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NOVEL ISOFORM OF Ca^{2+} CHANNEL IN RAT FETAL CARDIOMYOCYTES

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SUMMARY

1. Single cardiomyocytes of 18-day-old rat fetuses were isolated to characterize the cardiac Ca^{2+} channels in the fetal period, using whole-cell voltage clamp (Na^+, K^+-free external solution and K^+-free internal solution), and depolarizing test pulses from a holding potential (HP) of −87 mV were applied.

2. The Ca^{2+} current was completely blocked by 2 mM-CO^{2+}, but not completely blocked by the dihydropyridine (DHP) Ca^{2+} antagonist nifedipine. Nifedipine (3 μM) decreased the amplitude of the current (at −7 mV) by 65·9 ± 3·4 % (n = 20). At a HP of −47 mV, nifedipine decreased the Ca^{2+} current to about the same degree. Diltiazem (1 μM) did not block the nifedipine-resistant current which remained.

3. Nitrendipine, another DHP Ca^{2+} antagonist, had effects on the Ca^{2+} current similar to those of nifedipine.

4. The DHP-resistant current was not blocked by T-type channel blockers (Ni^{2+}, tetramethrine) or an N-type blocker (ω-conotoxin).

5. In conclusion, rat fetal cardiomyocytes may have a unique type of Ca^{2+} channel ($I_{Ca(fe)}$), which decreases in amplitude and becomes less prominent during subsequent development.

INTRODUCTION

Using the patch clamp method, two types of Ca^{2+} channels having different single channel conductances have been found in myocardial cells (Bean, 1985; Nilius, Hess, Lansman & Tsien, 1985; Mitra & Morad, 1986; Bonvallet, 1987; Hagiwara, Irisawa & Kameyama, 1988; Hirano, Fozzard & January, 1989; Hess, 1990; Bois & Lenfant, 1991), and three types of Ca^{2+} channels have been reported in sensory neurons (Nowycky, Fox & Tsien, 1985). The dihydropyridine (DHP)-sensitive L-type Ca^{2+} channel contributes to long-lasting current at large depolarizations (high threshold). The T-type Ca^{2+} channel produces a transient current activated at small depolarizations (low threshold). The N-type Ca^{2+} channel also produces a transient current, but is activated at large depolarizations. The T- and N-type channels are

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insensitive to DHPs. The L-type channels inactivate much more slowly than the T-type channels. It has been reported that the N-type Ca^{2+} channel is not present in cardiac muscle cells (McCleskey, Fox, Feldman, Cruz, Olivera, Tsien & Yoshikami, 1987). L-type current is dominant in adult cardiomyocytes.

Resting potential, action potential shape, and pacemaker activity change dramatically during development (Sperelakis, 1989). Kawano & DeHaan (1989) reported that T-type current was dominant in embryonic chick heart cells, but other reports showed that L-type current was dominant in embryonic chick heart cells (Anderson, Patmore & Spedding, 1988; Kristof, Shrier & Clay, 1990; Tohse & Sperelakis, 1990; Wahler, Rusch & Sperelakis, 1990). On the other hand, there is no information about the types of Ca^{2+} channels present in cardiomyocytes of fetal mammals. Therefore, in the present study, we recorded Ca^{2+} current from cardiomyocytes of 18-day-old rat fetuses. In addition to the L-type Ca^{2+} current, a novel type of Ca^{2+} current was observed, which had some properties different from those of the T-type and N-type currents. A preliminary report of this work has appeared as an abstract (Tohse & Sperelakis, 1991).

METHODS

Cell preparation

Freshly isolated single cells were prepared from ventricles of 18-day-old rat fetuses. The pregnant rat (18 days after impregnation) was anaesthetized with ether for a few minutes and then decapitated. The abdomen was opened and five of the fetuses were removed. They were also decapitated and the hearts were dissected. The hearts were rinsed in oxygenated normal Ca^{2+}–Tyrode solution (composition given in Table 1), and then immersed in Ca^{2+}-free Tyrode solution. The ventricles were dissected from the atria after the spontaneous contractions had ceased or slowed. The ventricles were enyzmatically digested for 50 min (at 37 °C) with the Ca^{2+}-free Tyrode solution containing collagenase (1 mg/ml). After digestion, the tissues were rinsed at least three times in modified Krebs–bicarbonate (KB) solution at room temperature (Table 1, Tohse, Nakaya, Hattori, Endou & Kanno, 1990). The cells were mechanically agitated for better dispersion using a Pasteur pipette. The cell suspension was stored in a refrigerator (4 °C) until used.

Patch clamp recording

Whole-cell patch clamp recording was made using a patch clamp amplifier (EPC-7, List, Germany) and standard techniques. The patch pipettes had resistances of 2–4 MΩ (when filled with the internal solution). The liquid junction potential between the internal solution and the normal Tyrode solution was $-7{\cdot}4 \pm 0{\cdot}5$ mV (mean ± S.E.M., $n = 9$). Therefore, all values of potentials given were corrected by -7 mV. The cells were put in a perfusion chamber placed on an inverted microscope (Nikon, Japan), and constantly perfused with external solution at room temperature (22–25 °C). Current and voltage signals were analysed on a IBM-AT personal computer using the pCLAMP analysis program (Axon Instruments, USA). Current density was calculated using the measured membrane capacitance. The capacitative currents were measured using ramp pulses (5 V/s).

All data are presented as means ± S.E.M. The steady-state activation and inactivation curves, and the inactivation time course of the Ca^{2+} current, were fitted by the method of least squares. Statistical analyses were performed using Student's paired and non-paired t test; $P < 0{\cdot}05$ was defined as significant.

Experimental solutions and drugs

All experiments were carried out in cells superfused with Na^+- and K^+-free external solution. The internal (pipette) solution contained (mM): caesium glutamate, 110; CsCl, 20; $MgCl_2$, 3; ATP-Na_2, 5; PCr-Na_2, 5; EGTA, 10; HEPES, 5; and pH adjusted to 7·2 with CsOH. Therefore, the Ca^{2+}

TABLE 1. Compositions of solutions

	Tyrode	KB	Na^+, K^+ free
NaCl	143	—	—
KCl	5·4	40	—
KOH	—	20	—
K-glutamate	—	50	—
KH_2PO_4	—	20	—
$CaCl_2$	1·8	—	1·8
$MgCl_2$	0·5	3	0·5
TEA-Cl	—	—	150
4-AP	—	—	3
Glucose	5·5	10	5·5
HEPES	5	10	5
pH (titre)	7·4 (NaOH)	7·4 (KOH)	7·4 (CsOH)

Concentrations are expressed in mM. Ca^{2+}-free Tyrode solution was made by removing $CaCl_2$ from the Tyrode solution. 10 mM-taurine and 1 mM-EGTA were added to the KB solution.

TABLE 2. Blockers used to block the Ca^{2+} channels

L-type channel	DHPs (nifedipine, nitrendipine), diltiazem
T-type channel	Ni^{2+}*, tetramethrine
N-type channel	ω-Conotoxin

* Ni^{2+} has a non-specific effect.

current was isolated from the Na^+ and K^+ currents. The compositions of external solution are given as in Table 1.

Nifedipine (Bayer, Germany), nitrendipine (Bayer, Germany), and tetramethrine (Sumitomo Chemical, Osaka, Japan) were dissolved in ethanol to provide 10 mM stock solutions (Table 2). Diltiazem (Sigma, USA) and tetrodotoxin (TTX, Sigma, USA) were dissolved in distilled water to make 10 mM stock solutions. ω-Conotoxin (ω-CTX) was obtained from Sigma (USA).

RESULTS

Nifedipine-resistant Ca^{2+} current

In the Na^+- and K^+-free external solution, and K^+-free internal solution, to eliminate fast Na^+ current and K^+ current, a transient inward current was elicited by depolarizing test pulses above -57 mV from a holding potential (HP) of -87 mV (Fig. 1). The inward current was completely blocked by external perfusion with Co^{2+} (2 mM, Fig. 1*A*), indicating that this inward current was carried by Ca^{2+}. Therefore, the capacitative and leakage currents were subtracted by measuring the currents in the absence and presence of Co^{2+}. Figure 1*B* shows the peak current–voltage relationship for the Co^{2+}-sensitive Ca^{2+} current. The activation threshold of the current was -57 mV, and the maximal current was observed at -7 mV.

Figure 2 shows effects of nifedipine (3 μM) on the Ca^{2+} current. Figure 2*A* and *B* shows selected tracings of the Ca^{2+} current elicited by the test pulses from a HP of -87 mV. At each test potential, nifedipine decreased the current amplitude (Fig. 2*B*), but did not block it completely (compare *B* to *A*). As shown by the peak current–voltage curves in Fig. 2*C*, the activation threshold and potential for maximal amplitude of nifedipine-resistant (DHP-resistant) current were similar to those of the control.

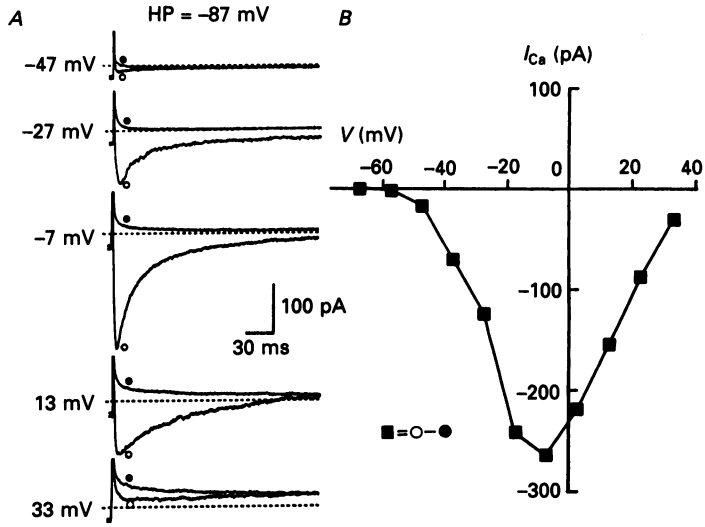

Fig. 1. Ca^{2+} current recorded in an 18-day-old fetal rat cardiomyocyte by the whole-cell voltage clamp technique. *A*, superimposed current tracings elicited by 300 ms depolarizing test pulses to −47, −27, −7, 13 and 33 mV from a holding potential of −87 mV. The tracings are shown until 200 ms after the start of test pulses. ○, control currents at each potential. ●, currents after external application of 2 mM-Co^{2+}. Differences between the two tracings indicate the Ca^{2+} current. *B*, current–voltage relation of the Co^{2+} -sensitive current (Ca^{2+} current). Amplitudes of peak currents at each potential were plotted (■). The threshold potential was at −57 mV. The maximal current was observed at −7 mV.

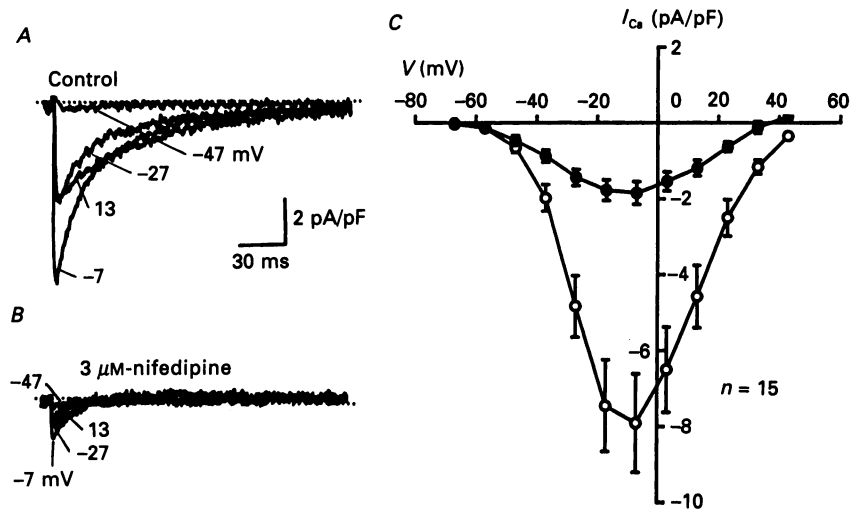

Fig. 2. Effects of 3 μM-nifedipine on the Ca^{2+} current in fetal rat cardiomyocytes. *A* and *B*, selected current tracings elicited by 300 ms depolarizing pulses to −47, −27, −7 and 13 mV from a holding potential of −87 mV; 150 ms segment only is shown. Capacitive and leakage currents were subtracted by tracings in presence of Co^{2+} (2 mM). The current calibration is given as current density. In the presence of 3 μM-nifedipine, small inward currents remained at each potential. *C*, current–voltage (current density) relationship of data from fifteen cells. Data points are shown as means ± S.E.M. As shown nifedipine (3 μM) did not completely block the Ca^{2+} current (at all potentials) (○, control; ●, nifedipine). The nifedipine-resistant current had a threshold potential and potential for maximal current similar to those of the control current.

The incomplete blocking of the Ca^{2+} current by nifedipine was not due to the voltage-dependent action of DHPs (Bean, 1984; Sanguinetti & Kass, 1984), since a small Ca^{2+} current remained in the presence of nifedipine even at the depolarized HP of −47 mV compared with a HP of −87 mV (Fig. 3*A* inset). This DHP resistance of

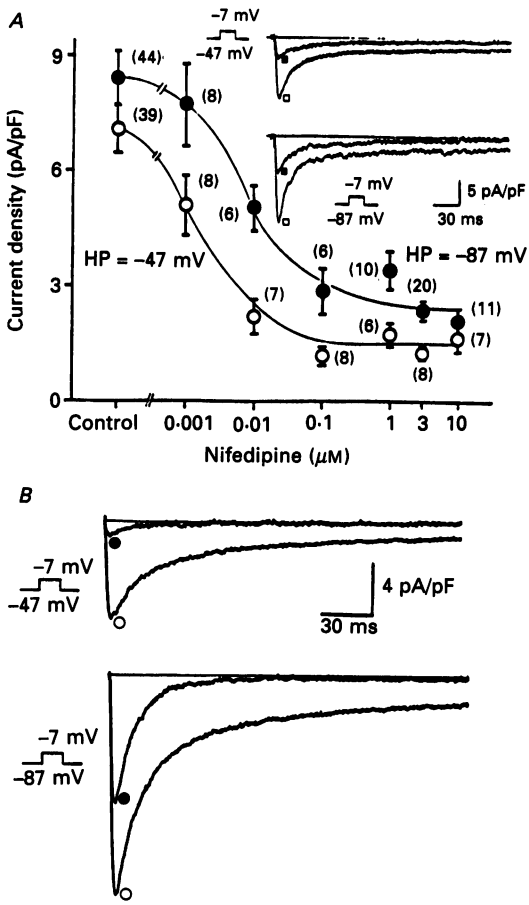

Fig. 3. Voltage dependence of the effects of DHPs on the Ca^{2+} current. *A*, inset; current tracings elicited by 300 ns depolarizing pulses to −7 mV from holding potentials of −47 mV (upper traces) and −87 mV (lower traces). Even with the more depolarized holding potential (−47 mV), 3 μM-nifedipine (■), did not completely block the Co^{2+}-sensitive current (□, control). *A*, concentration–response relations for nifedipine at two holding potentials. Data are shown as mean ± S.E.M. Numerals in parentheses indicate the number of cells for each data point. At high concentration of nifedipine, a small current remained at both holding potentials. This is the nifedipine-resistant current. *B*, effects of 3 μM-nitrendipine on the Ca^{2+} current in one cardiomyocyte. At two holding potentials (HP) of −47 mV (upper traces) and −87 mV (lower traces), nitrendipine gave incomplete block of the Ca^{2+} current, like nifedipine.

the Ca^{2+} current was confirmed by concentration–response relations for nifedipine at the two HPs (Fig. 3*A*). In the range of 0·001–0·1 μM, nifedipine decreased the Ca^{2+} current at both HPs of −47 and −87 mV. However, nifedipine did not produce further decrease in the Ca^{2+} current above 0·1 μM at the two HPs. This residual

current is the DHP-resistant Ca^{2+} current. Nifedipine (3 μM) decreased the total Ca^{2+} current by $77{\cdot}1 \pm 4{\cdot}7$ % ($n = 8$) and $65{\cdot}9 \pm 3{\cdot}4$ % ($n = 20$), at the HPs of -47 and -87 mV, respectively. Therefore, the DHP resistance of the Ca^{2+} current cannot be explained by the voltage-dependent action of DHPs.

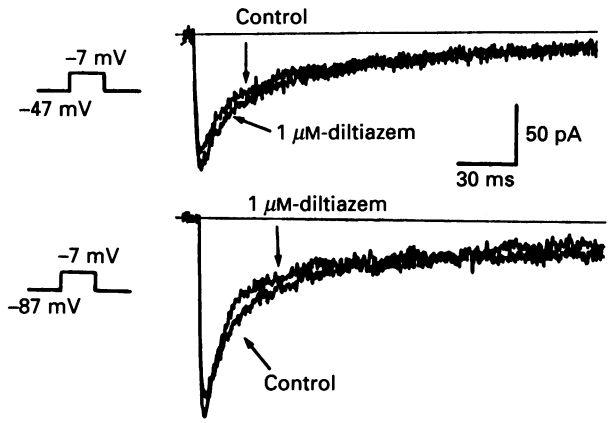

Fig. 4. Effects of diltiazem (1 μM) on the DHP-resistant current. Ca^{2+} current remained in the presence of 3 μM-nifedipine. Diltiazem did not produce further decrease in the Ca^{2+} current at both holding potentials: -47 mV (upper traces) and -87 mV (lower traces). This finding supports the view that the DHP-resistant current is a different type of Ca^{2+} current from the conventional L-type current.

Nitrendipine (3 μM), another DHP Ca^{2+} antagonist, also failed to completely block the Ca^{2+} current (Fig. 3*B*). The decrease in total I_{Ca} was $82{\cdot}4 \pm 2{\cdot}7$ % and $45{\cdot}4 \pm 1{\cdot}3$ % at the HPs of -47 and -87 mV, respectively ($n = 5$). Therefore, this remaining current is resistant to both DHPs.

Diltiazem, a non-DHP blocker of L-type Ca^{2+} current, was also used to determine whether the DHP-resistant current was L-type current. In the presence of 3 μM-nifedipine, diltiazem (1 μM) had no effect on the DHP-resistant current at the HPs of -47 and -87 mV (Fig. 4). Similar findings were observed in four other cells. Therefore, these findings support the view that the DHP-resistant current is not an L-type Ca^{2+} current.

Electrophysiological properties of the DHP-resistant current

To obtain the steady-state inactivation curves for the DHP-sensitive and DHP-resistant currents, the voltage protocol given in the inset of Fig. 5 was used in the absence and presence of 3 μM-nifedipine. Conditioning pulses (duration of 2 s) were applied between -97 and -17 mV from a HP of -87 mV, and then the test pulse (duration of 300 ms) was applied to -7 mV, following a brief 5 ms return to the HP (-87 mV). The peak currents elicited by the test pulses were normalized by the maximum current ordinate for each condition (with and without nifedipine), and plotted against the conditioning potential abscissa. The normalized values represent the mean of eight cells (DHP sensitive) and five to seven cells (DHP resistant). DHP-sensitive current was obtained by subtracting the current recording with nifedipine from that without nifedipine. Both inactivation curves were fitted to the Boltzmann

equation (Fig. 5). The best fit gave slope factors (k) of 6·8 and 10·7 mV, and half-inactivation potentials ($V_{0.5}$) of −42·0 and −58·4 mV for the DHP-sensitive and DHP-resistant currents, respectively.

Figure 6 shows the time course of inactivation of DHP-sensitive and DHP-resistant currents. Both currents were fitted by double-exponential curves (Fig. 6*A*

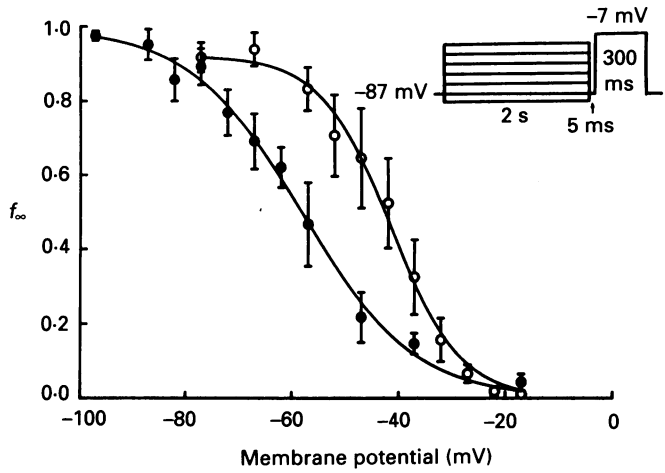

Fig. 5. Steady-state inactivation (f_∞) curves of the DHP-sensitive current (○) and the DHP-resistant current (●). Nifedipine (3 μM) was used for analysis. The inset shows the voltage protocol for measuring f_∞. Conditioning pulse duration was 2 s; test pulse duration was 300 ms. Test pulses were applied to −7 mV from various conditioning pulse levels. There was a 5 ms interval between the end of the conditioning pulse and the beginning of the test pulse in which the membrane potential was returned to the holding potential of −87 mV. Data are shown as mean ± S.E.M. (n = 5–8). Both curves were fitted by the Boltzmann equation: $f_\infty = 1/[1+(\exp(V-V_{0.5})k)]$. The half-inactivation potential ($V_{0.5}$) was −42·0 and −58·4 mV for DHP-sensitive current and DHP-resistant current, respectively. The slope factors (k) were 6·8 (DHP sensitive) and 10·7 (DHP resistant). Note the 16 mV shift of the curve in the hyperpolarized direction compared with that for the DHP-sensitive current.

and *B*). The time constant for the fast component of the DHP-resistant current was similar to that of the DHP-sensitive current at every potential (Fig. 6*C*). The time constant for the slow component of the DHP-resistant current was slightly faster than that of DHP-sensitive current at every potential, but the differences were not statistically significantly except that at −27 mV ($P < 0.05$, Fig. 6*D*). Therefore, these results suggest that the DHP-resistant channel may be a different type of Ca^{2+} channel from the T-type channel, because the T-type channel has faster inactivation than that of the L-type channel (Bean, 1985; Hagiwara *et al.* 1988).

Pharmacological evidence for a novel type of Ca^{2+} channel

The results presented above with the DHPs and diltiazem indicate that the DHP-resistant current is a different type of Ca^{2+} current from the L-type current. In addition, analyses of the kinetic properties of the DHP-resistant current suggested that the DHP-resistant current may not be the T-type current. Therefore, further pharmacological analyses were done to classify the DHP-resistant current.

Ni^{2+} at a low concentration (< 100 μM) is known to be a blocker of the T-type channels (Hess, 1990). In cardiac cells, Ni^{2+} at a concentration of 40 μM completely blocks the T-type current of rabbit sino-atrial node cells (Hagiwara *et al.* 1988). As shown in Fig. 7*A*, Ni^{2+} (40 μM) failed to completely block the DHP-resistant current. In six cells, Ni^{2+} decreased the DHP-resistant current by only 48·6±7·2%.

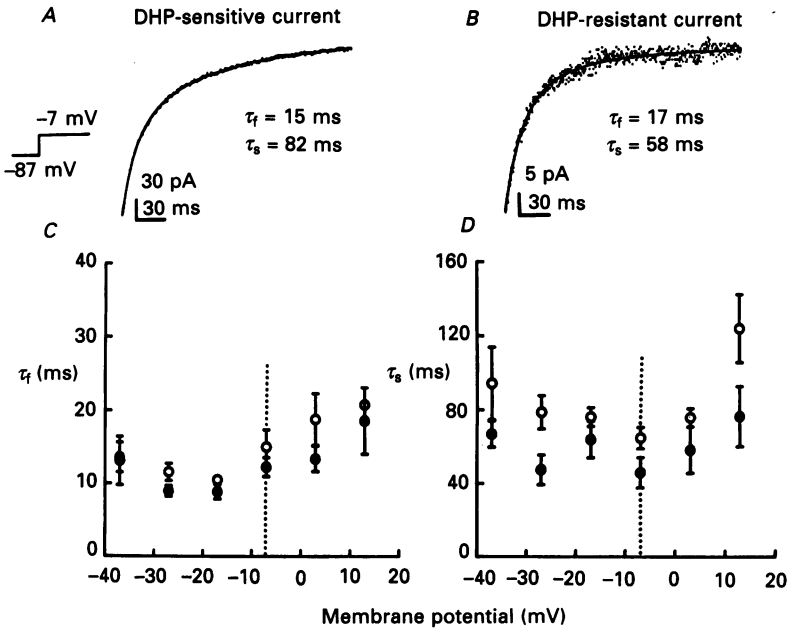

Fig. 6. Time course of inactivation of the DHP-sensitive current (*A* and *C*) and DHP-resistant currents (*B* and *D*). *A* and *B*, representative examples of theoretically fitted curves from the data points for DHP-sensitive (*A*) and DHP-resistant (*B*) currents. Both currents were well fitted by double two-exponential curves: $I = A + B\exp(-t/\tau_f) + C\exp(-t/\tau_s)$. The fast time constant (τ_f) and the slow time constant (τ_s) are indicated for each current. The voltage pulse protocol used is shown in panel *A*. *C* and *D*, voltage dependency of the fast (*C*) and the slow (*D*) time constants for the DHP-sensitive current (○) and the DHP-resistant current (●). Data are shown as mean±S.E.M. (n = 6–15). At most potentials, there is no significant difference between the fast and slow time constants of the DHP-sensitive and DHP-resistant currents.

Tetramethrine is another type of T-channel blocker, and blocks the T-type current of the rabbit sino-atrial node cells at a concentration of 0·1 μM (Hagiwara *et al.* 1988). However, the DHP-resistant current was only slightly decreased by tetramethrine, even at a concentration of 1 μM. In five cells, tetramethrine decreased the current by an average of 13·7±6·5%. In the cell illustrated in Fig. 7*B*, there was no effect. Therefore, these results indicate that the DHP-resistant current is a different type of Ca^{2+} current from the T-type Ca^{2+} current.

ω-Conotoxin (ω-CTX) is known to be a blocker of the N-type Ca^{2+} channels (McCleskey *et al.* 1987). In the present study, ω-CTX (10 μM) did not block the DHP-resistant current. In fact, there was a slight stimulation (Fig. 7*C*). Similar results were observed in another six cells. Therefore, the DHP-resistant current is not the N-type Ca^{2+} current.

It is possible that the DHP-resistant current is carried by Ca^{2+} through the fast Na^+ channels (Tytgat, Vereeke & Carmeliet, 1990). Therefore, an effect of tetrodotoxin (TTX, 10 μM) on the DHP-resistant current was examined (Fig. 7*D*). As TTX did not decrease the DHP-resistant current ($n = 4$), the DHP-resistant current was not the Ca^{2+} current through the fast Na^+ channels.

Therefore, these pharmacological experiments indicate that the DHP-resistant Ca^{2+} current is a novel type of Ca^{2+} current.

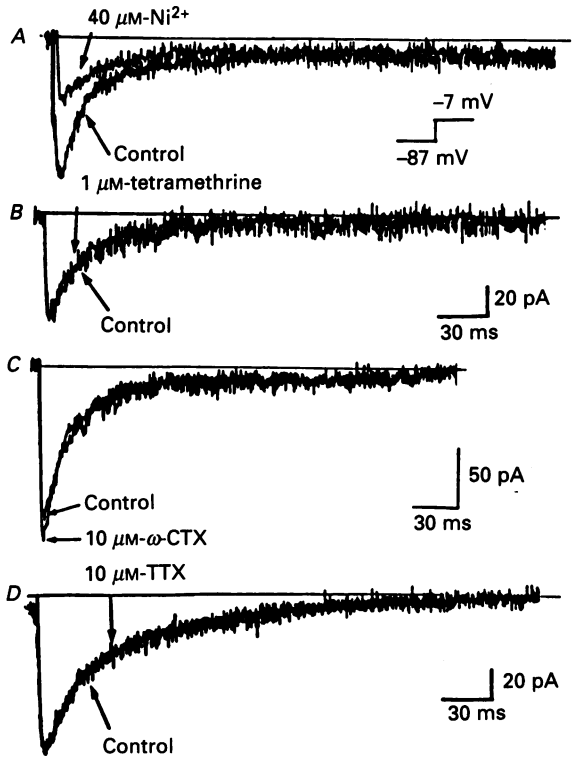

Fig. 7. Effects of blockers of T-type (*A* and *B*) and N-type (*C*) Ca^{2+} currents on the DHP-resistant current in the presence of 3 μM-nifedipine. *A*, 40 μM-Ni^{2+} did not completely abolish the DHP-resistant Ca^{2+} current. *B*, 1 μM-tetramethrine had no effect on the DHP-resistant Ca^{2+} current. *C*, ω-conotoxin (ω-CTX, 10 μM) did not depress the DHP-resistant Ca^{2+} current, but actually stimulated it slightly. Although 10 μM-ω-CTX blocks the L-type as well as N-type Ca^{2+} current, the DHP-resistant current was not blocked by the toxin. *D*, TTX (10 μM) had no effect on the DHP-resistant current.

DISCUSSION

Many authors have reported the presence of the L- and T-type currents in cardiac myocytes (Bean, 1985; Nilius *et al.* 1985; Mitra & Morad, 1986; Bonvallet, 1987; Hagiwara *et al.* 1988; Hirano *et al.* 1989; Hess, 1990; Bois & Lenfant, 1991), and the absence of N-type channels (McCleskey *et al.* 1987). In the present study, on rat fetal

(18 day) cardiomyocytes, the major Ca^{2+} current was L-type and the remainder was a novel type of Ca^{2+} current, different from the T-type and N-type currents. The L-type current observed in the rat fetal cardiomyocytes was blocked by nifedipine and nitrendipine. However, these DHPs at relatively high concentrations (up to 10 μM) did not completely abolish the fetal Ca^{2+} current at HPs of -47 and -87 mV. At depolarized HPs (-50 to -30 mV), various DHPs (at concentrations of 0·1–2 μM) completely block the Ca^{2+} current of adult cardiomyocytes (Kass, 1982; Mitchell, Powell, Terrar & Twist, 1983; Bean, 1984; Hume, 1985; Kawashima & Ochi, 1988; Hagiwara *et al.* 1988; Osaka & Joyner, 1991). Since, in the present study, nifedipine (10 μM) and nitrendipine (3 μM) did not abolish the fetal Ca^{2+} current at a HP of -47 mV, these findings indicate that the DHP-resistant current was not caused by (a) an insufficient concentration of DHPs for maximal effect, or (b) voltage-dependent effects of DHPs. The concentration–response curves for nifedipine at the two HPs (Fig. 3*A*) support this view. At concentrations above 1 μM, nifedipine did not produce further decrease in the Ca^{2+} current at the two HPs, leaving a residual current. Diltiazem had no effect on this DHP-residual current. Therefore, this DHP-residual current seems to be a different type of current from the L-type current.

The DHP-resistant current has quite different properties from those of the T-type current. The threshold potential and the potential which occurs for maximal current of the T-type current are more negative, compared with those for the L-type current (Bean, 1985; Hagiwara *et al.* 1988). These properties should produce a 'hump' at negative potentials in the *I–V* curve of the combined Ca^{2+} currents. In the present study, the DHP-resistant current had the same potentials for threshold and for maximal current as those of the L-type current. Therefore, there was no 'hump' in the *I–V* curve in rat fetal Ca^{2+} current (Figs 1 and 2). In addition, the DHP-resistant current has a time course of inactivation similar to that of the L-type current (Fig. 3*A* inset), whereas the T-type current has faster inactivation (Nilius *et al.* 1985; Bean, 1985; Hagiwara *et al.* 1988). Pharmacological analysis also supports the idea that the DHP-resistant current is different from the T-type current. The specific T-type channel blocker, tetramethrine (Hagiwara *et al.* 1988), did not block the DHP-resistant current (Fig. 7*B*). The non-specific T-type Ca^{2+} channel blocker Ni^{2+} (40 μM) partially blocked the DHP-resistant current (Fig. 7*A*).

The DHP-resistant current was not N-type current, because ω-CTX, a blocker of the N-type Ca^{2+} channels, did not decrease it (Fig. 7*C*). Therefore, the DHP-resistant current may be a novel type of Ca^{2+} current found in fetal cells. We propose to name this new Ca^{2+} current the fetal-type Ca^{2+} current: $I_{Ca(fe)}$. Recently, a high-threshold current, which was insensitive to DHPs and ω-CTX, was reported in neurons (Llinás, Sugimori, Lin & Cherksey, 1989; Sah, Regan & Bean, 1989; Mogul & Fox, 1991).

The DHP-resistant current may change during development in rat heart. In adult rat heart cells, there is no T-type current (Richard, Tiaho, Charnet, Nargeot & Nerbonne, 1990), and nifedipine completely abolished the Ca^{2+} current (Mitchell *et al.* 1983). We also confirmed that nifedipine (3 μM) abolished completely the Ca^{2+} current in adult rat ventricular cells ($n = 5$, authors' unpublished observations). These findings indicate that adult cells possess only the L-type Ca^{2+} current. Therefore, the DHP-resistant current that was observed in fetal rat heart cells disappears during development.

Since potentials for threshold and maximal current, and inactivation kinetics of the DHP-resistant current, are similar to those of the L-type current, the molecular structures for the voltage-dependent gating mechanism of the fetal Ca^{2+} channel may be similar to those of the L-type channel. During heart development, the fetal Ca^{2+} channel may be replaced by newly synthesized DHP-sensitive channels. Recently, Kojima, Ishima, Taniguchi, Kimura, Sada & Sperelakis (1990) reported that nitrendipine binding sites increase in developing rat hearts from fetus to adult, consistent with the loss of DHP-resistant Ca^{2+} channels and gain of DHP-sensitive Ca^{2+} channels. An increase in nitrendipine binding was also demonstrated for embryonic chick hearts during development (Marangos, Sperelakis & Patel, 1984). There is an analogy of the present findings with an isoform change in the fast Na^+ channels of rat skeletal muscle that occurs during development and denervation, in which the fetal/denervated isoform of the α-subunit (SkM2) is a TTX-insensitive form of the fast N^+ channel (Kallen, Sheng, Yang, Chen, Rogart & Barchi, 1990). The SkM2 mRNA level is highest in early development, but declines rapidly with age as SkM1 (TTX-sensitive isoform) mRNA increases. The SkM2 isoform has a longer cytoplasmic loop joining domains 1 and 2. The TTX-insensitive isoform predominated in adult rat cardiac muscles.

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