40. RECENT ADVANCES ON THE MECHANISM OF ACTION OF BRAZILIAN SCORPION VENOM — *T. SERRULATUS*

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The mechanism of the pronounced systemic hypertension produced by scorpion venom is not yet completely understood. Most of the theories relate the hypertensive response to a sympathetic stimulation, which, according to the different authors could be: *peripheral* (1, 2) (post ganglionic endings of the sympathetic system), *central* (3) (spinal cord and sympathetic preganglionic neurons) or both (4, 5). However, no attempt was made to suggest whether the sympathetic stimulation produced by the venom is a direct one or mediated by released catecholamines. On the other hand, it is known that the venom induces a contraction of the guinea-pig ileum through the release of acetylcholine (6, 7).

Since the venom seems to interfere with both components of the autonomic nervous system, we decided to investigate its actions on the isolated guinea-pig heart which gives opposite responses whether adrenergic or cholinergic agents are tested.

Isolated hearts from eighteen guinea-pigs prepared according the Langendorff method, were used.

The typical effect of the venom * is a bradycardia followed by a conspicuous increase in the force and frequency of the heart beatings; these effects are achieved in doses ranging from 5-10 mcg which to correspond to a final concentration of the order of 2×10^{-6} to 1×10^{-6} g/ml. In a few hearts a higher dose (20-40 mcg) was necessary to produce the bradycardia which usually precedes the stimulating effect (Fig. 1). We have shown that the cardiostimulating effect is indirect and due to the release of catecholamines since it was blocked by D.C.I. (9) and bretylium (2×10^{-5} g/ml/min.) and was absent in the reserpine treated animals ** (Fig. 2).

In our experiments no potentiation of the cardiac effects of epinephrine by the venom was seen indicating no interference by the venom in the mechanisms of inactivation of catecholamines.

The bradycardic response to the venom is better seen when the cardiostimulating effect is blocked by D.C.I. bretylium or previous reserpinization. The bradycardia is cholinergic in nature since it is blocked by atropine (2×10^{-5}

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* The whole venom, kindly supplied by Dr. W. Bücherl — Instituto Butantan, São Paulo, was obtained by electrical stimulation(8).

** Three guinea-pigs received 2 mg/kg of reserpine divided in two doses of 1 mg given intraperitoneally 48 and 24 hs. before the experiments.

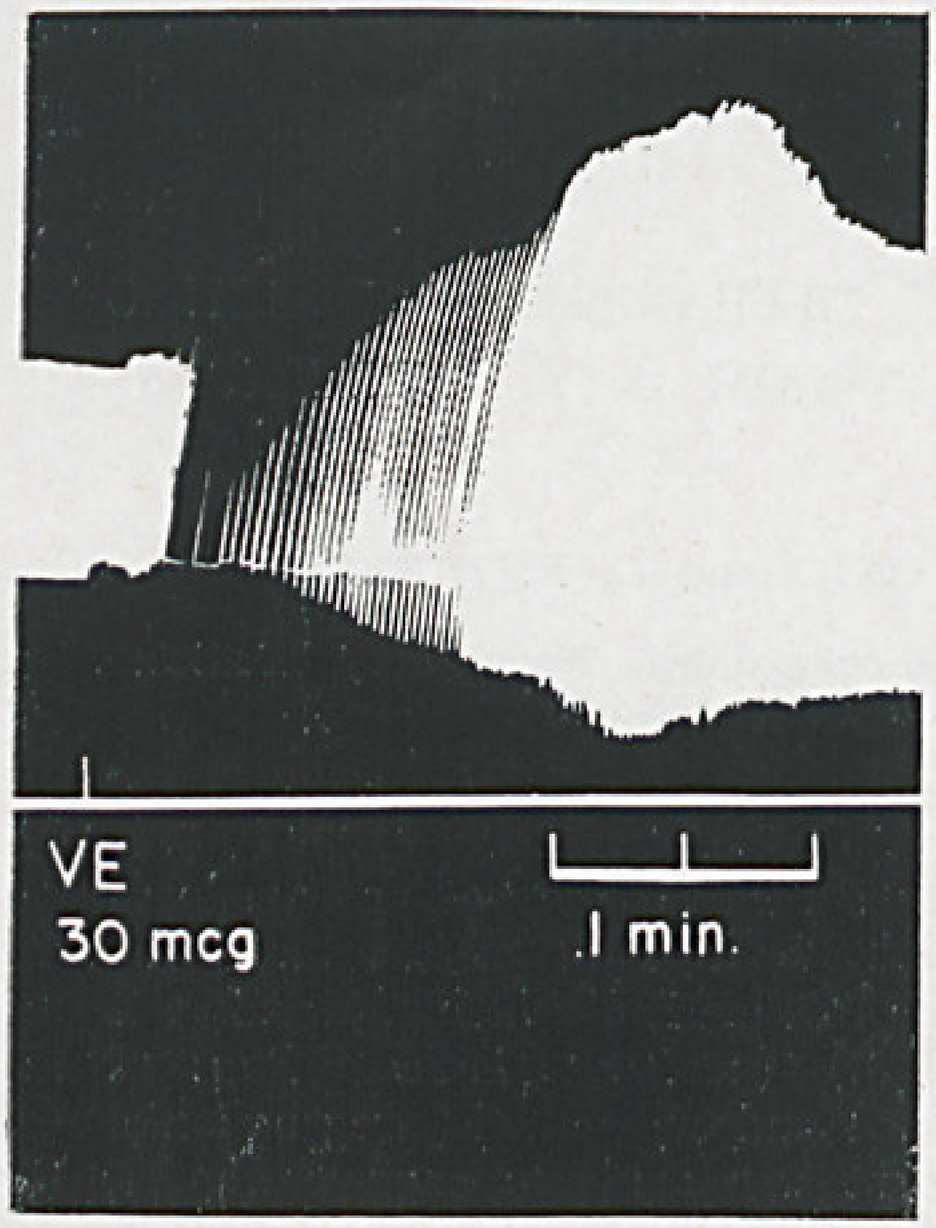

Fig. 1 — Normal guinea-pig heart — Effect of 30 mcg of scorpion venom (*VE* — final concentration 6×10^{-6} g/ml) on the isotonic force of contraction and frequency of heart beating. Notice the bradycardia preceding the cardiac stimulation.

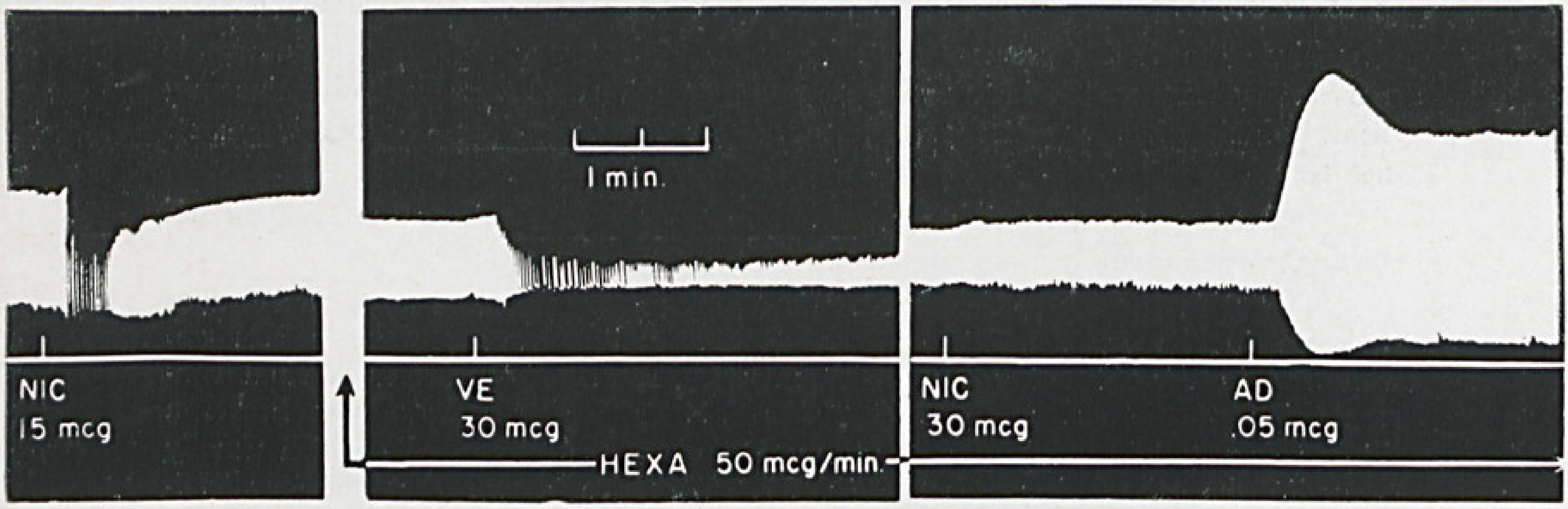

Fig. 2 — Reserpinized guinea-pig heart — No cardiac stimulation is observed with 15 mcg of nicotine (*NIC* — final concentration 3×10^{-6}) or 30 mcg scorpion venom (*VE* — final concentration 6×10^{-6}). Notice that hexamethonium (1×10^{-5} g/ml/min) blocks the bradycardic effect of nicotine but not that of the venom. At *AD*, epinephrine (final concentration 1×10^{-8} g/ml).

g/ml) and potentiated by prostygmine (4×10^{-6} g/ml). Moreover, the bradycardic action of the venom seems to be indirect since it is significantly reduced by hemicholinium (4×10^{-5} g/ml/min.) in agreement with this view is the observation that the contraction of the guinea-pig ileum elicited by the venom is markedly depressed by morphine (6) and cocaine (10). The site of action of the venom is post ganglionic since it was not affected by hexamethonium (1×10^{-5} g/ml/min.) in doses which abolished the response to nicotine (Fig. 2).

The site of action of the cardiostimulating effect of the venom is not at the sympathetic ganglion because it was not affected by either hexamethonium or atropine. On the other hand the effect of the venom is bigger and more resistant to tachyphylaxis than that elicited by tyramine; also the effect of the venom but not of tyramine is blocked by hemicholinium (4×10^{-6} g/ml/min.).

It should be noted that the only drug able to block simultaneously the cholinergic and the adrenergic effects of the venom is hemicholinium, which is a compound known to interfere with the synthesis of acetylcholine (11). Such result strongly suggests the participation of acetylcholine in both effects of the venom, i.e., bradycardia followed by cardiac stimulation. This hypothesis is strengthened by our observation that prostygmine 1×10^{-7} g/ml is able to potentiate the cardiac stimulation in the atropinized heart (Fig. 3). When we con-

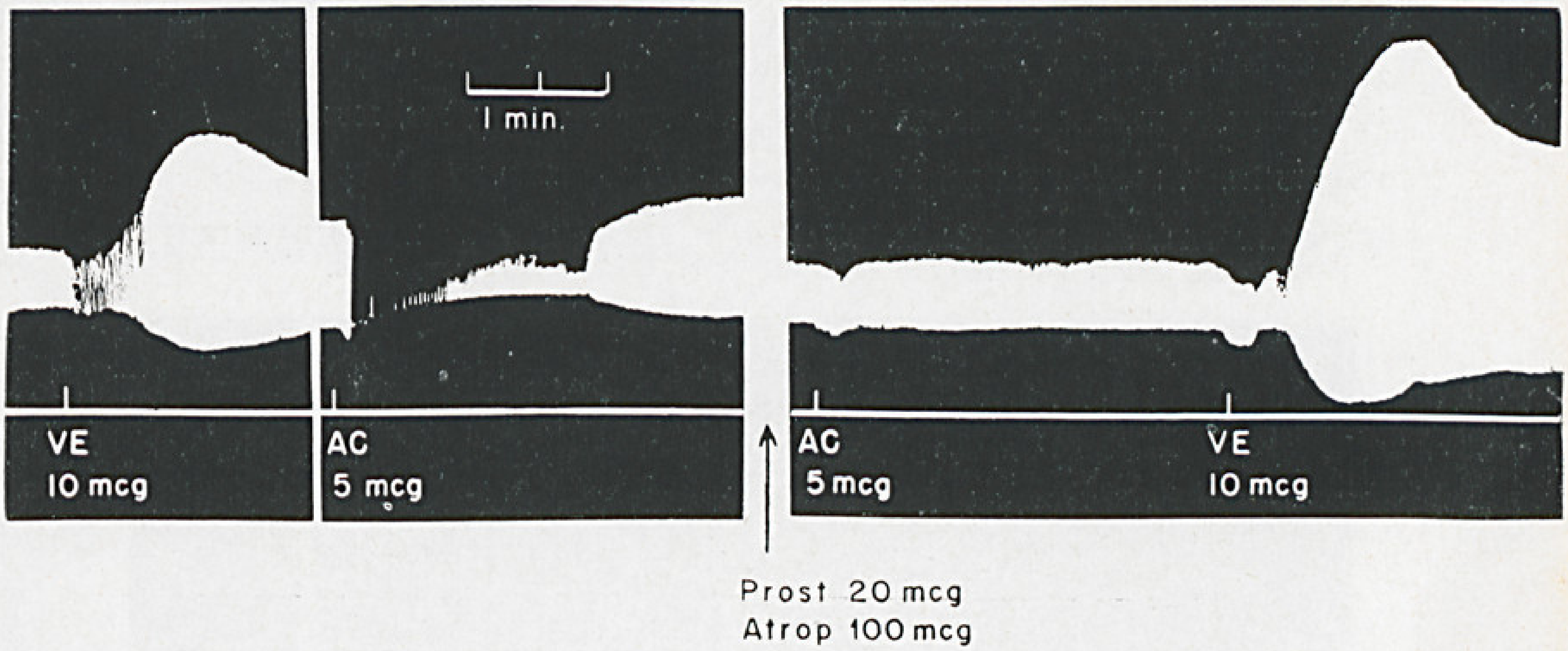

Fig. 3 — Normal guinea-pig heart — Cardiac effects of 10 mcg of scorpion venom (*VE* — final concentration 2×10^{-6} g/ml) and acetylcholine (*AC* — final concentration 1×10^{-6} g/ml) before and after the simultaneous administration of prostygmine (4×10^{-6} g/ml) and atropine (2×10^{-5} g/ml). Notice the increase of the cardiostimulant effect of the venom after treatment of the atropinized heart by prostygmine.

sider all these facts they agree with and give further support to the theory proposed by Burn and Rand (12) of the existence of a "cholinergic link" at the sympathetic nerve endings. According to the theory of Burn and Rand, the sympathetic post ganglionic stimulation produces a small discharge of acetylcholine which in turn releases norepinephrine.

Therefore, the cardiac stimulating effect of the scorpion venom resembles that of sympathetic nerve ending stimulation in the same way as it has been described and assumed for guanethidine (13).

References

1. HASSAN, A., and MOHAMMED, A. H., *Lancet*, 1, 1001, 1940.

2. MOHAMMED, A. H., *Lancet*, 2, 364, 1942.

3. DEL POZO, E. C., ANGUIANO, L. G., and GONZALES, Q. J., *Rev. Inst. Salubr. Enfer. trop.*, **5**, 227, 1944.

4. RAMOS, A. O., and CORRADO, A. P., *Ann. Fac. Med. Univ. S. Paulo*, **28**, 81, 1954.

5. FREIRE-MAIA, L., and FERREIRA, M. C., *Mem. Inst. Oswaldo Cruz*, **59**, 11, 1961.

6. DINIZ, C. R., and VALERI, V., *Arch. int. Pharmacodyn.*, **71**, 1, 1959.

7. TORRES, J. M., and DINIZ, C. R., *Ciência e Cultura*, **16**, 197, 1964.

8. BÜCHERL, W., *Mem. Inst. Butantan*, **25**, 53, 1953.

9. ANTONIO, A., DINIZ, C. R., and CORRADO, A. P., *Ciência e Cultura*, **15**, 270, 1963.

10. DINIZ, C. R., and GONÇALVES, J. M., in E. E. BUCKLEY, and N. PORGES (Editors), *Venoms*, Amer. Ass. Advanc. Sci., Washington, 1956, p. 131.

11. BURN, J. H., and RAND, M. J., *Ann. Rev. Pharmacol.*, **5**, 163, 1965.

12. BURN, J. H., and RAND, M. J., *Nature*, **184**, 163, 1959.

13. COSTA, E., and BRODIE, B. B., in H. E. HIMWIDCH, and W. HIMWIDCH (Editors), *Biogenic Amines*, Elsevier. Pub. Co., Amsterdam, 1964.