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EFFECTS OF GRAYANOTOXIN-I ON THRESHOLD INTENSITY AND COMPOUND ACTION POTENTIAL OF FROG SCIATIC NERVE

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Effects of (Grayanotoxin-I) GTX-I, one of the second group of sodium channel toxins, have been investigated on threshold stimulus voltage and upstroke velocity of isolated frog sciatic nerve. GTX-I was isolated from toxic honey obtained from the Black Sea Region of Turkey, and used in the experiments at the concentrations of 3.50^{-5}, 3.10^{-4}, 3.10^{-3} M. The threshold stimulus voltage and upstroke velocity of CAP were decreased and conduction velocity of frog sciatic nerve preparations was increased by GTX-I. The effects were dose-dependent. As a results, it is suggested that the decrease of threshold stimulus voltage and upstroke velocity of CAP and the increase of conduction velocity of frog sciatic nerve is mainly due to an increase in resting membrane permeability to sodium ions.

Key words: *Grayanotoxin, Sodium permeability, depolarization, frog sciatic nerve, action potential*

INTRODUCTION

Toxic honey has been known since ancient times. Xenophon in the Anabasis reported the poisoining of his troop by the honey of Rhododendron ponticum flowers. Rhododendrons belong to the family Ericaceae, which contains a number of well-known toxic substances (1). Honey from Grouse Mountain, British Columbia, Canada, which causes the same type of poisoining, contains grayanotoxins (GTXs) (2, 3). GTXs (GTX-I, II and III), mainly GTX-I occuring in Ericaceae plants are the compounds responsible for poisoining (2, 4). One of the food intoxications encountered in Turkey is that caused by toxic honey, produced by bees which collect nectar from the flowers of Rhododendron species which grow especially in the Black Sea Region of Turkey (3—6).

Pharmacological and physiological studies have demonstrated that GTXs and toxic honey containing GTXs or one of them, have a wide range of systemic effects including hypotension, arrhythmias, respiratory depression, nausea, vomiting, dizziness, and induced amyostatic postures indicating a central nervous system effect (1, 5—10).

GTXs are some of the group II sodium channel toxins (11—13). These toxins, including GTXs, batachotoxin, veratridine and aconitine, depolarize excitable membranes through a specific increase in resting permeability to sodium ions. This effect is antagonized by tetrodotoxin (TTX) (14—19). GTX-I and α-dihydrograyanotoxin-II a devivative of GTX-II, have been shown to depolarize squid axon membranes, frog and rat skeletal muscle fibers, guinea-pig atria, SA node and atrial myocardia of rabbit (9, 10, 16, 18, 20—23).

In the present study, in order to investigate whether GTX-I is also effective on frog sciatic nerve, the effects of GTX-I were studied on threshold stimulus voltage, conduction velocity and upstroke velocity of a compound action potential (CAP) of isolated frog sciatic nerve.

MATERIAL AND METHOD

The experiments were performed on the sciatic nerves of Rana temporaria-frogs weighing 65—80 g, at room temperature (15—17°C), using a modified Ringer's solution, which was composed of NaCl 117 mM, KCl 2.5 mM, NaH_2PO_4 1 mM, Na_2HPO_4 1 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 1.8 mM. The pH of the solution was maintained at 7.2 with 5% tris (24). GTX-I, which was isolated with Scott's method (2) from toxic honey samples obtained from Black Sea Region of Turkey (25) and identified with thin-layer chromatographic method (26) using GTX-I, II and III standards from T. Terai (Japan), was used. For the isolation of GTX-I samples of toxic honey (50 g), which were known to contain GTX-I, were shaken with 400 ml methanol-water (1:3 v/v) until they become homogeneous. The mixture filtered through Whatman no: 40 filter paper, was adjusted to pH = 6.5 with addition of 0.1 N sodium hydroxide and then was extracted with 5 × 30 ml chloroform. This procedure was repeated until 500 g toxic honey was utilized. The combined chloroform phases were evaporated to dryness by a rotavapor (Buchi RE 111), not exceeding 50°C. The residue was washed two times with about 1 ml of petroleum ether for purification, and the purified residue was used as the stock of GTX-I. GTX-I solutions were prepared by diluting the stock of GTX-I with the modified Ringer's solution at the concentrations of 3.10^{-5} M, 3.10^{-4} and 3.10^{-3} M. Ten isolated frog sciatic nerves were studied for each one of these concentrations. Also five preparations were examined as the time control group by using Ringer's solution without GTX-I.

The sciatic nerves removed; partially desheated and preincubated in modified Ringer's solution for 30 minutes were placed in the fluid-electrode chamber, containing eight 5 mm sections, as was described by Nakanishi's (27, 28). Vaseline was used for insulation of leakage points between chambers. Modified Ringer's solution was placed in each section except the third, fourth and fifth sections. Third and fifth section was filled with mineral oil and the fourth section was used for application of GTX-I solution. The nerves were stimulated through Ag-AgCl electrodes, by square wave pulses of 0.5 ms duration every two minutes to determine the threshold intensity and stimulus

voltage for maximum amplitude of monophasic compound action potential (CAP). In our experiments, we used a stimulator-isolator complex consisting of Nihon-Kohden SEN-3201 stimulator and Nihon-Kohden SS-201 J isolator deliver pulse to the nerve and to trigger the pulse on the oscilloscope. CAPs were amplified (Harvard AC-DC preamplifier) and displayed on an oscilloscope (Trio Digital Memoryscope MS 1650 B). The parameters of CAP were evaluated with a Volcraft Digi-Scope converter 500 and recorded with a Epson, LX-810 model printer. The block diagram of the system is shown in *Fig. 1.*

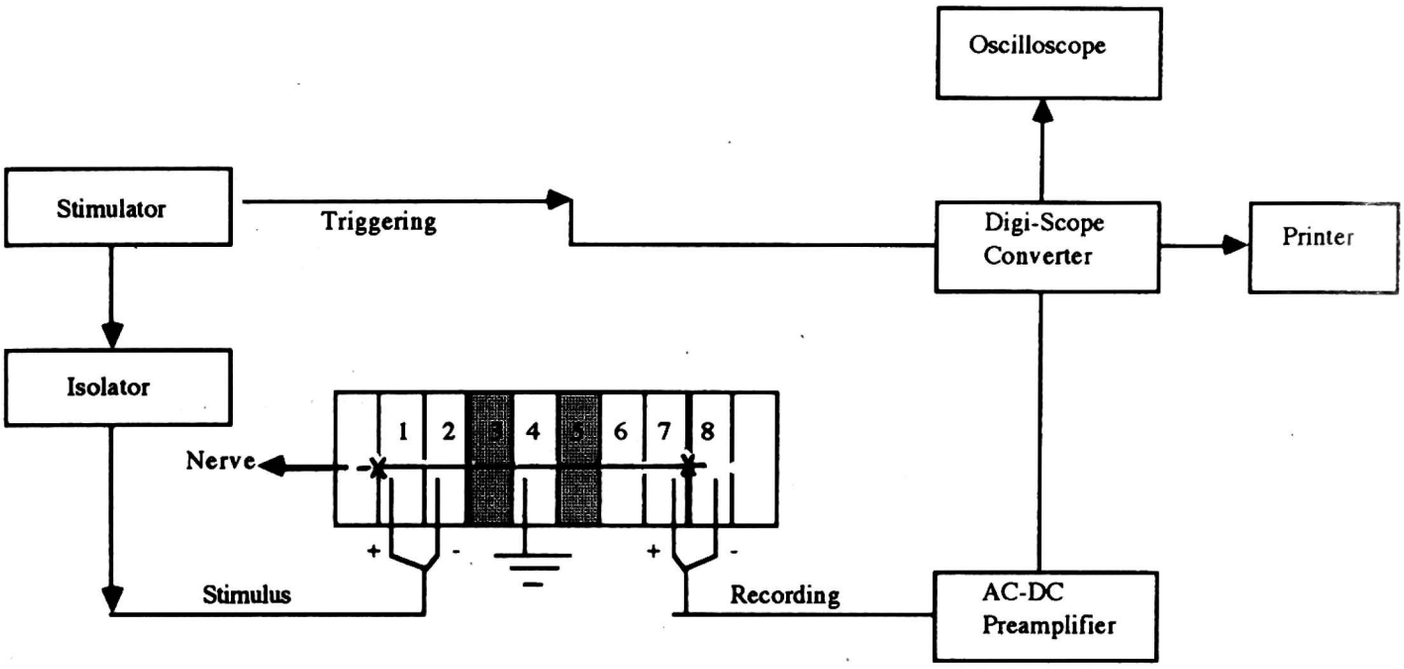

Fig. 1. A diagram of the system for recoroding monophasic compound action potential of isolated frog sciatic nerve.

The threshold stimulus voltage determinations were carried out at 0, 15, 25, 35, 40, 55, 63, 71, 80 minutes.

Monophasic CAPs produced by supramaximal pulses were recorded before and after application of GTX-I, which was done at the fifteenth minute of the experiment and the changes, which were produced in the threshold stimulus voltage, upstroke velocity (dv/dt) of CAP and conduction velocity of isolated frog sciatic nerve with GTX-I, were determined. In order to determine the conduction velocity of nerve, the distance between the stimulus electrode and first recording electrode was divided by the time from stimulus atrifact to maximum CAP (29). Then the nerve was washed with GTX-I free modified Ringer's solution, three times with 8-minute intervals. The parameters were redetermined 9 minutes after the last washing. These determination intervals of data were chosen based on the previous studies (9, 20). Data were analyzed for statistical significance using one-way analysis of variance and modified t-test (30).

RESULTS

The effect of GTX-I on a compound action potential (CAP) is illustrated in *Fig. 2.* As shown in *Fig. 3;* threshold stimulus voltage of the time control group increased in time. No such changes were observed in the conduction velocity and upstroke velocity of CAP of nerve in the time control group.

GTX-I significantly decreased the threshold stimulus voltage (*Fig. 4*) and upstroke velocity of CAP (*Tab. 1*) of frog sciatic nerve. Additionally the conduction velocity of nerve was increased by GTX-I (*Tab. 2*). These changes were reversible with washing. Moreover, the begining of the effect and its strength were proportional to the concentration of GTX-I.

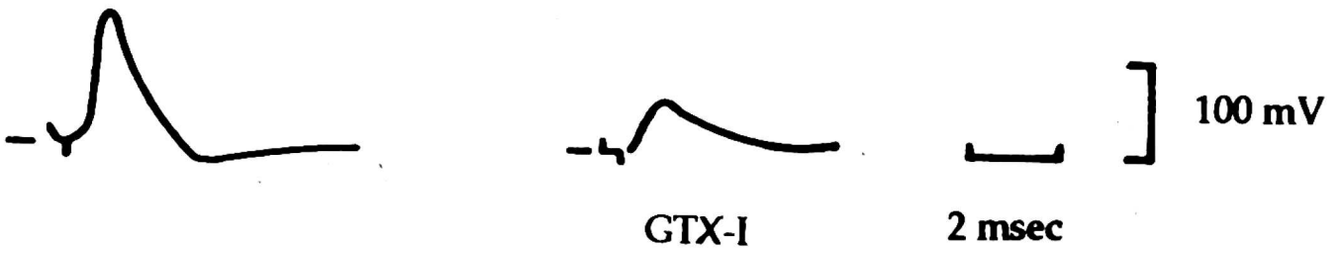

Fig. 2. Representative compound action potentials recorded from isolated frog sciatic nerve before and after application of GTX-I.

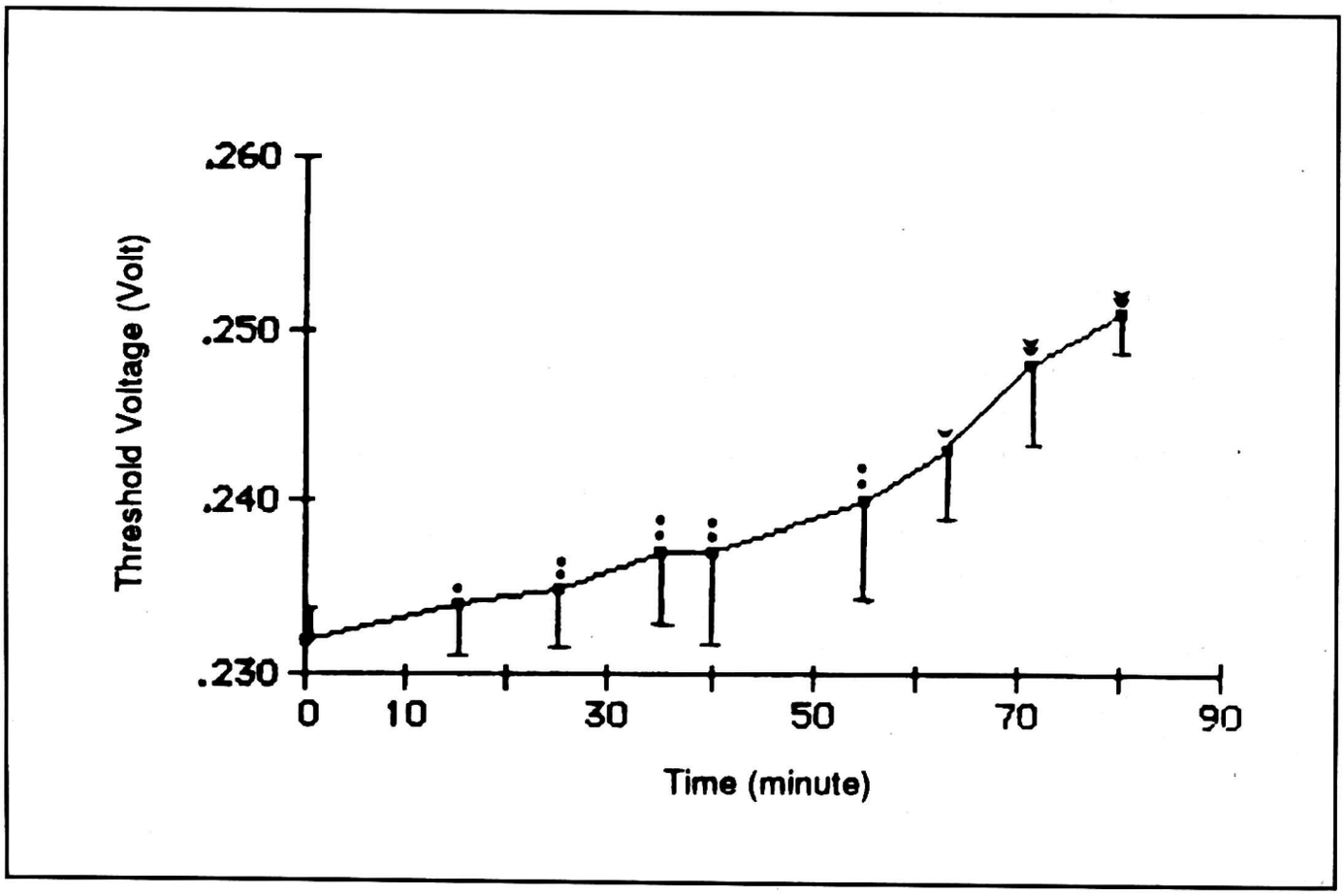

Fig. 3. The increase in the threshold stimulus voltage of isolated frog sciatic nerves in the time control group being related to time (*$p < 0.05$ when compared with the values at the beginning time, **$p < 0.01$ when compared with the values at the fifteenth minute, **$p < 0.01$, *$p < 0.05$ when compared with the values at the fifty-fifth minute. Each point represents the mean of five experiments. Vertical line represents SD).

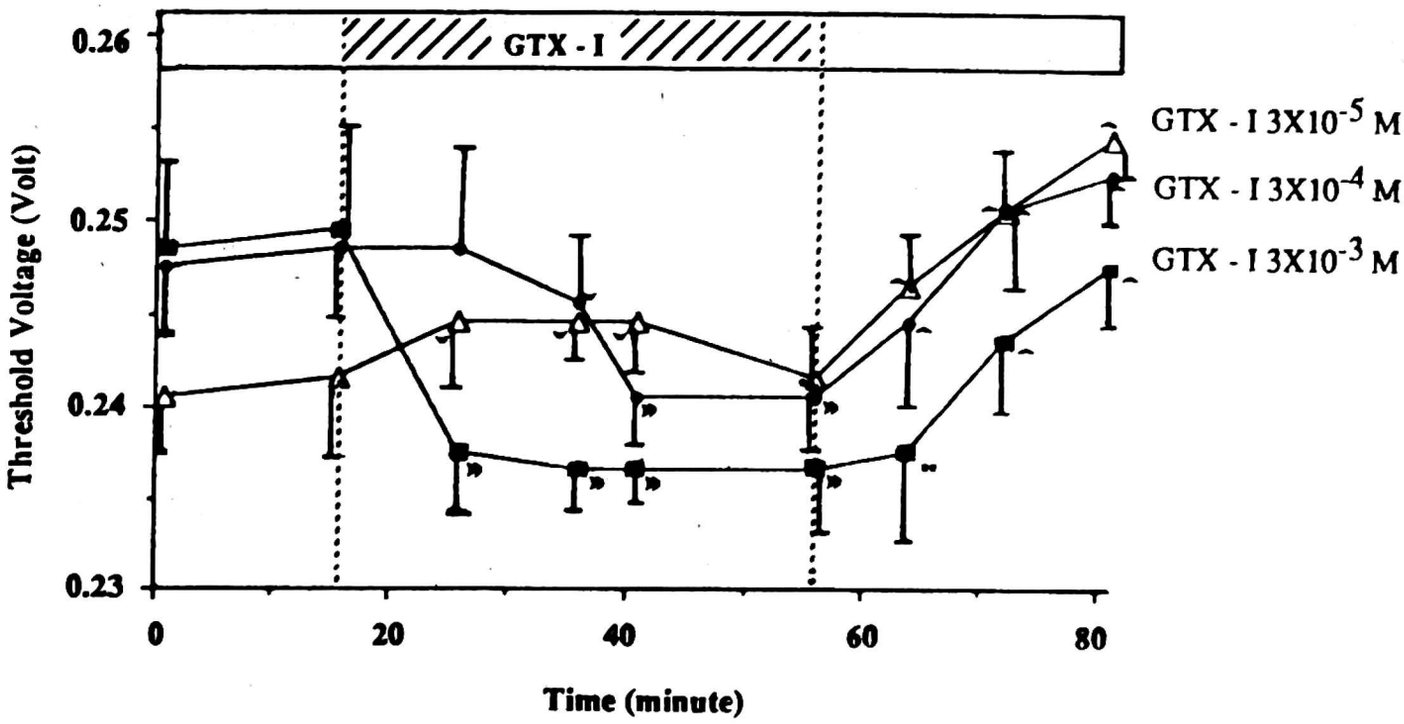

Fig. 4. Changes in the threshold stimulus voltage of isolated frog sciatic nerves produced by different concentrations of GTX-I and after washing with modified Ringer's solution (* $p < 0.01$, ** $p < 0.001$ when compared with the values before GTX-I, ** $p < 0.05$, * $p < 0.001$ when compared with the values under the effect of GTX-I, * $p < 0.05$ when compared with the preceding value. Each point represents the mean of ten experiments. Vertical line represents SD).

Table 1. Changes in the upstroke velocity of CAP of isolated frog sciatic nerves produced by application of GTX-I and washing with modified Ringer's solution (Data are given as the mean $\pm$ SD of ten experiments).

	Upstroke Velocity of Compound action Potential (V/sec)		
	Before GTX-I	Under the effect of GTX-I	After washing
GTX-I 3.10^{-5} M	91.2 ± 9	87 ± 9	92 ± 10
		$p < 0.05$	$p < 0.05$
		$p > 0.05$	
GTX-I 3.10^{-4} M	93 ± 10	79 ± 13	92 ± 13
		$p < 0.05$	$p < 0.05$
		$p > 0.05$	
GTX-I 3.10^{-3} M	90.2 ± 11	68 ± 11	90 ± 13
		$p < 0.05$	$p < 0.05$
		$p > 0.05$	

Table 2. Changes in the conduction velocity of isolated frog sciatic nerves produced by application of GTX-I and after washing with modified Ringer's solution (Data are given as the mean ± SD of ten experiments).

	Conduction Velocity (m/sec)		
	Before GTX-I	Under the effect of GTX-I	After washing
GTX-I 3.10^{-5} M	28.96 ± 4.81	32.60 ± 4.77	28.79 ± 4.23
	$p < 0.01$	$p < 0.01$	
	$p > 0.05$		
GTX-I 3.10^{-4} M	29.22 ± 4.98	37.61 ± 5.35	28.96 ± 4.98
	$p < 0.001$	$p < 0.001$	
	$p > 0.05$		
GTX-I 3.10^{-3} M	28.74 ± 4.77	45.77 ± 6.06	28.16 ± 4.22
	$p < 0.001$	$p < 0.001$	
	$p > 0.05$		

DISCUSSION

The present study clearly demonstrated that threshold stimulus voltage and upstroke velocity of CAP of frog sciatic nerve were decreased and conduction velocity of nerve increased by GTX-I. It is suggested that the decrease in the threshold stimulus voltage by GTX-I was mainly due to a decrease in resting membrane potential induced by GTX-I. This is consistent with previous studies showing that GTX-I exerts a substantial depolarizing action upon a variety of excitable cell membranes such as squid axon, skeletal muscle fiber and some cardiac tissue cells due to opening of some of the Na^+ channels at the normal resting membrane potential (16—19, 31). The increase in the threshold stimulus voltage control group may be a result of the rundown of the nerve.

The variation in upstroke velocity of CAP may be explained by the change in number of Na^+ channels per unit area of frog sciatic nerve membrane, as Seyema has also reported that maximum rate of rise of action potential in the myocardium could vary widely among regions of the heart (18). Increase in the conduction velocity of nerve may be explained by a reduction in the amount of depolarization required to reach threshold level. Also greater Na^+ permeability would lead to a faster excitation of adjacent regions and greater conduction velocity (32). It has been shown that GTX-I decreased the peak Na^+ current in amplitude while increasing the slow Na^+ current (19, 21). These

electrophysiological effects of GTX-I explain the decrease in threshold stimulus voltage and upstroke velocity of CAP even though conduction velocity of isolated frog sciatic nerve increased in our study.

The dose dependent reversible effects of GTX-I on the isolated frog sciatic nerve which were observed in our study, are consistent with previous investigations (9, 16—19, 21, 31). Akera et al have reported that GTX-I produced a dose-dependent positive inotropic effect in electrically driven guinea-pig atrial preparations. With higher GTX-I concentrations, the initial rate of development of positive inotropic effect was greater, and the time to reach the plateau of the positive inotropic response was approximately 20 minutes (9). It has been shown, that the time required to produce half-maximal depolarization of axonal membranes were approximately 30 and 60 minutes for α-2H-GTX-II and GTX-I, respectively (16, 21) and the changes were reversible.

As a result, it is concluded that a fraction of voltage dependent Na^+ channels responsible for generation of action potential in frog sciatic nerves was modified by GTX-I, permitting the Na^+ current to flow at voltages close to the resting membrane potential.

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