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Heart Atria Granularity Effects of Changes in Water–Electrolyte Balance (40584)[1]

ADOLFO J. DE BOLD

Department of Pathology, Queen's University and Hotel Dieu Hospital, Kingston, Ontario, K7L 3H6, Canada

Cardiocytes of the mammalian heart atria contain numerous storage granules referred to as specific atrial granules (1). These suggest a secretory role for atrial cardiocytes. Reports indicate that granularity of the atria is altered in some experimental situations such as adrenal regeneration hypertension (2, 3), adrenalectomy (4), and sodium chloride administration (3, 5), as well as in other situations of altered water–electrolyte balance (5). It has been suggested that these findings point to a role for atrial granules in the mechanism of volume sensitivity of the atria (5).

A method to quantitatively assess granularity of the heart atria has been recently developed in this laboratory (6). The present report shows that in some experimental situations, heart atria granularity does appear to respond to changes in water–electrolyte balance.

Materials and methods. The different experimental procedures and related data are summarized in Table I. Sprague–Dawley rats were used exclusively. Female rate were used in the adrenal regeneration hypertension (7) experiments and males in the remainder of the investigations. All animals were housed in single cages in a temperature and humidity-controlled room with a light–dark cycle (12:12 hr) for at least 1 week prior to experimentation, as well as during the experimental periods. Surgical procedures were carried out under Amytal Sodium anesthesia (15 mg/100 g body wt) as was tissue and blood sampling at the end of the experimental periods. Blood samples were taken by left ventricular puncture just prior to removing the heart for histological purposes and were used to determine hematocrit values. Blood pressure determinations in the adrenal regeneration experiments were carried out weekly under light ether anesthesia by tail plethysmography.

Morphometry to assess atria granularity was carried out using a method developed in this laboratory and described in detail elsewhere (6). Briefly, dissected right auricles were fixed in a 3% glutaraldehyde solution in 0.1 *M* phosphate buffer, pH 7.4, for 1.5 h, dehydrated in a graded series of alcohol and infiltrated in JB 4 plastic resin (Polysciences, Inc., Warrington, Pa.). Prior to embedding, the auricles were divided into four approximately equal slices. The second and third slices were embedded and one 1-μm section from each block was obtained. The sections were mounted on glass slides and stained with lead–hematoxylin–tartrazine (8). One randomly chosen field in each section was photographed using an oil immersion objective at a final magnification of 312.5. A grid of reference points was superimposed on the projected photographs to compute the quantity: percentage volume of cardiocytes occupied by granules = number of reference points falling on granulated areas $\times$ 100/total number of points falling on cardiocytes.

The above sampling scheme differs from the one previously reported (6) in that the number of sections and fields used for quantitation is smaller. It was shown in our previous work that the contribution to overall error of the factors sections and fields is comparatively small. Thus, in the present investigation it was possible to reduce the number of sections and fields while maintaining an acceptable level of precision.

The total number of morphometric measurements in each of the experimental groups was: one field $\times$ two sections $\times$ number of animals per group (Table II).

Statistical analysis was performed using a one-way analysis of variance and nested classification. In cases where statistically significant ($P < 0.05$) differences were detected between groups and the experiment consisted of more than two groups, the statistical significance of the difference between group means was tested using an unpaired t test

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TABLE I. SUMMARY OF EXPERIMENTAL PROCEDURES TESTED ON THEIR EFFECT ON HEART ATRIA GRANULARITY

Procedure	Group	Body weight ± SEM (g)	Drinking fluid	Food	Duration
Adrenal regeneration hypertension	Normal control	74 ± 1	Tap water	Rat chow *ad libitum*	
	1% NaCl	70 ± 2	1% NaCl	Rat chow *ad libitum*	6 weeks
	Unilateral nephrectoadrenalectomy	77 ± 1	Tap water	Rat chow *ad libitum*	
	Adrenal enucleation	83 ± 2	1% NaCl	Rat chow *ad libitum*	
Bilateral adrenalectomy	Normal control	307 ± 6	Tap water	Rat chow *ad libitum*	
	1% NaCl	305 ± 5	1% NaCl	Rat chow *ad libitum*	
	Sham operated	307 ± 6	Tap water	Rat chow *ad libitum*	5 days
	Sham + 1% NaCl	309 ± 5	1% NaCl	Rat chow *ad libitum*	
	Adrenalectomy	309 ± 4	Tap water	Rat chow *ad libitum*	
	Adrenalectomy + 1% NaCl	301 ± 5	1% NaCl	Rat chow *ad libitum*	
Desoxycorticosterone injection (1 mg/100g/day)	Normal control	121 ± 1	Tap water	Rat chow *ad libitum*	
	1% NaCl	122 ± 2	1% NaCl	Rat chow *ad libitum*	3 weeks
	DOC	123 ± 1	Tap water	Rat chow *ad libitum*	
	DOC + 1% NaCl	119 ± 2	1% NaCl	Rat chow *ad libitum*	
Desoxycorticosterone injection (2.5 mg/100g/day)	Normal control	118 ± 2	Tap water	Rat chow *ad libitum*	
	2% NaCl	115 ± 3	2% NaCl	Rat chow *ad libitum*	3 weeks
	DOC	109 ± 2	Tap water	Rat chow *ad libitum*	
	DOC + 2 % NaCl	108 ± 3	2% NaCl	Rat chow *ad libitum*	
Water deprivation	Normal control	120 ± 2	Tap water	Rat chow *ad libitum*	5 days
	Water deprivation	121 ± 3	None	Rat chow *ad libitum*	
Sodium restriction	Normal control	125 ± 3	Tap water	Sodium control[a] *ad libitum*	
	Sodium restricted	127 ± 2	Distilled water	Sodium deficient[a] *ad libitum*	3 weeks
	Pair fed	128 ± 2	Tap water	Sodium control[a] pair feeding	

[a] Teklad Test Diets, Madison, Wis. Average sodium content for sodium-deficient diet: 0.01%. Control diet: 0.6%.

after arranging the means according to their values. In the desoxycorticosterone (DOC) experiments the data was also subjected to an analysis of variance using a 2 × 2 factorial design. In addition, a linear regression analysis was carried out in all experiments between the percentage granule values and hematocrit values.

Results. Percentage granule values obtained in the different investigations are sum-

TABLE II. ATRIAL GRANULARITY (PERCENTAGE VOLUME OF CARDIOCYTES OCCUPIED BY GRANULES) RESULTS

Procedure	Group	% Granules	% Change[a]	Hematocrit
Adrenal regeneration hypertension	Normal control (8)[b]	3.18 ± 0.18		45 ± 0.4
	1% NaCl (8)	2.91 ± 0.21	−8	46 ± 0.5
	Unilateral nephrectoadrenalectomy (15)	3.26 ± 0.16	+2	45 ± 1
	Adrenal enucleation (13)	3.60 ± 0.29	+13	45 ± 1
Bilateral adrenalectomy	Normal control (6)	2.40 ± 0.22		49 ± 0.4
	1% NaCl (8)	2.24 ± 0.15	−7	45 ± 0.8
	Sham (6)	2.71 ± 0.27	+13	47 ± 0.6
	Sham + 1% NaCl (6)	2.47 ± 0.33	+3	45 ± 1.3
	Adrenalectomy (16)	2.66 ± 0.18	+11	50 ± 1.0
	Adrenalectomy + 1% NaCl (8)	2.24 ± 0.14	−7	49 ± 1.1
Desoxycorticosterone injection	Normal control (10)	2.70 ± 0.20		44 ± 0.6
	1% NaCl (10)	2.41 ± 0.20	−11	44 ± 0.4
	DOC (10)	2.44 ± 0.16	−10	44 ± 0.6
	DOC + 1% NaCl (10)	2.37 ± 0.18	−12	47 ± 0.6
Desoxycorticosterone injection	Normal control (10)	2.74 ± 0.20		46 ± 0.5
	2% NaCl (10)	2.46 ± 0.17	−10	46 ± 1.2
	DOC (10)	2.22 ± 0.17	−19	47 ± 0.7
	DOC + 2% NaCl	2.03 ± 0.18*	−26	48 ± 0.8
Water deprivation	Normal control (10)	2.54 ± 0.15		44 ± 1
	Water deprivation (10)	3.62 ± 0.20***	+42	54 ± 1***
Sodium restriction	Normal control (8)[c]	2.95 ± 0.17		45 ± 0.5
	Sodium restricted (8)[c]	3.67 ± 0.24**	+24	52 ± 0.6
	Pair fed (8)[c]	2.38 ± 0.21	−19	52 ± 0.6***

[a] $\frac{\%\text{ granules in control group} - \%\text{ granules test group}}{\%\text{ granule test group}} \times 100.$

[b] Number of animals in group; Average daily food consumption was: control: 18 ± 0.4 g; sodium restricted: 17 ± 0.6 g.

[c] Pair-fed animals were offered an amount of control diet equal to the amount of sodium-deficient diet consumed by its pair mate during the previous 24 hr.

* $P < 0.05$; ** $P < 0.005$; *** $P < 0.001$.

marized in Table II. In this table a percentage change of granularity values of test groups with respect to their control groups has been included to facilitate the visualization of direction and relative magnitude of change.

Significant changes in atrial granularity were observed in animals water deprived, on a sodium-deficient diet, and in animals receiving DOC at a concentration of 2.5 mg/100 g body wt/day and drinking a 2% NaCl solution. No significant interaction between the NaCl and DOC treatments was detected using a 2 × 2 factorial analysis of variance. The administration of NaCl alone had no significant effect on granularity although a decrease in granularity was observed in all animals so treated (Table II). Neither adrenalectomy nor adrenal regeneration hypertension significantly affected atrial granularity. In the latter experiments, all but one of the animals after adrenal enucleation showed blood pressures higher than 140 mm Hg. Statistically significant differences for hematocrit values (Table II) were found only in the sodium-restriction experiment and in the water-deprivation experiment. In both groups, higher granularity values often corresponded to high hematocrit values. Linear regression analysis showed that a significant correlation exists between hematocrit and granulation in the water-deprivation experiments (Fig. 1).

Discussion. Although a relatively large number of experimental procedures have been tested in their ability to affect the granularity of the heart atria (3), very few working hypotheses have emerged regarding the physiological role of atrial-specific granules. Undoubtedly, the complex anatomical distribution of atrial granules has made it difficult to distinguish between normal variations in atrial granularity and changes attributable to a given experimental procedure in cases where evaluations have been carried out on a subjective basis. We have found (6) for example, that as much as a near doubling in granularity as detected by the morphometric method used in the present investigations is not detectable by subjective microscopic evaluation.

Experimental procedures known to affect water–electrolyte balance have been reported by several authors to affect atrial granularity. Martinez-Palomo and Bencosme (2) and Bencosme and Berger (3) reported that adrenal regeneration hypertension increases the number of granules found in paranuclear zones of cardiocytes although no statistical significance was ascribed to this increase; bilateral adrenalectomy has been reported to lead to atrial degranulation in the hamster (4) and, more recently, Marie *et al.* (5) reported that sodium overloading and sodium and DOC overloading result in a decrease in right atrial granulation, whereas, full water restriction and sodium restriction led to hypergranulation.

The results of the present investigation demonstrate significant changes in granulation in water deprivation, in one of the NaCl/DOC overload experiments, and in sodium restriction. Furthermore, the results of this investigation indicate that hypergranulation is often accompanied with high hematocrit values. This relationship proved statistically significant in the water restriction experiments in which the most pronounced changes in hematocrit were observed.

Discrepancies between some of the results presented here and other works discussed above may well represent species or strain differences and/or differences in experimental procedures, including differences in the site of sampling. Differences in the approach used to measure atrial granularity should also be taken into account as sampling schemes in past investigations of atrial granularity differ (6) from the random type used in this work. Nevertheless, the above-discussed investigations as well as the present one, all suggest that atrial specific granules are likely related to water–electrolyte balance and this appears as a useful working hypothesis to further define the physiological role of these organelles.

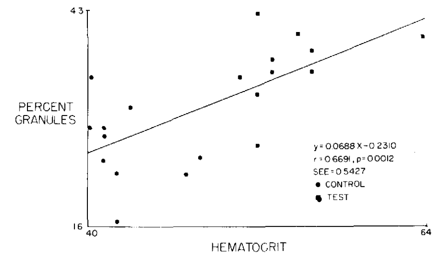

FIG. 1. Relationship between heart granularity and hematocrit in water-deprivation experiment.

Summary. Atrial granularity was measured using a new morphometric method in rats subjected to procedures known to alter water–electrolyte balance. It was found that water deprivation and sodium deficiency lead to atrial hypergranulation, whereas, the combined administration of desoxycorticosterone (2.5 mg/100 g body wt/day) and 2% NaCl in the drinking water leads to degranulation. A statistically significant correlation was found between atria granularity and hematocrit values in water deprivation; animals with higher hematocrit had more granules. The findings point to a relationship between atrial-specific granules and the regulation of water–electrolyte balance in these situations.

1. Jamieson, J. D., and Palade, G. E., J. Cell Biol. **23**, 151 (1964).
2. Martinez-Palomo, A., and Bencosme, S. A., Fed. Proc. **25**, 476 (1966).
3. Bencosme, S. A., and Berger, J. M., *in* "Methods and Achievements in Experimental Pathology" (E. Bajuz and G. Jazmin, eds.), Vol. 5, p. 173. Karger, Basel (1971).
4. Cantin, M., and Huet, M., Fed. Proc. **32**, 876a (1973).
5. Marie, J.-P., Guillemot, H., and Hatt, P.-Y., Path. Biol. **24**, 549 (1976).
6. de Bold, A. J., J. Mol. Cell. Cardiol. **10**, 717 (1978).
7. Skelton, F. R., Proc. Soc. Exp. Biol. Med. **90**, 342 (1955).
8. de Bold, A. J., and Bencosme, S. A., Stain Technol. **50**, 203 (1975).

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