Short communication

Effect of *Tityus* γ Toxin on the Activation Process in Sodium Channels of Frog Myelinated Nerve

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Recently it has been established that γ toxin of *Tityus serrulatus* scorpion venom has a very high affinity to Na^+ channels and can be successfully used as a marker during their purification from excitable membranes (Barhanin et al. 1983; Grishin 1983). There has, however, been some controversy concerning the effects of the γ toxin on properties of Na^+ channels in different tissues. Thus, in neuroblastoma cells the γ toxin induces a shift in the voltage depencence of Na activation to more negative potentials, E (Barhanin et al. 1983). By contrast, in frog skeletal muscles the γ toxin causes a partial block of Na^+ channels with no changes in the voltage dependence or kinetics of sodium currents, I_{Na} (Barhanin et al. 1984). In the present paper we give a short description of the effects of γ toxin* on I_{Na} in frog node of Ranvier.

Application of 0.15—0.30 μmol/l γ toxin to voltag-clamped nodal membrane caused an irreversible reduction in the maximum Na^+ conductance, g_{Na}, accompanied by a negative shift in the voltage dependence of Na^+ channels activation and a decrease in the slope of g_{Na}—E curve. The voltage shift in Na^+ channels activation was transiently enhanced by strong depolarizing pulses, and decayed slowly ($\tau \approx 20$ s at 5—6 °C) to its initial value. Fig. 1*A* illustrates toxin-induced changes in I_{Na}—E relation without (■) and with (▲) conditioning pulsing to $E = +60$ mV. In some experiments, the voltage shift of g_{Na} was absent before pulsing and it always appeared after conditioning depolarizing pulses (Fig. 1 *B*). The steepness factor, k, of the g_{Na}—E curve (number of mV required to give an e-fold change of g_{Na}) was increased by the toxin treatment combined with repetitive pulsing from ≈ 7 to ≈ 11, indicating a reduction in the effective gating charge of the Na^+ channel. The toxin did not abolish complete inactivation of the channels, and, as a rule, had no effect on the reversal potential after conditioning pulsing.

Qualitatively all these effects of the γ toxin are similar to those of toxins III and IV from the scorpion *Centruroides sculpturatus* (Hu et al. 1983). The latter toxins, however, induced only a transient shift in the g_{Na}—E relation, whereas γ

* Toxin γ was extracted by Dr. E. V. Grishin from the venom of the scorpion *Tityus serrulatus* using the procedure described by Barhanin et al. (1982).

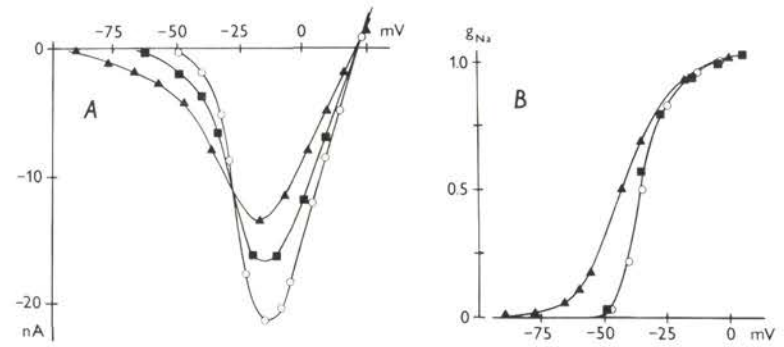

Fig. 1A. Effect of 0.2 μmol/l γ toxin on I_{Na}—E curve in the node of Ranvier of the frog *Rana ridibunda*. ○, Ringer without toxin. ■, Ringer + γ toxin, test pulses (10 ms) without conditioning pulses. ▲, the same solution, each test pulse was preceded by 10 conditioning depolarizing pulses ($E = 60$ mV, 30 ms) duration, separated by 30- ms intervals. The interval between the last conditioning depolarizing pulse and the test pulse was also 300 ms. The duration between test pulses used in measuring I—E relation was about 1 min. Holding potential, $E_h = -100$ mV. Ionic composition of the Ringer solution was as follows (in mmol/l): 112 NaCl, 2.5 KCl; 2.0 $CaCl_2$, 5 Tris buffer, 2 $NaHCO_3$, pH 7.2. The cut ends of the fibre were in the solution of 114 CsF. Fibre 2.3.83, 9 °C. ***B.*** Transient shift in the voltage dependence of normalized sodium conductance, g_{Na}, after conditioning pulsing of the nodal membrane treated with 0.2 μmol/l γ toxin. ○, Ringer without toxin; ▲, Ringer + γ toxin. Each test pulse was preceded by 7 conditioning pulses ($E = 60$ mV, 10 ms) separated by 500-ms intervals. ■, 5 min after the end of conditioning pulsing. Duration of test pulses 10 ms, $E_h = -100$ mV. g_{Na} was normalized to its maximum. In this experiment, the toxin treatment without conditioning pulsing did not induce a shift in g_{Na}—E relation (not illustrated). Fibre 25. 3. 83 8°C.

toxin caused both 'tonic' and 'transient' changes in the voltage dependence of Na channels activation (see Fig. 1*A*).

Application of γ toxin to the node of Ranvier pretreated with batrachotoxin (BTX) increased the negative voltage shift of g_{Na} caused by BTX (Khodorov et al. 1975), inducing a steady-state inward I_{Na} at $E_h = -120$ mV. The slope of g_{Na}—E curve was decreased, but the maximum g_{Na} remained unchanged (not illustrated). These results are in keeping both the finding that γ toxin and BTX interact with two separate receptors in the Na^+ channel (Barhanin et al. 1982). A transient shift in the g_{Na}—E relation induced by toxin after a conditioning membrane depolarization suggests that the affinity of the Na^+ channel 'voltage sensor' to this toxin raises during channel activation, and decreases after its closing. Persistent activation of BTX-modified Na channels stabilizes γ toxin interaction with the 'voltage sensor'.

References

Barhanin J., Giglio J., Leopold P., Schmid A., Sampaio V., Lazdunski M. (1982): *Tityus serrulatus* venom contains two classes of toxins J. Biol. Chem. **257**, 12553—12557

Barhanin J., Pauron D., Lombet A., Norman R., Vijverberg P., Giglio J., Lazdunski M. (1983): Electrophysiological characterization, solubilization and purification of the *Tityus* γ toxin receptor associated with the gating component of the Na^+ channel from the rat brain. The EMBO Journal **2**, 915—920

Barhanin J., Ildefonse M., Rougier O., Sampaio S., Lazdunski M. (1984): *Tityus* γ toxin, a high affinity effector of the Na^+ channel in muscle, with a selectivity for channels in the surface membrane. Pflügers Arch. **400**, 22—27

Grishin E. V., Kiyatkin N. I., Kovalenko V. A., Pashkov N. N., Shamotienko O. G. (1983): Identification and isolation of sodium channel components. In: III Soviet-Swiss Symposium Biol. Membranes Structure and Function. Abstr. p. 26

Hu S., Meves H., Rubly N., Watt D. (1983): A quantitative study of the action of *Centruroides sculpturatus* toxins III and IV on the Na currents of the node of Ranvier. Pflügers Arch. **397**, 90—99

Khodorov B. I., Peganov E. M., Revenko S. V., Shishkova L. D. (1975): Sodium currents in voltage-clamped nerve fiber of frog under a combined action of batrachotoxin and procaine. Brain Res. **84**, 541—546

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