Reflex Chronotropic and Inotropic Effects of Calcium Channel-Blocking Agents in Conscious Dogs

Diltiazem, Verapamil, and Nifedipine Compared

HARUAKI NAKAYA, ARNOLD SCHWARTZ, AND RONALD W. MILLARD

SUMMARY. In chronically instrumented, conscious dogs, rapid injection of equihypotensive doses of three calcium channel-blocking agents, verapamil (250 μg/kg), diltiazem (200 μg/kg) and nifedipine (50 μg/kg), produced disparate chronotropic and inotropic responses. Although they all decreased mean arterial pressure by about 10%, heart rate (93 ± 4 beats/min) was markedly increased to 175 ± 12 with nifedipine, to 163 ± 15 with verapamil, and only slightly increased to 118 ± 7 with diltiazem. Contractile responses measured before (left ventricular dP/dt max, 2749 ± 131 mm Hg/sec) and during left ventricular ejection (endocardial dimension dD/dt max, 57 ± 4 mm/sec) were increased by 24% and 14% with nifedipine, decreased by 26% and 22% with verapamil, and were unchanged with diltiazem. These chronotropic and inotropic responses to rapid intravenous administration of the three drugs were increased in a dose-dependent manner. Similar results also were observed after slow infusion of these drugs. To determine the extent to which autonomic reflexes participated in these cardiac responses, propranolol (0.5 mg/kg) or propranolol plus atropine (0.1-0.2 mg/kg) was administered prior to injection of each calcium channel-blocking agent. Propranolol abolished the positive inotropic response to nifedipine and potentiated the negative inotropic response to verapamil. Positive chronotropic responses to verapamil, nifedipine, and diltiazem were attenuated by propranolol plus atropine. These results suggest that equihypotensive doses of the three prominent calcium channel-blocking agents exert different degrees of autonomic reflex activation in awake, unsedated dogs. These reflexes, which modulate the direct effects of calcium channel-blocking agents on chronotropic and inotropic variables of the heart, may have important clinical implications. (Circ Res 52: 302-311, 1983)

CALCIUM CHANNEL-BLOCKING agents, represented by verapamil, diltiazem, and nifedipine, the drugs introduced recently in the United States, are known to be extremely useful for treatment of angina pectoris, certain cardiac arrhythmias, and hypertension (Ellrodt et al., 1980; Henry, 1980; Schwartz et al., 1981; Schwartz, 1982). Although these drugs have completely dissimilar chemical structures and physico-chemical properties, they do share the common property of inhibiting transmembrane calcium movement from extracellular to intracellular spaces in both cardiac and vascular smooth muscle (Fleckenstein, 1977). By this mechanism, all three drugs produce in vitro, dose-dependent, direct depression of myocardial contractility and sinus node automaticity, as well as relaxation of isolated coronary and peripheral blood vessels (Taira, 1979; Millard et al., 1982; Schwartz et al., 1981). In anesthetized open-chest dogs, verapamil produces dose-dependent negative chronotropic and inotropic responses consistent with the in vitro observations (Mangiaridi et al., 1978). In contrast to this, intravenous administration of verapamil in conscious dogs produces tachycardia (Newman et al., 1977; Walsh et al., 1981). Similarly, nifedipine and diltiazem are known to cause positive chronotropic response in conscious dogs (Cross et al., 1979; Walsh et al., 1981). Thus, it appears that the direct actions of these calcium channel-blocking agents on cardiac tissues can be significantly modulated in conscious animals, presumably by systemic hypotension-induced baroreceptor activation of autonomic reflexes.

The present study was undertaken to compare cardiac chronotropic and inotropic responses elicited by three clinically useful calcium channel-blocking agents—diltiazem, nifedipine, and verapamil—in chronically instrumented conscious dogs. To determine the contribution of vagal and sympathetic reflexes to these cardiac responses, experiments were performed before and after selective and combined pharmacological autonomic blockade.

Methods

Animal Preparation

Adult mongrel dogs of either sex weighing 16-28 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The animals then were intubated and ventilated by a fixed volume positive pressure respirator (model 607, Harvard Apparatus) with a mixture of the room air and oxygen. An aseptic thoracotomy was performed at the left 5th intercostal space. A precalibrated solid state pressure transducer (Konigsberg P6, Konigsberg Inst.) was implanted.
through a stab wound in the left ventricle apex for measurement of pressure and its first derivative, dP/dt. Global left ventricular mechanics were measured as minor axis dimension with two 3 MHz piezoelectric crystals (diameter 4 mm) implanted through transmural stab incisions so that they rested on the anterior and the posterior endocardial wall. Heparin-filled Tygon catheters (0.40 'i.d. X 0.70' o.d., formulation 5-54-HL, Norton Plastics) were secured in the left atrial appendage and in the descending thoracic aorta via the femoral artery to measure left atrial and systemic arterial pressures, respectively. All catheters and leads were directed subcutaneously and exteriorized at the dorsal, mid-cervical region of the neck. All dogs were allowed to recover at least 2 weeks after operation before experiments were initiated.

Hemodynamic Measurements

The solid state pressure transducers were precalibrated at 37°C against a mercury manometer before implantation. To correct the zero drift of the pressure cell, the left ventricular end-diastolic pressure (LVEDP) was adjusted equal to the mean left atrial pressure, and systolic left ventricular pressure was matched to systolic thoracic arterial pressure. Left ventricular dP/dtmax (LVdP/dtmax) was obtained by electronic differentiation of the left ventricular pressure pulse. Arterial and left atrial pressure were measured with calibrated strain gauge manometers (model P23Db, Gould, Inc.). Minor axis of left-ventricular internal dimension was determined by electronic measurement of the acoustic impulse transit time between the two crystals (Triton Instruments) placed at opposing endocardial sites. The transit time (distance) is measured 800 times/sec, providing a voltage output proportional to distance. The measurements have a sensitivity of 0.04 mm and calibrations are stable for more than 2 hours. In this manner, the left ventricular end-diastolic dimension (LVEDD), end-systolic dimension (LVESD), and maximal shortening velocity (dD/dtmax) were obtained from the records. LVdP/dtmax was synchronous with LV end-diastolic pressure and the R wave of the electrocardiogram. LVESD was at the nadir of the dimension excursion and occurred synchronous with aortic valve closure and the end of the T-wave on the electrocardiogram. Percent systolic shortening (%SS) was calculated according to the formula LVdP/dtmax/LVEDD x 100%. All variables including electrocardiogram (limb lead II) were stored on a magnetic data recorder (model 5600C, Honeywell Instruments) and recorded at 50 mm/sec paper speed on a rectilinear multichannel recorder (model 280, Brush/ Gould, Inc.). Heart rate was derived by multiplying by three the manually counted R waves of the electrocardiogram for 20 seconds centered on each time point reported.

Experimental Protocol

In eight dogs, three calcium channel-blocking agents, verapamil (250 µg/kg), diltiazem (200 µg/kg), and nifedipine (50 µg/kg), were administered intravenously over 1 minute on separate days, and time course changes of hemodynamics were observed for 30 minutes. These doses of the calcium channel-blocking agents were selected to reduce the mean arterial pressure (MAP) by approximately 10%. The same doses of three calcium channel-blocking agents were infused slowly over 10 minutes using a Harvard infusion pump (model 901) in four dogs. The changes of hemodynamic responses were determined at the end of the infusion and compared with those after rapid injection.

A dose-response study was also performed with these agents in four dogs. Incremental doses of verapamil (30, 100, 300, 1000 µg/kg), diltiazem (30, 100, 300, 1000 µg/kg) or nifedipine (3, 10, 30, 100 µg/kg) were given intravenously over 1 minute at intervals of 10 minutes.

To determine the role of efferent sympathetic nerve activation in the cardiovascular responses elicited by the rapid injection of these calcium channel-blocking agents, propranolol (0.5 mg/kg) was administered intravenously 10 minutes before the injection of calcium channel-blocking agents in five dogs. The effectiveness of the propranolol treatment was confirmed by the elimination of the tachycardia and increase in LV dP/dtmax induced by a rapid intravenous injection of isoproterenol (0.3 µg/kg). On a different day, these dogs were also pretreated with the combination of atropine sulfate (0.1-0.2 mg/kg) and propranolol in order to assess the effect of combined sympathetic and parasympathetic blockade on the responses observed after the rapid injection of these calcium channel-blocking agents. This dose of atropine has been shown to eliminate hypotension associated with reflex tachycardia following intravenous 10 µg/kg of acetylcholine (Barron and Bishop, 1981) in conscious dogs. Two dogs were pretreated with 0.2 mg/kg of atropine alone before the injection of the calcium channel-blocking agents, to clarify sympathetic contributions to inotropic state changes induced by these agents.

One of the three calcium channel-blocking agents was selected randomly and given on each day. In the dose-response studies, at least 48 hours were allowed to elapse between experiments.

Drugs

The following drugs were used: verapamil HCl (Knoll Pharmaceutical Co.), diltiazem HCl (Marion Labs, Inc.), nifedipine (Pfizer Inc.), propranolol HCl (Ayerst Labs), atropine sulfate (Eli Lilly), isoproterenol HCl (Winthrop Lab.), and acetylcholine (Smith, Miller and Path). Nifedipine was dissolved in 15% ethyl alcohol, 15% polyethylene glycol, and 70% saline in a dark room and injected, using a syringe shielded from the light. It was confirmed that this solvent did not exert any appreciable hemodynamic responses.

Statistical Analysis

All data in tables and figures are presented as mean values ± se. Changes from baseline values were analyzed by paired t-test. Comparisons between responses to calcium channel-blocking drugs before and after selective and combined autonomic blockades and for the dose-response data were made by analysis of covariance using general linear models (GLM). In addition, paired comparisons were performed employing Duncan's multiple range test. These analyses were made using the GLM procedure with Duncan's multiple range test on an Amdahl-470 computer as provided by the SAS Institute, Inc. Statistical significance was reached when probability estimates fell below the 5% level.

Results

Cardiac Responses after Equihypotensive Doses of Calcium Channel-Blocking Agents

Rapid Intravenous Injections

The time course of chronotropic, dromotropic, and inotropic responses after rapid injection of these calcium channel-blocking agents is summarized in Tables 1 and 2. Verapamil, diltiazem, and nifedipine...
Global Cardiac Inotropic Responses in Conscious Dogs

<table>
<thead>
<tr>
<th>Control</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>97 ± 5</td>
<td>85 ± 4‡</td>
<td>95 ± 3</td>
<td>94 ± 5</td>
</tr>
<tr>
<td>SAP (mm Hg)</td>
<td>135 ± 7</td>
<td>116 ± 6‡</td>
<td>123 ± 5‡</td>
<td>122 ± 4</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>75 ± 8</td>
<td>69 ± 4‡</td>
<td>81 ± 3</td>
<td>79 ± 4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>93 ± 7</td>
<td>163 ± 15‡</td>
<td>129 ± 9</td>
<td>114 ± 10‡</td>
</tr>
<tr>
<td>P-R interval (msec)</td>
<td>106 ± 6</td>
<td>129 ± 8‡</td>
<td>144 ± 11‡</td>
<td>144 ± 9</td>
</tr>
<tr>
<td>SS (%)</td>
<td>17.7 ± 3.3</td>
<td>13.7 ± 2.5‡</td>
<td>14.1 ± 2.7</td>
<td>14.7 ± 3.0‡</td>
</tr>
<tr>
<td>dD/dt max (mm/sec)</td>
<td>58 ± 7</td>
<td>54 ± 6‡</td>
<td>47 ± 5‡</td>
<td>50 ± 7‡</td>
</tr>
</tbody>
</table>

* During 30 minutes after rapid intravenous injection of equihypotensive doses of verapamil, diltiazem, and nifedipine.
‡ P < 0.05 vs. control value.

Results are expressed as mean ± se. n = 8 in each group. V = verapamil, 250 μg/kg; D = diltiazem, 200 μg/kg; N = nifedipine, 50 μg/kg. MAP = mean arterial blood pressure; SAP = systolic arterial blood pressure; DAP = diastolic arterial blood pressure; HR = heart rate.

Global Cardiac Inotropic Responses in Conscious Dogs

<table>
<thead>
<tr>
<th>Control</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>dP/dt_{max} (mm Hg/sec)</td>
<td>2766 ± 236</td>
<td>2034 ± 222‡</td>
<td>2162 ± 190‡</td>
<td>2251 ± 190‡</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>43.2 ± 2.5</td>
<td>42.2 ± 2.0</td>
<td>43.1 ± 2.4</td>
<td>43.1 ± 2.4</td>
</tr>
<tr>
<td>SS (%)</td>
<td>16.6 ± 3.3</td>
<td>18.1 ± 3.7</td>
<td>16.9 ± 3.3</td>
<td>17.0 ± 3.4</td>
</tr>
<tr>
<td>dD/dt max (mm/sec)</td>
<td>58 ± 7</td>
<td>54 ± 6‡</td>
<td>47 ± 5‡</td>
<td>50 ± 7‡</td>
</tr>
</tbody>
</table>

* During 30 minutes after rapid intravenous injection of equihypotensive doses of verapamil, diltiazem, and nifedipine.
‡ P < 0.05 vs. control value.

Results are expressed as mean ± se. n = 8 in each group. V = verapamil, 250 μg/kg; D = diltiazem, 200 μg/kg; N = nifedipine, 50 μg/kg. \( \text{dP/dt}_{\text{max}} \) = left ventricular maximum rate of pressure development; \( \text{LVEDD} \) = internal minor axis, left ventricle end diastolic dimension; SS = systolic shortening fraction; \( \text{dD/dt}_{\text{max}} \) = left ventricle maximum rate of internal minor axis diameter decrease.
The magnitude of positive chronotropic responses was less pronounced than achieved only by nifedipine. The magnitude of positive inotropic response to nifedipine is illustrated by the representative tracing in Figure 4. Prolongation of the P-R interval can be seen clearly in the ECG record after 300 μg/kg verapamil or diltiazem but not after 30 μg/kg nifedipine (Fig. 3). Three of four dogs studied developed Wenckebach-type atrioventricular conduction block after 1000 μg/kg of verapamil and diltiazem, which returned to 1:1 conduction within 30 minutes.

Rapid intravenous injections of the calcium channel-blocking agents were made in four conscious dogs. Only one drug was administered on each day. Doses greater than those reported in Figure 5 produced either a precipitous drop in blood pressure (nifedipine) or complete atrioventricular block (diltiazem and verapamil).

**Dose-Dependent Cardiac Responses**

Representative records obtained on different days from the same dog demonstrate that the three calcium channel-blocking agents produce disparate chronotropic and inotropic responses (Fig. 3). Verapamil produced a marked decrease in left ventricular dP/dt max; diltiazem had no demonstrable effect, whereas nifedipine increased dP/dt max. The time course of the inotropic response to nifedipine is illustrated by the representative tracing in Figure 4. Prolongation of the P-R interval can be seen clearly in the ECG record after 300 μg/kg verapamil or diltiazem but not after 30 μg/kg nifedipine (Fig. 3). Three of four dogs studied developed Wenckebach-type atrioventricular conduction block after 1000 μg/kg of verapamil and diltiazem, which returned to 1:1 conduction within 30 minutes.

**Intravenous Infusions**

Slow infusion of the same doses of the three calcium channel-blocking agents, as used in the rapid injection studies, reduced mean arterial pressure by 7-9% at the end of infusion of each drug (Fig. 2). Although this pressure reduction was not different among the drugs, a significant hypotension was achieved only by nifedipine. The magnitude of positive chronotropic responses was less pronounced than that after rapid injection. However, each drug still produced a significant tachycardia. The increase in heart rate after diltiazem (9 ± 2 beats/min) was significantly less than that which occurred after nifedipine administration (35 ± 11 beats/min) (P < 0.05).

The tachycardia caused by verapamil was not significantly different from that produced by nifedipine. No significant changes were produced in dP/dt max by any of the drugs. The decrease in dD/dt max after verapamil infusion (~3.4 ± 1.6 mm/sec) was significantly different from the increase after nifedipine (2.9 ± 2.7 mm/sec). The P-R interval was prolonged after verapamil by 19 ± 5 msec (P < 0.05) at the end of 10-minute infusion, whereas diltiazem and nifedipine did not significantly change the P-R interval.
Blood Pressure

Mean arterial blood pressure was significantly decreased from baseline (91 ± 3 mm Hg) by nifedipine at doses between 10 and 100 μg/kg, and by diltiazem and verapamil at doses between 30 and 300 μg/kg. Nifedipine produced significantly greater hypotension (−11 ± 2, −16 ± 1 mm Hg) than either diltiazem (−4 ± 1, −7 ± 2 mm Hg) or verapamil (−6 ± 1, −7 ± 3 mm Hg) at both 30 and 100 μg/kg.

Heart Rate

Heart rate was significantly increased from baseline (87 ± 6 beats/min) by nifedipine at doses from 3 to 100 μg/kg and by verapamil at doses from 30 to 300 μg/kg. All three calcium channel-blocking agents produced a dose-dependent tachycardia. At 30 μg/kg, the tachycardia caused by nifedipine (+67 ± 11 beats/min) was significantly greater than that produced by verapamil (+7 ± 1 beats/min) or diltiazem (+5 ± 3 beats/min). At 100 μg/kg, this difference remained as nifedipine increased heart rate by 104 ± 13 beats/min while verapamil and diltiazem produced increases of only 17 ± 1 and 13 ± 6 beats/min, respectively.

P-R Interval

Only verapamil at 100 and 300 μg/kg produced a significant prolongation of the baseline P-R interval (100 ± 2 msec). The P-R interval was prolonged by verapamil and diltiazem and shortened by nifedipine in a dose-dependent fashion. The responses to nifedipine were different from verapamil at 30 μg/kg and from both verapamil and diltiazem at 100 μg/kg.

$\frac{dP}{dt_{\text{max}}}$

The positive left ventricular $\frac{dP}{dt_{\text{max}}}$ was depressed from baseline (2639 ± 88 mm Hg/sec) by verapamil in a dose-dependent fashion, reaching significance at doses of 100 and 300 μg/kg. Nifedipine, as shown in Figure 4, produced a biphasic effect on $\frac{dP}{dt_{\text{max}}}$. Both the initial depression of $\frac{dP}{dt_{\text{max}}}$ and the subsequent augmentation by nifedipine showed a dose dependency. Significant depression of $\frac{dP}{dt_{\text{max}}}$ was achieved only with nifedipine at 100 μg/kg. No significant dose dependency could be demonstrated on $\frac{dP}{dt_{\text{max}}}$ for diltiazem over the dose range examined. A slight but significant decrease in $\frac{dP}{dt_{\text{max}}}$ was seen after diltiazem, 300 μg/kg. At a dose of 100 μg/kg, nifedipine produced significantly greater depression of $\frac{dP}{dt_{\text{max}}}$ (−771 ± 190 mm Hg/sec) than did diltiazem (−130 ± 47 mm Hg/sec). In contrast to the sustained depression of $\frac{dP}{dt_{\text{max}}}$ caused by verapamil at this dose,
Figure 5. Dose-response curves for verapamil (V, ●), diltiazem (D, ○), and nifedipine (N, A) in four conscious dogs. Data are expressed as change from baseline control values with symbols indicating mean value, bars representing ±1 standard error of the mean value, and asterisks (*) denoting significant differences (P < 0.05) from baseline values. Standard error bars are occasionally smaller than symbols. Dashed lines are for dP/dt max increases seen with high doses of nifedipine and diltiazem. MAP = mean arterial blood pressure; HR = heart rate; PR = P-R interval of the electrocardiogram; dP/dt max = maximal rate of left ventricular pressure rise.

The inotropic responses as reflected by left ventricular dP/dt max were different at 2 minutes following intravenous injections of the three calcium antagonists. β-Blockade potentiated the negative inotropic response to verapamil significantly and abolished the positive inotropic response produced by nifedipine. The response to diltiazem was unchanged, since this drug itself did not alter dP/dt max. The addition of atropine to effect combined blockade did not modify the response to any calcium channel-blocking agents that had occurred with β-blockade alone. The depression of dP/dt max caused by verapamil in intact, unblocked dogs and in dogs with either β-blockade or combined autonomic blockades was significantly greater than that caused by diltiazem or nifedipine in identical conditions.

The calcium channel-blocking agents were administered to two dogs after 0.2 mg/kg of atropine alone to elucidate more clearly sympathetic contributions to cardiac inotropy. The positive inotropic response to
TABLE 3
Maximal Heart Rate, Blood Pressure, and dP/dtmax Responses

<table>
<thead>
<tr>
<th></th>
<th>Verapamil (250 μg/kg)</th>
<th>Diltiazem (200 μg/kg)</th>
<th>Nifedipine (50 μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>2 min</td>
<td>Baseline</td>
</tr>
<tr>
<td>MAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td>97 ± 5</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td>85 ± 4†</td>
</tr>
<tr>
<td>Prop§</td>
<td></td>
<td></td>
<td>95 ± 5</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>83 ± 4†</td>
</tr>
<tr>
<td>Prop + atr†</td>
<td></td>
<td>100 ± 5</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td>93 ± 7</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td>163 ± 15†</td>
</tr>
<tr>
<td>Prop</td>
<td></td>
<td></td>
<td>80 ± 10</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>121 ± 9†</td>
</tr>
<tr>
<td>Prop + atr†</td>
<td></td>
<td>148 ± 11</td>
<td>153 ± 17</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dP/dtmax</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td>2766 ± 223</td>
</tr>
<tr>
<td>(n = 8)</td>
<td></td>
<td></td>
<td>2034 ± 222†</td>
</tr>
<tr>
<td>Prop</td>
<td></td>
<td></td>
<td>2802 ± 210</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td>1626 ± 165†</td>
</tr>
<tr>
<td>Prop + atr†</td>
<td></td>
<td>2317 ± 240</td>
<td>2365 ± 132</td>
</tr>
<tr>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* To equihypotensive doses of verapamil, diltiazem, and nifedipine before and after propranolol (Prop) and propranolol plus atropine (atracor). t P < 0.05 vs. baseline value.
§ Propranolol 0.5 mg/kg; † propranolol, 0.5 mg/kg plus atropine; ‡ 0.1-0.2 mg/kg or ‡ 0.2 mg/kg.
MAP = mean arterial blood pressure, mm Hg; HR = heart rate, beats/min; dP/dtmax = maximum rate of left ventricular pressure development, mm Hg/sec.

nifedipine was markedly enhanced after atropine, whereas the response to diltiazem and verapamil was unchanged.

Discussion

By definition, the calcium channel blockers inhibit the "calcium current" associated with the "slow channel," resulting in negative chronotropic, negative dromotropic, and negative inotropic actions in isolated cardiac tissues (Fleckenstein, 1977; Henry, 1980; Millard et al., 1982; Schwartz et al., 1981). Vascular smooth muscle is also relaxed, due probably to the inhibition of transmembrane calcium movement by these drugs. Each of the three prototype drugs, viz, diltiazem, nifedipine, and verapamil, exhibit different potencies on vascular and cardiac muscle (Schwartz et al., 1981; Lathrop et al., 1982). In conscious animals, the direct cardiac actions of these calcium channel-blocking agents appear to be significantly modified by the activation of autonomic reflexes. In this study we evaluated the contribution of autonomic reflexes to cardiac responses produced by three calcium channel-blocking agents. The reduction in mean arterial blood pressure achieved by rapid injection of the three calcium channel-blocking agents was approximately equal (hereafter called equihypotensive) and could not be shown to be statistically different in the intact conscious dogs. Although the hypotension produced was not significantly changed by pretreatment with propranolol or propranolol combined with atropine, the systemic blood pressure responses observed may have been influenced by changes of inotropic state and heart rate, resulting from both direct and reflex-mediated actions of the calcium-blocking drugs.

Consistent with a recent report (Walsh et al., 1981), our results show that diltiazem has little effect on left ventricular contractile performance as assessed by LV dP/dtmax, whereas verapamil produces direct myocardial depression. Walsh et al. (1981) reported that intravenous nifedipine (30 μg/kg) produced a negative inotropic response which was comparable to effects produced by 170 μg verapamil per kg body weight. Our observations, however, with a slightly higher nifedipine dose (50 μg/kg), consistently revealed a positive inotropic response. This discrepancy in the two studies may stem from different injection rates and observation periods. Walsh et al. (1981) injected the calcium channel-blocking agents very rapidly...
Nakaya et al. / Reflex Effects of Ca++ Blockers

(over 10 seconds) and obtained the responses 30 seconds after the injection. In the present study, nifedipine injected intravenously over 1 minute exerted a significant positive inotropic response within 2–3 minutes which lasted 15–30 minutes, and was abolished completely by pretreatment with a β-adrenergic blocking drug. This indicates that sympathetic activation probably contributed to the inotropic response. That sympathetic tone was augmented after nifedipine is also supported by the observation that positive inotropic responses to nifedipine were potentiated by atropine pretreatment. Muscarinic blockade is known to augment the positive inotropic response to sympathetically activated by intravenous sympathomimetic infusions (Vatner et al., 1979).

We should like to stress that propranolol has a potential membrane-stabilizing action (Tarr et al., 1973) which may reduce baroreceptor reflexes (Schultz and Zehr, 1981), and can produce direct myocardial depression (Liang and Hood, 1974). However the dose of propranolol we used to produce β-adrenergic blockade in the present study is lower than that required to exert the aforementioned direct actions. Also, although we demonstrated adequate blockade of the cardiovascular effects of acetylcholine and isoproterenol by the respective blocking agents, it is possible that the autonomic blockades were less effective against endogenous agonists released at autonomic nerve endings in the heart. Complete pharmacological blockade of muscarinic and β-adrenergic receptors is incompatible with conduct of studies in conscious animal preparations.

Equihypotensive doses of the three calcium channel-blocking agents also produced different degrees of tachycardia. Heart rate was markedly increased by nifedipine (86%) and verapamil (75%), and slightly by diltiazem (28%) at their maximum values. A review of previously reported clinical studies reveals that intravenous administration of either verapamil or nifedipine frequently causes tachycardia (Vincenzi et al., 1976; Lydtin et al., 1975; Rowland et al., 1979), whereas diltiazem produces little effect on heart rate (Oyama, 1979; Mitchell et al., 1982). However, such differences of positive chronotrophic action as we report here cannot be explained solely by the different potency of their direct blocking action on slow inward current of sinus node pacemaker cells.

Verapamil slowed the atrioventricular conduction more than diltiazem, suggesting that verapamil, more than diltiazem, suppressed the slow inward current in the atrioventricular node in these doses. On the assumption that these calcium channel-blocking agents inhibit the slow channel of sinus cells in a parallel fashion, verapamil had been expected to elicit less tachycardia than diltiazem. However, the reflex tachycardia observed after rapid injection of verapamil was significantly greater than that produced by diltiazem. There are several possibilities which might explain this effect. The first is that sinus nodal and atrioventricular nodal cells may have different sensitivities to the calcium channel-blocking agents. However, comparative studies using canine isolated, blood-perfused sinoatrial node and atrioventricular node preparations have shown that diltiazem and verapamil exerted the same potency of negative dromotropic effect on the atrioventricular node and that verapamil exerted more negative chronotropic effect on sinus node preparations than diltiazem when equivalent doses were compared (Taira, 1979). In the present study, both drugs exerted a similar degree of negative chronotropic action after β-adrenergic and parasympathetic blockade were combined. The second explanation may be that changes in contractile state induced by calcium channel-blocking agents, especially by verapamil, might have reflexly increased the efferent sympathetic outflow to the heart. It has been postulated that vagal afferent nerves originating from ventricular mecha

\[ \Delta \text{MAP} \text{ (mm Hg)} \]

\[ \Delta \text{HR} \text{ (beats/min)} \]

\[ \Delta \text{dP/dt}_{\text{max}} \text{ (mm Hg/kg/sec)} \]

**Figure 6.** Blood pressure (MAP), heart rate (HR), and inotropic (dP/dt_{max}) responses in the conscious dog to verapamil (V, 250 μg/kg, iv), diltiazem (D, 200 μg/kg, iv), and nifedipine (N, 50 μg/kg, iv) before (open bars, n = 8) and after propranolol (0.5 mg/kg, iv, hatched bars, n = 5) and propranolol combined with atropine (0.1 to 0.2 mg/kg, iv, shaded bars, n = 5). All values represent mean changes from baseline data. Lines on each bar represent one standard error of the mean value. Asterisks (*) denote significant (P < 0.05) difference from the baseline values. Those responses of significant difference (P < 0.05) between drugs after the same autonomic blockade pretreatment are shown by brackets above the respective data. Significantly different responses (P < 0.05) to any drug among the control and two autonomic blockade conditions are shown by brackets below the respective data.
a depression of ventricular contractility reduces the vagal afferent activity (Thoren et al., 1976; Thoren, 1977). A third possibility is that calcium channel-blocking agents may interact with autonomic nervous system mechanisms directly. In the present study, the tachycardia response caused by the calcium channel blocking agents, was attenuated by β-adrenergic blockade and was completely abolished by the combination of β-adrenergic and muscarinic blockade. It is generally recognized that baroreceptor-induced changes in heart rate are mediated by reciprocal adjustments in both sympathetic and parasympathetic cardiac efferent activities (Thames and Kontos, 1970). Although there are several reports showing that high concentrations of verapamil inhibit catecholamine release from the adrenal medulla (Pinto and Trifaro, 1976; Hiwatari and Taira, 1978), and from sympathetic nerve terminals (Gothert et al., 1979), it is unclear whether these calcium channel-blocking agents can alter acetylcholine release from vagal nerve terminals. In addition, it is noteworthy that a recent study suggested that verapamil inhibits the binding of specific radioligands to muscarinic receptors as well as to α-adrenergic receptors (Karliner et al., 1982, Atlas and Adler, 1981). A final possibility is that the calcium channel-blocking agents might change baroreceptor sensitivity. Kunze (1979) reported that when the calcium concentration of the perfusate to the isolated carotid sinus was altered, the threshold of the baroreceptor reflex was affected. Levels of both sodium and calcium are important for the carotid sinus pressure threshold. The interference of afferent sensitivity through calcium channel blocker inhibition of calcium fluxes may participate in this paradoxical chronotropic response. Further studies are needed to clarify the underlying mechanisms by which calcium channel-blocking agents produce inotropic and chronotropic responses in awake, unsedated dogs that are both disparate and appear to contradict the observed effects in vitro. The results presented here indicate that the autonomic nervous system participates significantly in those responses and may be of importance in the clinical effects of these agents.

We thank Drs. Victoria Hertzberg and Peter Gartside for their invaluable advice and assistance in data analysis. Computer analysis of the data was made possible through the efforts of Mr. Neil Krandall. The authors should also like to recognize Msrs. Mark Hardin and Randall Kratzer, and Ms. Victoria Rapien for their technical assistance. We are particularly appreciative for the surgical preparations made by Msrs. John Erickson, and Billy Joe Rice. Illustrations were prepared by Ms. Gwen Kraft. The skillful assistance of Ms. Janet Simons in manuscript preparation is greatly appreciated.

This study was completed with the partial support of grants from the National Institutes of Health, Bethesda, Maryland (ROI- HL 23558 and PO1-HL 22619-CORE 1). In addition, generous support (H.N.) was provided by Tanabe Pharmaceutical Company; diltiazem was supplied by Dr. Ronald K. Browne, Marion Laboratories, Kansas City, Missouri.

A preliminary report of these findings was made at the 54th Scientific Sessions of the American Heart Association held November 17, 1981, in Dallas, Texas.
Nakaya et al./Reflex Effects of Ca++ Blockers

Hakone Symposium. New Drug Therapy with a Calcium Antagonist, edited by RJ Bing. Amsterdam, Excerpta Medica, pp 169-189

Pinto JFB, Trifaro JM (1976) The different effects of D-600 (methylxverapamil) on the release of adrenal catecholamines induced by acetylcholine, high potassium or sodium deprivation. Br J Pharmacol 57: 127-132


Thoren PN, Donald DE, Shepherd JT (1976) Role of heart and lung receptors with nonmedullated vagal afferents in circulatory control. Circ Res 38 (suppl II): 2-9


INDEX TERMS: Baroreceptor reflex • Vasodilators • Heart size • Cardiac conduction • Autonomic nervous system • Dose-response relationships
Reflex chronotropic and inotropic effects of calcium channel-blocking agents in conscious dogs. Diltiazem, verapamil, and nifedipine compared.

H Nakaya, A Schwartz and R W Millard

Circ Res. 1983;52:302-311
doi: 10.1161/01.RES.52.3.302

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/52/3/302

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/