Gastric Secretion, Ed. Sachs, G. et al., Academic Press, N.Y., pp. 111-130, 1972). "Stat" titration to a pH of 4.5 underestimates the H^+ ion secretory rate but whether it does so consistently merits study. (Supported by USPHS grant AM 19020.)

NEUROGENIC CONTRIBUTIONS TO VASOMOTOR CONTROL IN *Squalus acanthias*

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Previous work from our laboratory suggested that vasomotor tone is present in the dogfish, *Squalus acanthias* (Opdyke et al., Comp. Biochem. Physiol. 42A:611-620, 1972). Further evidence is presented here with an analysis of possible neurogenic contributions to overall peripheral vasomotor control. Experiments were conducted to determine the level of neurogenic control by observing changes in blood pressure and heart rate under the following circumstances: 1) Freezing the brain to eliminate contributions to vasomotor tone by the higher central nervous system; 2) Administration of atropine, a cholinergic neuro-effector junction blocking agent; 3) Administration of the general ganglionic blocking agent, hexamethonium; and 4) Administration of the alpha receptor blocking agent, phentolamine.

It was also of interest to study the effect of angiotensin II (AII) under each of the above conditions. AII has been shown previously to elicit a pressor response in dogfish (Opdyke and Holcombe, Am. J. Physiol. 231:1750-1753, 1976). The possibility that a neural mechanism is involved in AII action has been explored here.

Male and female dogfish, weighing 1-6 kg were caught by trawl line in Frenchman Bay, Maine and kept in live cars 2-5 days before use. An indwelling Intramedic PE 60 catheter was placed in the caudal artery to monitor phasic dorsal aortic pressure (DAP) and heart rate (HR) with a P23AA Statham transducer and an Electronics-for-Medicine IR 4 recorder. All injections of AII, atropine, hexamethonium and phentolamine were made through this catheter. Fish undergoing brain-freeze operations were first injected with 15 mg/kg pentobarbital sodium intravenously which rendered the fish quiescent during the surgical procedures. In addition, procaine was infiltrated generously at the site of the cranial operation. Dogfish receiving only atropine, hexamethonium or phentolamine injections were unanesthetized.

All experiments were performed with the fish belly down in a V-shaped trough with the gills bathed in cold running seawater through tubes inserted in each spiracle. In the brain-freeze series, the brain was completely exposed from the anterior edge of the forebrain to the spino-medullary junction. Liquid nitrogen was applied liberally to all exposed surfaces of the brain and freezing occurred instantaneously. The brain was kept frozen throughout the remainder of the experiment by frequent applications of liquid N_2.

Data from experiments dealing with the maintenance of vasomotor tone in the dogfish were summarized in Table 1. In 5 fish there was no significant lowering of either systolic or diastolic pressure nor any change in heart rate as a result of freezing the brain. The previously reported effect of atropine on heart rate (Burger and Bradley, J. Cell. Comp. Physiol. 37-38:389-402, 1951) was again confirmed in 5 fish. Hexamethonium mimicked the effect of atropine by giving a highly significant elevation of heart rate from 21.6 ± 5.0 to 33.4 ± 2.2 beats/min.

Phentolamine injections were carried out on 13 fish following injection of either atropine or hexamethonium, or as a single initial injection. Phentolamine caused no significant change in HR in any case, but it did induce a highly significant decrease in both systolic pressure (from 31.3 ± 5.6 to 16.6 ± 2.5 Torr and diastolic pressure (from 26.3 ± 5.6 to 14.4 ± 2.8 Torr) in all trials.

Table 2 summarizes results of Angiotensin II (Beckman Instrument, Palo Alto, California) injections (20-40 µg) in the brain-freeze experiments. AII gave a highly significant elevation of both

TABLE 1

Effect of Brain-freeze and Autonomically Active Drugs on Blood Pressure and Heart Rate in *Squalus acanthias*

TREATMENT	N	CONTROL Mean ± 1 SE	RESPONSE Mean ± 1 SE
Brain-freeze	5		
Systolic pressure		23.2 ± 4.5	23.2 ± 4.6
Diastolic pressure		21.6 ± 3.6	21.8 ± 4.0
Heart rate		32.9 ± 5.9	31.8 ± 5.8
Atropine 0.1 mg/kg	5		
Systolic pressure		44.2 ± 8.9	40.3 ± 10.0**
Diastolic pressure		30.8 ± 6.4	33.7 ± 7.6*
Heart rate		17.6 ± 5.3	39.6 ± 5.7***
Hexamethonium 10 mg/kg	5		
Systolic pressure		39.3 ± 5.1	33.0 ± 3.5***
Diastolic pressure		33.1 ± 3.8	29.4 ± 3.2*
Heart rate		21.6 ± 5.0	33.4 ± 2.2***
Phentolamine 0.4 mg/kg	13		
Systolic pressure		31.3 ± 5.6	16.6 ± 2.5***
Diastolic pressure		26.3 ± 5.6	14.4 ± 2.7***
Heart rate		29.8 ± 8.7	30.4 ± 7.8

Pressures in Torr; heart rate in beats/min; *p < 0.05; **p < 0.01; ***p < 0.005

TABLE 2

Responses to Angiotensin II Before and After Freezing the Dogfish Brain

TREATMENT	N	CONTROL Mean ± 1 SE	RESPONSE Mean ± 1 SE
Angiotensin II before brain-freeze	5		
Systolic pressure		28.9 ± 7.1	37.1 ± 9.9***
Diastolic pressure		25.3 ± 4.7	31.2 ± 6.0***
Heart rate		27.2 ± 7.8	29.2 ± 8.1
Angiotensin II after brain-freeze	9		
Systolic pressure		23.8 ± 4.5	30.5 ± 6.5***
Diastolic pressure		22.2 ± 4.1	28.1 ± 5.3***
Heart rate		30.4 ± 5.1	30.0 ± 9.9

Pressures in Torr; heart rate in beats/min: *** p < 0.005

systolic and diastolic pressures in the dogfish with unfrozen, but exposed, brains. There was no significant change in heart rate. AII also induced an equally significant elevation of blood pressure in fish following brain freezing, since both unfrozen and frozen brain trials resulted in an 128% increase in systolic pressure and nearly the same rise in diastolic pressure. Tests for significance were carried out with Student's paired t-test for two normal populations whose means are unknown.

Since a decrease in blood pressure was not observed upon freezing the brain with liquid N_2, this indicates that the higher central nervous system is not necessary for maintenance of vasomotor tone in *S. acanthias*. This is in direct contrast to the mammalian control system and implies that vasomotor control is exerted at a lower neurogenic level, if, indeed, vasomotor control is neurogenically mediated in this species.

Hexamethonium, a potent ganglionic blocker, was used to determine the contribution of the spinal ganglia toward generation of vascular tone. Hexamethonium gave only a slight decrease in DAP. The reduction in pulse pressure was due largely to the increase in HR. The fact that hexamethonium mimicks, in part, the effect of atropine, a parasympathetic (vagal) blocker, indicates that in the dogfish hexamethonium is acting primarily on parasympathetic-type ganglia. Atropine gave no decrease in mean DAP (pulse pressure diminished, due to an increase in HR, as before). Since hexamethonium decreased systolic and diastolic pressures, but atropine did not, this suggests that some degree of sympathetic-type vasomotor control exists.

Phentolamine acts primarily on the vascular alpha adrenergic receptors and causes a marked decrease in DAP by inducing arteriolar vasodilation. The action of this drug suggests that major control of vasomotor tone lies either below the level of the spinal ganglia, or, that control is not neurogenic and is due to other factors. The conclusion made by others, as well as ourselves, that tonus is maintained by circulating catecholamines released from chromaffin tissue (which is abundant in this species) is likely to be correct.

A higher central nervous system link does not appear to be necessary for AII to exert a pressor response, since the pressor response is not blocked by brain-freeze. In addition, AII action is not blocked by hexamethonium, but it is effectively eliminated by phentolamine. This lends support to previous evidence presented by our group that AII acts through the direct release of catecholamines from chromaffin tissue. Higher neurogenic control seems to be absent in the dogfish, both in respect to the maintenance of vasomotor tone and as a factor in the pressor response to angiotensin II. Supported by USPHS Grant No. 18868.

CARDIAC FUNCTION IN A NEW DOGFISH ISOLATED HEART PREPARATION

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A new type of dogfish heart preparation has been developed in which cardiac output of the isolated heart can be observed under controlled conditions without physical disturbance to the heart itself. Cardiac output in this preparation can be determined either from the product of heart rate and stroke volume, or by direct measurement. It can be compared to values for cardiac output obtained from intact fish by indicator dilution methods (Murdaugh et al. Am. J. Physiol. 209:723-725, 1965). The maximum cardiac stroke volume and cardiac output theoretically possible in an intact dogfish was approximated by measuring both pericardial cavity volume and empty heart displacement volume. Comparisons of cardiac outputs calculated for the resting intact fish and cardiac outputs measured from the isolated heart of the same fish show a large discrepancy at any pre- or after-load.

Fish weighing between 4.2 and 7.0 kg were submerged in a trough of seawater. All subsequent dissections and cannulations were performed under water in order to avoid aspiration of air into the cardiac chambers. All experimental procedures were also made with the preparation completely submerged in a bath of seawater. The surface level of the bath was used as the zero hydrostatic pressure reference plane for pressure measurements. The sequential steps in making the preparation are now described. The head was severed with a single quick cut just anterior to the spiracles (approximate level of the spino-medullary junction). This exposed a length of the ventral aorta anterior to the pericardial wall. A second cut was made posterior to the last gill slit. This exposed the posterior cardinal sinus, the Ducts of Cuvier and the posterior wall of the pericardial cavity. Fluid-filled Foley balloon catheters (Fr. 18, 5 ml) were inserted into the Ducts of Cuvier and inflated. The hepatic veins and lateral abdominal veins were securely clamped. This permitted control of inflow into the sinus venosus by perfusion through the Foley catheters. Another cannula, connected to a