Combined Negative Inotropic Effects of Calcium Entry Blockers and Isoflurane on Canine Isolated Heart Muscles

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The combined negative inotropic effects of isoflurane and calcium entry blockers (verapamil, diltiazem, nifedipine, nicardipine) were studied utilizing isolated heart preparations of ventricular muscles from dogs. All of these calcium entry blockers exerted dose-dependent decreases in maximal velocity of shortening (Vmax), maximal developed isometric force (Fm), and the maximal first derivative of Fm (maximal dF/dt). Dose-dependent decreases of these variables of muscle mechanics were augmented in isoflurane-depressed myocardium. At equimolar concentrations, direct myocardial depression was demonstrated in the following order of severity: nifedipine > diltiazem = verapamil > nicardipine. Percent depressions of Vmax, Fm and maximal dF/dt were significantly greater in muscles when calcium entry blockers were combined with 1MAC isoflurane than in muscles of calcium entry blockers alone. These data suggest that the negative inotropic effects of verapamil, diltiazem, nifedipine, and nicardipine were potentiated by isoflurane. (Key words: calcium entry blockers, isoflurane, isolated myocardium, negative inotropism)

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Recently, calcium entry blockers (CEBs) have been widely used in the treatment for supraventricular tachyarrhythmia, myocardial ischemia, or acute hypertension in clinical practice[1–3]. By inhibiting calcium entry into the cell, these agents exert depression of myocardial contractility, systemic vasodilation, negative chronotropy, and prolongation of AV conduction[4–6]. Isoflurane produces a dose-dependent depression of myocardial contractility by interfering with subsequent stores of calcium in myocardial excitation-contraction coupling[7,8]. The cardiovascular effects of CEBs and isoflurane have been extensively studied in both intact animals and humans[9–13]. Although there is a report on interaction of diltiazem and isoflurane in isolated heart muscles[14], there is no data concerning the combined effects of other CEBs and isoflurane. Therefore, we evaluated the combined effects of either verapamil, diltiazem, nifedipine or nicardipine and isoflurane in isolated preparations of ventricular muscles obtained from dogs.

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Methods

Forty muscles were equally divided into four drug groups, verapamil, diltiazem, nifedipine, and nicardipine without isoflurane. Another 40 muscles were also equally divided into the four drug groups combined with isoflurane administration. The apparatus for muscle preparations, isotonic and isometric measurements of muscle me-

Table 1. Effects of calcium entry blockers on Vmax, Fm, and maximal dF/dt

	Vmax ($ML \cdot sec^{-1}$)	Fm ($g \cdot mm^{-2}$)	maximal dF/dt ($g \cdot mm^{-2} \cdot sec^{-1}$)
Control	1.56 ± 0.12	5.21 ± 0.35	14.42 ± 1.03
Verapamil			
10^{-7}M	1.48 ± 0.03	4.59 ± 0.26	14.20 ± 0.41
3×10^{-7}M	1.42 ± 0.03	4.63 ± 0.37	13.35 ± 0.91
10^{-6}M	1.26 ± 0.04*	4.07 ± 0.37*	12.05 ± 1.10*
3×10^{-6}M	1.09 ± 0.07*	3.36 ± 0.36*	9.19 ± 1.05*
10^{-5}M	0.82 ± 0.05*	2.58 ± 0.22*	8.38 ± 0.93*
3×10^{-5}M	0.67 ± 0.04*	1.67 ± 0.03*	5.64 ± 0.79*
10^{-4}M	0.49 ± 0.04*	1.16 ± 0.03*	3.78 ± 0.29*
Control	1.39 ± 0.09	5.37 ± 0.52	15.25 ± 0.90
Diltiazem			
10^{-7}M	1.34 ± 0.12	5.04 ± 0.29	13.98 ± 0.95
3×10^{-7}M	1.26 ± 0.11	4.77 ± 0.43	13.12 ± 1.21
10^{-6}M	1.16 ± 0.12*	4.33 ± 0.65*	12.31 ± 1.38*
3×10^{-6}M	1.03 ± 0.12*	3.72 ± 0.61*	10.98 ± 1.13*
10^{-5}M	0.71 ± 0.10*	3.00 ± 0.47*	9.15 ± 1.23*
3×10^{-5}M	0.58 ± 0.11*	2.03 ± 0.28*	6.48 ± 1.19*
10^{-4}M	0.47 ± 0.10*	1.46 ± 0.13*	5.29 ± 0.48*
Control	1.52 ± 0.15	5.42 ± 0.40	15.38 ± 1.08
Nifedipine			
3×10^{-8}M	1.45 ± 0.12	5.02 ± 0.60	14.70 ± 1.92
10^{-7}M	1.33 ± 0.11*	3.90 ± 0.51*	13.21 ± 1.67*
3×10^{-7}M	1.16 ± 0.14*	3.51 ± 0.49*	10.98 ± 1.50*
10^{-6}M	0.88 ± 0.09*	2.23 ± 0.28*	8.03 ± 0.88*
3×10^{-6}M	0.54 ± 0.05*	1.13 ± 0.13*	6.15 ± 0.60*
Control	1.35 ± 0.07	4.46 ± 0.34	13.10 ± 1.01
Nicardipine			
10^{-7}M	1.27 ± 0.03	3.97 ± 0.30	12.10 ± 0.90
3×10^{-7}M	1.21 ± 0.04	3.57 ± 0.38	11.45 ± 1.20
10^{-6}M	1.15 ± 0.06	3.34 ± 0.26	10.83 ± 1.00
3×10^{-6}M	1.03 ± 0.04*	3.13 ± 0.29*	9.94 ± 0.93*
10^{-5}M	0.97 ± 0.07*	2.67 ± 0.24*	9.05 ± 0.99*
3×10^{-5}M	0.84 ± 0.05*	2.32 ± 0.11*	8.03 ± 0.81*
10^{-4}M	0.69 ± 0.04*	2.05 ± 0.06*	7.26 ± 0.80*

Means ± SEM are shown, N = 10 for each group.
*significant difference compared to control value.
Vmax = maximal velocity of shortening at 0.5 $g \cdot mm^{-2}$.
Fm = maximal developed force.
dF/dt = the first derivative of Fm.

chanics, and recording system are described in detail in our previous report[15]. Muscles were quickly excised from the right ventricles of adult mongrel dogs anesthetized with intravenous pentobarbital 30 $mg \cdot kg^{-1}$. The ventricular muscles were perfused in modified Krebs-Henseleit solution equilibrated with 95% O_2-5% CO_2 at temperature of 35°C. The modified Krebs-Henseleit solution was composed of NaCl 119 mM, KCl 4.8 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 24.8 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, and glucose 5.6

Table 2. Combined effects of calcium entry blockers and isoflurane on Vmax, Fm, and maximal dF/dt

	Vmax ($ML \cdot sec^{-1}$)	Fm ($g \cdot mm^{-2}$)	maximal dF/dt ($g \cdot mm^{-2} \cdot sec^{-1}$)
Control	1.56 ± 0.12	5.21 ± 0.35	14.42 ± 1.03
Isoflurane	1.24 ± 0.09**	3.74 ± 0.46**	12.03 ± 1.47**
Verapamil			
10^{-7}M	1.02 ± 0.07	2.87 ± 0.38	8.94 ± 1.48
3×10^{-7}M	0.95 ± 0.13*	2.50 ± 0.48*	7.93 ± 1.09*
10^{-6}M	0.83 ± 0.08*	2.14 ± 0.29*	7.07 ± 1.05*
3×10^{-6}M	0.71 ± 0.03*	1.82 ± 0.23*	6.20 ± 0.86*
10^{-5}M	0.58 ± 0.06*	1.46 ± 0.21*	5.05 ± 0.41*
3×10^{-5}M	0.42 ± 0.08*	1.14 ± 0.04*	3.46 ± 0.29*
10^{-4}M	0.22 ± 0.03*	0.62 ± 0.03*	1.00 ± 0.11*
Control	1.39 ± 0.09	5.37 ± 0.52	15.25 ± 0.90
Isoflurane	1.13 ± 0.11**	3.55 ± 0.42**	11.69 ± 1.07**
Diltiazem			
10^{-7}M	0.92 ± 0.19	3.11 ± 0.36	9.91 ± 1.16
3×10^{-7}M	0.82 ± 1.00*	2.79 ± 0.49*	8.85 ± 1.03*
10^{-6}M	0.68 ± 0.07*	2.15 ± 0.27*	7.32 ± 1.06*
3×10^{-6}M	0.58 ± 0.05*	1.72 ± 0.26*	5.80 ± 0.69*
10^{-5}M	0.44 ± 0.08*	1.29 ± 0.18*	4.73 ± 0.36*
3×10^{-5}M	0.32 ± 0.05*	1.03 ± 0.05*	3.05 ± 0.23*
10^{-4}M	0.16 ± 0.06*	0.43 ± 0.05*	1.23 ± 0.17*
Control	1.47 ± 0.06	5.53 ± 0.39	14.89 ± 1.03
Isoflurane	1.08 ± 0.09**	3.31 ± 0.23**	11.04 ± 1.29*
Nifedipine			
3×10^{-8}M	1.00 ± 0.09	2.60 ± 0.54	8.19 ± 1.00
10^{-7}M	0.68 ± 0.07*	1.82 ± 0.39*	6.25 ± 0.95*
3×10^{-7}M	0.41 ± 0.06*	1.21 ± 0.14*	4.17 ± 0.83*
10^{-6}M	0.33 ± 0.06*	0.41 ± 0.19*	2.88 ± 0.68*
Control	1.43 ± 0.08	4.76 ± 0.38	13.09 ± 1.02
Isoflurane	1.09 ± 0.08**	2.93 ± 0.35**	10.00 ± 0.89**
Nicardipine			
10^{-7}M	0.94 ± 0.09	2.65 ± 0.37	9.39 ± 1.04
3×10^{-7}M	0.85 ± 1.00*	2.29 ± 0.26*	8.00 ± 0.93*
10^{-6}M	0.73 ± 1.01*	1.84 ± 0.29*	7.03 ± 0.82*
3×10^{-6}M	0.63 ± 0.06*	1.68 ± 0.19*	6.64 ± 0.72*
10^{-5}M	0.52 ± 0.09*	1.39 ± 1.04*	6.17 ± 0.79*
3×10^{-5}M	0.47 ± 0.09*	1.25 ± 1.00*	5.28 ± 0.55*
10^{-4}M	0.31 ± 0.06*	1.08 ± 0.08*	3.56 ± 0.61*

Means ± SEM are shown, N = 10 for each group.
*significant difference compared to isoflurane value.
**significant difference compared to control value.
Vmax = maximal velocity of shortening at 0.5 $g \cdot mm^{-2}$.
Fm = maximal developed force.
dF/dt = the first time derivative of Fm.

mM. The lower end of a muscle was attached to a stainless steel wire which passed through a mercury seal at the bottom of the muscle chamber and was connected to a force

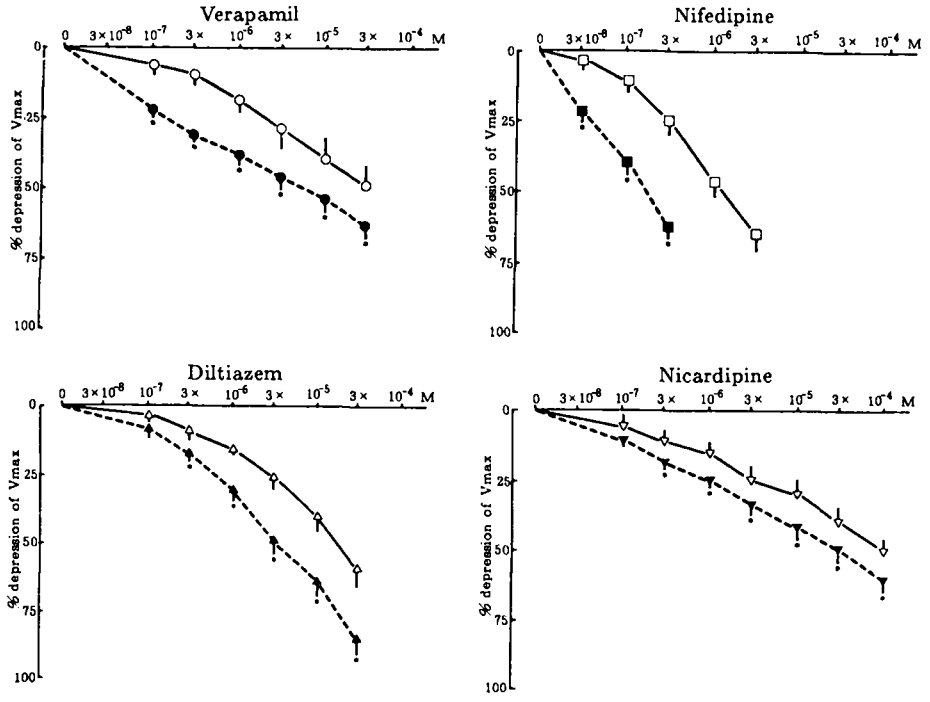

Fig. 1. Percent depression of Vmax by calcium entry blockers with or without isoflurane. Bars indicate ± SEM.
*significant difference between two groups.

○—○ : Verapamil ●···● : Isoflurane and Verapamil
△—△ : Diltiazem ▲···▲ : Isoflurane and Diltiazem
□—□ : Nifedipine ■···■ : Isoflurane and Nifedipine
▽—▽ : Nicardipine ▼···▼ : Isoflurane and Nicardipine

transducer. The upper end of the muscle was attached by a silver wire to an isotonic lever arm. Displacement of the lever was measured by a rotary variables differential transducer. Muscles were stimulated at 0.2 Hz by parallel platinum electrodes delivering 5 msec squre-wave pulses at voltages 20% above threshold. The muscle length was set by a small preload and kept constant throughout the procedure for each muscle study. Maximal velocity of shortening (Vmax) was approximated by the values of the velocity of shortening at 0.5 g·mm^{-2}. Maximal developed force (Fm) was obtained by the maximal afterload. First derivative of muscle length and force of muscle contraction with time (dl/dt and dF/dt) obtained by R-C differentiators were recorded on a multichannel recorder at a paper speed of 100 mm·sec^{-1}. Vmax was expressed in units of muscle length per second (ML·sec^{-1}), and force was expressed in grams per unit of cross-sectional area (g·mm^{-2}).

After a muscle preparation was equilibrated for 60 min, control of both isotonic and isometric measurements were made. Verapamil, diltiazem, and nicardipine diluted in saline were directly administered to bath solution from a concentration of 10^{-7} M to 10^{-4} M in a stepwise fashion. Nifedipine was administered from a concentration of 3 × 10^{-8} M to 3 × 10^{-6} M. Determinations of Vmax, Fm and maximal dF/dt were repeated at least 10 min after stabilization of contraction height at each concentrations. In another set of experiments, 1 MAC (human) isoflurane were administered into the perfusate by a calibrated vaporizer. After control measurements with isoflurane were made, dose-response studies of each CEB were similarly made. At the end of each experiment, the weight and length of the mus-

Table 3. The concentration of calcium entry blockers that caused a 50% decrease of contractility (DC_{50}) with and without isoflurane

	Vmax	Fm	maximal dF/dt
Verapamil	1.8×10^{-5}M	1.1×10^{-5}M	1.7×10^{-5}M
Isoflurane and Verapamil	1.6×10^{-6}M	2.1×10^{-7}M	7.6×10^{-7}M
Diltiazem	1.7×10^{-5}M	1.4×10^{-5}M	1.9×10^{-5}M
Isoflurane and Diltiazem	9.2×10^{-7}M	2.7×10^{-7}M	7.6×10^{-7}M
Nifedipine	1.2×10^{-6}M	5.6×10^{-7}M	9.3×10^{-7}M
Isoflurane and Nifedipine	6.6×10^{-8}M	3.0×10^{-8}M	5.0×10^{-8}M
Nicardipine	1.0×10^{-4}M	2.2×10^{-5}M	1.0×10^{-4}M
Isoflurane and Nicardipine	4.3×10^{-6}M	4.2×10^{-7}M	2.3×10^{-6}M

cle were measured, and cross-sectional area was calculated. All data were expressed by means ± SEM. Student's t-tests for paired and unpaired data were used. Values were considered significant when a P value was less than 0.05.

Results

The average length and cross-sectional area of 80 muscles, 10 for each experimental groups, were 7.5 ± 0.5 mm and 1.23 ± 0.14 mm^2, respectively. There were no significantly differences in both muscle lengths and cross-sectional areas between experimental groups.

The mean values for Vmax, Fm and maximal dF/dt by administration of either verapamil, diltiazem, nifedipine, or nicardipine alone are summarized in table 1. All CEBs decreased Vmax, Fm, maximal dF/dt dose-dependently. Significant decreases in Vmax were obtained at 3×10^{-6} M for nicardipine, at 10^{-6} M for verapamil and diltiazem and at 10^{-7} M for nifedipine. The mean values of Vmax, Fm, and maximal dF/dt by administration of each CEBs combined with isoflurane are summarized in table 2. 1 MAC isoflurane decreased Vmax, Fm and maximal dF/dt to 70 to 75% of the control value. Dose-dependent decreases of these variables of muscle mechanics were augmented in isoflurane-depressed myocardium as shown in the table. Figure 1 shows comparisons of percentage depressions of Vmax either by CEBs alone or by CEBs combined with isoflurane. Percentage depressions were calculated from the control values for muscles without isoflurane. Depressions of Vmax were all significantly greater when CEBs were administered in isoflurane-depressed muscles than CEBs alone.

Table 3 shows the concentration of CEBs that caused a 50% decrease in contractility (DC_{50}) either with or without isoflurane. DC_{50} of Vmax by verapamil was 1.8×10^{-5} M in muscles without isoflurane and 1.6×10^{-6} M in muscles with isoflurane, respectively. Similarly, the DC_{50} of diltiazem, nifedipine and nicardipine decreased from 1.7×10^{-5} M, 1.2×10^{-6} M, and 1.0×10^{-4} M in without isoflurane preparations to 9.2×10^{-7} M, 6.6×10^{-8} M, and 4.3×10^{-6} M in muscles with isoflurane.

Discussion

Results of the present study showed that either verapamil, diltiazem, nifedipine, or nicardipine has a direct dose-dependent negative inotropic effect on isolated ventricular muscles from dogs. Reductions in Vmax, Fm and maximal dF/dt were directly propotional to increases in concentrations of CEBs. Figure 2 shows a comparison of the percent depression of CEBs on Vmax and Fm at 3×10^{-7} M, which is believed to be the highest therapeutic concentration of each CEB, and severity of direct myocardial depression was in the following order: nifedipine > diltiazem = verapamil > nicardipine.

Another finding of the present study

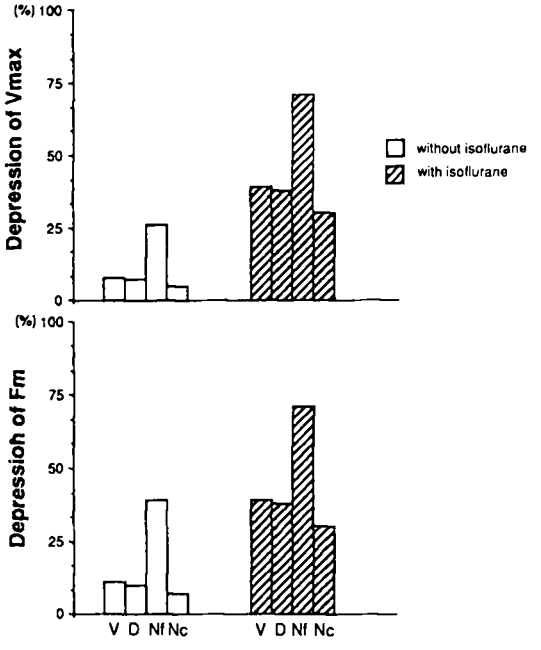

Fig. 2. Percent depression of Vmax (upper panel) and Fm (lower panel) by each calcium entry blocker of 3×10^{-7}M with and without isoflurane.

V: Verapamil, D: Diltiazem, Nf: Nifedipine, Nc: Nicardipine.

is that dose-dependent decrease in Vmax, Fm and maximal dF/dt were augmented in isoflurane-depressed myocardium. Therefore, when isoflurane-depressed myocardium was challenged further by another negative inotropic effect of CEB, the combined total depression was greater than caused by CEB alone. This means that the negative inotropic effect of CEBs was potentiated by isoflurane. These findings were in accordance with those reported by Nakata for halothane and enflurane[16]. Although the precise mechanism by which volantile anesthetics produce negative inotropism have not been well elucidated, isoflurane appears to reduce both the slow channel calcium entry and the amount of calcium in the intracellular stores, leading to decrease available calcium for contraction[17,18]. Furthermore, isoflurane decreases myofibrillar ATPase[8,19]. Alternatively, CEBs are characterized primarily as slow calcium current inhibitor in the cell membrane, although intracellular effects have also been suggested[4–6]. It seems unlikely that the action of CEBs on the excitation-contraction coupling or contractile machinery in muscles depressed with isoflurane are different from their action in muscles without isoflurane. One plausible explanation may be that the primary sites of isoflurane might be different from those of halothane and enflurane. Another possibility may be that CEBs could depress myocardial contractility at intracellular site. Indeed, it has been postulated that the myocardial depression of isoflurane is different from halothane and enflurane[17], and that the negative inotropism of isoflurane was potentiated in muscles from experimentally produced congestive heart failure[7]. However, these explanations are only speculative and further experiments are needed to clarify the underlying mechanisms.

In isolated myocardial preparations, the combined effects of CEBs and inhalation anesthetics have been studied by other investigators. The combination of diltiazem and halothane, enflurane or isoflurane caused additive depression in guinea pig papillary muscle[14]. Halothane and verapamil caused additive depression in rabbit right papillary muscles, and recovery was prolonged[20]. In contrast to these additive effect of CEBs and volantile anesthetics, Nakata demonstrated that the negative inotropism of nifedipine was potentiated by both halothane and enflurane[16]. Marshall, et al.[21] showed a low halothane-high nifedipine combination at the ED_{75} level of myocardial depression was a supra-additive by isobographic analysis. However, these findings should be interpreted cautiously because the magnitude and pattern of muscle mechanics might be different by either frequencies of stimulation, bath temperature or animal species.

Recent studies suggest that isoflurane depresses myocardial contractility to less extent than halothane and enflurane in vivo and in vitro experiments[17,22]. The cardiovascular effect of CEBs were altered with isoflurane concentrations and alteration in baroreceptor and reflex sympathetic tones in vivo[9–13].

Therefore, conclusion of the present study may not be directly applicable to clinical anesthesia. However, CEBs should be carefully administered during isoflurane anesthesia, particularly in patients with impaired myocardial function, reduced sympathetic activity, or receiving beta blockers.

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