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Functional Disorders in the Peripheral Neuromuscular System induced by Gonyautoxins in Rabbits

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ABSTRACT. The effects of gonyautoxins (GTXs) on the neuromuscular system in rabbits were examined. M waves were evoked by electric stimulation on the tibial nerve and recorded mainly from the plantar interosseous muscle. Intravenous administration of GTXs decreased the amplitude and prolonged both latency and duration of M waves, while showed little effect on the motor nerve conduction velocity. These results suggest that GTXs have inhibitory effects on the peripheral neuromuscular system, mainly on the neuromuscular transmission.—**KEY WORDS:** evoked electromyography, gonyautoxins, peripheral neuromuscular disorder.

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Gonyautoxins (GTXs), the main components of paralytic shellfish poison in Japan, are usually accumulated in the midgut glands of the toxic bivalves like scallop, mussel, clam, which had taken the planktons *Proto-gonyaulax* spp. [11]. Some cases of GTXs intoxication have been reported in Japan since 1975 [9, 11]. The major clinical manifestations of paralytic shellfish poisoning in human beings are nervous disorders varying from a slight tingling and numbness around the lips to the complete paralysis of the muscles of the extremities and also respiratory muscles leading to death [3, 5, 7].

In the previous papers [2, 8], the authors analysed discharge intervals of a single motor unit using the $\bar{\tau}$ -S curve method [14, 15] and the Nomura's method of time series analysis [12] to investigate the effects of GTXs on antigravity activity of muscles in rabbits on a board inclined forward. In these studies it was found that the intravenous administration of GTXs enlarged the range of distributions of $\bar{\tau}$ -S correlation points and prolonged the period of R-type fluctuation whereas GTXs

did not change the period of H-type fluctuation. These findings suggest that GTXs may have inhibitory effects on the motor system in the spinal cord. However, the hypofunction of the spinal motor system is due not only to the suppression of the spinal reflex but also to the inhibition of the activity of the peripheral neuromuscular system [12, 13]. The present study was designed to examine the peripheral actions of GTXs on the neuromuscular system in rabbits by means of evoked electromyography. The effects of GTXs were assessed with some features of M waves and motor nerve conduction velocity (MCV).

GTXs were extracted from the midgut glands of the toxic scallop collected in Ofunato Bay (Iwate Prefecture) in 1980 by the method described previously [1]. The collected toxin was confirmed to be a mixture of GTX₁, GTX₂ and GTX₃ by electrophoresis and prepared as a solution of 50 MU/ml. GTXs in an intravenous dose of 5.0 or 7.0 MU/kg were employed for the present experiment [2, 8].

Nineteen Japanese white rabbits of either

sex, weighing 2.2 to 2.7 kg, were anesthetized by the intravenous injection of 27.5 mg/kg pentobarbital sodium. They were fixed on a stereotactic apparatus in a sitting posture with the left hind limb a little stretched. The tibial nerve was exposed at the caudal region of the left thigh (proximal stimulation site) and also at the region immediately proximal to the left tarsal joint (distal stimulation site). The nerve was moistured with warm (34.8 to 36.2°C) Ringer's solution. The rectal temperature ranged from 37.8 to 38.7°C. Two sets of stimulating electrodes, consisting of two silver needles insulated except the tips (Unique Medical, UM2-5050), were applied to the nerve at the above mentioned sites. Rectangular pulses with intensity of approximately 130% of the maximal stimulus and 0.1 msec duration were delivered from an electronic stimulator (Nihon Kohden, S-7272B). A bipolar needle electrode insulated except the tips with an interelectrode distance of 3 to 5 mm was inserted into the muscles of the plantar surface of the hindpaw, mainly the plantar interosseous muscle innervated by the tibial nerve. The electrode was placed so that M wave with the maximal amplitude could be recorded. The ground electrode consisting of hypodermic needle was positioned subcutaneously at the lateral region about 3 cm distal to the left tarsal joint. M waves were transiently stored in a memory of an oscilloscope (Nihon Kohden, VC-10) through a preamplifier with a time constant of 0.03 sec (Nihon Kohden, AVB-10), and then recorded on a X-Y plotter (Yokogawa Electric, PRO-12) before and over 4 hours after the intravenous administration of GTXs. Action potential of the tibial nerve and M wave evoked by the stimulation at the proximal stimulating site were monitored simultaneously. The voltage levels which elicited action potential and M wave with the minimal amplitude were taken as the respective threshold levels. The experiment was carried out at room temperature of 26.0 to 30.0°C.

After the experiment, the tibial nerve between the two stimulation sites was exposed and the distance between the proximal and the distal stimulating cathode (D_1) was measured along the nerve. The mean and standard deviation of D_1 were 82.5 and 2.9 mm *in situ*, respectively. MCV was determined as D_1/D_2 , where D_2 is the difference between the latency periods of M waves evoked by stimulating the proximal site and the distal site.

Prior to the experiment, we confirmed in three rabbits that M waves in response to a given stimulus did not change in their amplitude, latency and duration over 4 hours under pentobarbital anesthesia as long as the stimulating and the recording electrodes stayed in their original positions.

Changes induced by GTXs in M waves in response to the stimulation at the distal site are shown in Fig. 1. The mean peak-to-peak amplitude of M waves are plotted as a function of time in Fig. 2. Since the maximal amplitude of M waves varied from rabbit to rabbit due to the position of the recording electrodes, the amplitude before the GTXs administration was taken as 100%. The amplitude decreased to the lowest values of 72.5 and 63.9% of the control 30 to 40 min after the administration of 5.0 and 7.0 MU/kg GTXs, respectively, and then returned gradually to the control level in 3 to 4 hours. Fig. 3 represents change in the latency of M waves evoked by the stimulation at the distal site. The latency before the GTXs administration averaged 2.77 msec. The latency was increased by the GTXs administration reaching the maximum 30 to 40 min after and lasted for about 1 hour. The prolongation was 0.45 and 0.62 msec in average at the doses of 5.0 and 7.0 MU/kg, respectively. Thereafter, the latency decreased gradually to the control level within 3 to 4 hours. The recovery time was approximately 1 hour longer than that for the amplitude. The time course of the GTXs-induced increase in the latency of M waves

Fig. 1. Typical recordings of M waves before and after the intravenous administration of GTXs in a dose of 7.0 MU/kg.

M waves evoked by the supramaximal stimulation of the tibial nerve at the distal stimulation site were recorded from the muscles of the plantar surface of the hindpaw, mainly the plantar interosseous muscle, innervated by the tibial nerve. Similar results were observed in the rabbits administered with 5.0 MU/kg GTXs.

evoked by the stimulation at the proximal site was similar to that shown in Fig. 3. The duration of M waves was also prolonged by the administration of GTXs and recovered to the control level in about 4 hours.

Table 1 shows the effect of GTXs on MCV. MCV before the administration of GTXs averaged 49.2 m/sec which was not significantly altered by the intravenous administration of GTXs ($p > 0.01$). In addition, the threshold of M wave (0.28 to 0.33 V before the GTXs administration) was increased to 0.66 to 0.89 V by GTXs, whereas the threshold of action potential of the tibial nerve was not changed.

Evoked M waves are useful for diagnosing the disease in the lower motor neurons, neuromuscular junctions or skeletal muscle fibers [4, 6, 10]. The waveform, which mainly depends on the position of the electrodes, is usually monophasic, biphasic or triphasic [4, 6, 10]. The amplitude is mainly determined by the number of the responding muscle fibers if the position of the electrodes is not altered. The latency represents the conduction time of the impulses along the nerve and across the neuromuscular junction. The duration represents the electrical excitation time of the muscle fibers. Polyphasic waveform, decrease in amplitude or prolongation of du-

Fig. 2. Change in the amplitude of M waves by the intravenous administration of GTXs in a dose of 5.0 or 7.0 MU/kg.

M waves were recorded as described in Fig. 1. Each point represents mean and standard deviation. The amplitude of M wave before the GTXs administration (shown at zero point on the abscissa) was taken as 100%.

Fig. 3. Change in the latency of M waves by the intravenous administration of GTXs in a dose of 5.0 or 7.0 MU/kg.

M waves were recorded as described in Fig. 1. Each point represents mean and standard deviation. The latency before GTXs administration is shown at zero point on the abscissa.

Table 1. Effect of GTXs on MCV

Dose	control MCV (m/sec)*	after GTXs MCV (m/sec)*
5.0 MU/kg	48.64±3.12	48.28±3.75
7.0 MU/kg	49.78±3.51	48.76±4.18

* mean±standard deviation.

ration are frequently observed in myopathy, neuritis, reinnervation after nerve injury, and so forth [4, 6, 10]. Measurement of MCV contributes to diagnosis of disorders in the peripheral nerve. Though abnormal conduction velocity of the motor nerve does not always indicate disorders in the motor neurons, decrease in MCV is frequently observed in defects of the lower motor neuron [4, 6, 10].

In the present experiment, M waves were decreased in amplitude and prolonged in latency and duration by the GTXs administration, while MCV was scarcely affected. These results suggest that GTXs may suppress the peripheral neuromuscular system, possibly the neuromuscular transmissions.

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要 約

ウサギ末梢神経—筋系の gonyautoxins による機能障害 (短報): 田場典治・勝田新一郎¹⁾・知見憲次 (神戸大学農学部家畜管理学教室, ¹⁾神戸大学医学部第2生理学教室)——麻痺性貝毒 gonyautoxins (GTXs) のウサギ末梢神経—筋系に対する作用を, 誘発筋電図を指標に検討した。GTXs の静脈内投与により, M 波の振幅の縮小, 潜時および持続時間の延長が認められたが, 運動神経伝導速度には変化はなかった。このことから, GTXs は, 神経筋接合部に対して抑制的作用を有すると考えられた。