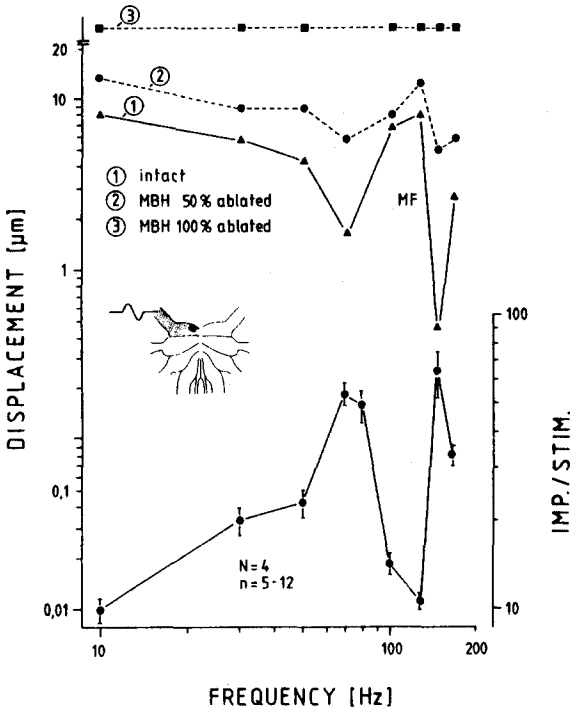

Figure 3. Top: Threshold curves of interneuron in the ganglion of the 1st right leg determined for vibratory stimulation of the tarsus with all metatarsal bridge hairs (*MBH*) intact, and 50% or 100% of them ablated. Bottom: Response of interneurons to tarsal vibration given as the number of impulses per stimulus (displacement 12 dB above threshold; duration 300 ms); note y-axis on right side. Inset: Ventral view of subesophageal ganglionic mass; site of recording.

tarsal displacement. Due to the lack of spontaneous activity the neuron's threshold response could easily be identified. The resulting threshold curves show lowest values at 70 Hz and at 150 Hz which is also reflected by corresponding maxima of the response upon stimulation by suprathreshold vibrations (fig. 3).

Ablation of half of the bridge hairs lowers threshold sensitivity of the interneuron by about 20 dB (at 150 Hz). Upon ablation of all of them the interneuron does not respond to tarsal vibration at all (fig. 3). The number of hairs stimulated may be an indication to the central nervous system of stimulus strength. It is also noteworthy that the sensitivity peaks of these interneurons coincide well with frequencies prominently contained in the male courtship vibrations of the same species and prey signals relevant to it[3,4].

A comparison of the metatarsal bridge hairs' primary sensitivity with that of the metatarsal organs and of the pretarsal slits is not possible on the basis of the interneuron responses. It will be interesting to see, whether they are tuned to specific small ranges of frequencies like the present and the other vibration sensitive interneurons known[10] or whether they show high pass characteristics in the biologically most relevant range of frequencies like the other two identified receptors for substrateborne vibrations in spiders[3].

The present finding of a third type of vibration sensitive receptor in spiders once more calls for an evaluation of the potentially different roles of these receptors in behavior as suggested by differences in absolute sensitivity and tuning. The lowest thresholds found for the slits of the metatarsal organ show a sensitivity in this organ which is higher by at least two powers of ten than that of any other primary sensory neuron or vibration sensitive central neuron so far described. Nevertheless, spider vibration sensitivity is not the result of the metatarsal organ's activity alone.

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2 To whom reprint requests should be sent. New address: Institut für Zoologie, Universität Wien, Althanstr. 14, A-1090 Wien, Austria.

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Selectivity of alterations in skeletal fibers in chronic Chagas' disease of the mouse

P. M. Cabeza Meckert and R. P. Laguens

Catedra de Patologia II, Facultad de Ciencias Medicas, Universidad Nacional de la Plata, 1900 La Plata (Argentina),
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Summary. In mice chronically infected with *Trypanosoma cruzi,* the masseter muscle (rich in type II fibers) was devoid of inflammatory infiltrates and parasites. In contrast, other muscles, composed of type I and II fibers, showed a decrease of type I fibers, parasites and lesions, suggesting that in *T. cruzi* infection type I muscle fibers are selectively damaged.
Key words. Trypanosoma cruzi; Chagas' disease; skeletal muscle; tissue tropism.

Trypanosoma cruzi, the ethiological agent of Chagas' disease, is an intracellular parasite that infects and multiplies selectively in macrophages, glial and nervous cells and smooth, skeletal and heart muscle[1,2]. Although this cell and tissue selectivity can be dependent on the parasite, which exists in either reticulotropic or myotropic strains[3], the reasons for this preferential tissue tropism are unknown. Recently, it has been reported that in the course of acute infection of the mouse with the Brasil strain of *T. cruzi,* the percentage of infected type I skeletal muscle fibers was nearly five-fold higher than that of type II, suggesting that the heart and muscle alterations characteristic of Chagas' disease are de-

termined by a selective tropism towards a particular type of muscle fiber[4]. In order to determine whether this tropism is shared by other strains of the parasite, and to establish whether during the chronic infection type I muscle fibers were more affected than type II, a systematic study was carried out, involving a determination of the number of parasitized fibers, the intensity of inflammatory infiltrations and the number of type I and II muscle fibers remaining. The investigation was performed on different skeletal muscles from mice chronically infected with two strains of *T. cruzi* with different tissue tropism.

Chronic infection was induced in female 3-month-old BALB/c mice by inoculating by the i.p. route either 25 trypomastigotes of the reticulotropic[5] Tulahuen strain, or 2×10^5 trypomastigotes of the myotropic[6] CA_1 strain. Three months after infection animals were killed and the masseter, rhomboideus cervicis, iliocostalis lumborum and rectus femoris muscles were removed and fixed in Bouin's fluid or snap frozen in liquid N_2 for histological and histochemical studies, respectively. The same procedure was performed in control non-infected mice of matching age and sex. In 3 consecutive transverse sections, stained with hematoxylin and eosin, the total number of muscle fibers was counted and the percentage containing nests of amastigotes or surrounded by inflammatory infiltrates was determined. NADH dehydrogenase and myofibrillar ATPase activities were investigated in cryostat transverse sections[7]. The relative proportions of type I and II fibers was determined by counting 200 fibers for each muscle.

In animals infected with either of the two strains, the result of the histological study was similar. The masseter was consistently devoid of inflammatory infiltrates. Of the remaining muscles, the most affected was the rectus femoris, which showed a large number of infiltrates. The quantitative determination of the number of muscle cells surrounded by inflammatory infiltrates indicated that the rhomboideus cervicis was less affected than the iliocostalis lumborum and the latter less than the rectus femoris (table 1). The search for parasitized fibers could be done only in animals infected with the CA_1 strain, because in those injected with the Tulahuen strain the number of fibers containing nests of amastigotes was very low, preventing an accurate numerical estimate. The number of parasitized fibers was higher in the rectus femoris than in the rhomboideus cervicis and iliocostalis lumborum. In the masseter no intracellular parasites were observed (table 2).

The enzymatic study of the muscles from non-infected mice showed striking differences between the masseter and the three remaining muscles. The masseter appeared to be composed only of type II fibers. In the other muscles, type II fibers represented less than half of the fibers. The same study performed on the muscles of chagasic mice showed a significant decrease of type I fibers, especially in the rectus femoris where they represented one fourth of the total number of fibers (table 3).

These results show a definite difference between the lesions present in several skeletal muscles in the mouse model of chronic Chagas' disease. The masseter was devoid of inflammatory infiltrates, which represented a constant pathological feature in the other examined muscles. Apparently this difference was correlated with the distribution of the fiber types in the different muscles. The masseter appeared to be composed only of type II (rapid strong contraction, short duration) fibers[8], whereas in the other muscles only less than one half of the muscle cells were of this type. This observation suggests that type I (slow contraction, tension sustained[8] fibers) could be the targets for the muscle alterations found in experimental Chagas' disease. This interpretation is supported by the fact that in the rectus femoris muscle of chagasic mice a relative decrease of type I fibers was also observed.

The reason for this selective fiber alteration could not be established in the present study. One possible explanation is that there is a selective tropism of *T. cruzi* towards type I fibers, which are then progressively destroyed during the chronic infection. The fact that in mice infected with the CA_1 strain no parasitized fibers could be seen in the masseter, and the well-known tropism of *T. cruzi* towards the heart muscle cells, which share histochemical characteristics with type I fibers, support this interpretation.

However, the possibility of an immune mechanism, as has been postulated for the heart damage in Chagas' disease[9], cannot be excluded, especially on account of the small number of fibers containing nests of amastigotes in the affected muscles.

An alternative hypothesis which cannot be ruled out is that the constant and rhythmical movement of the masseter which occurs in rodents, and the fast and more sudden movements of the other muscles examined, could result in differences in the vascularization or blood supply. In addition, the decrease in the number of type I fibers in the rectus femoris of chagasic mice could be due to neurogenic atrophy, a condition recently described in chagasic patients[10].

Although at the present time no satisfactory explanation exists for the observed selectivity of skeletal muscle lesions,

Table 1. Number of muscle fibers surrounded by inflammatory infiltrations in chronic Chagas' disease

Muscle	Tulahuen strain	CA_1 strain
Masseter	0%	0%
Rhomboideus cervicis	2.16% ± 1.2 (SD)	14.6% ± 1.5 (SD)
Iliocostalis lumborum	3.24% ± 1.8 (SD)	19.8% ± 3.2 (SD)
Rectus femoris	5.3% ± 0.9 (SD)	22.3% ± 4.8 (SD)

Determinations were made on 6 chagasic mice for each *T. cruzi* strain.

Table 2. Number of fibers containing nests of amastigotes in CA_1 strain-infected chagasic mice

Masseter	Rhomboideus cervicis	Iliocostalis lumborum	Rectus femoris
0	3.6% ± 1.4 (SD)	4.1% ± 0.9 (SD)	6.3% ± 1.8 (SD)

Counts were performed in muscles from 6 mice.

Table 3. Percent of type II skeletal muscle fibers in muscles from normal and chagasic mice

Muscle	Normal	Tulahuen-infected	CA_1-infected
Masseter	100%	100%	100%
Rhomboideus cervicis	40.6% ± 9.8 (SD)	55% ± 8 (SD)	47% ± 7.4 (SD)
Iliocostalis lumborum	31.8% ± 8.3 (SD)	62% ± 14 (SD)	48% ± 15.8 (SD)
Rectus femoris	33.4% ± 11.8 (SD)	73% ± 21 (SD)	63% ± 12.6 (SD)

Counts were done on the total number of fibers in the mid-zone of muscles from 6 normal mice and 6 mice infected with Tulahuen or CA_1 strains of *Trypanosoma cruzi*.

this observation opens new avenues for research into the complex relationships which exist between protozoan parasites and their mammalian hosts.

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Tetrodotoxin slightly shortens action potential duration in ventricular but not in atrial heart muscle

H. Jakob and H. Nawrath*

Pharmakologisches Institut der Universität Mainz, D-6500 Mainz (Federal Republic of Germany), 10 June 1987

Summary. Tetrodotoxin (TTX), at concentrations significantly decreasing maximal upstroke velocity (dV/dt_{max}) of the action potential, exerted variable effects on action potential duration (APD) in different myocardial preparations. APD was virtually unchanged by tetrodotoxin in the guinea pig atrium, but slightly shortened in the guinea pig ventricle at maximally effective concentrations. In the human ventricle, both dV/dt_{max} and APD were reduced in the same concentration range of TTX. These results suggest that a TTX-sensitive sodium current significantly contributes to the repolarization phase of the action potential in ventricular but not in atrial heart muscle.
Key words. Atrial and ventricular myocardium; tetrodotoxin; window current; action potential configuration.

TTX has been reported to decrease APD in cardiac Purkinje fibers [1], at concentrations where an effect on dV/dt_{max} is not yet observed [2]. This finding was taken as evidence for the existence of a sodium window current possibly due to the significant overlap of the steady state activation and inactivation curves of the sodium system [3]. Experiments with lidocaine yielded similar results [4,5]. More direct evidence for a significant contribution of a sodium current to the repolarization phase in cardiac Purkinje fibers was presented by the demonstration of a persisting sodium current upon depolarization [6-8]. TTX shortens the action potential duration in Purkinje fibers to a much greater extent than in the working myocardium [2,9]. We show here the effects of TTX on intracellularly recorded action potentials from guinea pig heart (atrium and ventricle) and from human ventricular preparations obtained after cardiac surgery.

Methods. The preparations were obtained from freshly stunned guinea pigs and from human patients undergoing open heart surgery for mitral valve replacement (for details see Eckel et al. [10]). Right atrial and ventricular trabeculae and left human ventricular papillary muscle preparations were electrically driven at 1 Hz in Tyrode's solution (composition in mmol/l: NaCl, 136.9; KCl, 5.4; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9; $CaCl_2$, 1.8; glucose, 5.6) bubbled with 95% O_2 and 5% CO_2 at 37 °C (pH 7.4). Action potentials were recorded intracellularly with conventional microelectrodes techniques and evaluated for duration at 20% and 90% of repolarization, APD_{20} and APD_{90}, respectively. The first time derivative (dV/dt) of the action potential was obtained by electronic differentiation and evaluated for dV/dt_{max}.

Results and discussion. In guinea pig atrial heart muscle, TTX decreased dV/dt_{max} in a concentration-dependent way, whereas APD remained virtually unchanged (figs 1a, 2a and 2b). In the guinea pig ventricle, dV/dt_{max} was decreased by TTX in the same concentration range and APD_{90} was slightly reduced at higher concentrations (figs 1b, 2c and 2d). In human ventricular heart muscle, both dV/dt_{max} and APD_{90} were reduced in the same concentration range (figs 1c and 3). In all preparations, the upstroke of the action potential was gradually decreased by cumulatively increasing concentrations of TTX and finally completely abolished, albeit at very high concentrations. In contrast, the effect of TTX on the repolarization phase was relatively weak (ventricular preparations) or virtually absent (atrial preparations). This shows that the contribution of a TTX-sensitive current to the repolarization phase of the action potential is relatively large in Purkinje fibers [1-3], relatively small in ventricular heart muscle [2,9] (this paper) and not significant in the atrium (this paper).

There are two possibilities to explain the quantitatively different results. First, a sodium window current may be differ-

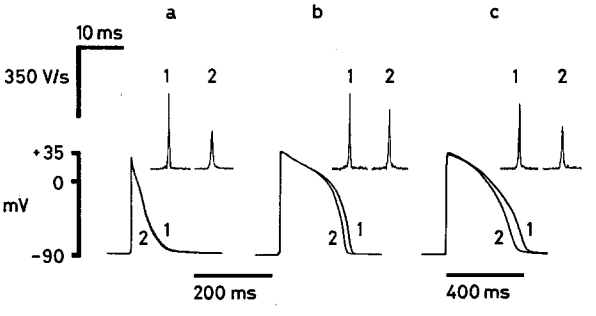

Figure 1. Effects of TTX 3×10^{-6} mol/l on dV/dt_{max} and APD in guinea pig atrium (a), guinea-pig ventricle (b) and human ventricle (c). Original records under control conditions (1) and 5 min after the addition of TTX (2) were superimposed (AP) or depicted side by side (dV/dt).