Mem. Inst. Butantan
49 (1):25-33, 1987

SPIDER VENOMS ACTING ON THE SODIUM CHANNEL *

Oswaldo VITAL BRAZIL **

ABSTRACT: Several toxins — animal, plant and microbial toxins act on the sodium channel. They may block it, slow down its inactivation or produce a persistent activation of it. The venom of spiders of the suborder Labidognatha, Ctenidae family, genus *Phoneutria* and of the suborder Orthognata, Dipluridae family, genus *Atrax* contain toxins that induce activation and/or slow down of the sodium channel. We have investigated the mechanism of action and the effects produced by *Phoneutria nigriventer* venom at the rat phrenic nerve-diaphragm muscle preparation. It was found that the venom caused a non-uniform depolarization of the diaphragm muscle fiber membrane which was abolished by tetrodotoxin or reduction of the sodium concentration in the bath fluid. The increase in the frequency of the miniature end-plate potentials induced by the venom was also suppressed by tetrodotoxin. On the other hand, the duration of action potentials was not increased by the venom. These results indicated that *Phoneutria* venom activates the voltage-dependent sodium channel in muscle and nerve cell membrane. All efects of venom on the phrenic nerve diaphragm muscle preparation can be explained on the basis of its action in the sodium channels. Sutherland studied the action of atraxotoxin, the main toxin from *Atrax robustus* venom, in the chicken *biventer cervicis* preparation. It was found that atraxotoxin induces spontaneous phasic contractions and enhances the response of the muscle to indirect stimulation. The spontaneous contractions were abolished by gallamine, succinylcholine, lowered calcium or elevated magnesium and by tetrodotoxin. This last observation suggests that atraxotoxin produces the spontaneous contractions by activating the sodium channel in nerve terminals.
KEYWORDS: *Phoneutria nigriventer* venom, *Atrax robustus* venom, sodium channel.

INTRODUCTION

The discovery of the mechanism of action of venoms and their toxins presents twofold interest. It permits to clear the pathophysiology of the en-

* Minireview presented in the Spider Venom Satellite Symposium held at Scottsdale, Arizona, in May 23th, 1986.

** Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas, P.O. Box. 6111, Campinas, São Paulo, Brazil.

venomations they produce and to improve their treatment, and/or to introduce in research new venoms or toxins that may become invaluable tools in physiological, pharmacological or pathophysiological investigations. The knowledge of the mechanism of action of such toxins as tetrodotoxin, saxitoxin, toxins from scorpions and some sea anemones, veratrum alkaloids, batrachotoxin and many others that act on the sodium channel has been of great usefullness from one or both points of view referred to. The study of the spider venoms acting on the sodium channel is only in its beginning. *Phoneutria nigriventer* venom activates the sodium channel in muscle and motor nerves [7,8] and may also slow down its inactivation in spinal nerve roots [3]. Sutherland experiments suggest that the main toxin from *Atrax robustus*, an Australian Dipluridae spider, activates also the sodium channel. Venoms of many other spiders from the Ctenidae or Dipluridae families probably act likewise activating and/or slowing down the sodium channel. They evoke signs and symptoms in experimental animals similar to those produced by *Ph. nigriventer* venom [13].

Phoneutria nigriventer venom

Ph. nigriventer (Ctenidae, Labidognatha), an aggressive wandering solitary spider from South America is responsible for most accidents of araneism in center eastern and southern Brazil. Its neurotoxic venom is very potent (Table I). The signs and symptoms it evokes in experimental animals or observed in human accidents are excruciating pain irradiating from the site of introduction, cramps, tremors, tonic convulsions, paralysis, salivation, diarrhea, sudoresis, priapism, tachycardia, arrhythmias and visual disturbances [4,5,10]. It does not produce local edema or necrosis, nor blood coagulation or hemolysis. The venom toxic components are polypeptides having molecular weight between 4000 and 6000 daltons [10,6].

TABLE 1

50% Lethal Dose to Mice of Some Arthropod Venoms Which Acts in the Sodium Channel

ARTHROPOD	Route of injection	LD 50 mg/kg
Leiurus quinquestriatus	subc.	0.25[a]
Androctonus australis	subc.	0.32[a]
Phoneutria nigriventer	subc. i.v.	0.67[b] 0.38[c]
Tityus serrulatus	i.v.	0.66[d]
Buthacus occitanus	subc.	0.90[a]
Centruroides sculpturatus	subc.	1.12[a]

a. Zlotkin et al., 1978.
b. Bucherl, 1983.
c. Fontana and Vital Brazil, 1985.
d. Vital Brazil et al., 1973.

We have investigated the mode of action of *Ph. nigriventer* venom at the isolated rat phrenic nerve-diaphragm preparation [14,7,8]. At the concentration of 5 ug/ml, the venom induced a tonic contraction with superimposed small phasic contractions in unstimulated diaphragms, both effects being suppressed by d-tubocurarine (Figure 1. I). Therefore, these effects must be ascribed to a presynaptic action of the venom producing acetylcholine release. In indirect stimulated diaphragms, the venom at concentrations of 1.0, 5.0 and 25.0 ug/ml produced the following effects: 1st.) a dose dependent tonic contraction of short duraction; 2nd.) small spontaneous phasic contractions, specially evident at venom concentrations of 5 ug/ml; 3rd.) an increase in twitch tension and delay in twitch relaxation; 4th.) a dose-dependent blockade of neuromuscular transmission at venom concentrations of 5.0 and 25.0 ug/ml, an effect partially antagonized by calcium but not by neostigmine or 4-aminopyridine (Figure 1, II, III and IV).

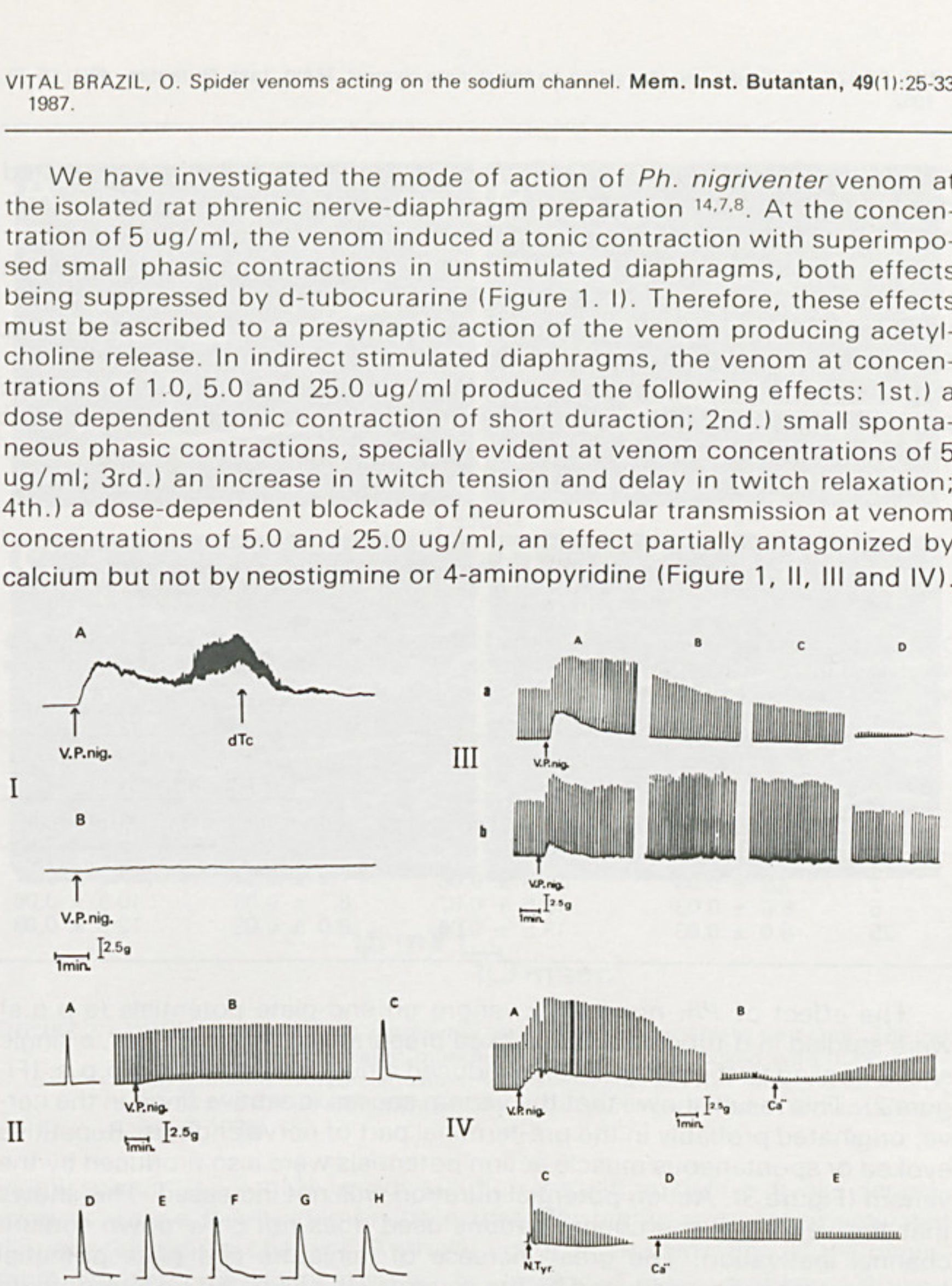

FIGURE 1 – Effects of *Phoneutria nigriventer* venom on muscle contraction. I. Unstimulated rat diaphragm. A, Addition of the venom, 5 ug/ml, and 14.6 uM d-tubocurarine to the bath. B, Addition of the venom, 5 ug/ml, to the bath containing a d-tubocurarine-treated rat diaphragm. II, III and IV, Indirectly stimulated rat diaphragms with maximal shock of 0.1 Hz and 0.2 ms. II, Effects of 1 ug/ml of the venom. A, Before venom addition to the bath; C, 10; D, 20; E, 70; F, 100; G, 140; H, 170 min after venom addition to the bath (paper speed: A, C, D, E, F, G, 5 cm/s; B. 0.02 cm/s). III, Effect of 25 (a) and 5 ug/ml (b) venom. B, C and D, 10, 20 and 40 min after venom addition to the bath. IV, Effect of calcium on venom-induced neuromuscular blockade. A, Addition of 25 ug/ml venom to the bath; B, addition of 10 mM $CaCl_2$; C, wash of the preparation with Tyrode solution; D, addition of 10 mM $CaCl_2$; E, 50 min after D. (Fontana & Vital Brazil, 1985).

In curarised directly stimulated diaphragm, the small phasic contractions did not occur. Therefore, they are due entirely to acetylcholine relea-

se. The tonic initial contraction only appeared in the d-tubocurarine treated diaphragms with the use of 25.0 ug/ml of venom. However, it was significantly smaller than that occuring in non-curarized preparations. The increase in twitch tension was smaller in directly stimulated diaphragms than in the indirectly stimulated ones when 1 and 5 ug/ml of venom were employed (Table 2). Twitch relaxation time was smaller in the curarized directly stimulated diaphragms. In summary, it can be said that at lower concentrations, *Phoneutria* venom presynaptic action is more important than the postsynaptic action in the genesis of the effects evoked at the rat phrenic nerve-diaphragm preparation.

TABLE 2

Effect of Phoneutria Nigriventer Venom on Twitch Tension

Data are reported as the increase in tension (grams) from the baseline to the peak of the twitches of rat phrenic nerve-diaphragm preparations. The directly stimulated preparations were blocked with 14.6 uM d-tubocurarine.

Venom (ug/ml)	Indirectly stimulated rat diaphragms		Directly stimulated rat diaphragms	
	Before venom	After venom	Before venom	After venom
1	7.3 ± 0.03	11.5 ± 0.08	7.0 ± 0.04	7.5 ± 0.07
5	8.0 ± 0.03	13.5 ± 0.07	8.7 ± 0.03	10.5 ± 0.06
25	8.0 ± 0.03	13.5 ± 0.06	8.0 ± 0.03	12.6 ± 0.03

The effect of *Ph. nigriventer* venom on end-plate potentials (e.p.p.s) were studied in d-tubocurarine blocked preparations. After venom, a single shock applied to the phrenic nerve induced a burst of repetitive e.p.p.s. (Figure 2). This result shows that the venom causes repetitive firing in the nerve, originated probably in the pre-terminal part of nerve endings. Repetitive evoked or spontaneous muscle action potentials were also produced by the venom (Figure 3). Action potential duration was not increased. This shows that the venom at the concentrations used does not slow down sodium channel inativation. The great increase of miniature end plate potential (m.e.p.p.) frequency induced by the venom was prevented by tetrodotoxin if added to the bath before venom. When added to the bath after the venom had greatly increased the frequency of the m.e.p.p.s., tetrodotoxin decreaseds it to normal levels (Figure 4). This effect shows that the increase in m.e.p.p. frequency is due to depolarization of the membrane of nerve endings resulting from activation of the sodium channel. The effect of *Phoneutria* venon on the muscle resting membrane potential was studied in preparations blocked by either d-tubocurarine or α-bungarotoxin and in unblocked diaphragms. The results did not differ significantly. Depolarization in five distinct regions of the diaphragm was investigated. It was found that *Ph. nigriventer* venom induced an unequal depolarization of the diaphragm muscle fiber membrane that was blocked by tetrodotoxin. End-plate and adjacent regions (R2 and R1, Figure 5) were much more depolarized by the venom than extrajunction regions (R4 and R5, Figure 5) of the diaphragm.

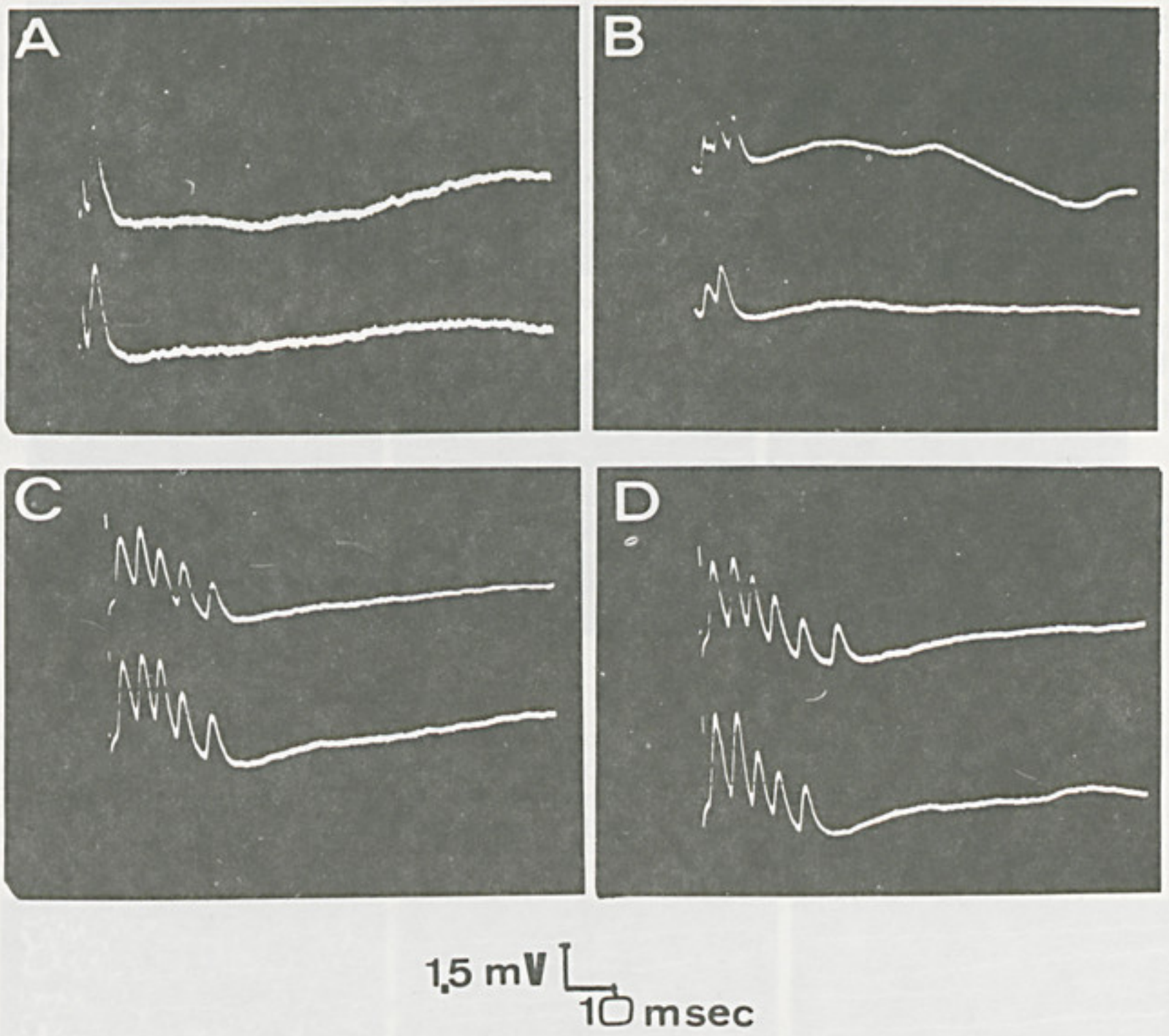

FIGURE 2 – Effect of *Phoneutria nigriventer* venom on the end-plate potential. The rat phrenic nerve-diaphragm muscle preparation was blocked with 0.73 uM d-tubocurarine. Nerve stimulation with single shocks. A, Control; B, C and D, 25, 30 and 40 min after addition of 5 ug/ml venom to the bath (Fontana & Vital Brazil, 1985).

In low sodium (17.2 mM) Tyrode solution the venom did not evoke depolarization. These results demonstrate that *Ph. nigriventer* venom activates the muscle sodium channel. A similar unequal depolarization of the diaphragm muscle fiber membrane is produced by veratrine [15,16] and crotamine [15], which activate also the sodium channel. This phenomenon may be due to a greater density of sodium channels at the end-plate region of the diaphragm or to a non-uniform distribution of activatable sodium channels by these toxins along the membrane of the diaphragm muscle fibers. The first hypothesis is favored by the findings that the maximum rate of rise of the action potential is greater at the end-plate than at extrajunctional regions [9,12,2] and that sodium-current density determined with the use of the loose patch voltage-clamp technique is much higher at regions immediately adjacent to the end-plate than at regions away from it [1].

In conclusion we may say:

1st.) *Ph. nigriventer* venom activates the sodium channel in nerve and muscle cell membrane. Its action in the sodium channel accounts for the effects produced in the rat phrenic nerve-diaphragm muscle preparation

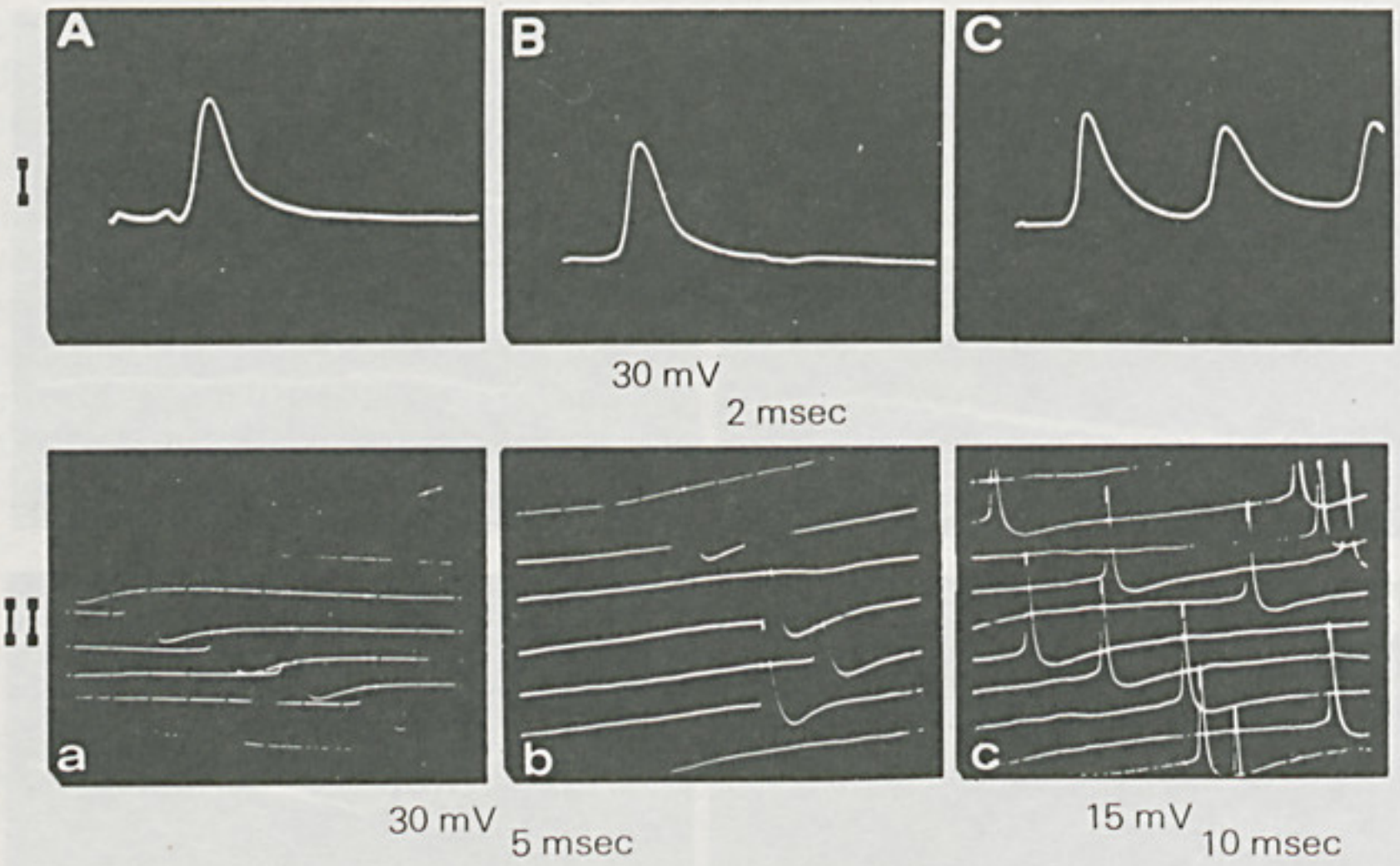

FIGURE 3 — Discharges of repetitive action potentials produced by *Phoneutria nigriventer* venom. I. Effect of 5 ug/ml venom on action potential evoked by nerve stimulation with a single shock II, Spontaneous discharges of action potentials in preparations treated with 1.0 (a), 5.0 (b) and 25.0 (c) ug/ml venom (Fontana & Vital Brazil, 1985).

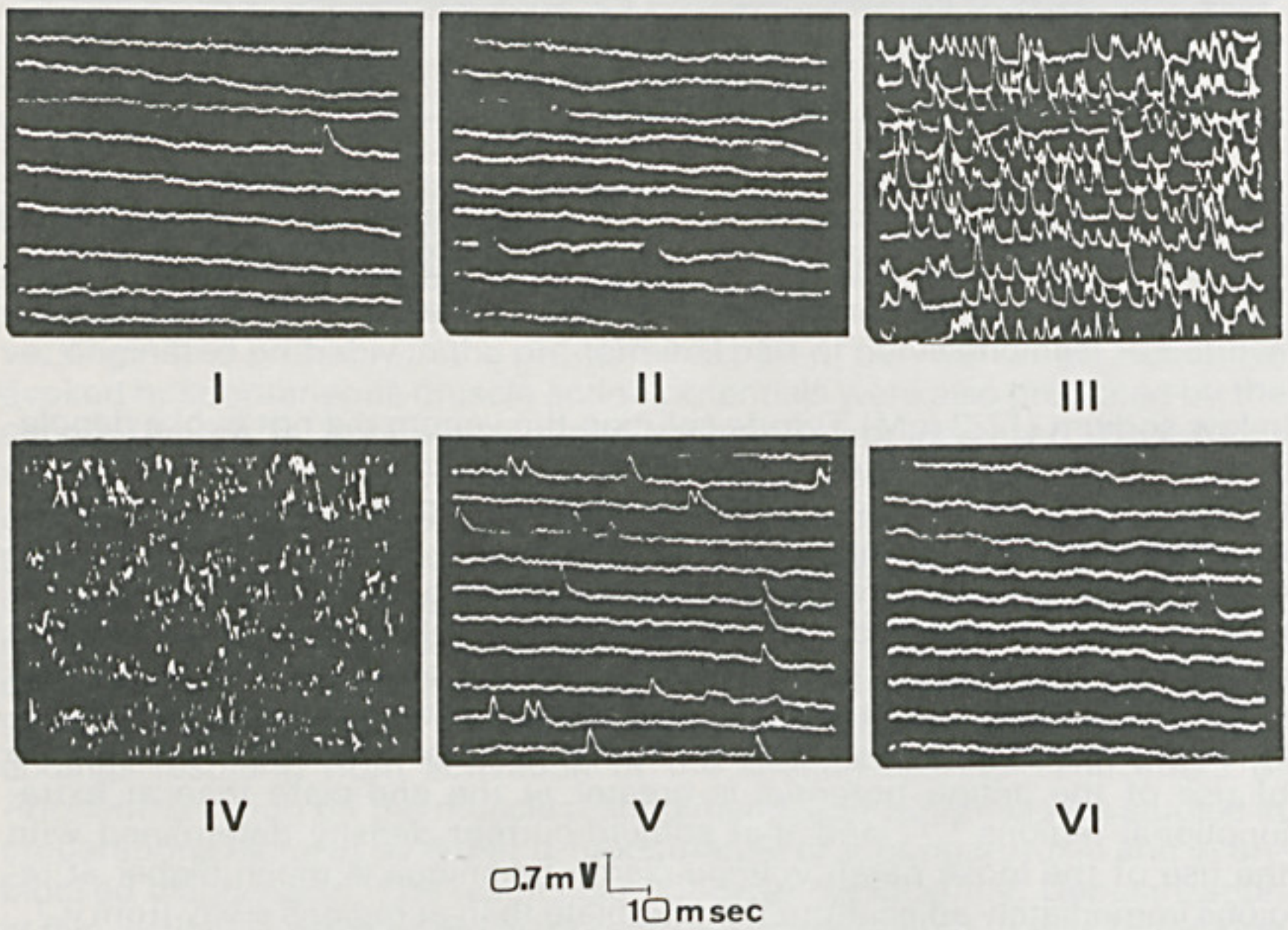

FIGURE 4 — Effect of *Phoneutria nigriventer* venom on the miniature end-plate potentials at the rat phrenic nerve-diaphragm muscle preparation. I, Control; II, III and IV, 10, 20 and 30 min after the addition of 5 ug/ml venom to the bath; V and VI, 5 and 15 min after the addition of 3 uM tetrodotoxin to the bath (Fontana & Vital Brazil, 1985).

and may be responsible for all signs and symptoms observed in experimental or clinical envenomation.

2nd.) *Ph. nigriventer* venom induces bursts of repetitive action potentials which may appear after an evoked action potential or spontaneously. Spontaneous action potential generation denotes the venom acts also reducing the threshold potential of the excitable cell membrane, that is, the threshold potential becomes more negative under the action of *Ph. nigriventer* venom.

3rd.) Neurotransmitter release produced by evoked or spontaneous bursts of repetitive action potentials is the main or unique cause of such effects as spontaneous phasic or tonic contractions, increase in twitch tension and delay in twitch relaxation.

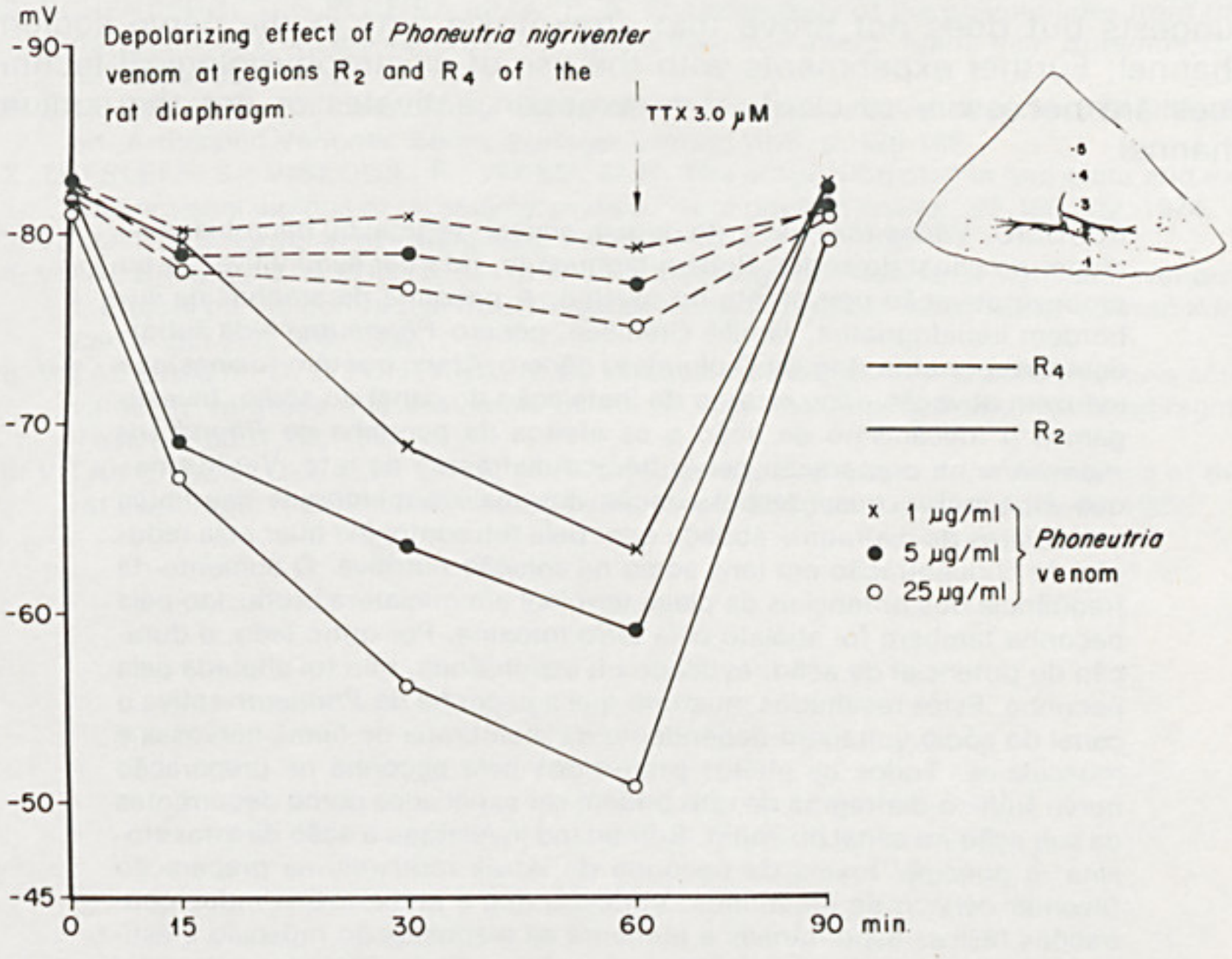

FIGURE 5 — Depolarization produced by *Phoneutria nigriventer* venom at the end-plate (R 2) and an extrajunctional region (R 4) of the rat diaphragm. The preparation was blocked with d-tubocurarine (14.6 uM). Three concentrations (1.0, 5.0 and 25 ug/ml) of venom were use. Tetrodotoxin (3.0 uM) was added to the bath 60 min after venom. Each point in the curves is the mean of three experiments.

Atrax robustus Venom

The Orthognatha spiders of the Dipluridae family, genus *Atrax* occur in southeastern Australia. *A. robustus*, a species from the central coastal region of New South Wales and Blue Mountain region to the west is responsible for severe human accidents. However, fatalities from them are very low. The signs and symptoms of envenomation are severe local pain lasting

for hours or even days, nausea and vomiting, abdominal pain, diarrhea, salivation, lacrimation, sweating, hypertension, dyspnea, local and generalized muscle fasciculations. Muscle twitching may be prolonged and violent. Hypotension in some patients preceeds cardiac arrest.

Sutherland [11] studied the action of atraxotoxin, the main toxin from *A. robustus* venom, in the chicken *biventer cervicis* preparation. He found that atraxotoxin induces spontaneous phasic contractions and enhances the response of the muscle to indirect stimulation. The spontaneous contractions were abolished by gallamine, succinylcholine, lowered calcium or elevated magnesium. These results show that the contractions were produced by acetylcholine release caused by discharge of action potentials in the nerve, very probably originated in the pre-terminal part of the nerve endings. The effect of tetrodotoxin abolishing the spontaneous contractions suggests but does not prove that atraxotoxin acts in the nerve sodium channel. Further experiments with the use of electrophysiological techniques are necessary to clarify if atraxotoxin activates or not the sodium channel.

RESUMO: Várias toxinas — de origem animal, vegetal ou microbiana — atuam no canal do sódio. Podem bloqueá-lo, retardar sua inativação ou produzir ativação persistente do mesmo. A peçonha de aranhas da subordem Labidognatha, família Ctenidae, gênero *Phoneutria* e da subordem Orthognatha, família Dipluridae, gênero *Atrax*, contém toxinas que induzem ativação e/ou retardo da inativação do canal do sódio. Investigamos o mecanismo de ação e os efeitos da peçonha de *Phoneutria nigriventer* na preparação nervo frênico-diafragma de rato. Verificamos que a peçonha causa despolarização desigual da membrana das fibras musculares de diafragma abolida quer pela tetrodotoxina quer pela redução da concentração em íons sódio na solução nutritiva. O aumento da freqüência dos potenciais da placa terminal em miniatura produzido pela peçonha também foi abolido pela tetrodotoxina. Por outro lado, a duração do potencial de ação, evocado ou espontâneo, não foi alterada pela peçonha. Estes resultados mostram que a peçonha de *Phoneutria* ativa o canal do sódio voltagem-dependente da membrana de fibras nervosas e musculares. Todos os efeitos produzidos pela peçonha na preparação nervo frênico-diafragma de rato podem ser explicados como decorrentes de sua ação no canal do sódio. Sutherland investigou a ação da atraxotoxina, a principal toxina da peçonha de *Atrax robustus*, na preparação *biventer cervicis* de pintainhos. Verificou que a atraxotoxina induz contrações fásicas espontâneas e aumenta as respostas do músculo à estimulação indireta. As contrações espontâneas foram abolidas pela galamina, pela succinilcolina, pela redução na concentração de cálcio ou elevação da de magnésio na solução nutritiva e pela tetrodotoxina. Esta última observação sugere que a atraxotoxina produza as contrações espontâneas ativando o canal do sódio nas terminações nervosas.

PALAVRAS-CHAVE: peçonha de **Phoneutria nigriventer**; peçonha de **Atrax robustus**, canal do sódio.

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Recebido para publicação em 08/10/1986 e aceito em 20/2/1987.

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