Rev. Inst. Med. trop. São Paulo
37 (1): 59-62, janeiro/fevereiro, 1995.

CARDIAC β-RECEPTORS IN EXPERIMENTAL CHAGAS' DISEASE (1)

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SUMMARY

Experimental Chagas' disease (45 to 90 days post-infection) showed serious cardiac alterations in the contractility and in the pharmacological response to β adrenergic receptors in normal and *T. cruzi* infected mice (post-acute phase).

Chagasic infection did not change the β receptors density (78.591 ± 3.125 fmol/mg protein and 73.647 ± 2.194 fmol/mg protein for controls) but their affinity was significantly diminished (Kd = 7.299 ± 0.426 nM and Kd = 3.759 ± 0.212 nM for the control) $p < 0.001$. This results demonstrate that the alterations in pharmacological response previously reported in chagasic myocardium are related to a significantly less β cardiac receptor affinity. During this experimental period serious cardiac cell alterations take place and functional consequences will be detected in the chronic phase.

KEYWORDS: Chagas' disease; β receptors.

INTRODUCTION

The clinical course of Chagas' disease includes an acute and chronic phase separated by an indeterminate period [8, 14, 15]. This stage of clinical quiescence, is characterized by seropositivity in the absence of obvious parasitemia.

All the stages of this trypanosomiasis, which have been widely studied in our lab, were reproduced in Albino Swiss mice inoculated with low number of *T. cruzi*. Cardiac contractility was altered in different degrees along the experimental period (from 2 days to 180 days post-infection -p.i.-). Similar results were observed in the pharmacological response to β adrenoceptor agonists and antagonists and in the histopathological studies [4, 5, 6]. We could not detect the presence of α cardiac adrenoceptors in this experimental model [12].

During the period between 45 to 90 days p.i, not only significantly less cardiac ability to develop tension, but also "atypical response" to β adrenergic agents were detected. Norepinephrine had no effect or it induced negative inotropic one on this damaged myocardium.

These findings allowed us to propose that changes in cardiac β receptors number or affinity could partly explain the results described. With this in mind present paper studies the number and affinity of cardiac β receptors during the post-acute phase of experimental Chagas' disease.

(1) This work was supported by a grant from SECyT. Universidad Nacional de Córdoba (R.D. 455/92).
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MATERIALS AND METHODS

Animals. A set of 300 three month old outbread Albino Swiss mice were used. They were divided in two groups: non-infected and infected with *T. cruzi* mice.

Parasites. Trypomastigote forms of *T. cruzi* Tulahuen strain were employed. The strain was maintained in 3 month old Albino Swiss mice by weekly passages.

Experimental Infection. It was produced with blood from parasited animals. The number was determined by counting in a Thomas Chamber, the parasited blood was then diluted with 15% of standard bovine albumin, in sterile saline solution to obtain a suspension of 90 parasites/ml. The animals were intraperitoneally inoculated with 0.5ml of the suspension. The animals were used at 75 days p.i. Parasitemia was examined in fresh blood from tails of mice and found positive in all the animals used from 9 days p.i until 37 days p.i. Parasitemia was negative from 37 days p.i in advance on. The immunofluorescence test was positive in all the infected animals.

Tissue used. Strips of right ventricles from infected and non-infected mice were used. The hearts were quickly removed and right ventricles were dissected, washed and immediatly frozen in liquid N_2 until they were used.

Determination of cardiac β adrenergic receptor binding. β adrenergic receptor binding was performed in hearts from both groups under study. A pool of 10 ventricles were homogenized in 10 volumes of ice cold homogenization buffer (mM composition: Sucrose 250; ClMg 1 and TRIS ClH 20; pH 7.4). Homogenates were centrifuged at 2000 g for 10 min. Pellets were rehomogenized and centrifuged at 30.000g for 30 min. 2 times with ClK 0.6M in homogenization buffer and 2 times in homogenization buffer only. The final pellet was suspended in incubation buffer (mM composition: Cl_2 Mg 12.5; EDTA 1.5; TRIS ClH 75; pH 7.65) in a volume of 1ml/g of wet tissue.

H-3/dihydroalprenolol (H-3/DHA, specific activity 3.515.10 ^{15} Bq/Mol from NEN, USA) was used as radioligand in β adrenergic receptors binding assays. Experiments were perfomed in triplicate with 100 µl of membrane suspension (480 µg protein) and H-3/DHA (2.4-11.5 nM) incubated at 37°C for 10 min in a final volume of 1000 µl. The incubation was concluded by adding 3 ml of cold incubation buffer to each tube and rapidly filtering the contents under reduced pressure through Whatman GF/B filters. The filters were dried and transferred to vials to count radioactivity in Aquasol (Universal LSC cocktail - NEN). Specific binding was defined as the difference in radioactivity bound in the absence or presence of propranolol 10^{-6}M. Dissociation constant (K_D) and maximum H-3/DHA binding were determined by Scatchard analysis using GraFit (Erithacus Software Limited).

Statistical analysis. Data were compared by analysis of variance and differences between groups were determined by Student's t test. Significance level was set at 0.05.

RESULTS

The possibility that *T. cruzi* infection during the post-acute stage of Chagas' disease may alter cardiac density of affinity of β adrenergic receptor was examined. Fig. 1 and Table 1 show the analysis of myocardium cell membranes prepared from uninfected or infected with *T. cruzi* animals.

In membranes prepared from uninfected mice, β adrenergic receptors density was found to be 73.647 ± 2.194 fmol/mg protein. When β adrenergic receptors density was determined in myocardial membranes from infected mice (78.591 ± 3.125 fmol/mg protein) no significant differences with the control group were found.

TABLE 1

Specific bindings of H-3 DHA to cardiac β adrenergic receptors.

Group	Dissociation Constant (nM)	Maximum binding (fmol/mg protein)
Control	3.759 ± 0.212	73.647 ± 2.194
Indeterminate	7.299 ± 0.426*	78.591 ± 3.125

Values are means ± S.E.

* Significantly difference from control ($p < 0.001$)

On the other hand the affinity in chagasic mice membranes was altered showing a Kd = 7.299 ± 0.426 nM and a Kd = 3.759 ± 0.212 nM in the control preparations ($p < 0.001$).

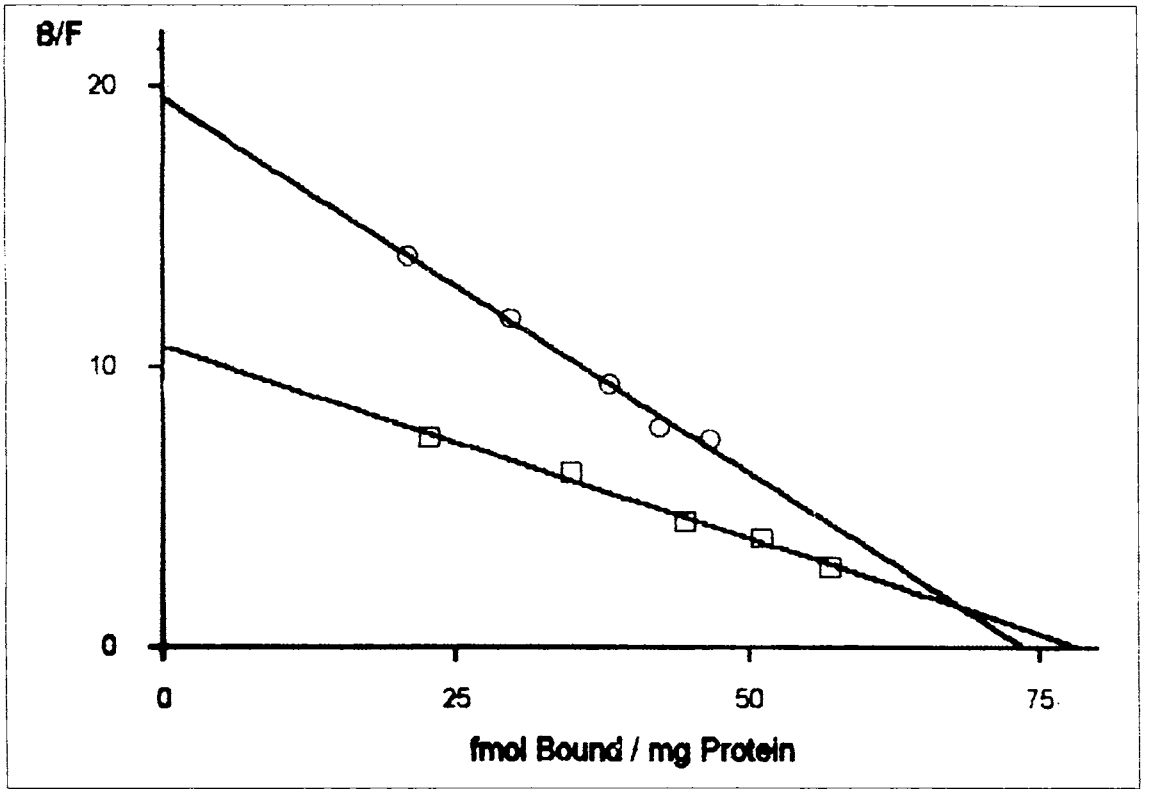

Fig. 1 - β - adrenergic receptors in mice myocardium infected with *T. cruzi*. Scatchard plots showed significant changes in the affinity with similar binding sites. ○: membranes from uninfected; □: membranes from infected.

DISCUSSION

In previous works [4, 5] we demonstrated that the period between the acute and chronic phase of experimental Chagas' disease, described in human host as silent, resulted a period of maximum cardiac disorder in our experimental model, when the ability to develop tension and the pharmacological response studied.

The present paper analizes density and affinity of cardiac β receptor during this phase. Our results show that chagasic infection does not change the β receptors density but that their affinity was significantly diminished. This explains in part, the abnormal cardiac response to adrenergic agents previously reported [4].

The cardiac histopathologic characteristics of this stage, in our experimental model, was the perivascular mononuclear infiltrates and isolated fibrotic areas which probably induced a defficient irrigation in cardiac cells [2]. They would receive a reduced oxigen supply and this would also provoke a reduction in pH cardiac cells. This mechanisms could modify cardiac β receptors affinity [7, 9] or elements of β receptors complex [10, 11].

Besides, STERIN-BORDA et al. [1, 3, 13] described that antibodies from chagasic patient sera interact with cardiac β receptors and that this fact could be related to the lower receptors affinity for the ligand here described.

The present paper shows that during the post-acute phase of Chagas' disease serious cardiac cell alterations take place. Such changes could lead to functional consequences which will be detected in the chronic phase.

RESUMO

Receptores beta cardíacos na doença de Chagas experimental

Estudaram-se os receptores beta cardíacos de camundongos infectados pelo *Trypanosoma cruzi* na fase pós-aguda da doença de Chagas para estabelecer em que medida os mesmos contribuem a gerar

respostas anômalas às catecolaminas observadas nestes miocardios. Utilizara-se 3-H/DHA para a marcação dos receptores beta cardíacos dos camundongos normais e dos infectados na fase pós-aguda (45 a 90 dias pós-infecção).

O número dos sítios de fixação foi similar nos dois grupos, 78.591 ± 3.125 fmol/mg. Proteína nos chagásicos e 73.647 ± 2.194 fmol/mg. Proteína no grupo controle. Em vez disso, a afinidade verificou-se significativamente diminuida no grupo chagásico (Kd = 7.299 ± 0.426 nM) respeito do controle (Kd = 3.759 ± 0.212 nM) $p < 0.001$.

Os resultados obtidos demonstram que as modificações observadas na estimulação adrenérgica do miocárdio chagásico se correlacionam com a menor afinidade dos receptores beta cardíacos e que estas alterações exerceriam uma parte determinante para as consequências funcionais que são detectadas na fase crônica.

ACKNOWLEDGEMENTS

The authors thank Pr. D. Arce A. for allowing the use of the liquid scintillation counter.

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Recebido para publicação em 27/06/1994.
Aceito para publicação em 10/10/1994.