Interaction between Diltiazem and Halothane or Enflurane in the Canine Blood-perfused Papillary Muscle and Sinoatrial Node Preparations Cross-circulated by Chronically Instrumented Conscious Donor Dog

Masanobu MANABE, Shigeru MOTOMURA* and Keitaro HASHIMOTO*

Interaction of cardiovascular effects of diltiazem with those of halothane or enflurane was estimated in the canine isolated papillary muscle and sinoatrial node preparations perfused by arterial blood of the chronically instrumented conscious and halothane- or enflurane-anesthetized donor dog, into which diltiazem was infused i.v. at a rate of 20 μg/kg/min for 60 min. One hour after diltiazem infusion, in the conscious donor dog, mean arterial pressure (MAP) and heart rate (DHR) were decreased to 84 ± 3 and 84 ± 2% and PQ interval (PQ) was prolonged to 148 ± 5%, while in the isolated preparations, developed tension (DT) of the papillary muscle and sinoatrial rate (SAR) were decreased to 68 ± 3 and 74 ± 3% and blood flow (BF) was increased to 155 ± 5% (n=10). On the other hand, halothane (0.8%) anesthesia per se decreased MAP, DHR, DT and SAR to 89 ± 8, 84 ± 3, 79 ± 3 and 89 ± 5% (n=7) of each basal value in conscious state 20 min after the inhalation. During halothane anesthesia, the same dose of diltiazem infused decreased MAP to 74 ± 4 (n=7), DHR to 66 ± 4 (n=6), DT to 62 ± 7 (n=7) and SAR to 69 ± 1% (n=3) of each value suppressed by halothane itself. Meanwhile, enflurane (1.7%) anesthesia itself decreased MAP, DHR, DT and SAR to 81 ± 3, 85 ± 2, 81 ± 2 and 88 ± 2% (n=10) of each basal value in conscious state 30 min after enflurane inhalation. During enflurane anesthesia, diltiazem decreased MAP to 74 ± 3 (n=10), DHR to 67 ± 3 (n=8), DT to 45 ± 5 (n=10) and SAR to 74 ± 6% (n=3) of each value under enflurane anesthesia alone. PQ interval of the donor dog heart was prolonged by halothane alone to 111 ± 5% (n=7) and by enflurane alone to 110 ± 2% (n=10) of the value before each anesthesia, and then diltiazem prolonged PQ interval to 160 ± 8% (n=6) and 174 ± 10% (n=8) of each value suppressed by the anesthetic itself during halothane- or enflurane-anesthesia, respectively. The second degree AV conduction block was induced in 1 of 7 halothane- and in 2 of 10 enflurane-anesthetized donor dogs, respectively. The sinus arrest was induced by diltiazem in 4 of 7 sinoatrial node preparations under halothane and in 7 of 10 ones during enflurane anesthesia. Moreover, plasma concentration of diltiazem 60 min after the start of infusion was 556 ± 121 ng/ml in conscious dogs and tended to increase to 752 ± 101 ng/ml in enflurane anesthetized donor dogs (n=4), but there was no significant difference between two values ($0.05<P<0.1$). These results indicate that effects of diltiazem could be potentiated during halothane or enflurane anesthesia by elimination of compensatory reflex noted in conscious state, and that the negative inotropic effect of diltiazem was enhanced by enflurane anesthe-

*Department of Anesthesiology and *Department of Pharmacology, Yamanashi Medical college, Tamaho, Yamanashi, Japan*

Address reprint requests to Dr. Manabe: Department of Anesthesiology, Yamanashi Medical College, Tamaho, Yamanashi, 409-38 Japan

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sia due to unknown mechanisms which probably include a slight but insignificant increase in plasma concentration. (Key words: pharmacokinetic interaction, plasma concentration, compensatory reflex, cardiac contractility, sinus arrest, AV conduction block, diltiazem, halothane, enflurane)

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Calcium (Ca) antagonists have been frequently introduced for maintaining blood pressure and for preventing coronary vasospasm and certain cardiac arrhythmias during inhalation anesthesia[1,2]. However, since inhalation anesthetics such as halothane and enflurance per se have potent cardiovascular depressant effects, it is very important to know how inhalation anesthetics modify the cardiovascular effects of Ca antagonists, such as marked hypotension, bradycardia, atrioventricular (AV) conduction disturbance, and depression of cardiac contractility[3–5]. In order to analyze the interaction between inhalation anesthetics and Ca antagonists, it is necessary to estimate effects of the inhalation anesthetic itself by anesthetizing conscious subjects, since elimination of the compensatory reflex mechanisms by the anesthesia could modify the effects of Ca antagonists observed in the conscious subjects[4,5]. In this regard, there have been several reports concerning the effects of verapamil, nifedipine and diltiazem during halothane anesthesia[6], the effects of verapamil under halothane, enflurane, and isoflurane anesthesia[7], and the effects of diltazem during isoflurane[8] and enflurane anesthesia[9]. Unfortunately, there have been no experiment using conscious animals as a control for defining reflex modifications which would be eliminated in the anesthetized animals. Nevertheless, Kapur et al.[7] reported that cardiac contractility was more markedly decreased by verapamil during enflurane anesthesia than during halothane or isoflurane anesthesia. Recently, Merin and co-workers[10–13] reported detailed experiments on interaction between verapamil and inhalation anesthetics by carefully comparing effects of verapamil in conscious dogs with those during halothane, enflurane and isoflurane anesthesia. In their experiments, plasma concentration of verapamil was increased by inhalation anesthesias, especially by enflurane, compared with those in conscious state[11,12] and more depressant cardiovascular effects were induced by verapamil with enflurane than with halothane or isoflurane anesthesia[12,13]. Furthermore, Kapur et al.[8,9] reported higher plasma levels of diltiazem in enflurane anesthetized dogs compared to isoflurane anesthetized dogs for the same infusion rate, which accompanied with severer hypotension, but no difference in cardiac contractility.

In the present experiments, we focused on the potentiation by enflurane anesthesia of the cardiovascular depressant effects of diltiazem, especially on the cardiac contractility, because the enhanced depressive effects on the LVdP/dt during enflurane anesthesia were reported to be induced by verapamil[7,11], but not by diltiazem[8,9]. For this purpose, we prepared the isolated papillary muscle[14] and sinoatrial node preparations[15] perfused with heparinized arterial blood of the chronically instrumented conscious[16–18] and halothane or enflurane anesthetized donor dog to which diltiazem was intravenously infused. Since the papillary muscle and sinoatrial node preparations were excised, the direct inotropic and chronotropic effects of the agents, either diltiazem or inhalation anesthetics, can be obtained without any neural effect by reflex. Meanwhile, in the conscious donor dog the effects of the drug modified by the compensatory reflex mechanisms could be obtained. In this model, we observed the enhancement of the negative inotropic effects of diltiazem during enflurane anesthesia but not under halothane anesthesia[19]. Thus, we measured arterial plasma concentration of

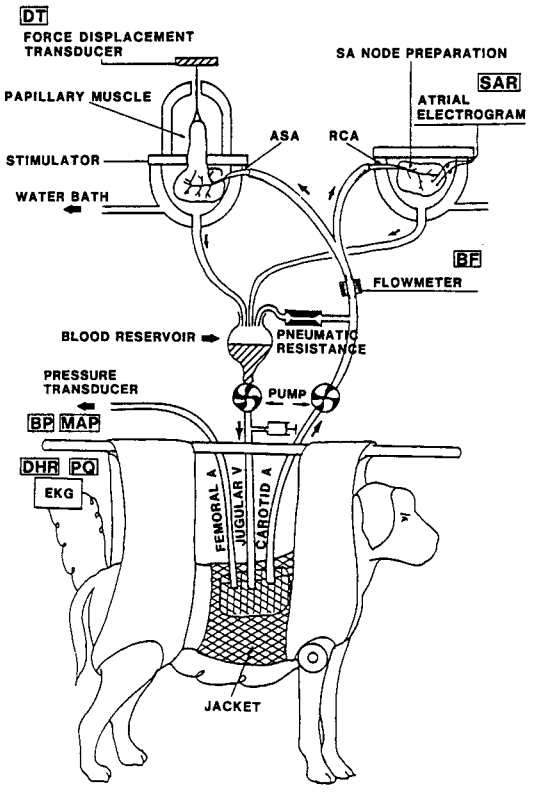

Fig. 1. A schematic representation of cross-circulation diagram of the isolated papillary muscle and sinoatrial node preparations by the chronically instrumented conscious donor dog. BP: systemic blood pressure, MAP: mean arterial pressure, DHR: heart rate of the donor dog, PQ: PQ interval, DT: developed tension of the papillary muscle, SAR: sinoatrial rate of the sinoatrial node preparation, BF: blood flow through the preparations, ASA: the anterior septal artery, RCA: the right coronary artery, EKG: the lead II of electrocardiogram.

diltiazem infused into conscious or enflurane anesthetized donor dog.

Methods

Experiments were carried out on 27 canine isolated, blood-perfused papillary muscle[14] and sinoatrial node preparation,[15] which were first cross-circulated by chronically instrumented conscious donor dogs.[16–18] in figure 1 is shown a schematic representation of cross-circulation diagram of the canine isolated papillary muscle and sinoatrial node preparations by the chronically instrumented conscious donor dog.

The papillary muscle and sinoatrial node preparations were obtained from a mongrel dog of either sex, weighing 8–14 kg, anesthetized with sodium pentobarbital (30 mg/kg· i.v.), given sodium heparin (500 *U*/kg i.v.) and exsanguinated. The heart was excised and plunged into cold Tyrode's solution kept at about 4°C, then the preparations were made. The papillary muscle preparation consists of the anterior papillary muscle of the right ventricle attached to the interventricular septum. The anterior septal artery, a nutrient artery to the papillary muscle was directly cannulated. The sinoatrial node preparation consists of the right atrium and the sinus node artery was cannulated through the right coronary artery. The each preparation was placed in each double-wall glass jacket maintained at 38°C by circulating warm water. The both papillary muscle and sinoatrial node preparations were simultaneously perfused through each cannulated artery with heparinized arterial blood from the donor carotid artery at a constant perfusion pressure of 120 mmHg with a Cole–Parmer Masterflex peristaltic pump and a Starling pneumatic resistance placed parallel to the perfusion system. Venous blood from the preparations and excess blood passing through the pneumatic resistance was collected in the blood reservoir and pumped by another peristaltic pump back into the jugular vein of the donor dog. The rate of blood flow (BF) through the anterior septal and right coronary arteries was measured with an electromagnetic flowmeter (Nihon-Kohden, MVF-1100) using a 2 mm cannulating flow probe. The papillary muscle preparation was electrically driven by a stimulator (MEC, ME-6021) and an isolation unit (MEC, ME-6217) with rectangular pulses of 1–3 V (about 20% above the threshold voltage) at 5 msec duration at a fixed rate of 2 Hz (120 beats/min) through bipolar silver-silver stimulating electrodes sutured onto the endocardium of the ventricular septum close

to the base of the papillary muscle.

Tension developed (DT) by the papillary muscle was measured with a force displacement transducer (Dia Medical, DMT-20). The muscle was loaded with a 2 g weight. The sinoatrial rate (SAR) of the sinoatrial node preparation was measured with a cardiotachograph (San-ei Instruments, 1321) triggered by bipolar atrial electrograms obtained from the right atrium close to the sinoatrial node.

Chronically instrumented donor dogs of either sex, weighing 15–23 kg, were obtained as follows: General anesthesia (thiopental, 15 mg/kg i.v. for inducton and halothane, 1% for maintenance) was given for placement of fluid-filled Tygon catheters in the carotid artery (for supplying arterial blood to the preparations) and the jugular vein (for returning venous blood), and of a polyethylene catheter in the femoral artery (for monitoring blood pressure). The catheters were passed subcutaneously to the back of the animal, brought through the skin at the neck above the scapulae, and placed in the pocket of the dog jacket. All fluid-filled catheters were irrigated each day with heparinized saline solution. Studies were conducted on the dogs one week after surgical operation. All dogs had been previously trained to stand in a sling in an unsedated conscious state during the experiments. The blood pressure of the donor dog (BP and mean arterial pressure, MAP) was measured at the cannulated femoral artery with an electromanometer (San-ei Instruments, 1257). The heart rate of the donor dog (DHR) was measured with cardiotachograph (San-ei Instruments, 1321) triggered by the R wave of lead II of the ECG. The PQ interval was measured directly from the ECG recorded with a paper speed of 100 mm/sec every 5 min.

All experiments began with cross-circulation by chronically instrumented conscious donor dogs for an initial 60 min stabilization period. After stabilization, in 10 dogs, diltiazem was intravenously and continuously infused into the conscious donor dog at the cannulated jugular vein at a rate of 20 μg/kg/min with an infusion pump (Terumo) for a period of 60 min. The volume of infusion was fixed at 50 ml/hr. In remaining 17 dogs, after stabilization thiamylal sodium (10 mg/kg) was injected intravenously for induction of anesthesia and intubation of a tracheal catheter. After lying the dog on an animal bed at left-side position and after all parameters reached their steady levels, i.e., approximately 15–20 min after injection of thiamylal, inhalation anesthesia started. The animal was ventilated with a dog respirator (Harvard Apparatus, model 607). In 7 dogs, inspired concentration of halothane was first fixed at 1.0% with a vapolizer, FLUO-V (Igarashi Ika Kogyo), and then finely adjusted to the concentration of 0.8 $\pm$ 0.1% (n=7) at which respiratory changes in DHR and BP were eliminated. Similarly, in 10 dogs enflurane anesthesia started at 2.0% of inspired concentration and then adjusted at 1.7 $\pm$ 0.2% (n=10) with a vaporizer, ENFLUWICK (Muraco, Type 200). After 20 and 30 min of exposure to 0.8% of halothane and 1.7% of enflurane, respectively, the effects of halothane and enflurane per se were determined. Then, the same dose of diltiazem was infused by the same procedure as described in the case of conscious donor dog. Plasma concentration of diltiazem was measured by a gas chromatographic method[20]. Blood samples were obtained from the arterial blood entering the anterior septal artery of the papillary muscle preparation during continuous and intravenous infusion of diltiazem in each 4 of 10 conscious or enflurane-anesthetized donor dogs, respectively.

Diltiazem hydrochloride (CRD-401 in an ampoule for injection, Tanabe, Osaka) was dissolved in 0.9% of saline to make a concentration of 10 mg/ml and desired concentrations in the 50 ml syringe were obtained by dilution of the original solution. Inhalation anesthetics used were halothane (Fluothane®, Takeda Pharmac Co. Osaka, Japan) and enflurane (Ethrane®, Dainippon Pharmac Co. Osaka, Japan).

Statistical analysis for all parameters was done for defining the effects of halothane

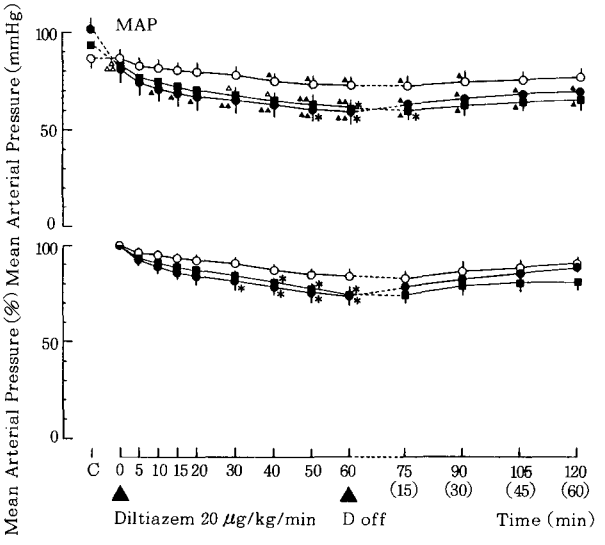

Fig. 2. Time courses of the effects on mean arterial pressure (MAP) of the donor dog of diltiazem infused i.v. into conscious (open circles), and enflurane- (solid circles) and halothane-anesthetized (solid squares) donor dog. In the upper panel are shown absolute values of MAP and in the lower panel the normalized values as percents of the value just before dilitiazem infusion 30 min after the start of inhalation of enflurane. All values are mean ± SE. In the absolute values, basal values at time C were not significantly different each other when the chronically instrumented donor dog was awake before any anesthesia, as shown in table 1. Effect of 1.7% of enflurane or those of 0.8% of halothane anesthesia per se was determined at 0 time, i.e., 30 or 20 min after the start of inhalation of enflurane or halothane and just before diltiazem infusion. The value at 0 time was compared with that at time C by paired *t*-test (significant differences were shown by open triangles: △; $P<0.05$, △△; $P<0.01$). At 0 time, diltiazem infusion started at a rate of 20 μg/kg/min and at 60 min the infusion was ceased, then followed up for one hour. Recovery time after ceasing was expressed in parenthesis. Solid triangles mean that the absolute values were significantly different from the value at time 0 (▲; $P<0.05$, ▲▲; $P<0.01$). In both absolute and normalized values, asterisks mean that the values were significantly different from the corresponding values in conscious dog at each measuring time (*; $P<0.05$, **; $P<0.01$).

Table 1. Basal values of conscious donor dogs and isolated heart preparations in conscious state before anesthesia

	Conscious Experiment (n=10)	Halothane Anesthesia (n=7)	Enflurane Anesthesia (n=10)
Mean Arterial Pressure (MAP, mmHg)	87±5	92±4	101±7
Donor Heart Rate (DHR, beats/min)	137±6	136±7	130±7
PQ Interval (PQ, msec)	92±4	95±6	93±3
Developed Tension (DT, g)	3.2±0.3	3.1±0.5	3.6±0.4
Sinoatrial Rate (SAR, beats/min)	107±6	98±5	97±4
Blood Flow (BF, ml/min)	7.4±0.4	6.8±0.8	6.9±0.7

or enflurane per se (dependent *t*-test), for determining the effects of diltiazem in conscious state or during halothane or enflurane anesthesia (dependent *t*-test), for comparing the effects of diltiazem between in conscious state and under halothane or enflurane anesthesia (independent *t*-test), and for comparing the plasma concentrations of diltiazem between in conscious state and during enflurane anesthesia (independent *t*-test). The values during infusion of diltiazem were normalized as percents of the values at the start of diltiazem infusion in conscious state and under halothane or enflurane anesthesia, and then compared by using independent *t*-tests. P-values of less than 0.05 were considered significant.

Results

1. *Effects of diltiazem infused inravenously into the chronically instrumented conscious donor dog*

In table 1 are shown the basal values

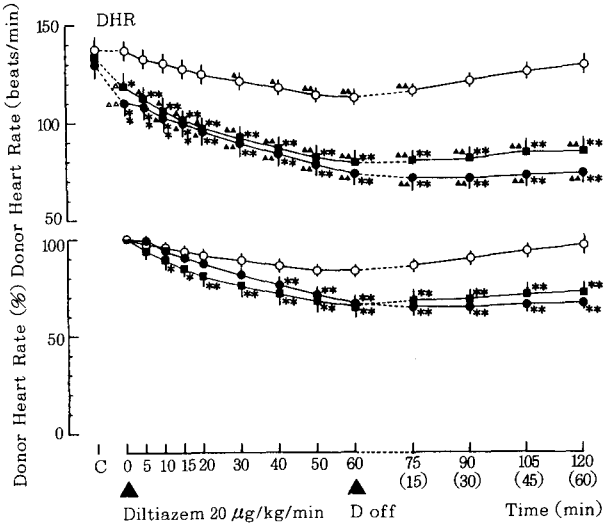

Fig. 3. Time courses of the effects on heart rate of the donor dog (DHR) of diltiazem infused i.v. into conscious (open circles), and enflurane- (solid circles) and halothane-anesthetized (solid squares) donor dog. All symbols and expression are the same as those in figure 2.

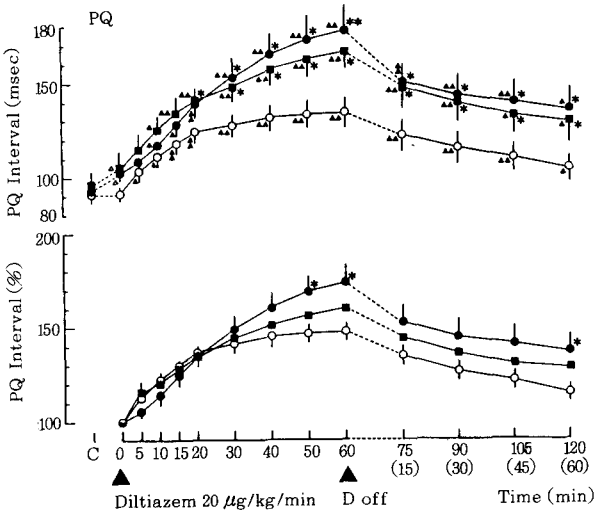

Fig. 4. Time courses of the effects on PQ interval (PQ) of the donor dog heart of diltiazem infused i.v. into conscious (open circles), and enflurane- (solid circles) and halothane-anesthetized (solid squares) donor dog. All symbols and expression are the same as those in figure 2.

of mean arterial pressure (MAP), heart rate (DHR) and PQ interval (PQ) of the conscious donor dog, and developed tension (DT) of the papillary muscle, sinoatrial rate (SAR) of the sinoatrial node preparation and blood flow (BF) through the cannulated nutrient arteries of the isolated preparations perfused wtih arterial blood of the chronically instrumented conscious donor dog. There is no significant difference between the corresponding basal values in conscious states before anesthetizing with halothane or enflurane.

When diltiazem was continuously infused intravenously into the conscious donor dog at a fixed rate of 20 μg/kg/min for a period of 60 min, MAP and DHR were progressively decreased and PQ was prolonged in the conscious donor dog (figs. 2–4), whereas DT and SAR were gradually decreased and BF was increased in the isolated heart preparations (figs. 5–7). The values were averaged from 10 experiments before and 5, 10, 15, 20, 30, 40, 50 and 60 min after the start of the infusion. The normalized values of all parameters as percents of the value just before diltiazem infusion were shown in the lower panel of each figure (figs. 2–7). After 1 hour diltiazem infusion, MAP was 84 ± 3% of the value before diltiazem infusion, and DHR, 84 ± 2%, PQ, 148 ± 5% DT, 68 ± 3%, SAR, 74 ± 3% and BF, 155

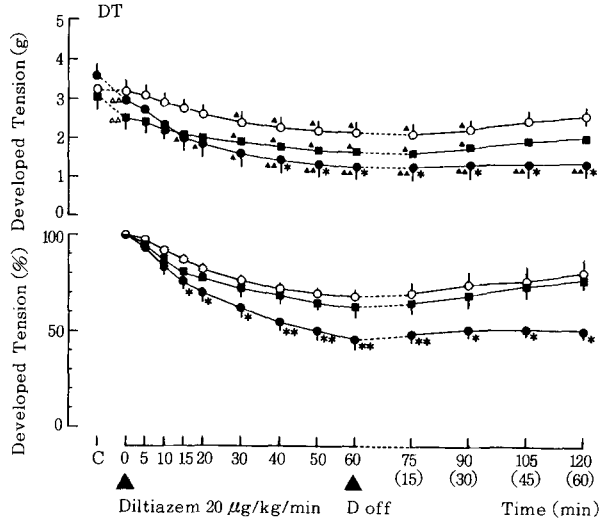

Fig. 5. Time courses of the effects on developed tension (DT) of the papillary muscle of diltiazem infused i.v. into conscious (open circles), and enflurane- (solid circles) and halothane-anesthetized (solid aquares) donor dog. All symbols and expression are the same as those in figure 2.

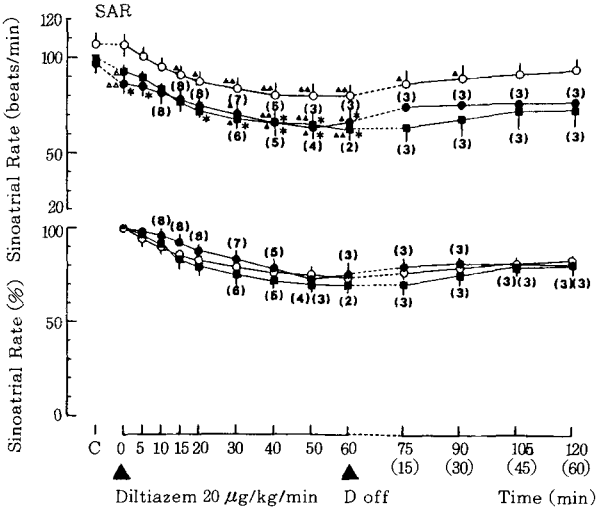

Fig. 6. Time courses of the effects on sinoatrial rate (SAR) of the sinoatrial node preparation of diltiazem infused i.v. into conscious (open circles), and enflurane- (solid circles) and halothane-anesthetized (solid squares) donor dog. All symbols and expression are the same as those in figure 2.

± 5%, respectively (figs. 2–7, n=10 for all parameters). The decrease in DHR of the conscious donor dog was significantly smaller than that in SAR of the sinoatrial node preparation ($P<0.05$).

2. *Effects of diltiazem infused intravenously into the halothane-anesthetized donor dog*

The chronically instrumented conscious donor dog was anesthetized with 10 mg/kg of thiamylal sodium, i.v., for induction. This dose of thiamylal itself slightly decreased MAP, DT and SAR, and increased DHR, PQ and BF, but these effects were transient and all values returned to the basal values within 15 min. During this period, although the animals sometimes reawake, tracheal intubation was quickly done and ventilation with 1.0% of inspired concentration of halothane started. The inspired concentration was finally adjusted to the level at which respiratory changes of DHR and BP were eliminated, 0.8 ± 0.1% (n=7). Twenty min after the start of halothane anesthesia, MAP became 89 ± 8% of the basal value in conscious state before anesthesia with thiamylal and halothane, and DHR, 84 ± 3%, PQ, 111 ± 5%, DT, 79 ± 3%, SAR, 89 ± 5% and BF, 129 ± 7%, respectively (figs. 2–7, n=7 for all parameters). All values were significantly

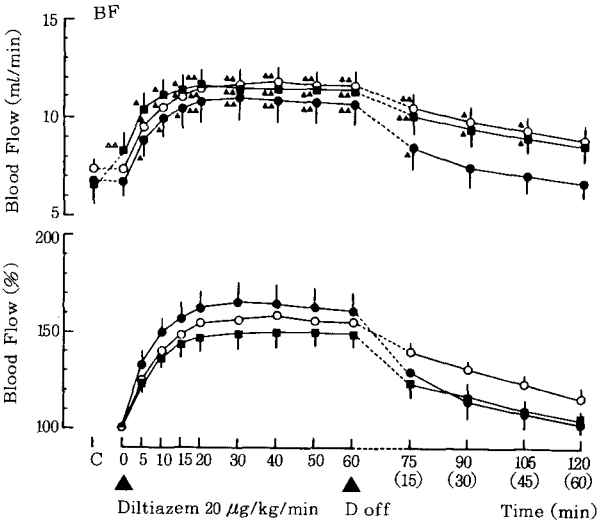

Fig. 7. Time courses of the effects on blood flow (BF) through the isolated heart preparations of diltiazem infused i.v. into conscious (open circles), and enflurane- (solid circles) and halothane-anesthetized (solid squares) donor dog. All symbols and expression are the same as those in figure 2.

different from the basal values in conscious state ($P<0.01$ for PQ, DT and BF; $P<0.05$ for MAP, DHR and SAR). Then, the same dose of diltiazem was infused intravenously into the halothane anesthetized donor dog for the same period of 60 min with the same procedure as done into the conscious donor dog. The MAP, DHR, DT and SAR progressively decreased and the PQ and BF increased from each value decreased or increased by halothane itself (figs. 2–7). For comparison of the effects of diltiazem during halothane anesthesia, all values under enflurane anesthezia were normalized as percents of the values 20 min after halothane anesthesia, i.e., just before diltiazem infusion (each lower panel of figs. 2–7). Sixty min after diltiazem infusion during halothane anesthesia, the normalized MAP was 74 ± 4% of the value just before diltiazem infusion during halothane anesthesia (n=7), and DHR, 66 ± 4% (n=6), PQ, 160 ± 8% (n=6), DT, 62 ± 7% (n=7), SAR, 69 ± 1% (n=3), and BF, 143 ± 6% (n=7), respectively (figs. 2–7). Since the second degree atrioventricular (AV) conduction block was induced in 1 of 7 halothane-anesthetized donor dogs 56 min after infusion, DHR and PQ under halothane anesthesia at 60 min were averaged from 6 animals. The decrease in DHR by diltiazem during halothane anesthesia was significantly greater than that in conscious state when compared either by absolute values ($P<0.01$) or by normalized values ($P<0.01$). The MAP and PQ were also significantly depressed during halothane anesthesia than did in conscious state ($P<0.05$ for both MAP and PQ in each absolute value, and $P<0.01$ for MAP in the normalized value). On the other hand, the changes in DT, SAR and BF by diltiazem under halothane anesthesia were not significantly different from those in conscious state when they were compared by normalized values, although the sinus arrest was induced in 4 of 7 sinoatrial node preparations and both DT and SAR were significantly small in their absolute values during halothane anesthesia because their basal values for normalizing under halothane anesthesia had been depressed by halothane itself. The sinus arrest was induced at the averaged time of 41 ± 5 min (n=4) after the beginning of diltiazem infusion with the SAR of 64 ± 4 beats/min (n=4), the averaged SAR was obtained from 6 preparations at 30 min, 5 at 40 min, 3 ones at 50 and 60 min after diltiazem infusion under halothane anesthesia.

All parameters tend to return to the values before diltiazem infusion, but the rates of recovery of DHR and PQ during halothane anesthesia were significantly slower than those in conscious state even in their normalized values (figs. 4 and 5).

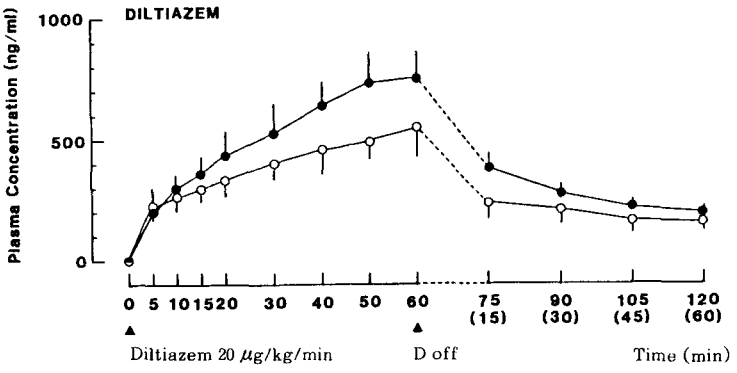

Fig. 8. Time courses of arterial plasma concentrations of diltiazem continuously infused i.v. at a rate of 20 μg/kg/min into conscious (open circles) and enflurane-anesthetized (solid circles) donor dogs. The values during enflurane anesthesia was not significantly greater than in conscious state even at the time 50 or 60 min after diltiazem infusion.

3. *Effects of diltiazem infused intravenously into the enflurane-anesthetized donor dog*

By the same procedures as halothane anesthesia, the chronically instrumented conscious donor dog was anesthetized with 1.7 ± 0.2% of enflurane (n=10). Thirty min after the start of enflurane inhalation, MAP became 81 ± 3%, DHR, 84 ± 2%, PQ, 110 ± 2%, DT, 81 ± 2%, SAR, 88 ± 2%, and BF, 100 ± 7% of each conscious basal value (figs. 2–7, n=10 for all parameters). All values, except BF, were significantly different from the basal values in conscious state ($P<0.01$ by *t*-dependent test for absolute values of all parameters). Meanwhile, the changes in all parameters, except BF, by 1.7% of enflurane per se were very similar to those by 0.8% of halothane itself, though the decrease in MAP was slightly, but not significantly, greater during enflurane anesthesia than under halothane anesthesia. Then, the same dose of diltiazem was infused intravenously into the enflurane anesthetized donor dog for the same period of 60 min with the same procedure as done into the conscious or halothane- anesthetized donor dog. The MAP, DHR, DT and SAR progressively decreased and the PQ and BF increased from each value decreased or increased by enflurane itself (figs. 2–7). For comparison of the effects of diltiazem during enflurane anesthesia, all values under enflurane anesthesia were normalized as percents of the values 30 min after enflurane anesthesia, i.e., just before diltiazem infusion (each lower panel of figs. 2–7). Sixty min after the start of diltiazem infusion during enflurane anesthesia, the normalized MAP was 73 ± 4% (n=10) of the value just before diltiazem infusion during enflurane anesthesia, and DHR, 67 ± 3% (n=8), PQ, 174 ± 10% (n=8), DT, 45 ± 5% (n=10), SAR, 74 ± 6% (n=3), and BF, 161 ± 10% (n=7), respectively (figs. 2–7). Since the second degree AV conduction block was induced in 2 of 10 enflurane anesthetized donor dogs 52 and 56 min after the start of diltiazem infusion, the values of DHR and PQ at 60 min were calculated from 8 donor dogs. In the donor dogs, the decreases in MAP and DHR and the increase in PQ by diltiazem during enflurane anesthesia were greater than those in conscious state ($P<0.01$ for DHR and $P<0.05$ for MAP and PQ), but these changes were almost identical to those under halothane anesthesia (figs. 2–4). In the isolated papillary muscles, however, the decrease in DT by diltiazem under enflurane anesthesia was significantly greater than not only that in conscious state but also that under halothane anesthesia ($P<0.01$, fig. 5), despite that 1.7% of enflurane or 0.8% halothane per se similarly decreased DT. In 7 of 10 sinoatrial node preparations, sinus arrest was induced at averaged time of 29 ± 7 min (n=7) after the start of diltiazem infusion during enflurane anesthesia with the SAR of 65 ± 5 beats/min (n=7). Thus, the averaged SAR was calculated from reduced numbers of sinoatrial node preparations, resulted in 3 preparations remained at 60 min.

All parameters tend to return to the values before diltiazem infusion, but the rates

of recovery of DHR, PQ and especially DT during enflurane anesthesia were significantly slower than those in conscious state even in their normalized values (figs. 4, 5 and 6).

Arterial plasma concentration of diltiazem obtained from conscious or enflurane-anesthetized donor dogs was progressively increased, as shown in figure 8. Sixty min after the infusion, plasma concentration was 556 ± 121 ng/ml in the conscious dog and 742 ± 156 ng/ml during enflurane anesthesia, the latter of which was slightly greater than the former, but there was no significant difference ($0.1>P>0.05$, n=4).

Discussion

The model used in the present experiments is very suitable for determining the cardiovascular effects of anesthetics, either intravenous or inhalation anesthetics.[16,17] In the canine isolated, blood-perfused heart preparations the direct effects of the agents which were given even into the donor dog could be observed, while in the donor animal the systemic effects of the agents which would be modified by neurohumoral compensatory reflex mechanisms could be observed. In the present experiments, halothane or enflurane per se decreased MAP, DHR, DT and SAR and increased PQ, while BF was increased by halothane but not by enflurane per se, when the effects of halothane or enflurane were determined 20 or 30 min after the start of inhalation. These results, except DHR, are generally consistent with previous reports by Merin and co-workers[10–12]. According to their results, the heart rate could be increased by halothane or enflurane anesthesia via compensatory reflex triggered by marked hypotension[10–12]. Meanwhile, the decrease in DHR by halothane or enflurane per se obsered in the present experiments may be due to the direct effect of halothane or enflurane itself on the sinoatrial node. Although the basal DHR in conscious donor dogs was higher in the present experiments than heart rate of the conscious dog reported previously[10–12], probably due to increased sympathetic nervous tone, the percent decrease in DHR was almost the same as that in SAR of the sinoatrial node preparation. Electrophysiological studies[21–23] showed that halothane or enflurane suppressed the slow inward Ca current of cardiac cells, that underlies the mechanisms of negative chronotropic (decreases in DHR and SAR), negative dromotropic (prolongation of PQ interval), and negative inotropic effects (decrease in DT) of halothane or enflurane itself as well as Ca antagonists. Even in the studies by Merin and co-workers[10–12], PR interval was prolonged by halothane or enflurane itself. Thus, it is important to estimate how does the Ca antagonistic actions of diltiazem and enflurane interact quantitatively with each other.

The cardiac contractility determined by the DT of the isolated papillary muscle preparation was almost equally decreased in the conscious state and under haothane anesthesia, when the values were normalized. On the other hand, during enflurane anesthesia diltiazem caused more marked decrease in DT than during halothane anesthesia, despite that enflurane per se caused an almost similar decrease in DT to that by halothane itself, even when the values were normalized. In other words, the negative inotropic effects of diltiazem was potentiated by enflurane anesthesia, while the interaction between diltiazem and halothane may be additive. Predominant decrease in cardiac contractility by a Ca antagonist, verapamil, during enflurane anesthesia was reported by Kapur et al.[7], in which the LVdP/dt in the in situ dog heart was decreased accompanying with marked hypotension. In their experiments, however, halothane anesthesia did not potentiate the negative inotropic effects of verapamil[7]. On the other hand, Kapur et al.[8,9] reported that although the LVdP/dt as well as MAP was markedly decreased by diltiazem during enflurane anesthesia, the decrease was not different from that during isoflurane anesthesia, while severer hypotension was enhanced during enflurane anesthesia compared with that during isoflurane anesthesia. In the present experiments, however, it was clearly demonstrated that

the direct effect of diltiazem on the DT of the papillary muscle was enhanced by enflurane anesthesia. Since the same degree of normalized decreases in DT of the papillary muscle and in SAR of the sinoatrial node preparation was obtained in the isolated preparations both under conscious state and during halothane anesthesia, the significantly enhanced decrease in DT under enflurane anesthesia means the potentiation of the direct negative inotropic effect of diltiazem by enflurane anesthesia. Thus, the compensatory reflex which could be eliminated by anesthesia might be at least one of the reasons for the absence of difference in the effects of diltiazem on cardiac contractility between enflurane and isoflurane anesthesia in the experimnts by Kapur et al.[8,9] On the other hand, Merin and co-workers[10–13] reported that cardiodepressive effects of verapamil were enhanced not only during enflurane anesthesia but also under halothane and isoflurane anesthesia. Then, they proposed that the enhanced suppressive hemodynamic effects of verapamil by inhalation anesthetics was due to pharmacokinetic interaction, i.e., an increase in plasma concentration of verapamil during the inhalation anesthesia resulting from the reduced hepatic blood flow, i.e., reduced hepatic elimination of verapamil. The same explanation was proposed for diltiazem by Kapur et al.[9], since the plasma concentrations of diltiazem in enflurane anesthetized dogs was higher than those in isoflurane anesthetized dogs for the same diltiazem infusion rates[8,9]. In the present experiments, however, arterial plasma concentration of diltiazem tended to be greater during enflurane anesthesia than in conscious state, for example, 752 ± 101 ng/ml (n=4) versus 556 ± 121 ng/ml (n=4) at the end of diltiazem infusion, but there was no significant difference in the values at any time between the two conditions, as shown in figure 1. It might be due to large variation of plasma concentrations among the individuals, though the infusion rate was fixed. Nevertheless, it is very probable that enhanced negative inotropic effects of diltiazem during enflurane anesthesia might be due to the increase in plasma concentration, since the pharmacokinetic properties of diltiazem is similar to those of verapamil[4].

Chronotropic effects of diltiazem obtained from the changes in SAR of the isolated sinoatrial node preparation and in DHR of the donor dog, in conscious state and during halothane or enflurane anesthesia indicate that the change in SAR reflects the direct effect of diltiazem either when the donor dog was anesthetized or not. The SAR was suppressed by halothane or enflurane itself and was further decreased by diltiazem infusion during halothane or enflurane anesthesia to the critical level, which was identical among the experiments using halothane-, enflurane- and pentobarbital-anesthetized donor dogs[3], and then sinus arrest occurred. Thus, the SAR under halothane or enflurane anesthesia just before diltiazem infusion may be important for sinus arrest, and the sinus arrest could be avoided when the SAR is high enough under halothane or enflurane anesthesia before diltiazem infusion. Nevertheless, increased incidence of the sinus arrest during enflurane anesthesia may be at least in part due to a slight, but insignicant, increase inplasma concentration of diltiazem during enflurane anesthesia by pharmacokinetic interaction, as discussed above on the enhanced negative inotropic effect. On the other hand, in the donor dog the change in DHR by diltiazem was smaller in conscious state than that under halothane or enflurane anesthesia, even when the values were normalized. Nonetheless, the normalized changes in DHR by diltiazem under halothane or enflurane anesthesia were almost similar to the normalized change in SAR by diltiazem in conscious state. These results suggest that during halothane or enflurane anesthesia the direct effect of diltiazem is unmasked even in the donor dog, while in the conscious dog the direct effect of diltiazem was modified to be smaller by probably compensatory reflex mechanisms. In addition, an increase in plasma concentration of diltiazem during enflurane anesthesia may contribute to the enhanced decrease in DHR during enflurane

anesthesia.

Similarly, the compensatory reflex might modify severe prolongation of PQ interval, high incidence of the second degree AV conduction block, and further marked decrease in MAP by diltiazem infusion in the halothane- or enflurane-anesthetized donor dog compared with those in the conscious donor dog, even when the values were normalized. The direct effect of diltiazem on the AV node, which is a prominent property as a Ca antagonist[3–5], was unmasked during halothane or enflurane anesthesia, since the second degree AV conduction block was never induced in conscious state. Furthermore, plasma concentration of diltiazem was well related to prolongation of PQ interval[6,8,9,24,25]. Thus, direct PQ prolongation by halothane or enflurane itself[26,27], increases in plasma concentration of diltiazem[9], which was not statistically significant in the present results, and potentiation of the effects of diltiazem by inhalation anesthetics due to unknown mechanisms, could contribute to the second degree AV conduction block by diltiazem during halothane or enflurane anesthesia.

In conclusion, diltiazem must be carefully used with the inhalation anesthetic, since severe AV nodal conduction disturbance, sinus arrest, and depression of cardiac contractility could be induced depending on the doses. Furthermore, since halothane or enflurane per se caused marked hypotension, it must be carefully noted that the absolute value of MAP was further decreased by diltiazem under halothane or enflurane anesthesia.

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