Disturbances in the A-V Conduction System in Chagas’ Myocarditis in the Dog

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ABSTRACT

Atrioventricular (A-V) conduction and the functional refractory period (FRP) of the A-V system were studied in 13 dogs chronically infected by T. cruzi that had ECG disturbances indicative of myocarditis. Histopathologic findings at the A-V node and the bundle of His were correlated with the FRP and with conduction times of the A-V propagation system. Retarded conduction was related to inflammatory and fibrotic processes localized at the bundle of His. At relatively slow rates of stimulation, conduction time was prolonged, but the ventricle was capable of following high atrial rates. With augmented FRP, mononuclear cell infiltrates were observed in the A-V node or in the septal muscle around the node. This suggests that the node was included in the inflammatory process. No correlation was found between inflammatory infiltrates of the bundle of His and changes in the FRP. At relatively slow rates of stimulation, normal A-V conduction time corresponded to prolonged FRP. A-V conduction became slow as the atrial rate increased. A point was reached when the ventricle could not follow the moderately increased atrial rates.

Excitability of cardiac tissues was so markedly altered in 5 dogs with severe inflammatory infiltrates that a similar study of the A-V system could not be made.

ADDITIONAL KEY WORDS

functional refractory period Trypanosoma cruzi V-A conduction testing stimuli driving stimuli summation phenomena

Ever since Chagas’ original work (1) most investigators have stated that atrioventricular (A-V) block occurs frequently in Chagas’ myocarditis. Cossio (2) first suggested that conduction is the physiologic property of the heart most affected in this disease, as manifested by partial or total block. Laranja et al. (3) found that A-V block occurred in experimental Chagas’ myocarditis. Pifano and co-workers (4) demonstrated the relationship between disturbances in excitability, the functional refractory period (FRP) of cardiac tissues, and the histologic changes produced by the inflammatory process. Rodríguez and Anselmi (5) found disturbances in A-V conduction during the acute phase of the myocardiopathy. In the chronic phase, A-V and ventriculoatrial (V-A) conduction were normal during relatively slow rates, but were easily blocked when there was a moderate increase in frequency.

In the present investigation, A-V and V-A conduction time and the FRP of the A-V system were studied in dogs with natural and experimental infection by Trypanosoma cruzi. The findings thus obtained have been correlated with a histopathologic study of the A-V node and the bundle of His.

Materials and Method

We studied 25 mongrel dogs with known T. cruzi infection. Eleven had the naturally occurring disease (N numbers); they were from a region in which Chagas’ disease is endemic and had T. cruzi forms in their blood. Fourteen (I numbers) were raised in the animal house and were inoculated with T. cruzi strains that had been kept in successive generations of mice by the technique described in a previous paper (6). In all the

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dogs myocardial damage was demonstrated by serial electrocardiographic (ECG) studies.

In thirteen animals we studied impulse propagation in both the A-V and V-A systems, the FRP of the atrial and ventricular muscles, and the intensity-duration curves of these same tissues. The dogs were anesthetized with intraperitoneal Nembutal (35 mg/kg) and ventilated by a Harvard pump through an intratracheal cannula. The chest was opened by a midline section of the sternum.

Conduction through the A-V node was studied by the technique of Rosenblueth and associates (7). Two stimulators (Grass, model S4-G) were coupled to discharge rectangular pulses twice the threshold through electrodes placed in the right atrial appendage (A-V conduction) or in the trabecular zone of the right ventricle (V-A conduction). One stimulator (the driver) discharged at a constant rate that was greater than that of the spontaneous rhythm, while the other interpolated additional stimuli at a rate one-third or one-fourth that of the former, with progressively increasing delays of 5 msec. We shall designate the two pulses as $S_1$ (the driving stimuli) and $S_2$ (the testing stimuli). The atrial recording electrodes were placed near the sinus node, and the ventricular in the trabecular zone of the right ventricle. Recordings were made with a Schwarzer electroencephalograph at speeds of 100 mm/sec. Figure 1 shows the method employed in all experiments: each artifact of $S_1$ is followed by an atrial response ($a_1$) which propagates through the A-V conduction system to produce a ventricular response ($V_1$). $S_2$ discharges are given after four $S_1$ stimuli in order to avoid summation phenomena. In Figure 1 the first five $S_2$ are not followed by an atrial response since the tissue is in its FRP. The sixth $S_2$ is followed by an atrial response ($a_2$) which measures the FRP of atrial tissue. This atrial response ($a_2$) is not followed by a ventricular one since the supraventricular impulse finds the A-V conduction system in its FRP. Finally, the last $S_2$ is followed by an atrial response...
(a2) which propagates through the A-V conduction system to give a ventricular response (V2). The time elapsed between the ventricular responses V1 and V2 measures the FRP of the A-V conduction system.

The A-V system was studied in 10 healthy dogs as controls. The mean values obtained in these animals were taken as normal reference values. They were 62 to 100 msec (mean 80 msec) for A-V conduction time and 210 to 260 msec (mean 240 msec) for functional refractory period.

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The following technique was employed for the histopathologic study of the node and the bundle of His. The outlet of the coronary sinus was located and a block of tissue (1.5 X 1.5 X 0.8 cm) was cut from this site. The blocks were fixed in 10% formalin and embedded in paraffin. Sections 7 \( \mu \) thick were cut and stained with hematoxylin-eosin, periodic acid Schiff, and Gomori trichromic stains. One section in five was examined, except in those areas where lesions appeared; in these all sections were studied. From 280 to 400 sections were studied from each block.

**Results**

Three of the 25 dogs chronically infected by T. cruzi died in heart failure; 4 died in cardiac arrest or ventricular fibrillation immediately after anesthesia. In 5 other dogs, the A-V conduction system was not studied because of profound alterations in the excitability of the cardiac tissues. Histologic studies in these 12 animals showed severe inflammatory infiltrates consisting of foci of mononuclear cells distributed throughout the myocardium and muscle fibers with severe degenerative changes and more or less abundant plaques of fibrous tissue (Fig. 2A).

In the other 13 dogs, conduction properties and FRP of the A-V and V-A system were studied; histologic studies of the A-V node, the bundle of His and the superior portion of the interventricular septal muscle were then made.

The results are shown in Table 1. Two dogs with experimental infestation (I2, I3) and 3 with natural infection (N1, N4, N5) had normal A-V conduction time; none of these had microscopic lesions in the bundle of His.

A-V conduction time was prolonged in the other 8 animals; values ranged between 105 and 147 msec. Five of these dogs had experimental infection (I1, I4, I5, I6, I7) and 3 had natural infection (N2, N3, N6). Seven of the animals with prolonged A-V conduction had mononuclear cell infiltrates in the bundle of His which produced separation of fibers (Fig. 2D); in some there was fiber destruction with fibrous tissue proliferation (Fig. 2C). In 1 dog (I8) the bundle of His was not identified and an inflammatory infiltrate occupied the entire thickness of the superior portion of the septal mass. Figure 3 shows the A-V conduction curves obtained in 6 dogs. The abscissa represents the milliseconds between two atrial responses (A1-A2). The ordinate shows the conduction time in milliseconds between the atrial response A2 and its corresponding ventricular response V2.

Figure 4 shows the V-A conduction curves obtained in 3 dogs with experimental T. cruzi infection (I1, I4, I7). The abscissae represent the milliseconds between two ventricular responses (V1-V2) and the ordinate, the milliseconds between the ventricular response (V2) and its corresponding atrial response (A2).

Of the 13 dogs studied, 9 had a normal FRP of the A-V system. In 7 of these (I2, I3, I4, I5, N4, N6, N8) no inflammatory lesions were found at the A-V node. Three (I4, I5, N8) had an inflammatory infiltrate in the bundle of His. One (I4) had a severe cellular infiltrate throughout the entire thickness of the superior septal mass, which did not permit identification of the A-V node. In another dog (N8) mononuclear infiltrates were distributed through the region of the A-V node and in the periphery. Among these were areas of healthy tissue.

Four dogs had an altered FRP of the A-V system. In 2 (I1, I2), an inflammatory reaction was found in the superior portion of the interventricular septum which prevented identification of the A-V node. In a third dog (N3) the A-V node could not be identified although there were no lesions of the superior interventricular septum. In the fourth dog (N7), mononuclear cell infiltrates in the periphery and center of the A-V node formed sparse clusters. The fibers showed severe degenerative changes and a moderate increase in connective tissue.
Myocarditis in T. cruzi infection. A: interstitial mononuclear cell infiltration distributed throughout the myocardium; abundant fibrous tissue and muscular fibers with degenerative changes; Gomori's trichrome, ×567. B: 1, bundle of His; 2, interatrial septum; 3, interventricular septum; 4, aortic annulus; Gomori's trichrome, ×20. C: inflammatory infiltration, degenerated myocardial fibers, and proliferation of connective tissue in the bundle of His; Gomori's trichrome, ×567. D: mononuclear infiltrates in the bundle of His which produced separation of fibers; PAS, ×567.
**TABLE 1**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>A-V conduction time (msec)</th>
<th>Functional refractory period (msec)</th>
<th>Histopathologic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>I₁</td>
<td>145</td>
<td>301</td>
<td>Scant infiltration by small lymphoid cells throughout the superior portion of the interventricular septum. Diffuse infiltration at the level of the bundle of His, with separation of the muscle fibers.</td>
</tr>
<tr>
<td>I₂</td>
<td>62</td>
<td>233</td>
<td>Absence of lesions in the A-V node, the bundle of His and the superior portion of the interventricular septum.</td>
</tr>
<tr>
<td>I₃</td>
<td>90</td>
<td>238</td>
<td>Absence of lesions in the A-V node, the bundle of His and the superior portion of the interventricular septum.</td>
</tr>
<tr>
<td>I₄</td>
<td>147</td>
<td>226</td>
<td>Interventricular septum with diffuse focal inflammatory infiltrates, more abundant in the right septal subendocardium (superior portion) where fibers show degenerative processes. Moderate lymphocytic infiltration around and inside the bundle of His. No lesions in the A-V node.</td>
</tr>
<tr>
<td>I₅</td>
<td>122</td>
<td>272</td>
<td>Scant, infrequent lymphocytic infiltrates dispersed in the thickness of the septal mass (superior portion). A-V node and bundle of His not identified.</td>
</tr>
<tr>
<td>I₆</td>
<td>145</td>
<td>254</td>
<td>Severe mononuclear cell infiltration (especially histiocytes and lymphocytes) in large and numerous clusters dispersed in the endocardium and thickness of the superior septal mass. Abundant fibroblastic proliferation. Diffuse inflammatory infiltrates in the bundle of His.</td>
</tr>
<tr>
<td>I₇</td>
<td>147</td>
<td>241</td>
<td>Discrete lymphocytic infiltrates around and inside the bundle of His. Discrete inflammatory infiltrates in the superior portion of the interventricular septum. No lesions in the A-V node.</td>
</tr>
<tr>
<td>N₁</td>
<td>85</td>
<td>230</td>
<td>Mononuclear infiltrates (histiocytes and lymphocytes) dispersed throughout the A-V node and its periphery. The infiltrates are moderately dense, leaving healthy areas between them. Fibers show pronounced changes.</td>
</tr>
<tr>
<td>N₂</td>
<td>105</td>
<td>280</td>
<td>Mononuclear infiltrates predominantly of histiocytes and lymphocytes at the periphery and center of the A-V node. Infiltrates are thin and form clusters where fibers show severe structural changes (atrophy). Discrete increase in collagenous fibers. Scarcely mononuclear cell infiltrates dispersed among the fibers of the bundle of His, especially at the bifurcation where the left branch originates. Partial destruction of some fibers at the level of the infiltrates.</td>
</tr>
<tr>
<td>N₃</td>
<td>110</td>
<td>289</td>
<td>Discrete, diffuse inflammatory infiltrates in the interstitium of the bundle of His. The A-V node was not identified.</td>
</tr>
<tr>
<td>N₄</td>
<td>94</td>
<td>223</td>
<td>Absence of lesions in the A-V node, the bundle of His and the superior portion of the interventricular septum.</td>
</tr>
<tr>
<td>N₅</td>
<td>73</td>
<td>240</td>
<td>Absence of lesions in the A-V node, the bundle of His and the muscle in the superior portion of the interventricular septum.</td>
</tr>
<tr>
<td>N₆</td>
<td>105</td>
<td>230</td>
<td>Dense infiltrates at the periphery of the bundle of His, less dense in the center. Fiber degeneration. Absence of fibrous tissue.</td>
</tr>
</tbody>
</table>
FIGURE 3
Lengthening of the $A_2V_2$ delay when the interval between pairs of stimuli applied to the atrium was gradually decreased. Abscissa = $A_1A_2$ interval in msec; ordinate = $A_2V_2$ delay in msec.

FIGURE 4
Lengthening of the $V_2A_2$ delay to pairs of stimuli applied to the right ventricle with gradual decrease between interstimuli intervals.

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Figure 5 shows the changes in V1-V2 intervals when pairs of stimuli were applied to the atrium, with a variable delay in S2. Long intervals between atrial responses (A1-A2) corresponded to long intervals between ventricular responses (V1-V2). As the A1-A2 interval decreased, the V1-V2 interval was similarly reduced. A particular stage was reached when the A1-A2 interval continued to decrease without a concomitant reduction in the V1-V2 interval. When the reduction of the A1-A2 interval reached a certain point, A2 conduction was blocked because the A-V tissue was in FRP.

The curves corresponding to the FRP in the 4 dogs with changes in this property can be seen in Figure 5. Three of them (I1, I3, N2) showed a descending slope at 45° to the abscissa. The fourth (N4) shows a 35° angle to the same axis. The descending branch of the curve is above that of controls and the horizontal branch begins early (at A1-A2 intervals of more than 230 msec and with V1-V2 intervals higher than those of control animals).

Discussion

A close relationship was found between impulse propagation characteristics at the level of the A-V conduction system and the histologic condition of the A-V node and the bundle of His.

In 5 dogs without histologic changes in the bundle of His, A-V conduction was found to be normal. Four of these dogs had no histologic lesions in the A-V node and one (N1) showed diffuse inflammatory infiltrates in the entire region of the A-V node and its periphery, with zones of healthy tissue which could explain the normalcy of A-V conduction.

In seven of 8 dogs with an increased A-V conduction time, there were inflammatory alterations in the bundle of His; in 1 the node and the bundle were unidentifiable, but diffuse infiltrates were found in the superior portion of the interventricular septum. Four of the 8 dogs did not have lesions in the A-V node. This suggests that the delayed conduction time was related to the histologic changes of the bundle of His.
Hoffman (8) found that conduction in excitable tissues is slowed when stimulus is delivered before repolarization is completed, and the action potential of the A-V nodal cells lasts about 150 to 200 msec. In our experiments, the slowing of A-V conduction began earlier in dogs with long FRP; a block in the A-V conduction is produced with long intervals (210 to 270 msec) between stimuli. On the other hand, in those with short FRP the slowing of the A-V conduction began later, with short intervals (160 to 190 msec) between stimuli.

We believe that the histologic changes in the bundle of His had very little effect on the FRP alterations, since two dogs with histopathologic changes in this area but without changes in the A-V node had slow A-V conduction time and normal FRP.

Like Scher and co-workers (9), we found that the minimum interval between atrial stimuli necessary to block A-V conduction in normal dogs was 160 msec. It is important to emphasize that when the FRP of the A-V system is normal, the ventricle can attain high atrial frequencies, even in the presence of slow A-V conduction time. This was found to be the case in 2 dogs with prolonged A-V conduction time (147 msec) together with A-V propagation at short intervals of the atrial stimuli (curves A1-A2, A2-V2). However, when the FRP is prolonged, A-V conduction is altered in the sense that the ventricle can not follow high atrial frequencies, as A-V conduction is blocked when the A1-A2 interval is shortened.

References
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