Alteration of The Voltage-dependence of the Twitch Tension in Frog Skeletal Muscle Fibres by a Polyether, Bistramide A

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Abstract. Bistramide A, a toxin isolated from *Lissoclinum bistratum* Sluiter, was applied to frog skeletal muscle fibres. Micromolar concentrations of toxin decreased the amplitude of the twitch tension. Bistramide A shifted the activation and the steady-state inactivation characteristics of the tension on the voltage axis towards more positive and negative membrane potentials respectively. The data suggest that Bistramide A binds to the activated and inactivated state of the dihydropyridine-receptor which senses the T-tubule membrane potential.

Key words: Skeletal muscle — Voltage clamp — Twitch tension — Bistramide A

Introduction

Bistramide A, an amided cyclic polyether toxin isolated from a New-Caledonian ascidian *Lissoclinum bistratum* Sluiter (Gouiffes et al. 1988 a; Foster et al. 1992), is highly toxic with a rapid effect on the central nervous system leading to paresthesia and loss of muscle tone (Gouiffes et al. 1988 b). In frog skeletal muscle, Bistramide A inhibited the peak sodium current (I_{Na}). The activation curve of I_{Na} was unchanged while the steady-state inactivation curve of I_{Na} was shifted along the voltage axis towards more negative membrane potentials. Bistramide A has been suggested to inhibit Na channel at rest and in the inactivated state (Sauviat et al. 1992). In frog heart muscle, micromolar concentrations of the toxin competed with external Ca for a common site and inhibited the cadmium-sensitive calcium current and the correlated phasic tension in intact fibres while the maximum calcium activated force and the calcium-sensitivity of contractile proteins were sensitive to nanomolar concentrations of the toxin in skinned fibres (Sauviat et al. 1993). The aim of the present work was to contribute to the analysis of Bis-

tramide A by studying its effects on the voltage-dependence of the twitch tension in frog skeletal muscle fibres

Materials and Methods

Voltage-clamp experiments were performed at 10–12 °C on fine cut-end skeletal muscle fibres (100–200 μm in diameter, 5–6 mm in length) isolated from sartorius muscle of *Rana esculenta* The double sucrose gap voltage-clamp technique with vaseline seals was used (Pater and Sauviat 1987) Starting from a holding potential of −80 mV, the potential of the test gap was displaced in rectangular steps at a rate of 0 2 Hz, positive potentials corresponded to depolarizations Twitch tension was recorded by means of a transducer system (Pixie 8101) applied to the fibre, in the test gap, by means of a thin glass lever (Sauviat et al 1991) Transmembrane potentials, currents and tension were displayed on a digital oscilloscope (Nicolet 310), recorded on the mass storage device of a desk-top computer (Hewlett Packard 9826) and plotted on a plotter (Hewlett Packard 7470) Each Figure is representative of six experiments unless stated otherwise Calculations are expressed as mean values ± S E M, (n) indicates the number of preparations tested

The composition of the Ringer solution was in mmol/l NaCl, 110 5, KCl, 2 5, $CaCl_2$, 2 , the pH of the solution was maintained at 7 3 with HEPES buffer (5 mmol/l) The composition of the "internal" solution which bathed the cut-ends of the fibres was in mmol/l, K aspartate, 120, ATP, 5, phosphocreatine, 5, $MgCl_2$, 2, HEPES buffer, 5, pH 7 3 (Pater and Sauviat 1987) The toxin was dissolved in absolute ethanol at a concentration

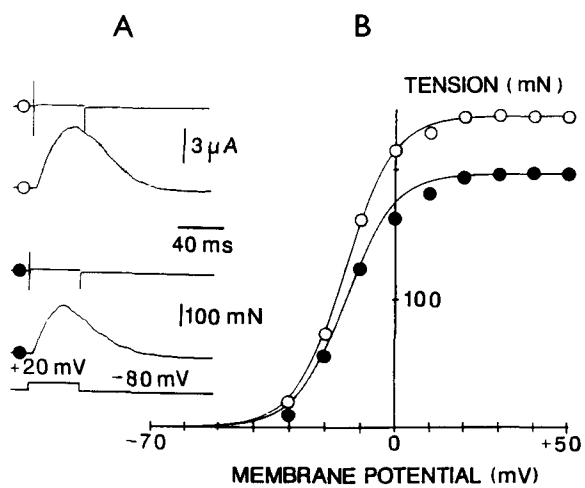

Figure 1. Effect of 2 8 μmol/l Bistramide A (●) on the membrane current and the peak tension recorded in the Ringer solution (○) on voltage-clamped frog skeletal muscle fibre *A* Membrane current (upper trace) and evoked peak tension (lower trace) recorded for a depolarizing clamp step (100 mV amplitude, 45 ms duration) applied from a −80 mV holding potential before and after 5 minutes of the toxin application *B* Tension-membrane potential relationships Experimental data were fitted according to equation 1 with $V_{1/2} = -15$ mV, $k = 7$ (control) and $V_{1/2} = -14$ mV, $k = 7$ (Bistramide A)

of 1.4 mmol/l kept at 4 °C and appropriately diluted just before use. Control solutions contained the same amount of ethanol as did the test solution.

Results

Fig. 1*A* shows that Bistramide A reduces the peak tension which develops a few milliseconds after the start of the depolarizing step. The reduction occurred as a shortening of the time-to-peak value (control: 35 ms; Bistramide A: 27 ms) and an acceleration of the time constant of the relaxation phase of the contraction (control: 37 ms; Bistramide A: 28 ms). The inhibition of the peak tension was dose-dependent and reached $26 \pm 8\%$ ($n = 8$) and $38 \pm 9\%$ ($n = 6$) in the presence of 2.8 μmol/l and 5.6 μmol/l Bistramide A respectively. Tension-membrane potential relationships recorded in Ringer solution show that the amplitude of the peak tension increases in the membrane potential range −30 mV to +20 mV and then reaches a plateau at more positive potentials (Fig. 1*B*). Experimental data were best fitted by a Boltzmann equation

$$T = T_{\max}/(1 + \exp(V_{1/2} - V_m)k) \tag{1}$$

where T is the tension, $T_{\max}$ is the maximum tension, $V_{1/2}$ is the membrane potential at which $T = 0.5$, V_m is the membrane potential and k is the slope factor. Bistramide A reduces the peak tension whatever the membrane potential investigated (Fig. 1*B*). $V_{1/2}$ and k (-16 ± 6 mV and 6 ± 1 ($n = 8$) in the absence,

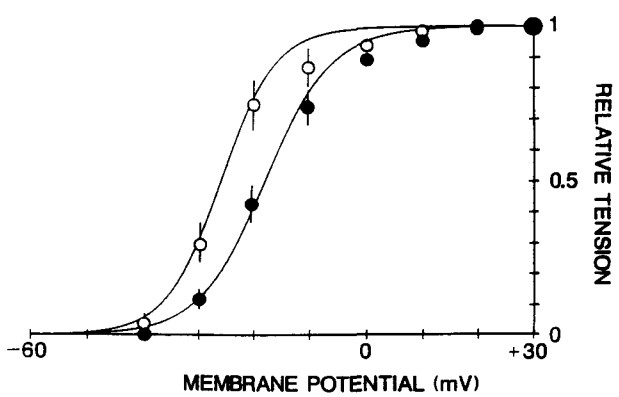

Figure 2. Activation curves for tension-membrane potential recorded in the absence (○) and in the presence (●) of 2.8 μmol/l Bistramide A in Ringer solution. Tension-membrane potential curves recorded as in Fig. 1*B* were normalized. The curves through the experimental points were generated using equation 2 with $V_{a\,1/2} = -25$ mV, $k_a = 5$ (control); $V_{a\,1/2} = -18$ mV and $k_a = 6$ (Bistramide A). The S.E.M. for $n = 6$ are either within the dimensions of the symbols or are represented by the vertical bars.

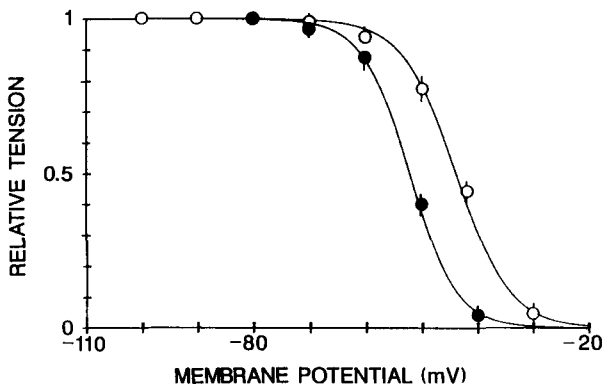

Figure 3. Steady state inactivation curves for tension recorded in Ringer solution before (○) and after (●) treatment with 2.8 μmol/l Bistramide. Tension was evoked using a depolarizing test step (110 mV amplitude, 45 ms duration) applied from a −80 mV holding potential. The holding potential was changed in 10 mV steps in a range of −110 mV to +30 mV. The peak tension was recorded one minute after each change of HP. The tension (T_i) was the ratio between the tension recorded at more depolarized membrane potential and the maximum tension recorded at more negative membrane potential. The curves through the experimental points were generated using equation 3 with $V_{1/2} = -52.7$ mV, $k_i = 8$ (control); $V_{a\,1/2} = -43.5$ mV, $k_i = 7$ (Bistramide A). The S.E.M. for $n = 6$ are either within the dimension of the symbols or are represented by the vertical bars.

-17.6 ± 4.3 mV and 7.2 ± 1.6 ($n = 8$) in the presence of the toxin) were not markedly modified. Activation curve of the tension (Fig. 2), reported as normalized tension-membrane potential curve for the maximum tension developed in the control solution was best fitted using Boltzmann equation

$$T_a = 1/(1 + \exp(V_{a\,1/2} - V_m)/k_a) \tag{2}$$

with T_a being the normalized tension ratio between tension and tension recorded at the plateau of the tension-membrane potential curve, $V_{a\,1/2}$ being the membrane potential at which $T_a = 0.5$, V_m the membrane potential, and k_a being the slope factor. Bistramide A shifts the activation curve by about 8 mV towards more positive membrane potentials (Fig. 2). The steady state inactivation curve for tension (Fig. 3) recorded for a given test potential in Ringer solution by changing the holding potential was described by a Boltzmann equation

$$T_i = 1/(1 + \exp(V_m - V_{i\,1/2})/k_i) \tag{3}$$

where T_i is the tension ratio between tension and the maximum tension recorded at a conditioning membrane potential V_m, $V_{i\,1/2}$ is the membrane potential at

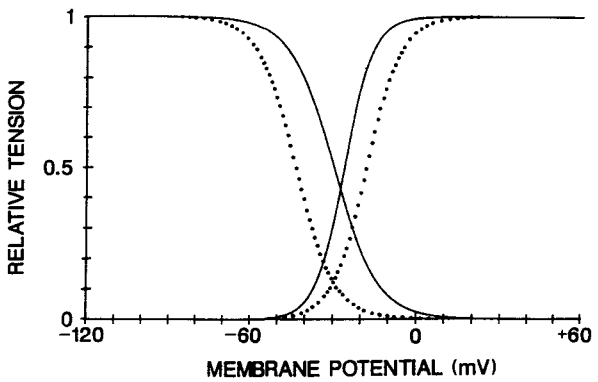

Figure 4. Overlap of activation (right traces) and inactivation (left traces) curves for tension recorded in Ringer solution (continuous line) and in Ringer solution containing 2.8 μmol/l Bistramide A (dotted lines). Same curves as in Figs. 2 and 3.

which $T_1 = 0.5$, and k_1 is the slope factor. The curve was shifted towards more negative membrane potentials by about 9 mV in the presence of the toxin (Fig. 3). In Ringer solution, activation and steady-state inactivation membrane potential curves overlap in the membrane potential range -60 mV to $+20$ mV and intersect at -28 mV, with an amplitude of the maximal relative tension of 0.43 (Fig. 4). In the presence of Bistramide A, curves overlap in the membrane potential range -50 mV to 0 mV and intersect at -30 while the magnitude of the relative tension (0.12) is decreased by 70%.

Discussion

The present results show that Bistramide A decreases the twitch tension of frog skeletal muscle fibres and shifts the voltage-dependent characteristics of the tension along the voltage axis.

In skeletal muscle, twitch tension is generated by the passage of the action potential (AP) along the membrane of the transverse (T)-tubule system which is followed by an increase in free myoplasmic calcium concentration that activates troponins and allows cross-bridge cycling. A dihydropyridine receptor (DHP-receptor), which senses the T-tubule membrane potential (voltage sensor), has been identified as a voltage-dependent L-type Ca channel responsible for the Ca inward current (Ashley et al. 1991; Rios and Pizarro 1991; Dulhunty 1992). Activation and inactivation characteristics of the tension reflect the voltage-dependence of the DHP-receptor and can be moved along the voltage axis by altering the surface charges on the T-tubule membrane or by specific binding to charged groups near

the voltage sensor (Dulhunty 1992). Reducing free extracellular Ca shifts the inactivation curve (Lüttgau and Spiercker 1979; Lüttgau et al. 1987) but leaves the activation curve unchanged (Brum et al. 1988 a, b; Pizarro et al. 1989) suggesting that Ca ions are directly and specifically involved in the inactivation mechanism of the voltage sensor. Dihydropyridine derivatives also shift the steady-state inactivation curve of both the tension and the Ca current towards more negative membrane potentials (Pizarro et al. 1989). Bistramide A, a highly lipophilic toxin, is able to bind to proteic sites. According to Sauviat et al. (1993), in frog heart Bistramide A inhibits L-type Ca channels and competes with extracellular Ca for a common site. In the present study, the observation that Bistramide A shifts the inactivation curves of the twitch tension towards more negative potentials along the voltage axis suggests that the toxin binds to the inactivated state of the DHP-receptor as shown for nifedipine (Neuhaus et al. 1990), perhaps by lowering the affinity for Ca of a site or sites in the voltage-sensing molecule as it has been suggested for nifedipine (Brum et al. 1988 a). The shift of the activation curve towards more positive membrane potentials suggests that Bistramide A binds to the active state of the receptor. In skeletal muscle, Bistramide has already been shown to bind to the inactive but not to the active state of the Na channel (Sauviat et al. 1992). In conclusion, the reduction of the overlap area between activation and inactivation curves resulting from the binding of the toxin to the DHP-receptor might account for the loss of muscle tone induced by Bistramide A (Gouiffes et al. 1988 b).

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