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Research Articles

Influence of Bistramide A on the twitch tension in rat atrial heart muscle

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Abstract. Bistramide A, a new toxin isolated from the Urochordate *Lissoclinum bistratum* Sluiter, was applied to rat auricular heart muscle bundles. At a stimulation frequency of 0.2 Hz, the toxin induces a dose-dependent reduction of the stimulated twitch tension force; it decreases $\dot{V}_{max}$ and shortens the duration of the plateau and the slow repolarizing phase of the action potential. In the control solution, switching from a stimulation frequency of 0.2 Hz to 1 Hz decreases the force with which a positive potentiation develops either at a maintained high frequency or after switching from 1 Hz to 0.2 Hz. Bistramide A reduces both the force evoked at 1 Hz and the potentiation. The data suggest that Bistramide A blocks Na^+ conductance; inhibits Ca^{++} channels in a time- and frequency-dependent manner; reduces Na^+-Ca^{++} exchange activity; but does not modify the ability of the sarcoplasmic reticulum to be refilled although the rate of Ca^{++} accumulation is decreased.
Key words. Bistramide A; mechanical activity; action potential; rat heart muscle.

Electrocardiograms from mice and rats showed that the amided cyclic polyether Bistramide A, isolated from a New Caledonian Urochordate *Lissoclinum bistratum* Sluiter[1,2] exerts a negative chronotropic effect, reduces cardiac rhythm and causes an inversion of T-wave[3]. Bistramide A inhibits the Na^+ conductance in a dose-dependent manner and is more effective on inactivated Na^+ channels in frog skeletal muscle[4]. The toxin decreses the calcium sensitivity of contractile proteins prior to block of the Ca^{++} current in frog heart[5]. It shifts the steady-state activation and inactivation of the dihydropyridine receptor in frog skeletal muscle towards more positive and more negative membrane potentials respectively[6]. The aim of the present work was to study the effect of Bistramide A on the excitation-contraction coupling of rat heart muscle in which internal Ca^{++} stores play a major role.

Materials and methods

Left auricles were isolated from the heart of adult male rats (Wistar 300–400 g) anesthetized with ether. Auricular bundles (0.6 to 0.9 mm in length, 0.3 to 0.5 mm in diameter) were isolated and placed in an experimental chamber (0.08 ml in volume) through which oxygenated Tyrode solution at 35 °C flowed at 5 to 6 ml min^{-1}.
Mechanical activity was recorded by means of a platinum wire attached to one end of the bundle by means of a natural silk thread and fixed by the other end to a Gould-Statham device. The electrical activity was measured using the conventional microelectrode technique (20–30 Mohms resistance; less than ± 3 mV tip potential). Bundles were electrically stimulated with square pulses (5 ms duration, 2 times threshold intensity) delivered at 0.2 Hz through bipolar platinum electrodes (earth isolated using an optoelectric coupling device).
The composition of the Tyrode solution was (mM): NaCl, 140; KCl, 5.7; $CaCl_2$, 2; $MgCl_2$, 0.25; Hepes (NaOH) buffer, 5; pH 7.35; gassed with 100% O_2. Bistramide A was dissolved in absolute ethanol at a concentration of 1.4 mM, kept at 4 °C and diluted appropriately just before use. Control solution contained the same amount of ethanol as the test solution.
Each figure represents five to six experiments unless otherwise stated. Calculations are expressed as mean values ± SE, and n corresponds to the number of bundles tested. The data were analyzed statistically using the unpaired Student's *t*-test and a two-way analysis of variance with repeated measures. Differences were considered significant at $p < 0.05$. Transmembrane potentials and mechanical activity were displayed on an oscilloscope (Nicolet 310) and recorded in its mass storage, on an X-Y plotter (Ifelec), or on a curviligne recorder and plotted on an X-Y plotter (Hewlett Packard 7470).

Results

Bistramide A decreases the peak force of the stimulated tension (fig. 1A) in a frequency-dependent manner (64 ± 6% and 87 ± 3% of the control value at stimulation frequency of 0.2 Hz and 1 Hz [n = 9] respectively). The time to peak of the tension is not markedly changed while the time constant of the relaxation phase of the contraction is decreased (table 1). This reduction

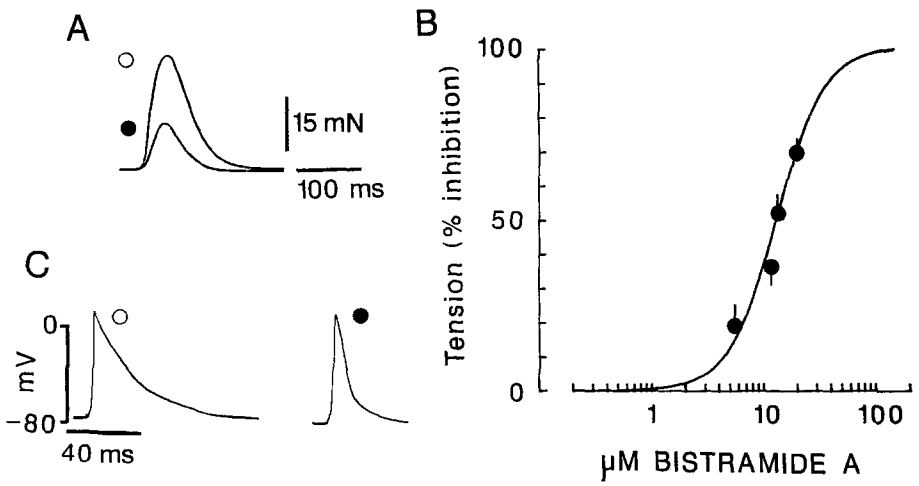


Figure 1. Effect of 10 minutes application of 12 μM Bistramide A (●) treatment on the twitch tension and the action potential (AP) of rat auricle recorded in Tyrode solution (○) at a frequency of 0.2 Hz.
A Stimulated twitch contraction recorded on the same bundle.
B Log-concentration-response relationship for the effect of Bistramide A on the amplitude of the contraction (ordinate scale). Results are expressed as % of the tension recorded in the absence of drug. The curve fitting experimental data was drawn according to the modified Langmuir equation ($Y = 100\ X^m/(K^m + X^m)$) with a dissociation constant $K_D = 14$ μM and a stoichiometry of 2. Vertical bars indicate S.E. values of the means of 5 experiments.
C AP recorded on two different cells from the same auricle.

Table 1. Effect of Bistramide A (14 μM) on the stimulated tension and the action potential (AP) parameters, recorded at 0.2 Hz, using a microelectrode technique in rat auricular bundles

Parameters	Control	Bistramide A
tension (%)	100.0	64.0 ± 6*
t_p (ms)	62.0 ± 7	57.0 ± 5
t_{relax} (ms)	51.0 ± 7	44.0 ± 6*
Total AP (mV)	91.0 ± 4	89.0 ± 5
RP (mV)	−74.0 ± 3	−72.5 ± 2
OS (mV)	17.6 ± 3	13.7 ± 6
APD_0 (ms)	6.6 ± 2	3.6 ± 1**
APD_{50} (ms)	15.5 ± 3	8.2 ± 2**
APD_{20} (ms)	41.0 ± 4	18.0 ± 4**
APD_{10} (ms)	60.0 ± 9	29.0 ± 3**
$\dot{V}_{max}$ (V/s)	180.0 ± 17	139.0 ± 24*

Tension (% of control), the time required for the tension to reach its peak value (t_p), the time constant of the relaxation phase (t_{relax}, measured by semi-logarithmic plot of the falling phase of the tension), were recorded. Total AP, RP, OS, APD_0, APD_{50}, APD_{20}, APD_{10}, $\dot{V}_{max}$, corresponded to: the total amplitude, the resting membrane potential, the overshoot, the duration measured at a membrane potential of 0 mV, 50 mV, 20 mV, 10 mV more positive than the RP, and the first derivative of the initial depolarizing phase respectively. The number of fibres tested was 9 (tension) and 12 (AP) from 9 different hearts. *$p < 0.05$; **$p < 0.001$ when compared with control.

of the tension induced by Bistramide A is dose-dependent (fig. 1B). The Hill plot of the data (log [% inhibition/(100-% inhibition)]) versus toxin concen- tration gives an apparent dissociation constant of 14 μM and a stoichiometric parameter of 2, which suggests that at least two molecules of toxin are involved in the inhibition of the tension as already reported in the frog heart[5]. Figure 1C shows that Bistramide A does not alter the membrane resting potential, but does decrease $\dot{V}_{max}$ and shortens the duration of both the plateau and the slow final phase of repolarization of the action potential (table 1).

Increasing the frequency of stimulation from 0.2 Hz to 1 Hz in the Tyrode solution produces an initial fast decrease of the peak tension amplitude, which developed with a time constant of about 8 s and reached its maximum within ten beats (fig. 2A). This frequency-dependent reduction of the peak tension is then followed by a phase of slow recovery, reaching a steady-state within 2 min. When the frequency of stimulation is lowered to 0.2 Hz, a positive staircase occurs. The force reaches a maximum in about 30 s and then declines to control value (fig. 2A). Figure 2B shows that, in a solution containing 12 μM Bistramide A (a concentration which inhibits the tension by about 36% at 0.2 Hz), the increase in the frequency of stimulation from 0.2 Hz to 1 Hz leads to an initial reduction of the tension followed by a slow and weak recovery (fig. 2Ba). The time constant of the initial reduction is significantly lower in the presence of toxin (4.1 ± 1 ms, $n = 6$; $p < 0.01$) than in the control solution (7.8 ± 0.5 ms, $n = 10$), but a larger number of beats is required to reach the maximal value. Upon lowering the frequency of stimulation to 0.2 Hz, the peak tension recovers its control magnitude within 30 s and does not exhibit a positive potentiation. Results are summarized in table 2. When a 1 Hz stimulation train is interrupted by a period of rest, the peak tension exhibits a clear potentiation decay which was beat-dependent either at 1 Hz or at 0.2 Hz (fig. 2Bb).

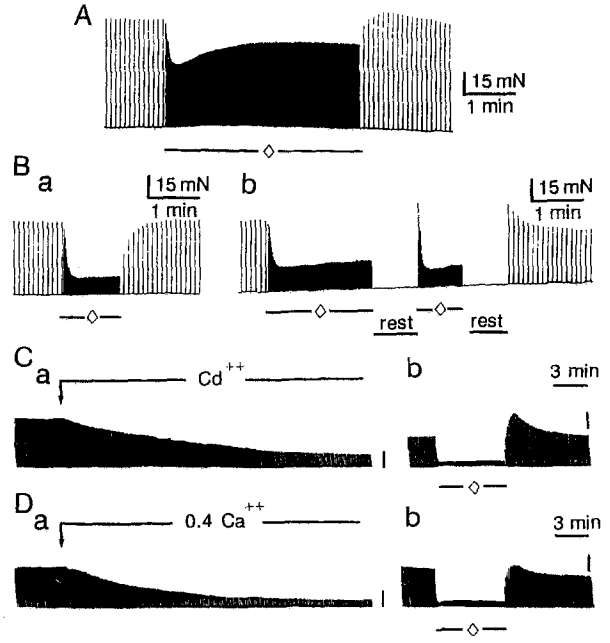
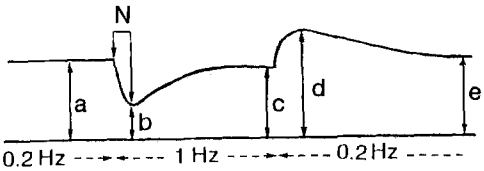


Figure 2. Effect of an increase in the frequency of stimulation from 0.2 Hz to 1 Hz (◇) on the contraction of rat auricular bundles under different solutions.
A Tyrode solution.
B (a) Tyrode solution containing 14 μM Bistramide A. (b) Effect of a 50 second resting period (rest) on the force recorded in the Tyrode solution containing Bistramide A. The preparation was stimulated at 1 Hz and then a rest period was applied; at the end of the second rest period the fibre was driven at 0.2 Hz.
C (a) Addition of 50 μM Cd^{++} to the Tyrode solution. (b) Effect of an increase in frequency of stimulation from 0.2 to 1 Hz on the force recorded in control solution containing Cd^{++}.
D (a) Reduction of the Ca^{++} concentration from 2 mM to 0.4 mM in the Tyrode solution. (b) Effect of the increase in frequency of stimulation from 0.2 to 1 Hz on the force recorded in control solution containing 0.4 mM Ca^{++}. In *C* and *D*, vertical scales: (a) 20 mN and (b) 10 mN.

In the following experiments, the effects of inorganic cation Cd^{++} (a L-type Ca^{++} channel blocker) and low Ca^{++} solutions have been tested on the peak twitch tension to compare the effects of the toxin under different conditions. At a stimulation rate of 0.2 Hz, the peak tension decreases either by Cd^{++} addition (fig. 2Ca) or by lowering external Ca^{++} (fig. 2Da). In both solutions, increasing the frequency of stimulation from 0.2 Hz to 1 Hz induces an initial decrease of the peak tension and a positive potentiation occurs when the frequency of stimulation is returned to 0.2 Hz (figs. 2Cb and 2Db). The reduction of the peak tension is about two times smaller in the presence of toxin ($36 \pm 6\%$, $n = 10$; $p < 0.02$) than in the solution containing Cd^{++} ($70 \pm 4\%$, $n = 6$; $p < 0.01$) or in the solution depleted in Ca^{++} ($81 \pm 2\%$, $n = 5$; $p < 0.001$). The maximal reduction of the peak tension following an increase in the frequency of stimulation to 1 Hz, and the amplitude of the slow recovery phase are of the same order of magnitude, while the number of beats required to reach the maximal reduction is increased (table 2). In the presence of Bistramide A, a positive potentiation does not develop on switching from 1 Hz to 0.2 Hz (fig. 2Ba) while a potentiation does develop in the solution containing Cd^{++} (fig. 2Cb) or depleted in Ca^{++} (fig. 2Db). Results are summarized in table 2. Figure 3A shows that Bistramide A decreases the amplitude of the positive potentiation induced by successive lowering of the stimulation frequency after application of a 1 Hz stimulation rate. Figure 3B shows that the amplitude of the potentiation is significantly decreased by the toxin in the frequency range 0.5 Hz to 0.1 Hz.

Table 2. Effect of change in the frequency of stimulation from 0.2 Hz to 1 Hz on the stimulated twitch tension recorded in the Tyrode solution in the absence and in the presence of (12 μM) Bistramide A, and in the Tyrode solution containing 50 μM Cd^{++} or depleted in Ca^{++} (0.4 mM)

Parameters	Control	Bistramide A	Cd^{++}	0.4 Ca^{++}
maximal amplitude of the force at 1 Hz (%)	55 ± 4	69 ± 3**	77 ± 4**	80 ± 4*
slow recovery phase (%)	79 ± 11	42 ± 7**	28 ± 3***	36 ± 7*
post potentiation (%)	28 ± 5	-	33 ± 7**	23 ± 1*
N	9 ± 1	17 ± 2*	16 ± 1*	14 ± 1*



Measurement of the different parameters is shown in the insert. The maximal reduction of the contraction force, the amplitude of the slow recovery phase at 1 Hz, and the post-potentiation were measured as the ratio b/a, (c-b)/a and (d-e)/e respectively. N was the number of beats required for the tension to reach the maximal reduction at 1 Hz. Parameters are expressed as mean values and the SE of $n = 10$ (control and Bistramide A); $n = 5$ (0.4 Ca^{++} solution); $n = 6$ (solution containing 50 μM Cd^{++}) respectively. *$p < 0.01$; **$p < 0.02$; ***$p < 0.001$ when compared with control.

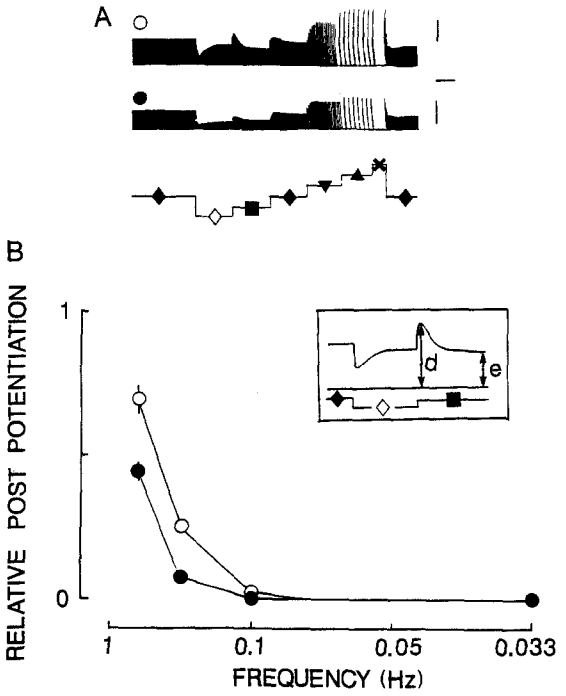


Figure 3. Post-potentiation induced by successive changes in the frequency of stimulation in rat auricular bundles bathed in Tyrode solution in the absence (○) and in the presence of 12 μM Bistramide A (●).

A Starting from 0.2 Hz (◆) the frequency of stimulation (lower trace) was first increased to 1 Hz (◇)until the contraction (upper traces) reached a steady state. The frequency was then successively lowered back to 0.5 Hz (■); 0.2 Hz (◆); 0.1 Hz (▼); 0.033 Hz (▲); 0.016 Hz (x) and finally to 0.2 Hz. Horizontal scale 1 min; vertical scales 15 mN.

B Relationship between the amplitude of the relative post-potentiation (ordinates) and the frequency of stimulation (abscissae). As shown in the insert, the normalized potentiation was represented as the ratio (d-e)/e in which (d) is the maximal tension and (e) the steady state of the tension recorded at a given frequency; lower trace: frequency of stimulation (same symbols as in A). *$p < 0.05$. The points and vertical bars are mean and SE of the different bundles associated with each point (control: $n = 10$; Bistramide A: $n = 7$).

Discussion

The present study shows that Bistramide A reduces the peak tension in a frequency-dependent manner, decreases $\dot{V}_{max}$ and shortens the duration of the action potential (AP) in rat auricular heart muscle.

The action potential of rat auricle consists of a spike, caused by the fast inward Na^+ current, followed by a short plateau generated by the inward Ca^{++} current (I_{Ca}) and a slow final phase of repolarization attributed to the development of the outward K^+ current and to the electrogenicity ($3Na^+/1Ca^{++}$) of the Na^+-Ca^{++} exchange[7-10]. The short plateau duration recorded in the presence of Bistramide A suggests that I_{Ca} is decreased by the substance. Bistramide A has already been shown to inhibit I_{Ca} in frog heart[5] and to bind to the dihydropyridine receptor which senses the T-tubule membrane potential in frog skeletal muscle[6]. The observation that the reduction of the peak tension at high frequency of stimulation (1 Hz) in the presence of Bistramide A is of the same magnitude as the decrease recorded at low frequency (0.2 Hz) in the Tyrode solution containing Cd^{++} or in solution depleted in Ca^{++}, suggests that Bistramide A decreases the Ca^{++} influx in a frequency-dependent manner.

The contraction of auricular rat heart at low frequency depends mostly on the functional state of the sarcoplasmic reticulum (SR), whereas at high frequency the systolic force is more dependent on the Ca^{++} influx entering the cells via Ca^{++} channels[11]. In control rat heart, the potentiation developing during a period of stimulation maintained at high frequency can be explained as the result of competition between Na^+ and Ca^{++} for the transport sites of the Na^+-Ca^{++} exchange. The increase in internal Na^+ concentration due to high frequency stimulation promotes Ca^{++} influx via the exchange and thus contributes to the increase in force[9]. The reduction of the potentiation by Bistramide A might be a consequence of the reduction in Na^+ influx (decrease of $\dot{V}_{max}$ by blockade of the Na^+ current[1,4]) by Bistramide A which alters the Na^+-Ca^{++} exchange activity and so reduces the subsequent Ca^{++} influx, contributing to a decrease in the potentiation.

In control heart, switching from high to low frequency of stimulation allows a relatively large diastolic Ca^{++} influx via the Na^+-Ca^{++} exchange, permitting a more significant Ca^{++} loading of the SR that leads to an increase in the contractions[9]. This potentiation still develops when I_{Ca} is partially blocked by nifedipine but is abolished by caffeine, suggesting that the Ca^{++} accumulation in the SR plays a major role in this phenomenon[8]. The potentiation is not observed in the presence of Bistramide A. Bistramide A inhibits I_{Ca} and reduces the inward Ca^{++} influx via the Na^+-Ca^{++} exchange; in addition, the toxin shortens the AP duration increasing thus the duration of the late diastolic Ca^{++} extrusion via the Na^+-Ca^{++} exchange. These effects of Bistramide A lead to an incomplete diastolic reloading of the SR which may affect the frequency-induced potentiation. From this point of view, Bistramide A differs from the Ca^{++} blocker nifedipine, which: 1) reduces the duration of the plateau and does not alter the slow final phase of the AP[7]; and 2) presents a marked positive potentiation when the frequency of stimulation was lowered[8]. The reduction of potentiation by Bistramide A in the presence of a post-rest potentiation either at 0.2 Hz or at 1 Hz indicates that the ability of the SR to be refilled is not altered by the toxin and does not depend on the previously applied frequency of stimulation. This suggests that Bistramide A does not modify the loading capacity but may slow the kinetics of Ca^{++} accumulation in the SR.

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