Depression of Atrial Rate, Atrioventricular Nodal Conduction, and Cardiac Contraction by Diltiazem and Volatile Anesthetics in Isolated Hearts

Lori A. Gallenberg, Ph.D.,* David F. Stowe, M.D., Ph.D.,† Jure Marijlic, M.D.,‡ John P. Kampine, M.D., Ph.D.,§ Zeljko J. Bosnjak, Ph.D.¶

The direct effects of isoflurane, halothane, and enflurane alone or combined with diltiazem were examined in 49 isolated perfused guinea pig hearts. Recording electrodes were placed in the right atrium and left ventricular septal wall to measure spontaneous atrial rate and atrioventricular conduction time (AVCT). The right atrium was paced at 3–7 Hz (n = 10) to examine rate-dependent effects on AVCT, Wenckebach's periodicity, and ventricular response rates with atrioventricular (AV) block. Isovolumetric left ventricular pressure (LVP) was measured with a saline-filled balloon placed through the mitral valve. Hearts were perfused with oxygenated Krebs–Ringer's solution at 55 mmHg equilibrated with low or high concentrations of isoflurane (0.7 and 1.5%), halothane (0.5 and 1%), or enflurane (1.1 and 2.2%). Hearts were also perfused with a low or high concentration of diltiazem (75 and 150 ng/ml) alone and during anesthetic exposure. Significant findings of combined exposure were as follows: 1) the low isoflurane, halothane, or enflurane concentration plus a low or high diltiazem concentration decreased LVP compared with control and diltiazem alone; low isoflurane plus the high diltiazem concentration decreased LVP more than isoflurane alone. The high isoflurane, halothane, or enflurane concentration plus low or the high diltiazem concentration decreased LVP from control, anesthetics and diltiazem alone. Diltiazem plus halothane or enflurane decreased LVP more than diltiazem plus isoflurane. 2) Diltiazem plus low or high isoflurane, halothane, or enflurane concentrations decreased spontaneous atrial rate from control and the agents alone, except the high isoflurane concentration plus the low diltiazem concentration was not greater than that of isoflurane alone. Diltiazem plus halothane or enflurane decreased atrial rate more than diltiazem plus isoflurane. 3) Low and high diltiazem concentration plus low isoflurane, halothane, or enflurane concentrations did not prolong AVCT more than the individual agents alone, but low or high diltiazem plus high isoflurane, halothane, or enflurane concentrations increased AVCT more than each anesthetic alone. In nonpaced hearts, AV block occurred only with high diltiazem plus low enflurane (23%) concentrations and the high enflurane concentration (31%). 4) In hearts paced at 5 and 6 Hz, AVCT increased above controls during a low or high concentrations of diltiazem, during enflurane, and during the low or high concentration of diltiazem plus enflurane; AVCT increased more with the low concentration of diltiazem plus enflurane than with the low diltiazem concentration alone. At 6 Hz, the high diltiazem concentration alone and low or high concentrations of diltiazem plus enflurane caused AV block; AV block was greater during the high concentration of diltiazem plus enflurane than with enflurane alone. Wenckebach's periods were increased over control for the high concentrations of diltiazem and low or high concentrations of diltiazem plus enflurane. At 6 Hz, ventricular escape rates during AV dissociation were slower with the high concentration of diltiazem plus enflurane than with low or high diltiazem concentrations, or enflurane alone. At 7 Hz, ventricular rates were slowed more with the low or high concentration of diltiazem plus enflurane than with low concentrations of diltiazem or enflurane alone. These results demonstrate that, in general, diltiazem plus the high concentration of isoflurane, halothane, or enflurane slow atrial rates, prolong AVCT, and depress left ventricular contractile function more than the agents alone. During atrial pacing, both enflurane and diltiazem increase AV nodal refractoriness (prolonged Wenckebach's periodicity) and slow the ventricular escape rate with AV dissociation; the combined effect of the low concentrations of diltiazem plus enflurane is greater than that of either alone. (Key words: Anesthetics, volatile; enflurane; halothane; isoflurane. Animal: guinea pig. Drugs: calcium channel blocker; diltiazem. Heart: atrial rate; atrioventricular block; atrioventricular conduction interval; functional refractory period; isolated; left ventricular pressure; perfused; Wenckebach's periodicity.)

CALCIUM CHANNEL-BLOCKING DRUGS are widely used to treat heart and circulatory disorders because of their negative chronotropic, inotropic, and vasodilatory effects.1 These agents prevent the inward movement of calcium ions across cardiac cell membranes by specifically blocking voltage-gated calcium channels activated by depolarization.2,3 The selective cardiovascular effects of these agents suggest that they have distinct sites of action on the calcium channel receptor.4,5 Diltiazem, a benzothiazepine derivative, is a calcium channel blocker used primarily for the treatment of coronary vasospastic disease and angina pectoris. In both in vitro and in vivo studies,1-6 diltiazem causes comparatively less depression of the myocardium than that caused by verapamil or nifedipine. The negative chronotropic and dromotropic effects of diltiazem on sinoatrial (SA) and atrioventricular (AV) nodal tissues appear less than those of verapamil.
and similarly to or less than those of nifedipine in vitro. These effects, in vivo, are modified because of different autonomic reflex responses brought on by their different peripheral vasodilatory effects.5,6

Volatile anesthetics, like calcium channel blockers, produce direct negative chronotropic and inotropic effects. In vivo6,10-12 and in vitro11-17 studies indicate that, like the calcium channel-blocking drugs, the commonly used volatile anesthetics have qualitatively different effects on the heart. At doses that give equivalent anesthetic effect, enflurane delays AV conduction more than does isoflurane or halothane10,17-19 and halothane and enflurane directly depress myocardial contractile function more than does isoflurane.16,17 Potential mechanisms of action for volatile anesthetics in cardiac cells are as follows: 1) a slowing of calcium flux through voltage-gated calcium channels11,15,16,20; 2) a change in calcium flux between myoplasm and sarcoplasmic reticulum or other intracellular pools16,21,22; and 3) a direct depression of contractile protein function.11,23

Diltiazem may be approved for intravenous use for treating supraventricular dysrhythmias (pending Food and Drug Administration approval). Knowledge of its acute cardiac depressant effects during general anesthesia is important. Furthermore, many patients receiving a volatile anesthetic during surgery may be taking oral diltiazem chronically. Indeed, severe sinus node and AV node depression have been reported in patients receiving chronic diltiazem therapy who were anesthetized with enflurane.24 Additive or potentiated negative effects of anesthetics and diltiazem on cardiac automaticity, conduction, and contractility could occur if volatile anesthetics and diltiazem share one or more of the same pathways in their mechanism of action.

These studies examined the combined direct effects of diltiazem and isoflurane, enflurane, or halothane in the isolated, perfused guinea pig heart. We were most interested in the direct acute cardiac effects of these agents at therapeutically relevant plasma concentrations, and the underlying cause of AV dissociation with diltiazem and anesthetic exposure was also investigated.

** Materials and Methods **

To avoid extrinsic mechanical, nervous, and humoral factors that are difficult to control in in vivo experiments, the isolated heart preparation was used to compare the direct cardiac effects of diltiazem and volatile anesthetics and to examine in more detail the effects of diltiazem and enflurane on the AV node. Forty-nine Hartley English short-haired, albino guinea pigs (250-500 g) of either sex were injected intraperitoneally with ketamine (20 mg/kg) and heparin (1,000 units) and were decapitated when unresponsive to noxious stimulation. Dose-response studies from our laboratory demonstrate a return to control values within 5 min after drug discontinuation in guinea pigs and no effect on spontaneous atrial rate 30 min after intraperitoneal injection.25,26 This protocol has been approved by the Animal Care and Use Committee of the Medical College of Wisconsin and the Zablocki VA Medical Center. Each heart was rapidly isolated and perfused with oxygenated (97% O2 + 3% CO2) Krebs-Ringer's solution as described previously.17,28 Solution pH was 7.41 ± 0.02, PCO2(gas)2 was 38 ± 2 mmHg, and PO2(gas)2 was 500 ± 10 mmHg (Radiometer® ABL-2; Medtron, Chicago, IL). Perfusate and organ bath temperatures were maintained at 36.8 ± 0.1 °C with a thermostatically controlled recirculating water bath. Perfusion pressure was constantly maintained at 55 mmHg by a 75-cm column. Inflow perfusion pressure was monitored at the level of the aorta with a pressure transducer (Statham).

Silver Teflon®-coated bipolar electrodes (125 mm diameter; Cooner Wire Company, Chatsworth, CA) were placed in the right atrial appendage and right ventricular conus. Electrode signals were amplified 1,000 fold and were continuously displayed on an image oscilloscope (Tektronix® 5113; Tektronix, Inc., Rolling Meadows, IL). Spontaneous atrial rate was determined from the right atrial (A-A) beat-to-beat interval, and AV conduction time (AVCT) was determined from the right atrial-to-left ventricular septal (A-V) beat-to-beat interval. In one group the right atrium was paced (2-ms duration at twice threshold current) for 30-s intervals at 120, 180, 240, 300, 360, and 420 beats per min (2-7 Hz). Wenckebach's periodicity was determined as the paced A-A interval (in milliseconds = 1,000/Hz) at which atrial to ventricular conduction became dissociated (Mobitz type I block). AV nodal functional refractory period (AVFRP) was not measured directly but was assumed to correlate closely with the Wenckebach's period (see discussion). A-A interval, ventricular (V-V) beat-to-beat interval, and A-V interval were calculated continuously by laboratory-designed, eight-bit calibrated digital timers that measure the time between successive atrial or ventricular pulses and the time between pulses generated by the A-V interval. The analog output of the timers displayed intervals in milliseconds.

Left ventricular pressure (LVP) was measured with a transducer connected to a saline-filled latex balloon (Hugo Sachs Electronik, Germany) inserted through the left atrium and mitral valve into the left ventricle. The balloon volume was adjusted by a syringe and screw-clamp to set the balloon pressure equal to 0 mmHg during diastole.
Peak LVP generated by the isovolumetric contractions was used to assess contractile function. All experimental variables were continuously recorded on a direct writing polygraph (Astro-Med® MT9500R; Astro-Med, Inc., West Warwick, RI) and on audio tape (Vetter® D1; AR Vetter Company, Rebersburg, PA) during the last minute of each control or treatment period for later playback on the polygraph.

Diltiazem (Sigma Chemical Company, St. Louis, MO) was dissolved in Krebs-Ringer's solution, divided into aliquots to assure uniform concentrations, and frozen for daily use. Diltiazem was administered for 10-min intervals at calculated perfusate concentrations of 75 and 150 ng/ml (0.18–0.36 μM), designated, respectively, as low and high concentrations of diltiazem. The high concentration of diltiazem used has less than the 50% contractile inhibitory dose in vitro and approximately double the peak free plasma concentration in vivo after a 120-mg oral dose in humans. The anesthetics, isoflurane, halothane, and enflurane, were administered at rodent equivalent concentrations of approximately 0.7 and 1.4 minimal alveolar concentrations (MACs). These anesthetic concentrations, designated as low and high, were delivered at total gas flows of 3 l/min and were equilibrated with perfusate at 0.5 and 1 volumes percent for halothane (Draeger® Vaporizer; North American Draeger Company, Telford, PA); 1.1 and 2.2% for enflurane (Ohio® Vaporizer, Ohmeda, Madison, WI); and 0.7 and 1.5% for isoflurane (Flotec 3® Vaporizer, Cyprane, England). Delivered anesthetic concentrations were determined by gas chromatography from perfusate samples collected after exposure to each level of anesthetic at the aortic inflow port and sealed in air-free glass vials. Concentrations for each anesthetic (low and high, respectively) were as follows: isoflurane, 230 ± 10 and 510 ± 30 μM; halothane, 200 ± 10 and 460 ± 20 μM; and enflurane, 380 ± 10 and 670 ± 30 μM. Anesthetic concentrations measured during control and recovery periods after exposure to anesthetics were not significantly different from zero.

**PROTOCOL AND STATISTICAL DESIGN**

At the end of a 30-min stabilization period, during which no more than two single premature atrial or ventricular beats per min were observed, initial control values were recorded. The protocol for diltiazem closely resembled that described previously by our laboratory for verapamil with these volatile anesthetics. Briefly, after a 10-min control and a 10-min exposure to a low or high concentration of isoflurane, halothane, or enflurane, each heart was perfused with a solution containing a low or high concentration of diltiazem for 10 min each in addition to the anesthetic (fig. 1). The anesthetic chosen, the anesthetic concentration, and the order of the combined administration of diltiazem and anesthetic were randomized in each heart preparation. Thirteen hearts constituted each of these three anesthetic groups. An additional group of ten hearts were paced and exposed to the low or the high concentration of diltiazem plus the high concentration of enflurane only; in this group changes in AV nodal delay with atrial pacing, incidence of AV dissociation, Wenckebach's periodicity, and ventricular rates were also measured. Posttreatment control values after the various treatments were not statistically different from pretreatment control values except where mentioned.

All data are reported as mean ± standard error of the mean (SEM). Individual heart measurements of atrial rate, AVCT, and peak LVP during control and treatment periods were averaged, and statistical inference was evaluated by analysis of variance. At each pacing rate Wenckebach's periodicity was analyzed by differences in ventricular rates among treatment groups compared with the drug-free pacing controls. When variance was significant, treatment and postcontrol mean values were evaluated for significance of difference from initial control means (P < 0.05) by Fisher's least significant difference test. This comparison of means test was applied several times to determine individual effects and combined within-group effects of both concentrations of diltiazem and volatile anesthetics, as well as among group effects of the three anesthetics (see key in tables and figures). The observed incidence of AV dissociation in spontaneous and paced hearts during treatment periods was tested by chi-squared analysis; the expected frequency of AV dissociation during control was assumed to be zero. Statistical computations were done with the use of a software program (StatView 512+, Brainpower Inc., Calabasas, CA) on an Apple SE-30® computer (Apple Computers, Inc. Cupertino, CA).
Table 1. Effects of Diltiazem Alone for Each Preanesthetic Group

<table>
<thead>
<tr>
<th>Group</th>
<th>Anesthetic</th>
<th>Peak LVP (mmHg)</th>
<th>Atrial Rate (beats per min)</th>
<th>AVCT (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ISO</td>
<td>90 ± 3</td>
<td>198 ± 6</td>
<td>61 ± 3</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>88 ± 3</td>
<td>202 ± 6</td>
<td>60 ± 2</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>92 ± 2</td>
<td>203 ± 5</td>
<td>63 ± 2</td>
</tr>
<tr>
<td>Low diltiazem (75 ng/ml)</td>
<td>ISO</td>
<td>86 ± 4</td>
<td>181 ± 5*</td>
<td>63 ± 2</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>85 ± 3</td>
<td>170 ± 6*</td>
<td>64 ± 2</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>87 ± 3</td>
<td>179 ± 5*</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>High diltiazem (150 ng/ml)</td>
<td>ISO</td>
<td>81 ± 3*</td>
<td>166 ± 5*†</td>
<td>66 ± 2*</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>84 ± 3</td>
<td>164 ± 5*</td>
<td>65 ± 2*</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>81 ± 3*</td>
<td>158 ± 5*†</td>
<td>68 ± 2*</td>
</tr>
<tr>
<td>Diltiazem postcontrol</td>
<td>ISO</td>
<td>77 ± 4*</td>
<td>192 ± 6</td>
<td>61 ± 2</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>80 ± 4*</td>
<td>194 ± 5</td>
<td>61 ± 2</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>79 ± 3*</td>
<td>202 ± 5</td>
<td>65 ± 2</td>
</tr>
</tbody>
</table>

Data are means ± SEM; n = 13 for each group.
Control values for hearts in each preanesthetic group and the effects of only low and high diltiazem in each group on peak left ventricular pressure (LVP), atrial rate, and atrioventricular conduction time (AVCT). Column 2 indicates the anesthetic to which hearts were exposed at other times in the experimental series. ISO = isoflurane; HAL = halothane; ENF = enfurane.
* P < 0.05 versus control for each variable.
† P < 0.05 versus low diltiazem for each variable.

Results

Individual Effects of Diltiazem and Anesthetics

Table 1 shows the effects of diltiazem alone on peak LVP, atrial rate, and AVCT for hearts in each of the three anesthetic control groups; no anesthetic was present. Drug-free control values, taken just before perfusion with diltiazem, were not significantly different among the three anesthetic groups. For all hearts (n = 39), during drug-free perfusion mean isovolumetric peak LVP was 90 ± 3 mmHg, mean spontaneous atrial rate was 200 ± 6 beats per min, and mean AVCT was 62 ± 2 ms. The low diltiazem concentration (75 ng/ml) alone significantly decreased atrial rate from control but did not change AVCT or peak LVP in each of the groups. The high diltiazem concentration (150 ng/ml) alone decreased atrial rate compared with the drug-free control and with the low diltiazem concentration in the propofol and preflurane groups. The high diltiazem concentration also increased AVCT and decreased peak LVP in the preflurane and preflurane groups compared with controls. In the postdiltiazem control period, atrial rate and AVCT, but not LVP, returned to the initial drug-free control values in each anesthetic control group; this effect on LVP appeared to be dependent on prior exposure to the combination of diltiazem and anesthetics (i.e., when the diltiazem-alone series randomly followed a diltiazem-anesthetic series). Under drug-free conditions in this preparation, peak LVP decreased only 5–10% after a 3-h perfusion period (unpublished observations).

Table 2. Effects of Anesthetic Exposure Alone

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre-ISO</th>
<th>Peak LVP (mmHg)</th>
<th>Atrial Rate (beats per min)</th>
<th>AVCT (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>ISO</td>
<td>92 ± 3</td>
<td>200 ± 5</td>
<td>60 ± 2</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>88 ± 2</td>
<td>201 ± 6</td>
<td>61 ± 2</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>91 ± 3</td>
<td>204 ± 5</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>Low anesthetic</td>
<td>ISO</td>
<td>75 ± 4*</td>
<td>185 ± 4*</td>
<td>65 ± 3</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>62 ± 3*†</td>
<td>175 ± 6*†</td>
<td>64 ± 2</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>60 ± 3*‡</td>
<td>174 ± 5*‡</td>
<td>65 ± 2‡</td>
</tr>
<tr>
<td>High anesthetic</td>
<td>ISO</td>
<td>55 ± 4*†</td>
<td>171 ± 5*†</td>
<td>74 ± 3*†</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>33 ± 3*‡</td>
<td>162 ± 6*‡</td>
<td>74 ± 4*‡</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>37 ± 3*‡</td>
<td>158 ± 5*†</td>
<td>82 ± 3*‡</td>
</tr>
<tr>
<td>Anesthetic postcontrol</td>
<td>Post-ISO</td>
<td>75 ± 3*</td>
<td>197 ± 5</td>
<td>62 ± 2</td>
</tr>
<tr>
<td></td>
<td>HAL</td>
<td>81 ± 3*</td>
<td>194 ± 6</td>
<td>60 ± 2</td>
</tr>
<tr>
<td></td>
<td>ENF</td>
<td>85 ± 4</td>
<td>200 ± 7</td>
<td>63 ± 2</td>
</tr>
</tbody>
</table>

Data are means ± SEM; n = 13 for each group.
Control values for each group pre- and postanesthetic exposure and the effects of each anesthetic alone. Each heart was exposed to only one anesthetic.
Low: ISO 0.7%, HAL 0.5%, and ENF 1.1%; high: ISO 1.5%, HAL 1.0%, and ENF 2.2%.
ISO = isoflurane; HAL = halothane; ENF = enfurane.
* P < 0.05 versus control.
† P < 0.05 versus low anesthetic.
‡ P < 0.05, ENF or HAL versus ISO.
Table 2 shows the effects of anesthetic exposure alone (0 diltiazem) on peak LVP, spontaneous atrial rate, and AVCT. Control values, taken just before exposure to a given anesthetic, were not significantly different among the three anesthetic groups. Each heart was exposed to two concentrations of only one anesthetic. The low concentration of each anesthetic (0.7 MAC) significantly decreased peak LVP and atrial rate without changing AVCT as compared with preanesthetic controls. Halothane and enflurane significantly decreased peak LVP and atrial rate more than did isoflurane. The high concentration (1.4 MAC) of each anesthetic reduced peak LVP and atrial rate and increased AVCT compared with controls and the low concentration of each anesthetic. The decreases in LVP with halothane and enflurane, the decrease in atrial rate, and the increase in AVCT with enflurane were significantly greater than the changes produced by isoflurane. During the drug-free, postanesthetic control period, atrial rate and AVCT were not statistically different from preanesthetic controls; LVP returned to control in the enflurane group, but remained below the control value in the halothane- and isoflurane-exposed groups (P < 0.05).

**COMBINED EFFECTS OF DILTIAZEM WITH EACH ANESTHETIC**

The percent changes in LVP, atrial rate, and AVCT from predrug controls at two concentrations of each anesthetic, two concentrations of diltiazem, and postdrug control values are shown in figures 2–4. Figures 2A and B show changes in peak LVP before diltiazem exposure, during combined diltiazem and anesthetic exposure, and during the posttreatment control for each of the three anesthetic groups. Low concentrations of isoflurane, halothane, and enflurane alone (fig. 2A, table 2) significantly depressed isovolumetric peak LVP compared with drug-free control values (table 2). The low concentration of each anesthetic combined with either the low or the high diltiazem concentration produced a greater depression of LVP than did a low or high concentration of diltiazem alone (table 1), and the low concentration of isoflurane combined with the high concentration of diltiazem decreased LVP more than did the low concentration of isoflurane alone. The negative inotropic effects of halothane and enflurane, with or without diltiazem, were greater than those of isoflurane. Finally, posttreatment control percentage LVP values were decreased from pretreatment controls in all anesthetic test groups.

Figure 2B shows that the high concentration of each anesthetic significantly decreased LVP more than did the low concentration of each anesthetic with and without diltiazem. The high concentration of each anesthetic combined with the low or the high concentration of diltiazem depressed LVP more than did diltiazem alone or the anesthetic alone. High halothane and enflurane concentrations decreased LVP more than did the high isoflurane concentration independent of diltiazem treatment. The group exposed to the high concentration of isoflurane plus diltiazem did not return to predrug LVP control levels.

Fig. 2. Percent decrease in peak isovolumetric left ventricular pressure (LVP) from preanesthetic controls in each of the three anesthetic groups with low (A) and with high (B) concentrations of anesthetic alone (0 diltiazem), with each anesthetic plus 75 and 150 ng/ml diltiazem, and in the absence of drugs (P CNTRL). Thirteen hearts comprised each anesthetic group. Low: isoflurane (ISO) 0.7%, halothane (HAL) 0.5%, and enflurane (ENF) 1.1%. High: ISO 1.5%, HAL 1.0%, ENF 2.2%. Significant comparisons are noted (P < 0.05); a = each anesthetic alone or diltiazem alone versus respective initial drug-free controls; b = high diltiazem versus low diltiazem, with anesthetic; c = high versus low anesthetic with or without diltiazem; d = diltiazem plus anesthetic versus diltiazem alone; e = diltiazem plus anesthetic versus anesthetic alone; and f = enflurane or halothane versus isoflurane, with or without diltiazem.
Fig. 3. Percent decrease in spontaneous atrial rate from pretreatment controls in each of three anesthetic groups with low (A) and with high (B) anesthetic alone, with anesthetic plus low and high (180 and 360 nM) diltiazem, and in the absence of drugs (P CNTRL). See legend to figure 2 for abbreviations and statistical symbols.

The low concentration of halothane, enflurane, and isoflurane alone (0 diltiazem) significantly decreased atrial rate versus control as shown in figure 3A and table 2. The low concentration of enflurane and halothane plus the low or the high concentration of diltiazem and the low concentration of isoflurane plus the low concentration of diltiazem decreased atrial rate significantly more than did diltiazem alone (table 1). The low or the high concentration of diltiazem combined with the low isoflurane, halothane, and enflurane concentrations produced significant decreases in atrial rate compared with effects of each anesthetic alone. Low concentrations of halothane and enflurane plus low and high concentrations of diltiazem produced significantly greater decreases in atrial rate than did isoflurane plus the low or the high concentration of diltiazem.

Figure 3B shows that the high concentration of isoflurane, halothane, or enflurane decreased atrial rate more than did the low concentration. Also, the high concentration of isoflurane or enflurane plus the low concentration of diltiazem decreased the rate more than these combinations at the low anesthetic combination. Either concentration of diltiazem with the high concentration of each anesthetic decreased atrial rate significantly more than did the low or high concentration of diltiazem alone and more than the high concentration of halothane or

Fig. 4. Percent increase in atrioventricular conduction time (AVCT) from initial controls in hearts with spontaneous atrial rates in each of three anesthetic groups with low (A) and with high (B) concentrations of each anesthetic alone, with each anesthetic plus diltiazem, and in the absence of drugs (P CNTRL) *3 of 13 with AV block; **4 of 13 with AV block. See legend to figure 2 for abbreviations and statistical symbols.
enflurane alone. Also, the high concentration of diltiazem plus the high concentration of each anesthetic caused significantly greater decreases in atrial rate than did the low concentration of diltiazem with the high concentration of each anesthetic. High concentrations of halothane and enflurane produced greater decreases in atrial rate at each level of diltiazem than did isoflurane. Posttreatment control values were not significantly different from those of the predrug control period.

Figures 4A and B show changes in AVCT resulting from each of the three anesthetics alone and combined with diltiazem. AVCT was not significantly prolonged by the low concentration of isoflurane, halothane, or enflurane alone compared with control (fig. 4A, table 2). Furthermore, the combinations of low and high concentrations of diltiazem plus the low concentration of each anesthetic did not significantly prolong AVCT compared with diltiazem and anesthetic alone. However, the low concentration of enflurane plus the low or high concentration of diltiazem and the low concentration of isoflurane plus the high concentration of diltiazem significantly prolonged AVCT compared with control. The low concentration of enflurane plus the high concentrations of diltiazem produced a significant 23% incidence (3 of 13, \( P < 0.05 \)) of AV dissociation that occurred as a result of atrial slowing or AV block. During AV dissociation in these three nonpaced hearts, atrial and ventricular escape rates were slowed and regular (136 ± 8 and 158 ± 16 beats per min, respectively).

**FIG. 5.** Prolongation of AV conduction time with enflurane (ENF) (1.4 MAC) and low or high diltiazem (DIL) during atrial pacing at 3–7 Hz in ten hearts. Only data in which 1:1 A-to-V conduction were shown. Drug effects were compared to drug-free controls at each pacing rate. Pacing at 3 Hz during control periods, and at 7 Hz for treatment periods caused AV dissociation in some hearts. From 3 to 6 Hz, all control hearts exhibited 1:1 A-to-V conduction. Statistical significance symbols at \( P < 0.05 \): a = drugs alone or combined during pacing versus drug-free control; b = paced treatment versus diltiazem 75 mg/ml during pacing; c = paced treatment versus enflurane during pacing; d = paced treatment versus diltiazem 150 mg/ml during pacing.

**FIG. 6.** Percent incidence of AV dissociation caused by Wenckebach AV nodal block with enflurane (ENF) (1.4 MAC) and low or high diltiazem (DIL) during atrial pacing from 4 to 7 Hz. Enflurane alone did not significantly change the incidence of dissociation compared with the nontreated controls. At 6 Hz, high diltiazem alone and low and high diltiazem with enflurane produced significant AV dissociation; at 7 Hz, low diltiazem alone also caused AV dissociation. See legend to figure 5 for statistical symbols.

High concentrations of isoflurane, halothane, and enflurane alone and combined with diltiazem each produced significant increases in AVCT compared with their respective controls and with each low anesthetic concentration (fig. 4B). The high concentration of enflurane or halothane plus the low concentration of diltiazem increased AVCT more than did the high halothane or enflurane concentration alone. Only the high isoflurane concentration plus the high diltiazem concentration increased AVCT more than did the high isoflurane concentration alone. High isoflurane, halothane, and enflurane concentrations plus the low or the high diltiazem concentration significantly increased AVCT more than did the low or the high concentration of diltiazem alone. The combination of high diltiazem plus high enflurane concentrations caused a significant 31% incidence (4 of 13, \( P < 0.01 \)) of AV dissociation with slowed and regular atrial and ventricular rates (125 ± 15 and 145 ± 14 beats per min, respectively). Posttreatment control AVCT values were not significantly different from the initial control values for any anesthetic group.

Figures 5, 6, and 7 display the effects of low and high diltiazem concentrations plus only the high concentration of enflurane during atrial pacing on AV nodal delay, Wenckebach's periodicity, incidence of AV dissociation, and ventricular response rates during AV dissociation. In figure 5, only data for hearts exhibiting 1:1 A to V conduction are displayed. In the absence of any treatment,
AVCT increased 44% with an increase in atrial pacing rate from 4 to 7 Hz (rate dependency of AV nodal conduction). At the 4-Hz level, AVCT additively increased above the untreated control during low and high concentrations of diltiazem plus enflurane, and at 5- and 6-Hz levels during low and high concentrations of diltiazem alone, during enflurane alone, and during low and high concentrations of diltiazem plus enflurane. AVCT increased additively at the 3-6-Hz levels during the low concentration of diltiazem plus enflurane compared with the low concentration of diltiazem alone.

Figure 6 shows the incidence of AV dissociation produced during atrial pacing by the Wenckebach's type of AV block. Depending on atrial rate, Wenckebach's blocks were typically 2:1, 3:2, 4:3, and so on, for A to V conduction. At 6-Hz pacing, the high concentration of diltiazem alone and low and high concentrations of diltiazem plus enflurane caused significant AV block as compared with the drug-free control, and the high concentration of diltiazem plus enflurane caused a greater incidence of AV block than enflurane alone. In the absence of drugs, pacing at 7 Hz caused a significant incidence of AV block, but low and high concentrations of diltiazem and enflurane, alone or combined, caused a significantly greater incidence of AV block than the drug-free control at 7 Hz.

Figure 7 displays ventricular rate responses during atrial pacing. Data include ventricular rates during pacing below intrinsic atrial rates (left of line of identity), as well as during AV dissociation (right of identity line). The approximate Wenckebach's periods, measured as the A-A interval at which AV dissociation occurred, were as follows: less than 166 ms (>6 Hz) for controls; greater than 166 ms (6 Hz) for the low concentration of diltiazem alone and for enflurane alone; greater than 200 ms (5 Hz) for the high concentration of diltiazem alone and for the low concentration of diltiazem plus enflurane; and greater than 250 ms (4 Hz) for the high concentration of diltiazem plus enflurane. At 2 Hz, intrinsic atrial rates during controls were faster than atrial paced rates so that only about every other paced beat was captured and conducted; during drug treatments ventricular rates were slower than during controls. At 3 Hz, A to V conduction was 1:1 during drug treatment but not during controls. At increasing pacing rates (>4 Hz) above the intrinsic atrial rate, the incidence of AV block increased and the ventricular rates progressively slowed. At 6 Hz, the high concentration of diltiazem alone and low and high concentrations of diltiazem plus enflurane produced significant slowing of the ventricular rate during AV block; at 7 Hz the incidence of AV block was 100% with all treatments except enflurane alone; low and high concentrations of diltiazem alone and combined with enflurane significantly slowed ventricular rates to approximate A to V ratios of 2:1. When mean ventricular rates of only those hearts with AV block at 7 Hz (nonpaced control and enflurane ventricular rates of 333 ± 15 and 308 ± 16 beats per min, respectively) were compared, rather than those of all hearts as shown in figure 7, the statistical findings were not different. The high concentrations of diltiazem plus enflurane produced the greatest likelihood of AV block with ventricular automaticity as shown by the least degree of 1:1 A to V conduction over the atrial pacing range of 120–420 beats per min and the most narrow range of ventricular response rates (190–250 beats per min).

Discussion

The major aims of our study were to compare the single and combined direct effects of diltiazem with isoflurane, halothane, and enflurane on cardiac contraction, spontaneous SA rate, and AV nodal conduction and to investigate the type and mechanism of AV dissociation with these agents in the isolated perfused heart. Overall, we observed that 1) the anesthetic concentrations alone and the high concentration of diltiazem plus each anesthetic produced decreases in spontaneous atrial rate, dose-dependent decreases in LVP, and dose-dependent increases in AV conduction time; 2) for LVP and atrial rate the effects of enflurane and halothane were similar and of
greater magnitude than those of isoflurane; 3) in non-paced hearts only enflurane with diltiazem caused AV dissociation, producing a slower atrial than ventricular rate; 4) during rapid pacing (7 Hz), AV nodal delay and Wenckebach’s periodicity increased more with diltiazem, alone and with enflurane, than with enflurane alone; and 5) ventricular escape rates during Wenckebach’s AV block at 7 Hz were slower with the low or high concentration of diltiazem plus enflurane than with the low concentration of diltiazem or enflurane alone. Specifically, this study demonstrated the following:

1) Low and high concentrations of diltiazem alone decreased atrial rate compared with controls; however, only the high concentration of diltiazem alone also increased AVCT and decreased peak LVP. Each anesthetic alone dose-dependently decreased atrial rate and peak LVP; only the high anesthetic concentration alone increased AVCT; halothane and enflurane given alone decreased atrial rate and LVP more than did isoflurane alone. No anesthetic or diltiazem given individually caused AV block.

2) Low and high concentrations of diltiazem combined with high concentrations of each anesthetic decreased peak LVP more than diltiazem plus low anesthetic concentrations. Regardless of concentration, diltiazem plus halothane or enflurane depressed peak LVP more than did respective concentrations of the individual agents alone. Diltiazem combined with halothane or enflurane at either concentration decreased LVP more than did diltiazem combined with isoflurane.

3) Spontaneous atrial rate was decreased by the combination of diltiazem plus the low concentration of each anesthetic compared with control and anesthetics alone. All diltiazem plus low anesthetic concentration combinations but high diltiazem and low isoflurane concentrations decreased atrial rate more than did diltiazem alone. High anesthetic concentrations plus diltiazem decreased atrial rate more than did diltiazem alone and, except for low concentrations of diltiazem plus isoflurane, more than the anesthetics alone. Regardless of concentration, diltiazem plus enflurane or halothane produced similar depressions of atrial rate that were greater than those produced by diltiazem and isoflurane.

4) During the decreased atrial rate produced by the high concentration of each anesthetic with low or high concentrations of diltiazem, AVCT increased more than it did with the low level of each anesthetic plus diltiazem. AVCT increased more with high concentrations of isoflurane, halothane, or enflurane plus diltiazem than with diltiazem alone and, except for the low concentration of diltiazem plus isoflurane, more than the anesthetics alone. Along with the slowed spontaneous atrial rates, only the combination of enflurane and the high concentration of diltiazem caused AV block (three of 13 with the low enflurane concentration and four of 13 with the high enflurane concentration).

During atrial pacing, the high concentration of diltiazem, alone and combined with the high concentration of enflurane, dose-dependently increased the delay in AVCT and increased the Wenckebach’s period (AV nodal refractory period), indicated by an increased incidence of Mobitz type 1 AV block at a given pacing rate, and slowed the ventricular escape rate.

**Effects on Left Ventricular Pressure**

Peak isovolumetric LVP decreased significantly more when diltiazem was combined with high concentrations of isoflurane, halothane, or enflurane than when each agent was given alone. Isoflurane and diltiazem combined produced significantly less depression of LVP than did either halothane or enflurane with diltiazem, although additive or synergistic interactions between the anesthetics and diltiazem were not directly examined. Results from this study in the isolated heart are similar to those of other studies in vivo and in vitro. Decreases in myocardial contractility have been shown to occur with diltiazem in both isolated guinea pig atrium and ventricular muscle. Myocardial depression by diltiazem has also been demonstrated in the anesthetized, intact dog by a decrease in cardiac output and by a lack of change in systolic segment shortening in response to changes in preload or afterload. It has been suggested that the variable effects diltiazem produces on contractility result from differing sensitivities among cardiac tissues of various species. Because of its frequency-dependent blockade of calcium channels, the negative inotropic effect of diltiazem also may be attenuated as rate decreases.

Isoflurane depresses myocardial function less than either halothane or enflurane. Other in vivo and in vitro studies have demonstrated a greater depression of cardiac function by volatile anesthetics in combination with diltiazem as well as with other calcium antagonists. Studies from this laboratory indicate that peak isovolumetric LVP was depressed by verapamil, isoflurane, halothane, and enflurane in a dose-dependent manner. Verapamil (75 and 150 ng/ml) combined with each anesthetic, in the same concentrations as used in these studies, additively depressed LVP. Enflurane plus verapamil did not produce greater depression of LVP than did isoflurane or halothane plus verapamil (0.7 MAC: enflurane + 75 verapamil = -60%, enflurane + 150 verapamil = -78%; isoflurane + 75 verapamil = -52%, isoflurane + 150 verapamil = -70%; halothane + 75 verapamil = -58%, halothane + 150 verapamil = -70%; 1.4 MAC: enflurane + 75 verapamil = -72%).
enflurane + 150 verapamil = -85%; isoflurane + 75 verapamil = -65%, isoflurane + 150 verapamil = -78%; halothane + 75 verapamil = -70%, halothane + 150 verapamil = -82%). This appears to be dissimilar to the current findings with diltiazem in which diltiazem plus enflurane or halothane produced greater decreases in LVP than diltiazem plus isoflurane. All of these studies, including the current study, support the view that both of these classes of negative inotropic drugs alter multiple, and possibly common, calcium-sensitive sites in the excitation contraction coupling sequence.

During each of the 20-min drug-free postcontrol periods, atrial rate and AVCT returned to initial control values. LVP did not return fully to the initial control values in several posttreatment periods. We could not discern whether this was drug related, resulting from an undefined decrease in contractile function, or resulting from its measurement. Because systolic LVP decreases only 5–10% over a 3-h period in the untreated, isolated guinea pig heart (personal observations), it is possible that the lipid-soluble volatile anesthetics retard the washout of diltiazem, a relatively water-soluble drug. Nevertheless, the statistical inferences concluded about the drug effects of diltiazem and volatile anesthetics, alone and in combination, are similar when the effects are compared with the initial or postcontrol values. One unexplained exception is that the postcontrol mean for LVP in the isoflurane-exposed group was not statistically different from the mean LVP during low concentrations of isoflurane.

**Effects on Atrial Rate**

Our finding of a slowing of atrial rate by diltiazem alone is supported by an in vitro study. Our findings with volatile anesthetics agree with previous in vitro isolated cardiac and SA nodal studies from our laboratory. We have demonstrated that volatile anesthetics produce dose-dependent decreases in spontaneous sinus rate that are associated with depression of the SA nodal action potential upstroke. A greater direct decrease in sinus rate with equivalent MAC levels of enflurane, compared with isoflurane or halothane, has also been reported previously. Reduction of the calcium transients by anesthetics probably accounts for the observed decrease in the rate of automaticity. Diltiazem combined with halothane and enflurane produced a greater decrease in atrial rate than with diltiazem or the anesthetic given alone. We have reported similar findings for these anesthetics plus verapamil. The effectiveness of diltiazem in additionally reducing atrial rate in the presence of anesthetics may be blunted by the greater rate decrease with the high compared with the low anesthetic level, which suggests that the interaction of these agents in reducing atrial rate is rate dependent. Given the common features of the reported mechanisms of action of anesthetics and calcium channel blockers, the combined magnitude of these direct effects was expected.

**Effects on Atrioventricular Conduction and Block**

The high concentration of each anesthetic alone uniformly increased AVCT along with the significant decrease in spontaneous atrial rate. The greater increase in AVCT with enflurane (table 2) compared with halothane or isoflurane is similar to our previous finding in the isolated heart. In dogs anesthetized with greater than 1.2 MAC equivalents of isoflurane, halothane, and enflurane, AVCT also increases, but without a change in spontaneous heart rate. The high concentrations of diltiazem alone also increased AVCT along with the decrease in spontaneous atrial rate. Diltiazem has been found to prolong antegrade conduction through the AV node in isolated tissue and in humans during atrial pacing. In anesthetized dogs, diltiazem prolonged AVCT at plasma concentrations approximately one third to one half of those used in our study.

Other studies show that AV nodal conduction is significantly prolonged by verapamil with volatile anesthesics, especially enflurane, which can lead to AV dissociation, caused possibly by SA nodal arrest or exit block. Diltiazem, in combination with volatile anesthetics, has also been shown to produce a greater prolongation of the AV interval than diltiazem alone in conscious, chronically instrumented dogs. Moreover, severe AV nodal depression has been reported in a case report of a patient given oral diltiazem chronically while undergoing enflurane anesthesia. We found that diltiazem augmented, but did not potentiate, the increase in AVCT resulting from exposure to anesthetics. This is in contrast to our results with enflurane and verapamil in which AVCT was potentiated. In anesthetized dogs, isoflurane has been demonstrated to produce little if any additional increase in the PR interval over that resulting from diltiazem alone. The differential slowing of AVCT in the isolated guinea pig heart and the intact dog heart may be species related or result from extrinsic cardiac factors in vivo. It has been suggested that AVCT is altered more by diltiazem in the dog than it is in humans.

It is interesting that AV dissociation, as a result of ventricular escape with sinus bradydysrhythmia or of AV block in nonpaced hearts, developed in this protocol only when diltiazem was combined with enflurane, but not when it was combined with halothane or isoflurane. This may be a dose-related phenomenon, but in the same preparation we demonstrated previously that 2.2% en-
flurane with 75 ng/ml verapamil and 150 ng/ml verapamil produced, respectively, 25% and 67% incidences of AV dissociation; moreover, a potentiation of AVCT occurred with enflurane but not with halothane or isoflurane. In that study and the current study, AV dissociation with slowed ventricular escape rates occurred only during the additional decrease in spontaneous atrial rate caused by the combination of the anesthetic and calcium entry blocker. In the dog, enflurane has been shown to increase AVCT and AVFRP more than other available anesthetics and to cause AV dissociation when given in combination with verapamil. Overall, examination of our earlier study with verapamil suggests that atrial rate and AV conduction time are depressed to a lesser magnitude, and AV block occurs less frequently with diltiazem than with verapamil during exposure to the same concentrations of volatile anesthetics.

Diltiazem and enflurane accentuated the increase in AV conduction time produced by increasing the atrial pacing rate. At a slow pacing rate (3 Hz), hearts exposed to diltiazem plus enflurane exhibited 1:1 A to V conduction, whereas control hearts did not exhibit 1:1 conduction. At faster pacing rates (5–7 Hz), diltiazem and enflurane progressively increased the Wenckebach’s period and produced AV dissociation with a progressively slower ventricular response rate. During autonomic blockade in vivo, Wenckebach’s periodicity and AVFRP appear equivalent.

Our results aid in understanding the direct cardiac actions of volatile anesthetics and diltiazem and the mechanism of their effects on the conduction system. Depression of the SA and AV nodes with these drug combinations—causing slowing of automatic primary and secondary pacemakers, a delay of AV conduction, and increased refractoriness of the AV node—appears to underlie the occurrence of AV dissociation with slowed ventricular escape rates in vivo. A delay in conduction through the AV node and a decrease in AVFRP are conditions for reentrant AV nodal excitation. An increase in AVFRP and an increase in automaticity of AV junctional cells are conditions for ventricular escape rhythms. In the isolated heart, diltiazem alone or with the volatile anesthetics caused only AV dissociation with sinus bradycardia and AV junctional or ventricular bradydysrhythmia. At slow atrial rates, these agents could permit junctional escape beats during suppression of the dominant SA nodal pacemaker.

In summary, our experiments indicate the following: 1) that diltiazem and enflurane retard conduction between the atria and ventricles, most probably by prolonging the AVFRP as assessed by the increase in Wenckebach’s period; 2) that these drugs permit usurpation of AV junctional (ventricular) pacemakers; and 3) that they retard the rate of junctional (or ventricular) automaticity as shown by the slower, stabilized ventricular response rates during AV dissociation with increasing atrial pacing rates. Moreover, it appears that enflurane and diltiazem are each capable of producing these effects and that the effects are additive when combined.

Taken together, our in vitro study and in vivo studies by others suggest that there may be a higher probability of depressed myocardial contractile function, AV block, and slowed junctional rhythm during administration of diltiazem in patients undergoing general anesthesia, especially with enflurane. In patients with intrinsic sinus bradycardia, AV conduction disturbances, or myocardial dysfunction, these drugs may exacerbate the abnormalities. Moreover, in an overview with our earlier study, it appears that the degree of change in atrial rate, AVCT, and LVP during exposure to enflurane is less with diltiazem than with verapamil at the concentrations used.

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