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Inhibition of myocardial L- and T-type Ca^{2+} currents by efonidipine: possible mechanism for its chronotropic effect

Haruko Masumiya, Toshinori Shijuku, Hikaru Tanaka *, Koki Shigenobu

Department of Pharmacology, Toho University School of Pharmaceutical Sciences, Miyama 2-2-1, Funabashi, Chiba 274, Japan

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Abstract

Effects of efonidipine, a dihydropyridine phosphonate Ca^{2+} channel antagonist, on the guinea-pig heart were compared with those of nifedipine. In the sino-atrial node, 1 μM efonidipine produced increase in cycle length accompanied by prolongation of the phase 4 depolarization which was not prominent with 0.1 μM nifedipine. In ventricular myocytes, both efonidipine and nifedipine produced inhibition of the L-type Ca^{2+} current, nifedipine being tenfold more potent than efonidipine. Efonidipine also inhibited the T-type Ca^{2+} current at higher concentrations but nifedipine did not. Both Ca^{2+} channel antagonists had no or only a weak effect on K^+ currents. In addition, 40 μM Ni^{2+}, which selectively inhibited the T-type Ca^{2+} current, had no effect on myocardial Ca^{2+} transients and contractile force. In conclusion, efonidipine was shown to have inhibitory effects on both L- and T-type Ca^{2+} currents, which may contribute to its high negative chronotropic potency.

Keywords: Efonidipine; Ca^{2+} channel, T-type ; Chronotropism; Sinoatrial node

1. Introduction

Efonidipine hydrochloride (NZ105) is a 1,4-dihydropyridine phosphonate with antihypertensive action which is slow in onset and long lasting (reviewed by Masuda and Tanaka, 1994). The reflex tachycardia produced by efonidipine is less pronounced than that produced by equally hypotensive doses of nicardipine, a dihydropyridine carboxylate (Masuda et al., 1990; Sakai et al., 1991a). Efonidipine increases cardiac output and left ventricular maximum dp/dt with accompanying decreases in blood pressure and improvements in coronary blood flow (Sakai et al., 1991b). In vitro studies of efonidipine demonstrated that it has a potent vasodilating and negative chronotropic action but weak negative inotropic action (Masuda et al., 1995; Tanaka et al., 1996b); the (negative chronotropic potency)/(negative inotropic potency) ratio of efonidipine was especially high among various dihydropyridine Ca^{2+} channel antagonists. This selective chronotropic effect of efonidipine could explain why the reflex tachycardia produced by efonidipine is less pronounced than that elicited by other dihydropyridine Ca^{2+} channel antagonists, and also suggested that factors other than inhibition of the L-type Ca^{2+} current might be involved in its chronotropic effect.

The low threshold Ca^{2+} channel, or the T-type Ca^{2+} channel, has been recently demonstrated with voltage clamp recordings in a variety of cell types including neurons and sino-atrial node cells (Vassort and Alvarez, 1994; Huguenard, 1996; Ertel and Ertel, 1997). The T-type Ca^{2+} channel has characteristics different from the L-type Ca^{2+} channel such as low activation and inactivation voltage, smaller single channel conductance and insensitivity to dihydropyridine Ca^{2+} channel antagonists. The T-type Ca^{2+} current is considered to be involved in the repetitive firing of neurons and pacemaking of the sino-atrial node (Hagiwara et al., 1988). In our previous study, we performed microelectrode recordings of action potentials in rabbit sino-atrial node tissue, and found that efonidipine prolonged the late phase 4 depolarization (Masumiya et al., 1997). This phenomenon was similarly observed with 50 μM nickel, which is known to selectively inhibit T-type Ca^{2+} current. These results suggested the possibility that the high selectivity of efonidipine for negative chronotropism might be explained by inhibitory effects of the drug on T-type Ca^{2+} current.

* Corresponding author. Tel.: +81-474-72-2092; fax: +81-474-72-2113; e-mail: htanaka@phar.toho-u.ac.jp

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In the present study, we examined the effects of efonidipine on guinea-pig myocardium to obtain further information on the mechanisms for its high chronotropic potency. We examined whether efonidipine can induce prolongation of the pacemaker depolarization of guinea-pig sino-atrial node tissue to produce the unique action potential configuration observed in the rabbit. Since L- and T-type Ca^{2+} current can be easily separated in guinea-pig ventricular myocytes by voltage clamp experiments, we examined whether efonidipine has inhibitory effects on L- and T-type Ca^{2+} currents. In addition, we examined the effects of T-type Ca^{2+} current blockade by Ni^{2+} on the Ca^{2+} transient and contractile force to clarify whether the T-type Ca^{2+} current is involved in myocardial excitation contraction coupling.

2. Methods

2.1. Recording of action potentials

Transmembrane potential of sino-atrial node preparation was measured as described previously (Satoh, 1991; Masumiya et al., 1997). Hartley strain guinea-pigs of either sex, weighing 300–350 g, were exsanguinated and the heart was quickly removed. Tissues including the sino-atrial node were cut perpendicularly to the crista terminals into strips of about 1×3 mm. Preparations were pinned down horizontally on a silicon block in a 20 ml organ bath filled with oxygenated (95% O_2, 5% CO_2) and warmed (35.5 ± 0.5°C) physiological salt solution of the following composition (in mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 24.9 and glucose, 11.1. Action potentials were recorded with glass microelectrodes filled with 3 M KCl having resistance of 20–50 MΩ. The output of the microelectrode amplifier (MEZ-8201, Nihon Kohden, Tokyo) was monitored through a dual beam cathode ray oscilloscope (VC-11, Nihon Kohden, Tokyo) and fed into an AD converter (Analog Pro, Canopus, Kobe) attached to a computer (PC 9801 DA2, NEC, Tokyo) for analysis. The action potential parameters measured were cycle length, maximum diastolic potential, threshold potential, maximum rate of rise, overshoot, action potential amplitude, action potential duration at 50% amplitude and slope of the pacemaker (phase 4) depolarization. To obtain slope values, the earlier and the later half of the pacemaker depolarization phase were fitted by straight lines whose slopes were used as slope (early) and slope (late), respectively. The curved regions close to the maximum diastolic potential and threshold potential were not included in the fitting (Masumiya et al., 1997).

2.2. Cell isolation

Single guinea-pig ventricular myocytes were dissociated by enzymatic dispersion as previously described (Kato et al., 1996). Hartley strain guinea-pigs, weighing 300–400 g, were anesthetized with pentobarbital sodium (40–50 mg/kg i.p.) and the ascending aorta was cannulated in situ under artificial respiration. The heart was Langendorff-perfused with Tyrode's solution (gassed with 100% O_2 and warmed to 36°C) of the following composition (in mM): NaCl, 143; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; glucose, 5.5 and HEPES, 5 (pH 7.4). After the blood was washed out, the heart was perfused with Ca^{2+}-free Tyrode's solution for 15 min, and with the same solution containing 0.2 mg/ml collagenase (Yakult, Tokyo Japan) for about 10 min. Then collagenase was washed out with storage solution of the following composition (in mM): glutamic acid, 70; taurine, 15; KCl, 30; KH_2PO_4, 10; $MgCl_2$, 0.5; glucose, 11; HEPES, 10; ethylene glycol bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 0.5 (pH 7.4). Thereafter, the ventricle was dissociated by gentle pipetting and the single cells obtained were stored in the storage solution until used.

2.3. Voltage clamp experiments

Membrane currents of isolated ventricular cells were recorded with whole-cell voltage clamp. For L-type Ca^{2+} current measurements, the external solution was of the following composition (mM): NaCl, 142; CsCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; glucose, 5.5; HEPES, 5 (pH 7.2) and the patch pipette solution was of the following composition (mM): CsCl, 130; $MgCl_2$, 1; HEPES, 5; EGTA, 5 and ATP-Mg, 5 (pH 7.2, 21–23°C). For T-type Ca^{2+} current measurements, the external solution was of the following composition (mM): Tris, 137.6; $MgCl_2$, 1; $CaCl_2$, 5.4; glucose, 5; CsCl, 20 (pH 7.4) and tetrodotoxin (30 μM), and the pipette solution contained the following composition (mM): CsCl, 125; ATP-Mg, 5; EGTA, 15; tetraethylammonium chloride, 20; HEPES, 10 (pH 7.2). The amplitude of the peak L- and T-type Ca^{2+} current was determined as the difference between the peak inward current and the current at the end of the depolarizing pulse. For the measurements of tail current of the delayed rectifier K^+ current and inward rectifier K^+ current, 0.1 mM Cd^{2+} was added to the extracellular Tyrode's solution to eliminate the L-type Ca^{2+} current. The pipette solution was of the following composition (mM): KCl 130, HEPES 5, $MgCl_2$ 1, ATP-Mg 5. The K^+ currents were measured at 33–34°C. Data acquisition and analysis were performed with a patch-clamp amplifier (Axopatch-1D, Axon, Foster City, CA), a personal computer (Deskpro 386s; Compac, Houston, TX) and pCLAMP software (Axon). The patch pipettes had resistance of 2–4 MΩ when filled with the internal solutions. Steady-state capacitive current in response to a 1 mV/ms ramp depolarization from 0 to 5 mV was used to measure the cell membrane capacitance. The capacitive currents were cancelled in the membrane current recordings with the capacitance-neutralization device of the amplifier. Drugs were applied at 15 min after the rupture of

the cell membrane, when the current amplitudes were maintained constant. We confirmed with time control experiments that the changes in current amplitude during the experimental period was less than 5% of that just before drug addition.

2.4. Measurement of fluorescence intensity

Intracellular Ca^{2+} activity was recorded using confocal microscopy as described previously (Tanaka et al., 1996a). Experiments were performed under the whole-cell configuration at room temperature with a patch clamp amplifier (PC-one, Dagan) and stimulator (SEN-3301, Nihon Kohden). Ventricular myocytes were dialyzed with indo-1 via the patch-clamp pipettes. The electrodes had a resistance of 2 MΩ and contained the following solution (mM): KCl, 130; HEPES, 5; $MgCl_2$, 1; ATP-K_2, 5 and indo-1, 0.2 (pH 7.2). Action potentials were elicited at a frequency of 0.5 Hz under current clamp mode with current pulses 5 ms in duration. The cells were excited at 351 nm with a high-power Ar^+ laser, and the emission bands, at wavelengths of 400–440 nm and longer than 440 nm, were separated by dichroic mirrors and long pass filters, detected by parallel photo multipliers, and ratioed after correction of background fluorescence and shading. Two dimensional ratio images were obtained every 67 ms and the ratio values of the cytoplasmic region were averaged. Calibration of intracellular Ca^{2+} from ratio values were performed as described previously (Tanaka et al., 1996a).

2.5. Measurement of contractile force

The right ventricular papillary muscles were isolated from Hartley strain guinea-pigs of either sex, weighing 300–350 g. Preparations were placed horizontally in an organ bath containing physiological salt solution of the following composition (mM): NaCl, 135; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$,15 and glucose, 5.5 (pH 7.4). The solution was gassed with 95% O_2–5% CO_2 and maintained at 36.5°C. Preparations were driven by a pair of platinum plate electrodes (field stimulation) with rectangular current pulses (1 Hz, 5 ms, 1.2 × threshold voltage) generated from an electronic stimulator (Dia Medical System, DPS-165B). Developed tension was recorded isometrically with a force-displacement transducer (Nihon Koden TB-611T) connected to a minipolygraph (Nihon Koden RM-6100). The preparation equilibrated for at least 60 min before the start of the experiments.

2.6. Drugs

Nifedipine and $NiCl_2$ were purchased from Sigma (St. Louis, MO, USA). Efonidipine was provided by Nissan Chemical Industries. (Tokyo, Japan). Efonidipine and nifedipine were dissolved in 100% dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the preparation bath (< 0.3%), did not affect the electrophysiological and mechanical responses. All other chemicals were commercial products of the highest quality available.

2.7. Statistics

All data are presented as mean ± standard errors (S.E.M.). Statistical significance between means were evaluated by the unpaired and paired *t*-test. P values less than 0.05 were considered significant.

3. Results

3.1. Effects of efonidipine and nifedipine on the sinus node action potential

Effects of 0.1 μM nifedipine and 1 μM efonidipine on the action potential configuration of sino-atrial node tissue were compared. Typical traces in the absence and presence of drugs were presented in Fig. 1, and the summarized data of each action potential parameter were presented in Table 1. Both drugs prolonged the cycle length to about 120% of basal values, accompanied by changes in action potential parameters. The decreases in the slope of the phase 4 depolarization was greater with efonidipine, while the decrease in V_{max} and the shift of threshold potential to positive direction were greater with nifedipine.

3.2. Effects of efonidipine and nifedipine on L- and T-type Ca^{2+} currents

We isolated L- and T-type Ca^{2+} currents in our ventricular cardiomyocytes using the large difference in their inactivation voltage (Balke et al., 1992). L-type Ca^{2+} current was elicited every 10 s by 400 ms depolarizing test pulses from a holding potential of −40 mV to +10 mV, and T-type Ca^{2+} current was elicited every 20 s by 200 ms

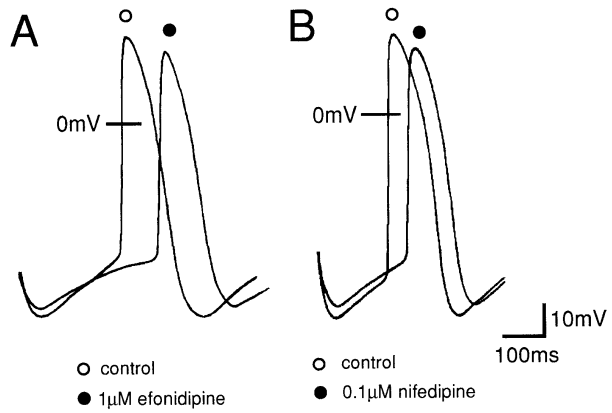

Fig. 1. Effects of efonidipine and nifedipine on the action potential of sino-atrial nodal tissue. Typical action potential traces obtained in the absence (open circles) and presence (closed circles) of 1 μM efonidipine (A) and 0.1 μM nifedipine (B).

Table 1
Effects of efonidipine and nifedipine on action potential parameters of guinea-pig sino-atrial node

	Efonidipine (1 μM)			Nifedipine (0.1 μM)		
	Before	After	Change	Before	After	Change
cycle length (ms)	316.3 ± 20.0	387.3 ± 37.8	71.0 ± 21.8	331.7 ± 24.5	409.6 ± 55.5	77.9 ± 32.7
maximum diastolic potential (mV)	−59.7 ± 0.6	−55.2 ± 2.2	4.6 ± 2.1	−63.5 ± 1.2	−57.8 ± 2.2	5.7 ± 1.9
slope (early; V/s)	0.10 ± 0.01	0.08 ± 0.01	−0.03 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	−0.01 ± 0.01
slope (late; V/s)	0.12 ± 0.01	0.07 ± 0.02	−0.05 ± 0.01[a]	0.10 ± 0.01	0.08 ± 0.01	−0.02 ± 0.01
threshold (mV)	−40.7 ± 1.4	−39.2 ± 2.2	1.5 ± 2.2[a]	−43.1 ± 0.9	−33.1 ± 2.7	9.9 ± 2.8
maximum rate of rise (V/s)	6.3 ± 0.8	5.4 ± 1.0	−0.6 ± 0.2	7.2 ± 1.3	5.2 ± 0.8	−1.9 ± 0.7
action potential overshoot (mV)	21.5 ± 2.3	15.6 ± 2.4	−6.0 ± 1.4	20.0 ± 1.5	16.3 ± 1.3	−3.7 ± 1.0
action potential amplitude (mV)	81.2 ± 2.1	70.7 ± 3.9	−10.5 ± 3.3	83.5 ± 1.5	74.1 ± 3.2	−9.4 ± 2.4
APD_{50} (ms)	95.4 ± 5.2	89.0 ± 3.3	−6.4 ± 3.9	97.4 ± 6.3	92.3 ± 4.2	−5.1 ± 5.8

APD_{50}: action potential amplitude at 50% repolarization. Values are the means ± S.E.M. of five to six experiments.
[a] indicates significant difference ($P < 0.05$) from corresponding changes observed in nifedipine treated preparations.

depolarizing test pulses from a holding potential of −80 mV to −20 mV (Fig. 2A). In the absence of drugs, the current density of the L-type Ca^{2+} current at +10 mV was -7.17 ± 0.35 pA/pF ($n = 40$) and that of T-type Ca^{2+} current at −20 mV was -1.35 ± 0.26 pA/pF ($n = 37$).

Nifedipine selectively inhibited L-type Ca^{2+} current; 0.1 μM nifedipine almost completely inhibited the L-type Ca^{2+} current while 10 μM had little effect on the T-type Ca^{2+} current. In contrast, efonidipine had inhibitory effects on not only L- but also T-type Ca^{2+} current; 0.1 μM efonidipine inhibited the L-type Ca^{2+} current to about half of control values and 10 μM inhibited the T-type Ca^{2+} current to about half of control values (Fig. 2B). Nickel (40 μM), which was reported earlier to selectively inhibit the T-type Ca^{2+} current in rabbit sino-atrial node cells (Hagiwara et al., 1988) completely inhibited the T-type Ca^{2+} current in guinea-pig ventricular cardiomyocytes with only a slight inhibition on the L-type Ca^{2+} current (Fig. 2Ac and f).

Concentration–response curves for the inhibitory effects of nifedipine and efonidipine on L- and T-type Ca^{2+} currents are shown in Fig. 2B. Nifedipine selectively inhibited L-type Ca^{2+} current while efonidipine had inhibitory effects on not only L- but also T-type Ca^{2+} current although at about 30-fold higher concentrations under the present voltage clamp conditions.

3.3. Effects of nifedipine and efonidipine on K^+ currents

To examine drug effects on the delayed rectifier K^+ current (I_K), membrane currents were recorded after 1.5 s voltage clamp pulses to various test potentials from a holding potential of −40 mV (Fig. 3A, B). Tail current amplitude (I_{Ktail}) was measured as the difference from the holding current level to the peak of the tail current on return to the holding potential. Nifedipine (1 μM) had a slight inhibitory effect on the I_{Ktail}, while efonidipine had

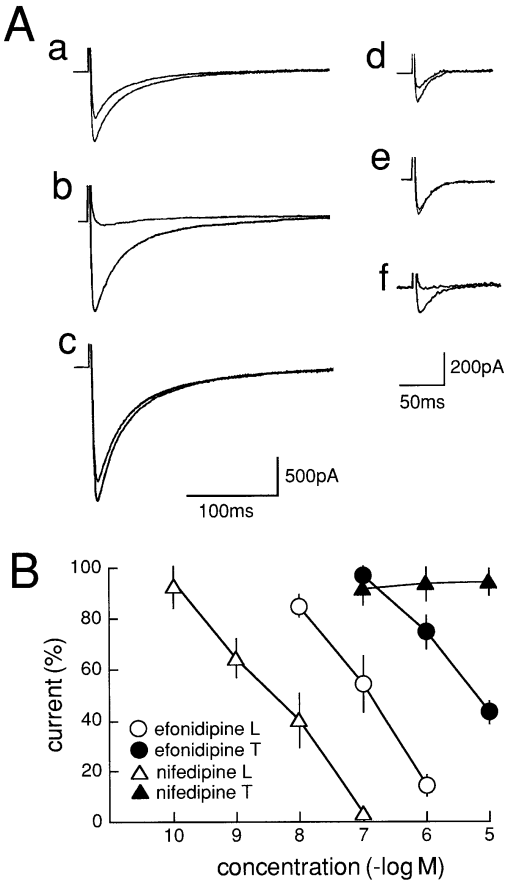

Fig. 2. Effects of efonidipine and nifedipine on the L- and T-type Ca^{2+} currents. (A) Typical current traces of L-type (a–c) and T-type (d–f) Ca^{2+} currents obtained in the absence (lower traces) and presence (upper traces) of 0.1 μM efonidipine (a), 0.1 μM nifedipine (b), 40 μM Ni^{2+} (c, f), 10 μM efonidipine (d) and 10 μM nifedipine (e). The scales for time and voltage on the left are for panels a to c and those on the right are for panels d to f. (B) Concentration–response curves for the inhibitory effects of efonidipine (circles) and nifedipine (triangles) on the L-type (open symbols) and T-type (closed symbols) Ca^{2+} currents. Peak inward current amplitude of the L- and T-type Ca^{2+} currents in the presence of drugs was expressed as a percentage of that in the absence of drugs. Symbols and vertical bars indicate the mean ± S.E.M. from five to six experiments.

no inhibitory effects at 10 μM, which were the concentrations to completely inhibit the L-type Ca^{2+} current.

To record the inward rectifier potassium current (I_{K1}), the membrane potentials were pulsed over a range from −120 mV to 0 mV in 10 mV increments for 200 ms from a holding potential of −40 mV. These currents were completely abolished by 2 mM Ba^{2+} (data not shown). Neither 10 μM nifedipine nor 10 μM efonidipine affected the I_{K1} (Fig. 3C, D).

3.4. Effects of T-type Ca^{2+} current blockade on myocardial contraction

To clarify whether the T-type Ca^{2+} current has any contribution to the contraction of cardiac muscles, we examined the effects of 40 μM nickel, which was shown to selectively inhibit the T-type Ca^{2+} current, on the intracellular Ca^{2+} transient (Fig. 4A) and force of contraction (Fig. 4B, C).

Ca^{2+} transients were evoked in ventricular cardiomyocytes loaded with indo-1 by depolarizing current application through patch electrodes at 0.2 Hz. Nickel (40 μM), which selectively inhibited the T-type Ca^{2+} current with little effect on the L-type Ca^{2+} current, had no effect on the Ca^{2+} transient; the peak amplitude of the Ca^{2+} transient at 3 min after the addition of 40 μM nickel was $100.3 \pm 3.1\%$ ($n = 4$) of initial values. Subsequent addition of 1 μM nifedipine markedly attenuated the Ca^{2+} transient; the peak amplitude of the Ca^{2+} transient at 3 min after the addition of 1 μM nifedipine was $32.8 \pm 7.5\%$ ($n = 4$) of initial values.

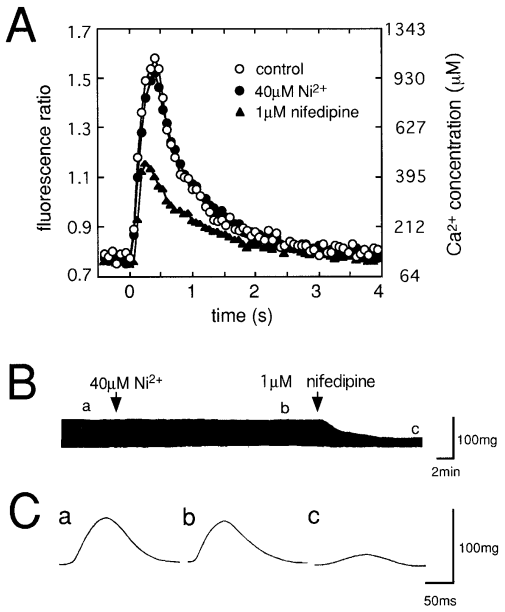

Fig. 4. Effects of L- and T-type Ca^{2+} current blockade on myocardial Ca^{2+} transient and contractile force. (A) Cytoplasmic Ca^{2+} transients of indo-1 loaded ventricular myocytes under control condition (open circles), 3 min after the application of 40 μM Ni^{2+} (closed circles) and 2 min (triangles) after the application of 1 μM nifedipine. (B) and (C) traces of contractile force obtained with right ventricular papillary muscle under control condition (a), after application of 40 μM Ni^{2+} (b) and 1 μM nifedipine (c). The rapid sweep traces in (C) were from the preparation shown in (B) at the time points indicated (a–c).

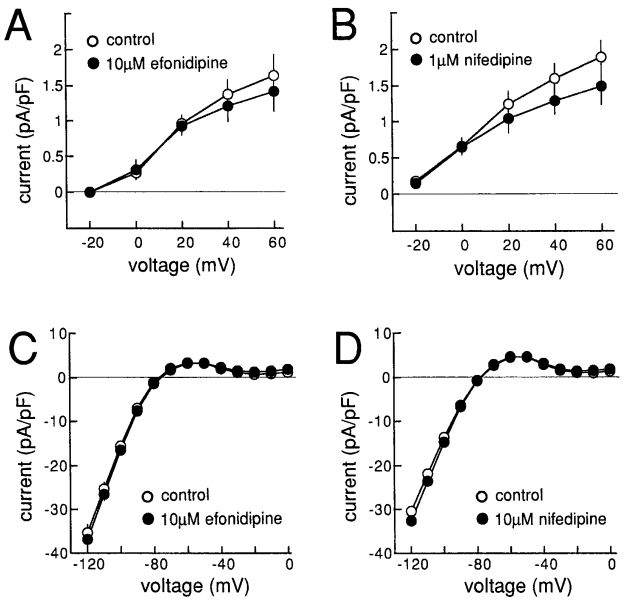

Fig. 3. Effects of efonidipine and nifedipine on the delayed rectifier and inward rectifier K^+ currents. Current–voltage relationship of the peak tail currents of the delayed rectifier was obtained in the absence (open circles) and presence (closed circles) of 10 μM efonidipine (A) and 1 μM nifedipine (B). Steady state amplitude of the inward rectifier was obtained in the absence (open circles) and presence (closed circles) of 10 μM efonidipine (C) and 10 μM nifedipine (D). Symbols and vertical bars indicate the mean ± S.E.M. from five experiments.

In right ventricular papillary muscle field stimulated at a rate of 1 Hz, 40 μM Ni^{2+} had no significant effects on force of contraction, while addition of 1 μM nifedipine markedly reduced the force of contraction. The contractile force after addition of 40 μM nickel and 1 μM nifedipine was $101.0 \pm 0.9\%$ ($n = 5$) and $17.2 \pm 2.1\%$ ($n = 5$) of initial values, respectively.

4. Discussion

We previously showed that the effect of efonidipine on the action potential configuration of the rabbit sino-atrial node tissue was unique among Ca^{2+} channel antagonists; efonidipine markedly prolonged the late phase 4 depolarization with relatively smaller effects on other action potential parameters (Masumiya et al., 1997). This could be attributed to inhibitory effects of efonidipine on both L- and T-type Ca^{2+} currents based on analogy with tetramethrin and Ni^{2+} which had inhibitory effects on the T-type Ca^{2+} current (Hagiwara et al., 1988, Satoh, 1995). The present study with guinea-pig sino-atrial node revealed that the decreases in the slope of the phase 4 depolarization

was greater with efonidipine when compared with nifedipine, suggesting that the unique effect of efonidipine on sino-atrial node action potential may be commonly observed in experimental animal species.

The T-type Ca^{2+} current is considered to contribute to the later half of the slow diastolic depolarization of sino-atrial node (Brown, 1982; Bean, 1985; Hagiwara et al., 1988; Doerr et al., 1989; Satoh, 1995). Although several non-dihydropyridine compounds have been reported to have inhibitory effects on the T-type Ca^{2+} current (Mishra and Hermsmeyer, 1994; Ertel and Ertel, 1997), dihydropyridine Ca^{2+} channel antagonists in general are reported not to or only weakly affect the T-type Ca^{2+} current (Vassort and Alvarez, 1994; Huguenard, 1996). However, some dihydropyridine Ca^{2+} channel antagonists, such as felodipine (Cohen et al., 1992) or niguldipine (Romanin et al., 1992) were reported to inhibit the T-type Ca^{2+} current, although effects on the sino-atrial node action potential have not been reported upon. In the present study, voltage clamp experiments with ventricular myocytes showed that efonidipine concentration-dependently inhibited not only the L-type Ca^{2+} current, but also the T-type Ca^{2+} current (Figs. 3 and 4). Thus, the unique change in the sino-atrial node action potential configuration by efonidipine in the rabbit (Masumiya et al., 1997) and guinea-pig (Fig. 1; Table 1), could be attributed to blockade of both L- and T-type Ca^{2+} currents by the drug. The relatively high affinity of efonidipine for T-type Ca^{2+} channels might reflect characteristics of its voltage dependence of binding to Ca^{2+} channels, which remains to be investigated.

Ca^{2+} influx through L-type Ca^{2+} channels is considered to be the primary pathway for triggering Ca^{2+} release from the sarcoplasmic reticulum and subsequent contraction of cardiac muscles. Little information is available on whether the T-type Ca^{2+} current is involved in myocardial excitation–contraction mechanisms. Our present results that 40 μM nickel had inhibitory effect on neither the Ca^{2+} transient nor the force of contraction (Fig. 4) indicate lack of contribution of T-type Ca^{2+} current to myocardial contraction. In fact, a non-dihydropyridine compound mibefradil (Ro 40-5967) was reported not to influence myocardial contractility at 1 μM (Osterrieder and Holck, 1989), a concentration at which complete inhibition of the T-type Ca^{2+} current was observed (Mishra and Hermsmeyer, 1994).

Ca^{2+} channel antagonists are widely used in the treatment of various cardiovascular disorders, such as angina pectoris, cerebral vasospasm and hypertension (Ellrodt et al., 1980; Laragh et al., 1987; Pepine et al., 1983). However, the hypotension induced by these drugs is often associated with baroreflex-mediated tachycardia. Investment of T-type Ca^{2+} current-inhibitory activity to dihydropyridine Ca^{2+} channel antagonists appears to be beneficial because such drugs would be expected to have intrinsic negative chronotropic activity through prolongation of the phase 4 depolarization of the sino-atrial node action potential which cancels the reflex acceleration of the heart rate. Judging from the present results (Fig. 4), inhibitory effects on contraction of the working myocardium would not be enhanced by the additional T-type Ca^{2+} current inhibitory activity. In fact, efonidipine, which was shown to inhibit both L- and T-type Ca^{2+} currents (Fig. 2), had been previously shown to have high (negative chronotropic potency)/(negative inotropic potency) ratio among several Ca^{2+} channel antagonists including nifedipine (Masuda et al., 1995; Tanaka et al., 1996b). When applied in vivo, the reflex tachycardia produced by efonidipine was less pronounced than that produced by equally hypotensive doses of nicardipine (Masuda et al., 1990; Sakai et al., 1991a).

One of the therapeutic goals for the treatment of cardiovascular disorders is a decrease in myocardial oxygen consumption through a decrease in heart rate (Sonnenblick and Skelton, 1971; Kobinger and Lillie, 1987; Kobinger, 1989). Bradycardia would be beneficial for patients with compromised coronary blood flow because it results in prolongation of the diastolic period, during which perfusion of the myocardium takes place. In coronary-ligated canine myocardium, efonidipine, but not nifedipine, was reported to attenuate the ischemia-induced decrease in ATP and energy charge potential through a decrease in heart rate (Yokoyama et al., 1997). Thus, investment to various drugs of bradycardiac activity through inhibition of T-type Ca^{2+} current might be beneficial because the drug could produce bradycardia without suppression of myocardial contractility.

5. Conclusions

In conclusion, efonidipine was shown to have inhibitory effects on not only L-type but also T-type Ca^{2+} current, which may explain its high selectivity for negative chronotropic effects. Investment of bradycardiac activity through inhibition of T-type Ca^{2+} current appears to be a promising strategy in the development of therapeutic agents for various cardiovascular disorders.

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