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Effects of Ca^{2+} channel antagonists on sinus node: Prolongation of late phase 4 depolarization by efonidipine

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Abstract

Effects of various Ca^{2+} channel antagonists on the action potential configuration of rabbit sino–atrial node tissue were examined with standard microelectrode techniques. All Ca^{2+} channel antagonists decreased the maximum rate of phase 0 depolarization ($\dot{V}_{max}$) and increased the cycle length. The potency order to increase the cycle length was nisoldipine = verapamil > nifedipine = clentiazem > efonidipine > diltiazem. The potency order to decrease $\dot{V}_{max}$ and to shift the threshold potential to a positive direction was the same as that to increase the cycle length, indicating that the major mechanism of negative chronotropism was inhibition of the L-type Ca^{2+} current. All Ca^{2+} channel antagonists except efonidipine shifted the maximum diastolic potential to the positive direction, decreased the action potential amplitude and prolonged the action potential duration. The effects of nifedipine were slightly weaker than those of other drugs when compared at equally bradycardiac concentrations. These differences may reflect differences in drug effects on currents other than the L-type Ca^{2+} current. A characteristic feature of efonidipine was selective suppression of the later phase of pacemaker depolarization with no effect on action potential amplitude and duration. Similar suppression of the later phase was observed with 50 μM Ni^{2+}, which is reported to inhibit the T-type, but not L-type, Ca^{2+} current. Thus, efonidipine appears to suppress selectively the later phase of pacemaker depolarization through inhibition of both L- and T-type Ca^{2+} currents, which may be the underlying mechanism for its reported potent negative chronotropic but weak inotropic activity.

Keywords: Sinus node; Efonidipine; Chronotropism; Action potential; Ca^{2+} channel antagonist; T-type Ca^{2+} channel

1. Introduction

Calcium ions play important roles in the excitation and contraction of various tissues including cardiac and smooth muscles. Ca^{2+} channel antagonists have been proven to have great therapeutic potential in the treatment of a wide range of cardiovascular disorders such as hypertension, angina pectoris and arrhythmia. One of the therapeutic goals for treatment of cardiovascular disorders is bradycardia (Kobinger and Lillie, 1987; Kobinger, 1989), which may result in long-term protection of the myocardium through a decrease in oxygen consumption (Sonnenblick and Skelton, 1971). Bradycardic effects would also be a desirable feature for Ca^{2+} channel antagonists, especially for dihydropyridine compounds, which are known to be accompanied by reflex tachycardia.

As the firing frequency of the sino–atrial node action potential is the determinant of heart rate, analysis of drug effects on the sino–atrial node action potential configuration would be essential for the understanding of chronotropic mechanisms and development of novel bradycardiac agents. The action potential of the sino–atrial node is known to be composed of several different membrane currents (Yanagihara et al., 1980; Brown, 1982; Noble, 1984; Hagiwara et al., 1988). However, relatively few reports deal with drug effects on the sino–atrial node action potential configuration (Molyvdas and Sperelakis, 1986; Satoh and Hashimoto, 1991; Satoh, 1991, 1993, 1995).

Ca^{2+} channel antagonists can be expected to posses intrinsic bradycardiac effects through inhibition of the L-type Ca^{2+} current (I_{CaL}), which largely contributes to the pacemaker depolarization of the sino–atrial node action potential. However, as Ca^{2+} channel antagonists may affect membrane currents other than I_{CaL} (Hume, 1985; Zernig, 1990), effects on the action potential configuration may differ among drugs. Therefore, in the present study, we compared in detail the effects of various Ca^{2+} channel

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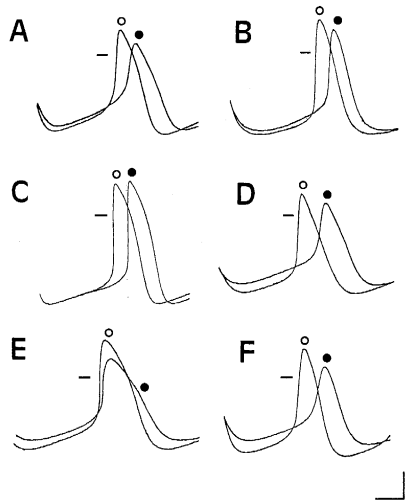

Fig. 1. Typical action potential traces of spontaneously beating sino–atrial node preparations before (open circles) and after (closed circles) the addition of 100 nM nifedipine (A), 10 nM nisoldipine (B), 100 nM efonidipine (C), 100 nM verapamil (D), 1 μM diltiazem (E), 100 nM clentiazem (F). Horizontal and vertical bars indicate 50 ms and 20 mV, respectively.

antagonists on the action potential configuration of rabbit sino–atrial node tissue using standard microelectrode techniques.

2. Materials and methods

Microelectrode experiments were performed as described previously (Satoh, 1991). Male rabbits weighing 2 to 3 kg were anesthetized with sodium pentobarbital (30 mg/kg) and the hearts were quickly isolated. Tissue including the sino–atrial node were cut perpendicularly to the crista terminalis into strips of about 1×3 mm. Preparations were pinned down horizontally on a silicon block in a 20 ml organ bath containing physiological salt solution of the following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 24.9; glucose, 11.1 (gassed with 95%O_2–5%CO_2, pH 7.4). The temperature of the organ bath was maintained at 35.5°C. After the preparations had been allowed to equilibrate for more than 1 h, penetration of microelectrodes filled with 3 M KCl (resistance, 20 to 50 MΩ) was made into dominant pacemaker cells. Criteria for pacemakers were (1) maximum diastolic potential less than −70 mV, (2) smooth transition between phase 4 depolarization and take-off of action potential and (3) low maximum rate of rise ($\dot{V}_{max}$) of action potential, less than 15 V/s. The output of the microelectrode amplifier (MEZ-8201, Nihon Kohden, Tokyo, Japan) was monitored through a dual-beam cathode ray oscilloscope (VC-11, Nihon Kohden) and fed into an AD converter (Analog Pro, Canopus, Kobe, Japan) attached to a computer (PC 9801 DA2, NEC, Tokyo, Japan) for the analyses. Action potential parameters measured were cycle length, maximum rate of rise, threshold potential, maximum diastolic potential, action potential amplitude, action potential duration at 50% amplitude, 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.

Effects of drugs were examined after a control period of 15–20 min. In some figures, drug-induced changes in parameters were plotted versus the % increase in cycle length in order to elucidate the qualitative differences in drug effects. All drugs were dissolved in dimethylsulfoxide, and small aliquots of drug solution were added to the organ bath to yield the desired final concentrations. Dimethylsulfoxide alone, less than 0.05%, had no effect on the parameters measured. Nisoldipine was supplied by Bayer Japan and efonidipine by Nissan Chem. All other drugs and chemicals were commercial products of the highest available quality of grade.

3. Results

3.1. Action potential configuration of SA node

Typical action potential recordings are presented in Fig. 1. Action potential parameters were consistent with those of previous reports. The mean ± S.E.M of action potential parameters from 48 preparations were: cycle length, 369 ± 7.8 ms; V_{max}, 9.8 ± 0.6 V/s; threshold potential, −39.9 ±

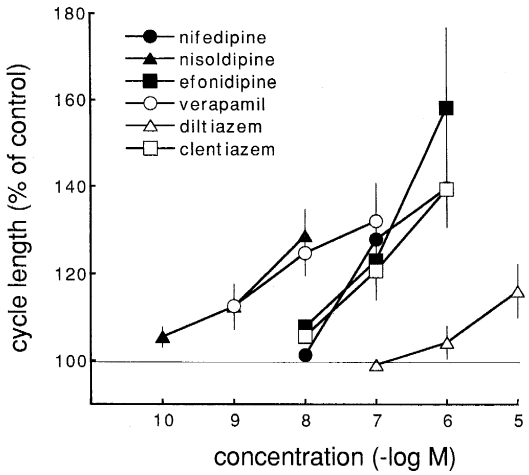

Fig. 2. Concentration–response curves for the effects of Ca^{2+} channel antagonists on cycle length. Cycle length in the presence of various concentrations of nifedipine (closed circles), nisoldipine (closed triangles), efonidipine (closed squares), verapamil (open circles), diltiazem (open triangles) and clentiazem (open squares) is expressed as a percentage of basal values. Each symbol with bars represents the mean ± S.E.M. of five to six experiments.

0.7 mV; maximum diastolic potential, -58.9 ± 0.3 mV; amplitude 81.9 ± 1.4 mV; action potential duration at 50% repolarization, 85.0 ± 1.9 ms; slope of the early phase 4 depolarization, 62.3 ± 2.8 mV/s; slope of the late phase 4 depolarization, 89.8 ± 3.5 mV/s.

3.2. Effect of drugs on cycle length

Typical action potential recordings before and after addition of drugs at concentrations which produced 20 to 30% increases in cycle length are presented in Fig. 1. All the Ca^{2+} channel antagonists examined increased the cycle length (decreased the heart rate; Fig. 2). The potency order was nisoldipine = verapamil > nifedipine = clentiazem = efonidipine > diltiazem. As the changes in action potential configuration induced appeared to be different between drugs, further analyses were performed to clarify the action potential parameters responsible for the observed changes in cycle length caused by each drug.

3.3. Effect on $\dot{V}_{max}$

All the Ca^{2+} channel antagonists examined decreased the $\dot{V}_{max}$, indicating inhibition of L-type Ca^{2+} channels (Fig. 3A; Table 1). The potency order was nisoldipine > verapamil > nifedipine = clentiazem > efonidipine = dilti-

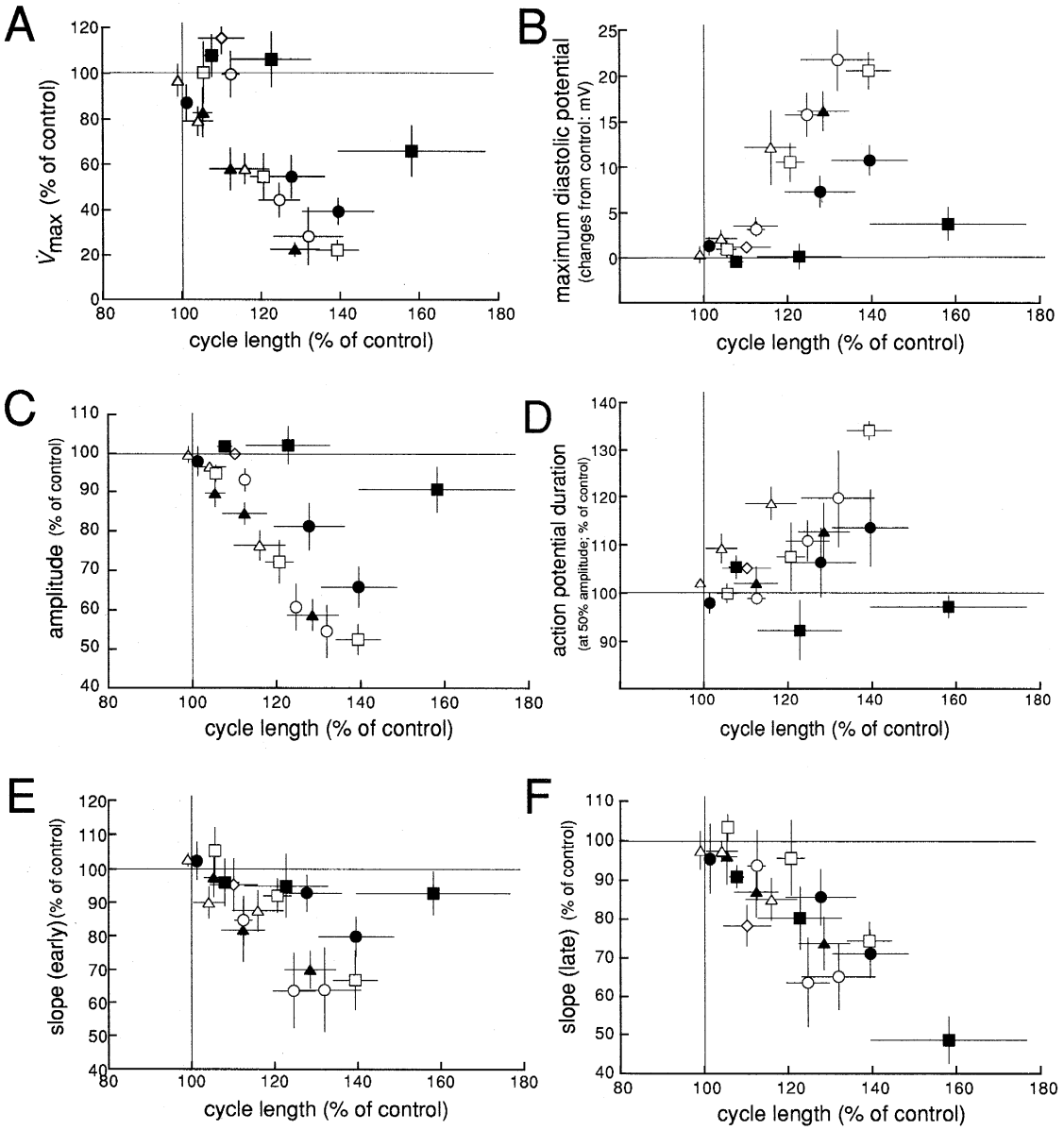

Fig. 3. Effects of Ca^{2+} channel antagonists on action potential parameters. $\dot{V}_{max}$ (A), maximum diastolic potential (B), action potential amplitude (C), action potential duration at 50% repolarization (D), slope of early phase 4 depolarization (E) and slope of late phase 4 depolarization (F) in the presence of various concentrations of nifedipine (closed circles), nisoldipine (closed triangles), efonidipine (closed squares), verapamil (open circles), diltiazem (open triangles), clentiazem (open squares) and 50 μM Ni^{2+} (open diamonds) were expressed as percentages of basal values and plotted against the corresponding cycle length expressed as a percentage of the basal value. Each symbol with bars represents the mean ± S.E.M. of five to six experiments.

Table 1
Effect of Ca^{2+} antagonists on action potential parameters

Drug	Concentration (μM)	$\dot{V}_{max}$ (%)	Positive shift in MDP (mV)	Action potential amplitude (%)	Positive shift in threshold (mV)	Action potential duration (%)	Slope (early) (%)	Slope (late) (%)
Nifedipine	0.01	87.1±7.8	1.3±1.0	97.9±3.8	2.4±1.5	97.9±2.1	102.4±5.5	95.5±8.7
	0.1	54.5±9.3	7.3±1.7	81.2±5.9	7.4±1.4	106.4±7.1	92.9±5.5	85.7±7.2
	1	39.3±5.7	10.8±1.6	65.8±5.2	7.8±1.4	113.6±8.0	79.9±5.8	71.1±6.7
Nisoldipine	0.0001	82.8±10.7	1.0±0.8	89.6±3.5	5.0±1.5	100.0±2.0	97.5±5.8	96.1±7.0
	0.001	58.0±9.2	3.6±0.9	84.5±2.7	3.6±0.8	102.0±3.6	81.7±9.3	87.1±6.5
	0.01	22.4±3.0	16.2±2.1	58.7±3.9	12.0±2.2	112.8±6.0	70.0±5.6	73.8±6.8
Efonidipine	0.01	107.6±9.1	−0.4±0.5	101.9±1.3	2.1±1.2	105.4±2.4	95.5±7.1	91.0±2.9
	0.1	107.1±10.7	0.2±1.2	102.2±4.9	6.2±1.4	95.5±5.6	95.0±9.4	80.3±8.0
	1	65.9±11.2	3.8±1.8	90.6±5.8	13.3±2.9	97.2±2.3	92.9±6.4	48.7±6.0
Verapamil	0.001	99.6±9.9	3.2±0.8	93.6±2.9	−0.7±1.8	98.9±0.9	84.6±7.3	93.8±8.8
	0.01	44.2±7.4	15.8±2.3	60.7±5.8	11.8±1.6	110.9±4.1	63.6±11.3	63.6±11.5
	0.1	28.3±12.6	21.8±3.4	54.6±6.6	18.5±2.3	119.8±10.1	63.9±12.7	63.1±8.5
Diltiazem	0.1	96.9±6.9	0.4±0.9	99.7±2.1	1.8±1.4	102.0±0.8	102.8±1.9	97.6±4.7
	1	79.8±6.2	2.2±0.9	96.5±1.0	2.6±1.4	109.3±3.0	90.0±4.7	97.5±2.5
	10	58.0±6.6	12.2±4.1	76.4±3.7	5.1±1.8	118.7±3.3	87.5±6.1	85.1±5.5
Clentiazem	0.01	100.5±13.1	1.0±0.6	94.8±2.1	0.6±1.5	99.9±0.9	105.4±6.5	103.3±3.3
	0.1	54.6±9.9	10.6±2.2	72.2±5.4	7.9±1.3	107.6±7.0	92.1±5.1	95.7±9.4
	1	22.1±4.4	20.6±2.0	52.5±3.9	12.4±3.1	134.2±1.9	66.8±8.9	74.5±4.8
Nickel	50	115.2±6.8	1.2±0.5	99.9±0.6	2.4±1.0	105.2±0.8	95.3±7.8	78.3±5.3

$\dot{V}_{max}$, action potential amplitude, action potential duration and slopes in the presence of drugs were expressed as percentages of control values. Shifts in the maximum diastolic potential (MDP) and threshold potential to the positive direction produced by drugs were expressed in mV. Values are the means±S.E.M. of five to six experiments.

azem, which was similar to that for the increase in cycle length. In the case of nisoldipine, nifedipine, clentiazem and diltiazem the concentration to produce a decrease in $\dot{V}_{max}$ was almost the same as needed to produce an increase in cycle length. In contrast, 1 nM verapamil and 100 nM efonidipine produced increases in cycle length with little effect on $\dot{V}_{max}$. The $\dot{V}_{max}$-cycle length plot for most drugs showed a close correlation between increase in cycle length and $\dot{V}_{max}$ (Fig. 3A). The points for 1 nM verapamil, 100 nM efonidipine and 1 μM efonidipine lie above the points for other drugs, indicating that the contribution of changes in other action potential parameters are larger for these drugs.

3.4. Effect on threshold potential

All the Ca^{2+} channel antagonists examined shifted the threshold potential to the positive direction (Table 1). The potency order was nisoldipine = verapamil > nifedipine = clentiazem = efonidipine > diltiazem, which was the same as that for the increase in cycle length.

3.5. Effect on maximum diastolic potential

All the Ca^{2+} channel antagonists examined, except efonidipine, decreased the maximum diastolic potential (Fig. 3B; Table 1). The potency order was the same as those for cycle length and $\dot{V}_{max}$. The effect of efonidipine was weak: 100 nM efonidipine, which increased cycle length by 20%, had no effect on maximum diastolic potential. The maximum diastolic potential-cycle length plot revealed that the effect of nifedipine on maximum diastolic potential was weaker than that of nisoldipine, verapamil, clentiazem and diltiazem at equally bradycardiac concentrations (Fig. 3). The data points for efonidipine located near the cycle length axis indicate minimum effect of the drug on the maximum diastolic potential.

3.6. Effect on action potential amplitude

All the Ca^{2+} channel antagonists examined except efonidipine decreased the action potential amplitude (Fig. 3C; Table 1). The potency order was the same as those for cycle length and $\dot{V}_{max}$. Efonidipine, even at 1 μM, which produced a 60% increase in cycle length, had no effect on amplitude. This is also apparent from the amplitude–cycle length plot (Fig. 3C) where the data points for efonidipine are located far above those for other drugs. The plot also suggests that the effect of nifedipine on action potential amplitude may be weaker than the effects of nisoldipine, verapamil and clentiazem at equally bradycardiac concentrations.

3.7. Effect on action potential duration

All the Ca^{2+} channel antagonists examined, except efonidipine, prolonged the action potential duration (Fig. 3D; Table 1). Efonidipine produced a slight shortening of the action potential. The action potential duration–cycle length plot (Fig. 3D) showed that the prolongation by diltiazem, clentiazem and verapamil was relatively strong when compared with that by nifedipine. Data points for efonidipine were at or just below the cycle length axis, indicating that efonidipine prolongs the cycle length through mechanisms other than prolongation of action potential duration.

3.8. Effect on slope

All Ca^{2+} channel antagonists prolonged the slope of phase 4 depolarization, but the selectivity for the early (Fig. 3E; Table 1) and late (Fig. 3F; Table 1) phases were different between drugs. Nisoldipine, nifedipine, verapamil, diltiazem and clentiazem decreased the slope of both phases equally (Fig. 4; Table 1). The potency order for these drugs was nisoldipine = verapamil > nifedipine = clentiazem > diltiazem, which was the same as that for cycle length and $\dot{V}_{max}$. Efonidipine had minimum effects on the slope of the early phase but markedly decreased that of the late phase (Fig. 3E, F, Fig. 4; Table 1). Similarly, 50 μM Ni^{2+}, which is reported to inhibit the T-type Ca^{2+} current (I_{CaT}), had little effect on the slope of the early phase but decreased that of the late phase (Fig. 3E, F, Fig. 4; Table 1). The slope–cycle length plot (Fig. 3E, F) revealed that inhibitory effects on the late phase but not on the early phase contribute to the increase in cycle length in the cases of efonidipine and Ni^{2+}, while effects on both phases are important for the other drugs examined. Typical

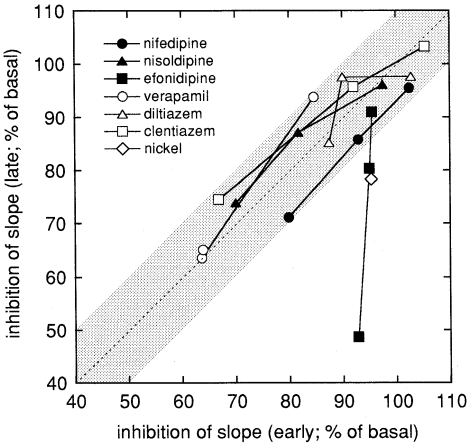

Fig. 4. Correlation of inhibitory effects on the early and late phase of pacemaker depolarization. Inhibition of slope (early) and slope (late) produced by various concentrations of nifedipine (closed circles), nisoldipine (closed triangles), efonidipine (closed squares), verapamil (open circles), diltiazem (open triangles) clentiazem (open squares) and 50 μM Ni^{2+} (open diamonds) expressed as percentages of basal values were plotted. While most of the drugs decreased slope (early) and slope (late) almost equally (shadowed region), efonidipine and 50 μM Ni^{2+} selectively decreased slope (late). Each symbol represents the mean of five to six experiments.

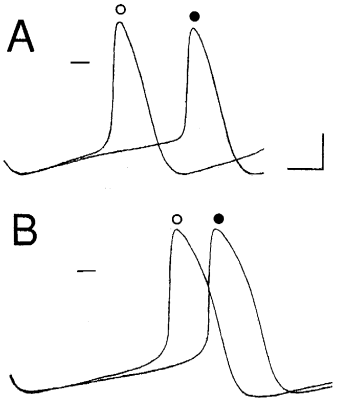

Fig. 5. Typical action potential traces of spontaneously beating sino–atrial node preparations before (open circles) and after (closed circles) the addition of 1 μM efonidipine (A) and 50 μM Ni^{2+} (B). Note that both agents prolonged the later phase of phase 4 depolarization with little effect on amplitude and action potential duration. Horizontal and vertical bars indicate 50 ms and 20 mV, respectively.

traces showing the effects of efonidipine and Ni^{2+} are presented in Fig. 5.

4. Discussion

All the Ca^{2+} channel antagonists examined in the present study decreased the $\dot{V}_{max}$ of action potentials (Figs. 1 and 3A). The potency order of Ca^{2+} channel antagonists for decreasing $\dot{V}_{max}$, as well as that for shifting the threshold potential to a positive direction was the same as that for increasing cycle length (Figs. 2 and 3). The order was the same as that for inhibition of I_{CaL} in guinea-pig ventricular cardiomyocytes in our preliminary experiments (Masumiya et al., 1996). Thus, a major fraction of the increase in cycle length by Ca^{2+} channel antagonists could be attributable to inhibition of I_{CaL} and the resulting decrease in $\dot{V}_{max}$. However, the changes in action potential configuration were different between Ca^{2+} channel antagonists (Fig. 1) suggesting that factors other than inhibition of I_{CaL} may also be involved in the prolongation of cycle length.

The decrease in maximum diastolic potential, as well as the increase in action potential duration caused by diltiazem and verapamil might be related to inhibition of the delayed rectifier potassium current. In guinea-pig ventricular myocardium, diltiazem and verapamil slightly prolonged the time required for 90% repolarization of the action potential, while shortening was observed with nifedipine and nisoldipine (Nakaya et al., 1988). In frog atrial cells, the inhibitory effect of nisoldipine on the Ca^{2+} current was greater than that on the I_K, while diltiazem and verapamil inhibited these currents less selectively (Hume, 1985). In our preliminary experiments (Masumiya et al., 1996), 1 μM nifedipine, but not 1 μM efonidipine, had weak inhibitory effects on I_K.

Multiple membrane currents such as I_{Ca} (L- and T-types), I_K, I_f and a background inward current, are considered to contribute to the slow diastolic depolarization of SA nodal cells (Yanagihara et al., 1980; Brown, 1982; Noble, 1984; Hagiwara et al., 1988). The I_{CaT} is present in various excitable cells, including the rabbit SA node, and has been considered to play an important role in late phase 4 depolarization (Bean, 1985; Hagiwara et al., 1988; Doerr et al., 1989; Satoh, 1995). Ni^{2+} (10 to 100 μM), which selectively inhibits the I_{CaT}, was demonstrated to prolong the later half of phase 4 depolarization with little influence on other action potential parameters in rabbit SA nodal cells (Hagiwara et al., 1988; Satoh, 1995). Our present results with Ni^{2+} in SA nodal tissue preparations agree with these previous reports. The effect of efonidipine to prolong the late phase 4 depolarization was similar to that of Ni^{2+}, suggesting that efonidipine may have inhibitory effects on I_{CaT}. Dihydropyridine Ca^{2+} channel antagonists in general are reported to not or only weakly affect I_{CaT} (reviewed by Vassort and Alvarez, 1994). However, some dihydropyridine Ca^{2+} channel antagonists, such as felodipine (Cohen et al., 1992) or niguldipine (Romanin et al., 1992) were reported to inhibit I_{CaT}, although effects on the SA node have not been reported upon. Inhibition of Ca^{2+} current by efonidipine was confirmed in a cultured smooth muscle cell line (Tamura et al., 1991). In our preliminary study with isolated guinea-pig ventricular cells (Masumiya et al., 1996), efonidipine inhibited both I_{CaL} and I_{CaT} with roughly equal potencies while nifedipine selectively inhibited the I_{CaL}. Whether efonidipine affects other membrane currents which may be involved in phase 4 depolarization such as I_f (Maylie et al., 1981) or the recently reported sustained inward current (Guo et al., 1995) awaits further investigation.

Previous reports, including those from our laboratory (Masuda et al., 1995; Tanaka et al., 1996), demonstrated that the negative chronotropic potency of efonidipine, a dihydropyridine phosphonate, was much greater than its negative inotropic potency. This may be, at least partly, explained by the high potency of efonidipine to prolong the phase 4 depolarization. One of the therapeutic goals for the treatment of cardiovascular disorders is a decrease in myocardial oxygen consumption through a decrease in heart rate (Kobinger and Lillie, 1987; Kobinger, 1989). The expected benefit of a decreased heart rate for patients with compromised coronary blood flow would be pronounced when the bradycardia is mainly achieved by prolongation of the diastolic period, during which perfusion of the myocardium takes place. Thus, drugs with profiles similar to that of efonidipine, which prolongs the phase 4 depolarization phase with little influence on the action potential duration and amplitude, might be of benefit in the treatment of cardiovascular disorders. In vivo experiments revealed that the reflex tachycardia produced by efonidipine is less pronounced than that produced by equally hypotensive doses of nicardipine (Masuda et al.,

1990; Sakai et al., 1991b) and that 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., 1991a; reviewed by Masuda and Tanaka, 1994).

In summary, the present results indicated that, although the major mechanism of negative chronotropism by Ca^{2+} channel antagonists is inhibition of I_{CaL}, drug effects on other membrane currents may result in a differential action potential configuration of the sino–atrial node. Efonidipine appeared to selectively suppress the later phase of pacemaker depolarization through inhibition of I_{CaT}, which may be the underlying mechanism for its potent negative chronotropic but weak inotropic activity.

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