Novel Blockade of Ca^{2+} Current by Quinacrine in Smooth Muscle Cells of the Guinea Pig

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ABSTRACT—Effects of quinacrine on voltage-dependent Ca^{2+} channel current (I_{Ca}) were examined using whole cell voltage clamp in single smooth muscle cells isolated from vas deferens and urinary bladder and single cardiac myocytes from ventricle of the guinea pig. When I_{Ca} was elicited by depolarization from a holding potential of -60 to 0 mV for 150 msec every 15 sec in vas deferens myocytes, external application of quinacrine reduced the amplitude of I_{Ca} in a concentration-dependent manner in a range of $0.1 \sim 30$ μM , and the IC_{50} of quinacrine was 1.3 μM . The block was at least partly removed by washout. The block of I_{Ca} by 1 μM quinacrine in vas deferens myocytes greatly depended upon the activation potentials but only slightly on the holding potentials. Use-dependent development of the block was also observed. Addition of 300 μM quinacrine to the pipette-filling solution did not significantly affect I_{Ca} . The IC_{50} of quinacrine for I_{Ca} block in urinary bladder myocytes was 1.1 μM and comparable to that in vas deferens. On the other hand, IC_{50} for the block of I_{Ca} elicited by depolarization from -45 to 0 mV in cardiac ventricular myocytes was 5.6 μM . It is concluded that quinacrine is a potent blocker of L-type Ca^{2+} channels in two types of smooth muscle myocytes and that the potency appeared to be approximately five times higher than that in cardiac myocytes. The action of quinacrine may be due to the direct block of Ca^{2+} channels from outside of the cell membrane.

Keywords: Ca^{2+} channel, Quinacrine, Smooth muscle, Cardiac ventricle

Quinacrine is an antihelminthic and antiprotozoan agent (1). In basic medical research, quinacrine has often been used as an inhibitor of phospholipase A_2 (PLA_2) (2), while it has also been reported to have other effects such as the ability to block the action of calmodulin (3). Quinacrine blocks the coupling of somatostatin stimulation to the increase in M-current via formation of arachidonic acid metabolites (4). It has, however, been reported that high threshold voltage-dependent Ca^{2+} channel current (I_{Ca}) in neurons is directly blocked by quinacrine (5). Direct Ca^{2+} -blocking action of quinacrine has also been reported in several types of preparations (6–8). Moreover, direct inhibition of ionic channels by quinacrine is rather nonspecific. Voltage-dependent K^+ current in melanotrophs (9), Na^+ current in squid axon (10) and end-plate channel in skeletal muscle (11, 12), Ca^{2+} -activated K^+ channel in hepatocyte (13), inward rectifying K^+ current induced by noradrenaline in submucosal neuron (14) and 5-HT-induced inward current in nodose

ganglion neuron (15) are susceptible to quinacrine. Na^+ - Ca^{2+} exchange in cardiac myocytes is also inhibited by quinacrine (16).

In vas deferens smooth muscle cells, application of noradrenaline reduces L-type I_{Ca} (17) and releases arachidonic acid (AA) (18). Since AA per se inhibits L-type I_{Ca} (19), we tried to examine the possible involvement of endogenous AA, which may be released by activation of PLA_2 via α -adrenoceptor stimulation, in noradrenaline-induced reduction of I_{Ca} in vas deferens myocytes, using quinacrine as a PLA_2 inhibitor. It was, however, found that externally applied quinacrine very effectively blocks I_{Ca} ; the potency appeared to be much higher than that reported in hippocampal and sensory neurons (5). The selectivity of quinacrine to high threshold (L-type) Ca^{2+} channels versus T- and N-type Ca^{2+} channels has been reported in hippocampal neurons (5) and cardiac myocytes (6). The present study was undertaken to elucidate the possibility that the L-type I_{Ca} in smooth muscle cells is more preferentially blocked by quinacrine than that in cardiac myocytes and neurons.

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MATERIALS AND METHODS

Cell isolation

Single smooth muscle cells were enzymatically isolated from vas deferens of the guinea pig (Japan SLC, Hamamatsu). The procedure of cell isolation was the same as described previously (17). A few drops of cell suspension were placed in a recording chamber (0.5 ml) mounted on the stage of a phase contrast microscope (TMD; Nikon, Tokyo). Cells were continuously perfused with a HEPES-buffered solution (see *Solutions*) at 5 ml per min. Only relaxed cells that had lengths over 100 μ m were used for electrical recording.

Solutions

A HEPES-buffered solution (standard HEPES solution) having the following composition was used for electrical recordings as the external solution: 137 mM NaCl, 5.9 mM KCl, 2.2 mM CaCl_2 , 1.2 mM MgCl_2 , 14 mM glucose and 10 mM HEPES; The pH was adjusted to 7.4 with NaOH. When the Ba^{2+} current (I_{Ba}) was recorded, Ca^{2+} in the standard HEPES solution was replaced by equimolar Ba^{2+} .

The pipette-filling solution (pipette solution) contained: 140 mM CsCl, 4 mM MgCl_2 , 4 mM ATP- Na_2 , 5 mM EGTA, 10 mM HEPES; The pH was adjusted to 7.2 with KOH. Addition of 300 μ M quinacrine did not affect the pH of the solution. When the effects of a drug were tested, the drug was applied externally by an exchange of perfusion solution or internally by adding it to the pipette solution. When internally applied, effects of drugs reached a steady level in 5–10 min after the rupture of the patch membrane.

Electrical recording and data analysis

Whole cell voltage clamp was applied using the method originally introduced by Hamill et al. (20). CEZ-2300 amplifier (Nihon Kohden, Tokyo) was used. The resistance of the pipette ranged from 2 to 5 megaohm when filled with the pipette solution. The seal resistance formed between the cell membrane and the tip was approximately 30 gigaohm. The series resistance was between 4 and 8 megaohm and was partly compensated. All electrophysiological measurements were made at room temperature ($24 \pm 1^\circ\text{C}$). Data were stored and analyzed using menu-driven software as previously reported (21). The leak current at positive potentials was obtained by extrapolating the linear relationship between voltage and current in the range of $-80 \sim -50$ mV and was subtracted from the total current on the computer.

Drugs

Collagenase, quinacrine hydrochloride and ethylene-

glycol-bis-(β -aminoethyl ether)- N,N,N',N' -tetraacetic acid (EGTA) were obtained from Yakult (Tokyo), Aldrich (Milwaukee, WI, USA) and Dojin Kagaku (Kumamoto), respectively.

Statistics

Pooled data are presented as the mean $\pm$ S.E.M. Statistical significance between two groups was determined by Student's *t*-test. Statistical significance between multi-groups was determined after adjustment for multi-comparison with the Bonferroni correction.

RESULTS

Blockade of I_{Ca} by quinacrine in vas deferens myocytes

Figure 1 shows the effects of quinacrine on voltage-dependent I_{Ca} . I_{Ca} was elicited by depolarization from a holding potential of -60 to 0 mV for 150 msec once every 15 sec. The averaged peak amplitude of I_{Ca} under these conditions was 302 ± 20 pA ($n=19$). External application of 3 μ M quinacrine reduced the peak amplitude of I_{Ca} by 61% within 2 min as shown in Fig. 1A. The decrease in I_{Ca} amplitude by quinacrine was larger at the end of the 150-msec pulse than at the peak, especially when relatively low concentrations of quinacrine were applied (Fig. 1B). I_{Ca} , which was reduced by quinacrine, recovered partly after washout. The incomplete-recovery of I_{Ca} after washout may be, at least in part, due to run-down of I_{Ca} in vas deferens myocytes; the peak amplitude decreased by $38.1 \pm 5.5\%$ ($n=19$) for 10 min as run-down under the control conditions. The run-down of I_{Ba} through voltage-dependent Ca^{2+} channels under the same conditions was $28.5 \pm 9.7\%$ per 10 min ($n=7$). To resolve the effects of quinacrine on I_{Ca} from the run-down, the time-course of changes in peak amplitude of I_{Ca} or I_{Ba} just prior to the addition of quinacrine was fitted by a straight line (20–45% decrease per 10 min) and extrapolated to the period after the addition of the agent, as done in the previous study (19). If the decrease by run-down during 3 min just prior to the addition of quinacrine was larger than 15%, the data were discarded. When 300 μ M quinacrine was added to the pipette solution, the averaged peak I_{Ca} was 330 ± 57 pA ($n=8$, $P>0.05$ vs control; 302 pA) and the run-down of I_{Ca} for 10 min was $39.3 \pm 11\%$ ($n=7$) of that about 2 min after the start of recording and not significantly different from that in the absence of quinacrine in the pipette ($P>0.05$ vs $38.1 \pm 5.5\%$, $n=19$). The K^+ currents upon depolarization were completely blocked by diffusion of Cs^+ in the pipette solution within 2 min.

Use-dependence of quinacrine-induced block of I_{Ca} was examined in the experiments shown in Fig. 1C. Quinacrine (3 μ M) was externally applied just after the stimula-

Fig. 1. The block of Ca^{2+} channel current (I_{Ca}) by quinacrine and its use-dependence in single smooth muscle cell of guinea pig vas deferens. **A:** The peak amplitude of I_{Ca} elicited by depolarization from a holding potential of -60 to 0 mV for 150 msec at 0.067 Hz was plotted against time after the start of whole-cell current recording. Application of 3 and 10 μM quinacrine reduced the I_{Ca} amplitude in a concentration-dependent manner. The current reduced by quinacrine recovered partly after washout. Lower traces in "A", which are numbered from "1" to "5", were obtained at the respective time indicated on the time course. **B:** Two pairs of traces shown in "A" were peak-adjusted on the computer to denote the effect of quinacrine on the decay of I_{Ca} during depolarization. Note that the decay in "2" in the presence of 3 μM quinacrine was faster than that in "1" in the control. **C:** Use-dependent block of I_{Ca} by 3 μM quinacrine. The change in peak I_{Ca} amplitude was plotted against time. After I_{Ca} was elicited by depolarization from -60 to 0 mV at 0.067 Hz for 1.5 min, the stimulation was stopped and 3 μM quinacrine was applied. After 5 min, the stimulation was started again and I_{Ca} was recorded. Lower traces numbered from "1" to "4" were obtained at the respective time indicated on the time course. Note that the block of I_{Ca} developed progressively after restarting of stimulation in the presence of 3 μM quinacrine.

tion by depolarization pulses at 0.067 Hz was stopped. After the cessation for 5 min, the stimulation was started again. The peak amplitude of the first I_{Ca} after the ces-

sation was about $59.1 \pm 9.8\%$ ($n=4$) of that before the application. Then I_{Ca} was progressively reduced in the presence of quinacrine. The block of I_{Ca} by 3 μM quina-

Fig. 2. The concentration-dependent block of I_{Ca} and I_{Ba} by quinacrine. **A:** Original traces that denote the block of I_{Ca} and I_{Ba} by 1, 3 and 10 μM quinacrine. I_{Ca} and I_{Ba} were elicited by depolarization from -60 to 0 mV at 0.067 Hz. Quinacrine was added cumulatively with an interval of about 2 min, and the reduced I_{Ca} and I_{Ba} recovered to about 70% of the control by washout. **B:** The relationships between the concentration of quinacrine and the amplitude of I_{Ca} ($\circ$) and I_{Ba} ($\bullet$). The data obtained from experiments such as that shown in "A" were summarized. The current amplitude was corrected for the run-down (see text in details). The peak amplitude of I_{Ca} or I_{Ba} in the presence of quinacrine was normalized by the control amplitude before the application in each cell. A vertical bar on each symbol indicates S.E.M. The number of cells used for each point was 4–7. Dashed line was obtained by fitting the concentration-amplitude relationship to following equation: $I/I_{max} = 1 - (1/(1 + (K_d/[Drug])^n))$, where K_d is the apparent dissociation constant and n is the Hill-coefficient. The Hill-coefficient was 1.03 and 1.23 for I_{Ca} and I_{Ba} , respectively. Assuming a Hill-coefficient of unity, IC_{50} s of quinacrine for I_{Ca} and I_{Ba} block were determined from the K_d values as 1.26 and 1.29 μM , respectively.

crine reached the steady level within 3 min if the stimulation at 0.067 Hz continued after the application of quinacrine (Fig. 1A), indicating that the quinacrine-induced block is use-dependent.

Figure 2 illustrates the concentration-dependent block by quinacrine of I_{Ca} and I_{Ba} , which were activated by depolarization from -60 to 0 mV. The degree of the block was measured when the block at each concentration of quinacrine reached the steady level. When the external 2.2 mM Ca^{2+} was replaced by equimolar Ba^{2+} , the peak amplitude of I_{Ba} at 0 mV was increased by $\sim 20\%$ in Fig. 2. The averaged peak I_{Ba} was 544 ± 78 pA ($n=7$). The

peak amplitude of I_{Ba} in the presence of 1 μM quinacrine was $54.4 \pm 3.6\%$ ($n=6$) of that before the application. The decrease in I_{Ba} by 1 μM quinacrine was not significantly different from that of I_{Ca} ($P > 0.05$). The summarized data show concentration-response relationships for quinacrine-induced block of I_{Ca} and I_{Ba} in Fig. 2B. The relationships were well fitted by an equation assuming one to one binding of a quinacrine molecule to a channel (Hill coefficient: 1.03 and 1.23, respectively). The concentration of quinacrine required for the half-block of I_{Ca} and I_{Ba} (apparent dissociation constant) were 1.26 and 1.29 μM , respectively.

Fig. 3. Effects of quinacrine on the current-voltage relationship of I_{Ca} and the voltage-dependence of the block. **A:** Current traces elicited by depolarization from a holding potential of -60 mV to potentials in a range of $-40 \sim +50$ mV by 10 -mV steps in the absence (left) and presence of $1 \mu\text{M}$ quinacrine (right). **B:** Current-voltage relationships of I_{Ca} in the absence ($\circ$) and presence of $1 \mu\text{M}$ quinacrine ($\bullet$). Summarized data from 5 cells. A vertical bar on each symbol denotes S.E.M. The leakage current was subtracted on the computer. **C:** The dependence of I_{Ca} block by quinacrine on activation potentials. The decrease in peak amplitude of I_{Ca} after application of $1 \mu\text{M}$ quinacrine at each activation potential was normalized by the control I_{Ca} amplitude as the relative inhibition. Statistical significance against the relative inhibition at -30 and -20 mV ($n=5$, $P<0.05$, with the Bonferroni correction) are indicated by * and †, respectively.

The dependence of quinacrine-induced block of I_{Ca} on the activation potential was examined by changing the potentials in a range from $-40 \sim +50$ mV by 10 -mV steps in this order, after the steady block at 0 mV was observed. To avoid the run-down of I_{Ca} , one pulse was applied at each potential. External application of $1 \mu\text{M}$

quinacrine reduced I_{Ca} at any of the examined potentials as shown in the current-voltage relationship (Fig. 3B). The current was corrected for the leakage by assuming that the ohmic property of the current in the negative potential range of $-60 \sim -90$ mV can be extrapolated to the potential range of $-60 \sim +50$ mV. Summarized

Fig. 4. The dependence of I_{Ca} block by quinacrine on holding potentials. **A:** Current traces elicited by depolarization from a holding potential of -60 and -30 mV to +10 mV for 150 msec at 0.067 Hz are superimposed. Currents were recorded in the absence (left) and the presence (right) of 1 μM quinacrine. The currents elicited by depolarization from -30 mV are indicated by arrows. By changing the holding potential from -60 to -30 mV, I_{Ca} was decreased to $78.0 \pm 6.9\%$ in the control ($n=6$). **B:** Data obtained in the experiments shown in "A" from 5 separate cells are summarized. The relative block of I_{Ca} by 1 μM quinacrine at a holding potential of -30 mV was slightly but significantly larger than that at -60 mV (* $P < 0.05$).

results are illustrated in Fig. 3C, where the peak I_{Ca} amplitude at various potentials in the presence of 1 μM quinacrine was normalized by that recorded at corresponding potentials before the application and was plotted against potentials. It is clear that the block of I_{Ca} by 1 μM quinacrine was significantly dependent upon the activation potentials. The inhibition at -30 mV was significantly smaller than those at 0, +10, +20, +30 and +40 mV ($P < 0.05$), and the inhibition at -20 mV was significantly smaller than that at +40 mV ($P < 0.05$).

Figure 4 denotes the effect of changing the holding potential on quinacrine-induced block of I_{Ca} in vas deferens cells. When the holding potential was changed from -60 to -30 mV in the absence of quinacrine, the peak I_{Ca} at +10 mV was decreased to $78.0 \pm 6.9\%$, indicating that a substantial portion of the L-type Ca^{2+} channels are supposed to be inactivated at -30 mV. In the presence of

1 μM quinacrine, the peak I_{Ca} at +10 mV was $39.6 \pm 2.0\%$ ($n=6$) and $32.5 \pm 0.9\%$ ($n=5$, $P < 0.05$) of the corresponding control when the holding potential was -60 and -30 mV, respectively. Therefore, the quinacrine-induced block of I_{Ca} was slightly but significantly affected by the change in holding potentials.

Blockade of I_{Ca} by quinacrine in urinary bladder smooth muscle cells and cardiac ventricular myocytes

Figure 5A shows the effects of 3, 10 and 30 μM quinacrine on I_{Ca} , which was activated by depolarization from -45 to 0 mV for 150 msec once every 15 sec in the guinea pig cardiac ventricular myocytes. The holding potential was chosen to inactivate Na^+ channels (22). The run-down of peak I_{Ca} at 0 mV was $18.9 \pm 5.3\%$ for 10 min ($n=11$) under the control condition. I_{Ca} was concentration-dependently reduced by quinacrine. The recovery

Fig. 5. The block of I_{Ca} by quinacrine in guinea pig ventricular myocytes. **A:** The peak amplitude of I_{Ca} elicited by depolarization from a holding potential of -45 to 0 mV for 150 msec at 0.067 Hz was plotted against time. I_{Ca} was reduced by cumulative application of 3 , 10 and 30 μM quinacrine in a concentration-dependent manner. The reduced I_{Ca} recovered almost completely by washout. Lower traces numbered from "1" to "5" were obtained at the respective time indicated on the time course. The horizontal dotted line in the traces indicates the zero I_{Ca} level determined by the addition of 0.1 mM Cd^{2+} . **B:** Concentration- I_{Ca} amplitude relationships for quinacrine-induced block in the myocytes of vas deferens ($\circ$), urinary bladder ($\bullet$) and cardiac ventricle (∇) are illustrated. The number of cells used was $3-8$ for each point. The data for the vas deferens myocytes are the same as those shown in Fig. 2. Dashed lines were obtained by fitting the relationships to the equation shown in Fig. 2. The Hill-coefficient was 0.94 for both the ventricle and urinary bladder. The IC_{50} s of quinacrine for I_{Ca} inhibition in the myocytes from the cardiac ventricle and urinary bladder were determined from the K_d values as 5.56 and 1.12 μM , respectively.

of I_{Ca} after washout of quinacrine was more clearly observed in ventricular myocytes than in smooth muscle myocytes, at least partly because of the smaller run-down rate. The 50% removal of the block by 1 or 30 μM

quinacrine was obtained by washout for 0.61 ± 0.19 min ($n=3$) and 1.8 ± 0.2 min ($n=7$), respectively, in ventricular myocytes, whereas those in vas deferens myocytes were 2.8 ± 0.3 min ($n=7$, $P < 0.01$ vs 0.61 min) and

3.1 ± 0.5 min ($n=3$, $P < 0.05$ vs 1.8 min), respectively. The peak amplitude of I_{Ca} upon depolarization from -45 to 0 mV was $32.3 \pm 3.6\%$ ($n=8$) of the control in the presence of $10 \mu\text{M}$ quinacrine in ventricular myocytes. Under the same conditions, the I_{Ca} was $11.4 \pm 2.7\%$ ($n=6$, $P < 0.05$ vs ventricle) and $11.6 \pm 2.9\%$ ($n=5$, $P < 0.05$ vs ventricle; $P > 0.05$ vs vas deferens) in smooth muscle cells from vas deferens and urinary bladder, respectively. The averaged peak amplitude of I_{Ca} at 0 mV and the run-down rate in urinary bladder myocytes under the control conditions were 282 ± 58 pA and $36.4 \pm 7.1\%$ for 10 min ($n=6$), respectively. The concentration-response relationships of quinacrine-induced I_{Ca} block in cardiac ventricular myocytes and smooth muscle cells from vas deferens and urinary bladder are illustrated in Fig. 5B. The IC_{50} s of quinacrine for I_{Ca} block in ventricular myocytes and urinary bladder myocytes were 5.56 and $1.12 \mu\text{M}$, respectively.

DISCUSSION

The present study clearly indicates that quinacrine is a potent blocker of Ca^{2+} current in smooth muscle cells isolated from guinea pig vas deferens and urinary bladder. The IC_{50} of quinacrine for I_{Ca} block in these smooth muscle myocytes was about $1.2 \mu\text{M}$ and apparently lower than that reported for the inhibition of high-threshold I_{Ca} in rat hippocampal neurons ($30 \mu\text{M}$) (5) and slightly lower than that in guinea pig ventricular myocytes ($6 \mu\text{M}$) (present study). The inhibition of A-type K^+ current in melanotrophs (9) and other membrane currents or channels in various types of cells require higher concentrations of quinacrine (IC_{50} of 20 – $80 \mu\text{M}$ at pH 7.4 or 7.5) (10, 11, 13, 14). The difference in sensitivity to quinacrine of I_{Ca} s in smooth muscle myocytes, cardiac ventricular myocytes and hippocampal neuron may not be due to a difference in experimental conditions, since the conditions used in this study are almost identical to those used for hippocampal neurons (5), except the pH of the pipette solution and the holding potentials. The pH in the pipette solution does not affect the block of I_{Ca} , since quinacrine added to the pipette solution was not effective on I_{Ca} in these two types of cells. The difference in holding potential of -60 and -70 mV in this and previous studies, respectively, may neither affect significantly the IC_{50} because the block in vas deferens myocytes was only slightly affected by the change in the holding potential from -60 to -30 mV. The fact that quinacrine-induced block was only slightly affected by the change in holding potential further indicates the followings: 1) The decrease in I_{Ca} by quinacrine is due to the block of L-type Ca^{2+} channels, which are predominant in these two types of smooth muscles (17, 23 and as a review: 24). 2) Quinacrine may

not have high affinity to inactivated Ca^{2+} channels in either smooth muscle cells or neurons (5).

There are additional differences between quinacrine-induced block of I_{Ca} in smooth muscles and that in neurons (5). 1) Quinacrine at low concentrations (0.1 – $3 \mu\text{M}$) facilitates the decay time-course of I_{Ca} during depolarization in smooth muscle cells, whereas the kinetics of I_{Ca} in hippocampal neurons are not affected by quinacrine. 2) The use-dependent block of I_{Ca} by quinacrine that was clearly observed in smooth muscle cells has not been found in hippocampal neuron. 3) The block of I_{Ca} by quinacrine was voltage-dependent in the present study; there was a larger block at higher potentials. Such voltage-dependence of the block was not detected in hippocampal neurons. 4) It has been reported that the block by quinacrine is quickly removed by washout in hippocampal neurons, whereas that in smooth muscle was slow and appeared to be incomplete during washout for ~ 5 min even if a larger run-down is taken into consideration. These results clearly suggest that the difference between the block of I_{Ca} by quinacrine in smooth muscle cells and that in neurons is not only the difference in affinity to the channels but also that in the mechanisms of blockade itself or even that in alternatively spliced α_1 -subunits of L-type Ca^{2+} channels. The features listed above as 1), 2) and 3) suggest that the block of Ca^{2+} channels in smooth muscle cells may be an open-channel block (25, 26). The interaction between L-type Ca^{2+} channels in these smooth muscles and low concentrations of quinacrine may be distinctive from that between high threshold Ca^{2+} channels in hippocampal neurons and higher concentrations of quinacrine. It is interesting in this respect that the cardiac I_{Ca} had intermediate sensitivity to quinacrine and its block by quinacrine was easily removed by washout. At higher concentrations, quinacrine may also interact with channels in the resting state as has been reported in the quinacrine-induced block of the A-type K^+ current (9).

The effect of quinacrine is apparently due to direct block of L-type Ca^{2+} channels from the outside. Not only Ca^{2+} channels but also many other channels including ligand-operated channels are directly blocked by quinacrine (14, 15), suggesting very nonspecific effects of quinacrine on ion channels. Surprisingly, the involvement of PLA_2 inhibition in the inhibition of ligand operated ionic channels by quinacrine has been reported only in very limited preparations such as sympathetic neuron (4). Internally applied quinacrine can not reach the binding site in the Ca^{2+} channel, suggesting that only protonated quinacrine in external solution can bind to the site from the outside (5). This may also indicate that the effect of quinacrine on I_{Ca} is not due to the interaction with phospholipids and the change in the membrane structure

which has been demonstrated in erythrocytes and platelets (27). The efficacy of quinacrine for I_{Ca} block was comparable to that for I_{Ba} block, suggesting that the binding site of quinacrine may possibly be close to the outer mouth of L-type Ca^{2+} channels but not deep-inside of the channel including the energy well where the affinity of Ca^{2+} is higher than that of Ba^{2+} (28, 29).

The reason why L-type Ca^{2+} channels in the smooth muscle has higher susceptibility to quinacrine than that in hippocampal neurons is not clear. Although quinacrine-induced block of I_{Ca} in neurons was not re-examined in the present study, the IC_{50} obtained in cardiac ventricular myocytes appears to be comparable to that in the previous study (6). The gene that encodes the α_1 -subunit of L-type Ca^{2+} channels in smooth muscle of rat aorta is *CaCh2b*. The deduced amino acid sequence has about a 95% homology to that in heart, which is encoded by an alternative splice product, *CaCh2a* (see as a review: 30). However, the dihydropyridine Ca^{2+} -blocker nisoldipine inhibits I_{Ba} through the Ca^{2+} channel encoded by *CaCh2b* about tenfold more effectively than that encoded by *CaCh2a* (31), although, three L-type Ca^{2+} channel subtypes, *CaCh2a*, *CaCh2b* and *CaCh3* are expressed in the brain, and the predominant Ca^{2+} channel in hippocampal neurons is not completely clear. It is possible that quinacrine has higher affinity to Ca^{2+} channels encoded by *CaCh2b* than those encoded by *CaCh2a* or *CaCh3*, whereas the difference in other subunits (α , β , γ or δ) might also be involved. Although the pH dependence of the quinacrine-induced block of I_{Ca} in smooth muscle cells was not examined in the present study, it is also likely that single protonated form of quinacrine has the highest affinity to L-type Ca^{2+} channels as shown in hippocampal neurons (5).

In conclusion, the IC_{50} of about $1 \mu\text{M}$ for the block of L-type Ca^{2+} channels in smooth muscle is the lowest among the nonspecific direct effects of quinacrine on ionic channels including L-type Ca^{2+} channels in neurons and cardiac myocytes. This is a favorable feature for the use of quinacrine as a prototype to develop a new type Ca^{2+} blocker which has high potency and selectivity to smooth muscle cells.

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