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Cardiac Atria of BIO 14.6 Hamsters Are Deficient in Natriuretic Factor

Abstract. *The hearts of 220-day-old hamsters of the BIO 14.6 strain are deficient in atrial natriuretic factor; saline extracts of atria produce one-third the natriuretic and diuretic effects of extracts of atria from age-matched normal hamsters. BIO 14.6 hamsters are known to develop congestive heart failure with edema when they are about 200 days old, and the venous congestion and edema are preventable by parabiosis with normal hamsters. The humoral mediator, the deficiency of which causes venous congestion and edema in BIO 14.6 hamsters, may be atrial natriuretic factor.*

The BIO 14.6 strain of Syrian hamsters is subject to hereditary cardiomyopathy (*1–3*). The cardiac degeneration results in decreased mechanical myocardial performance at all ages (*2*). BIO 14.6 hamsters develop congestive heart failure with cardiac dilation and edema by about 200 days of age (*1–3*). Parabiosis of BIO 14.6 hamsters with normal hamsters prevents the development of venous congestion, heart failure, and edema but does not prevent the myocardial degeneration (*1*). The life-span of the parabiotic BIO 14.6 hamsters is nearly tripled (*1*). These observations imply that BIO 14.6 hamsters are deficient in a humoral mediator, which is present in normal hamsters and transmissible to BIO 14.6 hamsters. They also imply that the humoral mediator increases the ability of the BIO 14.6 animals to excrete salt and water; that is, the congestive heart failure and edema are not the result of cardiomyopathy per se, but, rather, the result of a deficient ability to excrete salt and water.

Exogenous aldosterone causes increased renal sodium reabsorption in normal human subjects (*4*). The kidneys escape from the influence of aldosterone within a few days, however, and sodium excretion returns to normal (*4*). Aldosterone also causes increased sodium reabsorption from the tubular fluid in sweat glands, but sweat glands do not escape (*4, 5*). Thus renal escape is not part of a general tachyphylaxis to aldosterone; rather, renal escape seems to represent activation of another mechanism that overrides the renal effect of aldosterone. The escape phenomenon has caused an intense search for a salt-losing hormone (*6*). But renal escape from the influence of aldosterone does not always occur, particularly not in human congestive heart failure (*7*). Human patients with congestive heart failure have elevated aldosterone concentrations and retain sodium. It is possible that the edema and extracellular fluid volume expansion of congestive heart failure reflect a deficiency of the normal natriuretic-diuretic system, which accounts for the failure of the kidneys to escape from the influence of aldosterone.

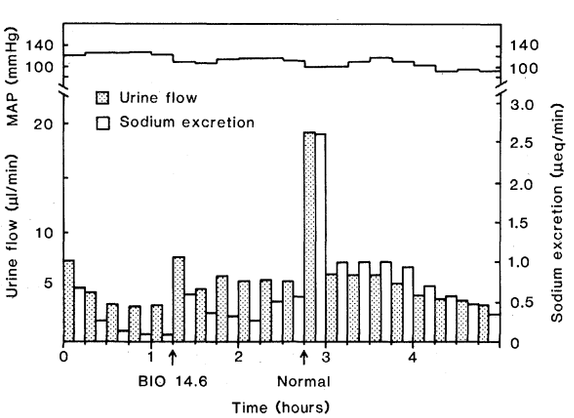

Fig. 1. Urine flow, sodium excretion, and mean arterial pressure in one assay rat during administration of BIO 14.6 and normal atrial extracts from pair 3 shown in Table 1. Each column represents the mean value for a 15-minute collection period. All collection periods are shown through the termination of the assay. Abbreviation: *MAP*, mean arterial pressure. The extracts (0.2 ml) were injected intravenously at times indicated by the arrows.

Since 1961 there has been a quest for a natriuretic hormone that could increase the renal excretion of salt and water (*6*). Natriuretic material (NM) has been extracted from the blood and urine of a variety of species under a variety of circumstances (*6, 8*). Typically the concentration of NM is increased when the volume of blood or extracellular fluid is expanded experimentally (*6, 9*). However, no known tissue origin or physiological role has been found for this natriuretic substance in blood and urine (*8*).

In 1956 Kisch (*10*) and in 1964 Jamieson and Palade (*11*) described specific (protein) secretory granules in mammalian cardiac myocytes in the atria, but not the ventricles. Twenty-five years passed before the observation was made that saline extracts of mammalian atria, but not ventricles, produce potent natriuretic and diuretic effects when injected intravenously (*12, 13*). This material—atrial natriuretic factor (ANF) (*14, 15*)—is not identical to NM extracted from blood and urine: (i) ANF has a molecular weight of about 5000 (*16*), and NM may be less than 1000 (*8*); (ii) ANF is sensitive to protease (*15, 17, 18*), whereas NM may be relatively insensitive (*8*); and (iii) ANF does not inhibit Na^+- and K^+-dependent adenosine triphosphatase (*18*), but NM does (*8*). Still, they may be part of the same system, much as renin, angiotensin II, and aldosterone are parts of a system for salt and water conservation (*19*).

We sought to determine whether BIO 14.6 hamsters are deficient in ANF. We speculated that failure to produce sufficient ANF by the degenerating hearts of BIO 14.6 hamsters is the cause of inadequate renal sodium and water excretion and of the syndrome of congestive heart failure.

Fourteen BIO 14.6 hamsters 220 days old were sorted into seven pairs. Each pair was then paired with two age-

Table 1. Urine flow, sodium excretion, and arterial pressure before, during, and after the administration of atrial extract. S.E.M., standard error of the mean.

Extract	Urine flow (μl/min)			Sodium excretion (μeq/min)			Mean arterial pressure (mmHg)		
	Control (before)	Atrial extract	Control (after)	Control (before)	Atrial extract	Control (after)	Control (before)	Atrial extract	Control (after)
				Normal extract					
1	5.19	17.25	10.04	0.081	1.061	1.040	110	109	110
2	3.03	14.64	4.03	0.166	2.176	0.646	105	94	98
3	5.57	19.11	6.03	0.532	2.580	0.985	110	96	101
4	6.30	35.45	7.71	1.501	7.012	2.310	107	104	103
5	4.85	15.49	5.32	0.426	2.296	1.049	98	100	93
6	4.74	22.07	6.87	0.478	3.743	1.423	119	120	119
7	5.13	22.85	7.03	0.990	4.547	1.822	115	115	100
Mean	4.97	20.98*	6.72	0.596	3.345*	1.325	109	105	103
S.E.M.	0.38	2.68	0.72	0.187	0.745	0.216	3	4	3
				BIO 14.6 extract					
1	5.54	8.98	7.43	0.355	0.406	0.756	111	107	101
2	4.98	8.63	7.37	0.940	1.490	1.568	90	84	86
3	3.27	7.75	5.33	0.081	0.594	0.326	123	108	108
4	3.52	9.84	8.67	0.103	1.064	0.901	109	104	106
5	5.35	9.65	5.48	0.552	1.332	0.909	85	84	83
6	6.81	21.91	8.07	1.165	4.172	1.648	140	138	138
7	5.94	7.10	6.55	0.824	0.917	0.677	90	92	86
Mean	5.06	10.55*†	6.99	0.574	1.425*‡	0.970	107	102	101
S.E.M.	0.48	1.93	0.48	0.159	0.480	0.180	8	7	7

*Significantly different from controls, $P < 0.01$. †Significantly different from normal, $P < 0.01$. ‡Significantly different from normal, $P < 0.05$.

matched random-bred normal control Syrian hamsters (BIO F1B) (*20*). Each animal in a group of four was anesthetized with pentobarbital (60 mg/kg, injected intraperitoneally) (*21*). The hearts were excised, placed in iced extraction fluid to remove blood, and dissected in a petri dish on ice. The atrial tissue was blotted, weighed, and placed in 10 volumes of cold extraction fluid (*22*) on ice, minced with iris scissors, homogenized with a motorized Teflon pestle in a glass mortar, placed in boiling water for 10 minutes, and then centrifuged at 12.8 $\times 10^3 g$ for 10 minutes. This extraction procedure is similar to that of deBold *et al.* (*12*). The supernatants were frozen immediately and encoded for subsequent assays.

The assay preparation used in this study is essentially the same as that used by other investigators (*12–18, 23*) who study natriuretic factor. Male Sprague-Dawley rats were anesthetized with Inactin (100 mg/kg, injected intraperitoneally). The trachea, bladder, one carotid artery, and one jugular vein were cannulated with polyethylene 240-, 90-, 50-, and 10-size tubing (Intramedic PE), respectively. Arterial pressure was recorded continuously. Physiologic saline was continuously infused (intravenously) at 55 μl/min. Urine was collected in weighed tubes at timed 15-minute intervals until urine flow rate was stable (usually about 1 hour), then an extract sample was administered at the beginning of the next urine collection period. The atrial extract supernatant was injected intravenously (0.2 ml over 30 to 45 seconds). Control collections after extracts were injected were made between assays of ANF activity. Urine flow rates and sodium excretion rates for the 30 minutes before, 15 minutes during, and 30 minutes after administration of extracts are shown in Table 1. Figure 1 typifies the mean urine flow and sodium excretion responses shown in Table 1 and also includes mean arterial pressure. A two-way analysis of variance and Duncan's test were used to evaluate these data ($\alpha = 0.05$). Both myopathic BIO 14.6 atrial extracts and normal control extracts caused significant increases in sodium and water excretion in the assay rats; the increases caused by normal atrial extracts were significantly greater than those caused by BIO 14.6 extracts.

It is tempting to speculate that this atrial humoral mechanism has evolved to complement the neural and other humoral mechanisms that regulate the volume of extracellular fluid. A humoral mechanism with longer time constants than the neural mechanism could be either a back-up system or the system primarily responsible for long-term adjustments of extracellular fluid volume and ultimately of mean arterial pressure. The neural mechanism consists of volume (stretch) receptors in the atria with afferent fibers running in the vagus nerves into the brainstem (*24*). Activation of these receptors results in decreased sympathetic nerve activity to the kidneys, renal vasodilation, and diuresis (*25*). In acute left-ventricular overload, these receptors also limit the renal vasoconstriction initiated by carotid sinus and aortic arch reflexes (*26*). Thus there is no question that the atria play a role in regulating extracellular fluid volume through neurally mediated natriuresis and diuresis (*27*). However, denervation of either the heart (*28*) or the kidneys (*8*) does not prevent natriuresis and diuresis in response to a volume load, which indicates that this reflex is not the only control mechanism. BIO 14.6 hamsters are deficient in ANF, and parabiosis prevents congestive heart failure and edema in them. These observations imply that the atria also regulate extracellular fluid volume by humorally mediated natriuresis and diuresis.

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20. Both the hamsters and the assay rats were allowed at least 2 weeks to recover from the stress of shipping before they were used. Both had free access to Purina Rat Chow and tap water. The atrial extracts were prepared from two hamsters at the same time to increase the yield. The average atrial weights were 31 and 39 mg for normal and BIO 14.6 hamsters, respectively. Thus the average weights of atrial tissue extracted were 62 mg and 78 mg for normal and BIO 14.6 hamsters, respectively. The extracts were produced in seven pairs, each of normal and myopathic tissue, to eliminate any systematic error that might have occurred if they had all been prepared at the same time.
21. The order of extract preparation was randomized to eliminate any systematic error associated with duration of anesthesia. The extracts were given a number code that was unknown to the person performing the assay to prevent inadvertent bias of the results.
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Microdifferential Holography and the Polysarcomeric Unit of Activation of Skeletal Muscle

Abstract. *Unbalanced holographic difference images of contracting skeletal muscle fibers reveal that activation affects the amplitude of the light scattered by individual myofibrils. The results suggest that the unit of activation is not the sarcomeric structural unit, but a monomyofibrillar segment containing 20 to 40 contiguous sarcomeres.*

For nearly two decades holographic interferometry has been the method of choice for analyzing the deformations of inanimate objects under stress (*1*, *2*). More recently the method has found its way into macroscopic biomechanics (*3*), but it has not been widely recognized that, with suitable modification, holographic interferometry can provide insight into the microscopic concomitants of the elementary life processes themselves. The power and potential scope of holographic methods in physiology are suggested by the novel images of contracting muscle presented in this report.

In our experiments isolated fibers or fiber pairs dissected from frog semitendinosus muscle were mounted on a microscope stage in Ringer solution at room temperature and transilluminated by a laser beam with a wavelength of 514.5 nm. Light passing through the microscope, the object wave, was directed to a holographic plate that was also illuminated by a coherent, collimated reference wave. The plate was doubly exposed by a pair of light flashes, submillisecond in duration, separated by 25 msec. During this interval the optical path traversed by the reference wave was shortened by a half-wavelength (*4*). Images reconstructed from such holograms reveal only the difference between the images that would be reconstructed from either exposure alone. Bright areas in such images may originate in submicroscopic motion of small structures, in gross translation, or in changes in the shape or index of refraction of the specimen. Differential control images of resting fibers proved vacuous, as expected, while nondifferential control images obtained without shortening the reference path were of normal appearance (Fig. 1A). When stimulated by pulses of transverse electrical current the fibers exhibited sharp response thresholds. The isometric tension curves, which peaked at about 10^5 N/m^2, were stable and repeatable for many hundreds of twitches. When carefully stored the fibers retained

Fig. 1. Holographic images of a portion of a fiber viewed through a ×10 microscope objective lens (numerical aperture, 0.30). The microscope was focused on the plane in which the lower edge of the fiber appeared sharp. Illumination was in the equatorial plane, passing obliquely at numerical aperture 0.22 from the lower portion of the field of view toward the upper portion. The upper edge of the fiber is therefore in partial shadow. The fiber was stimulated by 0.2-msec pulses of current between a pair of point electrodes lying immediately above and below the field of view near its left edge (fiber diameter, 90 μm; striation spacing, 2.7 μm; temperature, 22°C; and exposure length, 0.5 msec). (A) Nondifferential image of resting fiber. The three bright spots at the shadow's left edge and the four similar spots at extreme upper right are blood corpuscles or bits of capillary tissue closely applied to the fiber's surface. These seven spots are weakly imaged in (C) and brightly double-imaged in (D). (B to D) Differential images of the same portion of the fiber, stimulated at successively earlier times during the interval between exposure flashes. Background and static portions of the fiber and capillary tissue have been holographically subtracted from the images and appear black. Apart from the fourfold overexposure used to "burn in" the very bright image in (A), the holographic and photographic exposure and development parameters were the same for all four images.