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Interaction of Diltiazem and Volatile Anesthetics on Cardiac Function and Myocardial Metabolism in the Rat Heart-lung Preparation

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Abstracts

The direct effects of diltiazem on cardiac function and myocardial metabolism in the presence of the volatile anesthetics halothane (H), enflurane (E), isoflurane (I) and sevoflurane (S) were assessed in the isolated heart-lung (H-L) preparation. Wistar-ST rats were randomly divided into 5 groups (each group n=8) as follows: 1.Control (C) group; diltiazem (10^{-6}M) with no volatile anesthetics. 2. The H group; diltiazem and 1 % halothane. 3. The E group; diltiazem and 2.2 % enflurane. 4. The I group; diltiazem and 1.5 % isoflurane. 5. The S group; diltiazem and 2.5 % sevoflurane. Five minutes after the start of perfusion on the H-L preparation, 10^{-6}M of diltiazem was administered into the reservior. Thirty minutes after the start of perfusion, the hearts were freeze-clamped and myocardial ATP, ADP, AMP, lactate and glycogen were measured. Heart rate, cardiac output and LV dP/dt max in the E group decreased significantly by the administration of diltiazem. However, there were no significant differences in myocardial ATP, ADP, AMP, lactate and glycogen levels among the all groups. This result means that diltiazem under E has more cardiac depressant property than diltiazem under the other volatile anesthetics does, although diltiazem under all volatile anesthetics do not induce any deleterious effects on myocardial metabolism.

Key words : Diltiazem, Volatile anesthetics, Cardiac interaction, Myocardial metabolism

Introduction

Diltiazem is a benzothiazepine derivative with notable vasodilating and negative inotropic effects[1]. It interferes with a calcium-dependent slow curent across excitable cell membranes[2,3]. Volatile anesthetics also decrease free calcium availability for contraction and provably interfere with several different steps in the excitation-contraction coupling process[4,7]. Significant cardiac interactions are likely to occur because of similarity in pharmacological effects of these drugs. We knew of no other study of the direct effects of 4 volatile anesthetics and diltiazem on the isolated heart. Thus, it is interesting to investigate the direct cardiac effects of diltiazem in the presence of 1 MAC halothane, enflurane, isoflurane and sevoflurane in the isolated heart-lung preparation. This technique eliminates any confounding neurohumoral effects of *in vivo* studies and myocardial metabolism can be easily measured to study the relationship between function and metabolism.

Materials and methods

The experiment was performed in accordance

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with Guidelines for Animal Experiments, Yamanashi Medical University. The techniques used were almost identical to those used in our earlier study[8]. Briefly, 40 males Wister-ST rats, weighing 300-320 g, were randomly divided into five groups (each group n=8) as follows: 1. The control (C) group which received diltiazem (10^{-6}M) with no volatile anesthetics. 2. The halothane (H) group which received diltiazem and 1 % halothane. 3. The enflurane (E) group which received diltiazem and 2.2 % enflurane. 4. The isoflurane (I) group which received diltiazem and 1.5 % isoflurane. 5. The sevoflurane (S) group which received diltiazem and 2.5 % sevoflurane. All rats except in the C group were anesthetized with each volatile anesthetic in each group through the experiment and the C group rat was anesthetized with isoflurane only during the preparation. Tracheotomy was performed, and intermittent positive pressure ventilation through the tracheal tube inserted was adjusted to maintain $PaCO_2$ at 35-40 mmHg and PaO_2 at 300-400 mmHg with a 95 %O_2 + 5 %CO_2 gas mixture. The chest was opened and flooded with ice-cold saline, then the heart arrested. Cannulae were inserted into the aorta and the superior and inferior venae cavae. The cannula in the superior vena cava was used for the monitor of right atrial pressure.

The heart-lung preparation was perfused with a solution (25 ml) containing red blood cells collected from another rat and Krebs-Ringer bicarbonate buffer, with hematocrit and pH of 25 % and 7.4, respectively. The concentrations (mM) of the buffer constituents were: NaCl 127, KCl 5.1, $CaCl_2$2.2, KH_2PO_4 1.3, $MgSO_4$ 2.6, $NaHCO_3$ 15, glucose 5.5 and heparin. The perfusate blood pumped from the aorta passed through a pneumatic resistance and was collected in a reservoir kept at 37 ℃ and then returned to the inferior vena cava. In this model, no other organs except heart and lung were perfused, cardiac output was determined by the inflow to the inferior vena cava as long as the heart did not fail. and mean arterial pressure was regulated by the pneumatic resistance.

Heart rate was recorded from ECG with a bioelectric amplifier (AB-621G, Nihonkohden, Tokyo) and cardiac output was measured with an electromagnetic blood flow meter (MFV-1200, Nihonkohden, Tokyo). Arterial pressure and right atrial pressure were measured with transducers (TP101T and LPU-1.0A) and carrier amplifiers (AP-601G, Nihonkohden, Tokyo).

All hearts were perfused initially with a cardiac output of 30 $ml \cdot min^{-1}$ and systolic arterial pressure of 80 mmHg. In anesthetic groups, each anesthetics was added to the gas mixture using calibrated vaporizers throughout the experiment. Five minutes after the start of perfusion, 10^{-6}M of diltiazem, was administered into the reservoir. When right atrial pressure increased over 60 mmHg, the inflow from the reservoir was diminished. It was estimated as cardiac failure that cardiac output decreased under 20 $ml \cdot min^{-1}$.

Thirty minutes after the start of the experiment, the hearts were freezed by liquid nitrogen and freeze-dried for 6 days. An aliquot was extracted with perchloric acid and centrifuged at 3000 g. Myocardial high energy phosphates (ATP, ADP and AMP) were measured by the high lquid performance chromatography[9]. The lactate level was determined spectrophotometrically by a standard tecnique[10]. Another piece of freeze-dried sample was placed in 30 % KOH and digested at 100 ℃. Tissue glycogen was extracted, hydrolyzed and assayed as glucose equivalents[11]. The values are expressed as μ $mol \cdot g^{-1}$

Hemodynamic data within groups were analyzed by two way analyses of variance with repeated measures. The other data were analyzed by one way analysis of variance followed by the Dunnett test for multiple comparisons. A probability of $p < 0.05$ was regarded as statistically significant. The data are given as means ± S. D.

Results

Heart rate, cardiac output and LV dp/dt maximum in the E group significantly decreased by the administration of diltiazem at 10 minutes, but those in the other groups caused no chages at every mi-

nutes (Fig. 1-3). In spite of cardiac depression in the E group, there were no significant differences in myocardial ATP, ADP, AMP, lactate and glycogen levels among the all groups (Fig. 4,5).

Discussion

A volatile anesthetic agent, in general, interferes with calcium ion flux into myocardial muscle fibers as a calcium channel blocker does[12]. There are a number of cellular and subcellular sites at which anesthetics might act to depress cardiac contractility, including the sarcolemmma, sarcoplasmic reticulum, mitochondria, and contractile proteins[4]. Volatile anesthetics interfere with function of Ca^{2+} channels in the sarcolemmal membrane and also partially inhibit function of the sarcoplasmic reticulum as a modulator of changes in intracellular Ca^{2+} through several mechanisms[13~16]. Volatile anesthetics also may modify the responsiveness of contractile proteins, although this hypothesis remains somewhat controversial[13,17]. The anesthetics and calcium channel blockers act similarly to interfere with calcium ion flux across myocardial membranes[18,19].

Marijic et al said that the combination of either level of enflurane and verapamil had depressed cardiac function more than had done the same level of halothane or isoflurane with verapamil[20]. Gallenberg et al demonstrated that the combined administration of nifedipine and volatile anesthetics,especially enflurane, depressed atrial rate and contractility[21]. Kapur et al showed that the administration of verapamil during steady state inhalation anesthesia in the dog resulted in a significant depression of blood pressure, left ventricular dP/dt, and cardiac index during enflurane and isoflurane

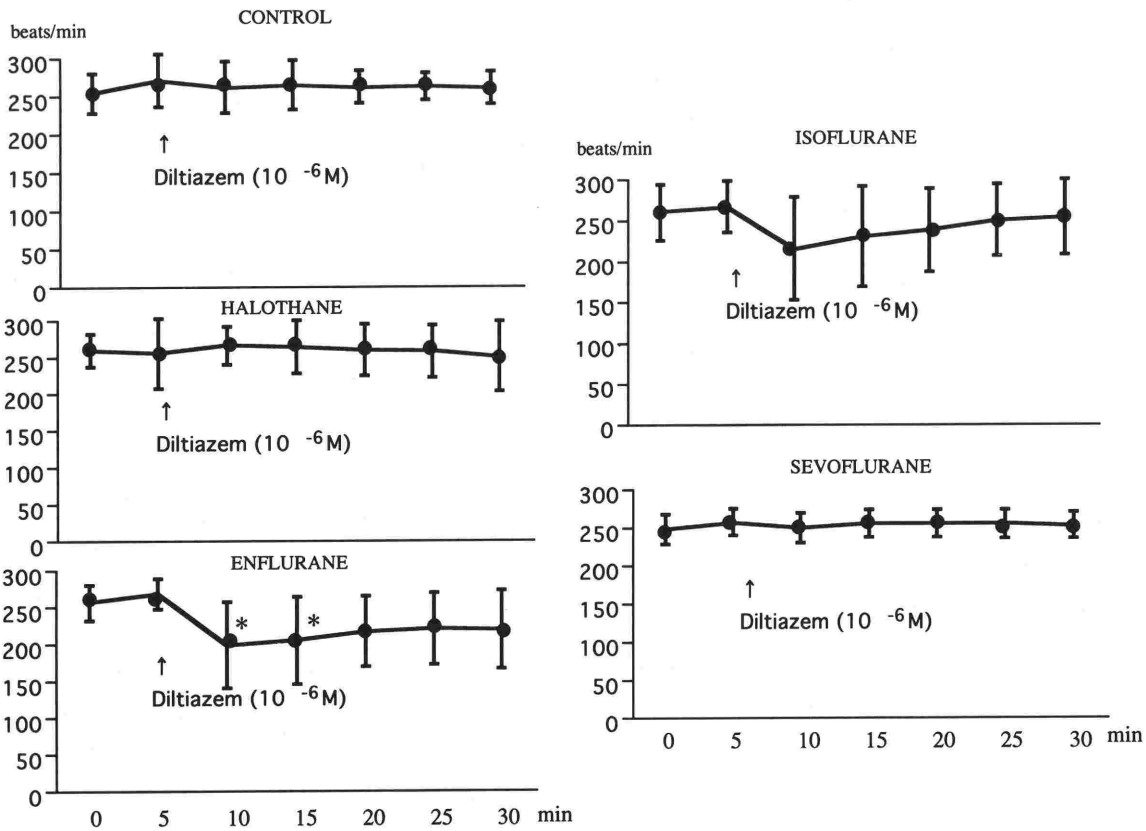

Fig 1. Changes in heart rate in five groups. Data are presented as means ± S.D.
*P<0.05 vs. values at 5 min.

anesthesia but not during halothane anesthesia, when compared with control anesthetic values. Moreover the hemodynamic values obtained with the enflurane-verapamil combination were the lowest among the three anesthetic groups[22]. Merin et al also described that the combination of oral verapamil with low doses of enflurane had been more depressant to the cardiovascular system of healthy dogs than had been the combination of verapamil and halothane or isoflurane[23]. We have previously reported that nicardipine caused significant myocardial depression in the presence of halothane or enflurane on the ischemic heart[24]. Manabe et al[25] reported that enflurane with diltiazem more depressed cardiac function than halothane with diltiazem did, and that the negative inotropic effect of diltiazem was enhanced by enflurane anesthesia due to unknown mechanisms which probably included a slight but insignificant increased in plasma concentration. Thus, these previous studies are agreeable with the present result that enflurane with diltiazem has more cardiac depressant property than the other volatile anesthetic with diltiazem does.

Enflurane had no influence on myofibrillar ATPase[26], nor on the Ca^{2+}-sensitivity of contractile proteins in cardiac skinned fibers[27], whereas halothane and isoflurane did[28]. In the presence of enflurane, the Ca^{2+} pump can accumulate more Ca^{2+} at lower ATP than halothane- or isoflurane-exposed sarcoplasmic reticulum[18]. Halothane and isoflurane appeared to decrease the calcium sensitivity of the contractile proteins in functionally skinned right ventricular rabbit papillary muscle[28], whereas enflurane did not[27]. Isoflurane's depressant effect on myofibrillar ATPase was less than of

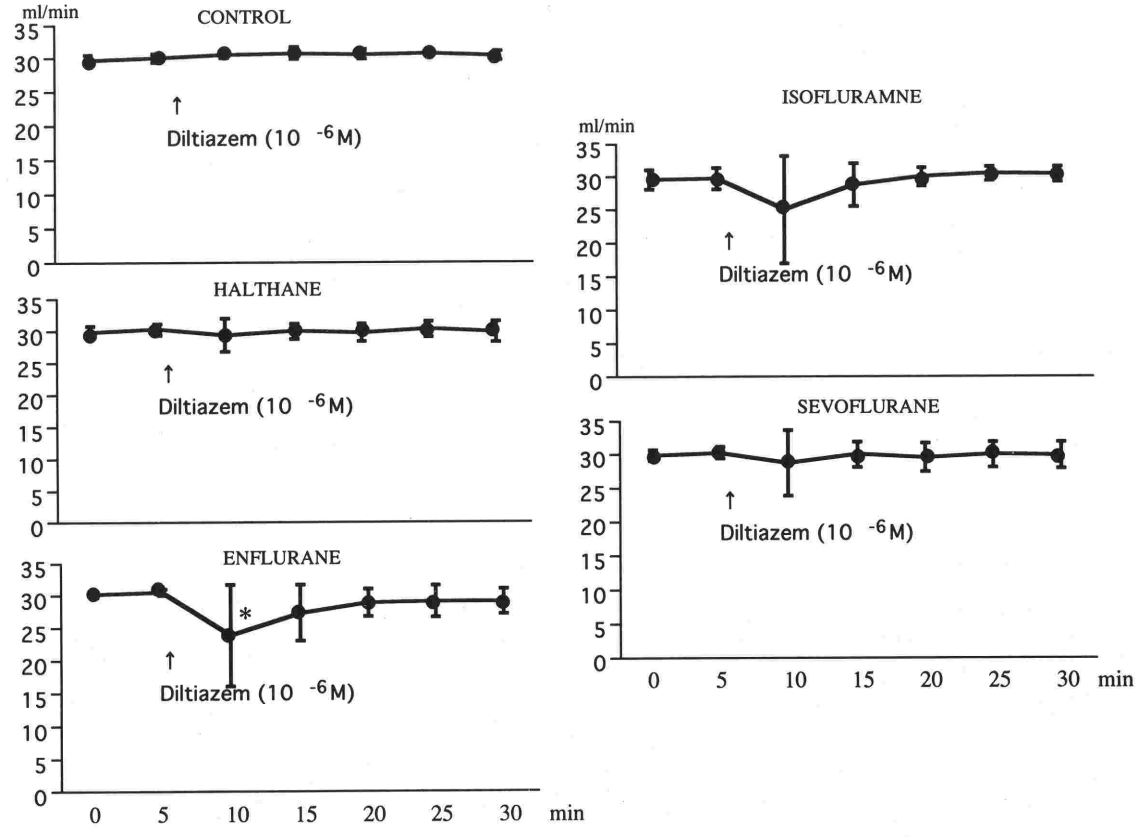

Fig 2. Changes in crdiac output in five groups. Data are presented as mean ± S.D.
*P<0.05 vs. values at 5 min.

halothane, whereas enflurane was reported to have no effect[29]. However, these reports cannot explain the reason why enflurane with diltiazem has more cardiac depressant property than the other volatile anesthetics with diltiazem do. Further studies are necessary to investigate this mechanism

The cardiac function decreased at 10 minutes in the E group. The recovery may be explained by the decrease in concentration of diltiazem which was induced by dilution in the perfusate by time. We confirmed in a preliminary study that 10^{-7} and 10^{-6}M of diltiazem did not depress cardiac function, although 10^{-5}M of diltiazem depressed this profoundly in the rat heart lung preparation. Therefore, we used 10^{-6}M of diltiazem because of above reason and the human plasma therapeutic concentration of diltiazem is about 3×10^{-7}M[30].

In spite of cardiac depression in the E group, there were no significant differences in myocardial ATP, ADP, AMP, lactate and glycogen levels among the all groups. We measured myocardial metabolites 30 minutes after the start of the experiment. The cardiac function in the E group recovered at this time. Therefore, it is likely that there was no differences in myocardial metabolites among the groups. It would be better if we had measured myocardial metabolites at the time of cardiac depression in the E group.

In conclusion, enflurane with diltiazem has more cardiac depressant property than the other volatile anesthetics with diltiazem do, although enfulrane with diltiazem induce no deleterious cahnges of myocardial metabolism. Attention should be paid when diltiazem is used during enflurane anesthesia.

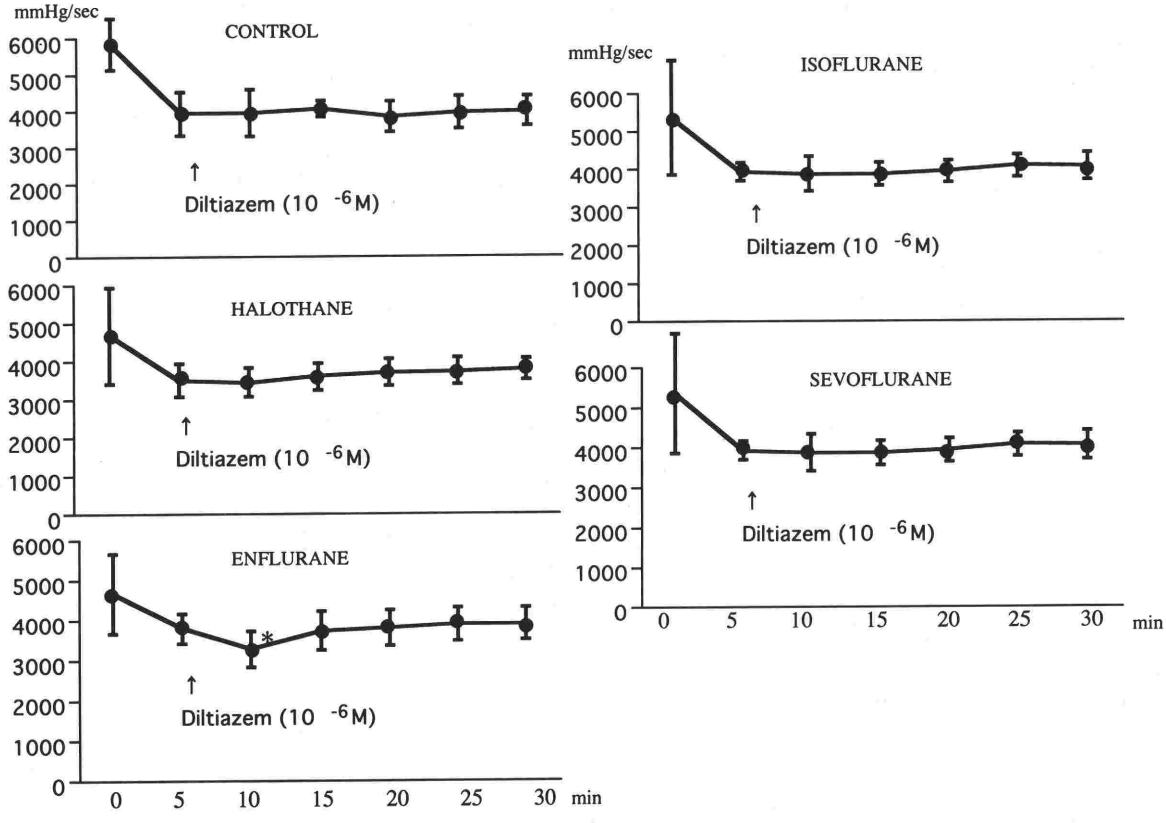

Fig 3. Changes in LV dp/dt max in five groups. Data are presented as mean ± S.D.
*P<0.05 vs. values at 5 min.

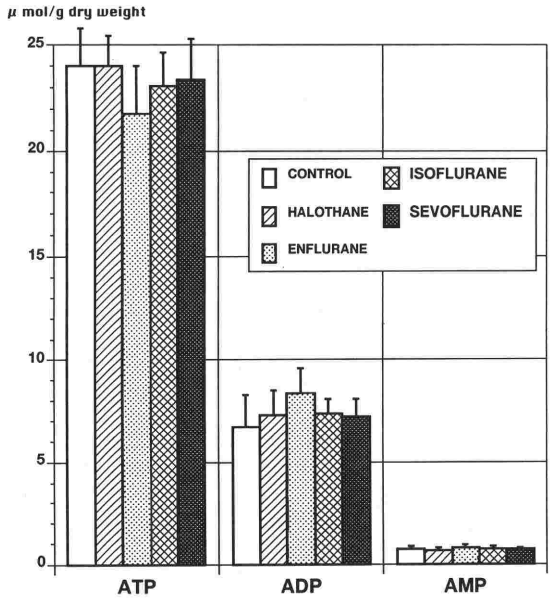

Fig 4. Myocrdial concentrations of ATP, ADP and AMP.

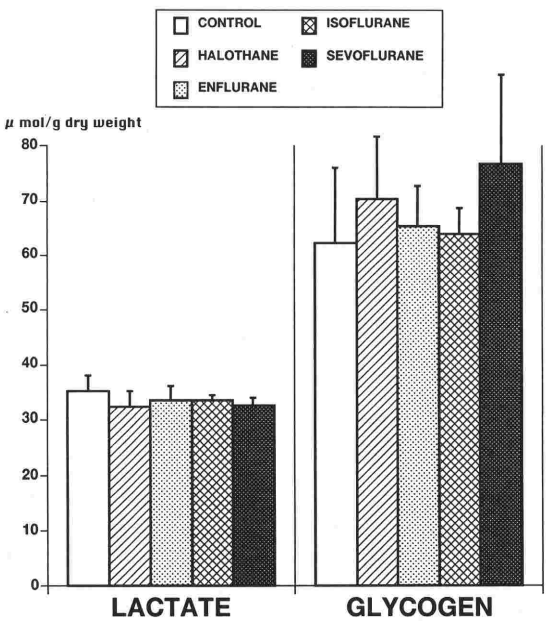

Fig 5. Myocrdial concentrations of lactate and glycogen.

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