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Inhibitory effect of aprindine on Na^+/Ca^{2+} exchange current in guinea-pig cardiac ventricular myocytes

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1 Using the whole-cell voltage clamp technique, the effect of aprindine on Na^+/Ca^{2+} exchange current (I_{NCX}) was examined in guinea-pig single cardiac ventricular myocytes and CCL39 fibroblasts expressing a dog cardiac Na^+/Ca^{2+} exchanger (NCX1).

2 I_{NCX} was recorded by ramp pulses from the holding potential of −60 mV with the external solution containing 140 mM Na^+ and 1 mM Ca^{2+}, and the pipette solution containing 20 mM Na^+, 20 mM BAPTA and 13 mM Ca^{2+} (433 nM free Ca^{2+}).

3 External application of aprindine suppressed I_{NCX} in a concentration-dependent manner. The IC_{50} values of outward (measured at 50 mV) and inward (measured at −100 mV) I_{NCX} components were 48.8 and 51.8 μM with Hill coefficients of 1.3 and 1, respectively.

4 Intracellular application of trypsin *via* the pipette solution did not change the blocking effect of aprindine, suggesting that aprindine does not affect the exchanger from the cytoplasmic side.

5 Aprindine inhibited I_{NCX} of a mutant NCX1 with a deletion of amino acids 247–671 in the large intracellular domain between the transmembrane segments 5 and 6 in a similar manner to that of the wild-type, suggesting that the site of aprindine inhibition is not in the large intracellular domain of NCX1.

6 A kinetic study indicated that aprindine was cooperatively competitive with KB-R7943, another inhibitor of NCX and that aprindine was a competitive inhibitor with respect to external Ca^{2+}.

7 We conclude that aprindine may modestly inhibit I_{NCX} in a therapeutic range of concentrations (around 2.5∼6.9 μM) possibly at an external or intra-membranous site of the exchanger.
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Abbreviations: DMSO, dimethylsulphoxide; I_{Ca}, L-type Ca^{2+} current; I_f, pacemaker current; I_k, delayed rectifier K^+ current; I_{KAch}, muscarinic acetylcholine receptor-operated K^+ current; I_{Na}, Na^+ current; I_{NCX}, Na^+/Ca^{2+} exchange current; I–V curve, current–voltage relation curve; KB-R7943, 2-[2-[4-(nitrobenzyloxy)phenyl]ethydil]isothiourea methanesulphonate; NCX1; cardiac Na^+/Ca^{2+} exchanger

Introduction

Aprindine hydrochloride is a class I-b anti-arrhythmic agent in Vaughan Williams classification, and is widely used to treat atrial and ventricular tachyarrhythmias (Kesteloot *et al*., 1973; Zipes *et al*., 1977; Fasola *et al*., 1977; Adams *et al*., 1984; Kodama *et al*., 1999). Aprindine suppresses the maximum upstroke velocity (Vmax) and duration (APD) of action potentials in Purkinje fibres and ventricular myocardium (Verdonck *et al*., 1974; Carmeliet & Verdonck, 1974; Steinberg & Greenspan, 1976). In electrophysiological experiments, aprindine acutely blocked the Na^+ current (I_{Na}) in guinea-pig ventricular myocytes (Sato *et al*., 1991). In addition to Na^+ channels, the drug inhibits various other ionic currents in the heart, such as the L-type Ca^{2+} current (I_{Ca}) (Tanaka *et al*., 1990; Shibasaki *et al*., 1991), a pacemaker current (I_f) (Tanaka *et al*., 1990, delayed rectifier K^+ current (I_K) (Shibasaki *et al*., 1991; Ohmoto-Sekine *et al*., 1999) and muscarinic acetylcholine receptor-operated K^+ current (I_{KAch}) (Ohmoto-Sekine *et al*., 1999).

Na^+/Ca^{2+} exchange is a major mechanism of Ca^{2+} extrusion in the heart (Matsuda *et al*., 1997; Blaustein & Lederer, 1999; Kimura, 2001). Recently we found that anti-arrhythmic drugs, such as amiodarone (Watanabe & Kimura, 2000) and bepridil (Watanabe & Kimura, 2001) suppressed I_{NCX} in guinea-pig cardiac ventricular myocytes. We also found that 2,3-butanedione monoxime (BDM) inhibited I_{NCX} (Watanabe *et al*., 2001). The inhibitory effects of these drugs on I_{NCX} were diminished in patch-clamp studies by trypsin applied intracellularly *via* the pipette solution, indicating that the site of inhibitory action of those drugs may be cytoplasmic. In the present study, we examined the effect of another anti-arrhythmic drug, aprindine, on I_{NCX} in guinea-pig ventricular cells and CCL39 fibroblasts expressing NCX1.

Methods

Cell isolation

All experiments were performed in compliance with the regulations of the Animal Research Committee of the School

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of Medicine, Fukushima Medical University. Guinea-pigs weighing 250–400 g were anaesthetized by intraperitoneal injection of pentobarbital. The chest was opened under artificial ventilation, the aorta was cannulated *in situ*, and the heart was mounted on a Langendorff perfusion system. After washing out the blood with Tyrode solution, the perfusate was changed to nominally Ca^{2+}-free Tyrode solution and then to one containing 0.01% w v^{-1} collagenase (Wako, Osaka, Japan) and 0.002% w v^{-1} alkaline protease (Nagase, Tokyo, Japan). After digestion for about 15–20 min, the enzymes were washed out by perfusing with a high K^+, low Cl^- solution (modified KB solution; Isenberg & Klöckner, 1982). The ventricular tissue was cut into the modified KB solution and gently shaken to isolate single ventricular cells. The cell suspension was stored in a refrigerator (4°C) for later use.

Cell cultures

CCL39 cells (American Type Culture Collection) with and without NCX1 transfection, were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 7.5% heat-inactivated foetal calf serum, 50 units/ml^{-1} penicillin, and 50 μg/ml^{-1} streptomycin. Two types of CCL39 fibroblasts stably expressing NCX1 were used. One was transfected with wild-type NCX1 and the other with a mutant NCX1 in which amino acids 247–671 of TM5-6 were deleted (Pan *et al*., 2000). The cells were cultured for 2 or 3 days on small pieces (2×8 mm) of a cover glass and were used for the whole cell voltage clamp experiments.

Patch-clamp recording

Whole-cell membrane currents were recorded by the patch-clamp method. Single cardiac ventricular cells and CCL39 fibroblasts were placed in a recording chamber (1 ml volume) attached to an inverted microscope (Nikon, Tokyo, Japan) and were superfused with the Tyrode solution at a rate of 5 ml min^{-1}. The temperature of the external solution was maintained at 36.0 ± 0.5°C. The Tyrode solution contained (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.33, glucose 5.5 and HEPES–NaOH (pH 7.4). The modified KB solution contained (in mM): KOH 70, l-glutamic acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, EGTA 0.2 and HEPES–KOH buffer 10 (pH 7.2). Patch pipettes were forged from 1.5 mm diameter glass capillaries with a micro-electrode puller (pp-83, Narishige, Tokyo, Japan). The pipette resistance was 2~3 MΩ when filled with the pipette solution which contained (in mM): NaCl 20, BAPTA 20, $CaCl_2$ 13 (free Ca^{2+} concentration 433 nM), CsCl 120, $MgCl_2$ 3, aspartic acid 50, MgATP 5 and HEPES 10 (pH 7.2 with CsOH). The extracellular solution contained (in mM): NaCl 140, $CaCl_2$ 2, $MgCl_2$ 1, ouabain 0.02, nifedipine 0.01, ryanodine 0.01 and HEPES–CsOH 5 (pH 7.2). The electrode was connected to a patch-clamp amplifier (TM-1000, Act ME, Tokyo, Japan). Recording signals were filtered at 2.5 kHz bandwidth, and the series resistance was compensated. Current signals were stored on-line and analysed by a computer (PC-9801RX, NEC, Tokyo, Japan) with non-commercial software called RAM5.

The current–voltage (I–V) relationship was obtained by ramp pulses (Kimura *et al*., 1987). The holding potential was set at −60 mV. The membrane was initially depolarized from −60 mV to 60 mV, then hyperpolarized from 60 to −110 mV and then depolarized back to −60 mV at a constant rate of 640 mV s^{-1}. The descending limb (from 60 to −110 mV) was plotted in the I–V relationship without capacitance compensation. The Ca^{2+} current (I_{Ca}), K^+ currents, Na^+–K^+ pump current and Ca^{2+} release channels of the sarcoplasmic reticulum were blocked by nifedipine, Cs^+, ouabain and ryanodine, respectively.

Drugs

Aprindine was a kind gift from Mitsui Co Ltd. (Tokyo, Japan). Ouabain, ryanodine and nifedipine were purchased from Sigma Chemical Co (St Louis, U.S.A.). KB-R7943 (2-[2-[4-1(4-nitrobenzyloxy) phenyl]ethyl] isothiourea methanesulphonate was a gift from Kanebo Co Ltd. (Osaka, Japan). Nifedipine and KB-R7943 were dissolved in dimethylsulphoxide (DMSO) and added to the extracellular solution so that the final concentration of DMSO was ⩽0.1%, which did not affect the Na^+/Ca^{2+} exchange current. Trypsin (2.5 μg ml^{-1}) (Difco laboratories, Detroit, MI, U.S.A.) was dissolved directly in the pipette solution. All the chemicals used were the highest grade available.

Data analysis

All data are presented as means ± s.e. (number of experiments). Student's *t*-test and analysis of variance were used for statistical analyses. A P value of less than 0.05 was considered significant. The concentration–response data were fitted and IC_{50} values and Hill coefficients were obtained using Delta Graph Professional (Polaroid Computing, Tokyo, Japan) on a Macintosh computer (Apple Computer, Mariani Avenue, Cupertino, CA, U.S.A.). Per cent inhibition of the outward I_{NCX} at various concentrations of aprindine was calculated using the following logistic equation:

$$\text{Per cent inhibition} = 100 \times 1/\{1 + (IC_{50}/[D])^{n_H}\} \quad (1)$$

where [D] is the concentration of aprindine, IC_{50} is the half-maximum inhibitory concentration of the drug and n_H is an empirical parameter describing the steepness of the fit and is equivalent to the Hill coefficient.

Results

Effect of aprindine on I_{NCX}

I_{NCX} was induced by 1 mM Ca^{2+} and 140 mM Na^+ in the external solution and 20 mM Na^+ and 433 nM free Ca^{2+} in the pipette solution. Under these ionic conditions, the reversal potential of the exchange current with a 3Na : 1Ca stoichiometry was calculated to be −50 mV. After establishing the whole-cell clamp mode with a holding potential, the external solution was changed from Tyrode solution to the control external solution. As shown in Figure 1A, the control external solution was then switched to one containing 300 μM aprindine. Aprindine immediately suppressed I_{NCX}. A high concentration (100 μM) of KB-R7943, a potent and selective inhibitor of I_{NCX} under these experimental conditions, was applied to completely inhibit I_{NCX}. Figure 1B illustrates the

I–V relationships obtained in the control (a), in the presence of aprindine (b) and in the presence of KB-R7943. Figure 1C illustrates the I–V curves of the aprindine-sensitive component (a-b) and the KB-R7943-sensitive component (b-c) obtained by subtraction. Both I–V curves reversed at about −50 mV, indicating that the aprindine-sensitive current is I_{NCX}. Aprindine at 300 μM inhibited I_{NCX} by 95% in this cell. The inhibition was reversible. The recovery after washout of 100 μM aprindine was 86.5±7.6% ($n=4$) and maximum recovery was observed at 52.5±12.5 s ($n=4$).

The concentration–response relation of aprindine is shown in Figure 2, in which it can be seen that it inhibited I_{NCX} in a dose-dependent manner. The current magnitude was measured at 50 mV for the outward component of I_{NCX} and the per cent inhibition was calculated assuming that 100 μM KB-R7943 completely inhibited I_{NCX}. The fitted sigmoidal curve yielded an IC_{50} of 48.8 μM with a Hill coefficient of 1.3 ($n=38$). The IC_{50} of the inward component measured at −100 mV was 51.8 μM with a Hill coefficient of 1.1 ($n=28$) (figure not shown). These results indicate that aprindine inhibited inward and outward I_{NCX} with equal potency.

Effect of trypsin on aprindine inhibition of I_{NCX}

We examined whether aprindine inhibited I_{NCX} from the cytoplasmic side of the membrane by including trypsin (2.5 μg ml^{-1}) in the pipette solution. We previously showed that BDM (2,3-butanedione monoxime) lost its inhibitory effect on I_{NCX} in the presence of trypsin (Watanabe *et al*., 2001). Therefore, 10 mM BDM was applied before aprindine to verify the effectiveness of trypsin. As shown in Figure 3, BDM did not significantly inhibit I_{NCX}, but subsequent application of 300 μM aprindine dramatically inhibited I_{NCX}. KB-R7943 at 100 μM was applied after aprindine. Aprindine at 300 μM inhibited I_{NCX} by 89.7±6.9% ($n=5$) after trypsin treatment and by 93.5±6.3% ($n=7$) without trypsin (Figure 2C). Since the inhibition by aprindine was not changed by trypsin, it is probable that aprindine affects the Na^+/Ca^{2+} exchanger from the extracellular or in the membrane and not from the cytoplasmic side.

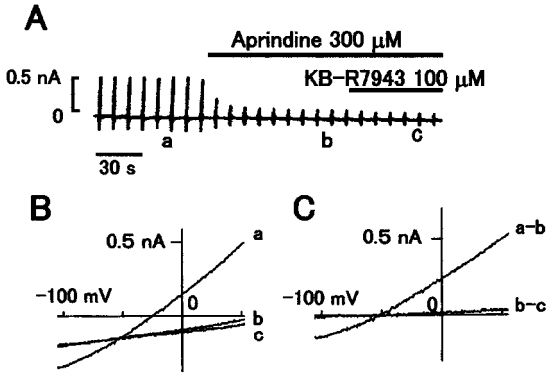

Figure 1 Effect of 300 μM aprindine on I_{NCX}. (A) Typical chart recording of membrane current. The bars above the current indicate when 300 μM aprindine and 100 μM KB-R7943 were applied externally. (B) I–V curves obtained at the time points corresponding to the labels in (A). (a) is the control and (b) in the presence of aprindine and (c) in the presence of KB-R7943. (C) Difference I–V curves between a and b (a-b) and between b and c (b-c) in (B).

Effects of aprindine on expressed I_{NCX}

To confirm that the site of inhibition of aprindine is not the cytoplasmic side, we tested the effects of aprindine on I_{NCX} with CCL39 cells expressing NCX1 or a mutant deleted of amino acids 247–671, which is a large portion of the long intracellular domain between the transmembrane segments (TM) 5 and 6, and contains the XIP region (He *et al*., 1997). I_{NCX} was induced by the same external and pipette solutions that were used in the experiment shown in Figure 1. As shown in Figure 4A and B, 100 μM aprindine inhibited both wild-type and mutant I_{NCX} by 70%. As summarized in Figure 4C, the inhibitory effect of 100 μM aprindine was not significantly different between the wild-type and the mutant NCX1 expressed in CCL39 cells and myocytes. The average I_{NCX} densities of the wild-type and mutant NCX1 measured

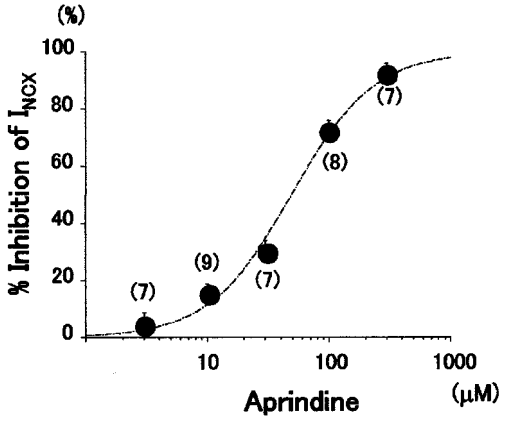

Figure 2 Aprindine concentration–inhibition curve. Average I_{NCX} values measured at +50 mV were fitted to a logistic equation. The IC_{50} of aprindine was 48.8 μM and the Hill coefficient was 1.3. Each point indicates mean±s.e. (number of cells).

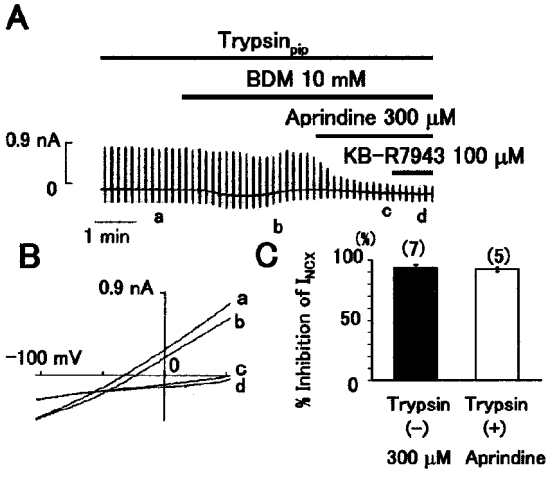

Figure 3 Effect of trypsin on inhibition by aprindine. (A) Typical chart recording of current. The pipette solution contained 2.5 mg ml^{-1} trypsin. BDM, a trypsin-sensitive inhibitor of I_{NCX}, did not inhibit I_{NCX}, indicating the presence of trypsin in the cell. (B) I–V curves obtained at the points a∼d in (A). (C) Summarized data of the inhibitory effect of 300 μM aprindine on I_{NCX} in the absence (left) and presence of trypsin (right) in the pipette solution. The values are means±s.e. $^*P<0.05$ based on unpaired *t*-test.

at +50 mV were 3.5±1.5 pA/pF ($n=5$) and 2.0±1.1 pA/pF ($n=6$), respectively. These results indicate that the long internal loop between TM5 and 6 was not involved in the inhibition by aprindine.

Mode of inhibition by aprindine with respect to KB-R7943

Watano *et al*. (1996) showed that KB-R7943 inhibits I_{NCX} competitively with respect to external Ca^{2+}. We found that the inhibitory effect of 100 μM KB-R7943 on I_{NCX} was trypsin-insensitive (Watanabe & Kimura, 2000; 2001; Watanabe *et al*., 2001) and in this study inhibition by aprindine was also trypsin-insensitive. Therefore, aprindine and KB-R7943 may affect the same site of the exchanger. We determined if aprindine and KB-R7943 inhibit I_{NCX} in a competitive or non-competitive manner by measuring I_{NCX} in the presence of various concentrations of KB-R7943. Figure 5A is a Dixon plot of the reciprocal values of normalized I_{NCX} at 0, 10, 30 or 50 μM aprindine obtained in the presence of 0, 1 or 3 μM KB-R7943. The three fitted lines crossed at a point to the left of the Y-axis and close to the X-axis. This indicates that aprindine and KB-R7943 were co-operative pure competitive inhibitors.

If aprindine and KB-R7943 were co-operative pure competitive inhibitors, aprindine should be a competitive inhibitor of I_{NCX} with respect to external Ca^{2+}. This was tested by applying 100 μM aprindine to inhibit I_{NCX} at three different concentrations of external Ca^{2+}. As shown in the Hanes–Wolf plot of the results in Figure 5B, the pair of fitted lines were parallel, indicating that aprindine is a competitive inhibitor with respect to external Ca^{2+}.

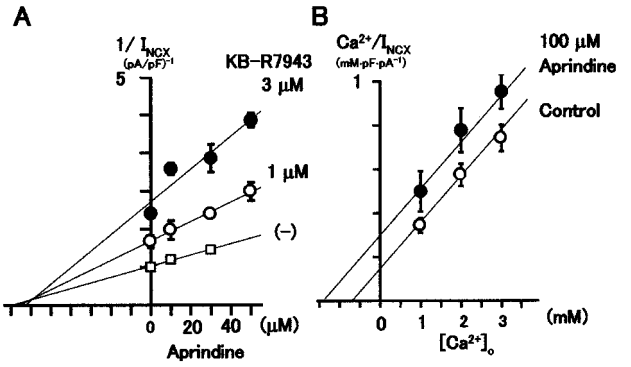

Figure 5 (A) Dixon plot of reciprocal or normalized I_{NCX} versus aprindine concentration under control conditions and in the presence of fixed concentrations of KB-R7943. The three fitted lines intersect at a point, indicating that aprindine and KB-R7943 are co-operative pure competitive inhibitors. (B) Hanes–Woolf plot for determining the mode of aprindine inhibition of I_{NCX} with respect to external Ca^{2+}. Parallel fitted lines indicate that aprindine is a competitive inhibitor with respect to external Ca^{2+}.

Discussion

In this study, we found that acute application of aprindine suppressed I_{NCX} in a concentration-dependent manner in guinea-pig cardiac ventricular cells. The IC_{50} values were 48.8 and 51.8 μM and the Hill coefficients 1.3 and 1.1 at 50 and −100 mV, respectively.

We explored the site of aprindine inhibition on the exchanger by using trypsin in the pipette solution and mutant NCX1 expressed in CCL39 cells. It has been shown that trypsin or α-chymotrypsin treatment removed intracellular regulation of the Na^+/Ca^{2+} exchange by cytoplasmic modulators such as Ca^{2+}, Na^+, ATP and protons (Hilgemann, 1990; Doering & Lederer, 1993; Espinosa-Tanguma *et al*., 1993). We previously showed that a calmodulin inhibitor W-7, BDM and anti-arrhythmic drugs such as amiodarone and bepridil inhibited I_{NCX}, and that the inhibition by those agents was attenuated by intracellular cymotrypsin or trypsin treatment *via* the pipette solution (Kimura, 1993; Watanabe *et al*., 2001; Watanabe & Kimura, 2000; 2001). From that we concluded that those drugs affected the exchanger from the cytoplasmic side. However, in this study, the inhibition of I_{NCX} by aprindine was trypsin-insensitive. In addition, aprindine inhibited a mutant NCX1 which had a deletion of amino acids 247–671 in the large internal domain between TM5 and 6. This indicates that aprindine affects the exchanger from the external side or intramembrane site and not the cytoplasmic side of NCX.

Recently, Chen *et al*. (2000) reported that digestion of scallop muscle membrane fractions with trypsin led to release of soluble polypeptides derived from the large cytoplasmic domain of a Na^+/Ca^{2+} exchanger. In the presence of Ca^{2+}, the major product was a ~37 kDa peptide, with an N-terminus corresponding to residue 369 of NCX1 processed polypeptide sequence according to Nicoll & Philipson (1991). In the absence of Ca^{2+}, ~16 kDa and ~19 kDa peptides were the major products. The 16 kDa fragment corresponded to the N-terminal part of the 37 kDa peptide. Polyclonal antibody raised against the 37 kDa peptide also bound to the ~16 kDa and ~19 kDa soluble tryptic peptides. Therefore, they concluded that the ~16 kDa and ~19 kDa peptides are the tryptic products of 37 kDa peptides.

Assuming an average residue mass of 110 Da, the ~16 kDa and ~37 kDa fragments were approximately 145

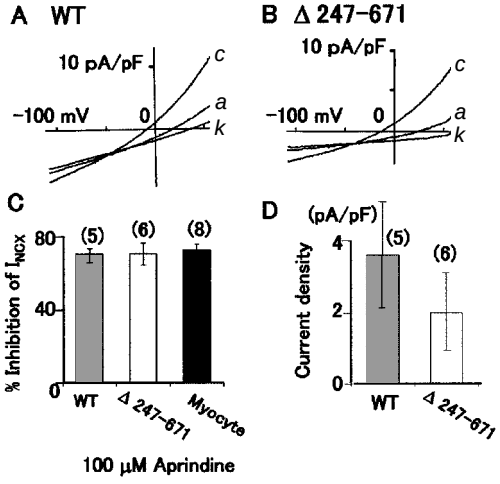

Figure 4 Effects of aprindine on I_{NCX} of wild-type (WT) NCX1 and a mutant with a deletion of amino acids 247–671 (Δ247–671) expressed in CCL39 cells. (A and B) I–V curves of control (*c*), in the presence of 100 μM aprindine (*a*) and 100 μM KB-R7943 (*k*). (C) Summary of the results of A and B. Per cent inhibition of WT, Δ247–671 and cardiac myocytes I_{NCX} by aprindine. (D) Comparison of I_{NCX} densities between WT and Δ247–671.

and 336 amino acids long and corresponded approximately to NCX1 amino acid sequences of 369–514 and 369–705, respectively. The large cytoplasmic domain of NCX1 consists of amino acids 218–764 (Nicoll *et al*., 1999; Iwamoto *et al*., 1999). The mutant we used was deleted of the 247–671 amino acid sequence. Therefore, the deleted sequence of NCX1 overlaps the domain clipped-off by trypsin. This strongly indicates that in cardiac myocytes a part of the large cytoplasmic domain of the exchanger is clipped off by trypsin. If this region is involved in the binding of an inhibitor of NCX, trypsin treatment should diminish its inhibitory effect. This was most likely the case for amiodarone and BDM, which were trypsin-sensitive NCX1 inhibitors. Consistent with this is the observation that the inhibitory effect of amiodarone was diminished in the deletion mutant NCX1. In the present study, aprindine was trypsin-insensitive and it inhibited the mutant and wild-type NCX1 equally. This suggests that the aprindine binding site is not in the cytoplasmic domain which is sensitive to trypsin.

Watano *et al*. (1996) showed that KB-R7943 inhibited I_{NCX} competitively with respect to external Ca^{2+}. Iwamoto *et al*. (2001) suggested that KB-R7943 affects the exchanger at its external side, because external application but not intracellular application of KB-R7943 inhibits NCX. However, this was challenged by Elias *et al*. (2001) who demonstrated that cytoplasmic application of KB-R7943 inhibited I_{NCX} in the giant-patch oocyte membrane expressing NCX1.1. If aprindine and KB-R7943 affect NCX at external sites, they might interact competitively. Therefore, we determined whether aprindine and KB-R7943 are competitive inhibitors. The Dixon plot of the data (Figure 5A) indicated that the three fitted lines intersected at a point to the left of the Y-axis and close to the X-axis. Since KB-R7943 is competitive with respect to external Ca^{2+}, this result suggests that aprindine and KB-R7943 are co-operative (or synergistic) pure competitive inhibitors, indicating that the two inhibitors may compete for different portions of the substrate binding site, or they may continue with the exchanger at specific sites in such a way as to distort the substrate binding site (Segel, 1964). This relation between aprindine and KB-R7943 was further supported by the finding that aprindine was a competitive inhibitor with respect to external Ca^{2+}. The Hanes–Woolf plot in Figure 5B clearly shows this. Iwamoto *et al*. (2001) found that the most important amino acid for KB-R7943 binding to NCX1 is Gly833 in the α-2 repeat re-entrant domain between TM7 and TM8. α-2 repeat together with α-1 repeat are assumed to form the ion transport pathway, because mutations of these regions reduce the affinity of the exchanger for extracellular Ca^{2+} (Iwamoto *et al*., 2000). Since we found that aprindine is a competitive inhibitor with respect to external Ca^{2+}, α-2 repeat may also be involved in aprindine binding.

With regard to action potentials, aprindine decreased the maximum upstroke velocity (Vmax) with an IC_{50} value of ~2 μM without affecting the resting potential in atrial and ventricular cells and Purkinje fibres of guinea-pigs, cats, cows and dogs (Verdonck *et al*., 1974; Carmeliet & Verdonck, 1974; Steinberg & Greenspan, 1976; Kodama *et al*., 1999). There are reports that the following ionic currents are affected by aprindine. I_{Na} of guinea-pig ventricular myocytes was inhibited with IC_{50} values of 37.7 μM at the holding potential (HP) of −140 mV, and 0.74 μM at −100 mV HP (Sato *et al*., 1991). In rabbit AV node, the Ca^{2+} current was blocked by aprindine in a voltage-dependent manner with an estimated dissociation constant of 10 μM and a Hill coefficient of 0.8 (Tanaka *et al*., 1990). Shibasaki *et al*. (1991) showed that 5 μM aprindine suppressed the calcium current (I_{Ca}) by 35% in guinea-pig atrial myocytes. Aprindine affects most types of K^+ channels in cardiac ventricular cells. In a double-microelectrode voltage-clamp study carried out in rabbit SA node and AV nodes, 1~4 μM aprindine inhibited the delayed rectifier K^+ current (I_K) (Tanaka *et al*., 1990). Aprindine at 3 or 5 μM inhibited the I_K with little influence on the inward rectifier K^+ current or Ca^{2+} current (Shibasaki *et al*., 1991; Ohmoto-Sekine *et al*., 1999). In addition, it inhibited I_{Kr} but not I_{Ks} (Ohmoto-Sekine *et al*., 1999). The ligand-gated K^+ currents were also susceptible to inhibition. Aprindine inhibited the carbachol-induced and GTPγS-induced I_{KAch} of guinea-pig atrial cells with the IC_{50} values of 0.4 and 2.5 μM, respectively (Ohmoto-Sekine *et al*., 1999). Aprindine at 1~4 μM also inhibited the pacemaker current (I_f) of rabbit SA node and AV node (Tanaka *et al*., 1990).

Acute and chronic clinical administration of aprindine results in plasma levels in the range of 0.9 to 2.5 μg ml^{-1}, which corresponds to 2.5 to 6.9 μM (Zipes *et al*., 1977; Vlay *et al*., 1985). The concentrations of aprindine that inhibit I_{Na}, I_{Ca}, I_{Kr} and I_{KAch} are in the therapeutic concentration range of the drug. As for I_{NCX}, the minimum concentration of aprindine required to block I_{NCX} by 5.4±3.2% ($n=7$) was 3 μM. Therefore, it is possible that aprindine might modestly inhibit I_{NCX} at therapeutic concentrations. Whether such modest inhibition of I_{NCX} is therapeutically beneficial or detrimental needs to be clarified. Further research is needed to elucidate the mechanism of inhibition of I_{NCX} by aprindine and its functional significance.

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