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Tetrandrine inhibits electrically induced $[Ca^{2+}]_i$ transient in the isolated single rat cardiomyocyte

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Abstract

The effect of tetrandrine on the electrically induced elevation of cytosolic Ca^{2+} concentration, $[Ca^{2+}]_i$, in the single isolated rat cardiomyocyte was studied with a fluorometric ratio method using fura-2 acetomethylester (fura-2/AM) as Ca^{2+} indicator. Tetrandrine (3–100 μM) concentration and time dependently inhibited the amplitude of the $[Ca^{2+}]_i$ transient without any significant effect on the resting level of $[Ca^{2+}]_i$. At high concentrations (60–100 μM), tetrandrine also prolonged the time to reach the peak ($t_{1.0}$) and the time to decline to 20% of the peak level ($t_{0.2}$) of the electrically induced $[Ca^{2+}]_i$ transient. The effect of tetrandrine was fast in onset and fully reversible upon washout. Tetrandrine (10 μM) partially inhibited the elevation of $[Ca^{2+}]_i$ in response to KCl-induced depolarization. Verapamil and diltiazem mimicked the effects of tetrandrine given at low concentrations, but not at high concentrations. At high concentrations, tetrandrine reduced the magnitude of the caffeine-induced $[Ca^{2+}]_i$ transient. Tetrandrine (100 μM) administered after thapsigargin, which itself decreased the amplitude and prolonged the duration of the electrically induced $[Ca^{2+}]_i$ transient, further decreased the amplitude of the $[Ca^{2+}]_i$ elevation. After ryanodine, which itself decreased the amplitude of the $[Ca^{2+}]_i$ transient, 100 μM tetrandrine not only further reduced the amplitude, but also prolonged the duration of the electrically induced $[Ca^{2+}]_i$ transient. These results provide evidence that in addition to its inhibitory effect on Ca^{2+} influx at the sarcolemma at the therapeutically relevant concentrations, tetrandrine at high concentrations may inhibit Ca^{2+} uptake into the sarcoplasmic reticulum.

Keywords: Tetrandrine; $[Ca^{2+}]_i$ transient; Heart muscle; Ca^{2+} antagonist; Ca^{2+} release; Cardiomyocyte

1. Introduction

Tetrandrine, a bis-benzylisoquinoline alkaloid purified from the Chinese medicinal herb *Radix stephania tetrandrae* has long been used for the treatment of angina and hypertension in China (Chang et al., 1956; Fang et al., 1986). Early studies showed that tetrandrine has negative inotropic (Zong et al., 1983) and antiarrhythmic (Dai et al., 1986) effects, and shortens the duration of the cardiac action potential (Zong et al., 1983). These pharmacologic actions on the cardiac muscle suggest that tetrandrine may act as a Ca^{2+} channel antagonist in cardiac tissue. More recent radioligand binding studies using cardiac sarcolemmal membrane vesicles demonstrated that tetrandrine interacts directly with the benzothiazepine-binding domain and allosterically modulates the dihydropyridine-binding domain in the same channel protein complex (King et al., 1988). Electrophysiological studies also showed that tetrandrine inhibits both T- and L-type Ca^{2+} channel currents in ventricular cells (Liu et al., 1992). Recently, Kwan and Wang (1993) observed that tetrandrine relaxed KCl-precontracted canine saphenous and mesenteric veins, but after washout of KCl, an unexpected transient contraction followed. This contraction upon washout of KCl was not observed with nifedipine or sodium nitroprusside as the relaxants. In addition, after the treatment with tetrandrine, the post-rest potentiation of myocardial contraction in the left atrium was depressed (Wang et al., 1987). These observations suggest that tetrandrine not only inhibits Ca^{2+} influx into the cells, but also alters the intracellular Ca^{2+} uptake or release. Despite the above physiological and biochemical studies, direct measurement of the effect of tetrandrine on the cytosolic Ca^{2+} concentration in cardiac myocytes is not available. The purpose of the present study was to examine the effects of tetrandrine on $[Ca^{2+}]_i$ transients in electrically stimulated adult rat cardiomyocytes using the fluorescent dye approach and to examine its

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pharmacological profile in conditions where intracellular Ca^{2+} was manipulated with sarcoplasmic reticulum modulators, caffeine, ryanodine and thapsigargin. The results demonstrate that $[Ca^{2+}]_i$ transients are concentration dependently and reversibly inhibited by tetrandrine and this effect appears to be mediated via actions on Ca^{2+} fluxes at the level of sarcolemma and sarcoplasmic reticulum depending on the concentration of tetrandrine used.

2. Materials and methods

2.1. Cell isolation

Ca^{2+}-tolerant cardiac ventricular myocytes were isolated from the hearts of adult Sprague-Dawley rats (200–250 g) using a collagenase perfusion method described previously (Dong et al., 1993). The hearts were rapidly removed from rats and perfused in a retrograde manner, at a constant flow rate (10 ml/min) with oxygenated Joklik modified Eagle's medium supplemented with 1.25 mM $CaCl_2$ and 10 mM HEPES, pH 7.2, at 37°C for 5 min followed by 5 min in the same medium without Ca^{2+}. Type I collagenase was added to this medium to a concentration of 125 U/ml with 0.1% (w/v) bovine serum albumin (BSA). After 35–45 min of collagenase digestion by perfusion, the atria were discarded and the ventricular tissue was dissociated by shaking in the collagenase solution for 5 min under oxygen at 37°C. Following a brief trituration, the residue was filtered through 250 μm mesh screens, sedimented by centrifugation at $100 \times g$ for 1 min and resuspended in fresh Joklik solution with 2% BSA. Typically, > 70% of the cells were rod-shaped and not permeate to trypan blue. Ca^{2+} concentration of the Joklik solution was increased gradually to 1.25 mM in 30 min.

2.2. Fura-2 loading

Ventricular myocytes were incubated with fura-2/AM (4 μM) by incubation for 25 min in Joklik solution supplemented with 1.25 mM $CaCl_2$. Unincorporated dye was removed by washing the cells twice in fresh incubation buffer. The loaded cells were maintained at room temperature (24–26°C) for 60 min before measurements of $[Ca^{2+}]_i$ to ensure complete hydrolysis of fura-2/AM in the cytosol.

2.3. Fluorescence measurement

Fura-2-loaded myocytes were transferred to a perfusion chamber at room temperature under an inverted microscope (Nikon) which was coupled to a dual excitation spectrofluorometer (Photo Technical International, NJ, USA). Myocytes were perfused with Krebs bicarbonate buffer (KB buffer) containing (mM) 118 NaCl, 5 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 1.25 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, with 1% dialyzed BSA and a gas phase of 95% O_2/5% CO_2. In low Na^+ and Ca^{2+}-free solution used for the experiment on the effect of caffeine, NaCl was replaced by an equimolar concentration of choline chloride and $CaCl_2$ was omitted. Cells were subjected to 0.2 Hz field stimulation with pulses from stimulator at 60 V for 15 ms duration. The emitted fluorescent light was filtered at 510 nm. Fluorescent signals obtained at 340 nm (F_{340}) and at 380 nm (F_{380}) excitation wavelengths were recorded in a computer for data processing and analysis. The F_{340}/F_{380} ratio represents $[Ca^{2+}]_i$ changes in the myocytes.

2.4. Drugs and chemicals

Fura-2/AM, verapamil, diltiazem, type I collagenase, ryanodine (Rya), thapsigargin and caffeine were purchased from Sigma (St. Louis, MO, USA. Tetrandrine was obtained from Jing-Hua Pharmaceutical (Zhejiang, China), > 98% purity and prepared as stock solution in 0.1 M HCl. Fura-2/AM was dissolved in dimethyl sulfoxide (DMSO) and other chemicals were dissolved in distilled water.

2.5. Statistical analysis

All values were presented as mean ± S.E.M. Student's paired or unpaired *t*-test was employed to analyze the change in parameters before and after drug treatment. Probability values less than 0.05 were considered statistically different.

3. Results

3.1. Effects of tetrandrine, verapamil and diltiazem on electrically induced $[Ca^{2+}]_i$ transient in isolated single rat cardiomyocyte

Fig. 1 shows typical $[Ca^{2+}]_i$ transients recorded in a fura-2-loaded cardiomyocyte with or without tetrandrine. Tetrandrine (3–100 μM) significantly reduced the ampli-

Table 1
Effects of tetrandrine on the temporal change in Ca^{2+} transient of isolated single rat cardiomyocytes electrically stimulated at 0.2 Hz

Tetrandrine (μM)	Before tetrandrine		After tetrandrine	
	$t_{1.0}$ (ms)	$t_{0.2}$ (ms)	$t_{1.0}$ (ms)	$t_{0.2}$ (ms)
30 μM	151 ± 12	830 ± 46	162 ± 15	853 ± 50
60 μM	160 ± 22	828 ± 47	188 ± 14	897 ± 42 [a]
100 μM	158 ± 8	803 ± 61	338 ± 54 [b]	1277 ± 166 [b]

Values are means ± S.E.M of 6 separate experiments. $t_{1.0}$ represents time required to reach the peak of the Ca^{2+} transient and $t_{0.2}$ stands for the time needed to decline to 20% of the peak value. [a] $P < 0.05$, [b] $P < 0.01$ vs. control.

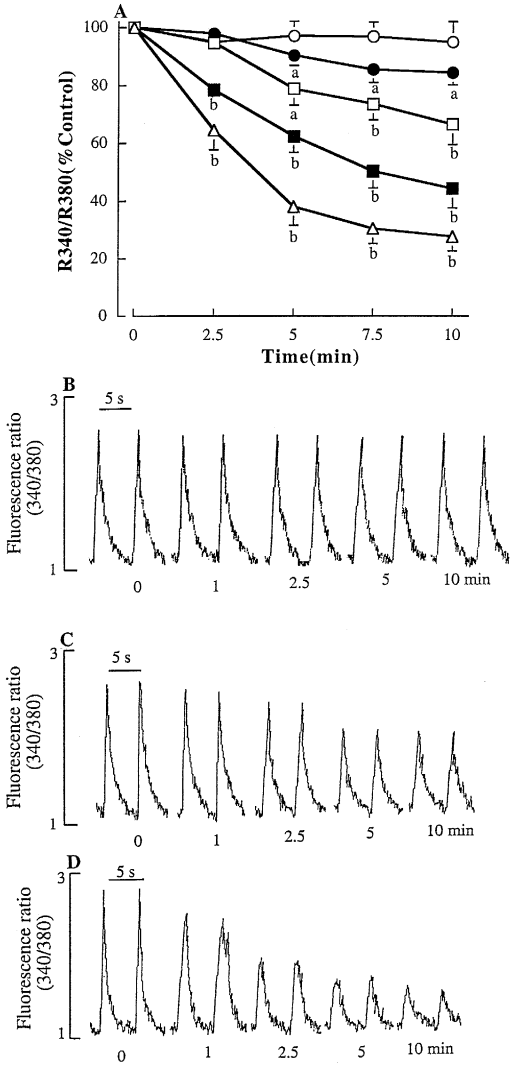

Fig. 1. $[Ca^{2+}]_i$ transients in single electrically stimulated (0.2 Hz) cardiomyocytes. $[Ca^{2+}]_i$ was measured in the fura-2-loaded cell in KB buffer. A: Effects of tetrandrine (1–100 μM) on the amplitude of the $[Ca^{2+}]_i$ transient. [a] $P < 0.05$, [b] $P < 0.01$ vs. 0 min. Each point is the mean ± S.E.M of 6 separate experiments. Tetrandrine concentrations: (○) 1 μM; (●) 3 μM; (□) 10 μM; (■) 30 μM; (△) 100 μM. Representative tracing showing the time-course of the control preparation (B), effect of 10 μM tetrandrine (C), and 100 μM tetrandrine (D) on the $[Ca^{2+}]_i$ transient over 10 min, respectively.

tude of the electrically induced $[Ca^{2+}]_i$ transient in a time- and concentration-dependent manner (Fig. 1A). The inhibitory effect was observed as early as 1 min after administration of tetrandrine and plateaued at about 10 min. Therefore, in all subsequent studies, fluorescence signals were measured in the presence of tetrandrine after 10 min incubation unless otherwise specified. At higher concentrations (60–100 μM), tetrandrine also significantly prolonged the time to reach the peak ($t_{1.0}$) and the time to decline from the peak to 20% of the peak value ($t_{0.2}$) (Fig. 1C and 1D; Table 1), but had no effect on the resting level of $[Ca^{2+}]_i$. Tetrandrine (1–100 μM) did not cause damage to the cardiomyocyte during the 10 min exposure period. The structural integrity was supported by the absence of leakage of fura-2 and full recovery of $[Ca^{2+}]_i$ transient after washout of the drug for 30 min.

Both verapamil and diltiazem at 10 μM inhibited the electrically induced $[Ca^{2+}]_i$ transient and the inhibitory effects were greater than that of tetrandrine (Fig. 2). Unlike tetrandrine, they did not affect $t_{1.0}$ or $t_{0.2}$ even at 100 μM (data not shown).

3.2. Effects of tetrandrine on increased $[Ca^{2+}]_i$ induced by KCl

To determine whether tetrandrine affected the voltage-dependent Ca^{2+} influx across the sarcolemma, the effect of tetrandrine on KCl-induced Ca^{2+} influx was studied. KCl when added to a final concentration of 40 mM further increased the amplitude of the electrically induced elevation of $[Ca^{2+}]_i$ (Fig. 3). Both verapamil and tetrandrine added before the administration of KCl reduced the response to the treatment by 71% and 55%, respectively (Fig. 3), indicating that these drugs act commonly on the L-type Ca^{2+} channel.

3.3. Effects of tetrandrine on caffeine-induced $[Ca^{2+}]_i$ transient

To test whether tetrandrine inhibits Ca^{2+} release from the sarcoplasmic reticulum, the effect of 100 μM tetrandrine on caffeine-induced $[Ca^{2+}]_i$ transient was examined, since caffeine is known to induce Ca^{2+} release from the ryanodine-sensitive Ca^{2+}-release channel in cardiac myocytes (Smith et al., 1988; Sttsapesan and Williams, 1990). When a quiescent cell in low Na^+ and Ca^{2+}-free solution was rapidly exposed to 10 mM caffeine, it elicited a $[Ca^{2+}]_i$ transient. This transient was not influenced by prior addition of 10 μM tetrandrine, but was significantly reduced at a higher tetrandrine concentration (100 μM). However, the $t_{0.2}$ value was not significantly affected by

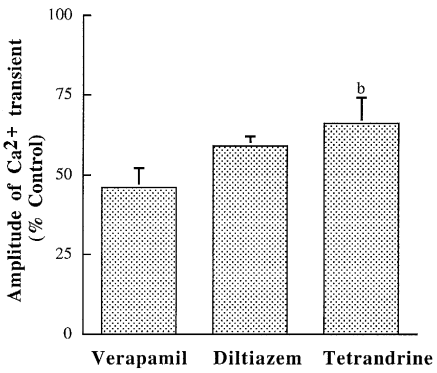

Fig. 2. Effects of 10 μM verapamil and diltiazem on the amplitude of electrically induced $[Ca^{2+}]_i$ transient. Drugs were added to the cardiomyocytes superfused with KB solution. Values are mean ± S.E.M of 6 individual cells. [b] $P < 0.01$ tetrandrine vs. verapamil. The amplitude of the $[Ca^{2+}]_i$ transient before drug treatment is 100%.

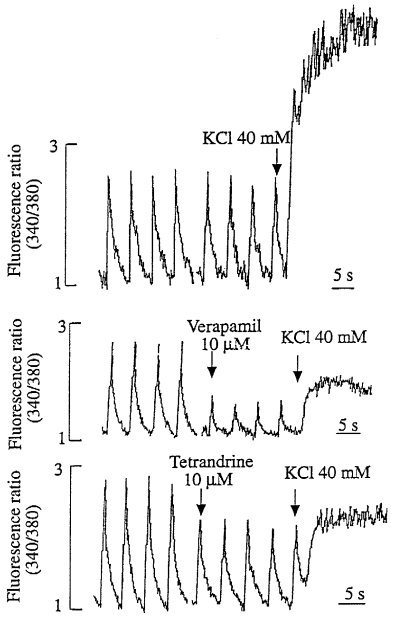

Fig. 3. Effects of verapamil and tetrandrine on electrically induced and KCl-induced elevation of $[Ca^{2+}]_i$. Electrically induced $[Ca^{2+}]_i$ transients were monitored in fura-2-loaded cardiomyocytes, and 40 mM KCl was then added as indicated. Each tracing represents a typical experiment from 6 cell preparations. (A) Response to KCl in the absence of any drug. (B) Response to KCl in the presence of 10 μM verapamil. (C) Response to KCl in the presence of 10 μM tetrandrine.

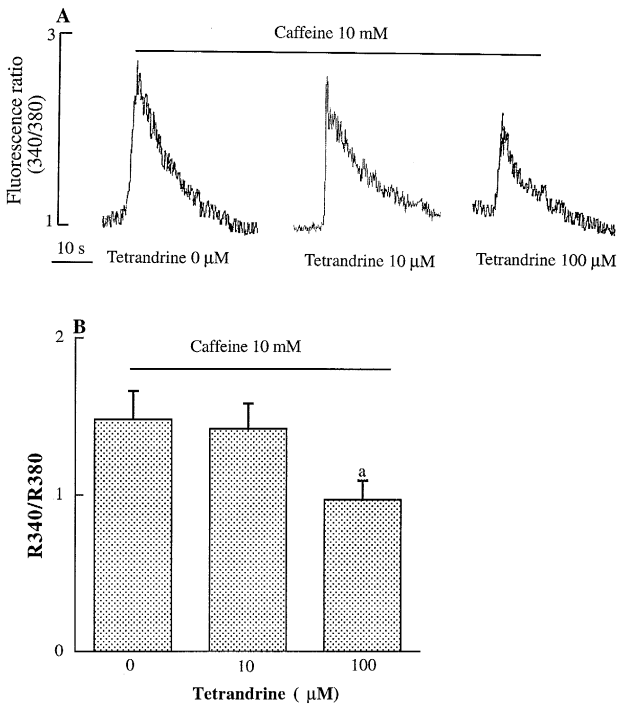

Fig. 4. Induction of $[Ca^{2+}]_i$ transients by caffeine (10 mM) in single quiescent cardiomyocytes following pretreatment with tetrandrine (10, 100 μM) for 20 min and superfused with a Ca^{2+}-free and low $[Na^+]_o$ solution. A: A representative control tracing. B: Amplitude of caffeine-induced $[Ca^{2+}]_i$ transients following tetrandrine pretreatment. Values are mean ± S.E.M of 6 individual cells. [a] $P < 0.05$ vs. control without tetrandrine.

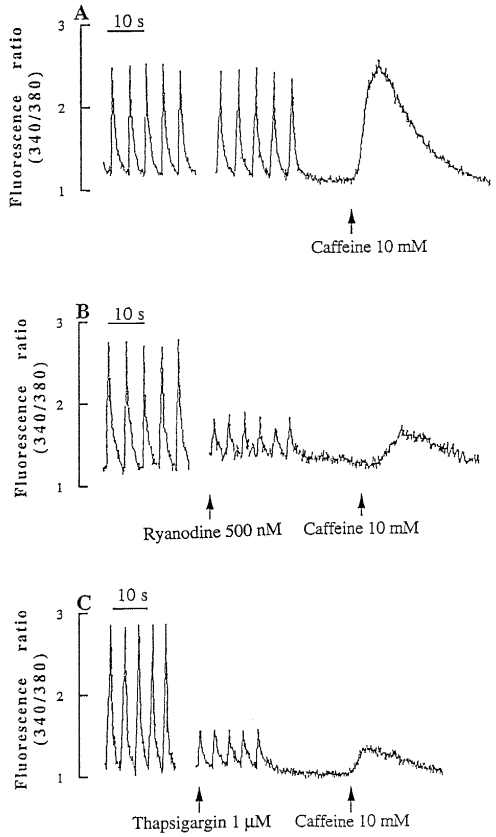

Fig. 5. Induction of $[Ca^{2+}]_i$ transients by caffeine (10 mM) in single quiescent cardiomyocytes following pretreatment with ryanodine (500 nM) or thapsigargin (1 μM) for 5–10 min and superfused with KB solution. (A) A representative control tracing. (B) A representative tracing for ryanodine pretreatment. (C) A representative tracing for thapsigargin pretreatment. Similar results were obtained with 4 individual cells in each case.

100 μM tetrandrine (from 17.0 ± 1.4 to 18.2 ± 1.7 s, $n = 6$, $P > 0.05$, see Fig. 4). Diltiazem at the same concentration did not influence the caffeine-induced $[Ca^{2+}]_i$ transient (not shown).

3.4. Effects of tetrandrine on electrically induced $[Ca^{2+}]_i$ transient following prior administration of ryanodine or thapsigargin

To further study the actions of a high concentration of tetrandrine on $[Ca^{2+}]_i$ transient in relation to the intracellular Ca^{2+} store, the effects of tetrandrine on the electrically induced $[Ca^{2+}]_i$ transient following prior administration of 500 nM ryanodine or 1 μM thapsigargin were studied. After addition of ryanodine or thapsigargin for 5–10 min, 10 mM caffeine induced only a very small $[Ca^{2+}]_i$ transient (Fig. 5). This suggests that prior addition of ryanodine or thapsigargin substantially depleted the sarcoplasmic reticulum Ca^{2+} pool. Ryanodine at the low concentration employed (500 nM) acts by keeping the Ca^{2+} release channel at the subconductance state thereby accelerating Ca^{2+} loss from sarcoplasmic reticulum (Marban and Wier, 1985). Application of 500 nM ryan-

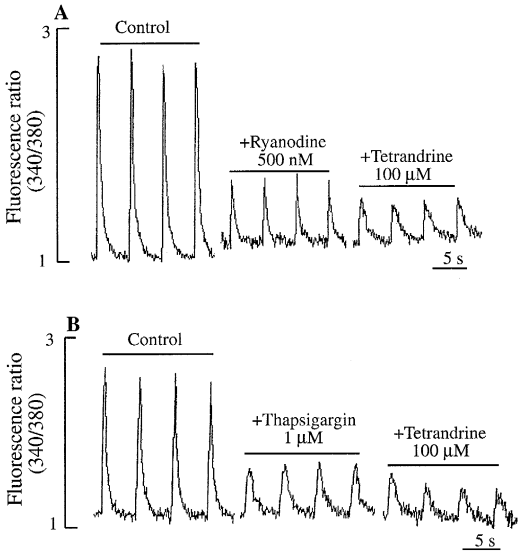

Fig. 6. Effects of tetrandrine (100 μM) on electrically induced $[Ca^{2+}]_i$ transient in isolated single cardiomyocytes following pretreatment with 500 nM ryanodine (A) or 1 μM thapsigargin (B). Similar results were obtained with 6 individual cells in each case.

odine increased the resting fluorescence ratio, and markedly decreased $t_{1.0}$ and the peak amplitude of the electrically induced $[Ca^{2+}]_i$ transient without affecting $t_{0.2}$ (Fig. 6A; Table 2). Subsequent rapid addition of 100 μM tetrandrine further decreased the amplitude, but only tetrandrine increased $t_{1.0}$ and $t_{0.2}$ and diltiazem did not (Table 2). The resting level ratio was however not affected.

Thapsigargin, a selective inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase pump in the cardiac myocytes (Wrzosek et al., 1992; Janczewski and Lakatta, 1993a) not only decreased the amplitude, but also increased the $t_{1.0}$ and $t_{0.2}$ of the electrically induced $[Ca^{2+}]_i$ transient. Unlike ryanodine, thapsigargin was without any effect on the resting fluorescence ratio (Fig. 6B; Table 2). Subsequent superfusion of 100 μM tetrandrine and diltiazem led to further reduction in the amplitude without any effect on $t_{1.0}$, $t_{0.2}$ and the resting level of $[Ca^{2+}]_i$ (Fig. 6B; Table 2).

4. Discussion

The present study is the first study on the effects of tetrandrine on intracellular Ca^{2+} of single rat cardiomyocytes using the direct fluorescence Ca^{2+} indicator method. An electrical stimulation applied to the cardiomyocyte elicits an increased influx of Ca^{2+}, which induces Ca^{2+} release from the sarcoplasmic reticulum via a Ca^{2+}-induced Ca^{2+}-release mechanism, leading eventually to contraction. It has been shown previously that the magnitude of the electrically induced $[Ca^{2+}]_i$ transient was directly related to contraction of the cardiac muscle (Janczewski and Lakatta, 1993b). In the present study, we observed that tetrandrine at 3–100 μM, known to reduce cardiac muscle contractility, duration of action potential (Zong et al., 1983) and voltage-dependent Ca^{2+} currents (Liu et al., 1992) also decreased the amplitude of the electrically induced $[Ca^{2+}]_i$ transient.

4.1. Tetrandrine acts primarily by inhibiting Ca^{2+} entry via L-type Ca^{2+} channels

The reduction in amplitude of the electrically induced $[Ca^{2+}]_i$ transient appears to be primarily due to the reduction in influx of Ca^{2+} and/or release of Ca^{2+} from the intracellular pool (Wier, 1990). We have directly demonstrated that tetrandrine affected the influx of Ca^{2+}. First, the inhibitory effects of tetrandrine on the electrically induced $[Ca^{2+}]_i$ transient were found to be similar to, though less potent than, those elicited by the classical L-type Ca^{2+} channel antagonists, verapamil and diltiazem. Second, tetrandrine attenuated the elevation of $[Ca^{2+}]_i$ induced by KCl, which is known to trigger an influx of Ca^{2+} via the L-type Ca^{2+} channels. Third, tetrandrine further decreased the amplitude of the $[Ca^{2+}]_i$ transient following prior treatment of the myocyte with either ryanodine or thapsigargin, that are commonly used to deplete the intracellular Ca^{2+} stores leading to Ca^{2+} influx in the presence of extracellular Ca^{2+} (Stewart et al., 1991; Hoffmann et al., 1992). These observations confirm the previ-

Table 2
Effects of 100 μM tetrandrine and diltiazem on the Ca^{2+} transient of isolated single rat cardiomyocytes electrically stimulated at 0.2 Hz after pretreatment with 500 nM ryanodine or 1 μM thapsigargin

	$t_{1.0}$ (ms)	$t_{0.2}$ (ms)	Peak amplitude (F_{340}/F_{380})	Resting level (F_{340}/F_{380})
Control	175 ± 17	858 ± 93	1.50 ± 0.21	1.02 ± 0.03
Ryanodine	137 ± 13 [a]	767 ± 62	0.51 ± 0.04 [b]	1.13 ± 0.04 [b]
Ryanodine + tetrandrine	330 ± 19 [b,c]	954 ± 71 [c]	0.32 ± 0.04 [b,c]	1.16 ± 0.04 [b]
Ryanodine + diltiazem	146 ± 18	799 ± 74	0.26 ± 0.04 [b,c]	1.14 ± 0.04 [b]
Control	179 ± 9	833 ± 67	1.41 ± 0.16	0.89 ± 0.06
Thapsigargin	237 ± 26 [a]	951 ± 65 [a]	0.97 ± 0.16 [a]	0.90 ± 0.07
Thapsigargin + tetrandrine	248 ± 30 [a]	958 ± 71 [a]	0.73 ± 0.11 [b,c]	0.91 ± 0.07
Thapsigargin + diltiazem	246 ± 31 [a]	962 ± 68 [a]	0.56 ± 0.14 [b,c]	0.92 ± 0.06

Values are means ± S.E.M of 6 separate experiments. $t_{1.0}$ represents time required to reach the peak of the Ca^{2+} transient and $t_{0.2}$ stands for the time needed to decline to 20% of the peak value. [a] $P < 0.05$, [b] $P < 0.01$ vs. control, [c] $P < 0.01$ vs. ryanodine or thapsigargin.

ous electrophysiological findings that tetrandrine elicited an inhibitory effect on inward Ca^{2+} current across the sarcolemma by blocking the voltage-dependent Ca^{2+} channel (both T-type and L-type) in cardiomyocytes (Liu et al., 1992) and are consistent with the radioligand binding study using isolated cardiac muscle sarcolemmal membrane fragments in which tetrandrine was demonstrated to interact with the diltiazem binding domain of the L-type Ca^{2+} channel protein (King et al., 1988). Furthermore, in vascular smooth muscle, tetrandrine inhibited the contractile response induced by cyclopiazonic acid (a selective sarcoplasmic reticulum Ca^{2+}-ATPase pump inhibitor, like thapsigargin) in the presence of Ca^{2+} (in rat aorta; Kwan et al., 1992) as well as thapsigargin- or norepinephrine-induced elevation of $[Ca^{2+}]_i$ resulting from entry of extracellular Ca^{2+} (in porcine coronary artery; Liu et al., 1995).

4.2. The inhibitory effect of tetrandrine on Ca^{2+} entry is not mediated via Na^+-Ca^{2+} exchange

Na^+-Ca^{2+} exchange may also be involved in the Ca^{2+} influx or extrusion from the cell during the contraction-relaxation cycle depending upon the direction of the Na^+ gradient (Ganitkevich and Isenberg, 1993). There is no direct evidence that tetrandrine affects Na^+-Ca^{2+} exchange in any tissue, though tetrandrine has been demonstrated to block rat myocardial Na^+,K^+-ATPase (Chen et al., 1991). The decay of the caffeine-induced $[Ca^{2+}]_i$ transient may be due to reuptake of Ca^{2+} by sarcoplasmic reticulum Ca^{2+}-ATPase, Ca^{2+} extrusion by sarcolemmal Ca^{2+}-ATPase, and/or Na^+-Ca^{2+} exchanger. Our present finding of the lack of effect of tetrandrine on the $t_{0.2}$ for the decay of caffeine-induced $[Ca^{2+}]_i$ transients in Na^+-free medium does not support the view that Na^+-Ca^{2+} exchange mediates the effect of tetrandrine. Furthermore, in the continuous presence of caffeine, ryanodine-sensitive Ca^{2+} release channels remain open and Ca^{2+} refilling of the leaky sarcoplasmic reticulum is impaired. Thus, the decay of $[Ca^{2+}]_i$ in the presence of 10 mM caffeine is mostly likely due to Ca^{2+} extrusion (Ganitkevich and Isenberg, 1993), which was not affected by tetrandrine. This is also supported by the fact that the $t_{0.2}$ value was not modified by tetrandrine following prior inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase with thapsigargin.

4.3. Tetrandrine at high concentration perturbs Ca^{2+} handling by sarcoplasmic reticulum

One interesting finding of the present study is the demonstration of the effect of tetrandrine on mobilization of Ca^{2+} from the intracellular pool based on two important pieces of evidence. Firstly, 100 μM tetrandrine attenuated the caffeine-induced $[Ca^{2+}]_i$ transient in Ca^{2+}-free medium, which is believed to be derived from the intracellular store via the Ca^{2+}-induced Ca^{2+} release. Secondly, 100 μM tetrandrine also prolonged the time to reach the peak ($t_{1.0}$) and the time to decline ($t_{0.2}$) to the basal level. Similar effects were observed as well with thapsigargin, but not mimicked by either verapamil or diltiazem. However, the lengthened $t_{1.0}$ and $t_{0.2}$ of $[Ca^{2+}]_i$ transient in the presence of thapsigargin or tetrandrine was not observed when another sarcoplasmic reticulum modulator, ryanodine, was employed. This is probably due to the fact that ryanodine depletes intracellular Ca^{2+} stores by keeping the Ca^{2+}-induced Ca^{2+} channel in a subconductance state and the sequestration of cytosolic Ca^{2+} by the sarcoplasmic reticulum Ca^{2+}-ATPase pump remains operative (Bers, 1987; Tatsukawa et al., 1993). Furthermore, we observed that following pretreatment with ryanodine, subsequent addition of tetrandrine prolonged both $t_{1.0}$ and $t_{0.2}$ of the electrically induced $[Ca^{2+}]_i$ transient, whereas no additive effect was seen with tetrandrine in thapsigargin-pretreated cardiac myocytes. Of particular interest is the finding that ryanodine, but not thapsigargin, caused an increase in the resting level of $[Ca^{2+}]_i$, though both of them caused the release of Ca^{2+} from sarcoplasmic reticulum. The observed difference between ryanodine and thapsigargin is conceivably related to their different mechanisms of action on the release of sarcoplasmic reticulum Ca^{2+}. Since the rate of Ca^{2+} release from the *leak channels* as a result of inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase pump by thapsigargin is kinetically slower than that from the Ca^{2+}-release channels locked in the subconductance state by ryanodine, the sarcolemmal Ca^{2+}-ATPase pump could effectively extrude the slowly released Ca^{2+} elicited by thapsigargin, thus maintaining a steady level of resting $[Ca^{2+}]_i$. On the other hand, the sarcolemmal Ca^{2+}-ATPase pump activity may not be able to keep up with the rapid and continuous release of Ca^{2+} elicited by ryanodine, thus leading to elevated resting $[Ca^{2+}]_i$. This interpretation is further supported by the finding of broadening of the $[Ca^{2+}]_i$ transient induced by thapsigargin but not ryanodine.

4.4. Other supporting evidence for intracellular mechanisms of tetrandrine

The fact that both thapsigargin and tetrandrine at high concentrations decreased the amplitude, but increased $t_{1.0}$ and $t_{0.2}$ of electrically induced $[Ca^{2+}]_i$ transient suggests that tetrandrine may inhibit the sequestration of Ca^{2+} by sarcoplasmic reticulum in a similar way as does thapsigargin. Previous studies in a number of cell types lended support to this suggestion. In human leukemic HL-60 cells, both tetrandrine and thapsigargin concentration-dependently caused release of Ca^{2+} from its intracellular store and tetrandrine inhibited thapsigargin-induced mobilization of Ca^{2+} in the presence and absence of external Ca^{2+}, suggesting that both drugs mobilize the same Ca^{2+} pool (Leung et al., 1994). In rat glioma C6 cells, high concentrations (100–300 μM) of tetrandrine inhibited the $[Ca^{2+}]_i$ as well as the elevation of IP_3 evoked by bombesin

(Takemura et al., 1995). In rat permeabilized vascular smooth muscle, tetrandrine decreased the sequestration of $^{45}Ca^{2+}$ into sarcoplasmic reticulum with an IC_{50} value of 77 μM (Su, 1993) and was consistent with the finding that tetrandrine inhibited the transient vascular smooth muscle contraction in Ca^{2+}-free medium induced by norepinephrine in swine coronary artery (Jia et al., 1985) or by phenylephrine in rat aorta (Kwan et al., 1992), a response known to be due to the release of Ca^{2+} from sarcoplasmic reticulum. On the other hand, Liu et al. (1995) did not observe any inhibitory effect of tetrandrine on the transient contractile response or the elevation of Ca^{2+} induced by norepinephrine in Ca^{2+}-free medium in the rat tail artery. Although in the study of rat tail artery by Liu et al. (1995), a low concentration of tetrandrine (10 μM) may preferentially inhibit Ca^{2+} entry and contraction induced by norepinephrine without an effect on the sarcoplasmic reticulum, Jia et al. (1985) have demonstrated that 10 μM tetrandrine inhibited the contractile responses of swine coronary artery in Ca^{2+}-free medium by about 50%. It is possible that the effect of tetrandrine may be tissue selective and further direct studies are needed to determine whether tetrandrine inhibits the uptake of Ca^{2+} by inhibiting the sarcoplasmic reticulum Ca^{2+}-ATPase pump in the cardiac muscle.

4.5. Clinical relevance of the effects of tetrandrine

The clinical dose of intravenously infused tetrandrine for the treatment of hypertension and arrhythmia has been reported to be in the range of 90–200 mg (Fang and Jiang, 1986; Dai et al., 1990) and the concentration of tetrandrine in the plasma may reach 30 μM (Fang et al., 1981). This concentration is similar to the lower range of concentration of tetrandrine used in this study. Therefore, the antihypertensive and antiarrhythmic effects of tetrandrine are most likely due to its Ca^{2+} antagonism at the cell membranes. Nevertheless, our present findings also point out that tetrandrine at higher concentrations (which may be toxicologically relevant) may aggrevate the depression of myocardium by interfering with the Ca^{2+} handling by the sarcoplasmic reticulum.

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