermic state. Since it is often difficult to distinguish between cardiac and peripheral drug actions, even when cardiac output is known, there are distinct advantages in using a perfusion preparation. Where drugs are given in intact animals, changes in cardiac output, due to direct cardiac action or to baroreceptor reflexes, render interpretations difficult (Opdyke and Opdyke, Bull. MDIBL 9:44, 1969). The series arrangement of gill and systemic resistances in the dogfish makes it necessary to monitor both ventral and dorsal aortic pressures (VAP and DAP). The varying condition of the fish, the relatively prolonged effects of some drugs, the relatively large doses that are required, and the occurrence of tachyphylaxis, make it very difficult to obtain dose-effect curves in intact preparations. Another significant problem in the intact fish is the rather large changes that occur very rapidly after minor volume loading (Figure 1).

A perfusion preparation incorporating a membrane lung was described last year (Bull. MDIBL 9:45, 1969). Venous drainage is obtained from the left ventricle and blood is returned at a controlled rate into the ventral aorta. Drugs are injected into the ventral aorta instead of the dorsal aorta to insure distribution in the systemic circulation of the fish. The membrane lung was deleted from the studies this year because the gas exchange capability was not needed.