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Effects of selective channel blocking agents on contractions and action potentials in K^+-depolarized guinea-pig atria

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1 Contractions and transmembrane action potentials were induced by 1 μM isoprenaline in K^+-depolarized guinea-pig left atria driven at 0.5 Hz.

2 The stability of these responses was significantly increased by doubling the extracellular glucose concentration to 22 mM.

3 Action potential overshoot increased by 28 mV per ten fold increase in extracellular calcium concentration suggesting that the inward current in this preparation is carried by Ca^{2+}.

4 In depolarized driven preparations, nanomolar concentrations of nifedipine and nisoldipine reduced contractility, maximum rate of depolarization (*dV/dt max*) and action potential height, whereas the fast channel blocking agents tetrodotoxin and mexiletine (in micromolar concentrations) produced little change. Nifedipine also rendered spontaneously beating depolarized right atrial preparations quiescent.

5 In concentrations which reduced *dV/dt* of normal action potentials, the sodium channel blocking agents quinidine and Org 6001 reduced the amplitude of contractions and reduced the maximum rate of phase 0 depolarization (*dV/dt*) of action potentials in depolarized tissue. These actions were reversed by Ca^{2+} and suggest calcium antagonistic activity. However action potential height was not reduced. Like bepridil, both drugs also reduced the frequency of spontaneous contractions in depolarized right atrial preparations.

6 Unlike Org 6001, quinidine failed to produce a shift in calcium log dose-response curves in driven depolarized preparations and induced positive inotropy in the presence of functional sodium channels.

7 Bepridil inhibited contractions in depolarized atria in the absence of a reduction in *dV/dt* suggesting that any calcium antagonistic action in atrial tissue is mainly located at an intracellular site.

8 In conclusion, action potentials elicited by isoprenaline in potassium-depolarized atria bathed in high glucose appear to be Ca^{2+} mediated. In concentrations which inhibit the inward Na^+ current, both quinidine and Org 6001 exhibit calcium channel blocking properties.

Introduction

Calcium entry blocking agents are a chemically heterogeneous group of compounds which offer potential therapeutic benefit in the treatment of cardiovascular disease (see Ellrodt *et al.*, 1980; Dargie *et al.*, 1981; Awan *et al.*, 1982, for recent reviews). Although the pharmacological profiles and precise sites of action may differ, these compounds all share the ability to inhibit the influx of calcium ions which occurs during membrane depolarization. Although there are many well-documented tests using vascular smooth muscle which can be used to identify calcium entry blockers (Kazda *et al.*, 1983; Hof & Vucrela, 1983), the situation is not so clear in cardiac muscle, where both Na^+ and Ca^{2+} play important roles in cell depolarization. Therefore, we have re-examined the suitability and selectivity of a test in cardiac muscle for calcium entry block, using selective Na^+ channel blockers (tetrodotoxin, quinidine and Org 6001), selective calcium entry blockers (nifedipine and nisoldipine) and a drug, bepridil, which is thought to block both Na^+ and Ca^{2+} channels (Vogel *et al.*, 1979; Kane & Winslow, 1980; Anno *et al.*, 1984).

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Methods

Normal atria

Left atria were removed from male guinea-pigs (Dunkin-Hartley strain) and mounted in a 50 ml organ bath containing modified Krebs solution (composition in mmol l^{-1}: NaCl 119, KCl 4.7, $MgCl_2$ 0.56, NaH_2PO_4 1.0, $NaHCO_3$ 25, $CaCl_2$ 2.5, glucose 22) gassed with carbogen and maintained at 35°C. A resting tension of 0.5 g was applied and the tissue stimulated at a frequency of 3 Hz with rectangular constant voltage pulses of 1 ms duration delivered at twice threshold voltage. Isometric contractions were obtained using a Grass FT 03 force displacement transducer coupled to a Devices MX2 pen recorder. After a stabilization period of 30 min, the stimulus frequency was reduced to 0.5 Hz and after new stabilization of the twitches, drugs were added in a cumulative manner to the organ bath.

Depolarized atria

Left atria were set up as described above, except that normal Krebs-Henseleit solution (glucose 11 mmol l^{-1}) was used in some experiments. After reducing the stimulation frequency to 0.5 Hz, the preparations were depolarized with additional (21.3 mM) KCl and contractions elicited by the administration of isoprenaline 1 μM. As in the case of normal atria, drugs were administered cumulatively, two concentrations being examined in any one preparation.

In other electrically-driven depolarized left atrial preparations, concentration-response curves were obtained to calcium, before and after 30 min incubation with the test drug. In these experiments, the basal bathing medium comprised (mmol l^{-1}): NaCl 119, KCl 26, $MgCl_2$ 0.56, NaH_2PO_4 1.0, $NaHCO_3$ 25, $CaCl_2$ 1.0, glucose 22, and contained isoprenaline 1 μM. Each tissue received only one concentration of test drug.

Contractions were also induced, using the methods described above, in spontaneously beating guinea-pig right atria, to examine the effects of the test drugs on frequency.

Electrophysiological studies

Guinea-pig left atria were pinned to the base of a recording chamber and superfused at a rate of 10 ml min^{-1} with Krebs-Henseleit solution containing 22 mM glucose gassed with carbogen and maintained at a temperature of 35 ± 0.5°C. Again, in some preliminary experiments, 11 mM glucose was used. The tissues were stimulated at a frequency of 1 Hz with rectangular pulses of 1 ms duration delivered at twice threshold voltage. After one hour equilibration, stimulation rate was reduced to 0.5 Hz. In some preparations normal action potentials were recorded. In others the superfusate was switched to one containing 26 mM potassium and 1 μM isoprenaline. Transmembrane action potentials were recorded using conventional microelectrode techniques. The parameters measured were resting membrane potential (RMP), action potential amplitude (A), the maximum rate of depolarization (dV/dt) and the times taken to reach 50% and 90% repolarization (APD_{50} and APD_{90}) levels.

A number (6–15) of action potentials were recorded before and 30–45 min after addition of drugs. Again, the cumulative method of drug addition was used.

Student's t test was used to detect the significance of differences.

Drugs used were nifedipine and nisoldipine (Bayer), bepridil HCl and Org 6001 HCl (3α-amino-5α-androstan-2β-ol-17-one HCl; Organon), mexiletine HCl and tetrodotoxin (Boehringer), quinidine sulphate (McCarthy) and isoprenaline bitartrate (Sigma). Isoprenaline solutions were made up in ascorbic acid (10^{-4} M) to prevent oxidation.

Results

Effect of glucose concentration and osmolarity

Preliminary observations in K^+-depolarized atria showed that with glucose concentrations of 11 mmol l^{-1} contractions could not always be elicited with isoprenaline, unless the stimulation voltage was increased 8–10 fold (from 2–8 V). These contractions were in any case poorly sustained (Figure 1). In the

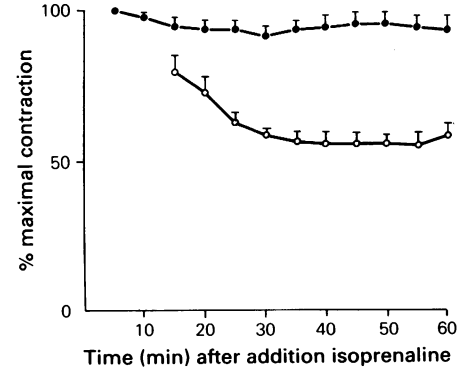

Figure 1 Effect of glucose concentration (22 mM glucose (●) and 11 mM glucose (○)) on the stability of isoprenaline-induced contractions in K^+ depolarized driven left atria. Results are expressed as % of the maximal contractions obtained after the addition of isoprenaline. Each point is the mean and vertical lines show s.e.mean of six observations.

Table 1 Electrophysiological effects of altering glucose concentration and osmolarity (by sucrose) on normal guinea-pig atria

Treatment	n	*RMP* (mV)	*APH* (mV)	APD_{50} (ms)	APD_{90} (ms)	dV/dt max (Vs^{-1})
Control (11 mM glucose)	69	80.4 ± 0.7	104 ± 1	31 ± 1	70 ± 1	180 ± 5
High glucose (22 mM)	52	81.5 ± 0.7	108 ± 1	38 ± 1***	79 ± 2***	184 ± 6
High glucose + sucrose (42.5 mM)	40	84.3 ± 0.7	107 ± 1*	30 ± 1	80 ± 1***	156 ± 7**

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, compared with control value.
Abbreviations used in this and subsequent Tables: RMP, resting membrane potential; APH, action potential height; APD_{50} and APD_{90}, times taken to reach 50% and 90% repolarizaton levels; *dV/dt max*, maximum rate of depolarization.

presence of double glucose concentration (22 mmol l^{-}) isoprenaline always restored contractions without the need for an increase in the stimulation voltage and these contractions were stable for at least 1 h (Figure 1). Likewise, stable transmembrane action potentials could be elicited in high-glucose medium for up to 3 h. Consequently all drug studies were conducted using salt solution containing 22 mmol l^{-1} glucose. This increase in glucose concentration produced only minor effects on transmembrane action potential duration in normal atria and no effect on *dV/dt max* was seen (Table 1). Further addition of 46 mmol l^{-1} sucrose (to mimic the increase is osmolarity produced by addition of 21.3 mmol l^{-1} KCl) caused a small increase in resting membrane potential, a small decrease (13%) in *dV/dt max* and restored APD_{50} to the value seen in normal glucose (Table 1). Action potential height and APD_{90} were not modified by addition of sucrose.

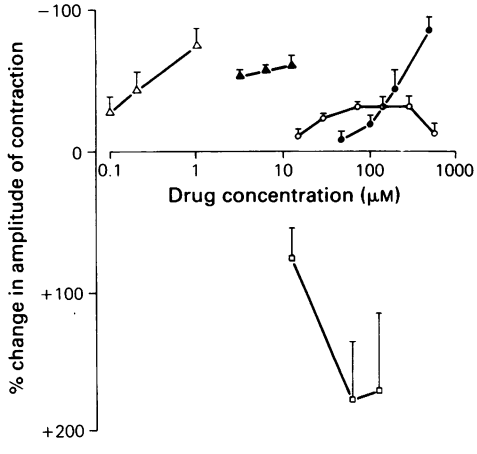

Figure 2 Log dose-response curves to nifedipine (△), bepridil (●), tetrodotoxin (▲), Org 6001 (○) and quinidine (□) for effects on normal contractions of driven left atria. Prior to drug addition, mean developed tension was 630 ± 60, 580 ± 120, 690 ± 270, 400 ± 50 and 330 ± 120 mg respectively. Each point is the mean, and vertical lines show s.e.mean of 4 to 6 observations.

Drug effects on contractions and action potentials in normal atria

Both nifedipine (0.1–1 μM) and bepridil (50–500 μM) produced a concentration-dependent depression of contractions of normal electrically-stimulated left atria (Figure 2). However Org 6001 (15–580 μM) produced only a modest (maximum 30%) depression of contractions, independent of concentration. These effects differed from those of tetrodotoxin, which reduced contractions by 50–60% at all concentrations (3–12 μM) studied. In contrast to these agents, quinidine (13–127 μM) produced marked positive inotropic effects and at higher concentrations (254 μM) rendered the tissue refractory to electrical stimulation.

Drug-induced changes in the action potential characteristics are summarized in Table 2. All the drugs known to inhibit the fast sodium channel (mexiletine, quinidine and Org 6001) produced concentration-dependent reductions in *dV/dt* together with a reduction in the height of the action potential (APH). The highest concentrations of mexiletine (116 μM) and quinidine (254 μM) used also caused a small decrease in RMP. In addition, all three drugs prolonged action potential duration although the smallest concentration of Org 6001 (29 μM) used decreased APD_{50} whilst APD_{90} was unchanged.

The calcium entry blocker, nisoldipine, decreased action potential duration and slightly decreased action potential height. *dV/dt* was essentially unchanged except at the highest concentration used (44.5 nM) where there was a slight increase despite a fall in RMP.

Bepridil also shortened APD_{50} and at the highest

Table 2 Effects of sodium and calcium entry blockers on normal action potentials

Drug (μM)	n	*RMP* (mV)	*APH* (mV)	APD_{50} (ms)	APD_{90} (ms)	dV/dt (V/s)
Mexiletine (3 preparations)						
0	54	75.9 ± 0.9	98.6 ± 1.3	51.4 ± 2.1	94.0 ± 2.8	151 ± 6
46.5	33	76.4 ± 1.1	94.6 ± 1.8	53.2 ± 2.1	94.9 ± 2.3	108 ± 6***
116.0	32	72.4 ± 1.1*	91.6 ± 2.0**	67.8 ± 1.2***	127.8 ± 4.5***	60 ± 6***
Quinidine (3 preparations)						
0	34	79.5 ± 0.8	104.8 ± 1.4	39.1 ± 1.3	88.5 ± 2.0	161 ± 8
127	34	80.3 ± 1.0	96.4 ± 1.7***	45.7 ± 1.8**	122.7 ± 3.5***	78 ± 4***
254†	18	74.8 ± 1.2**	67.6 ± 2.5***	66.9 ± 6.0***	170.9 ± 8.1***	21 ± 3***
Org 6001 (5 preparations)						
0	58	79.2 ± 0.7	104.7 ± 0.7	35.5 ± 1.0	80.2 ± 1.4	188 ± 7
29	58	81.4 ± 0.6*	103.5 ± 0.8	31.5 ± 0.8**	79.9 ± 1.1	148 ± 4***
72.5	58	79.7 ± 0.7	95.8 ± 0.7***	35.8 ± 0.6	93.1 ± 0.9***	98 ± 4***
290	54	79.6 ± 0.6	77.2 ± 1.4***	52.8 ± 1.4***	146.3 ± 4.0***	39 ± 3***
Nisoldipine (3 preparations)						
0	45	78.2 ± 0.7	105.8 ± 0.8	34.4 ± 0.3	82.4 ± 0.9	155 ± 5
0.0089	45	77.0 ± 0.7	102.3 ± 0.9**	30.5 ± 0.6***	83.7 ± 1.9	156 ± 4
0.0445	45	75.7 ± 0.6**	99.3 ± 0.9***	27.2 ± 0.9***	77.5 ± 2.1*	169 ± 5*
Bepridil (4 preparations)						
0	52	76.9 ± 0.5	102.4 ± 0.7	32.5 ± 1.8	82.5 ± 2.3	154 ± 5
5.0	43	76.9 ± 0.7	100.8 ± 1.0	24.1 ± 1.2***	79.8 ± 1.8	153 ± 4
10.0	47	75.7 ± 0.8	100.1 ± 0.8	24.3 ± 1.1***	77.5 ± 1.4	140 ± 5*

Each result is the mean ± s.e.mean of measurements taken from *n* cells.
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, denote significant differences from the appropriate control values.
†Two tissues failed to follow the driving stimulus.

concentration (10 μM) used produced a small but significant decrease in *dV/dt* and action potential height.

Effects on contractions in depolarized atria

All three agents known to possess calcium entry

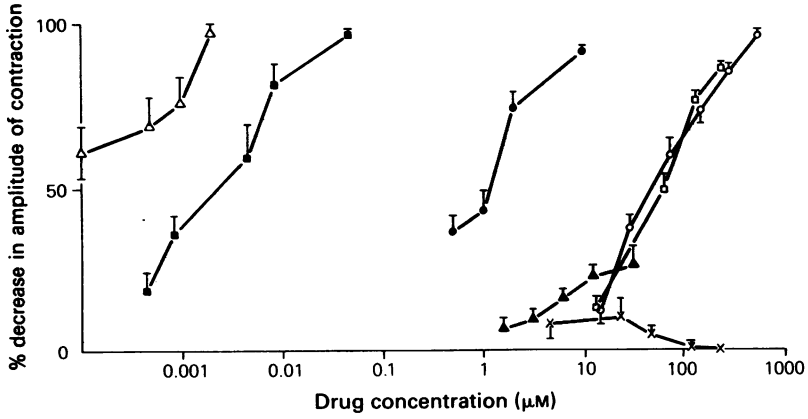

Figure 3 Log dose-response curves to nifedipine (△), nisoldipine (■), bepridil (●), tetrodotoxin (▲), mexiletine (×), Org 6001 (□) and quinidine (○) for inhibition of isoprenaline-induced contractions in K^+ depolarized left atria. Prior to drug addition, mean developed tension was 480 ± 70, 620 ± 80, 390 ± 50, 350 ± 40, 380 ± 40, 470 ± 30 and 390 ± 90 mg respectively. Each point is the mean, and vertical lines show s.e.mean of 5 to 6 observations.

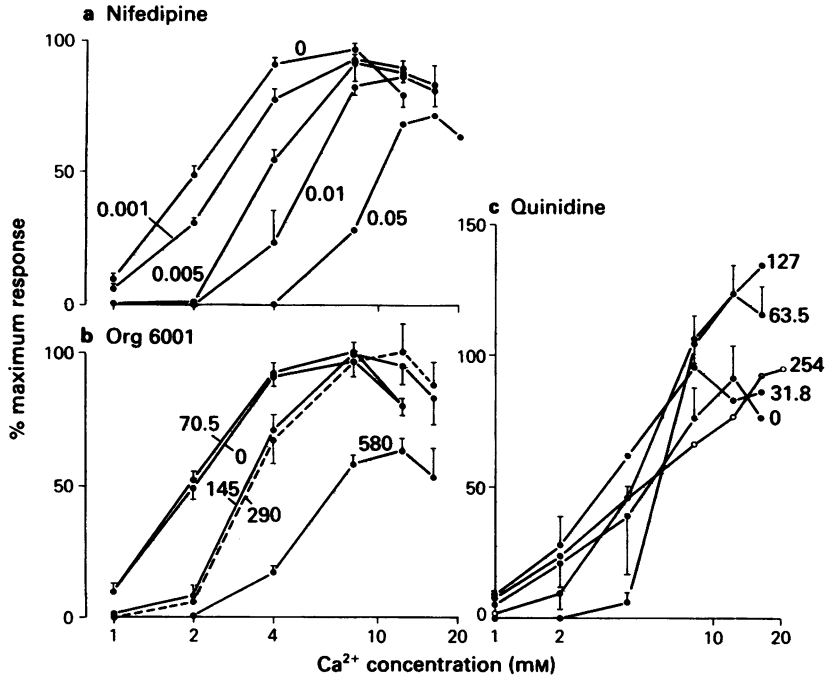

Figure 4 Effects of (a) nifedipine, (b) Org 6001 and (c) quinidine on log dose-response curves to calcium in K^+ depolarized driven left atria. Maximum control tension development in response to Ca^{2+} was 1460 ± 70, 1250 ± 140 and 1270 ± 100 mg for each drug studied, respectively. The concentrations (μM) of test drugs used are shown above the curves. Each point is the mean, and vertical lines show s.e.mean, of at least 4 observations.

blocking properties nifedipine (0.1–2.0 nM), nisoldipine (0.45–45 nM) and bepridil (0.5–10 μM), produced concentration-dependent reductions in the amplitude of contraction (Figure 3). The highest concentrations of these agents used also impaired the ability of the tissue to follow the driving stimulus. The fast-channel inhibitors fell into two distinct categories (Figure 3); whereas both tetrodotoxin (up to 31 μM) and mexiletine (4.7–233 μM) failed to modify substantially contraction amplitude, both quinidine (13–254 μM) and Org 6001 (15–580 μM) produced concentration-dependent negative inotropic effects. The EC_{50} values (with 95% fiducial limits) of the test drugs were (M): nisoldipine 2.1×10^{-9} (1.4–3.0); bepridil 1.0×10^{-6} (0.7–1.3); Org 6001 5.5×10^{-5} (4.5–6.6) and quinidine 5.6×10^{-5} (4.8–6.7).

The effects of nifedipine, Org 6001 and quinidine on the positive inotropic effects of calcium are shown in Figure 4. Both nifedipine and Org 6001 caused parallel shifts to the right of the calcium concentration-response curves without markedly depressing the maximal response. Calculated pA_2 values were 8.3 for nifedipine and 3.6 for Org 6001. In contrast, quinidine failed to inhibit calcium-invoked contractions and indeed with intermediate concentrations (64–127 μM), quinidine increased the maximum response to calcium. Thus, despite producing marked inhibition of contractions in depolarized atria, quinidine did not appear to act like a classical calcium entry blocker.

Electrophysiological effects in depolarized atria

According to the Nernst equation, if the membrane is highly selective for calcium then action potential overshoot should increase by 30 mV per ten fold increase in the external concentration of calcium. Using a calcium concentration range of 0.4 to 8 mM we found a 28.2 (± 2.6) mV increase per decade of external calcium in depolarized preparations, which is similar to values found by Pappano (1970) in guinea-pig atria (29 mV per decade) and by Kohlhardt & Haap (1980) in guinea-pig papillary muscle (28 mV per decade).

The electrophysiological effects of the calcium entry blockers nisoldipine and nifedipine and of bepridil on action potentials in depolarized electrically driven left atria are summarized in Table 3. Nisoldipine (0.9–45 nM) produced a concentration-dependent reduction in action potential height, overshoot and in the maximal rate of depolarization (dV/dt). Similar

Table 3 Effects of calcium entry blockers (nisoldipine and nifedipine) and bepridil on 'slow' action potentials in guinea-pig depolarized left atria

Drug (μM)	n	*RMP* (mV)	*APH* (mV)	*Overshoot* (mV)	dV/dt (V/s)	APD_{50} (ms)	APD_{90} (ms)	*CB* (%)
Nisoldipine (7 preparations)								
0	42	43.9 ± 0.8	55.0 ± 1.0	11.1	13.0 ± 0.5	41.4 ± 1.1	58.6 ± 1.4	
0.00089	13	43.5 ± 1.0	53.9 ± 1.1	10.4	12.7 ± 1.0	42.2 ± 1.5	55.5 ± 1.8	
0.00445	40	44.8 ± 0.8	51.2 ± 1.2*	6.4	10.1 ± 0.5***	41.9 ± 1.0	58.7 ± 1.3	
0.0089	30	46.1 ± 1.4	46.7 ± 2.5**	0.6	8.7 ± 0.7***	45.6 ± 0.6**	61.2 ± 1.4	17
0.0445	7	51.4 ± 1.1***	49.5 ± 1.6*	1.9	8.6 ± 0.9**	53.6 ± 0.5***	75.4 ± 1.7***	67
Nifedipine (1 preparation)								
0	15	46.0 ± 1.5	61.0 ± 2.2	15.0	14.0 ± 1.1	54.0 ± 2.4	88.0 ± 6.7	
0.0001	6	47.0 ± 3.6	54.0 ± 2.8	7.0	5.5 ± 0.4**	58.0 ± 1.4	88.0 ± 1.3	
Bepridil (8 preparations)								
0	73	44.3 ± 0.6	55.3 ± 0.8	11.0	11.0 ± 0.4	46.7 ± 0.8	67.1 ± 1.4	
1.0	25	46.5 ± 1.5	54.7 ± 1.4	8.2	10.1 ± 0.9	43.8 ± 0.9	68.0 ± 2.9	
2.0	39	42.3 ± 0.6	51.9 ± 1.1*	9.6	11.0 ± 0.6	44.5 ± 0.9	62.7 ± 1.4	
5.0	74	44.7 ± 1.0	56.5 ± 1.2	11.8	11.5 ± 0.4	44.1 ± 0.7	61.2 ± 1.0***	25†
10	16	41.2 ± 0.8**	56.1 ± 1.5	14.9	12.4 ± 0.7	45.2 ± 1.3	59.1 ± 1.0	100‡

Each observation is the mean ± s.e.mean of measurements taken from *n* cells.
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, denote significant differences from the appropriate control values.
†Conduction block (CB) overcome by increasing voltage.
‡Conduction block (CB) overcome by increasing voltage in one preparation; transient reversal in 2 preparations; complete block in 4 preparations within 30–60 min.

effects were seen with nifedipine (0.1 mM). The effects of both drugs were reversed by increasing extracellular calcium concentration to 7.5 mM. This is shown for nisoldipine in Figure 5. The highest concentrations of nisoldipine (> 8.9 nM) produced small but significant increases in action potential duration. At this concentration some tissues failed to follow every stimulus and these tissues were excluded from the electrophysiological analysis.

In contrast to nisoldipine and nifedipine, bepridil (1–10 μM) failed to modify action potential height, overshoot or *dV/dt* (Table 3). In addition, bepridil produced a concentration-dependent shortening of 90% repolarization time (APD_{90}). Failure to follow the stimulus was observed in 25 and 100% of preparations exposed to 5 and 10 μM bepridil, respectively. Increasing the stimulation voltage (by a factor of 1.7–2.7) restored the ability of the tissues to respond at a rate of 0.5 Hz when exposed to a concentration of 5 μM. However, only 1 of 5 preparations exposed to 10 μM bepridil responded with sustained 1:1 conduction. The remaining 4 preparations became quiescent after 30–40 min exposure to this concentration of bepridil despite voltage increases of up to 20 times the threshold voltage. It would therefore appear that in atrial tissue, the major effect of bepridil is one of

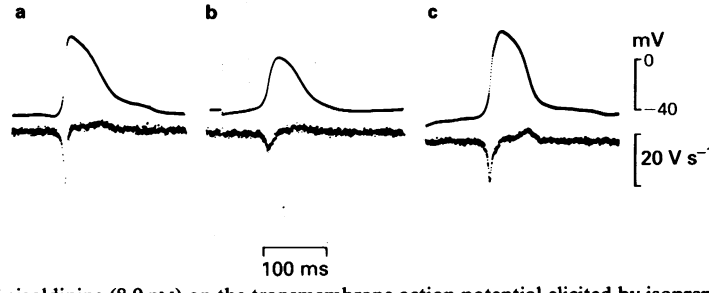

Figure 5 Effect of nisoldipine (8.9 nM) on the transmembrane action potential elicited by isoprenaline (1 μM) in K^+ depolarized driven left atria. (a) Shows a typical control action potential. (b) Shows the action potential 30 min after superfusion with nisoldipine and (c) shows the effect of the addition of Ca^{2+} 5 mM in the presence of nisoldipine.

Table 4 Effects of sodium channel blockers on 'slow' action potentials in guinea-pig atria

Drug (μM)	n	*RMP* (mV)	*APH* (mV)	*Overshoot* (mV)	dV/dt (V/s)	APD_{50} (ms)	APD_{90} (ms)	*CB* (%)
Mexiletine (3 preparations)								
0	38	41.6 ± 1.3	57.4 ± 1.5	15.8	11.9 ± 0.9	44.1 ± 2.2	58.0 ± 3.1	
46.5	19	47.8 ± 1.2	55.3 ± 1.9	7.5	12.6 ± 0.5	55.7 ± 3.2**	73.2 ± 3.8**	
116.0	27	43.0 ± 1.6	59.5 ± 1.3	16.5	11.6 ± 0.7	47.8 ± 1.3	65.1 ± 1.4:	
Quinidine (8 preparations)								
0	53	44.3 ± 0.7	51.5 ± 0.8	7.2	9.6 ± 0.5	45.4 ± 0.8	63.3 ± 0.8	
63.5	35	42.9 ± 0.8	52.4 ± 1.0	9.5	6.5 ± 0.3***	90.3 ± 2.7***	113.2 ± 3.0***	
127	57	41.4 ± 0.5**	50.5 ± 1.0	9.1	5.0 ± 0.3***	127.3 ± 3.3***	161.4 ± 4.5***	
254	7	37.9	33.7	− 4.2	6.4	121.7	151.8	80†
Org 6001 (8 preparations)								
0	52	40.9 ± 1.0	51.9 ± 1.1	11.0	10.9 ± 0.7	44.7 ± 1.1	66.1 ± 1.6	
29	23	42.5 ± 0.7	58.7 ± 1.5***	16.2	10.8 ± 0.5	59.2 ± 2.6***	81.7 ± 3.6***	
72.5	38	45.9 ± 1.1**	57.0 ± 1.9*	11.1	9.7 ± 0.7	49.6 ± 2.8	81.0 ± 4.6**	
290	48	45.3 ± 0.7**	56.3 ± 1.9*	11.0	6.9 ± 0.3***	67.6 ± 2.8***	111.3 ± 4.0***	

Each result is the mean ± s.e.mean of measurements taken from *n* cells.
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, denote significant differences from the appropriate control values.
† Denotes the presence of 2:1 conduction block (CB)

impaired conduction. Addition of 5 mM calcium in the presence of bepridil failed to reverse these effects and indeed conduction became further impaired. Thus, despite marked inhibition of contractions in depolarized preparations, we were unable to demonstrate any effect of bepridil on the accompanying action potentials.

The effects of the sodium channel blocking agents, mexiletine, quinidine and Org 6001 are summarized in Table 4. Apart from a modest prolongation of APD and an increase in RMP (only seen at the low concentration used, 46.5 μM), mexiletine was without effect on the action potential characteristics. In contrast, quinidine in concentrations (63.5–254 μM) similar to those required to reduce the amplitude of contractions, caused a dose-dependent reduction of *dV/dt* in the absence of a decrease in APH or overshoot. Indeed, the overshoot appeared to be increased (Figure 6 and Table 4). A small but significant decrease in RMP was also observed at the higher concentrations used (127–254 μM) and conduction block developed in 80% of preparations exposed to 254 μM quinidine. Unlike nisoldipine, nifedipine or bepridil, quinidine induced a marked prolongation of the action potential duration both at the 50 and 90% repolarization levels (Figure 6).

Org 6001 (29–290 μM) also reduced *dV/dt* in the absence of a decrease in APH or overshoot. Approximate EC_{50} values for the effects of quinidine and Org 6001 on *dV/dt* were 340 and 740 μM respectively, suggesting that quinidine is about twice as potent as Org 6001 in reducing *dV/dt*. In this case APH and overshoot were increased only at the lowest concentration tested (29 μM). Org 6001 also increased action

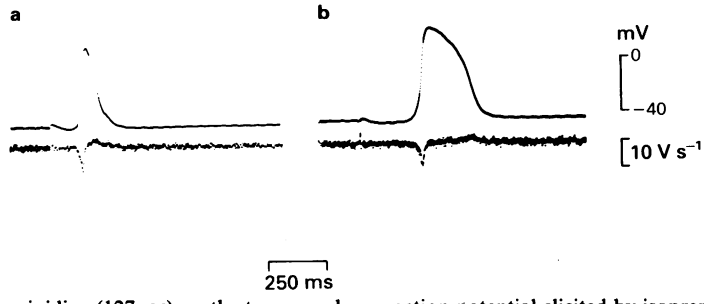

Figure 6 Effect of quinidine (127 μM) on the transmembrane action potential elicited by isoprenaline (1 μM) in K^+ depolarized driven left atria. (a) Shows a typical control action potential and (b) shows the action potential after 30 min exposure to quinidine.

Table 5 Effects of Ca^{2+} (5 mM) on responses to quinidine (Quin) and Org 6001 (Org)

Drug (μM)	n	*RMP* (mV)	*APH* (mV)	*Overshoot* (mV)	dV/dt (V/s)	APD_{50} (ms)	APD_{90} (ms)
0	20	43.5 ± 1.0	53.5 ± 1.0	10.0	12.6 ± 0.6	40.6 ± 0.9	57.5 ± 1.1
Quin (127)	19	42.1 ± 0.8	57.9 ± 0.9*	15.8	7.1 ± 0.4***	115.0 ± 1.7***	141.8 ± 2.0***
Quin (127) + Ca^{2+}	14	41.1 ± 1.5	67.2 ± 2.8***	26.1	12.6 ± 0.6	103.7 ± 1.7***	135.5 ± 2.9***
0	23	40.1 ± 1.9	56.2 ± 0.6	16.1	12.4 ± 1.1	44.6 ± 1.2	64.8 ± 2.5
Org (290)	23	44.1 ± 0.8	56.6 ± 1.1	12.5	6.5 ± 0.5***	62.5 ± 3.3***	106.2 ± 5.8***
Org (290) + Ca^{2+}	15	45.2 ± 1.3*	59.5 ± 2.7	14.3	11.5 ± 1.5	60.1 ± 2.0***	125.8 ± 10.1***

In one further preparation complete block by Org 6001 25 μg ml^{-1} was reversed by Ca^{2+}.
Each result is the mean ± s.e.mean of measurements taken from *n* cells in 3 preparations.
*$P < 0.05$ and ***$P < 0.001$, denote significant differences from the appropriate control values.

potential duration but this effect was less marked than that observed in response to quinidine. Unlike quinidine, Org 6001 caused a significant increase in RMP and conduction block was not observed.

The effects of quinidine and Org 6001 on *dV/dt* were reversed by addition of 5 mM Ca^{2+} (Table 5); however, the APD remained prolonged. Also, in the presence of quinidine (but not Org 6001), addition of calcium caused a marked increase in APH and overshoot compared with the effects of quinidine alone.

Effects on frequency of contractions in depolarized spontaneously beating right atria

In view of the effects of quinidine and Org 6001 on slow action potentials, the actions of these compounds on the spontaneous frequency of contractions in depolarized right atria were compared with those of nifedipine and tetrodotoxin. The results are summarized in Figure 7. The mean spontaneous frequency in these experiments was 0.81 ± 0.10 Hz which is close to the frequency of stimulation (0.5 Hz) used in driven preparations. Nifedipine, in concentrations (0.1–1.0 nM) which did not completely inhibit the amplitude of contraction (in driven left atrial preparations) rendered right atrial preparations quiescent. The effect was time-dependent (7–28 min) depending on the concentration used. Tetrodotoxin (3.1–31 μM) had no effect whatsoever whilst quinidine and Org 6001 induced a concentration-dependent reduction in spontaneous frequency (EC_{50} values were 19.9 and 31.3 μM respectively). Bepridil (0.5–5 μM) yielded less consistent results: a concentration-related reduction in spontaneous frequency was seen in some preparations whereas in others even the lowest concentration used rendered the tissue quiescent. The EC_{50} value calculated from preparations which did not become quiescent was 3.44 μM. If quiescent preparations were included this value fell to 0.64 μM.

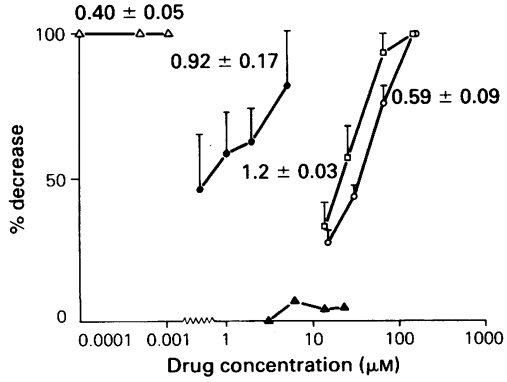

Figure 7 Log dose-response curves to nifedipine (Δ), bepridil (●), tetrodotoxin (▲), quinidine (□) and Org 6001 (○) for effects on the spontaneous frequency of K^+ depolarized atria. The mean control frequencies (Hz) in the absence of drugs are shown above the appropriate curves. Each point is the mean, and vertical lines show s.e.mean, of 4 to 7 observations.

Discussion

Pappano (1970) and Schneider & Sperelakis (1974) demonstrated that contractions could be restored in K^+ depolarized guinea-pig atria by isoprenaline (1 μM) but the tissues often failed to follow a driving stimulus of more than 0.04 to 0.1 Hz. In our study, increasing glucose concentration from 11 to 22 mM improved stability and allowed experiments to be performed using a higher stimulation frequency (0.5 Hz). The contractions would seem to be elicited by a slow inward calcium current since they were completely resistant to the fast Na^+ channel blocker tetrodotoxin and also to mexiletine (up to 200 μM). In addition the electrode properties of the membrane

agreed well with the predicted Nernst value for a Ca-electrode at 35°C (30 mV per decade).

Further evidence that we were dealing with responses mediated by a true slow calcium current was obtained from electrophysiological studies. Mexiletine, in concentrations (46.5–116 μM) which inhibited the fast inward current in normal atria, failed to modify the action potential characteristics of depolarized preparations suggesting that there is no fast sodium component in the action potential of these depolarized preparations.

In view of the above results, it was therefore not surprising to find that the specific calcium channel inhibitors, nifedipine (Fleckenstein *et al.*, 1972; Kohlhardt & Fleckenstein, 1977) and nisoldipine (Kass, 1982) both produced a marked inhibition of contractions elicited in depolarized preparations at concentrations which reduced dV/dt, amplitude and overshoot of the accompanying slow current action potentials. All these effects were reversed by increasing extracellular calcium concentration. Further experiments in depolarized right atrial preparations revealed that nifedipine was even more potent in abolishing slow impulse generation in nodal tissue than in inhibiting contractions of left atria.

In contrast to nifedipine and nisoldipine, bepridil in concentrations which exerted minimal effects on normal action potentials (decrease in APD_{50} and slight decrease in dV/dt) caused a pronounced decrease in the amplitude of contractions in depolarized atria without reducing dV/dt or action potential height. Indeed the only significant electrophysiological effect seen in depolarized preparations was a small decrease in APD_{90} at concentrations of 5–10 μM. These results are consistent with those obtained by Vogel *et al.* (1979) in potassium depolarized guinea-pig ventricular muscle stimulated with isoprenaline. These workers failed to detect a significant reduction in dV/dt or APH until concentrations of bepridil of 10 μM were reached whereas marked impairment of contractility was seen at concentrations of 5 μM. They concluded that bepridil, in addition to blocking slow membrane channels, may also exert an intracellular action. We also found an inability of depolarized atrial tissue to follow the driving stimulus in the presence of bepridil in concentrations of 5 μM and above, an action exacerbated by the addition of calcium. We conclude from our results that in atrial tissue, inhibition of slow current-mediated contractions may well result from an intracellular action (which is reversible by Ca^{2+}) and that any potential inhibition of slow membrane channels is masked by impairment of conduction. In this context, it is of interest that bepridil, unlike nifedipine, is known to be avidly taken up by cardiac cells (Pang & Sperelakis, 1983; Cramb & Dow, 1983). Other differences between bepridil and nifedipiné were also apparent. Although both agents produced concentration-dependent reductions in contractility in the absence of fast channel inhibition, the ratio of their potencies on normal and depolarized atria differed markedly (217 for bepridil compared with >3400 for nifedipine). Again nifedipine and bepridil differed in their effects on the frequency of slow contractions in spontaneously beating atria. Unlike nifedipine which abolished spontaneous impulse generation, bepridil produced a concentration-related inhibition of frequency in most preparations ($EC_{50} = 0.64$ μM) in concentrations similar to those required to inhibit contractions in depolarized left atria, an effect more similar to that observed in response to quinidine and Org 6001. Whether such graded frequency responses are the result of calcium channel inhibition, of an effect on potassium permeability or of an effect on conduction remains to be clarified.

Somewhat surprising results were obtained with quinidine and Org 6001 which are generally held to be fast Na^+ channel inhibitors (Vaughan Williams, 1975; Salako *et al.*, 1976; Marshall & Winslow, 1982). Both drugs inhibited the amplitude of contractions and reduced dV/dt in depolarized preparations in similar concentrations to those required to block fast Na^+ channels in normal atria. These effects were completely reversed by raising the extracellular calcium concentrations (to 5 mM), and suggest that both Org 6001 and quinidine are producing inhibition of the membrane calcium channel. A similar conclusion has been independently reached by Nawrath (1981) and Ducouret (1976) in voltage clamp studies with quinidine on cat papillary and frog atrial muscle. Org 6001 (but not quinidine) also produced an apparently competitive shift in calcium concentration-response curves. The effects of Org 6001 and quinidine on 'slow' action potentials differed from those of nifedipine or nisoldipine in that neither amplitude nor overshoot were reduced and there was a marked prolongation of action potential duration, which was not reversed by increasing extracellular calcium. This prolongation of action potential duration, which probably reflects effects of Org 6001 and quinidine on K^+ conductance (Nawrath, 1981), would allow more calcium to enter the cell during the pleateau phase and might mask any reduction in overshoot produced by a direct calcium channel inhibition. It is interesting to note that Ca^{2+} withdrawal *per se* also increases 'slow' action potential duration in K^+ depolarized guinea-pig papillary muscles treated with isoprenaline (Harman & Poole-Wilson, 1981).

Paradoxically, quinidine, unlike Org 6001 or nifedipine, failed to produce a shift in calcium concentration-response curves in depolarized preparations and indeed, in intermediate concentrations, increased the maximal contractile response produced by calcium. This effect in depolarized atria and the clearcut

positive inotropic effects in normal atria may well be due to the well-documented intracellular actions of quinidine to both inhibit calcium uptake by (Fuchs *et al.*, 1968; Dhalla *et al.*, 1978), and to release calcium from, the sarcoplasmic reticulum (Thorpe, 1973). Thus quinidine may produce two opposing actions, the end-response depending not only on concentration of drug, time of exposure and frequency of stimulation, but also on the absence or presence of both Na^+ and Ca^{2+} channels and extracellular calcium concentration (also see Kennedy & West, 1960).

The contrasting inotropic actions of quinidine (and Org 6001) on contractions in normal as compared with depolarized atrial preparations raise questions regarding the possible different mechanisms of electromechanical (E–C) coupling in the two situations. Wiggins (1981) has provided strong evidence to suggest that in potassium depolarized cardiac muscle, 'slow' action potentials induced by isoprenaline allow the muscle to utilize calcium directly from extracellular sources for contraction. In contrast, in non-depolarized muscle, with functional fast Na^+ channels, intracellular calcium stores are of primary importance. These fundamental differences in E-C coupling could explain the divergent effects of quinidine observed in the two preparations and would also account for the vastly greater potency (some 3000 fold) of nifedipine in depolarized preparations.

Whatever the exact mechanisms involved in E-C coupling it would seem that isoprenaline-induced contractions in previously depolarized guinea-pig left atria represent a useful model for identifying calcium antagonistic blocking properties of new drugs. However, accompanying electrophysiological measurements are necessary if the site(s) of action of test drugs are to be identified with any confidence.

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