

## NEURONAL COUNTING AND PARASYMPATHETIC DYSFUNCTION IN THE HEARTS OF CHRONICALLY *Trypanosoma cruzi* - INFECTED RATS

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### SUMMARY

Ten male Wistar rats, chronically infected with Colombian, São Felipe (12SF) and Y strains of *Trypanosoma cruzi* and ten non-infected control animals were submitted to the bradycardia responsiveness test, an assessment of heart parasympathetic function, after phenylephrine injection. Six chagasic animals showed heart parasympathetic dysfunction characterized by reduction in the index of bradycardia baroreflex responsiveness, as compared with the control group.

Microscopic examination of the atrial heart ganglia of chagasic rats showed ganglionitis, but no statistically significant reduction in the number of neurons.

**KEY WORDS:** Cardiac parasympathetic dysfunction; Experimental Chagas' disease; Neuroganglionitis; *T. cruzi*; Neuronal counting.

### INTRODUCTION

Previous works have demonstrated that in chronic chagasic patients<sup>2, 3, 13, 14, 15</sup> and rats chronically infected with *T. cruzi*<sup>6, 16</sup>, depression of the baroreflex autonomic control of cardiac rhythm is mainly due to disturbed efferent parasympathetic function as a consequence of neuroganglionitis in the heart.

However, no correlation was clearly yet established between the degree and nature of the lesions in cardiac intrinsic innervation and the reflex parasympathetic dysfunction. This correlation would have made possible an evaluation of the qualitative and quantitative involvement of intracardiac neurons in the production of the autonomic functional disturbance.

Thus, the present work aims to correlate cardiac denervation to the parasympathetic function in the rat model of chagasic infection. Following the

assessment of cardiac autonomic function based on baroreflex bradycardia responsiveness in rats chronically infected with three strains of *Trypanosoma cruzi*, counts were taken on the numbers of neurons in the ganglia of the hearts of these rats.

### MATERIAL AND METHODS

Ten male Wistar rats weighing between 50 and 80 grams were intraperitoneally inoculated with 4000 to 5000 blood forms per gram body weight of São Felipe (12SF), Y and Colombia strains of *T. cruzi*, at 25 to 30 days of age. Ten non-infected age-matched male rats were used as controls.

After the eighth month of observation, both infected and control animals were submitted in a conscious state to several intravenous bolus injections of 3 to 12 $\mu$ g of the pressor drug,

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phenylephrine, for evaluation of cardiac parasympathetic function. The autonomic function was expressed by the baroreflex bradycardia responsiveness estimated as the ratio between the maximum percent decrement of heart rate obtained from the blood pressure recording, and the maximum percent increment of systolic arterial pressure ( $-\Delta\% \text{HR}/\Delta\% \text{SAP}$ ) following phenylephrine injections, in relation to the control values. For each rat, a mean index of bradycardia was calculated from the multiple injections (6 to 10) of phenylephrine.

Methodological details on the present model of chagasic infection and on the cardiac autonomic function evaluation were described in previous works<sup>6,16</sup>.

After the autonomic function study, the rats were sacrificed, the hearts removed and sectioned sagittally in two halves and fixed with 10% neutral formaldehyde, and embedded, in paraffin. The entire paraffin block was serially sectioned and every

seventh 5 $\mu\text{m}$  section was mounted and stained with hematoxylin and eosin (HE). Additional intercalated sections were also mounted for other stains (trichrome, cresyl-violet), including immunostaining for identification of *T. cruzi* (immunoperoxidase-antimmunoperoxidase). Neurons were counted on every 7<sup>th</sup> section since the rat neuron size averages around 35 $\mu\text{m}$ .

Statistical comparisons between chagasic and control groups of rats were performed employing the non-parametric Mann-Whitney test, at a two-tailed significance level of 5% ( $p < 0.05$ ).

## RESULTS

After 8 months of infection most of the hearts examined histologically showed similar pathological alterations to that observed in human chronic Chagas' disease, especially in the indeterminate form<sup>6,9</sup>. The ganglia and nerve twigs in the atria of six hearts showed mild to intense (+ to +++) in-

TABLE

Index of baroreflex bradycardia responsiveness, cardiac ganglionic inflammatory lesions and neuronal counting in chagasic rats infected with Colombian (C), São Felipe (SF) and Y strains of *T. cruzi*, and in control rats.

CHAGASIC RATS				CONTROL RATS			
PROTOCOL	$\Delta\% \text{HR}/\Delta\% \text{SAP}$ (bpm/mmHg)	GANGLIONIC LESIONS	NEURONAL COUNT	PROTOCOL	$\Delta\% \text{HR}/\Delta\% \text{SAP}$ (bpm/mmHg)	GANGLIONIC LESIONS	NEURONAL COUNT
C2 EA 4.20	-0.79	none	2253	N1 ET 3.20	-0.84	none	2258
C5 EP 4.25	-0.04*	++	2321	N1 EV 3.60	-0.51	none	2443
C6 DA 4.36	-0.51	+	3827	N2 DEA 3.20	-0.51	none	3133
SF3 DEV 1.23	-0.45*	none	3354	N2 DT 3.24	-0.84	none	2418
SF4 DTEA 1.38	-0.26*	++	3194	N2 DPEA 3.22	-0.55	none	3444
SF5 DTEV 1.45	+0.03*	+++	3670	N2 EV 3.18	-0.47	none	5350
Y4 DT 2.26	-0.51	+	4896	N3 DT 3.26	-0.61	none	2902
Y4 EP 2.28	-0.55	none	3057	N3 ET 3.25	-0.78	none	3529
Y5 DA 2.35	-0.33*	none	4257	N3 DEV. 3.29	-0.83	none	4909
Y5 DP 2.34	-0.30*	+++	3083	N4 ET 3.31	-0.61	none	2476
Median	-0.39*		3274+		-0.61		3017
Lower quartile	-0.51		3057		-0.83		2443
Upper quartile	-0.26		3827		-0.51		3529

HR = heart rate

SAP = systolic arterial pressure

$\Delta\% \text{HR}/\Delta\% \text{SAP}$  = index of baroreflex bradycardia responsiveness

\* $p < 0.01$  compared with control group (Mann-Whitney test)

+ $p > 0.05$  compared with control group (Mann-Whitney test)

flammatory cell infiltration. There was a mononuclear infiltrate (macrophages, lymphocytes and plasma cells) surrounding neuronal degenerative changes (chromatolysis, vacuolation, hyperchromasia and retraction); necrosis and satellitosis were rarely observed (Figures 1 and 2). There were no morphological alterations in the remaining four

chagasic animals. Although amastigote forms of *T. cruzi* were noted in the myocardium of some rats, they were systematically absent in the ganglia of all animals.

On the other hand, there was no statistically significant reduction ( $p > 0.05$ ) in the number of atrial neurons in chagasic rats (median = 3274) as compared with control rats (median = 3017).

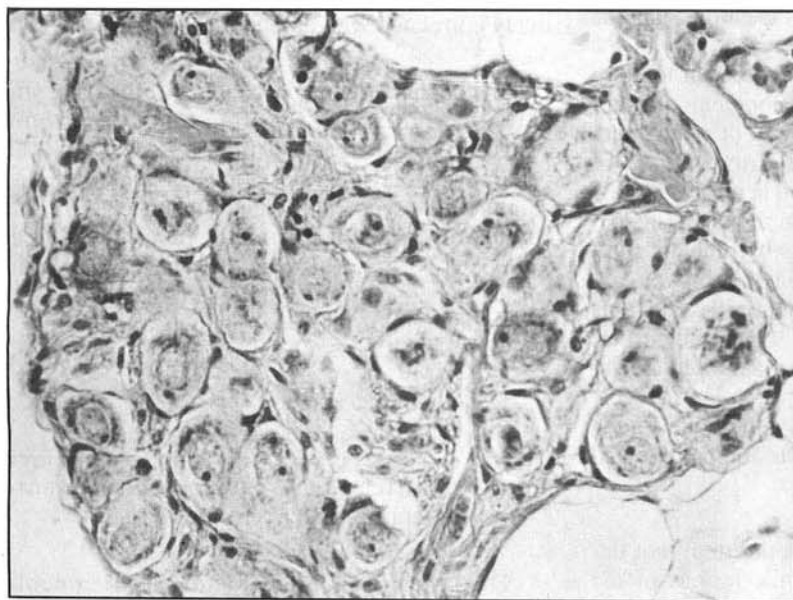


Fig. 1. Control rat. Normal aspect of atrial cardiac ganglia. HE 200x.

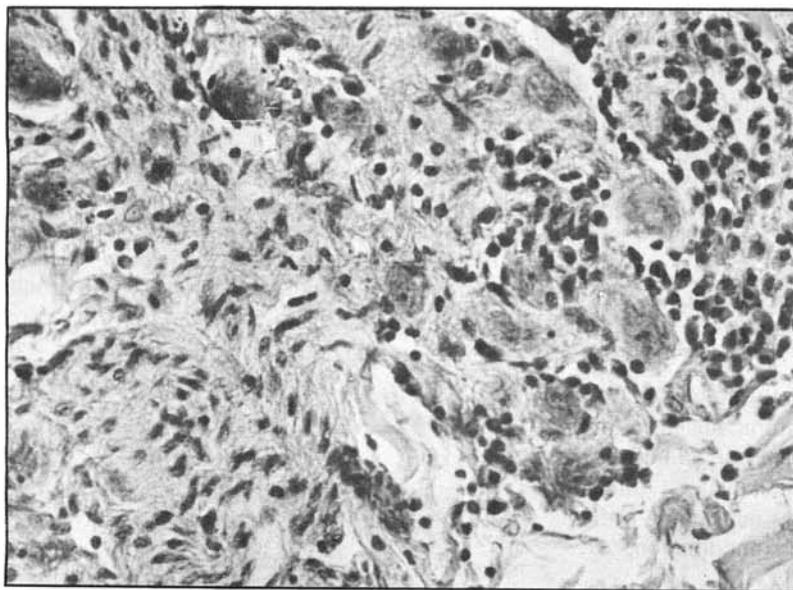


Fig. 2. Chronic chagasic rat. Mononuclear infiltration (plasma cells chiefly) of atrial cardiac ganglia; degenerative changes of ganglion cells. HE 200x.

With respect to the autonomic function, a significant ( $p < 0.01$ ) reduction in the index of bradycardia baroreflex responsiveness was observed in the chagasic group of rats (median = -0.39% bpm/mmHg) as compared with the control group (median = -0.61% bpm/mmHg). In 60% (6/10) of the chagasic rats the bradycardia index was higher than the upper quartile of the control group (in relative values), i.e. parasympathetic dysfunction was observed. Of these chagasic rats, 66.6% (4/6) exhibited neuroganglion inflammatory lesions in a variable degree. There were no lesions in parasympathetic cardiac plexus of two chagasic rats with depressed bradycardia responsiveness (SF3 DEV and Y5 DA). In two others rats with mild ganglionitis there was no change in the baroreflex response (C6 DA and Y4 DT).

The table lists the bradycardia responsiveness indices, the presence or absence of neuroganglion lesions and the neuronal counts for each chagasic and control rat.

## DISCUSSION

Our findings confirm other reports<sup>6,16</sup> that Wistar

rats chronically infected by São Felipe (12SF), Y and Colombian strains of *T. cruzi*, show parasympathetic cardiac dysfunction, characterized by reduced baroreflex bradycardic responsiveness after phenylephrine injection. These data also confirm experimentally those findings observed in chronic chagasic patients<sup>2, 3, 13, 14, 15</sup>. However, in chronic chagasic rats we did not find a significant reduction of neurons (denervation) in the cardiac ganglia as described in chagasics<sup>18, 19, 20, 21</sup> patients. In the rat, the cardiac denervation does not seem to be of fundamental importance in development of autonomic heart dysfunction, as well as of other functional and autonomic disturbances of Chagas' chronic cardiopathy<sup>1, 18</sup>. Therefore, the assessment of cardiac denervation in chagasic patients and in other animal models by time consuming counts of neurons, not always comparable<sup>24</sup>, may not be necessary. The qualitative lesions (ganglionitis and neuritis) may be sufficient for identifying the cause of cardiac autonomic dysfunction, as emphasized previously<sup>6, 16</sup>. Yet perhaps even, this is not necessary since one of the chagasic rats without any neural lesion has showed autonomic dysfunction.

The parasympathetic dysfunction may not depend exclusively on the ganglionic lesions of the heart because, as was shown in some rats in the present study, they may be absent. In rats, as well as in chronic chagasic patients, the cardiac autonomic dysfunction might be explained also by lesions in others segments of the parasympathetic nervous system, as suggested on the basis of the abnormal ventilatory response<sup>13</sup> due to involvement of the bulbar vasomotor center and of the efferent sympathetic pathway. A significant reduction in the number of neurons in the dorsal vagal and hypoglossal nuclei of chronic chagasics with and without megaesophagus has been noted<sup>20</sup>. In rats (infected with the same strains of *T. cruzi*, lesions were observed also in the cerebral cortex, the brain stem and the medulla oblongata<sup>12</sup>).

Others hypotheses have been formulated to explain baroreflex depression in chronic chagasic infection, such as block to the adrenergic stimuli by EVI antibody and immunoglobulins reacting to the plasmatic membrane of the Schwann cells<sup>17</sup> with alteration of the baroreceptor reflex modulation.

On the other hand, in hypertensive cardiopathy in which reduced baroreflex sensitivity was noted<sup>8</sup>,

no lesions and/or significant neuronal reduction was found<sup>21</sup>.

Finally, cardiac autonomic dysfunction exists in diabetes mellitus, which is also associated with lesions in ganglia and nerves (sympathetic and parasympathetic) in the heart and elsewhere<sup>5, 10, 22</sup>. However, these alterations have not been quantitatively correlated with autonomic dysfunction.

In conclusion, the mechanisms of the parasympathetic autonomic dysfunction in chronic chagasic cardiopathy and its role in the pathophysiologic manifestations of the heart disease do not seem sufficiently clarified<sup>14, 23</sup>. The chronic *T. cruzi* infection in the rat can be an useful model<sup>6, 9, 16</sup> for understanding chagasic and others cardiopathies producing lesions in cardiac intrinsic innervation and autonomic dysfunction.

## RESUMO

### Contagem neuronal e disfunção cardíaca parassimpática em ratos cronicamente infectados pelo *Trypanosoma cruzi*

Dez ratos machos Wistar cronicamente infectados pelas cepas Colombiana, São Felipe (12SF), e Y do *Trypanosoma cruzi*, foram submetidos, após 8 meses de infecção, juntamente com dez animais controles, ao teste da resposta bradicárdica barorreflexa pela injeção endovenosa de fenilefrina. Seis ratos chagásicos exibiram disfunção cardíaca parassimpática, caracterizada pela depressão do índice da resposta bradicárdica barorreflexa.

Embora o estudo histológico dos corações chagásicos mostrasse lesões dos gânglios atriais, a contagem dos neurônios em cortes seriados, não apresentou redução numérica significativa dos mesmos.

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