Comparison of the Effects of Calcium Channel Blockers and Antiarrhythmic Drugs on Digitalis-induced Oscillatory Afterpotentials on Canine Purkinje Fiber

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SUMMARY

We studied the effects of Ca channel blockers and 3 antiarrhythmic drugs on the digitalis-induced oscillatory afterpotential (OAP). The OAP was observed in Purkinje fibers stimulated by pulse trains, with cycle lengths ranging from 1,000 to 300 msec. The Ca channel blockers verapamil, diltiazem and nifedipine (2.0×10^{-6} M) depressed OAP significantly and abolished triggered activity. Verapamil was more effective than diltiazem. However, nicardipine and nitrendipine (2.0×10^{-6} M) had no depressant effects on OAP or triggered activity. The antiarrhythmic drugs procainamide (1.0×10^{-4} M), mexiletine (1.0×10^{-5} M) and propranolol (1.0×10^{-4} M) depressed both OAP and triggered activity. There were no significant differences in the depressant effects between the Ca^{2+} antagonists (except for nitrendipine and nicardipine) and the other antiarrhythmic drugs. The OAP coupling interval was prolonged by verapamil, diltiazem, propranolol, procainamide and mexiletine. Although the APD50 was shortened by verapamil, diltiazem and nifedipine, it was prolonged by propranolol. It is concluded that nifedipine, verapamil, diltiazem, procainamide, mexiletine and propranolol may be effective for digitalis-related arrhythmia.

Additional Indexing Words:

Oscillatory afterpotential Digitalis Ca channel blocker Mexiletine Propranolol

CALCIUM channel blockers have recently been used in clinical practice for their antihypertensive, antianginal and antiarrhythmic effects. These drugs are sometimes used in combination with digitalis. Among the calcium channel blockers, verapamil has been reported to significantly depress ouabain-induced oscillatory afterpotentials (OAPs).[1] These OAPs

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have been proposed as a potential model for arrhythmias because they may change into triggered activity when supra-threshold is reached.[2)-4)] Nifedipine[5)] and diltiazem[6)] have been reported to depress the amplitude of ischemia-induced OAPs. No reports are available about the effects of nifedipine and diltiazem on digitalis-induced OAPs on canine Purkinje fibers. In addition, the effects of nicardipine and nitrendipine on OAP have not been reported in the literature.

The antiarrhythmic drugs procainamide, quinidine,[7)] lidocaine,[1)] disopyramide[8)] and ethmozin,[7)] are known to significantly depress digitalis-induced OAPs. Mexiletine has been reported to abolish OAPs,[9)] and propranolol was reported to depress isoproterenol potentiation of ouabain-induced OAPs.[10)] This study compared the effects of calcium channel blockers, propranolol and mexiletine, on digitalis-induced OAP.

Materials and Methods

Seventy mongrel dogs of either sex, weighing 7 to 15 kg were used under sodium pentobarbital anesthesia (30 mg/kg i.v.). After initial artificial respiration (Harvard Apparatus, model 607), the heart was removed quickly and bathed in Tyrode's solution. The false tendons of both ventricles were excised and one was placed in a 3 ml lucite tissue chamber.

The false tendon in the chamber was perfused with modified Tyrode's solution, aerated with 95% O_2 and 5% CO_2. The millimolar composition of the Tyrode's solution was: NaCl 125, KCl 4.0, $NaHCO_2$ 24.6, $MgCl_2$ 0.5, $CaCl_2$ 1.8 and glucose 5.5. All experiments were performed at a temperature of 36.5 ± 0.5°C and a pH of 7.3.

The preparations were impaled with glass microelectrodes filled with 3M KCl having tip diameters less than 1 micrometer and a resistance of 10–20 MΩ. The preparations were stimulated at 1 Hz using rectangular pulses with 4 msec duration generated by a digital stimulator through an isolator (ME Commercial, ME-6012). Transmembrane potentials were amplified (Teledyne Philbrick, 1035–12, 1026) and displayed on an oscilloscope (type 564B, Tektronix, Inc.) for photographic recording (model PC-28B Continuous Recorder, Nihon Kohden Co.).

Data were simultaneously stored on a digital data recorder (TEAC, DR2000A) at a sampling rate of 20 KHz.

Measurement of oscillatory afterpotential (Fig. 1)

The preparations were stimulated at a basic cycle length of 1,000 msec for more than 30 min. Five minutes before the drug exposure, the basic

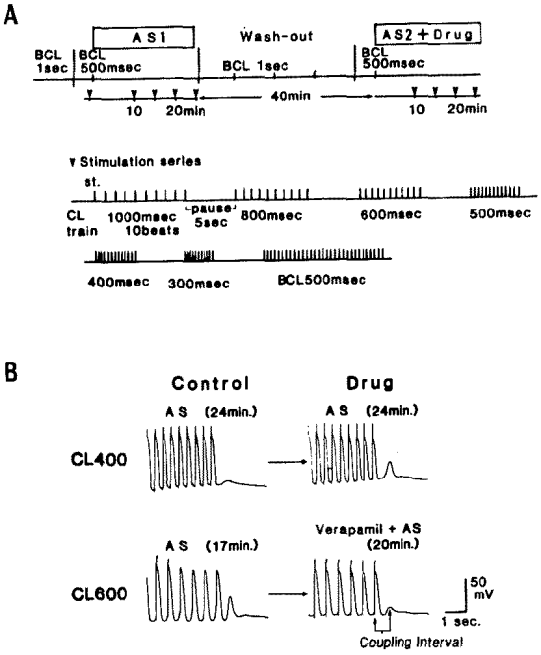


Fig. 1. Protocol of OAP measurement.

Panel A shows stimulation protocols to induce oscillatory afterpotentials (OAPs). At the time indicated by an arrow, pulse trains were given as shown in the " Stimulation series " (for details of the experiment, see the text). Panel B shows an example of an OAP during the first superfusion with 2.4×10^{-7} M acetylstrophanthidin (AS1) (" Control ") and during the second perfusion period with either AS alone (AS2) or with a drug (AS2+Drug) (" Drug "). In the upper part of panel B, AS was superfused for 24 min during stimulation at a cycle length of 400 msec. In the lower part of panel B, AS was given in combination with 2.0×10^{-6} M verapamil at a cycle length of 600 msec. CL=cycle length of the pulse train; BCL=basic cycle length; AS=acetylstrophanthidin, 2.4×10^{-7} M.

cycle length of the stimulus was changed to 500 msec. This cycle was maintained throughout the drug exposure period. The preparation was superfused for 15 to 25 min with Tyrode's solution containing 2.4×10^{-7} M acetylstrophanthidin (AS1). During this time, the stimulation was stopped for 5 sec every 5 min. When OAPs with an amplitude of more than 3 mV appeared during the pause in stimulation, the preparation was driven at cycle lengths of 1,000, 800, 600, 500, 400 and 300 msec, with trains of 10 stimuli; an intervening quiescent period of 5 sec was placed after each stimulation. The fibers were then washed for more than 40 min and stimulated with a basic cycle length of 1,000 msec. After the fiber was stimulated with a basic cycle length of 500 msec for 5 min, it was superfused with Tyrode's solution

containing AS alone or with either a calcium channel blocker (verapamil, diltiazem, nicardipine, nitrendipine or nifedipine), propranolol, mexiletine or procainamide for 15 to 25 min. The stimulation at the basic cycle length of 500 msec was continued with 5 sec pauses every 5 min for the same length of time as it had been stimulated before OAPs appeared at the first experiment (AS1). The same series of stimulation used as for AS1, independent of the appearance of OAPs, was used in the second experiment (AS2). The experiments using nifedipine, nitrendipine and nicardipine were performed in a dark room because of their photosensitive properties. The OAPs were developed in 107 of the 168 preparations; 99 of the 107 preparations were used for the experiments.

The oscillatory afterpotentials with an amplitude less than 40 mV were described as OAP. When their amplitudes were more than 40 mV, they were counted as triggered activities.

Measurement of action potential characteristics

Maximum upstroke velocity ($\dot{V}$max), amplitude of the action potential, 50% of action potential duration (APD50) and 90% of APD (APD90) were recorded by the digital data recorder (TEAC, DR-2000A). The data were analyzed with an HP 1000 minicomputer.

Statistical analysis

Statistical analyses were performed with an IBM 4341LO9 system using an SAS package by the paired and unpaired Student's t-test, and analysis of variance. When an F value was statistically significant after the analysis of variance, the least significant difference (LSD) method was used for further comparison.

Drugs

Verapamil HCl (M.W. 454.59), diltiazem HCl (M.W. 450.98) and mexiletine (M.W. 215.72) were prepared as solutions of 10^{-4} M/10 ml distilled water. Nifedipine (M.W. 346.34), nicardipine (M.W. 515.99) and nitrendipine (M.W. 360.30) were prepared as solutions of 10^{-4} M/10 ml methyl alcohol. Procainamide (M.W. 216.0) was prepared as a solution of 10^{-4} M/10 ml distilled water. An ampule of propranolol HCl 1 mg/ml (M.W. 295.81) was used. All of these drugs were diluted in 1 litre of Tyrode's solution to their respective final concentrations (see below).

Results

Oscillatory afterpotentials and triggered activity

(1) Effects of acetylstrophanthidin (AS) and antiarrhythmic drugs (Fig. 2)

In order to examine preparation stability over the relatively long time course of these experiments, control preparations were superfused with AS alone on the same schedule as in the experiments testing the effects of other

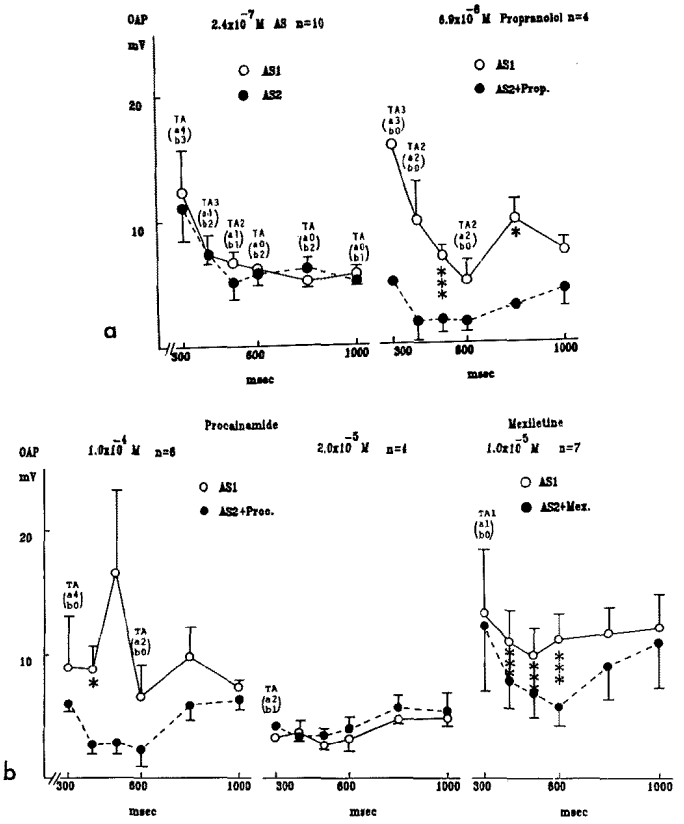


Fig. 2. OAP amplitude vs. cycle lengths of the pulse trains: Comparison between AS1 and AS2 periods, or AS1 and AS2 & antiarrhythmic drug superfusion periods.

The ordinate shows the amplitude of OAPs and the abscissa shows the cycle length (CL) of the pulse trains. Statistical significance was calculated by a paired t-test between the first AS (white circle, AS1) and the second AS period or the AS with drug period (black circle, AS2 or AS2+Drug) superfusion. * $p<0.05$, ** $p<0.02$, *** $p<0.01$. TA2=triggered activity developed in 2 preparations; a1=1 preparation developed TA during the first AS superfusion; b2=2 preparations developed TA during the second AS with drug superfusion; n=number of preparations.

drugs. The OAP amplitudes during the first AS superfusion (AS1) and the second AS superfusion (AS2) periods (Fig. 2a left panel) did not differ significantly at any cycle length. A triggered activity higher than 40 mV was observed in 6 preparations during the first AS superfusion and in 11 preparations during the second AS superfusion.

As shown in the right panel of Fig. 2a, the OAPs were significantly depressed by propranolol at two cycle lengths (CL) of the pulse trains, 500 and 800 msec. The triggered activities developed by the first AS superfusion in 7 preparations were abolished by propranolol in all preparations. Automaticity was developed by the first AS superfusion in 1 experiment; it was suppressed by the second AS+propranolol superfusion.

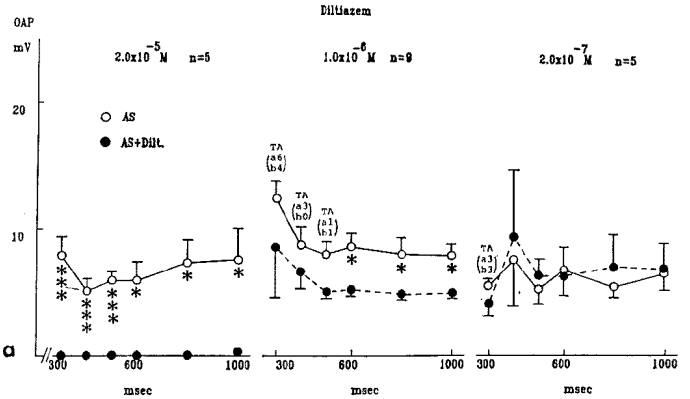
Procainamide (1.0×10^{-4} M) depressed the OAPs at a CL of 400 msec, and abolished the triggered activity which developed during the first AS superfusion in 6 preparations (Fig. 2b, left panel). Procainamide (2.0×10^{-5} M) did not affect OAPs.

Finally, mexiletine (1.0×10^{-5} M) depressed the OAPs significantly at CLs of 400, 500 and 600 msec (Fig. 2b, right panel). Triggered activities were abolished by mexiletine at a CL of 300 msec. Mexiletine also depressed automaticity in all 4 preparations.

(2) Effects of Ca channel blockers (Fig. 3)

Verapamil, diltiazem, nicardipine and nifedipine (1.0×10^{-5} M) depressed OAPs significantly at all cycle lengths. The triggered activities were also depressed by these drugs, except for 1 experiment in which nicardipine did not abolish the triggered activity at a CL of 300 msec. Verapamil and diltiazem depressed OAPs almost completely.

A lower dose of verapamil, diltiazem or nifedipine (2.0×10^{-6} M) also depressed OAPs significantly. Nicardipine and nitrendipine (2.0×10^{-6} M), though, did not show any significant depression of OAPs. The triggered



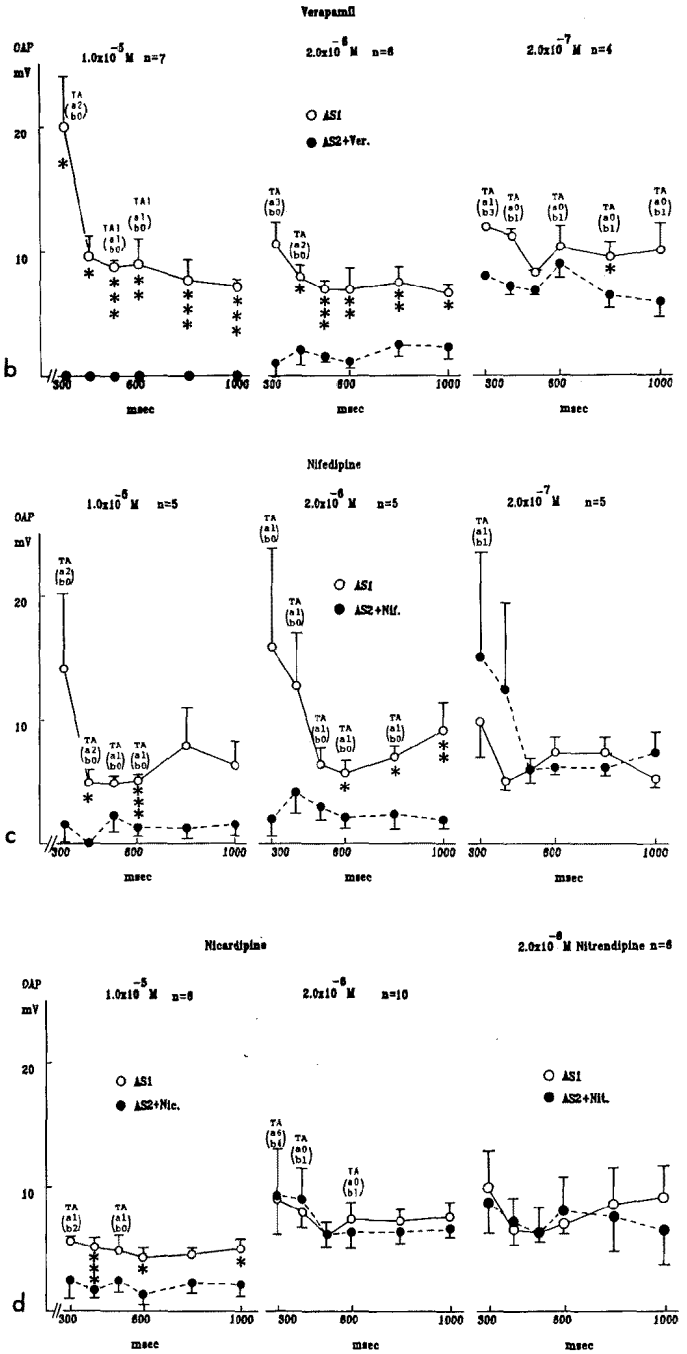


Fig. 3. OAP amplitudes vs. cycle lengths of the train pulses: Comparison between the AS1 and the AS2 & Ca channel blocker superfusion periods. Abbreviations are the same as in Fig. 2.

activity was abolished by verapamil in all 5 preparations which developed triggered activity. It was abolished by diltiazem in 5 of 10 preparations, and by nifedipine in all 5 preparations. The automaticity was also depressed in 1 preparation by verapamil, in 2 by diltiazem, in 1 by nifedipine and in 1 by nicardipine. However, diltiazem, nicardipine and nifedipine (2.0×10^{-7} M) did not depress triggered activity.

(3) Comparison of differences between OAP amplitudes of (AS1-AS2) and (AS1-AS2&drug) (Table Ia)

The differences between OAP amplitudes during the first AS and the second AS superfusion (AS1-AS2) were compared to those between OAP amplitudes during the first AS and the second AS in combination with drugs (AS1-AS2&drug). For statistical analysis, the least significant difference method (LSD) for paired comparisons when the F value in analysis of variance was significant. Among calcium antagonists, nifedipine (2.0×10^{-6} M) depressed OAP significantly more than AS at four CLs of pulse trains: 1,000, 800, 600 and 400 msec. Verapamil depressed OAP significantly at CLs of 1,000, 800 and 600 msec. Diltiazem depressed OAP significantly at CLs of 800 and 600 msec. The mean value of OAP amplitudes (AS1-AS2&drug) was in the following order; nifedipine>verapamil at CL 1,000, verapamil>nifedipine>diltiazem at CL 800 msec and verapamil>nifedipine>diltiazem at CL 600 msec. Nitrendipine and nicardipine did not show any significant differences.

Among the other antiarrhythmic drugs, procainamide (1.0×10^{-4} M)

Table Ia. Differences between OAP Amplitudes

CL of train pulse	⊿OAP			
	1,000 msec		800 msec	
	n	mean±SE (mV)	n	mean±SE (mV)
AS1 - AS2	9	−0.94±0.81	8	−1.71±1.29
AS1 - AS2&Prop	4	3.0 ±1.04	3	6.67±1.76*
AS1 - AS2&Proc	6	0.95±0.96	6	3.98±1.58*
AS1 - AS2&Mex	7	1.23±1.11	7	2.64±1.32*
AS1 - AS2&Ver	6	4.43±1.32*	6	5.27±1.32*
AS1 - AS2&Dil	9	1.27±1.47	9	3.51±1.05*
AS1 - AS2&Nic	10	0.76±1.11	9	0.66±0.92
AS1 - AS2&Nif	5	7.22±1.64*	4	4.63±1.43*
AS1 - AS2&Nit	6	2.63±2.96	6	0.87±2.99

* Significant difference by LSD in analysis of variance, compared to (AS1-AS2). F values were significant in all cycle lengths (CL) of the train pulses. ⊿OAP=differences of OAP amplitudes between the first AS superfusion (AS1) and the second AS (AS2) alone or AS2 in combination

and mexiletine (1.0×10^{-5} M) were effective at CLs greater than or equal to 500 msec. Propranolol (6.9×10^{-6} M) depressed OAPs significantly over the range of CLs. Propranolol was the most effective at a CL of 800 msec, mexiletine at CL 600 msec and procainamide at 500 msec. It was difficult to put the drugs in the order of efficacy.

Propranolol, procainamide, mexiletine, verapamil, diltiazem and nifedipine depressed OAPs compared to control (AS1-AS2). Nicardipine and nitrendipine were not effective during the early stages of stimulation at 1,000 msec and 800 msec.

Coupling interval (Figs. 1, 4, 5, Table Ib)

The coupling interval between the last beat of the train pulse and the OAPs was measured as illustrated in Fig. 1b. The coupling interval was shortened by the second AS superfusion, although there were no significant changes (Fig. 4a, left panel). Procainamide (1.0×10^{-4} M) significantly prolonged the coupling interval at CL 300 and 400 msec. Nitrendipine (2.0×10^{-6} M) shortened the coupling interval significantly at CL 300, 400 and 1,000 msec. Nifedipine also had a tendency to shorten coupling intervals.

Differences between the coupling intervals between the first and the second AS alone or AS with drug superfusion were tested by LSD and analysis of variance (Table Ib). The differences were significantly different in the groups only at CLs of 600 and 400 msec. Verapamil, diltiazem, nicardipine, propranolol, procainamide and mexiletine also prolonged the

of (AS1-AS2) and those of (AS1-AS2&Drug)

amplitude

600 msec		500 msec		400 msec		300 msec	
n	mean±SE (mV)	n	mean±SE (mV)	n	mean±SE (mV)	n	mean±SE (mV)
8	−0.34±1.07	8	2.06±3.20	7	0.59±1.16	4	3.40±3.02
2	3.15±0.15*	3	4.97±0.49	3	17.13±8.83*	3	27.0 ±8.50*
4	4.43±1.49	6	13.82±5.88*	6	6.12±1.97	2	3.0 ±5.0
7	5.66±1.18*	6	3.02±0.50	6	3.28±0.69	4	1.25±2.13
6	5.70±1.64*	6	5.45±0.80	4	5.88±2.19	3	9.63±2.66
9	3.30±1.27*	7	1.01±1.73	5	2.14±1.65	2	4.25±3.05
9	1.79±0.77	10	−0.12±1.24	9	−1.13±2.72	4	−0.35±0.94
4	3.60±1.06*	4	3.33±1.41	4	8.68±4.14*	4	13.95±6.70
6	−0.97±2.13	6	1.77±1.35	6	−0.47±1.62	6	3.95±2.49

with Ca antagonist or antiarrhythmic drug; Prop=propranolol 6.9×10^{-6} M; Proc=procainamide 1.0×10^{-4} M; Mex=mexiletine 1.0×10^{-5} M; Ver=verapamil 2.0×10^{-6} M; Dil=diltiazem 2.0×10^{-6} M; Nic=nicardipine 2.0×10^{-6} M; Nif=nifedipine 2.0×10^{-6} M; Nit=nitrendipine 2.0×10^{-6} M.

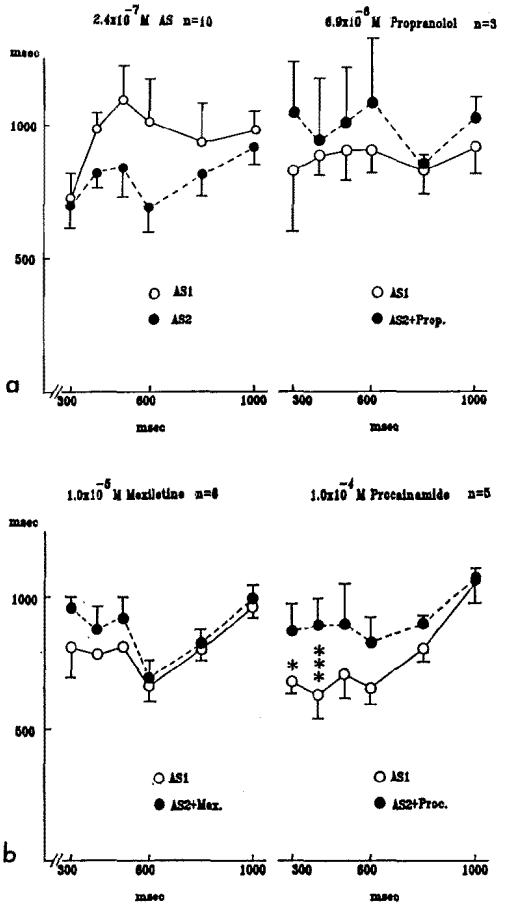


Fig. 4. Coupling interval vs. cycle lengths of the pulse trains: Comparison between AS1 and AS2 periods, or AS1 and AS2 & antiarrhythmic drug superfusion periods.

Coupling intervals were calculated as shown in Fig. 1, panel B. The abscissa shows cycle lengths of the train pulses and the ordinate shows coupling interval. Abbreviations are the same as in Fig. 2.

coupling intervals significantly compared to AS alone at a CL of 600 msec. At a CL of 400 msec, diltiazem, procainamide and mexiletine significantly prolonged the coupling interval. However, nicardipine and nitrendipine did not show any significant difference at any CL.

Action potential characteristics (Tables II, III)

Since the effects of calcium channel blockers and antiarrhythmic drugs on action potentials are reported to develop within 10 min,[9),11)-15)] action potential characteristics were measured after a 10 min-superfusion. Per-

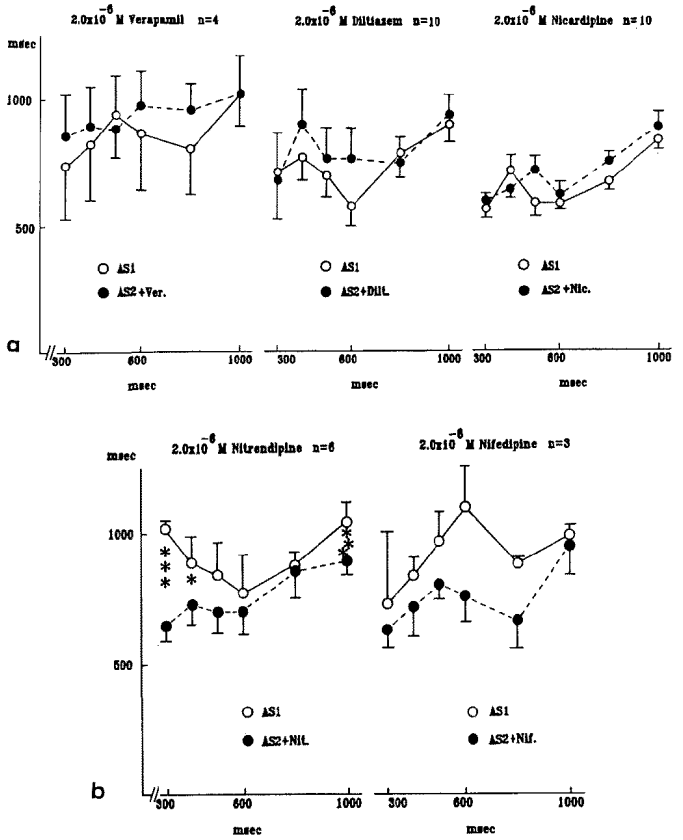


Fig. 5. Coupling intervals vs. cycle lengths of the pulse trains: Comparison between AS1 and Ca channel blocker administration. Abbreviations are the same as in Fig. 2.

centile changes in maximum rate of rise ($\dot{V}$max), the amplitude of action potentials, action potential duration at 50% of amplitude (APD50) and APD at 90% of amplitude (APD90) were calculated after a 10 min-superfusion period with AS alone, or AS with other drugs. There was no significant change in $\dot{V}$max, amplitude or APD90 between the first AS superfusion and the second AS with any drug superfusion. APD50 was significantly prolonged by propranolol (6.9×10^{-6} M, $p<0.01$) and significantly shortened by nifedipine (2.0×10^{-6} M, $p<0.02$) and diltiazem (2.0×10^{-6} M, $p<0.05$). The maximum diastolic potentials (MDP) at the beginning of stimulation which elicited OAPs were compared between the first AS and the second AS superfusion with the drugs; the only significant changes were elicited by procainamide (1.0×10^{-4} M) and diltiazem (2.0×10^{-6} M).

Table Ib. Differences between Coupling Intervals of (AS2-AS1) and those of (AS2&Drug-AS1)

CL of train pulse	Coupling interval			
	600 msec		400 msec	
	n	mean±SE	n	mean±SE
AS2 - AS1	10	−225.9±101.9	10	−165.5± 48.6
AS2&Prop - AS1	3	173.3±181.8*	3	62.7±292.3
AS2&Proc - AS1	5	122.0± 63.7*	4	242.5± 44.4*
AS2&Mex - AS1	6	24.2± 27.1*	6	99.3± 42.0*
AS2&Ver - AS1	3	110.0±153.1*	3	70.0±123.4
AS2&Dil - AS1	9	96.7± 52.8*	8	130.6±113.6*
AS2&Nic - AS1	9	39.4± 59.6*	10	−72.5± 59.8
AS2&Nif - AS1	3	−335.7±266.6	3	−116.7±148.1
AS2&Nit - AS1	5	−71.8± 97.8	5	−162.0± 6.3

F value was significant only in the CL 600 and 400 msec groups. Abbreviations are the same as in Fig. 2a.

Table II. Maximum Diastolic Potential at the Beginning of the Train Pulse Which Developed OAP

		n	AS1 mean±SE (mV)	AS2 with drug mean±SE (mV)
Acetylstrophanthidin	2.4×10^{-7} M (AS)	10	76.00± 3.87	75.10±4.14a
Propranolol	6.9×16^{-8} M	3	68.43± 7.46	59.00±1.00
Procainamide	1.0×10^{-4} M	6	75.50± 4.94	57.33±5.40*
	2.0×10^{-5} M	3	76.00±11.14	72.67±3.37
Mexiletine	1.0×10^{-5} M	6	69.02± 6.13	71.67±3.37
Verapamil	1.0×10^{-5} M	7	86.49± 4.13	84.41±3.90
	2.0×10^{-6} M	6	76.50± 3.63	77.95±3.90
	2.0×10^{-7} M	4	75.50± 5.17	68.75±4.54
Diltiazem	1.0×10^{-5} M	5	72.60± 5.04	75.94±4.49
	2.0×10^{-6} M	9	80.33± 2.27	72.81±2.44*
	2.0×10^{-7} M	5	77.00± 4.96	74.26±2.95
Nicardipine	1.0×10^{-5} M	5	77.80± 1.43	70.00±5.32
	2.0×10^{-6} M	9	77.00± 3.90	75.41±3.88
Nifedipine	1.0×10^{-5} M	5	75.20± 6.41	71.20±5.46
	2.0×10^{-6} M	5	76.60± 4.55	62.40±9.57
	2.0×10^{-7} M	5	75.40± 1.36	76.20±5.07
Nitrendipine	2.0×10^{-6} M	6	78.50± 5.43	79.17±1.87

* statistical significance ($p<0.05$) by paired t-test. n=number of preparations; AS1=first AS superfusion; AS2=second AS superfusion. a: only AS was used in the second superfusion.

Discussion

It has been reported that verapamil, nifedipine and diltiazem depress the delayed after-depolarization (DAD, same as OAP).[1),5)] These drugs were studied separately, and the effects of nifedipine and diltiazem were examined on ischemia-induced DAD, but not on digitalis-induced DAD. In our experiments, the effects of calcium antagonists (verapamil, diltiazem, nifedipine and nicardipine) were dose-dependent. While 2.0×10^{-7} M levels of calcium antagonists did not depress OAP and triggered activity, 2.0×10^{-6} M verapamil, diltiazem and nifedipine depressed or abolished OAPs and triggered activity. However, 2.0×10^{-6} M nicardipine and nitrendipine did not depress OAP and triggered activity. Finally, 1.0×10^{-5} M concentrations of calcium antagonists depressed OAP and triggered activity; nitrendipine was not examined because of its insolubility at such high concentrations. Thus, the effects on OAPs and triggered activity seem to be in the following order: verapamil, (nifedipine)>diltiazem>nicardipine, nitrendipine. In our experiments, nifedipine seemed to be more potent than diltiazem. However, diltiazem and verapamil have a stronger use-dependent block than nifedipine,[16)] and these drugs are effective in shorter cycle length. Therefore, the depressant effects of these drugs may have been underestimated in the experiment, and we did not compare nifedipine and diltiazem directly.

The depressant effects of the antiarrhythmic drugs, lidocaine,[1)] procainamide, quinidine and ethmozin,[7)] mexiletine[9)] and disopyramide[8)] and propranolol[10)] have all been studied on OAPs. In our study, procainamide, mexiletine and propranolol depressed OAPs. Mexiletine also has use-dependent block.[16)] Therefore, the depressant effect of mexiletine on OAPs may be more potent than the effect of those in our data. Propranolol was reported to decrease only the potentiating effect of isoproterenol on digitalis-induced OAPs.[10)] In our study, we used propranolol at concentrations one order of magnitude higher than that of their study. Propranolol as well as nifedipine depressed OAPs over a wide range of cycle lengths of the pulse trains. There were no significant differences in OAP depressive effects between the calcium antagonists and antiarrhythmic drugs.

The mechanisms of development of OAPs have been discussed in the literature.[17)-20)] Digitalis inhibits Na^+-K^+ ATPase and blocks the Na-K pump. This causes an increase in intracellular Na^+ which leads to intracellular Ca^{++} overloading through the Na^+-Ca^{++} exchange mechanism. When intracellular Ca^{++} is overloaded, a transient inward current occurs and causes OAPs. Calcium channel blockers block the slow Ca current and indirectly

Table III. Effects of Drugs on

	n	Amplitude	
		Before mean±SE (mV)	Drug# mean±SE (%)
2.4×10^{-7} M Acetylstrophanthidin			
AS1	5	120.0± 3.0	−1.5±3.1
AS2	5	120.6± 3.3	−13.4±8.4
6.9×10^{-6} M Propranolol & AS2	4	101.8± 8.2	−6.7±2.2
2.0×10^{-5} M Procainamide & AS2	4	111.5± 8.3	−5.1±3.1
1.0×10^{-5} M Mexiletine & AS2	3	101.7±16.0	+1.6±4.7
2.0×10^{-6} M Verapamil & AS2	3	116.0± 8.7	−0.3±2.8
2.0×10^{-6} M Diltiazem & AS2	4	124.3± 3.8	−10.2±0.7
2.0×10^{-6} M Nicardipine & AS2	4	125.8± 1.0	−3.4±1.3
2.0×10^{-6} M Nifedipine & AS2	5	123.8± 2.9	−12.7±2.8
2.0×10^{-6} M Nitrendipine & AS2	4	112.3± 6.1	−9.1±1.7

percentile changes during AS or AS with drug superfusion for 10 min compared to data during superfusion in the Tyrode's solution.

decrease intracellular Ca^{++} concentrations. The order of the depressive effects of verapamil, diltiazem and nitrendipine on OAP, which we described above, are almost identical to their effects on the slow inward current.[21] However, class I antiarrhythmic drugs such as procainamide[7),14] and mexiletine[9] decrease OAPs by decreasing the fast Na^+ current which decreases intracellular Na^+ and leads to a decrease in intracellular Ca^{++} through Na^+-Ca^{++} exchange.[22),23] Propranolol might have decreased OAPs by antagonizing beta receptor stimulation of AS.[23]

Hewett[7] reported that prolongation of the coupling interval between the last pulse trains and the OAP was correlated to a decrease in OAP amplitude. In our data, the coupling interval was prolonged significantly by verapamil, diltiazem and nicardipine. However, nifedipine depressed OAPs but did not prolong the coupling interval significantly. It may be argued that the levels of intracellular Ca^{++} may have been different during the two AS superfusion periods because intracellular Ca^{++} is increased rapidly by AS. From this viewpoint, we cannot conclude that nifedipine shortens the coupling interval. Procainamide, mexiletine and propranolol, though, prolonged the coupling interval significantly.

Henning[24] reported that there was a positive relationship between the OAP amplitude and the action potential duration (APD). In our experiments, the APD50 was shortened by diltiazem and nifedipine as in Henning's report, but it was prolonged by propranolol. All of these drugs depressed the OAP amplitude significantly. We do not know why our data

Action Potential Characteristics

Vmax		APD50		APD90	
Before mean±SE (V/sec)	Drug# mean±SE (%)	Before mean±SE (mV)	Drug# mean±SE (%)	Before mean±SE (msec)	Drug# mean±SE (%)
410.4± 31.4	−21.6± 8.3	152.8±19.6	−1.8±3.3	227.0±17.1	+0.2±1.8
416.6± 42.8	−10.9±10.9	156.8±14.1	+5.7±3.2	222.5±15.0	−3.5±4.8
330.0± 78.6	−15.8± 3.9	165.3±12.9	+10.8±2.9*	273.2±14.5	+6.0±1.6
344.3± 67.8	−20.4± 8.3	166.1±18.2	+0.7±2.4	262.8± 3.7	+2.8±0.6
278.7±106.8	−14.4±10.0	180.0±35.1	−4.7±3.2	228.4± 7.8	−2.0±0.9
444.0± 8.0	−16.0± 0.4	162.3± 3.6	−10.1±1.4*	228.4± 4.3	+3.4±3.0
456.5± 62.7	−20.0± 5.4	152.7±12.0	−16.6±3.1*	222.9± 7.0	−4.4±2.2
461.5± 29.2	−10.0± 3.3	165.4±16.6	−9.4±4.5	233.5±16.6	−2.5±3.1
387.8± 70.0	−12.2± 4.9	187.0±20.5	−17.4±1.4*	257.8±23.1	−5.2±2.8
344.3± 49.3	−0.5± 5.2	159.9±17.3	−3.8±4.7	231.3±15.6	−0.6±3.2

* statistically significant ($p<0.05$) compared to the first AS superfusion by unpaired t-test. BCL was 500 msec.

contradicts Henning's reports.[24] Shortening of the APD50 by diltiazem[12] and nifedipine,[11] though, were consistent with other studies. David[15] reported that concentrations of propranolol less than 1.0×10^{-6} M did not influence APD, while a 1.0×10^{-4} M level shortened the APD. We used 6.9×10^{-6} M propranolol in combination with acetylstrophanthidin, which prolonged the APD50. Since toxic doses of acetylstrophanthidin were reported to stimulate the beta receptor of sympathetic nerve endings,[23] propranolol may have antagonized beta stimulation, resulting in an increased APD50.

Verapamil, nifedipine and diltiazem appear to be useful calcium antagonists for treating digitalis arrhythmia (OAP, triggered activity and automaticity). However, we must be careful in applying the results of the experiments to clinical practice. In particular, nifedipine may shorten the functional refractory period of the AV node and the AH interval in man and dog,[25),26] stimulate sympathetic activity by hypotension or may cause ventricular arrhythmias.[27]

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