

The mechanism of the inotropic action of striatoxin, a novel polypeptide toxin from a marine snail, in isolated cardiac muscle

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- 1 Striatoxin (StTX), a novel polypeptide from a marine snail, caused a dose-dependent increase in contractility in the isolated atria of guinea-pig and rat in the concentration-range of 2×10^{-9} to 3×10^{-8} M and 3×10^{-8} to 10^{-6} M, respectively.
- 2 In guinea-pig atria, the StTX-induced inotropic effect was inhibited by tetrodotoxin but not by cimetidine or chlorpheniramine. Practolol, propranolol or reserpine caused only partial block of this inotropic action.
- 3 In isolated single cells from rat hearts, StTX caused an increase in the degree and the rate of contraction.
- 4 In guinea-pig atria, StTX provoked action potentials with a plateau phase of long duration without affecting the maximum rate of rise, the amplitude of action potential and the resting membrane potential. This prolongation was also reversed by tetrodotoxin.
- 5 In guinea-pig cardiac myocytes, whole-cell patch-clamp experiments showed that StTX slowed Na channel inactivation without affecting the time course of channel activation. The voltage dependence of Na currents was not altered by StTX.
- 6 The residual currents, but not peak currents were markedly enhanced by StTX.
- 7 These results suggest that StTX causes prolongation of the action potential duration probably due to slowed inactivation of Na inward currents and enhanced residual currents and that this may result in an increase in Ca^{2+} availability in cardiac muscle cells. This could explain the cardiotoxic action of StTX.

Introduction

Cone shells are a rich source of valuable peptide toxins (Olivera *et al.*, 1985; Ohizumi *et al.*, 1986a,b). It was reported that the crude venoms of a fish-eating cone shell, *Conus striatus* caused a long-lasting positive inotropic action in guinea-pig isolated atria (Endean *et al.*, 1977b; 1979; Kobayashi *et al.*, 1982a), a gradual rise in the baseline and a decline in twitch tension induced by direct stimulation in rat and mouse isolated diaphragm (Freeman *et al.*, 1974; Endean *et al.*, 1976; Kobayashi *et al.*, 1982a) and a rhythmic contraction (Endean *et al.*, 1977a) or a biphasic response, consisting of a transient contraction followed by a relaxation (Kobayashi *et al.*, 1981) in the guinea-pig isolated ileum. Recently, striatoxin (StTX), a powerful inotropic glycoprotein has been isolated from the

venom of *C. striatus* as an active component (Kobayashi *et al.*, 1982b). The molecular weight of the toxin was approximately 25,000. There have been no pharmacological studies on StTX except our previous report using neuroblastoma cell (Gonoi *et al.*, 1987). The present study was undertaken to clarify the mechanism of the positive inotropic action of StTX by means of pharmacological, electrophysiological and biochemical techniques.

Methods

Mechanical responses of cardiac tissues

Male guinea-pigs (250–350 g) and male Wistar rats (280–300 g) were used. The left and right atria of guinea-pigs or rats were excised and mounted vertically in a 20 ml organ bath containing a Krebs-

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Ringer bicarbonate solution of the following composition (mM): NaCl 120, KCl 4.8, CaCl₂ 1.2, MgSO₄ 1.3, KH₂PO₄ 1.2, NaHCO₃ 25.2, and glucose 5.8 at pH 7.4, and were aerated with 95% O₂: 5% CO₂ at 30°C. The left atria were stimulated electrically at a frequency of 2 Hz, with square-wave pulses of 5 ms at 5 V. The method of preparing the tissues and the technique for measurement of contractions were performed as described previously (Kobayashi *et al.*, 1985b).

Mechanical response of cardiac cells

Cardiac myocytes were isolated from ventricular muscle of adult Wistar rats (250–300 g) by digestion with collagenase (Worthington, Corp., Freehold, NJ, U.S.A.) and were superfused at a flow rate of 0.2 ml min⁻¹ with medium of the following composition (mM): NaCl 120, KCl 5, MgSO₄ 1.2, CaCl₂ 2, NaHCO₃ 5, glucose 10, and N-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) (Sigma Chemical Company, Ltd. St. Louis, MO, U.S.A.); pH 7.4 at 37°C and were bubbled with O₂ as reported previously (Kobayashi *et al.*, 1985a). The beating activity of myocytes was measured either with a video recording system for counting the cardiac cells or with a high-speed movie camera (IVN-200, Photo-Sonics, Inc., Burbank, CA).

Intracellular microelectrode recordings

The left atria were excised from male guinea-pigs (250–350 g), cut into strips, approximately 3 mm in length and less than 1 mm in width and incubated in a 2 ml chamber that was continuously perfused at a rate of 3 ml min⁻¹ with Krebs-Ringer bicarbonate solution aerated with 95% O₂: 5% CO₂ and maintained at 30°C. In the K⁺-depolarization experiments, K⁺ concentration was elevated to 28 mM by replacing a part of Na⁺ with equimolar K⁺. A resting tension of 0.3 g was applied to each preparation. Preparations were stimulated through a bipolar extracellular electrode with square-wave pulses of supramaximal duration at a frequency of 1 Hz. Isometric contractions were measured by a force-displacement transducer. Before the application of drugs, the Ca²⁺ concentration was reduced to 0.3 mM in order to suppress the contraction of atrial muscle. The membrane potential was recorded by inserting a glass microelectrode filled with 3 M KCl and having a resistance from 10 to 20 MΩ, which was connected to a high input impedance pre-amplifier.

Voltage-clamp recordings

Single myocytes were isolated from atrial muscle of male guinea-pigs (250–300 g) by collagenase

digestion as described previously (Kobayashi *et al.*, 1987). The cells were superfused at 37°C with normal Tyrode solution containing (mM): NaCl 135, KCl 5.4, MgCl₂ 1, CaCl₂ 1, glucose 5 and HEPES 5 (pH 7.4). The myocytes were voltage-clamped by the whole-cell patch-clamp technique (Hamill *et al.*, 1981). Giga-ohm seals were obtained with fire-polished patch-clamp pipettes having resistances in the range of 1 to 3 MΩ filled with a solution containing (mM): Cs aspartate 110, NaCl 20, EGTA 5, ATP-tris(hydroxymethyl)aminomethane(Tris) 5 and HEPES-Tris 5 (pH 7.2). The superfusion medium was then changed to a modified Tyrode solution in which Ca²⁺ was omitted and K⁺ was replaced by equimolar Cs⁺. Under these conditions, currents flowing through Ca and K channels were minimal, and only inward Na channel currents were observed in response to depolarizing pulses. The ohmic leakage current and the capacitative currents associated with onset and offset of square pulses were compensated.

Assay of tissue cyclic AMP

The left atria were excised from male guinea-pigs weighing 300 to 350 g and cut into halves. The preparations were loaded with 0.3 g and electrically driven at a frequency of 2 Hz by rectangular pulses of 5 ms in duration and equilibrated with the Krebs-Ringer bicarbonate solution aerated with 95% O₂: 5% CO₂ at 30°C for 60 min. After incubation with the control or test solution, the atria were immediately frozen in liquid nitrogen. Extraction and purification of tissue cyclic AMP were done by the method of Endoh *et al.* (1982). The amount of cyclic AMP was measured by the sensitive radioimmunoassay method using a cyclic AMP assay kit (Yamasa Shoyu Company Ltd., Choshi, Chiba, Japan).

Preparation of sarcoplasmic reticulum (SR)

The heavy fraction of the fragmented SR was prepared from male rabbit (2–3 kg) white muscle as described by Kim *et al.* (1983). The pellet (the SR) was stored at 0°C and used within 3 days. The dog cardiac SR was prepared according to the method of Harigaya & Schwartz (1969). The purified cardiac SR was suspended in 3 ml of the buffer, quickly frozen in liquid nitrogen, and stored at –70°C.

Function of fragmented sarcoplasmic reticulum

The skeletal or cardiac SR was suspended in a mixture (final volume 1 ml) containing 50 μM CaCl₂, 100 mM KCl, 0.5 mM MgCl₂ and 50 mM 3-(*N*-morpholino)propanesulphonic acid (pH 7.0) and tem-

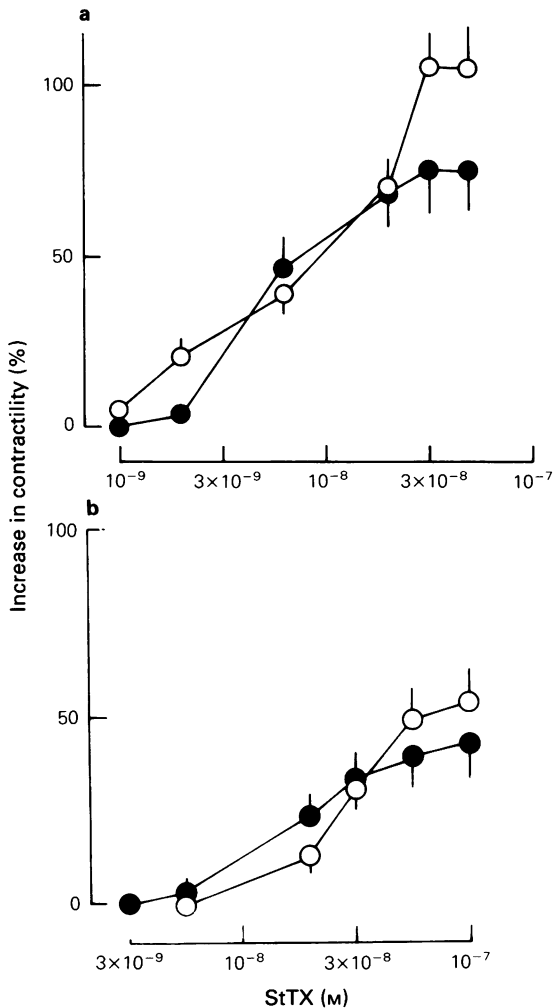


Figure 1 The log dose-ino-tropic response curves for striatoxin (StTX) in the left (O) and right (●) atria of guinea-pigs (a) and rats (b). The left atria was driven at a frequency of 2 Hz with square wave pulses of 5 ms at 5 mV. StTX was cumulatively applied.

perature was maintained at 30°C. The extravesicular Ca^{2+} concentration was measured by means of a Ca^{2+} electrode as described previously (Nakamura *et al.*, 1986).

Na^+ , K^+ -ATPase assay

Na^+ , K^+ -ATPase was prepared from the guinea-pig ventricular muscle according to the method of Pitts & Schwartz (1975). After 5 min preincubation with StTX or ouabain, the enzyme reaction was carried

out at 37°C for 15 min in 0.5 ml reaction media containing (mM): NaCl 100, KCl 20, MgCl_2 5, ATP 3 and Tris-HCl 50, pH 7.4 as described previously (Ohizumi & Yasumoto, 1983).

Cyclic AMP phosphodiesterase assay

Cyclic AMP phosphodiesterase assay was carried out by the method of Butcher & Sutherland (1962). Cyclic AMP phosphodiesterase (0.01 unit ml^{-1}) from bovine heart (Sigma Chemical Company, Ltd.) was incubated at 30°C in 1 ml of 40 mM Tris-HCl buffer (pH 7.4) containing 2 mM MgCl_2 , 0.2 mM cyclic AMP (Sigma Chemical Company, Ltd.) and 0.7 unit ml^{-1} 5'-nucleotidase (Sigma Chemical Company, Ltd.). After a 5 min preincubation with StTX or 1-methyl-3-isobutylxanthine, the reaction was started by the addition of cyclic AMP and terminated by the addition of one tenth volume of cold 55% trichloroacetic acid. The enzyme activity was estimated from the amount of inorganic phosphate during the 20 min incubation.

Reserpine pretreatment

Reserpine (2 mg kg^{-1} , i.p.) was twice administered to guinea-pigs 48 and 24 h before the experiments.

Statistical analysis of the data

The data are presented as means \pm s.e.mean. Statistical analyses were done by means of Student's *t* test.

Extraction and purification of StTX

Thirty specimens of *C. striatus* were obtained from reefs in Okinawa waters. Venom (150 mg) was stripped from the excised venom ducts and mixed with 20 mM phosphate buffer (pH 7.0). The extract of crude venom was centrifuged at 10,000 *g* for 10 min. The resulting supernatant was chromatographed on Sephadex G-100 and DEAE-Sephacel, monitored by the u.v. absorption at 280 nm and cardiotoxic activities, to yield StTX as an active principle. StTX was dissolved in distilled water and kept frozen as stock solution. The details of purification of StTX were previously reported elsewhere (Kobayashi *et al.*, 1982b).

Drugs

The following agents were used in the present study. Tetrodotoxin (TTX, Sankyo Company, Ltd., Tokyo, Japan), verapamil hydrochloride (Eisai Company, Ltd., Tokyo, Japan), practolol (Imperial Chemical

Table 1 Effects of striatoxin (StTX) on the developed tension of the guinea-pig left atria in the presence or absence of various drugs

Treatment ^a	Before StTX	Developed tension ^b (%)		
		StTX		
		(2×10^{-9} M)	(6×10^{-9} M)	(2×10^{-8} M)
None	100	121.2 ± 7.1	138.7 ± 6.9	170.0 ± 9.4
Tetrodotoxin (3×10^{-6} M)	24.1 ± 1.3*	24.3 ± 1.5*	26.7 ± 2.1*	30.1 ± 2.9*
Verapamil (3×10^{-7} M)	42.1 ± 1.2*	43.5 ± 6.1*	105.6 ± 6.0*	121.9 ± 6.7*
Practolol (3×10^{-6} M)	99.7 ± 2.4	107.3 ± 3.9	122.5 ± 7.3	134.4 ± 6.5*
Propranolol (3×10^{-6} M)	70.4 ± 9.3*	81.5 ± 4.6*	88.5 ± 7.0*	105.6 ± 7.8*
Cimetidine (2×10^{-6} M)	102.1 ± 8.3	115.0 ± 9.4	148.8 ± 10.6	179.2 ± 11.4
Chlorpheniramine (10^{-6} M)	108.4 ± 6.4	133.4 ± 8.5	150.3 ± 11.1	197.3 ± 11.5
Reserpine ^c	100	105.5 ± 3.0	115.1 ± 7.3**	135.5 ± 11.9**

^a Drugs were added 15 min before the cumulative application of StTX.

^b Mean ± s.e.mean ($n = 4$ to 6). The developed tension was expressed as a percentage of the contractile force obtained before the drug treatment. The contractile force before drug treatment was 956 ± 85 mg.

^c Reserpine (2 mg kg^{-1} , i.p.) was twice administered to guinea-pigs 48 and 24 h before the experiment.

* Significantly different from the corresponding control value, $P < 0.05$.

** Significantly different from the corresponding control value, $P < 0.1$.

Industries, Ltd., Macclesfield, Cheshire, England), propranolol (Sumitomo Chemical Company, Ltd., Osaka, Japan), cimetidine (Sigma Chemical Company, Ltd.), chlorpheniramine maleate (Sankyo Company Ltd.) and reserpine (apoplone; Daiichi-Seiyaku Company, Ltd., Tokyo, Japan). All drugs

were freshly dissolved in distilled water before the experiment.

Results

Mechanical response of cardiac tissues

StTX caused a powerful inotropic action on the guinea-pig isolated left and right atria at concentrations above 2×10^{-9} M. StTX induced arrhythmias only at concentrations above 2×10^{-8} M. Figure 1a shows the dose-response curve for StTX in the guinea-pig left and right atria. In either tissue, the contractile response increased with StTX concentrations in the range of 2×10^{-9} to 3×10^{-8} M and maximal responses were obtained with concentrations of 3×10^{-8} to 5×10^{-8} M. In rat isolated left and right atria StTX also induced a similar positive inotropic action at higher concentrations (2×10^{-8} to 10^{-7} M) (Figure 1b). The effects of treatment of guinea-pig left atria with various blocking agents on the inotropic action of StTX at different concentrations (2×10^{-9} , 6×10^{-9} and 2×10^{-8} M) were examined and the results are summarized in Table 1. In the presence of TTX (3×10^{-6} M), the inotropic action of StTX was nearly abolished, whereas that of isoprenaline (3×10^{-9} to 3×10^{-8} M) was not affected. Treatment with practolol (3×10^{-6} M), propranolol (3×10^{-6} M) or reserpine (2 mg kg^{-1} , twice) slightly inhibited the inotropic response to StTX. The marked inotropic action was observed in the presence of verapamil (3×10^{-6} M). In addition,

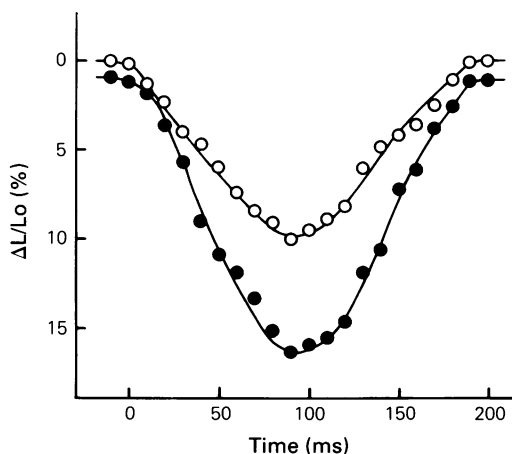


Figure 2 Effects of striatoxin (StTX) on the time course of contraction-relaxation cycles in a single beating action of cardiac myocytes. The degree of longitudinal contraction ($\Delta L/L_0$) was expressed as a ratio to the cell length in the resting state at time zero. (○) Control; (●) 2 min after application of StTX (3×10^{-8} M). The cardiac cell was electrically stimulated with 3 ms rectangular pulses of 15 V cm^{-1} at time zero.

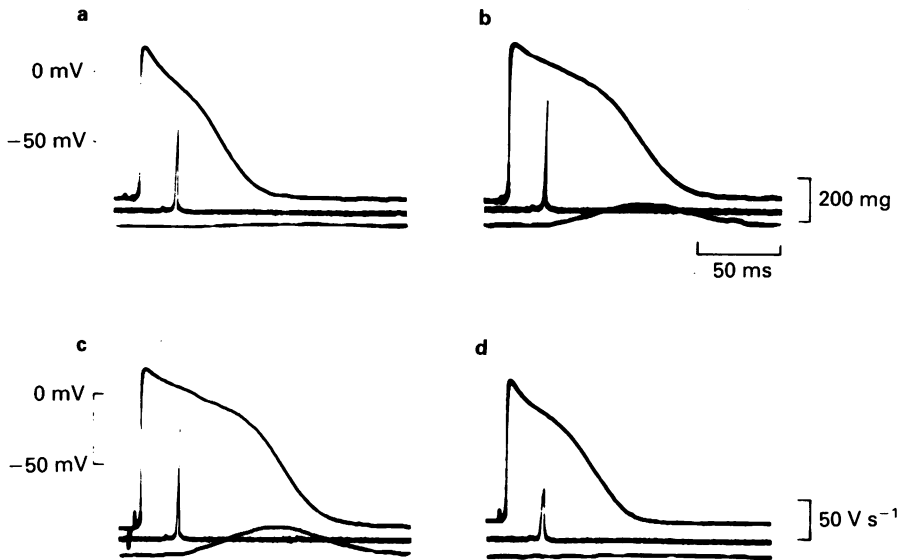


Figure 3 Typical effects of striatoxin (StTX) on the action potential of guinea-pig left atria. The preparation was stimulated at a frequency of 1 Hz. After 30 min exposure to a low-Ca (0.3 mM) Krebs-Ringer solution, StTX (2×10^{-8} M) was added. (a) Control; (b), 10 min after application of StTX; (c), 20 min after application of StTX; (d), 10 min after application of tetrodotoxin (10^{-6} M) in the presence of StTX. In each record, the top trace is membrane action potential; the middle trace gives dV/dt ; the bottom trace shows developed tension.

cimetidine (2×10^{-6} M) or chlorpheniramine (10^{-6} M) had no effect on the inotropic action of StTX.

Mechanical responses of cardiac cells

The effect of StTX on the time course of contraction-relaxation cycles in a single beating action of a myocyte were examined with a high-speed movie camera. As shown in Figure 2, the addition of StTX (3×10^{-8} M) caused an increase in the degree and

rate of longitudinal contractions of myocytes during 2 min. The cellular motion was changed into irregular beating 15 min after application of StTX.

Action potential experiments

The effect of StTX on the electrophysiological properties of guinea-pig left atria was examined. As shown in Figure 3 the duration of the action potential evoked by electrical stimulation was markedly prolonged after exposure to StTX (2×10^{-8} M). The

Table 2 Effects of striatoxin (StTX) and tetrodotoxin (TTX) on resting and action potentials of guinea-pig left atria^a

Treatment	Resting potential (mV)	Action potential amplitude (mV)	V_{max} ($V s^{-1}$)	Action potential duration		
				10% (ms)	50% (ms)	90% (ms)
Control	70.3 ± 1.8	86.0 ± 2.6	122.3 ± 5.8	9.1 ± 0.7	40.8 ± 1.7	71.7 ± 2.2
StTX (2×10^{-9} M) ^b	70.8 ± 2.1	86.5 ± 3.9	123.0 ± 9.5	16.1 ± 1.9*	67.6 ± 6.0*	102.6 ± 3.7*
StTX (2×10^{-9} M) + TTX (10^{-6} M) ^c	69.7 ± 1.9	78.0 ± 3.4	73.0 ± 6.9**	11.0 ± 1.4	42.4 ± 3.7**	74.7 ± 1.5**

^a All values are presented as mean ± s.e.mean ($n = 6$).

^b Measurements were made 20 min after the application of StTX.

^c TTX was added 20 min after the application of StTX. Measurements were made 10 min after the application of TTX.

* Significantly different from control values, $P < 0.05$.

** Significantly different from values after StTX (2×10^{-9} M) alone, $P < 0.05$.

application of TTX (10^{-6} M) reversed the prolonged duration of action potential to its control value (Figure 3 and Table 2), but had little influence on the action potential duration of untreated muscle. As shown in Table 2 the action potential duration at 10, 50 and 90% repolarization was markedly prolonged by StTX, whereas the resting membrane potential and the amplitude or the maximum rate of rise of action potential were not affected by the toxin. TTX reduced the maximum rate of rise of action potential in the presence (Table 2) or absence of StTX. In addition changes in the action potential duration and the contractility of atria by StTX or TTX may be correlated (Figure 3). Furthermore, in the high- K^+ (28 mM) depolarized atrial muscle, StTX (6×10^{-9} to 2×10^{-8} M) had little effect on both the contractile response and the slow action potential.

Voltage-clamp experiments

The effects of StTX on fast Na inward currents in single atrial cells were investigated by the whole-cell patch-clamp technique. Figure 4 shows a family of Na currents induced by depolarizing pulses from a holding potential of -100 mV to -50 , -20 and 10 mV before and after treatment with StTX (4×10^{-8} M) for 20 min. In the control cells, the fast Na current inactivated rapidly with a time constant of 0.9 ± 0.2 ms ($n = 5$) in response to a depolarizing pulse from -100 to -60 mV. After 20 min exposure to StTX, the transient current component was fol-

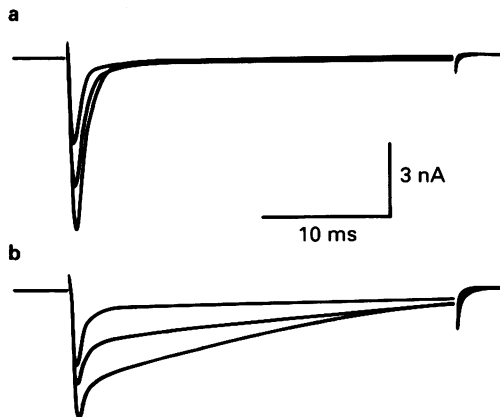


Figure 4 Families of Na currents in voltage-clamped guinea-pig myocytes before (a) and after (b) treatment with striatoin (StTX, 4×10^{-8} M) for 20 min. The holding potential is maintained at -100 mV and a family of Na currents was elicited by 30 ms depolarizing pulses to -50 , -20 and $+10$ mV. Almost the same results were obtained reproducibly from 4 different cells.

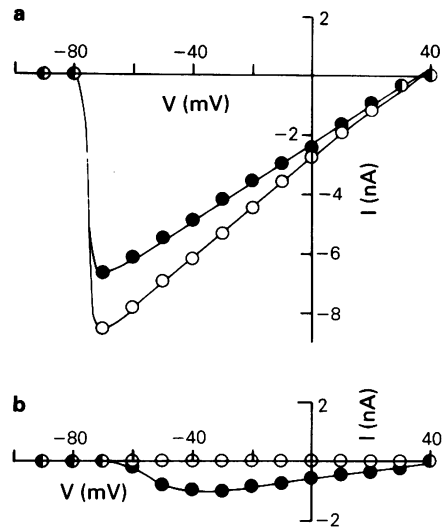


Figure 5 Effects of striatoin (StTX) on the current-voltage curves for peak currents (a) and residual currents (b) in guinea-pig cardiac myocytes. (O) Control; (●) 20 min after application of StTX (4×10^{-8} M). The holding potential is maintained at -100 mV and Na currents were elicited by 30 ms depolarizing voltage steps of 10 mV increment from -90 mV to $+40$ mV. Almost the same results were obtained reproducibly from 4 different cells.

lowed by a slowly decaying Na current component with a time constant of 16.7 ± 1.3 ms ($n = 5$) that was not present in the control. On the other hand, StTX had little effect on the time course of channel activation. Figure 5 shows the Na current-voltage curves for the peak transient current and the residual steady-state current at the end of a 30 ms pulse in the presence or absence of StTX (4×10^{-8} M). The peak current may contain some pipette series resistance artifact, although the leakage and capacitative currents were compensated. The residual current was markedly enhanced after application of StTX, whereas the peak current was slightly reduced by StTX from 8.5 ± 0.7 (control) to 6.6 ± 0.6 nA ($n = 5$).

Assay of cyclic AMP

The effects of StTX (2×10^{-9} to 3×10^{-8} M) and 1-methyl-3-isobutylxanthine (10^{-4} M) on the tissue cyclic AMP content of the atria were examined. The cyclic AMP content was increased by 1-methyl-3-isobutylxanthine, from 0.95 ± 0.05 to 2.33 ± 0.29 nmol g^{-1} tissue during 20 min incubation. But, the cyclic AMP content was not affected by StTX up to 6×10^{-9} M and was only slightly (approximately 25%) elevated by the toxin even at 3×10^{-8} M.

Functions of fragmented sarcoplasmic reticulum

Effects of StTX on functions of the skeletal or cardiac SR were examined by monitoring extravascular concentrations of Ca^{2+} . The Ca^{2+} uptake into either SR was initiated with the addition of ATP (0.5 mM) and creatine kinase (Sigma Chemical Company Ltd.) (0.1 mg ml^{-1}). After extravascular Ca^{2+} concentrations decreased to reach a plateau (submicromolar levels), StTX or caffeine was applied after 30 s. Caffeine (10^{-3} M) induced a marked Ca^{2+} release from skeletal or cardiac SR, whereas StTX did not induce Ca^{2+} release even at $3 \times 10^{-8} \text{ M}$. In addition StTX (2×10^{-9} to $3 \times 10^{-8} \text{ M}$) had no effect on the rate of Ca^{2+} uptake and the caffeine-induced release of Ca^{2+} .

Enzyme assay

Ouabain inhibited Na^+ , K^+ -ATPase prepared from guinea-pig ventricular muscle in a concentration-dependent manner (IC_{50} , $3 \times 10^{-6} \text{ M}$). 1-Methyl-3-isobutylxanthine caused a concentration-dependent inhibition of 3',5'-cyclic nucleotide phosphodiesterase activity (IC_{50} , $1.9 \times 10^{-5} \text{ M}$). However, neither enzyme was affected by StTX at concentrations of 10^{-9} to 10^{-7} M .

Discussion

StTX exerted a powerful positive inotropic action on the guinea-pig and rat isolated atria. This inotropic effect was markedly inhibited by a Na channel blocking drug (TTX), but was not affected by histamine receptor blocking drugs (cimetidine and chlorpheniramine). A Ca antagonist (verapamil), β -adrenoceptor blocking drugs (practolol and propranolol) and a catecholamine depleting agent (reserpine) blocked the inotropic action of StTX only slightly. In rat isolated cardiac myocytes, StTX caused an increase in the degree and the rate of contraction. These results suggest that the StTX-induced inotropic effect was mainly caused by a direct action, probably due to an excitation of TTX-sensitive Na channels and was partially attributed to an indirect action mediated through noradrenaline release from adrenergic nerve terminals.

In the guinea-pig atria, StTX markedly prolonged the action potential duration. This prolongation by StTX was reversed by TTX. The resting membrane potential was not affected by StTX, suggesting that StTX did not increase the Na^+ permeability at the normal resting potential. Furthermore, in the K^+ -depolarized atrial muscle StTX had no or little effect on either the contractile response or the slow action potential, suggesting that StTX does not affect the

Ca^{2+} permeability through the slow channels. These data suggest that in cardiac muscle StTX interacts with Na channels to increase Na^+ entry into myocardial cells, but does not influence Ca^{2+} influx through Ca channels.

Numerous electrophysiological studies on cardiac muscle cells suggest that the properties of the cardiac fast Na channels are closely similar to those in various excitable cells (Hamill *et al.*, 1981; Horn *et al.*, 1981; Cachelin *et al.*, 1983). It has been proposed that inactivation processes of Na channels proceed independently of its activation (Horn *et al.*, 1981). In the present experiment, StTX lengthened the action potential duration of cardiac muscle without affecting the maximum rate of rise and amplitude of action potential and the resting membrane potential. Whole-cell patch-clamp studies on cardiac myocytes indicated that StTX slowed the inactivation of fast Na inward currents without altering the time course of activation and enhanced residual currents. In addition, our recent patch-clamp experiments using N18 neuroblastoma cells revealed that StTX specifically delayed inactivation of Na inward currents (Gonoi *et al.*, 1987). These data suggest that the StTX-induced prolongation of the action potential duration may be attributed to delayed inactivation of Na channels and enhanced residual currents. Furthermore, prolongation of the action potential duration in cardiac muscle has been shown to increase the contractile force (Ochi & Trautwein, 1971). An increase in contractile responses of cardiac muscle by StTX and a reversal of this effect by TTX was closely correlated with prolongation of the action potential duration by StTX and its antagonism by TTX, respectively. Consequently, these observations suggest that the inotropic effect of StTX is caused by increasing Na^+ permeability of Na channels due to modification of channel kinetics and that this may result in an increase in Ca^{2+} availability in cardiac muscle cells.

It was reported that the positive inotropic action of ouabain, a Na^+ , K^+ -ATPase inhibitor, is a consequence of a transient increase in intracellular Na concentrations caused by an inhibition of the Na pump; the elevated Na concentration causes an increase in intracellular Ca by Na/Ca exchange (Aker & Brody, 1978). It has been shown that the positive inotropic effect of amrinone, a cardiotonic drug is caused by an elevation of the cyclic AMP content probably due to an inhibition of cyclic AMP phosphodiesterase (Endoh *et al.*, 1982). But, StTX caused no or little change in the activity of cardiac Na^+ , K^+ -ATPase and cyclic AMP phosphodiesterase and in the cyclic AMP content. These observations suggest that the major mechanism of the positive inotropic action of StTX cannot be explained by the inhibition of Na pump or the change in cyclic

AMP metabolism. Furthermore, it is possible that the stimulation of Ca^{2+} uptake by the SR may play an important role in the positive inotropic effect of a cardiotonic drug because a large pool of Ca for subsequent beats may be provided (Opie, 1982). However, StTX is without effect on functions of the cardiac SR; these observations may rule out the possible involvement of direct stimulation of the SR in the inotropic action.

It is well-known that voltage-sensitive Na channels have separated receptor sites 1–4 for neurotoxins (Catterall, 1986). In cardiac muscle, pharmacological properties of StTX may be similar to marine peptides such as toxin II (receptor site 3) (Ravens, 1976; Romey *et al.*, 1980), anthopleurin A (receptor site 3) (Shimizu *et al.*, 1979; Hashimoto *et al.*, 1980; Kodama *et al.*, 1981) and goniopora toxin (Fujiwara *et al.*, 1979; Noda *et al.*, 1984), since these

polypeptides caused an inotropic effect accompanied by prolongation of the action potential. However, in synaptosomes StTX had no effect on the binding of *Leiurus* α -scorpion toxin or saxitoxin to their receptor site 3 or 1 on Na channels and slightly enhanced the batrachotoxin binding to receptor site 2 (Gonoi *et al.*, 1987). Pharmacological properties of β -scorpion toxin (receptor 4) (Catterall, 1986) were quite different from those of StTX. These observations suggest that StTX may act at a new receptor site on Na channels to delay channel inactivation.

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