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NORADRENALINE CONTENT OF THE HYPOTHALAMUS AND BRAIN-STEM OF RATS INOCULATED WITH TRYPANOSOMA CRUZI

Ramon M. COSENZA, Conceição R. S. MACHADO and Angelo B. M. MACHADO

SUMMARY

Noradrenaline was assayed fluorimetrically in the hypothalamus and brain-stem of rats killed 20 and 32 days after inoculation with the Y strain of **T. cruzi** (300,000 trypomastigotes i.p.). In these animals and their normal controls the right atrial appendages were submitted to the glyoxilic acid fluorescence technique for the demonstration of noradrenergic nerves. The noradrenaline content of the brain-stem and hypothalamus of the infected animals was not significantly different from that of controls. In the atrial appendages, however, an almost complete noradrenergic denervation was observed. This result indicates that the mechanism involved in neuronal lesion in Chagas'disease discriminates between peripheral and central noradrenergic neurons.

INTRODUCTION

Lesions of the nervous system in the course of **T. cruzi** infection have been reported since the early works of CHAGAS [5]. In the peripheral nervous system both somatic [19] and visceral neurons [10] are involved. Central neurons are also affected and neuron death has been described in several locations in the human [3,4,11] and experimental disease [7,8,22].

Previous studies have shown that during the acute phase of experimental Chagas' disease in the rat there is an almost complete disappearance of the noradrenergic innervation of the heart [12], submandibular gland [14], ileum [17], and some areas of the male genital system [12]. Coherently with these findings the levels of noradrenaline are markedly reduced in the submandibular gland [14] and totally depleted in the heart [12,13].

It is now well established that the central nervous system (CNS) contains a complex system of noradrenaline-containing neurons [6] whose importance for the regulation of several functions has been recently emphasized [15]. The possibility of involvement of this system during the acute phase of Chagas' disease was now investigated by carrying out fluorimetric assays of noradrenaline in the CNS of rats inoculated with **T. cruzi.** The assays were performed in the brain-stem and hypothalamus, respectively, the locations of the cell bodies and the main projections of the central noradrenergic system.

MATERIAL AND METHODS

Thirteen male Holtzman rats, weighing from 117 to 175 g were used. Seven animals aged 28 days were inoculated i.p. with blood containing 300,000 trypomastigotes of the Y strain of **T. cruzi** and six littermates remained as control. The animals were killed by decapitation 19 to 20 days and 32 to 34 days after inoculation, always at the same hour in order to avoid circadian variations of noradrenaline content. The brain was quickly removed and

Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais. Caixa Postal 2486. 30000 Belo Horizonte MG, Brasil

trimmed to obtain two blocks containing respectivelly the hypothalamus and the brain-stem. The hypothalamus block was obtained by making frontal cuts through the optic chiasma and the mammilary bodies followed by an horizontal cut through the anterior commissure. The brain-stem block was obtained by removing the cerebellum and making a midcollicular cut followed by a cut at the level of the first cervical spinal nerve root.

The blocks were weighed, immersed in 0.8N perchloric acid and homogenized in a Sorvall Omni-mixer. Noradrenaline was then assayed fluorimetrically by the technique of ANTON & SAYRE [2].

All animals had the right auricular appendages removed, treated according to the glyoxilic acid induced fluorescence method [21] for the demonstration of catecholamines, in order to evaluate the degree of noradrenergic denervation of the heart. The Orthoplan (Leitz) fluorescence microscope equipped with the epi-illumination system (Ploemopack 21) were used.

RESULTS

The results are shown in Table I. The noradrenaline content of the brain-stem and hypothalamus of the infected rats were not significantly different from that obtained for the control animals. Moreover, there was no difference between the results obtained at days 19 to 20 and 32 to 34 after inoculation. By contrast, an almost complete disappearance of the noradrenaline containing terminals could be detected by the histochemical method performed in the atria of the infected animals at days 19 to 20 and 32 to 34 after inoculation, whereas the normal pattern of innervation could be seen in all control animals.

TABLE I

Noradrenaline content of the brain-stem and hypothalamus of rats inoculated with the Y strain of the **T. cruzi**

Days after inoculation	Group	Noradrenaline (μg/g) + S.D.	
		Brain-stem	Hypothalamus
19 to 20	Control (3)	0.30 ± 0.09	1.26 ± 0.20
	Infected (3)	0.38 ± 0.12	1.24 ± 0.17
32 to 34	Control (3)	0.40 ± 0.05	1.30 ± 0.18
	Infected (3)	0.36 ± 0.10	1.08 ± 0.14

DISCUSSION

In the conditions of our experiments the stores of noradrenaline in the brain-stem and hypothalamus were not modified during the acute phase of the experimental Chagas' disease. Since these areas contain, respectivelly, the main nuclei of origin and the main projections of the central noradrenergic system [15], these results indicate that this system is not affected during the acute phase of the disease. Nevertheless, the histochemical studies performed in the same infected animals in which the central assays of noradrenaline were performed, revealed an almost complete disappearance of the atrial noradrenergic innervation. This is in good agreement with previous results [12] demonstrating the depletion of the cardiac stores of noradrenaline together with the disappearance of its noradrenergic innervation during the acute phase of Chagas' disease. Indeed, involvement of the peripheral noradrenergic system during this phase of the disease has also been demonstrated for several organs [14,16,17].

The pathogenic mechanism of neuronal lesions in Chagas' disease has been a matter of considerable discussion [1]. Recent data favours the view that an autoimmune mechanism might be involved [20]. It has been demonstrated that serum from **T. cruzi** infected patients contains antibodies that cross-react with Schwann cells, thus recognizing peripheral but not central nervous structures [9]. This finding could explain why in our results the peripheral noradrenergic fibres were damaged while in the central nervous system they were spared. Nevertheless, other mechanisms are certainly involved, as there is no doubt that some types of central neurons are also damaged in Chagas' disease [4, 7,11,18,22]. The possibility that such damage could also be due to an immunological mechanism is supported by the recent finding of an IgM monoclonal antibody cytotoxic to mammalian neurons **in vitro** which labels **T. cruzi** and certain types of peripheral and central neurons [23]. Our results indicate that whatever mechanisms are involved in neuronal lesion in Chagas' disease they can discriminate between central and peripheral adrenergic neurons. Since peripheral adrenergic neurons are lesioned whereas central ones are spared, this discrimination appears

to be unrelated to the transmitter specificity of the neurons.

RESUMO

Conteúdo de noradrenalina do hipotálamo e tronco-encefálico de ratos inoculados com Trypanosoma cruzi

O conteúdo de noradrenalina do hipotálamo e do tronco encefálico de ratos controle e inoculados com a cepa Y (300.000 tripomastigotas, i.p.) de **Trypanosoma cruzi** foi medido pela técnica fluorimétrica de Anton e Sayre. Os animais foram sacrificados 20 e 32 dias depois da inoculação. Para avaliação do grau de desnervação simpática do coração dos animais infectados, a aurícula direita foi observada com microscópio de fluorescência após tratamento histoquímico pela técnica do ácido glioxílico.

O conteúdo de noradrenalina do hipotálamo e do tronco encefálico dos animais infectados não diferiu do medido nos animais controle. Contudo, um quase completo desaparecimento das fibras adrenérgicas foi observado no coração dos animais chagásicos, sugerindo que o mecanismo envolvido na lesão discrimina neurônios adrenérgicos centrais e periféricos.

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REFERENCES

1. ANDRADE, Z. A. & ANDRADE, S. G. **in** BRENER, Z. & ANDRADE, Z. A. (eds.) **Trypanosoma cruzi** e Doença de Chagas. Cap. 6. Rio de Janeiro, Guanabara Koogan, 1979.

2. ANTON, A. H. & SAYRE, D. F. — A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. **J. Pharmacol. Exp. Ther. 138:** 360-373, 1962.

3. BRANDÃO, H. J. S. & ZULIAN, R. — Nerve cell depopulation in chronic Chagas' disease. A quantitative study in the cerebellum. **Rev. Inst. Med. trop. São Paulo 8:** 281-286, 1966.

4. BRITO COSTA, R. & GALLINA, R. A. — Hipotálamo anterior na moléstia de Chagas humana. **Rev. Inst. Med. trop. São Paulo 13:** 92-98, 1971.

5. CHAGAS, C. — Les formes nerveuses d'une nouvelle trypanosomiase **(Trypanosoma cruzi** inoculé par **Triatoma megista)** (Maladie de Chagas). **Nouv. Iconogr. Salpêtrière 26:** 1-9, 1913.

6. DAHLSTROM, A. & FUXE, K. — Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in cell bodies of brain stem neurons. **Acta Physiol. Scand.** (Suppl. 232) **62:** 1-55, 1964.

7. JARDIM, E. — Moléstia de Chagas experimental no rato. Parasitismo do núcleo do II par craniano. **Rev. Inst. Med. trop. São Paulo 13:** 405-410, 1971.

8. JARDIM, E. — Moléstia de Chagas aguda experimental. Parasitismo do hipotálamo. **Arq. Neuro-Psiquiat.** (São Paulo) **29:** 191-197, 1971.

9. KHOURI, E. L.; RITACCO, V.; COSSIO, P. M.; LAGUENS, R. P.; SZARFMAN, A.; DIEZ, C. & ARANA, R. M. — Circulating antibodies to peripheral nerve in American trypanosomiasis (Chagas' disease). **Clin. Exp. Immunol. 36:** 8-15, 1979.

10. KÖBERLE, F. — Chagas' disease and Chagas' syndromes. The pathology of American trypanosomiasis. **Adv. Parasitol. 6:** 63-116, 1968.

11. LOPES, E. R.; TAFURI, W. L. & CHAPADEIRO, E. — Estudo morfológico e quantitativo dos núcleos dorsal do vago e hipoglosso em chagásicos crônicos com e sem megaesôfago. **Rev. Inst. Med. trop. São Paulo 11:** 123-129, 1969.

12. MACHADO, A. B. M.; MACHADO, C. R. S. & GOMES, C. B. — Depletion of norepinephrine in experimental acute myocarditis caused by **Trypanosoma cruzi. Experientia 31:** 1202-1203, 1975.

13. MACHADO, C. R. S.; MACHADO, A. B. M. & CHIARI, C. A. — Recovery from heart norepinephrine depletion in experimental Chagas' disease. **Am. J. Trop. Med. Hyg. 27:** 20-24, 1978.

14. MACHADO, C. R. S.; ALVES, J. B. & MACHADO, A. B. M. — Adrenergic innervation and noradrenaline content of the rat submandibular gland during the experimental **Trypanosoma cruzi** infection. (in press).

15. MOORE, R. Y. & BLOOM, F. E. — Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. **Ann. Rev. Neurosci. 2:** 113-168, 1979.

16. NOVAES, J. C. & MACHADO, C. R. S. — Adrenergic innervation of male genital system in rats with Chagas' disease. Rio de Janeiro, Anais Congr. Intern. Doença de Chagas, 1979, pág. 30.

17. RIBEIRO, J. E.; MACHADO, C. R. S. & MACHADO, A. B. M. — Adrenergic innervation of the small intestine in rats with Chagas' disease. VIII Reunião Pesq. Bas. Doença de Chagas. Caxambu, 1981, pág. 30.

18. RIBEIRO DOS SANTOS, R.; MARQUEZ, J. O.; VON GAL FURTADO, C. C.; RAMOS DE OLIVEIRA, J. C.;

MARTINS, A. R. & KÖBERLE, F. — Antibodies against neurons in chronic Chagas' disease. **Tropenmed. Parasitol. 30:** 19-23, 1979.

19. SANZ, O. P.; SICA, R. E. P.; BASSO, S. & FUMO, T. — Compromiso del sistema nervioso periférico en la enfermidad de Chagas. **Medicina** (Buenos Aires) **40** (Supl. 1): 231-233, 1980.

20. TEIXEIRA, A. R. L.; TEIXEIRA, M. L. & SANTOS-BUCH, C. A. — The immunology of experimental Chagas' disease. IV. Production of lesions in rabbits similar to those of chronic Chagas' disease in man. **Am. J. Path. 80:** 163-180, 1975.

21. DE LA TORRE, J. C. & SURGEON, J. N. — A methodological approach to rapid and sensitive monoamine histofluorescence using a modified glyoxilic acid technique. The SPG method. **Histochemistry 49:** 81-93, 1976.

22. VICHI, F. L. — Destruição de neurônios motores na medula espinhal de ratos na fase aguda da moléstia de Chagas. **Rev. Inst. Med. trop. São Paulo 6:** 150-154, 1964.

23. WOOD, J. N.; HUDSON, L.; JESSELL, T. M. & YAMAMOTO, M. — A Monoclonal antibody defining antigenic determinants on subpopulations of mammalian neurons and **Trypanosoma cruzi** parasites. **Nature** (London) **296:** 34-38, 1982.

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