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ORIGINAL CONTRIBUTION

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Effects of pre- and postnatal protein deprivation on atrial natriuretic peptide- (ANP-) granules of the right auricular cardiocytes

An ultrastructural morphometric study

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Abstract *Background* The atrial natriuretic peptide (ANP) is a peptide hormone that is mainly produced in the cardiocytes of the atria and auricles, where it is stored within secretory granules. *Aim of the study* The aim of the present work was to analyze the effects of pre- and postnatal protein malnutrition on weight gain, the size of the heart and the number and sizes of the ANP-granules in the cardiocytes of the rat's right auricle. This study was conducted on 21-day-old rats from mothers exposed to 73% protein restriction during the gestation and lactation and on age-matched control animals. At this stage, both control and protein-deprived animals were killed by a lethal intraperitonial injection of sodic pentobarbital. The weight and the size of the heart were determined and ANP-granules of auricular cardiocytes were examined by transmission electron microscopy and ultrastructural morphometry. *Results* Protein deprivation of the mother throughout pregnancy, and the mother and unweaned rat pups in the first 21 postnatal days reduced the weights of pups to about 60% of the normally fed group. The weight and size of the heart were also reduced, by about 50%. Despite this, perinatal malnutrition did not significantly affect the numbers of ANP-granules/field in the cardiocytes. However, there was a small but significant reduction in the sizes of granules. *Conclusion* It is concluded that ANP-granules are protected from loss even when there is a substantial reduction in body weight and organ size caused by protein deprivation.

Key words undernourishment – ANP – morphometry – ultrastructure

Introduction

Heart atria and auricular cardiocytes from a variety of animal species, including humans synthesize a peptide hormone called atrial natriuretic peptide (ANP) which is stored in granules [1]. The atrial and auricular walls distension under conditions of hypervolemia [2] or blood pressure increase would promote an increase in the secretion and circulation of ANP. The ANP is involved in the modification of the water-electrolyte balance like other hormones, anti-diuretic hormone and renin-angiotensin [3]. ANP stimulates natriuresis and diuresis by enhancing glomerular fil-

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tration rate and excretion of sodium and water [4] and inhibiting the secretion of aldosterone induced by angiotensin II [5], renin and vasopressin [6]. ANP is also involved in opposing the development of hypertension [7–11].

It is well known that malnutrition severely alters the structure of organs and tissues in development [12]. Particularly in the heart, its effects are mainly described in the ventricular myocardium where it determines myocardial edema [13, 14] and cardiocytes apoptosis [15]. At the ultrastructural level, alterations were seen in food restriction rat ventricular muscle including absence and/or disorganization of myofilaments and Z line, swollen mitochondria with disorganized cristae and a great quantity of collagen fibrils [16]. Undernourishment has been shown to induce important morphological changes in ANP-granules. The number of ANP-granules had increased significantly by the 20th day of gestation in the 22°C environmental groups of rats, but was decreased on the 20th day of gestation in the 33°C environmental groups [17]. A reduction of temperature from 37 to 27°C caused a decrease of ANP release by 64% in the rat heart. The number of granules in the cardiocyte increased during dehydration [3]. However, nothing is known on the changes in ANP-granules in cardiocytes in a situation of undernutrition. As the ANP-granules from the right auricle are important sources of ANP in the plasma [18], we have examined the ANP-granules morphologically and morphometrically in the right auricle in nourished and undernourished rats.

Materials and methods

Animals and feeding regimes

Young male and female Wistar rats (200–240 g body weight) were mated. After conception, which was assumed to have occurred when vaginal plugs or sperm were found, the females were placed in individual cages. The nourished mothers received an AIN-93G normal protein diet (protein, 18.7%; fat, 7%, carbohydrate, 20% and fiber, 5%) and the undernourished mothers an AIN-93G low protein diet (protein, 5%, fat, 7%, carbohydrate, 20% and fiber, 5%) [19].

The rats were maintained under standard conditions at 21°C, with a 12 h light-dark cycle, and all groups were supplied with water ad libitum. Following birth, the mothers and the pups received the same diet that the mother had during pregnancy. The animals were divided into two experimental groups. The first group was caged with the mothers and normal feed was supplied until the pups were killed at 21 days postnatal (N group, $n = 10$). The second group was deprived of protein during pregnancy and until 21 days postnatal ($D = 10$). After they were weighed, the animals were sacrificed with an overdose of ether and the anterior thoracic wall was opened. The heart was removed, washed in Krebs's solution, weighed and the right auricle was removed.

The investigation was conducted according to current legislation on animal experiments of the Biomedical Science Institute of the University of São Paulo.

Preparation of tissue samples for electron microscopy

For electron microscopic observations, fragments of the auricle of five animals from each group were fixed in 2% paraformadehyde-2.5% glutaraldehyde in 0.1 M cacodylate buffer for 2 h at 4°C and post-fixed in 1% osmium tetroxide in the same buffer for 2 h at 4°C. Then, the fragments were dehydrated through a graded series of ethanol and embedded in Araldite. Thin sections were double-stained with uranyl acetate and lead citrate and examined with a JEOL—transmission electron microscope.

For ultrastructural morphometry, the number and diameter of ANP-granules were determined [20]. Five electron micrographs per animal, chosen by systematic random sampling of squares, were taken at a final magnification of x7500 and the number of granules/field and the diameter of all granules present in the field was determined, using a computerized program (Axion Plus, Zeiss). A total of fifty fields were analyzed. The diameters of 1180 granules were measured.

Statistical analysis

All data are presented as means ± S.E.M. The results of morphometry were statistically analyzed by the Student's t test. The level of significance was set at $P < 0.05$.

Results

Ultrastructure

In samples of normally fed groups, the auricular cardiocytes contained two types of granules: A-granules, with a well defined membrane and electron-dense core and B-granules with an indistinct membrane and less electron-dense core (Fig. 1). Several ANP-granules were observed near the nucleus of the cardiocyte (Fig. 2). However, in the undernourished

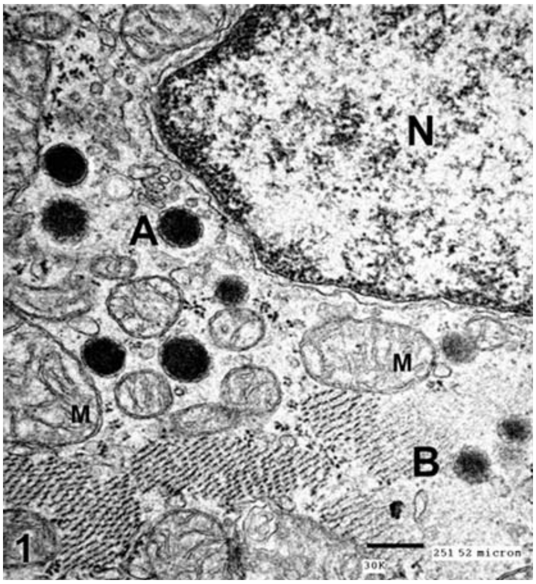

Fig. 1 Elecron micrograph of a right auricular cardiocyte in a nourished rat. Two types of granules can be seen: A-granules (A) with a well defined membrane and electron-dense core, and B-granules (B) with an indistinct membrane and less electron-dense core. N-Nucleus; M-Mitochondria

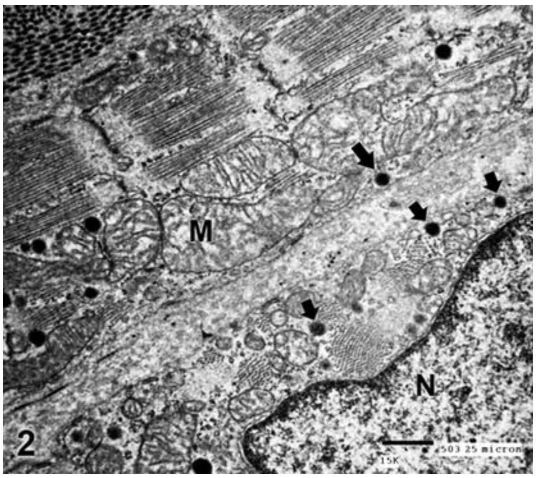

Fig. 2 Electron micrograph of a right auricular cardiocyte in a nourished rat. Several ANP-granules (arrows) are observed near to the nucleus. N-Nucleus; M-Mitochondria

group the cardiocytes were polymorphic, with numerous vacuoles, irregular myofilaments and sarcoplasmic content, polymorphic mitochondria with disorganized cristae and apparent reduction in the sizes of ANP-granules (Fig. 3).

Ultrastructural morphometry

Table 1 shows biometrical and morphometrical data. Rats of the normally fed group had body weights

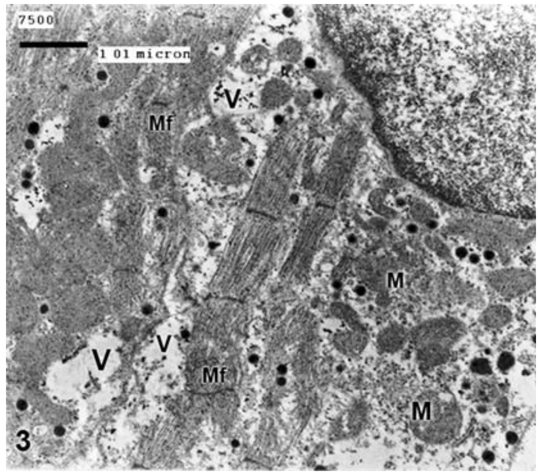

Fig. 3 Electron micrograph of a right auricular cardiocyte in an undernourished rat. Numerous vacuoles (V), irregular myofilaments (Mf), polymorphic mitochondria (M) and apparent reduction in the sizes of ANP-granules (arrows) are seen

Table 1 Body weight, heart weight, granule diameter and granule density of occurrence of normally fed and pre- and post-natal protein deprived rats at 21 days post-natal

	Normally fed to 21 days (n = 10)	Protein deprived to 21 days (n = 10)
Body weight (g)	48.40 ± 5.54	14.19 ± 0.98
Heart weight (g)	0.43 ± 0.05	0.14 ± 0.01
Granule diameter (nm)	156.35 ± 2.62	144.57 ± 2,25
Granule density (granule/field)	24.80 ± 6.82	25.6 ± 4.56
Nuclear volume (μm^3)	121 ± 11.5	124.8 ± 13.1

Data from nourished and undernourished rats. Values are means ± SEM

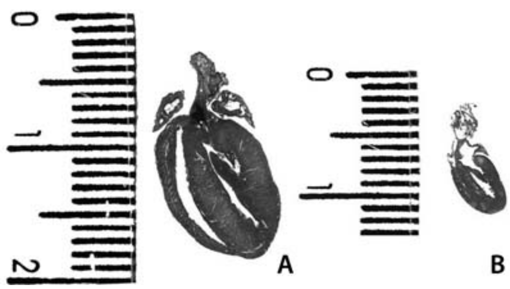

Fig. 4 Representative gross sections from nourished (A) and undernourished (B) rats at 21 days of age. Note the decrease of the heart dimensions in the undernourished rat

more than three fold of the protein-deprived animals at 21 days postnatal ($P < 0.001$). The weight of the heart was 32% of the control value ($P > 0.05$) and its size was 50% of the normally fed group at this time ($P < 0.05$) (Fig. 4). Despite this, the numbers of ANP-granules in the heart were not reduced. At 21 days postnatal the ANP-granule density of occurrence was greater by 3% in undernourished compared with

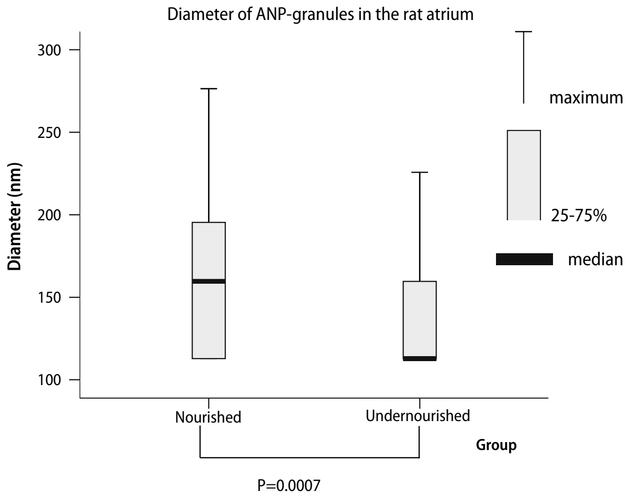

Fig. 5 Diameter of ANP-granules in the right auricular cardiocytes from nourished and undernourished rats

normally fed animals ($P > 0.05$). The diameter of the granules was 8% greater in the normally fed group, compared to the protein-deprived group ($P < 0.05$).

The distribution of granule sizes in the atrial cardiocytes of normally fed and undernourished groups is shown in the histogram in Fig. 5. In the undernourished group, compared to the normally fed group, there was an increase in the number of smaller granules.

Discussion

Different methods have been employed to induce experimental undernutrition [21–23]. The model used in this investigation [24] substantially reduced the body weight of the undernourished rats. The present study demonstrated that at 21 days of age, perinatally protein-deprived male rats showed somatic growth retardation. This confirms previous data in rats obtained by several authors [24–26]. Twenty-one-day-old protein restricted rats also showed reduced absolute weight of hearts. This is in accordance with previous works [24, 27], for other organs (kidneys and adrenals). The cytoplasm alterations observed in atrial cardiocytes are similar to that found in the ventricular cardiocytes [28]. Although the cardiocytes of the rats that were undernourished showed diffuse or less intense alterations in the cytoplasm, there was no sign of cellular appearance that might indicate dying cells. Taken together, these data show the strong influence of dietary restriction on somatic growth during the perinatal period [27].

Regulation of body fluid homeostasis within narrow limits depends on maintenance of water and electrolyte (particularly sodium) balance [24]. Sodium balance is controlled by the renin-angiotensin system [29] and aldosterone [30, 31]. Atrial natriuretic peptide (ANP) is also involved in the regulation of body fluid and electrolyte balance [3, 7]. The atrial and auricular walls distension under conditions of hypervolemia [2] or blood pressure increase would promote an increase in the secretion and circulation of ANP. ANP stimulates natriuresis and diuresis by enhancing glomerular filtration rate and excretion of sodium and water [4] and inhibiting the secretion of aldosterone induced by angiotensin II [5], renin and vasopressin [6]. ANP is also involved in opposing the development of hypertension [7–11].

Intrauterine growth retardation is a common complication of pregnancy and a significant cause of perinatal morbidity and mortality [24]. According to some authors [32] fetal malnutrition causes persistent endocrine changes leading to abnormal structure, function and disease in adult life. Furthermore, epidemiological evidence suggests that a low birth weight increases the risk of developing a number of adult-onset disorders, including non-insulin-dependent diabetes mellitus, hypertension, and cardiovascular diseases [32]. Regulation of body fluid homeostasis may also be affected by perinatal malnutrition as suggested by several studies of fetal growth restriction in humans or animals. In sheep, malnutrition during fetal life was associated with suppression of renal renin and renal angiotensin gene expression in fetuses [33]. In rats, perinatal protein restriction suppresses the newborn intrarenal renin-angiotensin system [34]. In another study, a low-protein diet pregnancy exhibited enhanced plasma aldosterone levels at four and eight weeks of age [35]. Taken together, these investigations show that perinatal undernutrition may affect the endocrine system.

This is the first investigation to examine ANP-granules in nourished and malnourished rats. The ANP-granules in the right auricle were examined by ultrastructural and morphometrical methods. ANP-granules were classified in two types according to their structure and morphology. The structural differences between A- and B-granules are caused by the sectioning level of the granules [36]. In the present study the A- and B-granules were considered the same. The numbers of ANP-granules/area in the atrial cardiocytes were not statistically different between normally fed and undernourished rats. Our experiments imply that there is considerable resilience of atrial cardiocytes, in relation to the number of ANP-granules, because ANP-granules did not diminish in number even though heart weights were halved. These results are in accordance with the observation that plasma level ANP was not affected by perinatal food restriction [24]. Other tissues and organs also demonstrated to be resistant to protein deprivation. In many regions of the central nervous system, severe undernutrition does not cause neuronal loss [37]. In the case of the peripheral nervous system, the same was observed for the neurons of the myenteric plexus of the large intestine [22, 38].

The diameters obtained for the undernourished group were significantly smaller than those of the normally fed group. In fact, the corresponding histograms showed that the distribution of the granule diameters from the undernourished rats was displaced towards smaller diameter sizes compared to data from normally fed animals. Further examination of ANP-granules and ANP plasma levels is required to explain the relationship between granule size and ANP synthesis.

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