HEART AUTONOMIC INNERVATION DURING THE ACUTE PHASE OF EXPERIMENTAL AMERICAN TRYPANOSOMIASIS IN THE DOG

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Abstract. Heart autonomic innervation was studied in dogs during the acute phase of the experimental infection with the Berenice-78 strain of Trypanosoma cruzi. A glyoxylic acid–induced fluorescence method for catecholamines and a thiocholine method for demonstrating acetylcholinesterase activity showed the sympathetic and the parasympathetic nerve fibers, respectively. At day 34 of infection, moderate-to-intense rarefaction of both cholinergic and noradrenergic nerve fibers occurred in the atria of all animals coincident with moderate to intense myocarditis. In the ventricles, sympathetic denervation was clearly present only when the inflammatory processes were moderate to intense. Preliminary results on the chronic phase indicate that normal autonomic innervation coexists with an incipient chronic fibrosing myocarditis.

Human or experimental American trypanosomiasis (Chagas’ disease) may cause extensive reduction in the number of autonomic neurons, mainly in the parasympathetic ganglia of the heart and digestive tract.1–3 Neuronal cell death occurs mainly or exclusively during the acute phase.4,5 However, the great plasticity of the peripheral neurons has been neglected in all studies showing ganglionic neuronal depopulation. Autonomic neurons have a high capability for axonal regrowth or sprouting. Reinnervation was proved to occur after procedures such as tissue culture, eye-anterior chamber transplants,6 chemical or physical damage of post-ganglionic nerves7 and heart transplantation.8 Re-establishment of functional neuro-effector junctions follows the axonal regeneration. Because of this plasticity, counting the neuronal cell bodies at the chronic phase of Chagas’ disease may not reveal the actual denervation state of an organ. This would be better assessed by studying the density of post-ganglionic fibers, neurotransmitter levels, or even enzymes involved in neurotransmitter synthesis or inactivation. Such methods have shown that severe-to-complete autonomic denervation of the heart is induced by the acute phase of Trypanosoma cruzi infection in rats.8,9 Recovery of the normal pattern of innervation occurs during the chronic phase. In organs of the digestive tract, the experimental disease also provoked reduction followed by recovery of the levels of choline acetyltransferase activity.10 At least for the post-ganglionic sympa-thetic innervation, the acute phase lesion is restricted to the nerve terminals.11 Therefore, axonal regrowth is responsible for the re-innervation. However, the autonomic denervation and re-innervation of the heart have been proved only in the rat model of the experimental disease with the Y strain of T. cruzi. In this model, signs of chronic myocarditis were not found up to 120 days postinoculation. Young dogs that survive the acute phase of T. cruzi infection (Berenice-78 strain) progress to the asymptomatic or indeterminate phase, and some of them proceed to the chronic cardiac disease.12 Thus, the dog seems to be a good model for studying the involvement of the autonomic cardiac innervation during the T. cruzi infection. In the present paper, both sympathetic and parasympathetic nerve fibers were histochemically studied in the atria and ventricles of T. cruzi-infected dogs (Berenice-78 strain), with the aim of verifying the occurrence of autonomic denervation during the acute phase.

MATERIALS AND METHODS

The animals used in this study were born and maintained in an appropriate kennel at the University of Ouro Preto. They had been vaccinated against distemper, parvovirus, leptospirosis, parainfluenzae, and infectious hepatitis (Master-guard-Plus; Salsbury/Solvay Saude Animal, Ltd., Campinas, Sao Paulo, Brazil). All were negative for antibodies to T. cruzi (immunofluorescence test) and received antihelminthic treatment (mebendazole; Univet, Sao Paulo, Brazil) prior to the experimental infection. Two-month-old mongrel dogs were inoculated intraperitoneally with the Berenice-78 strain13 of T. cruzi (2,000 trypomastigotes/kg of body weight). Age-matched dogs from the same litter were kept as controls. The dogs were killed by electrocution (220 V) under thionembutal (Abbott, Sao Paulo, Brazil) anesthesia (3.5%, 1 mg/kg of body weight) at days 27 (three infected plus one control) and 34 (five infected plus two controls) after infection. All had patent parasitemia. Previous studies have shown that the parasitemia becomes subpatent around day 40 after infection, with the peak of the parasitemic curve occurring around day 27.13

Fragments of the left auricular appendages, right atria, and left ventricles were used for histologic and histochemical studies. The post-ganglionic sympathetic innervation was studied in 32 μm–thick cryostat sections by using a modified glyoxylic acid–induced fluorescence method for catecholamines.14 Briefly, the sections obtained at −30°C were immersed in the glyoxylic acid solution for 30 sec. After drying and covering with oil, the slides were heated at 60°C for 30 min, and a coverslip was then applied. Fluorescence was examined under a Leitz (Wetzlar, Germany) Orthoplan microscope equipped with HBO 100 mercury lamp and epillumination. (Ploemopack 2.1; Leitz). The post-ganglionic parasympathetic nerve fibers were revealed by demonstrating the acetylcholinesterase (AChE) activity. A thiocholine method15 was applied to 16 μm–thick cryostat sections. The sections were incubated for 2 hr at 37°C in a medium containing acetylthiocholine iodide as substrate and 4 × 10−3 M tetraisopropyl pyrophosphamide (iso-OMPA; Sigma, St. Louis, MO) as an inhibitor of nonspecific cholinesterases.

For histologic studies, adjacent fragments were fixed in 10% buffered formalin at pH 7.0 and processed for paraffin
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FIGURE 1. Glyoxylic acid–induced fluorescence showing the noradrenergic nerve fibers in atrial sections of A, control and B, Trypanosoma cruzi–infected dogs at day 34 postinoculation. B shows a drastic reduction of the nerve terminals; some nerve trunks are seen at the lower right corner. Scale bar = 50 μm.

FIGURE 2. Noradrenergic innervation of the left ventricle of A, control and B, Trypanosoma cruzi–infected dog at day 34 postinoculation. The rarefaction of the fluorescent nerve terminals is clearly seen in B. Scale bar = 50 μm.

embedding. Four micron–thick sections were stained with hematoxylin and eosin.

Some littermate dogs were left for studies during the chronic phase. Unfortunately, only one of them survived, and this one was killed 16 months after infection. Fragments of the heart were processed as described, together with tissues of a control animal to obtain preliminary results during the chronic phase. In addition, one control and two other dogs infected via the conjunctiva with 2,000 trypomastigotes of the Berenice-62 strain16 were killed 51 and 53 months after inoculation. Both strains were isolated from a chagasic patient at different times, and they were maintained in mice by repeated blood passages.

RESULTS

Both atrial and ventricular myocardia of the control animals exhibited a rich noradrenergic innervation (Figures 1A and 2A). In the ventricle, however, this innervation showed a smaller density. At day 27 of the T. cruzi infection, the density of nerve fibers in atrial and ventricular tissues was similar to that observed in the control dog. At day 34, however, the hearts of all animals showed noradrenergic denervation in the atria and auricular appendages. Two of the five infected animals displayed a virtual absence of the varicose nerve terminals in the atria (Figure 1B) and auricular appendages; the ventricles showed moderate-to-intense denervation (Figure 2B). In the three other animals, the denervation was intense in the atria, moderate to intense in the auricular appendages, and slight or absent in the ventricles. Rarefaction of the vascular innervation accompanied the moderate-to-intense myocardial denervation. Some nerve trunks and thin branches of nonvaricose fibers accounted for the sparse distribution seen in hearts of infected dogs even when the denervation was virtually total.

Regarding the parasympathetic innervation, the atrial tissues of control animals displayed a rich plexus of AChE-containing nerve fibers (Figure 3A), but the ventricles were poorly innervated. Because of this, only the atria were stud-
FIGURE 3. Acetylcholinesterase-positive nerve fibers in the atria of control (A) and Trypanosoma cruzi–infected animals at day 34 of infection (B). B shows moderate (lower half) to intense (upper half) cholinergic denervation of the myocardium. The arteriole (v) in B is also less innervated than that in A. Scale bar = 83 μm.

FIGURE 4. Intense inflammatory process in the atrium of a Trypanosoma cruzi–infected dog killed at day 34 of infection. Scale bar = 50 μm.

ied in infected animals and the results were similar to those obtained for the noradrenergic innervation. However, the rarefaction of AChE-positive innervation was less pronounced (Figure 3B).

The inflammatory processes were absent or very discrete in the hearts of the animals killed at day 27 of infection. In contrast, at day 34 both atria and ventricles exhibited diffuse and moderate-to-intense myocarditis (Figure 4) in the two animals with moderate or intense autonomic denervation in all cardiac regions. In the three other animals, the inflammatory infiltrates were moderate to intense in the atria, discrete to moderate in the auricular appendages, and absent or very discrete in the ventricles. Amastigote nests were present mainly in atrial tissues.

All three infected animals killed during the chronic phase exhibited the normal pattern of both noradrenergic and cholinergic innervation in atrial tissues. The ventricular noradrenergic innervation was also similar with that of controls.

The heart of one of these animals (Berenice-62 strain, 51 months postinoculation) showed no appreciable inflammatory process and some very small areas with discrete fibrosis restricted to the left ventricle. The two other dogs showed clear signs of chronic chagasic myocarditis. However, the areas with inflammatory exudate and/or fibrosis were small and very sparse.

DISCUSSION

The heart of dogs in the acute phase of T. cruzi infection (Berenice-78 strain) showed rarefaction of both noradrenergic and AChE-positive nerve terminals only when moderate-or-intense myocarditis had been observed. Our previous studies in young rats revealed that the cardiac autonomic innervation can disappear (noradrenergic) or be severely reduced (cholinergic) at the end of the acute phase of the infection with Y strain. In the rat, the autonomic denervation process paralleled the acute myocarditis. The neuronal damage seems to be restricted to the varicose nerve terminals. Accordingly, the neuronal cell bodies and presynaptic nerve terminals inside the cervical sympathetic ganglia remained unaffected.

In the dog, our results indicate that the denervation process might also be related to the intensity of the inflammatory processes. The discrete myocarditis present at day 27 of the infection was accompanied by normal pattern of autonomic innervation. At day 34, the discrete myocarditis and normal noradrenergic innervation also characterized the ventricles of some animals. Moderate-or-intense myocarditis always coexisted with moderate-to-intense denervation at day 34 of infection. In the rat model, the myocarditis become more severe in the atria than in the ventricles at the middle of the acute phase. In the ventricular tissue, the myocarditis remains moderate for almost all acute phase and the sympathetic denervation was as severe as that observed in the
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atra. Moderate myocarditis lasting for two weeks may cause complete denervation.17 The disappearance of autonomic nerve terminals in another model of the experimental disease reinforces the need of studies aiming at elucidating the mechanism involved. Such studies are at present under investigation in the rat model. The first findings show that the damage is independent of complement-mediated lysis11 and occurs in rats immunosuppressed by gamma irradiation, favoring the participation of the radioresistant macrophages.20 It is important to remember that neuronal depopulation occurs in ganglia located in the wall of organs in which the inflammatory processes are supposed to be present during the acute phase of the human disease.

The cervical sympathetic ganglia of T. cruzi-infected dogs remain to be studied. Fortunately, the parasympathetic cardiac ganglia of dogs killed 37 days after inoculation with the Berenice-78 or Berenice-62 strains have been recently studied.21 In each animal infected with the Berenice-78 strain, most (63–87%) cardiac ganglia remained free of inflammatory processes. In the great majority of the infiltrated ganglia, the inflammation was classified as discrete. Indeed, moderate or intense inflammatory processes were rare. In dogs inoculated with the Berenice-62 strain, less than 2% of the cardiac ganglia showed discrete or moderate inflammatory processes. Consistent with this finding, histoquantitative studies showed no effect of the infection with either Berenice strain on the number of cardiac ganglia per dog and on the number of neurons per ganglion.21 Therefore, the rarefaction of the AChE-positive nerve fibers now detected in the atria of the dogs killed near the end of the acute phase may result mainly from local damage of the nerve terminals. The presence of nerve trunks and branches revealed by both histochemical techniques reinforced the occurrence of damage limited to the nerve terminals. The neuronal cell bodies, preganglionic fibers, and synapses are somehow protected by glial ensheathing and a partial hematoganglionic barrier, as discussed elsewhere.19 In nerve trunks, the axonal ensheathing by Schwann cell processes should also provide some protection. However, in the post-ganglionic nerve terminals, the varicosities are synaptic sites that are partially or totally devoid of Schwann cell processes. Therefore, there are reasons to consider such sites more sensitive to the hazardous factors related to heart parasitism and/or inflammatory processes of the acute phase.

Our preliminary findings on the chronic phase fail to show signs of autonomic denervation. Indeed, normal autonomic innervation coexisted with an incipient chronic fibrosing myocarditis. It is important to verify the involvement of the autonomic innervation in dogs exhibiting an advanced form of the chronic myocarditis as well as to confirm the recovery of the autonomic innervation during the chronic phase. Pharmacologic and physiologic tests in human chronic chagasic patients indicate parasympathetic dysfunction in those bearing chagasic cardiomyopathy.22–24 Catecholamines levels in the plasma and heart tissues of chronic chagasic patients have also been studied and showed conflicting results.25, 26 Therefore, the dog seems to be a good model for establishing whether the autonomic innervation fail to regenerate, remains unaltered, or is de novo reduced when chronic cardiomyopathy is an ongoing process.

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