

ATRIAL NATRIURETIC FACTOR IN EXPERIMENTAL ACUTE CHAGAS' DISEASE

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Many of the aspects regarding the pathogenesis of Chagas' disease have been extensively studied (S. A. Morris et al., 1990, *Circulation*, 82: 1900-1909). The manifestation of the chronic disease has been proposed to occur as a consequence of pathological events produced during the acute phase of the infection. However, the endocrine function of the heart has never been focused in this pathology. The atrial natriuretic factor (ANF) is a hormone produced mainly by atrial cardiocytes and stored within atrial "specific granules" (A. J. de Bold, 1985, *Science*, 230: 767-770). Its release into the circulation is mediated by atrial distention through a regulated pathway. The ANF has powerful actions in the control of extracellular fluid volume, by protecting the heart against increases in volume load, as was shown by the hyperstimulated ANF system found in a wide spectrum of heart diseases (T. Fujiwara et al., 1990, *Am. Heart J.*, 120: 612-618; G. Takemura et al., 1991, *Circulation*, 83: 181-190).

The aim of the present study was to investigate possible changes produced in atrial ANF stores in an experimental acute Chagas' infection in rats.

Wistar rats (28 ± 3 days of age) were infected intraperitoneally with 6×10^5 trypomastigotes of *Trypanosoma cruzi* X-1 strain (R. C. Cano & E. R. Rubiolo, 1985, *Comun. Biol.*, 3: 457). Another similar but not infected group was utilized as control. The acute phase of Chagas' disease was followed through parasitemic, serologic, histopathologic and electrocardiographic studies until 40 days post infection (p.i.).

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ANF stores were assessed through histochemical reactions and bioassays. ANF storage granules were selectively stained by the lead-haematoxylin-tartrazine technique (A. J. de Bold & S. A. Bencosme, 1975, *Stain Technol.*, 50: 203-205). Atrial granularity scores were obtained by a semiquantitative method (B. S. Edwards et al., 1986, *Mayo Clin. Proc.*, 61: 517-521). Bioactivity of atrial extracts was determined through bioassay in rats on day 40 p.i.. Atrial extracts were obtained and processed using Sep Pak cartridges as already described (I. R. Sarda et al., 1989, *Clin. Biochem.*, 22: 11-15). Diuretic and natriuretic activities of atrial extracts were assayed by a method similar to that described by De Bold et al. (1981, *Life Sci.*, 28: 89-94). Bioactivity values were expressed as the percent increase in excretion of sodium from 20-min urine samples before and after injection of the test materials, per kg of animal weight and per mg of wet tissue (%UNaxV/kgxmg w.t.).

ANF storage granules – Since day 20 of the infection, atrial tissue of chagasic rats showed a significant lower granularity of ANF as compared to controls (Table). The inflammatory infiltrates associated regions were practically depleted of ANF storage granules.

Bioactivity of atrial extracts – Both chagasic rat and normal control atrial extracts caused significant increases in sodium and water excretion in bioassay rats. However, the atrial extracts of chagasic rats showed a significantly lower bioactivity per mg of wet tissue than those of controls ($p < 0.03$, Table).

The present results indicate that experimental *T. cruzi* infection in the rat produces a significant alteration in atrial ANF stores consisting of a dramatic diminution of atrial ANF storage granules near day 20 after infection,

TABLE

ANF storage granules and bioactivity of atrial extracts in control and experimental *Trypanosoma cruzi* infected rats

Groups	Mean atrial granularity score			Atrial bioactivity (%UNaxV/kgxmg w.t.)
	0 ^a	20 ^a	40 ^a	40 ^a
Control	4.0 ± 0.3 (6)	4.2 ± 0.3 (6)	4.7 ± 0.2 (6)	361 ± 117 (8)
Infected	4.1 ± 0.2 (6)	2.7 ± 0.3 ^b (6)	2.8 ± 0.3 ^b (6)	91 ± 20 ^c (10)

%UNaxV/kgxmg w.t. = percent increase in excretion of sodium per kg of animal weight and per mg of wet tissue; ^a = days post infection; () = number of samples. Data are means ± SEM. ^b: p < 0.01 and ^c: p < 0.03 vs control

when the most aggressive parasitary action step in myocardial tissue took place as revealed by a severe myocarditis and the presence of amastigote nests (data not shown). This depletion of atrial ANF stores was more prominent in the inflammatory infiltrate foci associated regions, where specific granules were practically absent. On day 40 p.i., atrial granules of infected animals remained diminished despite the lower cardiac inflammatory process observed. Furthermore, the decrease of ANF stores was corroborated by a decreased atrial natriuretic activity per mg of wet tissue as compared to controls. Preliminary findings carried out in our laboratory suggested that the fall in the bioactivity of atrial extracts from chagasic animals shows a fall in the ANF concentration rather than in its biological activity (L. A. Piazza et al., 1991, *Mem. Inst. Oswaldo Cruz*, 86 (Suppl. I): 188).

In other cardiomyopathies, the drop of ANF stores reflects a hyperstimulated system due to the overload of the congestive heart failure present, this being a pathological event not commonly found during the acute period of Chagas' disease (UNDP/WORLD BANK/

WHO, 1986, *Mem. Inst. Oswaldo Cruz*, 81 (Suppl.): 185-186). The alteration of atrial ANF content found in this work may have been produced, at least in part, by the inflammatory process action over atrial fibers stretch, the main factor that regulates ANF synthesis and secretion (J. R. Dietz, 1987, *Am. J. Physiol.*, 252: R498-R502). A decrease in ANF stores is compatible with an increase in demand without a concomitant increase in synthesis and/or with normal demand with a decrease in synthesis. Nevertheless, the real situation remains to be elucidated.

It would be of significance to determine whether the observed changes in atrial ANF stores in the acute phase of the disease compromise the ability of the cardiovascular system to compensate for increases in volume load.

Our studies would be indicating a modification of the cardiac hormonal system in experimental Chagas' infection in rats, thus adding a new component probably involved in the poorly understood pathogenesis of Chagas' disease.