Trypargine Blocks the Sodium Channels Only from the Inside of Squid Giant Axon

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Summary A new tetrahydro- β -carboline, trypargine (TRG), specifically suppresses the Na current (I_{Na}) when applied to the internal surface of the squid axon membrane without affecting the K current (I_K). The binding of TRG to its receptor is potential-dependent and occurs at a site about halfway through the membrane electric field from the outside; the dissociation constant is 11 μ M at 0 mV.

Key words: trypargine, Na channel block, guanidinium group

It has been reported that the guanidinium group plays an important role in the suppression of I_{Na} both for external blocker (KAO and NISHIYAMA, 1965; HILLE, 1975) and for internal blocker (KIRSCH *et al.*, 1980; MORELLO *et al.*, 1980). Since TRG, isolated from the African frog *Kassina senegalensis* (AKIZAWA *et al.*, 1982), has a terminal guanidinium group, it was thought worthwhile to test whether TRG could exert an inhibitory effect on the Na channel. Moreover neurochemical investigation has made clear that tetra- β -carboline which is chemically analogous to TRG is present in the rat arcuate nucleus (SHOEMAKER *et al.*, 1978) or in the rat forebrain (HONECKER and ROMMELSPACHER, 1978). The finding that intraperitoneal administration of tetra- β -carbolines increased concentration of 5-HT in mouse and rat brain (McISAAC *et al.*, 1972) promotes the idea that β -carboline may serve as an endogenous neuromodulator or neurotransmitter in the 5-HT containing nerve endings (BUCKHOLTZ, 1980). In order to elucidate the mechanism of action of TRG to the nerve membrane, a pharmacological study of TRG was carried out on squid axons isolated from *Doryteuthis bleekeli* or *Sepioteuthis lessoniana*. A combined method for internal perfusion and voltage clamp was used in all experiments.

As shown in Fig. 1A (lower left figure) TRG suppressed the peak inward current in such a way that the degree of inhibition was enhanced as the membrane

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potential was stepped to more depolarized levels. The steady-state outward current was enhanced at potentials lower than about +50 mV and suppressed at potentials higher than +50 mV (Fig. 1A, see I-V curve).

Fig. 1

This curious behavior of the steady-state current in the presence of TRG strongly suggests that TRG specifically inhibits I_{Na} without affecting I_K . Since the inactivation of I_{Na} in the perfused axon is incomplete, the total membrane current measured at the end of a 6 msec step depolarization is mainly composed of inward-directed I_{Na} and outward-directed I_K at potentials lower than E_{Na} (54 mV). In contrast, both I_{Na} and I_K are outward at potentials higher than E_{Na} . If TRG specifically suppressed I_{Na} , the steady-state current would then be composed primarily of outward-going I_K . Thus, under the action of TRG, the total steady-state membrane current in response to step depolarizations of less than +54 mV should be larger than that in control, whereas the steady-state current should be smaller for depolarizations beyond 54 mV. This supposition is clearly supported by the data shown in Fig. 1A. To clarify the action of TRG on the individual current systems of the axonal membrane, the effect of TRG on either I_{Na} or I_K was checked in separate experiments. Figure 1B shows that TRG at a concentration of 1×10^{-5} M did not suppress I_K (upper column), but did suppress I_{Na} (lower column) in a frequency dependent manner. When applied externally,

Fig. 1. A. The effect of intracellular application of 1×10^{-4} M TRG on both transient and steady-state currents. The composition of the external solution, i.e., artificial sea water, (ASW) (in mM) is: NaCl 449, KCl 10, $CaCl_2$ 50, TRIS 30. Temperature-corrected pH was adjusted to 8.0. The standard internal solution (SIS) contains: NaF 50, K glutamate 320, K phosphate buffer, 15 sucrose 333. pH was adjusted to 7.3. Temperature was 12°C. Membrane currents were elicited by step depolarizations from a holding potential of -8.0 mV in increments of 20 mV. The frequency of stimulation was set arbitrarily. The upper left inset figure shows original records for the control, and the lower one, those for 1×10^{-4} M TRG. I-V curve for both transient and steady-state currents is presented to the right.

B. Selective action of TRG on I_{Na} . Upper graph: Lack of effect on I_K . I_K was recorded in a medium (ASW/SIS) containing 1×10^{-6} M tetrodotoxin (TTX) externally. Step depolarizations of 180 mV from the holding potential (-80 mV) were applied at a frequency of 1/sec. In the upper graph, internal application of 1×10^{-5} M TRG did not change the amplitude of I_K . The slight reduction of I_K with repetitive pulsing is most likely due to K^+ ion accumulation in the so-called Frankenhaeuser-Hodgkin space. Lower graph: Block of I_{Na} . In order to eliminate I_K and isolate I_{Na} , K-free ASW was used as the external perfusate and Cs-SIS, as the internal perfusate (composition in mM: NaF, 8.4; NaOH, 41.6; CsF, 41.6; CsOH, 208.4; sucrose, 500; and HEPES, 10). pH was adjusted to 7.3 by L-glutamic acid. The same pulse sequence was used. The solid line is a single exponential fitted to the data points with a time constant of 1.95 sec.

C. The dependency of TRG-induced suppression of I_{Na} on pulse duration in pronase-treated axon in a medium containing 1×10^{-5} M TRG. A train of depolarizing conditioning pulses of 180 mV having a duration of 1 msec (○) or 7 msec (△) were applied at a frequency of 1/sec. The extent of inhibition of I_{Na} was estimated by applying a single 80 mV depolarizing test pulse 1 sec after the last conditioning pulse. I_{Na} at 1 msec from the beginning of test pulse associated with each conditioning pulse was normalized to the value obtained without conditioning pulses. Since recovery from TRG-induced block occurred at the time constant of 18.5 ± 3.5 sec (mean $\pm$ S.E.) ($n=4$), the pause between each train of conditioning pulses was set at 2 min.

TRG at a concentration up to 1×10^{-4} M did not affect any current.

The gating mechanism of Na channel may regulate the accessibility of the drug to a receptor within the channel; hence, experiments were carried out using pronase-treated axons. In this condition, the availability of open Na channels can be determined by the length of the depolarizing pulses. When step depolarization with duration of 1 msec was applied at a frequency of 1/sec, the intracellular administration of TRG (at 1×10^{-5} M) suppressed I_{Na} in a frequency dependent manner (Fig. 1C). The time course of suppression of I_{Na} is very similar to that in the h-gate intact axon shown in Fig. 1B. On the other hand, when the duration of step depolarization extended to 7 msec again at a frequency of 1/sec, suppression occurred at the very first stimulation, and subsequent application of the stimulating pulses did not produce further suppression of I_{Na} (Fig. 1C). The time course of decline of I_{Na} during the first stimulating pulse followed a single exponential after asymptotic correction (Fig. 2A).

If one assumes that TRG binds to its receptor with a one-to-one stoichiometry when the Na channel is open, and thereby induces the occlusion of the Na channel, the process can be represented as: resting channel $\xrightleftharpoons{H-H}$ open channel $\xrightleftharpoons[k]{l}$ TRG occluded channel, where H-H indicates Hodgkin & Huxley (HODGKIN and HUXLEY, 1952) kinetics and k (ms^{-1}) is a function of the TRG concentration in the internal solution, namely, $k=b[\text{TRG}]$, where b is a constant in $\text{liters} \times \text{mole}^{-1} \times \text{ms}^{-1}$ (ARMSTRONG, 1969). The time constant from the falling phase of I_{Na} (in pronase-treated axons) is related to k and l by

$$\tau = \frac{1}{k+l}$$

The ratio of steady-state I_{Na} in the presence of TRG to that in the control is

$$\tau l = \frac{l}{k+l}$$

The rate constants were calculated from these two equations and plotted against the membrane potential on a semilogarithmic graph (Fig. 2 B-1 and B-2).

The dependency of the dissociation constant, $K_D(E)$, for TRG on the membrane potential was elucidated by the following procedure: (1) The degree of suppression of steady-state I_{Na} in response to a depolarizing pulse was calculated from $(I_{Na, \text{control}} - I_{Na, \text{TRG}})/I_{Na, \text{control}}$, and plotted against the TRG concentrations (i.e., 10^{-6} M, 10^{-5} M, 3×10^{-5} M and 10^{-4} M). (2) The relative suppression of I_{Na} is $1/(1 + K_D(E)/[\text{TRG}])$, (assuming that the drug binds to a receptor with one-to-one stoichiometry), where $K_D(E)$ is the apparent dissociation constant, and $[\text{TRG}]$, TRG concentration in M. (3) $K_D(E)$ was determined as the concentration at which half suppression of steady-state I_{Na} occurred. According to the model proposed by WOODHULL (1973), drug access to the receptor is via a hydrophilic pathway and the drug is bound to a receptor which experiences a fraction, δ , of the membrane field. The relationship between $K_D(E)$ and membrane potential is expressed as $K_D(E) = K_D \exp[(\delta - 1)E]$

Fig. 2. A. Time course of decay of sodium current due to the action of TRG in pronase-treated axon. The experiments were done in K-free ASW/Cs-SIS. A-1 and A-2: Semilogarithmic plot of the falling phase of I_{Na} in the presence of TRG (1×10^{-4} M) at two potentials. A-1 indicates the data for I_{Na} in response to a depolarizing pulse of 180 mV from -80 mV and A-2, that in response to a pulse of 80 mV. Arrows indicate time constants of decay of I_{Na} ; these were calculated to be 0.25 msec at +100 mV (A-1) and 1.28 msec at 0 mV (A-2). The membrane current and potential recorded on 35 mm film were projected on graph paper and measurements were made manually.

B. Relationship between the rate constants b (B-1) and l (B-2) and the membrane potential for TRG (1×10^{-4} M, 1×10^{-5} M). The wide range of data for I_{Na} was covered by preparing the solutions having various concentrations of sodium ion, where Na was replaced with tetramethylammonium. $\circ$; 50 mM [Na⁺]_o, $\triangle$; 225 mM [Na⁺]_o, $\square$; 449 mM [Na⁺]_o. The solid line for B-1 was fitted by $b = 2.15 \times 10^3 \times \exp(0.00142 \times V)$, where V (mV) is the size of the step made from a holding potential of -80 mV. The backward rate constant, l , was independent of the membrane potential during stimulating pulses.

$zFE/RT]$, where K_D ($11 \mu\text{M}$) is the dissociation constant at zero membrane potential, δ , the fraction of membrane potential acting on binding site, z , the valency of TRG, and E , the membrane potential, and F , R and T have their usual meanings. Because the pK_a for TRG is more than 12, almost all of TRG should be positively charged in the internal perfusate. When the gate of Na channel is open, TRG binds to a site calculated to be about halfway ($\delta=0.46$) through the membrane field from the outside.

Since an increase in the available 5-HT (e.g., as caused by increased synthesis or decreased reuptake) leads to a depression of firing rate in 5-HT containing cells (AGHAJANIAN and WANG, 1978), it has been proposed that the inhibitory action of tetrahydro- β -carboline to 5-HT containing cell may be due to the inhibition of MAO activity and the modification of transmitter release (BUCKHOLTZ, 1980). In taking account of the inhibitory action of TRG on the Na channel, another possible action of tetrahydro- β -carboline for the regulation of transmitter release of 5-HT containing cells is that tetrahydro- β -carboline may act as endogenous neuromodulator through the inhibition of Na channel which in turn reduces the degree of opening of Ca channel and modulates the transmitter release.

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