ELSEVIER

European Journal of Pharmacology 327 (1997) 233–238

ejp

Ionic mechanisms of tetrandrine in cultured rat aortic smooth muscle cells

Sheng-Nan Wu *, Tsong-Long Hwang, Chung-Ren Jan, Ching-Jiunn Tseng

Department of Medical Education and Research, Veterans General Hospital-Kaohsiung, 386 Ta-Chung 1st Road, Kaohsiung City, Taiwan

Received 12 December 1996; revised 25 February 1997; accepted 21 March 1997

Abstract

The ionic mechanism of tetrandrine, an alkaloid extracted from the Chinese medicinal herb *Radix stephania tetrandrae*, was investigated in A7r5 vascular smooth muscle cells. The nystatin-perforated whole-cell voltage-clamp technique was performed to examine the effects of tetrandrine on ionic currents. Tetrandrine (1–100 μM) reversibly caused an inhibition of L-type voltage-dependent Ca^{2+} current ($I_{Ca,L}$) in a concentration-dependent manner. Tetrandrine did not cause any change in the overall shape of the current–voltage relationship of $I_{Ca,L}$. The IC_{50} value of tetrandrine-induced inhibition of $I_{Ca,L}$ was 5 μM. In the presence of Bay K 8644 (3 μM) or cyclopiazonic acid (30 μM), tetrandrine still produced a significant inhibition of $I_{Ca,L}$. The inhibitory effects of tetrandrine on $I_{Ca,L}$ exhibited tonic and use-dependent characteristics. Moreover. tetrandrine (3 μM) shifted the steady-state inactivation curve of $I_{Ca,L}$ to more negative membrane potentials by approximately −15 mV. These results indicate that tetrandrine directly inhibits the voltage-dependent L-type Ca^{2+} current in vascular smooth muscle cells, which may predominantly contribute to the vasodilatory actions of tetrandrine.

Keywords: Smooth muscle cell, vascular; Tetrandrine; Ca^{2+} current

1. Introduction

Tetrandrine, an alkaloid extracted from the Chinese medicinal herb *Radix stephania tetrandrae*, possesses a wide spectrum of pharmacological activities. Tetrandrine was reported to inhibit vascular smooth muscle contraction evoked by high K^+ (Hu et al., 1983; Anselmi et al., 1994; Liu et al., 1995) as well as norepinephrine (Su, 1993; Liu et al., 1995; Wang and Lemos, 1995). Previous findings thus suggested that tetrandrine might be able to inhibit the voltage-dependent Ca^{2+} channels in various tissues, including vascular smooth muscle cells (King et al., 1988; Liu et al., 1992, 1995). In vascular smooth muscle, the vasoactive agents (e.g., norepinephrine, vasopressin or endothelin) can induce Ca^{2+} release from internal Ca^{2+} storage sites and Ca^{2+} influx through the sarcolemma, hence producing muscle contraction (Bolton, 1979; Somlyo and Himpens, 1989). Because nifedipine inhibited norepinephrine-induced vasoconstriction to a lesser extent (Kwan et al., 1992; Oriowo and Ruffolo, 1992), the mechanisms underlying tetrandrine-mediated inhibition of norepinephrine-induced vasoconstriction or increase in intracellular Ca^{2+} concentration still remain unclear.

In order to further understand the mechanisms of relaxing effects of tetrandrine in vascular smooth muscle cells, the actions of tetrandrine on ionic currents were examined in A7r5 aortic smooth muscle cells, using the nystatin-perforated whole-cell voltage-clamp technique (Horn and Marty, 1988). The A7r5 cell line is known to possess electrophysiological characteristics of vascular smooth muscle cells (Van Renterghem et al., 1988; Lo et al., 1995). These results strongly indicate that direct inhibition of the voltage-dependent Ca^{2+} channel mainly, if not entirely, involves the tetrandrine-induced relaxation of vascular smooth muscle.

2. Materials and methods

2.1. Cell preparation

Rat thoracic aorta smooth muscle cells (clonal cell line, A7r5) were obtained from American Type Culture Collection (CRL 1444, Rockville, MD, USA). The cells were maintained and subcultured in Dulbecco's modified Eagle

* Corresponding author. Tel.: (886-7) 3422121-1507; Fax: (886-7) 346-8056; e-mail: snwu@isca.vghks.gov.tw

PII S0014-2999(97)00075-7

medium (DMEM) supplemented with fetal bovine serum (10%), penicillin G (10 000 units/ml) and streptomycin (100 μg/ml) and equilibrated at 37°C with a humidified atmosphere of 5% CO_2-95% air. The culture medium was changed every 2–3 days. Cells were subcultured weekly after being detached from the flasks by using culture medium containing 1% trypsin. One-day preconfluent cultures were used for the experiments (Wu et al., 1995).

2.2. Current measurement and data analysis

In order to minimize the dialysis of cell constituents with the recording pipette and hence stabilize the ionic currents, the nystatin-perforated whole-cell voltage-clamp technique (Hamill et al., 1981; Horn and Marty, 1988; Wu et al., 1993) was used in the present study by means of a RK-400 patch-clamp amplifier (Biologic, Claix, France). The heat-polished patch pipette with the internal solution had a tip resistance of 3–5 MΩ. The membrane potential and ionic currents were continuously monitored with a storage oscilloscope (model 1602; Gould, Valley View, OH, USA) and recorded on digital audio tape using a digital tape recorder (model 1204; Biologic). The data were reproduced, low-pass filtered at 3 kHz (−3 dB) with a Bessel filter and later analyzed off-line using the pClamp software package version 6.03 (Axon Instruments, Foster City, CA, USA) after the analogue-to-digital conversion at a sampling frequency of 2.5–10 kHz.

The time course of activation or inactivation kinetics of $I_{Ca,L}$ was determined according to the one- or two-exponential model (Wu et al., 1993). The steady-state inactivation parameters of the L-type Ca^{2+} inward current ($I_{Ca,L}$) at various membrane potentials were estimated with the use of a double-pulse protocol (Lo et al., 1995). The conditioning voltage pulses (3 s in duration) to various membrane potentials between −80 and +30 mV were applied from a holding potential of −90 mV. At 10 ms after the end of each conditioning pulse, a test pulse to +10 mV (150 ms in duration) was applied to evoke $I_{Ca,L}$. The normalized amplitudes of $I_{Ca,L}$ (I/I_{max}) were then plotted at each conditioning potential (inactivation curve). The interval between the sets of double pulses was 60 s to prevent incomplete recovery of $I_{Ca,L}$.

To calculate the percentage inhibition of tetrandrine, each cell was depolarized from a holding potential of −40 mV to +10 mV, and the amplitude of $I_{Ca,L}$ during the application of tetrandrine was compared with the control value. The concentration-dependent effect of tetrandrine on the inhibition of $I_{Ca,L}$ was then fitted with the Hill equation. That is,

Percentage inhibition

$$= \left(E_{max} \times [\text{drug}]^n\right) / \left(IC_{50}^n + [\text{drug}]^n\right)$$

where [drug] represents the concentration of tetrandrine; IC_{50} and n are the concentration required for a 50% inhibition and Hill coefficient, respectively; E_{max} is tetrandrine-induced maximal inhibition of $I_{Ca,L}$.

All values are shown as mean ± S.E.M.. The paired Student's *t*-test or Duncan's multiple range test was used for the statistical analyses. Differences between values were considered significant when $P < 0.05$.

2.3. Solutions and drugs

The normal Tyrode's solution was as follows (in mM): NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5, *N*-[2-hydroxyethyl]piperazine-*N*′[2-ethanesulfonic acid] (HEPES)-NaOH buffer 5 (pH 7.4). The patch pipette solution contained (in mM): CsCl 130, ethylene glycol-bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) 0.1, $MgCl_2$ 2, Na_2ATP 3, guanosine-5′-triphosphate 0.1 and HEPES-CsOH buffer 5 (pH 7.2). To completely block K^+ current, tetraethylammonium chloride (10 mM) was added into normal Tyrode's solution. (*S*,*S*)-(+)-Tetrandrine (6,6′,7,12-tetramethoxy-2,2′-dimethylberbamam) was obtained from Aldrich (Milwaukee, WI, USA). *S*(−)-Bay K 8644 was purchased from Biomol (Plymouth Meeting, PA, USA). Tetraethylammonium chloride, [Arg^8]vasopressin and guanosine-5′-triphosphate were purchased from Sigma (St. Louis, MO, USA). Cyclopiazonic acid and nystatin were obtained from Research Biochemicals International (Natick, MA, USA). In perforated patch whole-cell recording experiments, polyene antibiotic nystatin was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 50 mg/ml, and then added to the internal pipette solution to yield a final nystatin concentration of 100 μg/ml (Horn and Marty, 1988). Tetrandrine was dissolved in DMSO (less than 0.01%) and made immediately prior to experiments. The time allowed for nystatin action was 125 ± 12 s ($n = 14$).

3. Results

3.1. Effect of tetrandrine on voltage-dependent L-type Ca^{2+} current

In A7r5 vascular smooth muscle cells, when the patch membrane was perforated with the aid of nystatin in whole-cell recording experiments, the stability of $I_{Ca,L}$ in normal Tyrode's solution containing 1.8 mM $CaCl_2$ remained constant for more than 20 min. Resting membrane potential of A7r5 cells used in the present study was −40 ± 5 mV ($n = 22$). As shown in Fig. 1, the effect of tetrandrine on $I_{Ca,L}$ was examined at various membrane potentials, and hence a current–voltage (*I*--*V*) relationship of $I_{Ca,L}$ was constructed. By comparing the two *I*–*V* curves shown in Fig. 1, it can be observed that the threshold potential (around −30 mV), the potential of maximum peak $I_{Ca,L}$ (around +10 mV), and the apparent reversal potential (around +40 mV) were essentially the

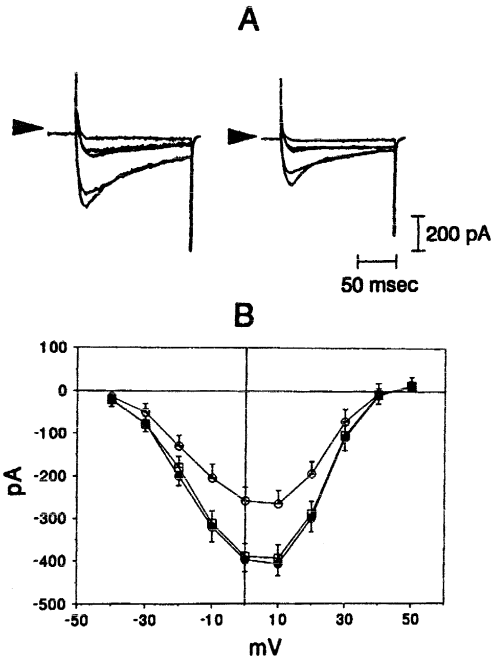

Fig. 1. The current–voltage relationships of $I_{Ca,L}$ in the absence and presence of tetrandrine. The cell was held at −50 mV, and the command voltage pulses to various membrane potentials with the duration of 150 ms were applied at 0.2 Hz. In A, the original traces are shown when the cell was depolarized from a holding potential of −50 mV to −40, −30, −10, +10 and +30 mV. The traces shown on the left are control, and those on the right were obtained 1 min after the addition of tetrandrine (10 μM). The arrows denote zero current level. In B, the current-voltage relationships of the peak $I_{Ca,L}$ in control (filled circles), after the application of tetrandrine (open circles) and after the washout of the drug (open squares) are plotted. Each data point represents the mean ± S.E.M. of 12 different cells.

same in the absence or the presence of tetrandrine (10 μM). In other words, tetrandrine (10 μM) was capable of suppressing the $I_{Ca,L}$ without changing its voltage dependence and the overall shape of the *I–V* curves for $I_{Ca,L}$. The inhibitory effect of tetrandrine on $I_{Ca,L}$ was completely reversible. Similar results were obtained from 12 different cells. In addition, when cells were held at −50 mV and the command pulses to 0 mV with a duration of 150 ms, tetrandrine (10 μM) did not produce any significant change in the kinetics of activation or inactivation of $I_{Ca,L}$ (control $\tau_{act} = 4 \pm 1$ ms, $\tau_{inact(f)} = 19 \pm 6$ ms, $\tau_{inact(s)} = 206 \pm 12$ ms; tetrandrine $\tau_{act} = 3 \pm 1$ ms, $\tau_{inact(f)} = 21 \pm 5$ ms, $\tau_{inact(s)} = 209 \pm 15$ ms ($n = 6$)).

Fig. 2 shows the relationship between the concentrations of tetrandrine and the percent inhibition of $I_{Ca,L}$. Tetrandrine (1–30 μM) inhibited $I_{Ca,L}$ in a concentration-dependent manner. The half-maximal concentration required for the inhibitory effect of tetrandrine was 5 μM; and 30 μM tetrandrine suppressed the amplitude of $I_{Ca,L}$ by 85%. These results demonstrate that tetrandrine has a Ca^{2+}-antagonistic effect in A7r5 cells in a concentration-dependent fashion.

Whether the effect of tetrandrine on $I_{Ca,L}$ was affected

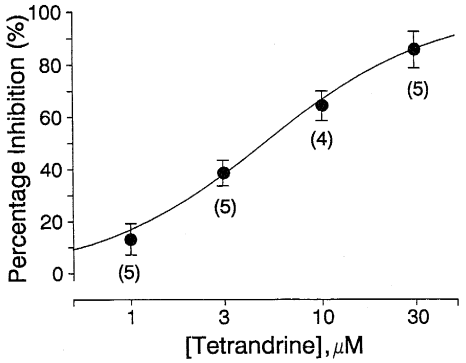

Fig. 2. Concentration-dependent inhibition of $I_{Ca,L}$ by tetrandrine. The relations between the percent inhibition of $I_{Ca,L}$ and the concentration of tetrandrine are illustrated. The cells were held at −40 mV, and the command voltage pulses to +10 mV were applied. Various concentrations of tetrandrine (1–30 μM) were examined. The amplitude of the peak $I_{Ca,L}$ during the application of tetrandrine was compared with the control value. Numbers in parentheses denote the number of cells. The smooth line represents best fits to the Hill equation as described in Section 2. The values for IC_{50} and maximally inhibited percentage of $I_{Ca,L}$ in the presence of tetrandrine were 5.0 μM and 96%, respectively. The Hill coefficient was 1.1.

by the presence of Bay K 8644, a specific L-type Ca^{2+} channel opener, or cyclopiazonic acid, an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, was also examined. Fig. 3 shows the comparison of tetrandrine's effect on the inhibition of $I_{Ca,L}$ between the absence and presence of Bay K 8644 (3 μM) or cyclopiazonic acid (30 μM). Bay K 8644 (3 μM) caused a 1.6-fold increase in the amplitude

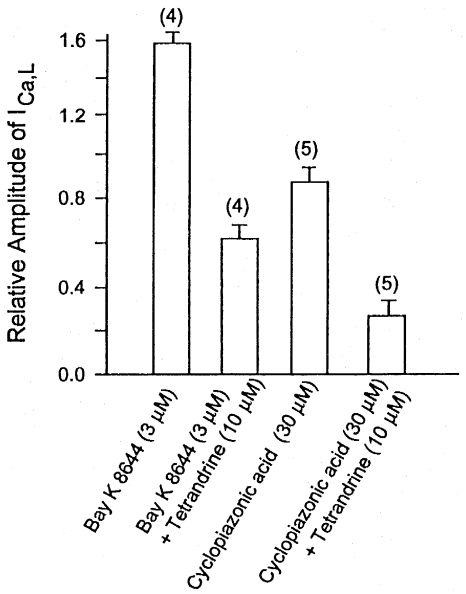

Fig. 3. The effect of tetrandrine on the inhibition of $I_{Ca,L}$ in the absence and presence of Bay K 8644 or cyclopiazonic acid. The cells were held at the level of −40 mV, and the command voltage pulses to +10 mV (150 ms in duration) were applied. The amplitude of $I_{Ca,L}$ in the control was considered to be 1.0 and the relative amplitude of $I_{Ca,L}$ after application of agent(s) was plotted. Numbers in parentheses denote the number of cells.

of $I_{Ca,L}$, whereas cyclopiazonic acid (30 μM) produced a reduction of $I_{Ca,L}$ by about 10%. When $I_{Ca,L}$ was augmented by Bay K 8644, tetrandrine (10 μM) still significantly reduced the amplitude of $I_{Ca,L}$ by about 60%. Moreover, in the presence of cyclopiazonic acid, the inhibitory effect of tetrandrine (10 μM) on $I_{Ca,L}$ still existed. These results indicate that the inhibitory effect of tetrandrine on $I_{Ca,L}$ is direct and not related to the function of Ca^{2+}-ATPase in the sarcoplasmic reticulum.

3.2. Voltage and use dependence of tetrandrine-induced inhibition of Ca^{2+} current

To further characterize the inhibitory effects of tetrandrine on $I_{Ca,L}$, we also examined the voltage dependence of the effect of tetrandrine on $I_{Ca,L}$. Fig. 4 shows the steady-state inactivation curve of $I_{Ca,L}$ in the absence and presence of tetrandrine (3 μM). The double-pulse protocol was applied. A 3 s conditioning pulse to various membrane potentials preceded the test pulse (150 ms in duration) to +10 mV from a holding potential of −90 mV. The relationships between the membrane potentials and the normalized amplitudes of $I_{Ca,L}$ with or without the application of tetrandrine (3 μM) were plotted and well fit by the Boltzmann equation using the non-linear regression analysis:

$$I = I_{max}/\{1 + \exp[(V - a)/b]\}$$

where I_{max} = the maximal activated $I_{Ca,L}$, V = the membrane potential in mV, a = the membrane potential for half-maximal inactivation and b = the slope factor of inactivation curve. In control, $a = -31.8 \pm 1.2$ mV, $b = 10.4 \pm 0.8$ mV ($n = 5$), whereas in the presence of tetrandrine (3 μM), $a = -46.7 \pm 0.9$ mV, $b = 10.9 \pm 0.6$ mV ($n = 4$). Thus, tetrandrine not only inhibited the maximal conductance of $I_{Ca,L}$, but also shifted the inactivation curve to hyperpolarized potentials by approximately −15 mV. However, tetrandrine did not produce any significant change in the slope of the curve. These results indicate that tetrandrine can inhibit the amplitude of $I_{Ca,L}$ in a voltage-dependent manner in A7r5 cells.

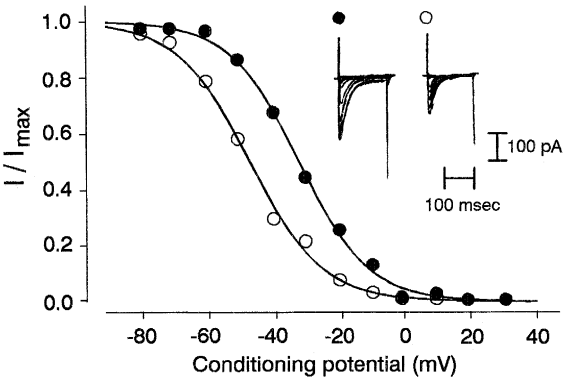

Fig. 4. Steady-state inactivation curve of $I_{Ca,L}$ in the absence and presence of tetrandrine. By the use of the double-pulse protocol, the steady-state inactivation parameters of $I_{Ca,L}$ were obtained in the absence and presence of tetrandrine (3 μM). The conditioning voltage pulses with a duration of 3 s to various membrane potentials between −80 mV and +30 mV were applied from a holding potential of −90 mV. At 10 ms after each conditioning pulse, a test pulse to +10 mV with a duration of 150 ms was applied to evoke $I_{Ca,L}$. The normalized amplitude of $I_{Ca,L}$ (I/I_{max}) was constructed against the conditioning pulse potential and the curves were well fit by the Boltzmann equation (see text for details). The insets show the original current traces. Closed circles, control; open circles, tetrandrine (3 μM).

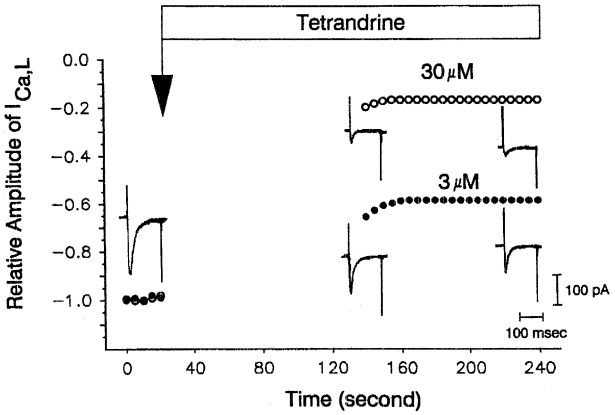

Fig. 5. Tonic and use-dependent block of $I_{Ca,L}$ by tetrandrine. The cell was held at −40 mV, and the command voltage pulses of +10 mV (150 ms in duration) were applied at 0.2 Hz. The protocol for the addition of tetrandrine is denoted by the bar and arrow. The alteration of the relative amplitude of $I_{Ca,L}$ during the addition of various concentrations of tetrandrine (3 μM and 30 μM) is shown. The amplitude of $I_{Ca,L}$ in the control was taken as −1.0. After the voltage steps were stopped, various concentrations of tetrandrine were added to the bath. After approximately 2 min of cessation in the tetrandrine (3 and 30 μM)-containing solution, the repetitive depolarizing pulses to +10 mV at 0.2 Hz were applied again. Note that under the application of tetrandrine, the amplitude of $I_{Ca,L}$ evoked by the first voltage step following a long pause had already been suppressed (i.e., tonic block), and during the repetitive stimuli, the amplitude of $I_{Ca,L}$ was reduced exponentially (i.e., use-dependent block). Insets show the original current traces of $I_{Ca,L}$ in each condition. The calibration bars are shown in the right-hand bottom corner.

The use-dependent characteristic of tetrandrine inhibition of $I_{Ca,L}$ was also studied. As shown in Fig. 5, the command voltage pulses from −40 mV to +10 mV (150 ms in duration) were applied at 0.2 Hz. Under control conditions, no decline in the amplitude of $I_{Ca,L}$ at this stimulation protocol was observed for 5 min. When the amplitude of $I_{Ca,L}$ remained constant in the normal Tyrode's solution, the depolarizing pulses were then stopped and the cells remained exposed to various concentrations (3 or 30 μM) of tetrandrine. After 2 min of cessation, repetitive depolarizing pulses to +10 mV at 0.2 Hz were applied to the cells again. The relative amplitude of $I_{Ca,L}$ with respect to that before the addition of tetrandrine was plotted in Fig. 5. Of note, in the presence of 3 μM tetrandrine, the amplitude of $I_{Ca,L}$ evoked by the first voltage step after a 2 min pause was already suppressed by $35 \pm 2\%$ ($n = 5$) (i.e., tonic inhibition). Following the repetitive pulse stimuli, the amplitude of $I_{Ca,L}$ was further

reduced to the constant level in an exponential fashion (i.e., use-dependent inhibition). The percentage inhibition of $I_{Ca,L}$ following the repetitive stimuli was increased to $45 \pm 2\%$ ($n = 5$). When the concentration of tetrandrine was increased to 30 μM, the amplitudes of $I_{Ca,L}$ at the first depolarizing pulse and during subsequent repetitive stimuli were significantly suppressed by $82 \pm 2\%$ and $88 \pm 1\%$ ($n = 6$), respectively. These results indicate that tetrandrine-mediated inhibition of $I_{Ca,L}$ consists of both tonic and use-dependent components.

4. Discussion

In the present study, we show that tetrandrine (1–100 μM) directly inhibits the L-type voltage-dependent Ca^{2+} current ($I_{Ca,L}$) in a concentration-dependent and voltage-dependent manner in A7r5 vascular smooth muscle cells. The inhibitory effect of tetrandrine on $I_{Ca,L}$ was not related to the function of sarcoplasmic reticulum Ca^{2+}-ATPase. These results indicate that the inhibition of the voltage-dependent L-type Ca^{2+} channel should be one of the ionic mechanisms underlying tetrandrine-induced relaxation in vascular smooth muscle cells.

In smooth muscle, it is known that high K^+ evokes contraction by depolarizing membrane potential and thereby opening voltage-dependent Ca^{2+} channels. The inhibition of high K^+-induced contraction by tetrandrine suggests that tetrandrine may act as a Ca^{2+} channel antagonist (Su, 1993; Liu et al., 1995). Our results provide direct evidence showing that tetrandrine inhibits the amplitude of $I_{Ca,L}$ in vascular smooth muscle cells. The IC_{50} value of tetrandrine required for the inhibition of $I_{Ca,L}$ was 5 μM in the present study. In rat tail artery, it has been recently reported that tetrandrine inhibited KCl-induced contraction with an IC_{50} value of 6 μM (Liu et al., 1995). This value was quite similar to the present data obtained from A7r5 vascular smooth muscle cells. Also, in bovine chromaffin cells, Weinsberg et al. (1994) showed that tetrandrine inhibited Ca^{2+} current (IC_{50} = about 10 μM).

Dihydropyridines (e.g., nifedipine), which are known to have preferential affinities for the inactivated state of Ca^{2+} channels, can produce a distinct negative shift of the steady-state inactivation curve. As shown in Fig. 4, tetrandrine not only reduced the maximal conductance of $I_{Ca,L}$, but it produced a negative shift in the steady-state inactivation curve as well. The present finding indicates that the effect of tetrandrine on $I_{Ca,L}$ is voltage dependent, and the extent of current inhibition caused by tetrandrine can be altered by membrane potential. These observations are consistent with previous findings that tetrandrine alone did not affect resting tension and cytosolic Ca^{2+} concentrations (Anselmi et al., 1994; Liu et al., 1995), but it preferentially inhibited the contraction during the K^+-induced depolarization. Thus, the sensitivity to tetrandrine in smooth muscles would be dependent on the preexisting level of resting membrane potential, the firing rate of action potential, or the concentration of tetrandrine used (Spedding et al., 1995), if tetrandrine action in vascular smooth muscle in vivo is the same as those on A7r5 subcultured cell lines shown in this study.

Tetrandrine was also found to exhibit use-dependent inhibition of $I_{Ca,L}$ (Fig. 5). Tetrandrine-induced inhibition of $I_{Ca,L}$ was observed at the beginning of the depolarizing pulse after a long pause. However, further inhibition was observed during subsequent repetitive pulses. The use-dependent characteristics of tetrandrine on $I_{Ca,L}$ in vascular smooth muscle cells suggest that when action potentials in vascular smooth muscle in vivo were fired more frequently, the action of tetrandrine on $I_{Ca,L}$ should be enhanced.

The action of tetrandrine has also been reported to be related to an increase in intracellular cyclic AMP level (He et al., 1989). However, a previous study by us has shown that agents which increase intracellular cyclic AMP level, such as calcitonin gene-related peptide or isoproterenol, barely affected the amplitude of $I_{Ca,L}$ in A7r5 vascular smooth muscle cells (Lo et al., 1995). Therefore, it is unlikely that the inhibitory effects of tetrandrine on Ca^{2+} current involve increase in cyclic AMP level.

It was reported that tetrandrine may activate α_2-adrenoceptors in blood vessels (Kwan and Wang, 1993). However, because norepinephrine or the activation of α_2-adrenoceptors was believed to cause an increase in $I_{Ca,L}$ in vascular smooth muscle cells (Nelson et al., 1988; Loirand et al., 1990; Hughes et al., 1996), it is unlikely that tetrandrine-mediated inhibition of $I_{Ca,L}$ is due to the binding to α_2-adrenoceptors. Also, tetrandrine-mediated α_2-adrenergic action in vascular smooth muscle could be minor, because this effect would be counteracted by its direct inhibition of $I_{Ca,L}$.

Several papers have shown that tetrandrine inhibits cyclopiazonic acid- or thapsigargin-induced Ca^{2+} release (Kwan et al., 1992; Liu et al., 1995). In the present study, we may not exclude these mechanisms by which tetrandrine produces vasodilatory activity. The present study showed that cyclopiazonic acid produced a slight inhibition of $I_{Ca,L}$ (Fig. 3). This effect could be due to the direct inhibition of $I_{Ca,L}$, or a Ca^{2+} inactivation process following cyclopiazonic acid-mediated inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase (Fabiato, 1985). It was also observed that tetrandrine action on the inhibition of $I_{Ca,L}$ remained effective in the presence of cyclopiazonic acid (Fig. 3). This result makes it unlikely that the action of tetrandrine on the sarcoplasmic reticulum Ca^{2+}-ATPase is directly responsible for the inhibition of $I_{Ca,L}$. Therefore, if similar results were found in vascular smooth muscle cells in vivo to those occurring in these A7r5 subcultured cell lines, tetrandrine should cause vasodilation by directly inhibiting voltage-dependent L-type Ca^{2+} currents. Tetrandrine, an antihypertensive agent of herbal origin, has been found to have an inhibitory effect on $I_{Ca,L}$ as well as the

above-mentioned actions, which makes it a very promising drug to relax vascular smooth muscle.

Acknowledgements

This work was partly supported by grants from Department of Health (DOH-85-CM-041), National Science Council (NSC-86-2314-B-075B-011), and Veterans General Hospital-Kaohsiung (VGHKS-86-66), Taiwan, ROC. The authors would like to thank Miss Hui-Fang Li for her technical assistance.

References

Anselmi, E., Gomez-Lobo, M.D., Blzaquez, M.A., Zafra-Polo, M.C., D'ocon, M.P., 1994. Influence of the absolute configuration on the vascular effects of tetrandrine and isotetrandrine in rat aorta. Pharmazie 49, 440–443.

Bolton, T.B., 1979. Mechanisms of action of transmitter and other substances on smooth muscle. Physiol. Rev. 59, 606–718.

Fabiato, A., 1985. Time and calcium dependence of activation and inactivation of calcium-induced release of calcium from the sarcoplasmic reticulum of a skinned canine cardiac Purkinje cell. J. Gen. Physiol. 85, 247–289.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J., 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. Pflüg. Arch. 391, 85–100.

He, F.C., Tang, R.Y., Yao, D.F., 1989. Effects of tetrandrine on vascular permeability and neutrophil function in acute inflammation. Acta Pharmacol. Sin. 10, 249–251.

Horn, R., Marty, A., 1988. Muscarinic activation of ionic currents measured by a new whole-cell recording method. J. Gen. Physiol. 92, 145–159.

Hu, W.S., Pan, X.B., Wang, T., Hu, C.G., Lu, F.H., 1983. Mode of action of tetrandrine on vascular smooth muscle. J. Tradit. Clin. Med. 3, 7–12.

Hughes, A.D., Parkinson, N.A., Wijetunge, S., 1996. α_2-Adrenoceptor activation increases calcium channel currents in single vascular smooth muscle cells isolated from human omental resistance arteries. J. Vasc. Res. 33, 25–31.

King, V.F., Garcoa, M.L., Himmel, D., Reuben, J.P., Lam, Y.K.T., Pan, J.X., Han, G.Q., Kaczorowski, G.J., 1988. Interaction of tetrandrine with slowly inactivating calcium channels. Characterization of calcium channel modulation by an alkaloid of Chinese medicinal herbal origin. J. Biol. Chem. 263, 82238–82244.

Kwan, C.Y., Wang, Z.Y., 1993. Tetrandrine: a vasodilator of medicinal herb origin with a novel contractile effect on dog saphenous vein. Eur. J. Pharmacol. 238, 431–434.

Kwan, C.Y., Deng, H.W., Guan, Y.Y., 1992. Tetrandrine is not a selective calcium channel blocker in vascular smooth muscle. Acta Pharmacol. Sin. 13, 385–390.

Liu, Q.Y., Karpinski, E., Pang, P.K.T., 1992. Tetrandrine inhibits both T and L calcium channel currents in ventricular cells. J. Cardiovasc. Pharmacol. 20, 513–519.

Liu, Q.Y., Li, B., Gang, J.M., Karpinski, E., Pang, P.K.T., 1995. Tetrandrine, a Ca^{2+} antagonist: effects and mechanisms of action in vascular smooth muscle cells. J. Pharmacol. Exp. Ther. 273, 32–39.

Lo, Y.C., Wu, S.N., Wu, J.R., Chen, I.J., 1995. Effect of capsaicin on membrane currents in cultured vascular smooth muscle cells of rat aorta. Eur. J. Pharmacol. 292, 321–328.

Loirand, G., Pacaud, P., Mironneau, C., Mironneau, J., 1990. GTP-binding proteins mediate noradrenaline effects on calcium and chloride currents in rat portal vein. J. Physiol. 428, 517–529.

Nelson, M.T., Standen, N.B., Brayden, J.E., Worley, J.F., 1988. Noradrenaline contracts arteries by activating voltage-dependent calcium channels. Nature 336, 382–385.

Oriowo, M.A., Rufolo, R.R. Jr., 1992. Activation of a single α_1-adrenoceptor subtype in rat aorta mobilizes intracellular and extracellular pools of calcium. Pharmacology 44, 139–149.

Somlyo, A.P., Himpens, B., 1989. Cell calcium and its regulation in smooth muscle. FASEB J. 3, 2266–2276.

Spedding, M., Kenny, B., Chatelain, P., 1995. New drug binding sites in Ca^{2+} channels. Trends Pharmacol. Sci. 16, 139–142.

Su, J.Y., 1993. Mechanisms of action of 7-*o*-ethyl tetrandrine in isolated vascular smooth muscle of rat aorta. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 445–451.

Van Renterghem, C., Romey, G., Lazdunski, M., 1988. Vasopressin modulates the spontaneous electrical activity in aortic cells (line A7r5) by acting on three types of ionic channels. Proc. Natl. Acad. Sci. USA 85, 9365–9369.

Wang, G., Lemos, J.R., 1995. Tetrandrine: a new ligand to block voltage-dependent Ca^{2+} and Ca^{2+}-activated K^+ channels. Life Sci. 56, 295–306.

Weinsberg, F., Bickmeyer, U., Wiegand, H., 1994. Effects of tetrandrine on calcium channel currents of bovine chromaffin cells. Neuropharmacology 33, 885–890.

Wu, S.N., Lue, S.I., Yang, S.L., Hsu, H.K., Liu, M.S., 1993. Electrophysiologic properties of isolated adult cardiomyocytes from septic rats. Circ. Shock 41, 239–247.

Wu, S.N., Yu, H.S., Seyama, Y., 1995. Induction of Ca^{2+} oscillations by vasopressin in the presence of tetraethylammonium chloride in cultured vascular smooth muscle cells. J. Biochem. 117, 142–148.