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CARDIAC SYMPATHETIC-PARASYMPATHETIC BALANCE IN RATS WITH EXPERIMENTALLY-INDUCED ACUTE CHAGASIC MYOCARDITIS

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SUMMARY

To clarify the mechanism responsible for the transient sinus tachycardia in rats with acute chagasic myocarditis, we have examined the cardiac sympathetic-parasympathetic balance of 29 rats inoculated with 200,000 parasites *(Trypanosoma cruzi)*. Sixteen infected animals and 8 controls were studied between days 18 and 21 after inoculation (acute stage). The remaining 13 infected animals and 9 controls were studied between days 60 and 70 after inoculation (sub-acute stage). Under anesthesia (urethane 1.25 g/kg), all animals received intravenous atenolol (5 mg/kg) and atropine (10 mg/kg). Acute stage: The baseline heart rate of the infected animals was significantly higher than that of the controls ($P < 0.0001$). The magnitude of the negative chronotropic response to atenolol was 4 times that of the controls ($P < 0.00001$). This response correlated with the baseline heart rate ($r = -0.72$, $P < 0.001$). The heart rate responses to the beta-blocker and to atropine, of the infected animals studied during the sub-acute stage, were not different from controls. These findings suggest that cardiac sympathetic activity is transiently enhanced and cardiac parasympathetic activity is not impaired, in rats with acute chagasic myocarditis. The transient predominance of cardiac sympathetic activity could explain, in part, the sinus tachycardia observed in the acute stage of experimentally-induced chagasic myocarditis.

KEYWORDS: Myocarditis; Chagas disease; Cardiac Autonomic System; Atropine; Beta-Blockers.

INTRODUCTION

Morphologic studies postulate that the cardiac parasympathetic neurons are destroyed in the *Trypanosoma cruzi*-induced myocarditis [17, 27]. This selective cardiac parasympathetic denervation should provoke a persistent sinus tachycardia [23]. However, in experimentally-infected rats, the heart rate closely parallels the histological course of the acute chagasic myocarditis [25] and corresponds to a transient sinus tachycardia [14].

A transient sinus tachycardia could be due to increase sympathetic influence or deficient parasympathethic activity [16, 19]. Recent studies indicate that, the efferent cardiac parasympathetic innervation, is functionally preserved in rats with acute chagasic myocarditis [6]. In view of these facts, we postulate that the transient sinus tachycardia of chagasic rats is probably due to a reversible predominance of cardiac sympathetic influences.

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MATERIALS AND METHODS

Forty six female "Wistar" rats approximately 4 months old were used. They were separated into two groups. Twenty nine rats were inoculated intraperitoneally with 200,000 parasites ("Y" strain) [3]. Seventeen rats were used as controls.

Between days 18 and 21, after inoculation (acute stage), 16 infected animals and 8 controls were anaesthetized with an intraperitoneal injection of urethane (SIGMA) (1.25 g/kg). The animals breathed spontaneously through tracheal cannulation. A femoral vein was also cannulated. Rectal temperature was maintained at 37 ± 0.5°C. The heart rate was recorded in a Grass 7 Polygraph. Standard limb leads were used. The electrocardiographic signal was digitalized, stored in a Radio Shack Computer and played back for analysis (interphase built in our laboratory).

The cardiac sympathetic-parasympathetic balance was studied as follows: cardiac sympathetic activity was indirectly assessed by beta adrenergic blockade [12]. The heart rate was recorded during 5 minutes before and after intravenous administration of a beta-blocker (atenolol, Roussell) (5 mg/kg). This was followed by parasympathetic blockade with intravenous atropine (Vargas) (10 mg/kg). The heart rate was then recorded during 5 minutes. At the end of the experiment the animals were sacrificed for histological studies. Between days 60 and 70, after inoculation (sub-acute stage), the remaining 13 infected animals and 9 controls were subjected to the same experimental procedure.

Statystical Analysis.

The magnitude of the negative chronotropic response to atenolol was obtained by substracting the mean post beta-blocker heart rate, from the baseline heart rate. This value was taken as an indirect evidence of cardiac efferent sympathetic activity.

The magnitude of the positive chronotropic response to atropine was obtained by substracting the mean post beta-blocker heart rate, from the post atropine heart rate. This value was taken as an indirect evidence of cardiac efferent parasympathetic activity [24].

The control and infected animals were compared by mean of analysis of variance. The possible relation, between the baseline heart rate and the negative chronotropic response to the beta-blocker, was assessed by standard correlation analysis [13].

RESULTS

Acute stage. The baseline heart rate of the infected animals was significantly higher than that of the controls. The magnitude of the negative chronotropic response, to the beta-blocker, was 4 times that of the controls. This response correlated with the baseline heart rate ($r = -0.72$, $p < 0.001$). In other words, the absolute decrement, in beats per minute, was more prominent in those infected animals with the fastest heart rate (Fig. 1). The heart rate response to atropine was similar in both groups of animals (Table 1).

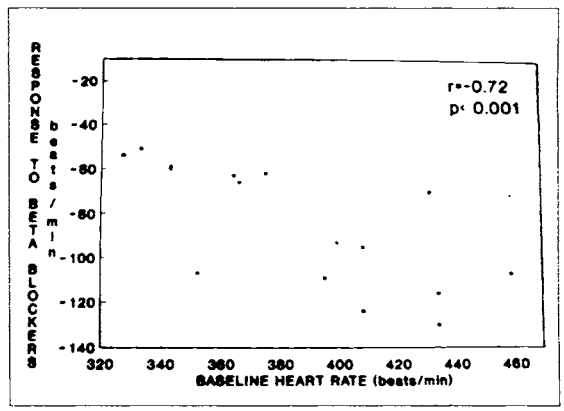

Fig. 1 - Acute Stage. Baseline heart rate (HR) and negative chronotropic response to beta-blockers. The negative chronotropic response was linearly and significantly related to the baseline heart rate. The absolute decrement (beats/min) was more prominent in those infected animals with the fastest heart rate. Dots represent each of the infected animals.

Sub-acute stage. The baseline heart rate and the heart rate response to the beta-blocker and to atropine, of the infected animals, were now similar to that of the controls (Table 1).

Histologic findings. The control animals had no gross cardiac abnormalities. The infected animals had very variable degrees of diffuse myocarditis. Parasites were present in the atria of all infected animals (acute stage). We made no attempt to count the number of neurons in the cardiac parasympathetic ganglia. No parasites were seen within ganglion cells. The hearts of the animals sacrificed between days 60 and 70 (sub-acute stage) were not examined.

DISCUSSION

Heart rate is normally under control of the cardiac autonomic nervous system. The parasympathetic and

TABLE 1

Cardiac sympathetic-parasympathetic balance in rats with acute chagasic myocarditis

	Acute Stage			Sub-acute stage	
	controls (=8)	infected (n=16)		controls (n=9)	infected (n=13)
Baseline heart rate	270 ± 22	385 ± 44	P < 0.00001	262 ± 40	273 ± 18 NS
Chronotropic response to beta-blockers	-16 ± 8	-85 ± 27	P < 0.00001	-23 ± 16	-25 ± 14 NS
Chronotropic response to atropine	28 ± 14	21 ± 17	NS	50 ± 22	41 ± 25 NS

Values represent M ± SD NS = Not significant

sympathetic divisions interact in a complex fashion [19]. The predominance of sympathetic activity increases heart rate. Similarly, a decreased or impaired parasympathetic activity can also increase heart rate. Cardiac sympathetic activity is temporarily enhanced during exercise, emotional stress, and in response to acute myocardial injury [20, 22].

Histological studies of acute *Trypanosoma cruzi*-infected rats at different periods after inoculation, have established the similarity of the myocardial lesions with those described in human studies. The inflammatory process begins 8 days after inoculation (acute stage), peaks between days 15 and 20 and subsides by day 25 after inoculation (sub-acute stage) [25].

The transient sinus tachycardia, described in experimentally-infected rats, closely parallels the histological course of the chagasic myocarditis [6, 14, 25]. To elucidate the mechanism responsible for these transient heart rate changes, we have examined the cardiac sympathetic-parasympathetic balance. The very prominent response to the beta-blocker, of the infected animals studied during the acute stage, is an indirect evidence of enhanced cardiac sympathetic activity [26]. Furthermore, the magnitude of the response correlated with the baseline heart rate. On the other hand, the heart rate response to the beta-blocker, of the infected animals studied during the sub-acute stage, was similar to that of the controls. An enhanced cardiac sympathetic activity is, therefore, limited to the acute stage of the chagasic myocarditis.

A diminished cardiac parasympathetic activity is an alternative explanation for the transient heart rate changes [7, 16, 20]. However, in both the acute and sub-acute stages of the chagasic myocarditis, the heart rate response to atropine was not different from that of the controls. These findings would indicate that the background cardiac parasympathetic activity was similar in both groups of animals. These results suggest that, in rats with experimentally-induced chagasic myocarditis, cardiac sympathetic activity is transiently enhanced and cardiac parasympathetic activity is not impaired [24]. Therefore, the sympathetic-parasympathetic balance is shifted towards a sympathetic predominance. This cardiac autonomic imbalance could explain, in part, the transient heart rate changes observed in the acute stage of experimentally-induced chagasic myocarditis.

The resting electrocardiogram has also been used as a tool to study myocardial damage in experimental Chagas' disease [1]. In this investigation, rats, which were studied 6 months after the acute stage of myocarditis, were found to have severe electrocardiographic abnormalities and sinus tachycardia. The resting heart rate of the infected animals, included in the study, was faster than the heart rate of our infected animals (sub-acute stage). In other words, it would appear that those infected animals did have a persistent sinus tachycardia. However, there are substancial differences between the two studies which could, in part, explain the faster heart rate of their infected animals. First of all, heart rate was measured 6 months after infection and the animals were not subjected to tracheal cannulation, during the general anesthesia with urethano. Therefore, the faster heart rate of their infected animals could be secondary to the respiratory conditions during the anesthesia. An additional explanation would be that, the faster heart rate was the expression of extensive myocardial damage. The presence of the electrocardiographic abnormalities would support this possibility. Finally, the faster heart rate would indeed represent a certain degree of cardiac parasympathetic damage. Nonetheless, the already

mentioned differences, in the experimental design, make this possibility very unlikely.

The anatomical and functional status, of the cardiac autonomic nervous system in Chagas' disease, still is the subject of an intense controversy [7, 8, 9, 10, 11, 27]. Most investigators postulate that, the parasympathetic and sympathetic divisions of the cardiac autonomic nervous system, are primarily and irreversibly damaged in the acute stages of Chagas' disease [4, 25]. However, the original description of cardiac neuronal depopulation, by professor Fritz Koberle, is based on morphologic studies of chagasic patients, who had died from persistent cardiac failure secondary to chronic Chagas' disease. The hearts of these patients were massively dilated, diffusely fibrotic and had a pronounced reduction in the number of cardiac vagal neurons [18]. In other words, the morphologic basis of the neurogenic theory of Chagas' disease is in part an extrapolation, of chronic and terminal histologic findings, to the acute stages of Chagas' disease. Moreover, we are not aware of follow up studies of children, with doccumented acute Chagas' disease, in whom persistent cardiac parasympathetic denervation was unequivocally demonstrated [15].

In our study, we made no attempt to count the number of neurons, in the parasympathetic ganglia, of our infected animals. The aim of the histological study was to prove that, the infected animals, had unequivocal histologic evidence of *Trypanosoma cruzi*-induced acute myocarditis.

Recent investigations have pointed out that cardiac parasympathetic denervation does not seem to be of fundamental importance in the development of cardiac autonomic dysfunction, in experimental Chagas' disease [5, 21, 28]. Furthermore, a detailed ultrastructural study, of murine cardiac parasympathetic ganglia, showed that the cardiac parasympathetic neurons remained normal throughout the acute stages of experimentally-induced chagasic myocarditis [29]. The authors concluded that their findings support the view that, parasympathetic dysfunction, in experimental Chagas' disease, may be of transient nature [6, 7, 8, 14].

RESUMO

O balanco autonômico cardíaco nas ratas com miocardite chagásica aguda experimental.

Com a finalidade de pesquisar o mecanismo responsável pela taquicardia sinusal transitória que ocorre nas ratas com miocardite chagásica aguda, foi estudado o balanço autonômico cardíaco em 16 ratas inoculadas com *Trypanosoma cruzi* por via intraperitoneal. Oito animais foram estudados aos 18 e 21 dias após-inoculação (Estádio agudo); os oito animais restantes foram estudados entre os dias 60 a 70 após inoculação (Estádio sub-agudo). Todos os animais em estudo bem como os controles receberam atenolol e atropina. No estádio agudo, a frequência cardíaca basal dos animais infectados foi significativamente maior que a dos controles. A resposta cronotrópica negativa pela administração de atenolol foi quatro vezes maior nos animais infectados. No estádio sub-agudo, a frequência cardíaca basal e a resposta cronotrópica ao atenolol e atropina foi similar nos dois grupos do estudo. Os nossos resultados sugerem que no estádio agudo da miocardite chagásica experimental, a atividade simpática encontra-se periodicamente aumentada.

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