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Effects of calcium antagonistic drugs on the electrical activity of rabbit sino-atrial node

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1 The effects of several calcium antagonistic drugs (slow channel blockers), mesudipine, FR-34235 (nilvadipine), nifedipine, and verapamil, were compared on the naturally-occurring slow action potentials (APs) of the isolated spontaneously-contracting sinoatrial (SA)-node of the rabbit.

2 Mesudipine, at 1×10^{-8} M, had little or no effect on the AP parameters and beating frequency. At 1×10^{-7} M, mesudipine depressed the amplitude, + $\dot{V}_{max}$ and frequency of the APs. A higher concentration of mesudipine (3×10^{-7} M) completely blocked the slow APs.

3 The concentration of FR-34235 required for 50% depression of $\dot{V}_{max}$ was 3×10^{-8} M, and complete block required 1×10^{-7} M.

4 The verapamil and nifedipine concentrations required for complete block were 1×10^{-6} M and 1×10^{-7} M, respectively.

5 The cells blocked by these drugs were depolarized to about -40 mV.

6 Cells blocked and depolarized by the drugs responded to intensive field stimulation with a transient after-hyperpolarization, followed by damped oscillations.

7 When $[Ca]_o$ was elevated from 1.8 mM to 5.4 mM, full block required a higher concentration of mesudipine (1×10^{-6} M) and FR-34235 (1×10^{-6} M).

8 The order of potency of the drugs tested was: nifedipine = FR-34235 > mesudipine > verapamil.

9 The effects of mesudipine, FR-34235, and nifedipine were easily reversible upon washout for 10 min, whereas those of verapamil required longer periods.

10 The results indicate that the slow channel blockers, mesudipine, FR-34235, nifedipine, and verapamil depress the APs and automaticity of the SA-node.

Introduction

Calcium antagonists (Ca^{2+} entry blockers) depress the slow inward current and produce excitation-contraction uncoupling in myocardial cells (Kohlhardt *et al.*, 1972; Shigenobu *et al.*, 1974; Kohlhardt & Fleckenstein, 1977; Vogel *et al.*, 1979). These drugs also depress action potentials (APs) and contraction of vascular smooth muscle (Haeusler, 1972; Harder & Sperelakis, 1978; 1981; Mras & Sperelakis, 1981a,b; Sperelakis & Mras, 1983). Verapamil and bepridil, in addition, block the slow Na^+ current of young embryonic chick hearts (Shigenobu *et al.*, 1974; Kojima & Sperelakis, 1983); the slow Na^+ channel may be a different channel type from the Ca^{2+} channel. The tissue specificity of these drugs and their mechanism of action are not similar (Scheuer & Kass, 1982). Bepridil may have a second site of action intracellularly, such as to depress the Ca^{2+} release from the sarcoplasmic reticulum (SR) (Vogel *et al.*, 1979). The blocking effects of these drugs depends on the stimulation frequency, i.e., they have a frequency- or use-dependency (Tritthart *et al.*, 1976; Kohlhardt & Fleckenstein, 1977; Vogel *et al.*, 1979). The frequency dependency of the dihydropyridine drugs is the least prominent. Analogues of nifedipine, such as mesudipine (Prous *et al.*, 1981), block the slow channels of guinea-pig papillary muscle and Purkinje fibres (Molyvdas & Sperelakis, 1983a,b).

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The study of the effects of slow channel blockers on the primary pacemaker is important because the slow inward current (I_{si}) is the only component of the action potential upstroke (Noma *et al.*, 1978), and drugs that depress I_{si} might be expected to have profound effects on the electrical activity of the sino-atrial (SA) nodal cells. The I_{si} is carried by both Ca^{2+} and Na^+ ions (Noma & Irisawa, 1976; Reuter, 1979), and has properties similar to those of the slow inward channel described by Shigenobu *et al.* (1974) in 3-day-old embryonic chick heart. Furthermore, it has been suggested that I_{si} participates in the pacemaker potential of the SA node (Yanagihara & Irisawa, 1980). The importance of I_{si} for the pacemaker potential has been questioned recently by other investigators, who present evidence that the current during the nodal pacemaker potential is a mixed current (Na-K) similar to that of Purkinje fibres, and that I_{si} participates in the final stage of the pacemaker potential (Brown & Di Francesco, 1980; Brown, 1982; Osterrieder *et al.*, 1982; Grant & Strauss, 1982). It was recently shown that nisoldipine, another analogue of nifedipine, depresses automaticity, and that acetylcholine depresses automaticity by depressing I_{si} (Shibata *et al.*, 1985). The purpose of the present study was to compare the effects of several slow-channel blockers, including mesudipine, FR-34235 (nilvadipine) (another dihydropyridine analogue of nifedipine), nifedipine, and verapamil, on isolated rabbit SA node electrical activity.

Methods

New Zealand rabbits weighing 2.0–2.5 kg were anaesthetized with sodium thiopentone (25 mg kg^{-1}), and the heart was quickly removed. The region of the right atrium bounded by the crista terminalis, the superior and inferior vena cavae, and the interatrial septum was dissected free from the adjacent tissues in Tyrode solution at room temperature. Two or three strips of the central region of the SA node were dissected with the help of a Zeiss dissecting microscope. The strips were cut perpendicular to the crista terminalis and were 4–7 mm long and 400–500 μm wide. Three ligatures 350–450 μm apart were applied, approximately 1–1.5 mm medial to the crista terminalis. The preparations that resulted were about 400 μm square (Noma & Irisawa, 1976; Seyama, 1979; Grant & Strauss, 1982).

These preparations were fixed with pins to the Sylgard resin (Dow Corning) base of a perfusion chamber (1.5 ml volume), and were allowed to recover and equilibrate for 1 h. The tissues were perfused with Tyrode solution at a rate of 5 ml min^{-1}. The composition of normal Tyrode solution (in mM) was: Na^+ 141, K^+ 2.7, Ca^{2+} 1.8, Mg^{2+} 0.76, Cl^- 124, $H_2PO^-_4$ 1.7, HCO^-_3 25 and glucose 11. In some experiments, the Ca^{2+} concentration was elevated to 5.4 mM. The temperature was maintained at 36.5 ± 0.5°C, and the medium was gassed with a mixture of 95% O_2 and 5% CO_2.

The preparations (when not spontaneously beating) were stimulated through field electrodes, positioned on either side of the preparation. Rectangular current pulses, 2 ms in duration, were applied at a frequency of 0.5 Hz.

The transmembrane potentials were recorded by microelectrodes filled with 3 M KCl (resistance of 20–30 MΩ). Ag:AgCl half-cells were used. The preamplifier (WPI Model 750) had a high input-impedance and had capacitance compensation. The action potentials (APs) were differentiated by an operational amplifier (Tektronix model 501). The traces were displayed on a storage oscilloscope (Tektronix model 5111), and photographed.

Following the recording of control APs, a calcium antagonistic drug was added. The effects of the experimental drugs, mesudipine (10^{-8}, 10^{-7}, and 3×10^{-7} M) and FR-34235 (nilvadipine) (10^{-8}, 3×10^{-8}, 10^{-7} M) on the SA node APs were compared with those of verapamil (10^{-6} M) and nifedipine (10^{-7} M). Nifedipine and mesudipine are photolabile, so care was taken to protect them from light. FR-34235 is not sensitive to light. The structure of mesudipine is given in the article by Prous *et al.* (1981). Nivaldipine is 5-isopropyl 3-methyl 2-cyano-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate.

Results

Effects of mesudipine on true pacemaker cells of sino-atrial node

In 1.8 mM $[Ca]_o$ The action of mesudipine on the normal slow action potentials (APs) of isolated SA node preparations, superfused with normal Ringer solution, is illustrated in Figure 1. Following a 20 min recovery period after the impalement, slowly-rising APs were recorded that sometimes were overshooting (a). Addition of mesudipine, 10^{-8} M, had no significant effect on the AP parameters and beating frequency (b). At 10^{-7} M, the AP amplitude and $+ \dot{V}_{max}$ were markedly depressed (c). Elevation of the mesudipine concentration to 3×10^{-7} M rapidly (within 3–6 min) depressed the amplitude and the $+ \dot{V}_{max}$ of the APs (d,e), and completely blocked spontaneous firing after 10–12 min (f). The cells remained quiescent and were depolarized to a resting potential of about 35–40 mV. Washout of the drug for 10–15 min restored the APs and automaticity almost to the normal (g).

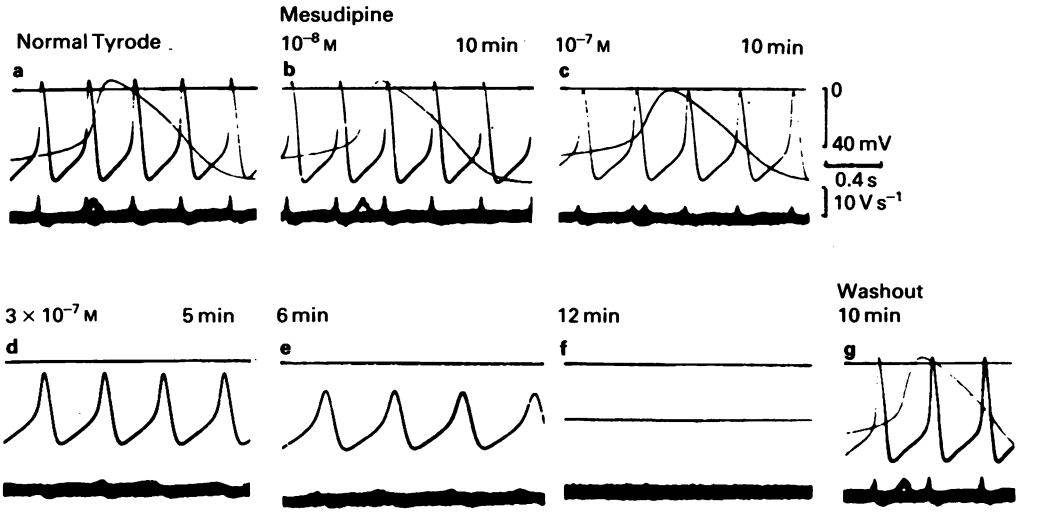

Figure 1 Effect of the calcium antagonistic drug, mesudipine, on the naturally-occurring slow action potentials (APs) of a spontaneously-firing isolated SA node of rabbit. (a) Control APs in normal Ringer solution. (b) Addition of 1×10^{-8} M mesudipine had little or no effect on the AP amplitude, $\dot{V}_{max}$, and slope of the diastolic depolarization. (c–f) Elevation of the mesudipine concentration to 1×10^{-7} M (c) and 3×10^{-7} M (d–f) depressed the amplitude and + $\dot{V}_{max}$ of the APs, and abolished (f) the APs within 12 min. (g) Washout of the drug restored the spontaneous APs within 10 min. Upper solid line represents zero potential. Lower trace is dV/dt, the peak excursion of which gives the maximal upstroke velocity (+ $\dot{V}_{max}$). Time calibration bar represents 40 ms for the superimposed faster sweeps (10 times faster). All records are from the same impalement.

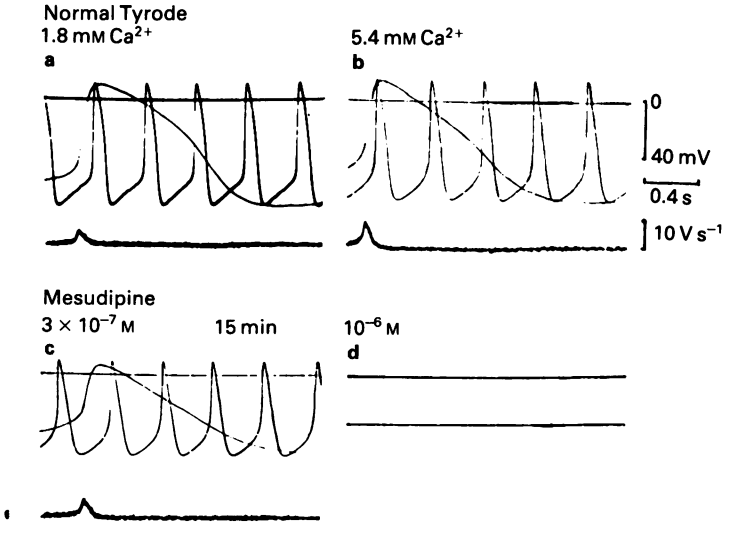

Figure 2 Partial antagonism of the effect of mesudipine on the slow APs of a spontaneously-firing SA-nodal cell by high (5.4 mM) $[Ca]_o$. (a) Control slow APs in normal $[Ca]_o$ of 1.8 mM. (b) Potentiated slow APs in elevated $[Ca]_o$ (5.4 mM). + $\dot{V}_{max}$ and amplitude of the APs were increased. (c) Addition of 3×10^{-7} M mesudipine depressed the amplitude and + $\dot{V}_{max}$ of the APs, but did not abolish them (as occurred in 1.8 mM $[Ca]_o$. (d) Increase in the mesudipine concentration to 1×10^{-6} M completely blocked the APs. Upper solid line is the zero potential. Lower trace is the dV/dt. Time calibration for the superimposed faster sweeps is 40 ms. All records are from the same impalement.

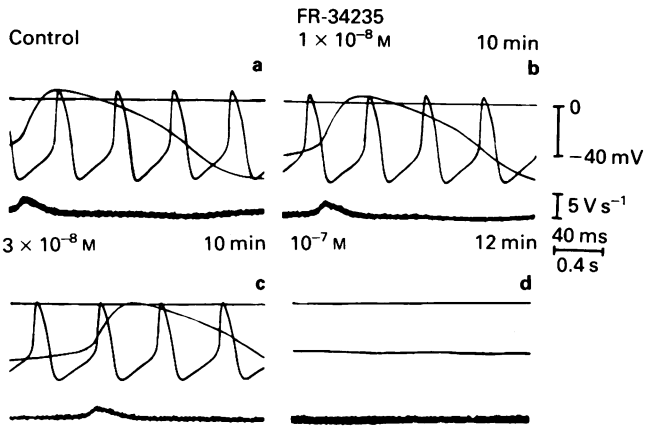

Figure 3 Effect of compound FR-34235 on the naturally-occurring slow action potentials (APs) of a isolated spontaneously-firing SA node of rabbit. (a) Control APs in normal Ringer solution. (b) Addition of 1×10^{-8} M FR-34235 had little or no effect on the AP amplitude and $\dot{V}_{max}$. (c–d) Elevation of the FR-34235 to 3×10^{-8} M (c) and 1×10^{-7} M (d) depressed the amplitude and $+\dot{V}_{max}$ of the APs (c), and abolished (d) the APs within 12 min. All records are from the same impalement.

In 5.4 mM $[Ca]_o$ In order to determine whether the blocking effect of mesudipine was a function of $[Ca]_o$, three experiments were performed in which the $[Ca]_o$ was increased to 5.4 mM before the addition of the drug. One of these experiments is illustrated in Figure 2. Mesudipine, at 3×10^{-7} M, diminished the AP amplitude and $+\dot{V}_{max}$ after 10 min of equilibration with the drug (c). Complete blockade of the APs occurred at 10^{-6} M (d). The cell remained quiescent and was depolarized to about −35 mV. The effects of mesudipine on SA node cells are summarized in Table 1. When $[Ca]_o$ was increased from 1.8 mM to 5.4 mM, the blocking effect of mesudipine was shifted 0.5 log unit to the right. Part of this effect may be due

Table 1 Summary of the effects of mesudipine on the action potentials of rabbit SA-nodal cells

Preparation	*Mesudipine Conc.* (M)	n	$+\dot{V}_{max}$ (Vs^{-1})	*Amplitude* (mV)	APD_{50} (ms)	*Frequency* (min^{-1})	*Slope of diastolic depol.* (mVs^{-1})
Pacemaker cells (1.8 mM $[Ca]_o$)	0	8	5.0 ± 0.5	79 ± 5	56 ± 4	197 ± 10	120 ± 20
	10^{-8}	8	4.1 ± 0.6	77 ± 5	56 ± 4	190 ± 10	110 ± 16
	10^{-7}	8	2.4 ± 0.5**	65 ± 4*	58 ± 5	173 ± 11	93 ± 20
	3×10^{-7}	8	0**	0**	0**	0**	0**
	Washout	4	4.0 ± 0.1	71 ± 2	57 ± 3	175 ± 2	90 ± 5
Pacemaker cells (5.4 mM $[Ca]_o$)	0	3	9.0 ± 1	73 ± 4	72 ± 4	187 ± 3	80 ± 5
	3×10^{-7}	3	5.6 ± 0.3	69 ± 3	68 ± 2	152 ± 9	66 ± 10
	10^{-6}	3	0**	0**	0**	0**	0**
Latent pacemaker cells (1.8 mM $[Ca]_o$)	0	5	9.0 ± 2.6	76 ± 9	69 ± 5	203 ± 25	81 ± 20
	10^{-7}	5	6.4 ± 2.1	68 ± 10	69 ± 6	172 ± 5	72 ± 10
	10^{-6}	5	0**	0**	0**	0**	0**

Data given as mean ± s.e. *n* gives the number of preparations. The data were obtained from 16 preparations. For each experiment, the same cell was studied throughout the duration of the experiment.
*$P < 0.05$; **$P < 0.01$.

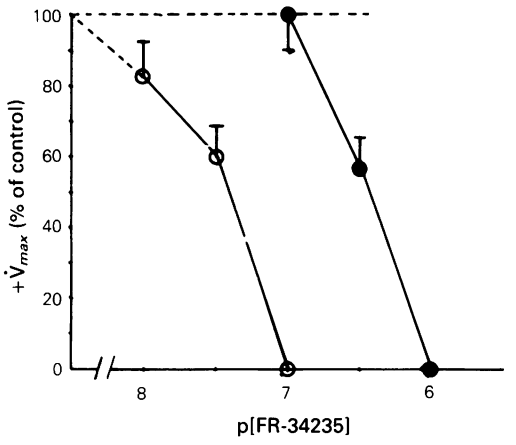

Figure 4 Summary of the effects of compound FR-34235 on the + $\dot{V}_{max}$ of naturally-occurring slow APs of isolated SA-node of rabbit in the presence of 1.8 mM Ca^{2+} (○) and 5.4 mM Ca^{2+} (●). In high $[Ca]_o$ the dose-response curve was shifted to the right.

to the increased electrochemical driving force for Ca^{2+} influx through any unblocked Ca^{2+} channels.

Effects of FR-34235 on true pacemaker cells

FR-34235 (nilvadipine), like mesudipine, is a dihydropyridine analogue, but FR-34235 is not photosensitive. The effects of FR-34235 on the APs of the SA-node were similar to those of mesudipine, and are illustrated in Figure 3, and summarized in Figure 4 and Table 2. Addition of 1×10^{-8} M FR-34235 slightly depressed the amplitude and + $\dot{V}_{max}$ of the APs. Increase of the concentration of FR-34235 depressed at 3×10^{-8} M (Figure 3c; Table 2) and blocked at 1×10^{-7} M (Figure 3d; Table 2). The cells, when blocked, became depolarized to a resting potential of about − 40 mV.

The effects of FR-34235 were antagonized by elevated Ca^{2+} concentration. When the Ca^{2+} concentration was increased from 1.8 mM to 5.4 mM, the dose-response curve for the FR-34235 was shifted to the right by about 1 log unit (Figure 4, Table 2).

Effects of nifedipine and verapamil on true pacemaker cells

In Figure 5, the effects of mesudipine (upper row) are compared with those of nifedipine (middle row), and verapamil (lower row). The effects of nifedipine and verapamil were tested on three preparations each. Nifedipine, at 10^{-7} M, blocked the action potentials and automatic activity of the SA node within 6 min. Nifedipine and FR-34235 were the most potent drugs tested. Mesudipine is less potent than nifedipine, but more potent than verapamil; FR-34235 was about equipotent with nifedipine. The blocking effects of mesudipine, nifedipine, or FR-34235 were readily reversible after 10 min washout of the drug; washout of verapamil for 20 min did not restore automatic activity.

Effect of mesudipine on latent SA node cells

SA node cells with + $\dot{V}_{max}$ higher than 10 Vs^{-1} and a discontinuous takeoff were considered as latent pacemaker cells. Such action potentials were recorded from preparations close to the crista terminalis or from preparations relatively large in size. When the impalement was close to the crista terminalis, the dose

Table 2 Summary of the effects of FR-34235 (nilvadipine) on the action potentials of rabbit SA-nodal cells

FR-34235 Conc. (M)	$[Ca]_o$ (mM)	n	+ $\dot{V}_{max}$ (Vs^{-1})	*Ampl.* (mV)	APD_{50} (ms)	*Frequency* (min^{-1})
0	1.8	7	3.0 ± 0.3	72 ± 3	87 ± 7	163 ± 12
1×10^{-8}	1.8	7	2.5 ± 0.3	70 ± 3	86 ± 8	155 ± 11
3×10^{-8}	1.8	7	1.8 ± 0.3*	58 ± 4	87 ± 8	138 ± 9
1×10^{-7}	1.8	7	0**	0**	0**	0**
0	5.4	3	7.0 ± 1.0	86 ± 5	84 ± 7	158 ± 9
1×10^{-7}	5.4	3	7.0 ± 0.7	86 ± 3	86 ± 6	107 ± 12
3×10^{-7}	5.4	3	4.0 ± 0.6*	68 ± 3	86 ± 3	73 ± 12**
1×10^{-6}	5.4	3	0**	0**	0**	0*

Data given as mean ± s.e. *n* gives the number of preparations for each experiment; the same cell was studied throughout the duration of each experiment.
Measurements were made after 10–12 min equilibration of the drug at each concentration.
Values that are statistically significantly different from the control value (no drug) are indicated by $*P < 0.05$ and $**P < 0.01$.

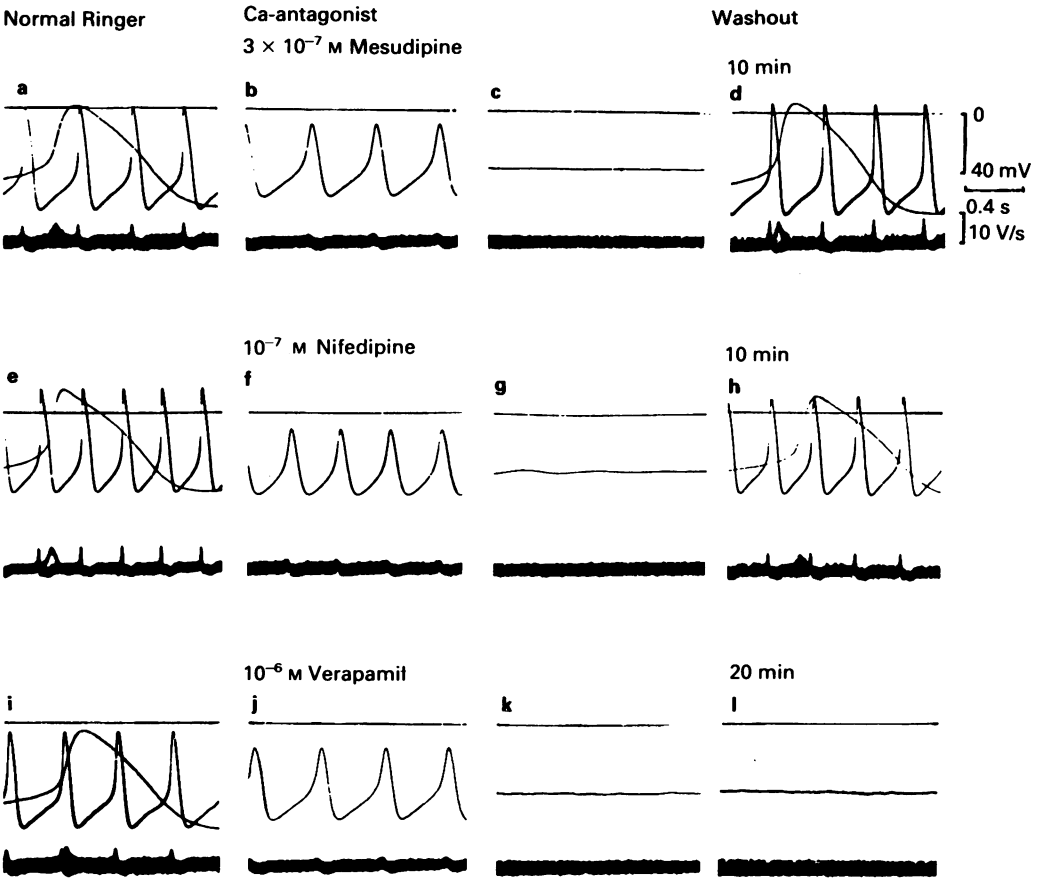

Figure 5 Comparison of the effects of mesudipine, nifedipine, and verapamil on the APs of isolated spontaneously-firing SA nodes of rabbit. Upper row (a–d): (a) Control APs in normal Ringer solution. (b–c): Addition of 3×10^{-7} M mesudipine depressed after 5 min (b) and abolished the slow APs after 12 min (c). (d) Washout of the drug restored the APs and automatic activity within 10 min. Middle row (e–h): (e) Control APs in normal Ringer solution. (f–g) Addition of 1×10^{-7} M nifedipine rapidly depressed the APs after 3 min (f) and abolished the APs after 6 min (g). (h) Washout of the drug restored the APs and automatic activity within 10 min. Lower row (i–l): (i) Control APs in normal Ringer solution. (j–k): Addition of verapamil 1×10^{-6} M depressed in 4 min (j) and completely abolished the electrical activity of the preparation within 6 min (k). (l) Washout of the drug for 20 min did not restore the APs.

of the drug required to block the AP was higher (Table 1). The blocking concentrations of mesudipine required, varied between 10^{-6} M and 10^{-5} M. Figure 6 illustrates one of these experiments. The control APs have a $+ \dot{V}_{max}$ of about $20\ Vs^{-1}$ (a). Addition of 10^{-7} M mesudipine depressed the $+ \dot{V}_{max}$ (b). Higher concentrations of the drug depressed both amplitude and $+ \dot{V}_{max}$ of the APs. The concentration of mesudipine required to block excitability was as high as 10^{-5} M (Figure 6e). In five other experiments, the automatic activity of latent cells was blocked at 10^{-6} M mesudipine (not illustrated, summarized in Table 1).

After-hyperpolarization with oscillations in cells completely blocked by calcium antagonistic drug

When the electrical activity of the isolated SA-node preparations was suppressed by a calcium antagonist, intensive field stimulation was applied to determine whether the preparations might be excitable. In all

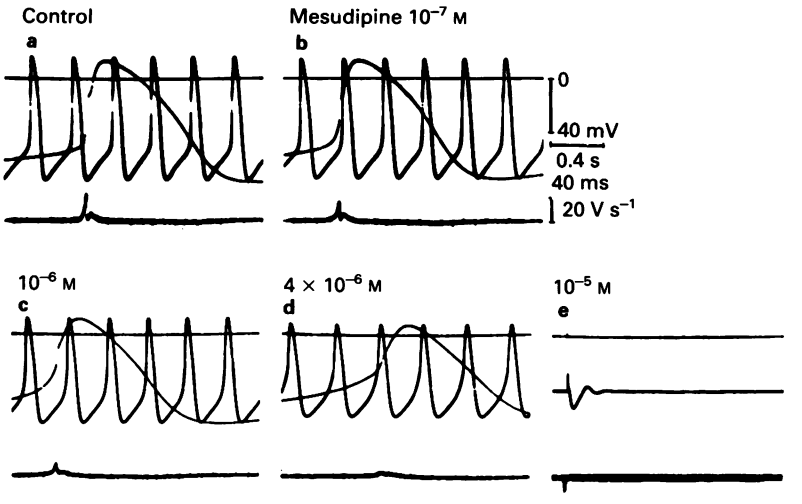

Figure 6 Lower sensitivity to mesudipine of the electrical activity of a latent pacemaker cell in a spontaneously-firing SA node preparation of rabbit. (a) Control APs in normal Ringer solution. (b–d) Addition of mesudipine to 1×10^{-7} M (b), 1×10^{-6} M (c), and 4×10^{-6} M (d) depressed the amplitude and the $+\dot{V}_{max}$ of the slow APs. (e) Elevation of the mesudipine concentration to 1×10^{-5} M completely abolished the APs. Upper solid line is the zero potential. Lower trace is the dV/dt. The dV/dt trace was turned on only for the fast sweep speed (40 ms calibration bar). A prominent step on the rising phase and a relatively high $+\dot{V}_{max}$ were the major criteria to identify latent pacemaker (driven by the true pacemaker cell). All records are from the same impalement.

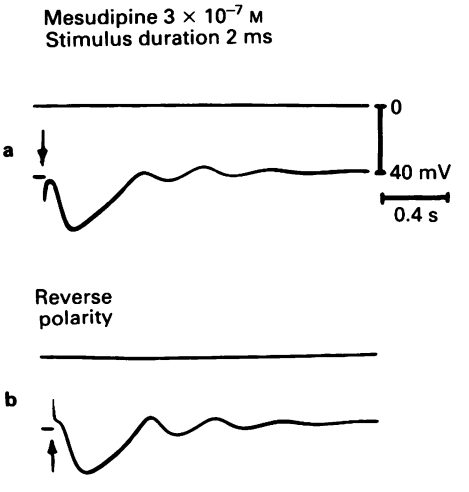

Figure 7 Intense field stimulation (duration of 2 ms) of an isolated SA-node preparation of rabbit in which APs were blocked by 3×10^{-7} M mesudipine, elicited a large after-hyperpolarization followed by small oscillations in E_m. This type of response occurred with either polarity of stimulation. (a) Stimulus of one polarity; stimulus artifact negative. (b) Reversed polarity; stimulus artifact positive. Both records are from the same impalement. Similar responses were obtained in the presence of other calcium antagonistic drugs used to block the APs.

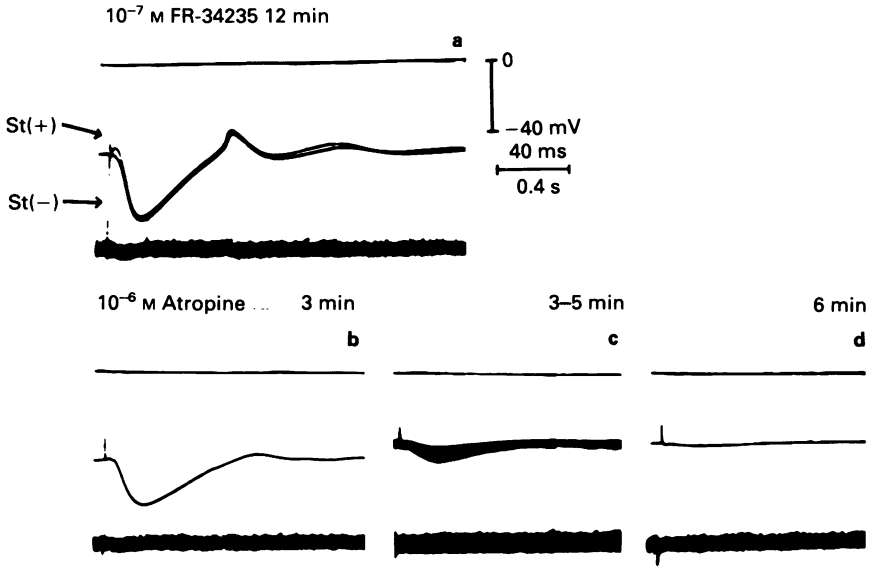

Figure 8 Effect of atropine on the afterpolarization induced by intense field stimulation. (a) Excitability was blocked by the dihydropyridine derivative Ca channel blocker FR-34235. Afterhyperpolarization followed by oscillations occurred following each stimulation. (b–d) Addition of 10^{-6} M atropine to the perfusate reduced the afterhyperpolarization within 3–5 min (b) and (c), and completely blocked the afterhyperpolarization after 6 min (d).

cases, a large after-hyperpolarization was observed. The hyperpolarizing response that occurred was independent of the polarity of the stimulation used. In most cases, this response was followed by small oscillations. Two examples of this phenomenon are illustrated for preparations that were blocked by mesudipine (3×10^{-7} M) (Figure 7) and by FR-34235 (Figure 8). Addition of 10^{-6} M atropine in the perfusate blocked the afterdepolarizations within 6 min (Figure 8 b–d).

Discussion

Our results indicate that mesudipine, like verapamil and nifedipine, depresses and blocks the naturally-occurring slow APs of the SA-node, and depresses automaticity. The ED_{50} for the blocking effect was about 1×10^{-7} M (Table 1). The effects of mesudipine are dose-related, and are antagonized by high Ca^{2+} concentration. The antagonism of the mesudipine effect with high $[Ca]_o$ is consistent with a mesudipine block of the Ca^{2+} entry. This antagonism of the drug effect by Ca^{2+} may be explained in part by competition between the drug and Ca^{2+} ion for the same binding site on or near the slow channel. Consistent with this possibility, Pang & Sperelakis (1982) reported that verapamil and bepridil (but not nifedipine and diltiazem) depress the binding of Ca^{2+} ions to isolated sarcolemmal membranes in a dose-dependent manner, verapamil being the more potent. In addition, the increase in electrochemical driving force $(E_m - E_{Ca})$ $[Ca]_o$ for an inward Ca^{2+} current through the unblocked slow channels would be a factor in the apparent reversal of the drug effect by Ca^{2+} ion.

The concentration of mesudipine that blocked excitability of the SA-node was almost the same as that which blocked the slow APs of guinea-pig papillary muscle and Purkinje fibres (3×10^{-7} M compared with 1×10^{-7} M) (Molyvdas & Sperelakis, 1983 a,b). The initial resting potential may be important for the blocking effect of mesudipine, since 'hyperpolarized' cells of the SA node close to the crista terminalis (latent cells) required much higher concentration for blockade (i.e., 10^{-6}–10^{-5} M).

The mesudipine concentration (3×10^{-7} M) that completely blocked the slow APs also depolarized the cells (E_m of 40–45 mV) to what appeared to be a second stable state, i.e., a second resting potential (Wiggins & Cranefield, 1976; Sperelakis, 1979). It is possible that this depolarization to the second stable resting potential is due to a lowering of K^+ conductance (g_K) produced by the drug. It has been reported that the calcium antagonists block the outward movement of K^+ through the Ca^{2+} channels (Lee & Tsien, 1983).

The faster washout of mesudipine and nifedipine compared with verapamil suggests that mesudipine

may act, like nifedipine, on the outer surface of the membrane, and may not have intracellular effects at the concentrations tested. Bepridil, verapamil and nitrendipine enter and accumulate inside the muscle cells (Pang & Sperelakis, 1983a,b). Furthermore, it has been shown recently that a quaternary ammonium derivative (D890) of methoxy verapamil (D600), which is less permanent because of its charge, has no effect when added to the outside of single cardiac myocytes, but blocks the slow channels when injected intracellularly (Hescheler *et al.*, 1982). These results support the hypothesis that verapamil and D600 enter the myocardial cell in the uncharged lipid-soluble form and block the slow channel perhaps by binding to the inner surface of the cell membrane.

The transient after-hyperpolarizing response of the SA-node cells to field stimulation after the slow APs were blocked by the drugs may be attributed to changes in K^+ conductance. This increase in K^+ conductance may be mediated by acetylcholine release from the parasympathetic nerve endings present in the preparations, because atropine blocked the after-hyperpolarizations (Goto *et al.*, 1983).

We conclude that the calcium channel blockers mesudipine, nifedipine, and verapamil are effective at low concentrations on the APs and automaticity of the SA-node.

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