Virchows Arch. A Path. Anat. and Histol. 376, 353–360 (1977)

Virchows Archiv A

Studies on the Vesicular Component of the Auerbach's Plexus and the Substance P Content of the Mouse Colon in the Acute Phase of the Experimental *Trypanosoma cruzi* Infection

H.O. Almeida
Faculdade de Medicina do Triangulo Mineiro, Uberaba, Brazil

W.L. Tafuri, J.R. Cunha-Melo, L. Freire-Maia, P. Raso and Z. Brener
Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Summary. A dramatic reduction in the total number of dense vesicles in Auerbach's plexus of the mouse colon was observed during the acute phase of experimental American Trypanosomiasis (Chagas' disease). A significant decrease in substance P activity of the colon of inoculated animals was also measured. It is suggested that this decrease in substance P activity could be related to the reduction in the number of dense vesicles in Auerbach's plexus.

Key words: Chagas' disease — Auerbach's plexus — Substance P.

Introduction

It has been demonstrated that experimental American Trypanosomiasis (Chagas' disease) causes lesions of cells and nerve fibers of Auerbach's plexus in the colon (Tafuri, 1971). Experimental evidence seems to indicate that the undecapeptide called substance P could be related to the granular vesicles of Auerbach's plexus (Tafuri et al., 1974a, b; Nilson et al., 1975). The amount of substance P varies at different levels in the intestine (Douglas et al., 1951; Pernow, 1953) and could be related to the disturbances in esophageal and intestinal motility observed in Chagas' disease (Hial et al., 1973). Substance P was recently synthesized by a solid-phase technique (Fisher et al., 1974).

This paper studies the possible changes in number, diameter and morphology of the vesicles in Auerbach's plexus and the substance P content of the colon of mice experimentally inoculated with *Trypanosoma cruzi.*

For offprints contact: Prof. Dr. W.L. Tafuri, Departamento de Anatomia Patológica e Medicina Legal, Faculdade de Medicina da Universidade Federal de Minas Gerais, Belo Horizonte – Brazil

Material and Methods

In order to study the vesicular component of Auerbach's plexus, nine albino mice, male, weighing 18–20 g, were intraperitoneally inoculated with 4000 trypanosomes per gram of body weight of the "Y" strain (Silva and Nussenzweig, 1953). Groups of three animals were killed six, nine and twelve dayes after the inoculation. Five normal mice of the same strain, sex and weight, were used as controls.

Electron Microscopy. Auerbach's plexuses of the colon of control and inoculated mice were prepared according to the method describ by Tafuri (1974a) and examined in a Zeiss EM9S2 electron microscope. The axonal vesicles of the plexus were classified as granular or dense vesicles (DV) and clear vesicles (CV). The former contain an electron-transparent halo between the membranes of the vesicles and its granular core, whereas the latter contain little or no electron dense material (Hager and Tafuri, 1959; Tafuri, 1964). The vesicles were counted in 200 transversely cut axons, in areas not directly related with inflammation; 100 in normal mice and 100 in inoculated animals. The area of each axon was measured by means of a planimeter and the number of vesicles per 100 μm^2 of axonal area was estimated. The maximal diameter of 400 dense vesicles in the normal and in the inoculated groups, and 400 clear vesicles in the same groups were measured, in order to provide data for the histograms.

Substance P Determination. For studies of substance P content of the colon, albino mice, male, weighing 18–20 g were inoculated with *T. cruzi*, using the same method employed for the study of the vesicular components of the Auerbach's plexus. Two groups of seven animal were killed seven and eleven days after the inoculation. Five normal mice of the same strain, sex and weight were used as controls.

Substance P was extracted and determined according to Pernow (1953), with the following modifications: The mice were killed by a blow on the head and exsanguination. The abdominal cavity was openned and the large intestine was dissected, removed and placed in a beaker containing Tyrode's solution. The muscular layers were weighed and chopped in smaller fragments after removal of the mucosa and boiled for 15 min in a glass tube containing 3 ml of Tyrode's solution and 0.2 ml of HCl 0.1 M. The extracted material was centrifuged at 0° C and $10{,}000 \times g$ for 10 min and the supernatant was neutralized with NaOH. This was followed by the addition of 0.5 ml of phosphate buffer to the solution. The supernatant was tested directly on isolated guinea pig ileum or rat duodenum, immersed in an organ-bath containing 10 ml of an aerated Tyrode's solution, at 37° C. To study pharmacological effects of bradykinin, substance P and the colon extrat on the isolated preparations, atropine (1×10^{-7} M) and promethazine (3×10^{-6} M) were added to the Tyrode's solution.

Drugs Used. The doses of atropine sulfate (Sigma), promethazine hydrochloride (Rhodia), acetylcholine chloride (Sigma) and histamine dihydrochloride (Carlo Erba) are expressed as the weights of the salts. Substance P (kindly provided by Prof. C.R. Diniz) and bradykinin (Batch BRS-640, Sandoz) were also used.

Statistical Analysis. The data were subjected to the χ^2 test, Z-test for differences in proportion or Student t-test. In all cases $P < 0.05$ indicates statistical significance.

Results

The total number of vesicles per 100 μm^2 of axonal area in the Auerbach's plexus of the colon was much smaller in mice inoculated with trypanosomes of the "Y" strain than in normal animals (Table 1). This decrease in the number of vesicles in inoculated mice was mainly due to a drastic reduction of dense vesicles (Table 1).

Differences in the diameters of the dense and clear vesicles, in normal and

Table 1. Number of vesicles in the Auerbach's plexus in colon of normal and *T. cruzi* inoculated mice (acute phase)

Type of vesicles	Number of vesicles per 100 μm^2 axonal area	
	Normal mice	Inoculated mice
Dense (DV)	1005	151
Clear (CV)	1243	1163[a]
Total	2248	1314
Ratio DV/CV	0.81	0.13

The mice were inoculated with 4000 trypanosomes per gram of body weight and killed 6, 9 and 12 days later. The vesicles were counted in 200 transversely cut axons, 100 in normal mice and 100 in inoculated mice.

To observe the independence between the number of dense and clear vesicles, in normal and inoculated mice, the χ^2 test was used ($\chi^2 = 446$, $P < 0.001$)

[a] Significantly different from normal mice (Z-test, $P < 0.01$)

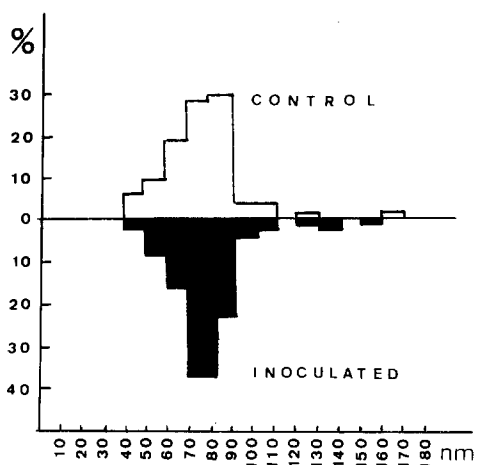

Fig. 1. Histogram comparing the percentual distribution of the maximum diameters of the dense vesicles, in the Auerbach's plexus of the mouse colon, in the control (normal) and in the group inoculated with *T. cruzi*

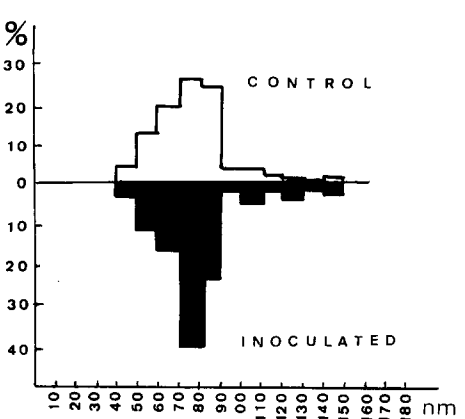

Fig. 2. Histogram comparing the percentual distribution of the maximum diameters of the clear vesicles (little or no electron dense material), in the Auerbach's plexus of the mouse colon, in the control (normal) and in the group inoculated with *T. cruzi*

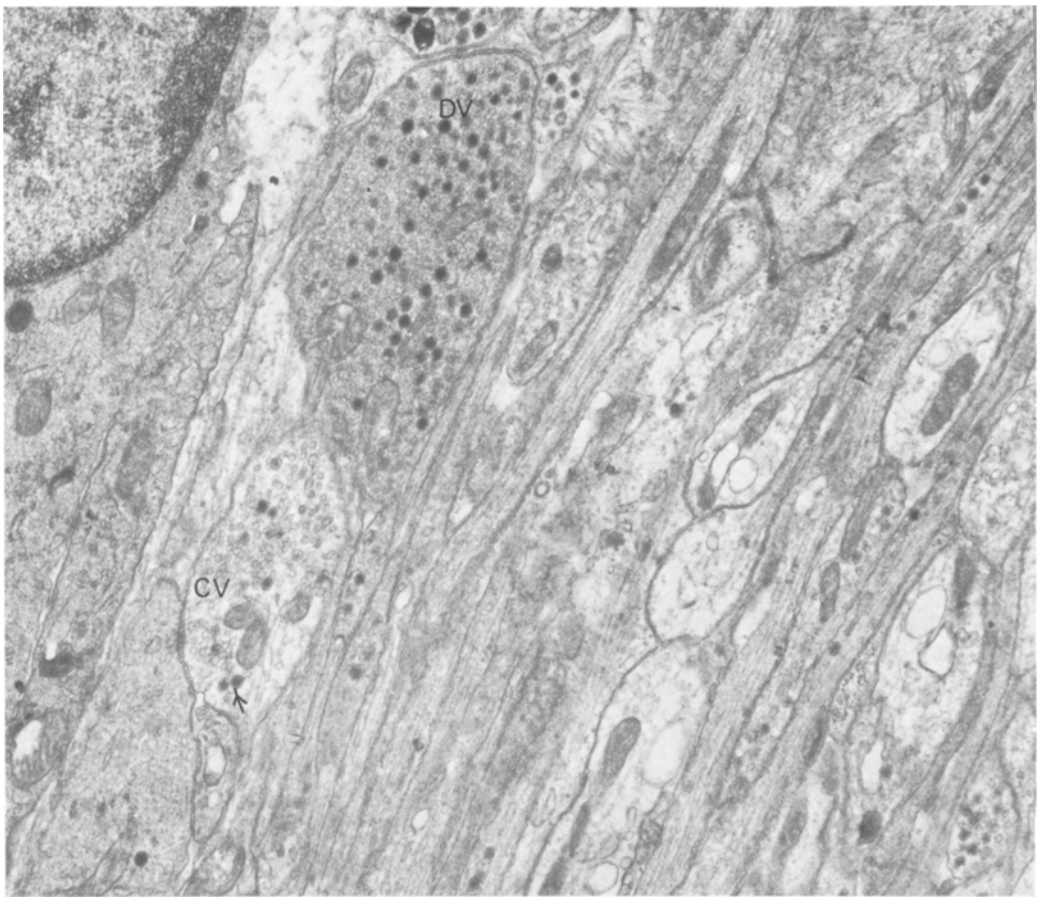

Fig. 3. Colon of normal mouse (Auerbach's plexus). Axon with prepoderance either of dense vesicles (*DV*) or agranular vesicles. The later are of the cholinergic type (*CV*). The arrow indicates the presence of granular vesicles inside of cholinergic axons. ×28,000

inoculated animals, were not observed, since the majority of vesicles had diameters from 70 to 90 nm (Figs. 1 and 2).

Abnormal and apparently normal groups of axons were frequently found side by side in the animals inoculated with *T. cruzi.* The lesions varied from a simple axonal swelling to lysis of neurofilaments and neurotubules, mitochondrial swelling, shrinkage and rupture of the membranes of the vesicles, with the release of the vesicular content (Figs. 3 and 4).

The colon extract of mice inoculated with *T cruzi* induced smaller contractions of the isolated guinea pig ileum than the extract obtained from normal animals. The experiments were carried out in the presence of atropine and promethazine (Figs. 5 and 6). Both extracts and substance P caused contraction of the isolated rat duodenum immersed in Tyrode's solution containing atropine and promethazine, whereas bradykinin relaxed it.

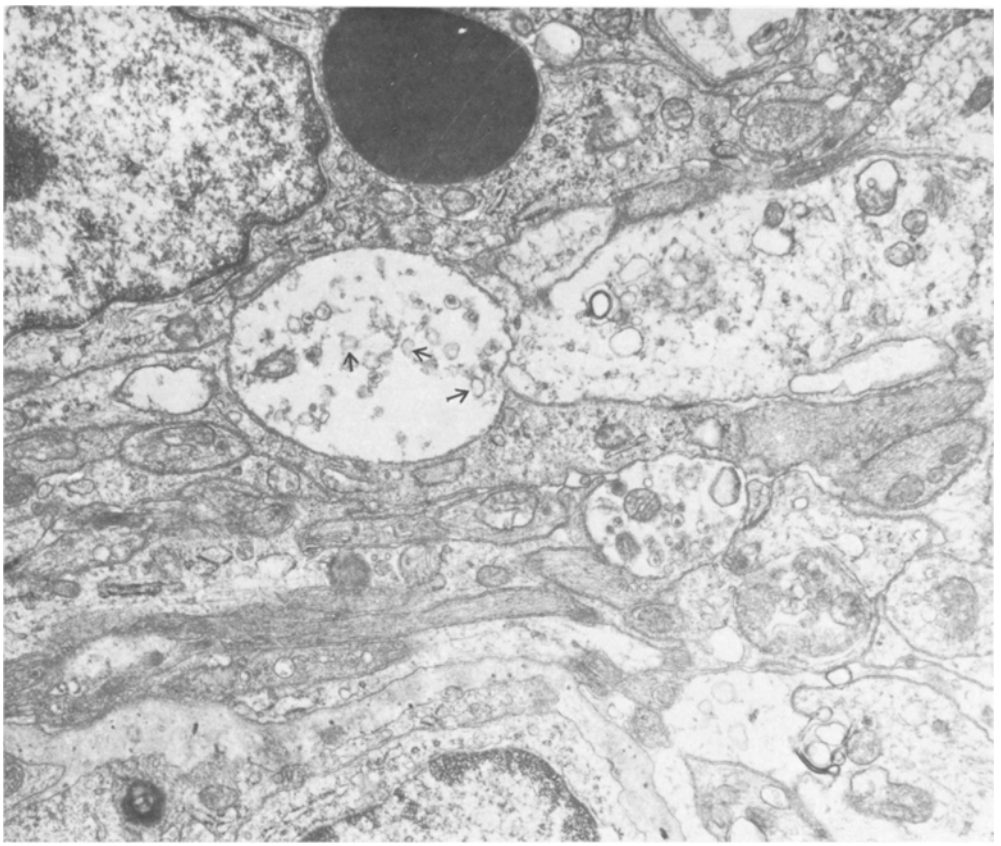

Fig. 4. Colon of mouse inoculated with *T. cruzi* and killed 12 days later. Auerbach's plexus. To observe axonal swelling with lysis of neurofilaments and neurotubules. The majority of the vesicles, still preserved, contain little or no electron dense material. The arrows show rupture of the membranes of the vesicles. ×28,000

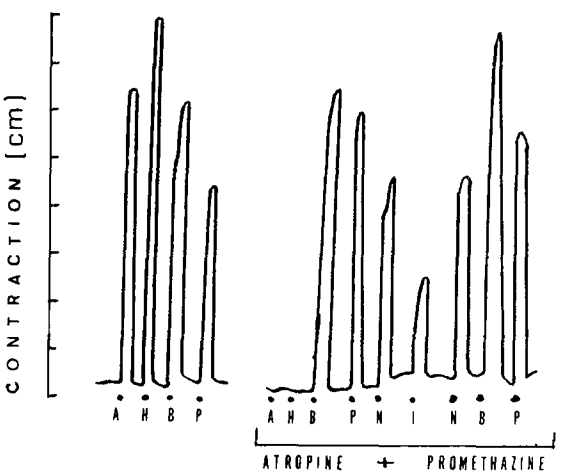

Fig. 5. Strip of guinea pig ileum immersed in Tyrode's solution, at 36° C. Acetylcholine (*A*, 1×10^{-8} M), histamine (*H*, $2{,}7 \times 10^{-7}$ M), bradykinin (*B*, 2×10^{-9} M), substance P (*P*, 2×10^{-8} M), colon extract of normal mice (*N*) and colon extract of mice inoculated with *T. cruzi* (*I*). At right, atropine (1×10^{-7} M) and promethazine (3×10^{-6} M) were added into the Tyrode's solution

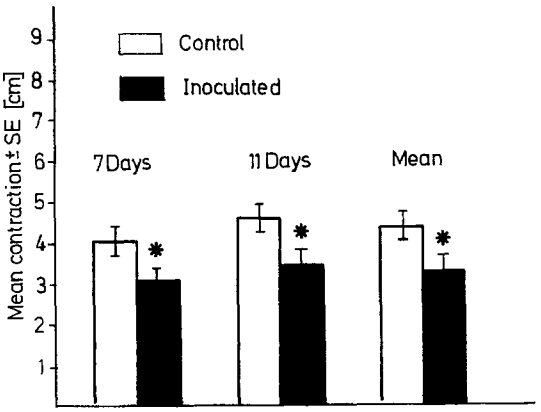

Fig. 6. Histogram comparing the amplitude of contraction of the isolated guinea-pig ileum, immersed in Tyrode's solution, at 36° C, induced by colon extract of normal mice (control) and mice inoculated 7 and 11 days before with *T. cruzi*. At right, it is shown the mean of the results obtained at 7 and 11 days of inoculation. $*P < 0.01$

Discussion

The lesions of cells and nerve fibers found in the autonomic nervous system of the mouse colon, during the acute phase of the American trypanosomiasis (Chagas's disease) have been previously described (Tafuri, 1969; Tafuri 1971). Many of these lesions were also observed in our material.

We have also shown a dramatic reduction in the number of dense vesicles per 100 μm^2 in the Auerbach's plexus of mice inoculated with *T. cruzi*, accompanying a slight reduction of clear vesicles, in comparison to the number found in normal animals. As a consequence of these different reductions, the ratio of dense vesicles over clear vesicles in the inoculated mice was 6.23 times smaller than in control animals.

The reduction in the number of the vesicles could be explained by an axonal swelling, resulting an increase in the axonal area. The rupture of the membranes of the vesicles could also lead to a decrease in the number of vesicles. However, swelling or rupture would only partly explain the reduction of the total number of vesicles; they do not explain the dramatic reduction of the ratio of dense vesicles to clear vesicles.

Alternatively lesions in the perikarion and in the nerve fibers of Auerbach's plexus, induced by the parasite and the inflammation, might explain, in part, the reduction of dense vesicles, if they lead to a decrease in its ability to synthesise chemical mediators. The release of chemical mediators from dense vesicles due to the presence of the parasite and/or as a consequence of inflammatory processes could also explain, in part, the reduction of dense vesicles.

It seems that the chemical mediators released from the dense vesicles in the acute phase of Chagas' disease could induce changes in the intestinal motility. One of these mediators is noradrenaline, which is present in the dense (granular) vesicles (Hager and Tafuri, 1959; Grillo and Pallay, 1962; Baumgarten, 1967; Tranzer et al., 1969). Recently, it was demonstrated that the noradrenaline

content of the rat heart was reduced to undectable values during the acute phase of experimental Chagas' disease (Machado et al., 1975).

In the present paper we have shown a dramatic reduction in the number of dense vesicles in the Auerbach's plexus of the mouse colon, during the acute phase of Chagas' disease. As noradrenaline is normally stored in these vesicles, we might conclude that there is also a reduction of the noradrenaline content in the mouse colon in this period of the disease.

We have also shown a reduction in substance P activity of the colon of mice inoculated with *T. cruzi*. Some investigations seem to indicate that substance P could also be stored in dense vesicles of Auerbach's plexus (Tafuri et al., 1974a, b; Nilson et al., 1975). Therefore, it seems likely that the decrease in substance P activity of the mouse colon in the acute phase of Chagas' disease could be related to the reduction in the total number of dense vesicles in the Auerbach's plexus.

Acknowledgements. Aided by "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq, Brazil). The authors are also indebted to CNPq for fellowships.

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Received July 4, 1977