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Cardiovascular effects of verapamil enantiomer combinations in conscious dogs

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Abstract

We examined the systemic and coronary hemodynamic effects of five combinations of *R*- and *S*-verapamil enantiomers (*R*/*S*; 100/0, 90/10, 80/20, 50/50, and 20/80%, respectively) in conscious dogs chronically instrumented for measurement of aortic and LV pressure, $+dP/dt$, subendocardial segment length, coronary blood flow velocity, and aortic blood flow. Dogs received escalating doses (0.1, 0.2, and 0.4 mg kg^{-1}) of each verapamil combination over 2 min at 30 min intervals on different experimental days and peak changes in hemodynamics were recorded 2 min after each dose. All verapamil combinations increased heart rate, mean aortic blood flow, and coronary blood flow velocity and decreased calculated systemic and coronary vascular resistance. Alterations in coronary hemodynamics were most pronounced with 20/80 *R*/*S* verapamil. Racemic and 20/80 *R*/*S* verapamil decreased mean arterial and left ventricular systolic pressure, in contrast to combinations with greater concentrations of the *R* enantiomer. Left ventricular function was unchanged during administration of 100/0, 90/10, and 80/20 *R*/*S* verapamil. Direct negative inotropic and lusitropic effects occurred with 50/50 and 20/80 *R*/*S* verapamil. The high dose of 20/80 *R*/*S* verapamil also increased left ventricular end-diastolic pressure and the regional chamber stiffness constant, consistent with diastolic dysfunction. The results indicate that combinations of *R*- and *S*-verapamil produce differential hemodynamic and left ventricular functional effects in conscious, unsedated dogs that are dependent on the relative ratio of these enantiomers.

Keywords: Ca^{2+} channel antagonist; Verapamil; Diastolic function; Isovolumic relaxation; Hemodynamics; Preload recruitable stroke work

1. Introduction

The chirality of the calcium (Ca^{2+}) channel antagonist verapamil confers important stereospecific differences in the cardiovascular actions of the optical isomers of the drug. Levo (−) verapamil produces negative chronotropic and inotropic effects, (Bayer et al., 1975b) delays atrioventricular node conduction, (Echizen et al., 1985; Echizen et al., 1988; Satoh et al., 1980) and causes systemic and coronary artery vasodilation (Satoh et al., 1980; Van Amsterdam and Zaagsma, 1988). In contrast, similar concentrations of dextro (+) verapamil produce vasodilation of the arterial and coronary vasculature but are relatively devoid of direct cardiac effects (Satoh et al., 1980; Van Amsterdam and Zaagsma, 1988). Commercially available verapamil consists of a racemic mixture of these optical isomers. The clinical efficacy of this drug has been widely demonstrated in the treatment of essential hypertension, supraventricular tachyarrhythmias, and ischemic heart disease (Aristizabal and Frohlich, 1994; Henry, 1980; Robertson and Robertson, 1996). The utility of racemic verapamil as an arterial and coronary vasodilator may be limited by the direct negative chronotropic, dromotropic, inotropic, and lusitropic (relaxation) effects of the drug, however (Walsh and O'Rourke, 1985). The present investigation examined the hypothesis that combinations of *R*- and *S*-verapamil [corresponding to the dextro (+) and levo (−) optical isomers, respectively] based primarily on the *R*-enantiomer preserve the beneficial vasodilator actions of this Ca^{2+} channel blocker without causing depression of myocardial contractility or left ventricular diastolic dys-

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function. Conscious, unsedated dogs were used to allow direct comparison of the systemic and coronary hemodynamic actions and left ventricular functional effects of verapamil enantiomer mixtures in the same animal without the confounding influence of baseline anesthetics or acute surgical intervention.

2. Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. All procedures conformed to the 'Guiding Principles in the Care and Use of Animals' of the American Physiological Society and were performed in accordance with the 'Guide for the Care and Use of Laboratory Animals,' [DHEW (DHHS) publication (NIH) no. 85-23, revised 1996].

2.1. Surgical preparation

The surgical implantation of instruments has been previously described in detail (Harkin et al., 1995). Briefly, in the presence of general anesthesia and using aseptic techniques, a left thoracotomy was performed in conditioned mongrel dogs for placement of instruments for measurement of aortic, left atrial, and intrathoracic pressures (heparin-filled catheters), subendocardial segment length (ultrasonic crystals), left anterior descending coronary blood flow velocity (precalibrated Doppler flow probe), and aortic blood flow (ascending thoracic aortic ultrasonic flow transducer; cardiac output minus coronary blood flow). A high fidelity, miniature micronanometer was placed in the left ventricle for measurement of continuous left ventricular pressure and the peak rate of increase and decrease of left ventricular pressure ($+dP/dt_{max}$ and $-dP/dt_{min}$, respectively). A hydraulic vascular occluder was placed around the inferior vena cava for abrupt alteration of left ventricular preload. All instrumentation was firmly secured, tunneled between the scapulae, and exteriorized via several small incisions. The pericardium was left widely open, the chest wall closed in layers, and the pneumothorax evacuated by a chest tube.

All dogs received fentanyl as needed for analgesia after surgery. Dogs were allowed to recover a minimum of 7 days prior to experimentation. Dogs were treated with intramuscular antibiotics (cephalothin (40 mg kg^{-1}) and gentamicin (4.5 mg kg^{-1})) and trained to stand quietly in a sling during hemodynamic monitoring. Segment length and coronary blood flow velocity signals were monitored by ultrasonic amplifiers. End-systolic (ESL) and end-diastolic segment length (EDL) were measured at 30 ms before $-dP/dt_{min}$ and immediately prior to the onset of left ventricular isovolumic contraction, respectively. Percent segment shortening (% SS) was determined using the equation: $\%\ SS = (EDL - ESL)100 \cdot EDL^{-1}$. Relative diastolic and mean coronary vascular resistances were calculated as the quotients of diastolic and mean arterial pressure to diastolic and mean coronary blood flow velocity, respectively. An estimate of myocardial oxygen consumption, the pressure work index, was determined using a previously validated formula (Rooke and Feigl, 1982). Hemodynamic data were continuously recorded on a polygraph and simultaneously digitized by a computer interfaced with an analog to digital converter for recording and subsequent analysis of left ventricular pressure–segment length waveforms and diagrams.

2.2. Experimental protocol

Each dog ($n = 8$; weight $= 25.7 \pm 0.6$ kg, mean $\pm$ S.E.M.) was fasted overnight, and fluid deficits were replaced prior to experimentation with 0.9% saline (500 ml). Maintenance fluids were continued at 3 ml kg^{-1} h^{-1} for the duration of each experiment. After the instrumentation was calibrated, baseline systemic and coronary hemodynamics were recorded. Left ventricular pressure, intrathoracic pressure, and segment length waveforms were also recorded for later off-line analysis of diastolic function. Regional myocardial contractility was evaluated using a series of left ventricular pressure–segment length diagrams generated by abrupt constriction of the inferior vena cava as previously described (Harkin et al., 1995). The slope of the regional preload recruitable stroke work relationship derived from these pressure–length diagrams was used to quantify myocardial contractility (Glower et al., 1985). The time constant of left ventricular isovolumic relaxation was calculated using the derivative method (Raff and Glantz, 1981). A regional chamber stiffness constant was derived from left ventricular pressure–segment length data between minimum ventricular pressure and the beginning of atrial systole using a monoexponential relation assuming a simple elastic model (Harkin et al., 1995).

Dogs were randomly assigned to receive five different combinations of *R*- and *S*-verapamil (Searle, St. Louis, MO) on different experimental days. Baseline systemic and coronary hemodynamics and left ventricular pressure–segment length waveforms and diagrams were recorded during control conditions. In one group of experiments, escalating doses (0.1, 0.2, and 0.4 mg kg^{-1}, i.v.) of 100% *R*-verapamil were administered over 2 min at 30 min intervals. Peak changes in systemic and coronary hemodynamics were recorded and left ventricular pressure–segment length waveforms and diagrams were obtained using the techniques described above 2 min after the administration of each dose of *R*-verapamil. In four other group of experiments, dogs received escalating doses (0.1, 0.2, and 0.4 mg kg^{-1}, i.v.) of 90% *R*- and 10% *S*-, 80% *R*- and 20% *S*-, 50% *R*- and 50% *S*-, or 20% *R*- and 80% *S*-verapamil mixtures over 2 min at 30 min intervals on separate days. Peak alterations in hemodynamics and indices of left ventricular systolic and diastolic function were

recorded as described above. Pure *S*-verapamil was not studied because pilot experiments demonstrated that this enantiomer reproducibly caused complete atrioventricular node conduction blockade in conscious dogs. Thus, the effects of five combinations of verapamil enantiomers on systemic and coronary hemodynamics, myocardial contractility, isovolumic relaxation, and regional chamber stiffness were studied in the same conscious, unsedated dogs on five different days.

2.3. Statistical analysis

Statistical analysis of the data within each group during the administration of combinations of *R*- and *S*-verapamil was performed by analysis of variance (ANOVA) with repeated measures, followed by use of Student's *t* test with Duncan's adjustment for multiplicity. Changes were considered to be statistically significant when the probability (P) value was < 0.05. All data are expressed as mean ± S.E.M.

3. Results

No differences in baseline systemic and coronary hemodynamics or indices of left ventricular function were observed between experimental groups during control conditions. *R*-verapamil caused significant ($P < 0.05$) increases in heart rate, rate–pressure product, pressure–work index, mean aortic blood flow, and diastolic and mean coronary blood flow velocity (Table 1). Reductions in systemic vascular resistance and diastolic and mean coronary vascular resistance were observed with *R*-verapamil consistent with peripheral and coronary vasodilation. Systolic, diastolic, and mean arterial pressure, left ventricular end-diastolic pressure, end-diastolic segment length, and end-systolic segment length were unchanged. A decrease in left ventricular systolic pressure occurred at the 0.4 mg kg^{-1} dose. *R*-verapamil did not alter myocardial contractility (preload recruitable stroke work slope, $+dP/dt_{max}$, and percent segment shortening), isovolumic relaxation (time constant and $-dP/dt_{min}$), or the regional chamber stiff-

Table 1
Hemodynamic effects of 100% *R*-verapamil in conscious dogs

	Control	Verapamil (mg kg^{-1})		
		0.1	0.2	0.4
HR (bpm)	79 ± 3	104 ± 6[a]	110 ± 6[a]	119 ± 7[a]
SAP (mmHg)	120 ± 4	122 ± 5	118 ± 3	112 ± 5
DAP (mmHg)	86 ± 3	89 ± 3	88 ± 3	82 ± 4
MAP (mmHg)	101 ± 3	101 ± 4	101 ± 3	95 ± 4
RPP (mmHg bpm · 10^3)	9.4 ± 0.4	12.5 ± 0.6[a]	13.0 ± 0.9[a]	13.5 ± 1.1[a]
LVSP (mmHg)	117 ± 5	117 ± 6	113 ± 5	109 ± 5[a]
LVEDP (mmHg)	9 ± 1	7 ± 2	7 ± 1	7 ± 1
$+dP/dt_{max}$ (mmHg s^{-1})	2304 ± 89	2371 ± 134	2270 ± 148	2289 ± 77
$-dP/dt_{min}$ (mmHg s^{-1})	−2172 ± 98	−2225 ± 134	−2194 ± 135	−2136 ± 126
DCBFV (Hz · 10^2)	45 ± 6	55 ± 8[a]	55 ± 7[a]	66 ± 9[a,b,c]
DCVR (mmHg Hz^{-1} · 10^{-2})	2.18 ± 0.30	1.91 ± 0.30	1.88 ± 0.33	1.40 ± 0.18[a,b,c]
MCBFV (Hz · 10^2)	27 ± 3	36 ± 5[a]	32 ± 4[a]	39 ± 5[a]
MCVR (mmHg Hz^{-1} · 10^{-2})	4.21 ± 0.52	3.32 ± 0.59[a]	3.49 ± 0.52[a]	2.70 ± 0.30[a,b]
EDL (mm)	20.8 ± 0.9	20.4 ± 0.8	20.2 ± 0.8	20.3 ± 0.8
ESL (mm)	15.3 ± 1.0	14.9 ± 1.0	15.0 ± 1.0	15.0 ± 1.0
SS (%)	26.8 ± 3.0	27.3 ± 3.2	25.9 ± 3.7	26.2 ± 3.2
M_w (mmHg)	85 ± 5	81 ± 6	90 ± 6	81 ± 6
L_w (mm)	14.4 ± 1.2	14.1 ± 1.0	14.5 ± 1.0	14.1 ± 1.3
τ (ms)	38 ± 2	36 ± 2	37 ± 2	35 ± 2
K (mm^{-1})	0.35 ± 0.07	0.23 ± 0.05	0.24 ± 0.03	0.29 ± 0.05
MAQ (l min^{-1})	2.4 ± 0.2	3.1 ± 0.5	3.1 ± 0.2[a]	3.7 ± 0.4[a]
SVR (dyn s cm^{-5})	3540 ± 360	2920 ± 390	2680 ± 240[a]	2380 ± 390[a]
SV (ml)	31 ± 2	29 ± 2	27 ± 2[a]	28 ± 2
PWI (ml min^{-1} 100 g^{-1})	9.0 ± 0.4	11.0 ± 1.0	11.5 ± 0.8[a]	12.8 ± 0.8[a,b]

Data are mean ± S.E.M.
[a] Significantly ($P < 0.05$) different from control.
[b] Significantly ($P < 0.05$) different from 0.1 mg kg^{-1} verapamil.
[c] Significantly ($P < 0.05$) different from 0.2 mg kg^{-1} verapamil.
Abbreviations: HR = heart rate; SAP, DAP, and MAP = systolic, diastolic, and mean aortic blood pressure, respectively; RPP = rate–pressure product; LVSP and LVEDP = left ventricular systolic and end-diastolic pressure, respectively; DCBFV and DCVR = diastolic coronary blood flow velocity and coronary vascular resistance, respectively; MCBFV and MCVR = mean coronary blood flow velocity and coronary vascular resistance, respectively; EDL and ESL = end-diastolic and end-systolic segment length, respectively; SS = segment shortening; M_w and L_w = preload recruitable stroke work slope and length intercept, respectively; τ = time constant of isovolumic relaxation; K = regional chamber stiffness constant; MAQ = mean aortic blood flow; SVR = systemic vascular resistance; SV = stroke volume; PWI = pressure work index.

Table 2
Hemodynamic effects of 90% *R*-, 10% *S*-verapamil in conscious dogs

	Control	Verapamil (mg kg^{-1})		
		0.1	0.2	0.4
HR (bpm)	85 ± 6	116 ± 10[a]	125 ± 11[a]	140 ± 8[a,b]
SAP (mmHg)	123 ± 3	119 ± 3	114 ± 2[a]	108 ± 5[a,b]
DAP (mmHg)	89 ± 3	89 ± 3	84 ± 2	83 ± 4
MAP (mmHg)	104 ± 3	102 ± 3	96 ± 1[a]	93 ± 4[a,b]
RPP (mmHg bpm · 10^3)	10.5 ± 0.8	13.8 ± 1.2[a]	14.1 ± 1.2[a]	15.1 ± 1.0[a]
LVSP (mmHg)	119 ± 4	115 ± 3	111 ± 3[a]	107 ± 4[a,b]
LVEDP (mmHg)	10 ± 1	8 ± 1[a]	8 ± 1[a]	7 ± 1[a,b,c]
$+dP/dt_{max}$ (mmHg s^{-1})	2351 ± 55	2301 ± 172	2192 ± 172	2197 ± 175
$-dP/dt_{min}$ (mmHg s^{-1})	−2203 ± 71	−2267 ± 117	−2166 ± 97	−2184 ± 88
DCBFV (Hz · 10^2)	49 ± 6	61 ± 10	70 ± 11[a]	86 ± 14[a,b,c]
DCVR (mmHg Hz^{-1} · 10^{-2})	2.00 ± 0.23	1.75 ± 0.27[a]	1.45 ± 0.24[a,b]	1.19 ± 0.21[a,b,c]
MCBFV (Hz · 10^2)	29 ± 4	38 ± 6[a]	40 ± 6[a]	50 ± 9[a,b]
MCVR (mmHg Hz^{-1} · 10^{-2})	3.94 ± 0.46	3.18 ± 0.47[a]	2.80 ± 0.38[a]	2.23 ± 0.34[a,b,c]
EDL (mm)	20.7 ± 0.8	20.1 ± 0.9[a]	20.2 ± 0.9[a]	19.9 ± 1.0[a]
ESL (mm)	15.1 ± 0.9	14.9 ± 1.0	15.0 ± 0.9	15.0 ± 1.0
SS (%)	27.1 ± 2.8	26.5 ± 3.2	25.8 ± 3.0	24.8 ± 3.2[a]
M_w (mmHg)	93 ± 4	81 ± 6	91 ± 6	86 ± 4
L_w (mm)	14.8 ± 1.0	14.1 ± 1.0	14.6 ± 0.9	14.3 ± 1.3
τ (ms)	38 ± 2	35 ± 2[a]	35 ± 2[a]	35 ± 1[a]
K (mm^{-1})	0.30 ± 0.04	0.26 ± 0.04	0.24 ± 0.03	0.28 ± 0.05
MAQ (l min^{-1})	2.5 ± 0.2	3.2 ± 0.5[a]	3.4 ± 0.5[a]	3.5 ± 0.5[a]
SVR (dyn s cm^{-5})	3430 ± 410	2670 ± 350[a]	2470 ± 390[a]	2130 ± 330[a,b,c]
SV (ml)	32 ± 3	28 ± 3[a]	27 ± 3[a]	24 ± 3[a,b]
PWI (ml min^{-1} 100 g^{-1})	8.9 ± 0.7	11.7 ± 1.3[a]	12.1 ± 1.3[a]	11.8 ± 0.9[a]

Data are mean ± S.E.M.
[a]Significantly ($P < 0.05$) different from control.
[b]Significantly ($P < 0.05$) different from 0.1 mg kg^{-1} verapamil.
[c]Significantly ($P < 0.05$) different from 0.2 mg kg^{-1} verapamil.
Abbreviations: see Table 1.

ness constant, indicating that this drug does not affect left ventricular systolic and diastolic function.

The 90% *R*-, 10% *S*- and 80% *R*-, 20% *S*-verapamil combinations produced hemodynamic effects (Tables 2 and 3) that were similar but not identical to those caused by pure *R*-verapamil. The 90% *R*-, 10% *S*- and 80% *R*-, 20% *S*-verapamil mixtures increased heart rate, rate–pressure product, pressure–work index, mean aortic blood flow, and diastolic and mean coronary blood flow velocity and decreased systemic and coronary vascular resistance. In contrast to the findings with *R*-verapamil alone, decreases in arterial pressures, left ventricular systolic and end-diastolic pressures, and end-diastolic segment length were observed when small percentages of *S*-verapamil were added to the *R* enantiomer. No changes in preload recruitable stroke work slope and $+dP/dt_{max}$ were observed with 90% *R*-, 10% *S*- and 80% *R*-, 20% *S*-verapamil. Small but significant decreases in the time constant of isovolumic relaxation and the regional chamber stiffness constant were also observed with 90% *R*-, 10% *S*- and 80% *R*-, 20% *S*-verapamil concomitant with tachycardia and decreases in left ventricular preload and afterload.

The 50% *R*-, 50% *S*- and 20% *R*-, 80% *S*-verapamil mixtures caused hemodynamic effects (Tables 4 and 5) that were different than combinations based primarily on the *R* enantiomer. Racemic and 20% *R*-, 80% *S*-verapamil increased heart rate, rate–pressure product, and pressure–work index, similar to the findings with other enantiomer combinations. However, racemic and 20% *R*-, 80% *S*-verapamil caused decreases in arterial and left ventricular systolic pressures that were greater in magnitude that those produced by 100% *R*-, 90% *R*-, 10% *S*- and 80% *R*-, 20% *S*-verapamil. Despite these decreases in arterial pressure, 50% *R*-, 50% *S*- and 20% *R*-, 80% *S*-verapamil produced greater increases in coronary blood flow velocity and decreases in calculated coronary vascular resistance than those observed with pure *R*-verapamil. In contrast to verapamil combinations based primarily on the *R* enantiomer, racemic and 20% *R*-, 80% *S*-verapamil caused dose-related decreases in preload recruitable stroke work slope (e.g., 89 ± 9 during control to 60 ± 8 mmHg at the 0.4 mg kg^{-1} dose of 20% *R*-, 80% *S*-; Table 5), $+dP/dt_{max}$, and percent segment shortening, suggesting a direct negative inotropic effect in spite of the presence of intact autonomic nervous system reflexes. Increases in the time constant of isovolumic relaxation (e.g., 39 ± 2 during control to 49 ± 2 ms at the 0.4 mg kg^{-1} dose of 20% *R*-, 80% *S*-; Table 5) and the regional chamber stiffness constant (e.g., 0.38 ±

Table 3
Hemodynamic effects of 80% *R*-, 20% *S*-verapamil in conscious dogs

	Control	Verapamil (mg kg^{-1})		
		0.1	0.2	0.4
HR (bpm)	76 ± 5	117 ± 9[a]	123 ± 4[a]	141 ± 6[a,b,c]
SAP (mmHg)	120 ± 5	119 ± 5	113 ± 4	108 ± 4[a,b]
DAP (mmHg)	85 ± 4	87 ± 3	83 ± 4	81 ± 3
MAP (mmHg)	100 ± 4	101 ± 4	96 ± 5	91 ± 3[a,b]
RPP (mmHg bpm · 10^3)	9.1 ± 0.8	13.8 ± 0.9[a]	13.9 ± 0.6[a]	15.3 ± 0.9[a]
LVSP (mmHg)	119 ± 6	120 ± 5	114 ± 6	109 ± 5[a,b]
LVEDP (mmHg)	10 ± 1	7 ± 1[a]	6 ± 1[a]	6 ± 2[a]
$+dP/dt_{max}$ (mmHg s^{-1})	2327 ± 104	2329 ± 140	2315 ± 146	2228 ± 164
$-dP/dt_{min}$ (mmHg s^{-1})	−2155 ± 111	−2232 ± 111	−2281 ± 173	−2188 ± 110
DCBFV (Hz · 10^2)	50 ± 7	62 ± 9	73 ± 11[a]	88 ± 16[a,b]
DCVR (mmHg Hz^{-1} · 10^{-2})	1.97 ± 0.30	1.70 ± 0.31	1.39 ± 0.23[a,b]	1.28 ± 0.30[a,b]
MCBFV (Hz · 10^2)	27 ± 4	36 ± 5	41 ± 6[a]	53 ± 10[a,b,c]
MCVR (mmHg Hz^{-1} · 10^{-2})	4.28 ± 0.59	3.27 ± 0.48[a]	2.77 ± 0.42[a]	2.47 ± 0.61[a,b]
EDL (mm)	21.3 ± 1.0	20.8 ± 1.0	20.0 ± 1.1[a,b]	19.9 ± 1.1[a,b]
ESL (mm)	16.3 ± 0.8	15.8 ± 0.9	15.5 ± 1.1[a]	15.5 ± 1.1
SS (%)	23.4 ± 2.2	24.2 ± 2.6	23.2 ± 2.9	22.0 ± 3.0
M_w (mmHg)	87 ± 12	80 ± 7	86 ± 9	85 ± 11
L_w (mm)	15.0 ± 0.9	14.7 ± 1.0	15.0 ± 1.0	14.8 ± 1.1
τ (ms)	39 ± 1	36 ± 2[a]	34 ± 2[a]	34 ± 1[a,b]
K (mm^{-1})	0.40 ± 0.07	0.29 ± 0.05[a]	0.24 ± 0.03[a]	0.28 ± 0.04[a]
MAQ (l min^{-1})	2.0 ± 0.2	2.7 ± 0.3[a]	3.0 ± 0.3[a]	2.7 ± 0.2[a]
SVR (dyn s cm^{-5})	4120 ± 540	3190 ± 460[a]	2690 ± 440[a]	2660 ± 310[a]
SV (ml)	28 ± 4	22 ± 3[a]	24 ± 3[a]	20 ± 2[a,b]
PWI (ml min^{-1} 100 g^{-1})	8.1 ± 0.7	11.1 ± 0.7[a]	10.9 ± 0.4[a]	11.1 ± 0.7[a]

Data are mean ± S.E.M.
[a]Significantly ($P < 0.05$) different from control.
[b]Significantly ($P < 0.05$) different from 0.1 mg kg^{-1} verapamil.
[c]Significantly ($P < 0.05$) different from 0.2 mg kg^{-1} verapamil.
Abbreviations: see Table 1.

0.07 during control to 0.61 ± 0.12 mm^{-1} at the 0.4 mg kg^{-1} dose of 20% *R*-, 80% *S*-; Table 5) also occurred with racemic and 20% *R*-, 80% *S*-verapamil.

4. Discussion

The present results indicate that combinations of *R*- and *S*-verapamil produce systemic and coronary hemodynamic effects in conscious, unsedated dogs that reflect a mixture of the cardiovascular actions of these enantiomers alone. All five combinations of *R*- and *S*-verapamil caused increases in heart rate that were probably mediated by baroreceptor reflex activation secondary to peripheral vasodilation. These findings support previous observations with racemic verapamil in conscious dogs (Millard et al., 1982; Nakaya et al., 1983; Walsh et al., 1981) and humans (Schmieder et al., 1987). In contrast to the findings with other *R*- and *S*-mixtures, however, tachycardia associated with 20% *R*-, 80% *S*-verapamil was significantly attenuated at the 0.4 mg kg^{-1} dose. *S*-verapamil has been shown to produce more potent negative chronotropic and dromotropic effects than the *R* enantiomer in vivo (Echizen et al., 1985, 1988). The present results with 20% *R*-, 80% *S*-verapamil may indicate expression of these direct actions on the sinus node and atrioventricular conduction system by the *S* enantiomer.

All combinations of verapamil enantiomers caused similar declines in systemic vascular resistance, confirming that both optical isomers of this Ca^{2+} channel blocker cause arterial vasodilation. Small but significant decreases in left ventricular end-diastolic pressure and end-diastolic segment length also occurred with most of the *S*-verapamil-containing mixtures, suggesting that these verapamil enantiomer combinations reduce left ventricular preload. These findings confirm previous results identifying the direct vasodilating actions of verapamil on vascular smooth muscle (Campbell et al., 1982; Fleckenstein, 1977; Henry, 1980). Reductions in systemic vascular resistance and left ventricular end-diastolic pressure were associated with decreases in mean arterial and left ventricular systolic pressures during administration of racemic and 20% *R*-, 80% *S*-verapamil but not combinations based primarily on the *R* enantiomer. Racemic and 20% *R*-, 80% *S*-verapamil-induced hypotension probably occurred because these mixtures produced more pronounced depression of myocardial contractility than *R* enantiomer-based combinations as well as declines in systemic vascular resistance. Increases in mean aortic blood flow were observed with all combinations of *R*- and *S*-verapamil as a result of decreases in

Table 4
Hemodynamic effects of 50% *R*-, 50% *S*-verapamil in conscious dogs

	Control	Verapamil (mg kg^{-1})		
		0.1	0.2	0.4
HR (bpm)	75 ± 5	107 ± 8[a]	137 ± 10[a,b]	136 ± 11[a,b]
SAP (mmHg)	121 ± 4	114 ± 6	105 ± 4[a,b]	96 ± 3[a,b,c]
DAP (mmHg)	87 ± 3	83 ± 4	80 ± 4	68 ± 4[a,b,c]
MAP (mmHg)	102 ± 3	97 ± 5	90 ± 4[a,b]	79 ± 4[a,b,c]
RPP (mmHg bpm · 10^3)	9.1 ± 0.7	12.0 ± 0.8[a]	14.4 ± 1.4[a,b]	13.0 ± 0.9[a]
LVSP (mmHg)	116 ± 4	111 ± 5	102 ± 4[a,b]	94 ± 4[a,b,c]
LVEDP (mmHg)	8 ± 1	7 ± 1	6 ± 1[a]	7 ± 1
$+dP/dt_{max}$ (mmHg s^{-1})	2398 ± 75	2224 ± 73	1947 ± 94[a,b]	1772 ± 115[a,b]
$-dP/dt_{min}$ (mmHg s^{-1})	−2151 ± 83	−2151 ± 114	−1988 ± 139[a,b]	−1719 ± 80[a,b,c]
DCBFV (Hz · 10^2)	50 ± 7	66 ± 10	92 ± 14[a,b]	103 ± 17[a,b]
DCVR (mmHg Hz^{-1} · 10^{-2})	1.97 ± 0.26	1.50 ± 0.23[a]	1.06 ± 0.19[a,b]	0.84 ± 0.17[a,b]
MCBFV (Hz · 10^2)	29 ± 5	41 ± 7	56 ± 9[a,b]	61 ± 9[a,b]
MCVR (mmHg Hz^{-1} · 10^{-2})	4.05 ± 0.56	2.89 ± 0.44[a]	1.96 ± 0.34[a,b]	1.58 ± 0.30[a,b]
EDL (mm)	20.5 ± 0.9	20.3 ± 0.7	19.7 ± 0.9[a]	20.0 ± 0.7
ESL (mm)	15.4 ± 0.9	15.5 ± 0.8	15.7 ± 1.0	16.3 ± 1.0[a,b,c]
SS (%)	24.9 ± 3.2	23.8 ± 3.3	20.4 ± 3.3[a,b]	18.3 ± 3.9[a,b]
M_w (mmHg)	91 ± 10	85 ± 9	77 ± 6	70 ± 5[a,b]
L_w (mm)	14.1 ± 1.0	14.2 ± 1.1	14.3 ± 1.1	14.1 ± 1.5
τ (ms)	39 ± 1	37 ± 1	40 ± 2	42 ± 1[a,b]
K (mm^{-1})	0.30 ± 0.03	0.30 ± 0.04	0.31 ± 0.06	0.54 ± 0.18
MAQ (l min^{-1})	2.4 ± 0.3	3.1 ± 0.5[a]	3.1 ± 0.4[a]	3.0 ± 0.4[a]
SVR (dyn s cm^{-5})	3530 ± 460	2780 ± 510[a]	2390 ± 350[a]	2230 ± 520[a,b]
SV (ml)	32 ± 2	29 ± 2	23 ± 3[a,b]	24 ± 2[a,b]
PWI (ml min^{-1} 100 g^{-1})	8.4 ± 0.9	10.6 ± 1.1[a]	11.3 ± 1.2[a]	9.6 ± 0.7

Data are mean ± S.E.M.
[a]Significantly ($P < 0.05$) different from control.
[b]Significantly ($P < 0.05$) different from 0.1 mg kg^{-1} verapamil.
[c]Significantly ($P < 0.05$) different from 0.2 mg kg^{-1} verapamil.
Abbreviations: see Table 1.

systemic vascular resistance and increases in heart rate despite simultaneous reductions in left ventricular preload and stroke volume.

All combinations of *R*- and *S*-verapamil increased coronary blood flow velocity and reduced coronary vascular resistance in conscious dogs. Despite producing more profound declines in coronary artery perfusion pressure, racemic and 20% *R*-, 80% *S*-verapamil caused greater increases in coronary blood flow velocity and decreases in coronary vascular resistance than those observed with pure *R*-verapamil. Nevertheless, significant increases in coronary blood flow velocity were also produced by the *R*-enantiomer alone. The changes in coronary hemodynamics produced by enantiomer mixtures were accompanied by baroreceptor reflex-mediated (Nakaya et al., 1983) increases in heart rate, rate–pressure product and pressure–work index. These results suggest that verapamil-induced increases in coronary blood flow velocity and decreases in coronary vascular resistance may have occurred in response to increased myocardial oxygen consumption. However, the coronary vascular actions of the 0.4 mg kg^{-1} dose of racemic and 20% *R*-, 80% *S*-verapamil were observed in the absence of changes in pressure–work index, suggesting that coronary vasodilation occurred independent of alterations in myocardial oxygen supply–demand relations. The present findings confirm and extend the results of previous studies (Lathrop et al., 1982; Satoh et al., 1980; Van Amsterdam and Zaagsma, 1988) and indicate that although both *R*- and *S*-verapamil reduce coronary vascular resistance, *S*-verapamil is a more potent coronary vasodilator than the *R* enantiomer. The present results also indicate that addition of small percentages of the *S* enantiomer to *R*-verapamil causes alterations in coronary hemodynamics that are similar to those produced by the racemate and the 20% *R*, 80% *S* mixture in the absence of negative inotropic and lusitropic effects.

Verapamil combinations based primarily on the *R* enantiomer did not alter indices of myocardial contractility. In contrast, racemic and 20% *R*-, 80% *S*-verapamil caused reductions in preload recruitable stroke work slope, left ventricular $+dP/dt_{max}$, and percent segment shortening, consistent with a direct negative inotropic effect. The slope of the regional preload recruitable stroke work relation derived from a series of differentially loaded left ventricular pressure–segment length diagrams has been shown to be a relatively heart rate- and load-insensitive index of myocardial contractility in vivo, (Glower et al., 1985) unlike isovolumic (e.g., $+dP/dt_{max}$) and ejection phase (e.g., percent segment shortening, left ventricular ejection fraction) indices of contractile state that are relatively

Table 5
Hemodynamic effects of 20% *R*-, 80% *S*-verapamil in conscious dogs

	Control	Verapamil (mg kg^{-1})		
		0.1	0.2	0.4
HR (bpm)	75 ± 4	139 ± 10[a]	127 ± 11[a]	112 ± 12[a,b]
SAP (mmHg)	122 ± 5	116 ± 5	103 ± 5[a,b]	87 ± 4[a,b,c]
DAP (mmHg)	88 ± 4	88 ± 4	74 ± 5[a,b]	60 ± 4[a,b,c]
MAP (mmHg)	104 ± 4	100 ± 5	86 ± 5[a,b]	71 ± 4[a,b,c]
RPP (mmHg bpm · 10^3)	9.3 ± 0.6	15.9 ± 1.0[a]	13.0 ± 0.9[a,b]	9.8 ± 1.1[b,c]
LVSP (mmHg)	119 ± 6	115 ± 4	103 ± 5[a,b]	87 ± 4[a,b,c]
LVEDP (mmHg)	9 ± 0	6 ± 1[a]	6 ± 1	10 ± 1[b,c]
$+dP/dt_{max}$ (mmHg s^{-1})	2375 ± 85	2288 ± 114	1863 ± 71[a,b]	1513 ± 139[a,b,c]
$-dP/dt_{min}$ (mmHg s^{-1})	−2173 ± 115	−2308 ± 156	−1884 ± 119[a,b]	−1481 ± 103[a,b,c]
DCBFV (Hz · 10^2)	50 ± 8	90 ± 14[a]	95 ± 15[a]	100 ± 17[a]
DCVR (mmHg Hz^{-1} · 10^{-2})	2.07 ± 0.33	1.17 ± 0.18[a]	0.98 ± 0.20[a]	0.79 ± 0.19[a,b]
MCBFV (Hz · 10^2)	28 ± 5	57 ± 9[a]	61 ± 10[a]	55 ± 7[a]
MCVR (mmHg Hz^{-1} · 10^{-2})	4.37 ± 0.62	2.15 ± 0.38[a]	1.77 ± 0.36[a]	1.50 ± 0.26[a]
EDL (mm)	20.8 ± 1.1	20.1 ± 1.1	20.4 ± 1.0	21.2 ± 0.8[b]
ESL (mm)	15.3 ± 0.9	15.1 ± 1.1	15.7 ± 0.9	16.5 ± 0.9[a,b,c]
SS (%)	26.4 ± 2.9	24.9 ± 3.4	23.4 ± 3.5[a]	22.2 ± 3.2[a,b]
M_w (mmHg)	89 ± 9	87 ± 7	62 ± 6[a,b]	60 ± 8[a,b]
L_w (mm)	14.1 ± 1.0	14.2 ± 1.2	13.0 ± 1.1	13.3 ± 0.9
τ (ms)	39 ± 2	36 ± 2	41 ± 2[a]	49 ± 2[a,b,c]
K (mm^{-1})	0.38 ± 0.07	0.26 ± 0.02	0.27 ± 0.06	0.61 ± 0.12[a,b,c]
MAQ (l min^{-1})	2.1 ± 0.4	3.2 ± 0.5[a]	2.7 ± 0.4[a]	2.2 ± 0.3[b]
SVR (dyn s cm^{-5})	4390 ± 610	2630 ± 400[a]	2600 ± 470[a]	2690 ± 600[a]
SV (ml)	29 ± 4	23 ± 4[a]	23 ± 3[a]	25 ± 4
PWI (ml min^{-1} 100 g^{-1})	8.5 ± 0.7	12.6 ± 0.7[a]	9.5 ± 1.0[a]	6.9 ± 0.5[b]

Data are mean ± S.E.M.
[a]Significantly ($P < 0.05$) different from control.
[b]Significantly ($P < 0.05$) different from 0.1 mg kg^{-1} verapamil.
[c]Significantly ($P < 0.05$) different from 0.2 mg kg^{-1} verapamil.

dependent on heart rate and ventricular loading conditions (Kass et al., 1987). The present results confirm the findings of several previous investigations (Bayer et al., 1975a,b; Ferry et al., 1985; Satoh et al., 1980; Van Amsterdam and Zaagsma, 1988) demonstrating that levoverapamil produces more potent negative inotropic effects than its optical isomer and is primarily responsible for the myocardial depression observed with the racemic mixture in the intact heart (Nakaya et al., 1983; Schulman et al., 1993; Singh and Roche, 1977; Vlietstra et al., 1983; Walsh et al., 1981). Despite these negative inotropic actions, cardiac output was increased by lower doses of racemic and 20% *R*-, 80% *S*-verapamil because concomitant reductions in systemic vascular resistance also occurred. However, the increases in cardiac output observed at lower doses were attenuated at the 0.4 mg kg^{-1} dose of 20% *R*-, 80% *S*-verapamil. This result suggests that direct depression of myocardial contractility by higher concentrations of the *S* enantiomer begins to impair left ventricular systolic performance despite simultaneous arterial vasodilation.

R-based verapamil combinations did not adversely affect indices of left ventricular diastolic function. In fact, increases in heart rate and decreases in left ventricular end-diastolic pressure and systemic vascular resistance produced by 90% *R*-, 10% *S*- and 80% *R*-, 20% *S*-verapamil resulted in modest reductions in the time constant of isovolumic relaxation and the regional chamber stiffness constant, consistent with an indirect improvement in diastolic function.(Gilbert and Glantz, 1989) In contrast, racemic and 20% *R*-, 80% *S*-verapamil caused increases in the time constant of isovolumic relaxation and decreases in the magnitude of $-dP/dt_{min}$, suggesting that the *S* enantiomer is responsible for a prolongation of the isovolumic relaxation phase of diastole. The present findings confirm and extend previous observations with racemic verapamil in isolated canine hearts (Gelpi et al., 1983) and conscious dogs (Walsh and O'Rourke, 1985). The 0.4 mg kg^{-1} dose of 20% *R*-, 80% *S*-verapamil also increased the regional chamber stiffness constant concomitant with an increase in left ventricular end-diastolic pressure and end-diastolic segment length, consistent with an increase in regional chamber stiffness. These results support previous observations demonstrating that intracoronary racemic verapamil increases left ventricular end-diastolic pressure in conscious dogs concomitant with direct negative inotropic and lusitropic effects (Walsh and O'Rourke, 1985). Thus, verapamil mixtures containing larger quantities of the *S* enantiomer may not only depress myocardial contractility but may also produce diastolic dysfunction in vivo. These

findings emphasize that reversal of diastolic dysfunction observed with verapamil in patients with heart failure resulting from pressure-overload hypertrophy, (Hess et al., 1986) hypertrophic cardiomyopathy, (Bonow et al., 1985) or coronary artery disease (Bonow et al., 1981; Setaro et al., 1990) probably occurs as a result of favorable alterations in heart rate and ventricular loading conditions and not because of direct positive lusitropic effects.

In summary, the results of the present investigation demonstrate that all mixtures of *R*- and *S*-verapamil produce baroreceptor reflex-mediated tachycardia, arterial and coronary vasodilation, and increases in mean aortic blood flow in conscious, unsedated dogs. The results also indicate that mixtures containing large percentages of the *S* enantiomer reduce arterial pressure and cause direct negative inotropic and lusitropic effects, in contrast to the findings with *R*-verapamil-based combinations. Thus, combinations of *R*- and *S*-verapamil produce systemic and coronary hemodynamic effects and left ventricular functional actions that are based on the relative proportion of each enantiomer.

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