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Direct Myocardial Effects of Halothane and Isoflurane

Comparison between Adult and Infant Rabbits

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Background: **Infants may be more sensitive than adults to myocardial depression by potent inhalation anesthetics. Most studies of cardiovascular effects of inhalation agents in infants are performed *in vivo* with multiple factors producing the observed effects. The purpose of this study was to determine if newborns are more sensitive than adults to the direct actions of halothane and isoflurane on global electrophysiologic, contractile and metabolic functions of the heart.**

Methods: **Direct myocardial effects of the agents were determined using isolated rabbit hearts perfused at constant pressure. Three doses of halothane and isoflurane were administered to 37 infant (3–8 days old) and 36 adult rabbit hearts. Heart rate and rhythm, atrioventricular conduction time, left ventricular function (systolic, diastolic, and developed pressures; maximum and minimum values of the differential wave (dP/dt); and time constant of isovolumic LV relaxation), coronary flow and O_2 consumption, and fractional O_2 extraction were measured and compared between age and anesthetic groups.**

Results: **Halothane was a more potent depressant of cardiac function than isoflurane and developmental differences were more evident with this agent. The most striking developmental differences in anesthetic effects were the significantly greater prolongation of atrioventricular conduction time and the time constant of isovolumic LV relaxation by halothane in infant compared with adult hearts. Infant hearts were also more sensitive to depression of left ventricular developed pressure and maximum value of the differential wave and to elevation of diastolic pressure by halothane. For both agents heart rate was less depressed in infants than in adults. There were no developmental differences in anesthetic effects on coronary flow and O_2 metabolism.**

Conclusions: **Developmental changes in myocardial physiology make the newborn less sensitive to direct depression of heart rate by halothane and isoflurane, but more sensitive to depression of contraction–relaxation and atrioventricular conduction by halothane. (Key words: Age factors. Anesthetics, volatile: halothane; isoflurane. Heart: atrioventricular node; diastole; oxygen consumption; rate.)**

INFANTS and small children are considered to have "increased sensitivity of the cardiovascular system to potent [inhalation] agents." [1] Most of our knowledge of the cardiovascular effects of inhalation agents in infants has been obtained *in vivo* from studies in which multiple factors (pharmacokinetic factors, autonomic reflexes, alterations in preload and afterload, and direct myocardial effects) interact to produce observed effects. These studies do not reveal which factors are responsible for "increased sensitivity" of newborns. Several *in vitro* studies have demonstrated that myocardial tension development, sarcoplasmic reticulum function, and contractile protein function are more sensitive to depression by inhalation anesthetics in newborns.[2–6] One such study, however, found the newborn to be less sensitive to depression (of contractile protein function) by halothane.[7]

The purpose of this study was to determine if newborn myocardium is "more sensitive" to the direct actions of halothane and isoflurane on global electrophysiologic, contractile and metabolic functions of the heart. Direct myocardial effects of the agents were determined using an isolated, perfused heart model that eliminates extrinsic mechanical, humoral, and auto-

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nomic influences. The whole isolated heart offers advantages over cardiac muscle fragments for examining global anesthetic effects because natural anatomy and function of the heart are maintained and the coronary circulation is used for delivery of nutritive substances. The isolated rabbit heart has been demonstrated to be suitable for study of developmental cardiac physiology including electrical, mechanical, and metabolic functions.[8-10] Isolated guinea pig hearts have been used to examine direct myocardial effects of inhalation anesthetics in mature hearts, but developmental changes in anesthetic effects have not previously been examined with the isolated heart model.[11,12]

Materials and Methods

This study was approved by the Animal Care Committee of the Medical College of Wisconsin and conforms to the *Guiding Principles in the Care and Use of Animals* as approved by the Council of the American Physiologic Society. Direct myocardial effects of halothane and isoflurane were determined by administering the agents to isolated, perfused hearts. Subjects were newborn (3–8-day-old) and adult (4-month-old) New Zealand White rabbits. The animals were anesthetized, a tracheostomy was performed and ventilation was mechanically controlled. Newborns were anesthetized by titration of intraperitoneal ketamine (86 ± 10 mg/kg [mean ± SD]) and xylazine (16 ± 2 mg/kg) and their blood was anticoagulated with 500 U intraperitoneal heparin. Adults were anesthetized by titration of intravenous thiamylyl (27 ± 8 mg/kg), xylazine (1.4 ± 0.4 mg/kg), and ketamine (11 ± 3 mg/kg) and their blood was anticoagulated with 1,000 U intravenous heparin. Lidocaine (1%) was infiltrated subcutaneously at the tracheostomy site. A thoracotomy was performed, the superior and inferior vena cavae were ligated, and the aorta was cannulated distal to the aortic valve. The heart was perfused retrogradely (Langendorff mode) with crystalloid solution initially at room temperature (24–25°C). It was then excised and suspended in and perfused with warm solution (37°C) that was maintained at constant temperature with a circulating heated H_2O system. Perfusion pressure measured at the root of the aorta was maintained constant at normal physiologic pressures for age: 80 mm Hg for adults and 45 mm Hg for newborns.[13]

The perfusate was a modified Krebs-Ringer solution containing (millimolar) Na^+ 140, K^+ 4.5, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 122, HCO_3^- 22, $H_2PO_4^-$ 1.2, glucose 11.5, mannitol 16 and ethylenediamine tetraacetic acid 0.05 plus insulin 5 U/l. O_2 and CO_2 (approximately 97% and 3%, respectively) were bubbled through the perfusate reservoir. CO_2 was adjusted to maintain *p*H = 7.40 with a total gas flow of approximately 3 l/min. Gas tensions were constantly monitored with a mass spectrometer (model 1100, Marquette Gas Analysis, St. Louis, MO) and *p*H of the perfusate was intermittently measured (ABL-3, Radiometer, Copenhagen, Denmark). The perfusate was filtered (5-μm pore, Cole Palmer, Chicago, IL) and did not recirculate.

Electrograms were recorded with pairs of bipolar electrodes (125-μm diameter Teflon-coated silver, Cooner Wire, Chatsworth, CA) placed on the right atrial appendage and on the right ventricle pulmonary conus. Signals were amplified (1,000–10,000 times) and filtered (100–1,000-Hz bandpass range). Heart rate and atrioventricular (AV) conduction time were measured by setting a voltage threshold on the electrogram signals such that levels exceeding the threshold generated rectangular voltage pulses. The time between successive pulses was measured using an eight-bit counter/timer with digital-to-analog output that provided a continuous reading of the interval with a resolution of 5 ms.

Isovolumic left ventricular pressure (LVP) was measured with a transducer (DTX, Spectramed, Oxnard, CA) connected by a short segment of 25-G polypropylene tubing to a saline-filled latex balloon (Hugo-Sachs Electronik, KG, March-Hugstetten, Germany; sizes 3–4 [30–60 μl] for infants and size 12 [1.3 ml] for adults). The balloon was inserted through the left atrium and mitral valve into the left ventricle. Minimum diastolic LVP was set between 5–10 mmHg by adjusting balloon volume. The first derivative of the pressure wave was continuously derived electronically with an analog differentiator (±30 volts/s range). Maximum and minimum LVPs (systolic and diastolic, respectively) and maximum and minimum values of the differential wave ($+dP/dt_{max}$ and $-dP/dt_{max}$, respectively) were determined with peak and trough detection software, and each wave was visually inspected for accuracy. Developed LVP was calculated as systolic LVP minus diastolic LVP. The time constant of the decrease in isovolumic LVP (*i.e.*, of isovolumic relaxation) (τ) was derived from the relation $P_t = P_0 e^{-t/\tau}$, where P_t = LVP at time t and P_0 = LVP at $-dP/dt_{max}$.[14,15] Pressure was recorded for 35–40 ms/beat beginning with t_0 at $-dP/dt_{max}$. Linear regression analysis was performed on the plot of ln P_t *versus* t, and τ was defined as the negative

reciprocal of the slope according to the equation $\ln P_t = -t/\tau + \ln P_0$. These calculations are based on the assumption of a zero pressure asymptote and correlation coefficients were required to be >0.99 in all calculations of τ.

Coronary flow (perfusate inflow) was measured with an ultrasonic flow probe (probe model 2N, meter model T106, Transonic Systems, Ithaca, NY). O_2 tension of coronary inflow was calculated as dry-gas O_2 tension (measured with mass spectrometry) minus H_2O vapor pressure at 37°C. O_2 tension of coronary effluent was measured by cannulating the main pulmonary artery and directing the entire effluent through a heated Clark electrode (SYS203FH with low flow chamber for infants and high flow chamber for adults, Instech Laboratories, Plymouth Meeting, PA). O_2 content of perfusate was calculated as the product of O_2 tension and O_2 solubility (24 $\mu l \cdot ml\ saline^{-1} \cdot 760\ mmHg^{-1}$). Myocardial O_2 consumption was calculated as the product of coronary flow and the difference in O_2 content between coronary inflow and effluent solutions. O_2 consumption was normalized to wet (blotted) heart weight. Fractional O_2 extraction was calculated as the ratio of O_2 consumption to O_2 delivery (delivery was calculated as the product of coronary flow and inflow O_2 content). Anesthetic concentration was measured in the gas delivered to the perfusate reservoir by mass spectrometry and in the coronary effluent by gas chromatography (Sigma 3B, Perkin-Elmer, Norwalk, CT). Anesthetic doses were based on coronary effluent concentrations and vaporizers were adjusted accordingly.

All directly measured electronic signals were recorded on magnetic tape (model D1, A. R. Vetter, Rebersburg, PA) and stored on floppy discs by a personal computer (model 310, Hewlett-Packard, Palo Alto, CA) equipped with a 12-bit analog-to-digital converter sampling at 10 Hz (100 Hz for calculation of τ) (AD 200, Infotek Systems, Anaheim, CA). Calculated variables were stored in floppy disc files also. Each reported value is the mean of a 10-s data segment (1-s for τ) that was visually inspected to assure absence of artifact. All directly measured signals (except O_2 tension) plus heart rate and AV conduction time were continuously monitored on a chart recorder (Dash 8, AstroMed, West Warwick, RI). Electrograms were also continually displayed on a digital oscilloscope (model 310, Nicolet Instrument, Madison, WI).

Experimental Protocol

Anesthetic gases were administered by bubbling through the perfusate reservoir with carrier gas (O_2–CO_2). Each heart was exposed to one of the anesthetic agents, which were alternated between hearts. Equianesthetic doses of the agents were based on adult rabbit MAC: 1.4% halothane (0.41 mM in saline at 37°C) and 2% isoflurane (0.44 mM).[16] Three doses of each anesthetic agent were administered in alternating order to define a dose-response pattern: low = 0.2 mM halothane and 0.22 mM isoflurane; medium = 0.41 mM halothane and 0.44 mM isoflurane; and high = 0.6 mM halothane and 0.66 mM isoflurane. Although MAC varies between infants and adults, all animals were given similar concentrations of agents to allow direct pharmacologic comparisons between age groups.

The hearts were allowed to stabilize for at least 30 min after the preparation was completed. The complete protocol included four sets of control measurements and three sets of measurements with the anesthetic agent. Hearts were equilibrated with each dose of anesthesia for at least 20 min before measurements and each dose was preceded and followed by a control, anesthetic-free state for at least 20 min. Measurements were made while the hearts beat spontaneously and then were repeated while the atrium was paced at a rate approximately 15% higher than the initial control rate. Infant hearts were paced at 284 ± 28 (mean ± SD) beats/min and adult hearts were paced at 238 ± 33 beats/min. Duration of experiments was 3.0 ± 0.4 h (mean ± SD) for infant and 3.3 ± 0.5 h for adult hearts. The preparations were stable over this period with mean values of final control measurements for all variables within 13% of initial control values.

Statistical Analysis

Initial control values were compared between infants and adults and between anesthetic agents in each age group by Student's *t* test (StatView, Abacus Concepts, Berkeley, CA). Because control values were different between age groups, values for anesthetic doses are reported as a percentage of the preceding control. To compare anesthetic effects between age groups (infants *vs.* adults) and between agents (halothane *vs.* isoflurane) repeated-measures analysis of variance was performed for each variable with anesthetic dose as the repeated measure and age and agent as between-group variables (SuperANOVA, Abacus Concepts, Berkeley, CA). Differences between age groups or agents were isolated with means contrasts. The incidence of non-

Table 1. Control Values

	Adults		Infants	
Heart rate (beats/min)	212 ± 6	(36)	251 ± 5‡	(37)
AV conduction time (paced) (ms)	93 ± 2	(36)	78 ± 1‡	(37)
Peak systolic LVP (mmHg)	134 ± 3	(36)	107 ± 3‡	(37)
Diastolic LVP* (mmHg)	8.1 ± 0.3	(36)	6.4 ± 0.3	(37)
Developed LVP† (mmHg)	128 ± 3	(36)	103 ± 3‡	(37)
(+) dP/dt_{max} (mmHg/ms)	2.34 ± 0.08	(36)	1.94 ± 0.06‡	(37)
(−) dP/dt_{max} (mmHg/ms)	−1.64 ± 0.04	(36)	−1.50 ± 0.05‡	(37)
τ (ms)	34.7 ± 0.9	(22)	31.5 ± 0.9‡	(20)
Coronary flow ($ml \cdot min^{-1} \cdot g^{-1}$)	7.9 ± 0.2	(36)	11.5 ± 0.4‡	(37)
O_2 consumption ($\mu l \cdot min^{-1} \cdot g^{-1}$)	107 ± 6	(9)	172 ± 14‡	(11)
O_2 extraction (%)	66 ± 3	(9)	65 ± 3	(11)

Values are mean ± SEM; n values are in parentheses.

τ = time constant of isovolumic relaxation.

* Diastolic LVP was set between 5 and 10 mmHg by adjusting LV balloon volume.

† Developed pressure is peak systolic pressure minus minimum diastolic pressure.

‡ $P < 0.05$ *versus* Adults.

sinus rhythm was compared between age and anesthetic groups by Fisher's exact test. Groups were considered to be significantly different if the probability of being similar was less than 5% ($P \leq 0.05$).

Results

Thirty-seven infant hearts were exposed to anesthetic agents, 19 to halothane and 18 to isoflurane. Infant rabbits were 6 ± 1 days (mean ± SD) old with body weight 121 ± 9 g and heart weight (wet-blotted) 0.60 ± 0.02 g. There were no differences in age, weight or heart weight between infants that received halothane and those that received isoflurane ($P \geq 0.35$). Thirty-six adult hearts were exposed to anesthetic agents, 20 to halothane and 16 to isoflurane. Adult rabbits were 4.2 ± 0.4 months old with body weight 3.0 ± 0.2 kg and heart weight (wet-blotted) 6.7 ± 0.7 g. There were no differences in age, weight, or heart weight between adults that received halothane and those that received isoflurane ($P > 7$).

Heart Rate

Hearts were considered to be in sinus rhythm if the primary pacemaker was atrial, the rhythm was regular, and AV conduction time was not less than two standard deviations from the mean during the initial control period (for adults, mean and mean minus 2 SD = 70 and 46 ms, respectively; for infants, mean and mean minus 2 SD = 63 and 49 ms, respectively). Five adult and one infant heart had nonsinus rhythm in the initial control period and were not included in heart rate analysis. Control heart rate was significantly higher for infants than for adults (table 1). Adult hearts exposed to halothane had a significantly higher rate of occurrence of nonsinus atrial and nodal rhythms (9 of 17) than other groups (1 of 14 for adults with isoflurane and 0 of 36 for infants with either anesthetic) ($P \leq 0.01$). When nonsinus rhythms occurred heart rate changes were relatively small (≤5%) and not dose-related (fig. 1). When sinus rhythm was maintained, both halothane and isoflurane produced a dose-related decrease in heart rate that was greater in adults than infants ($P \leq 0.03$) (fig. 1). Halothane effects were not significantly different from isoflurane effects in either age group ($P \geq 0.25$). Results are similar when analyzed using atrial beat-to-beat interval.

Atrioventricular Conduction Time

AV conduction time is heart rate dependent (increasing and decreasing in the same direction as heart rate), therefore anesthetic effects were determined during atrial pacing. Control AV conduction time was significantly greater for adults than for infants (table 1). Both agents produced a dose-related increase in AV conduction time in infants and adults ($P = 0.0001$) (fig. 2). Halothane produced a greater increase than isoflurane in both age groups ($P < 0.001$). Both agents produced a greater increase in infants than in adults ($P \leq 0.01$), but the difference between age groups was much greater with halothane than with isoflurane (AV con-

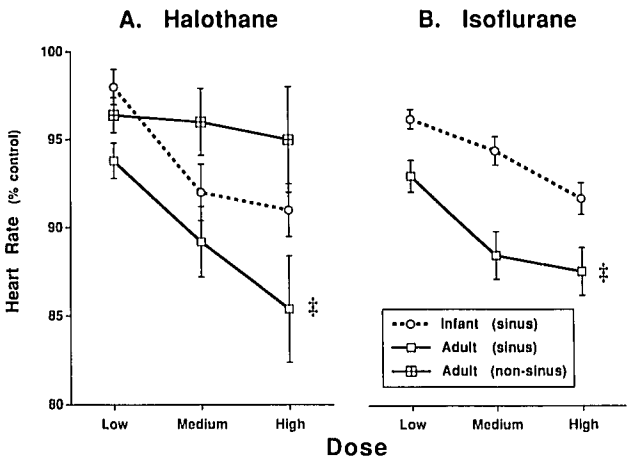

Fig. 1. Effects of (*A*) halothane and (*B*) isoflurane on spontaneous heart rate of infant and adult hearts. Only adult hearts exposed to halothane had a significant occurrence of nonsinus atrial and nodal rhythms. ‡When sinus rhythm was maintained, both halothane and isoflurane decreased heart rate significantly more in adults than infants. Halothane effects were not significantly different from isoflurane effects in either age group. (Values are mean ± SEM.)

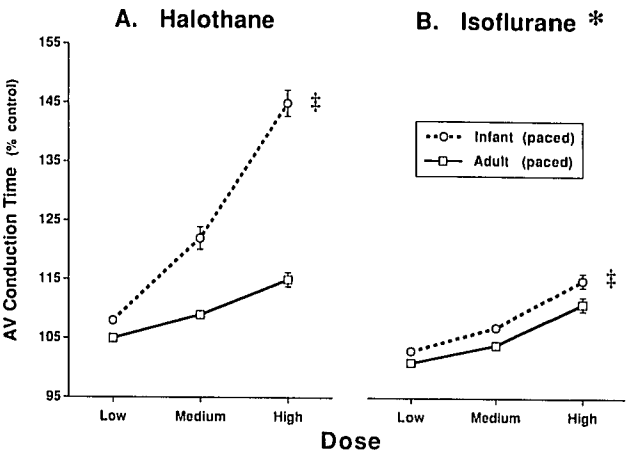

Fig. 2. Effects of (*A*) halothane and (*B*) isoflurane on atrioventricular conduction time during atrial pacing of infant and adult hearts. *Halothane produced a significantly greater increase than isoflurane in both age groups. ‡Both agents produced a significantly greater increase in infants than in adults. (Values are mean ± SEM.)

duction time increased 45% for infants *vs.* 15% for adults with high dose halothane, whereas the increases were 15% and 11%, respectively, with high dose isoflurane).

Left Ventricular Function

Control peak systolic LVP was significantly less for infants than for adults (table 1). Both agents produced a dose-related decrease in systolic LVP in both infants and adults ($P = 0.0001$) (fig. 3). Halothane produced a greater decrease than isoflurane in both age groups ($P = 0.0001$). Anesthetic effects were not different between infants and adults for either agent ($P > 0.60$). Results of data obtained during atrial pacing were similar to these results for spontaneously beating hearts.

Control minimum diastolic LVP was set between 5 and 10 mm Hg by adjusting left ventricular balloon volume (table 1). Both agents produced a dose-related increase in minimum diastolic LVP in both infants and adults ($P = 0.0001$) (fig. 4). Halothane produced a greater increase than isoflurane in infants ($P = 0.0001$), but the agents were not different in adults ($P = 0.19$). Halothane increased minimum diastolic pressure significantly more in infants than in adults ($P = 0.03$), but with isoflurane there was no difference between age groups ($P = 0.20$). Results for data obtained during atrial pacing were similar to these results for spontaneously beating hearts except that 2 of 19 adult hearts increased minimum diastolic pressure to very high levels (42–63 mm Hg) with the high halothane dose.

Developed LVP is systolic minus diastolic LVP. Control developed LVP was significantly less for infants than for adults (table 1). Both agents produced a dose-related decrease in developed LVP in both infants and

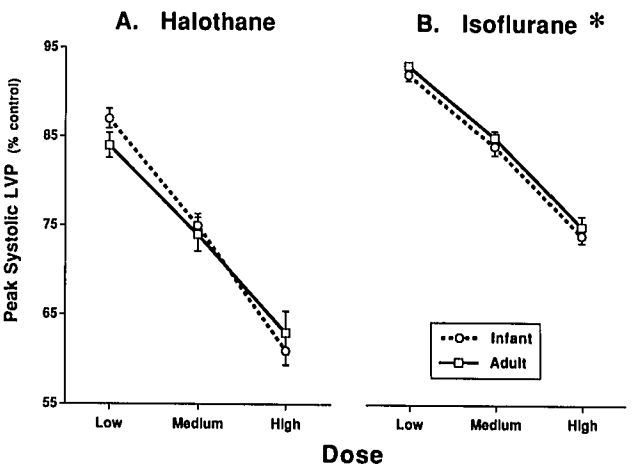

Fig. 3. Effects of (*A*) halothane and (*B*) isoflurane on peak systolic left ventricular pressure in infant and adult hearts. *Halothane produced a significantly greater decrease than did isoflurane in both age groups. Anesthetic effects were not different between infants and adults for either agent. (Values are mean ± SEM.)

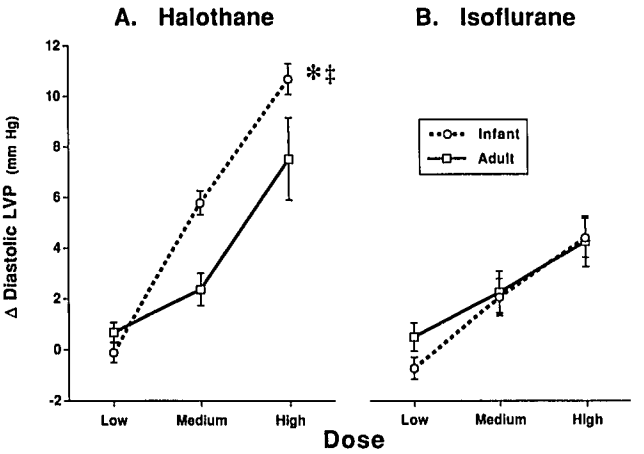

Fig. 4. Effects of (*A*) halothane and (*B*) isoflurane on diastolic left ventricular pressure in infant and adult hearts. *Halothane produced a significantly greater increase than did isoflurane in infants, but the effects of the agents were not different in adults. ‡Halothane produced a significantly greater increase in infants than in adults, but with isoflurane there was no difference between the age groups. (Values are mean ± SEM.)

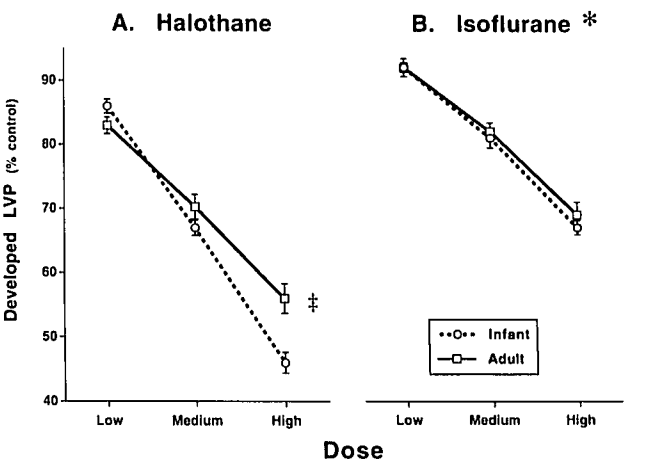

Fig. 5. Effects of (*A*) halothane and (*B*) isoflurane on developed left ventricular pressure in infant and adult hearts. *Halothane produced a significantly greater decrease than isoflurane in both age groups. ‡Halothane produced a significantly greater decrease in infants than in adults, but with isoflurane there was no difference between the age groups. (Values are mean ± SEM.)

adults (P = 0.0001) (fig. 5). Halothane produced a greater decrease than isoflurane in both age groups (P = 0.0001). Halothane produced a greater decrease in infant hearts than in adult hearts (P = 0.05). With isoflurane there was no difference between age groups (P = 0.90). Results of data obtained during atrial pacing were similar to these results for spontaneously beating hearts.

Control $+dP/dt_{max}$ was significantly less for infants than for adults (table 1). Both agents produced a dose-related decrease in $+dP/dt_{max}$ in infants and adults (P = 0.0001) (fig. 6). Halothane produced a greater decrease than isoflurane in both age groups (P = 0.0001). Halothane produced a greater decrease in infant hearts than in adult hearts (P = 0.03). With isoflurane there was no difference between age groups (P = 0.90). Results of data obtained during atrial pacing were similar to these results for spontaneously beating hearts.

Control $-dP/dt_{max}$ was significantly less for infants than for adults (table 1). Both agents produced a dose-related decrease in $-dP/dt_{max}$ in infants and adults (P = 0.0001) (fig. 7). Halothane produced a greater decrease than isoflurane in both age groups (P = 0.0001). Anesthetic effects were not different between infants and adults for either agent ($P \geq 0.08$ spontaneous rhythm, $P \geq 0.28$ paced). Results for data obtained during atrial pacing were similar to these results for spontaneously beating hearts.

Control τ was significantly less for infants than for adults (table 1). Both agents produced a dose-related increase in τ in both infants and adults (P = 0.0001) (fig. 8). Halothane produced a greater increase than isoflurane in infants (P = 0.0001), but the agents were not different in adults (P = 0.11). Halothane increased

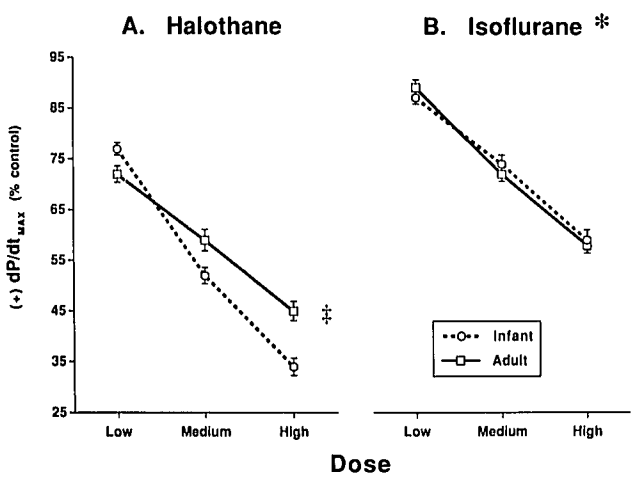

Fig. 6. Effects of (*A*) halothane and (*B*) isoflurane on the maximum value of the differential wave for left ventricular pressure ($+dP/dt_{max}$) in infant and adult hearts. *Halothane produced a significantly greater decrease than isoflurane in both age groups. ‡Halothane produced a significantly greater decrease in infants than in adults. With isoflurane there was no difference between age groups. (Values are mean ± SEM.)

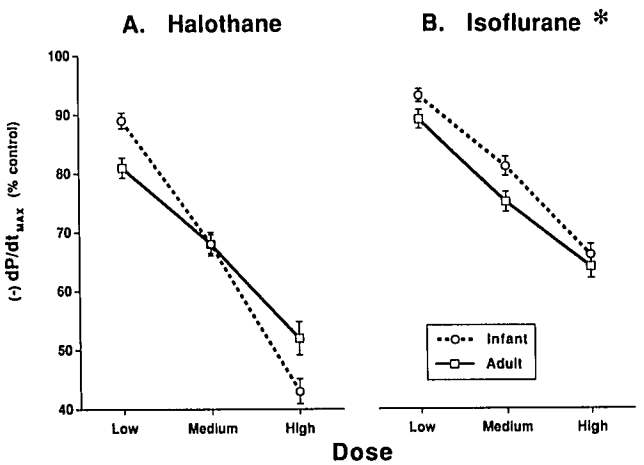

Fig. 7. Effects of (*A*) halothane and (*B*) isoflurane on the minimum value of the differential wave for left ventricular pressure ($-dP/dt_{max}$) in infant and adult hearts. *Halothane produced a significantly greater decrease than isoflurane in both age groups. For neither agent were anesthetic effects different between infants and adults. (Values are mean ± SEM.)

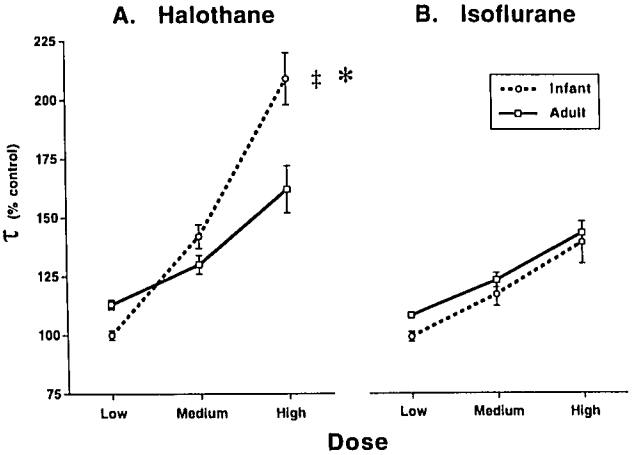

Fig. 8. Effects of (*A*) halothane and (*B*) isoflurane on the time constant of isovolumic relaxation (τ) in infant and adult hearts. *Halothane produced a significantly greater increase than isoflurane in infants, but the agents were not different in adults. ‡Halothane produced a significantly greater increase in infants than in adults, but with isoflurane there was no difference between the age groups. (Values are mean ± SEM.)

τ significantly more in infants than in adults ($P = 0.02$), but with isoflurane there was no difference between age groups ($P = 0.34$). Results for data obtained during atrial pacing were similar to these results for spontaneously beating hearts except that halothane produced a greater increase than isoflurane in adults ($P = 0.04$) and in infants.

Coronary Flow

Control coronary flow was significantly greater for infants than for adults (table 1). Both agents produced a dose-related increase in coronary flow in both infants and adults ($P = 0.0001$) (fig. 9). Anesthetic effects were not different between agents for either infants or adults ($P > 0.30$). Anesthetic effects were not different between infants and adults for either agent ($P > 0.45$). Results for data obtained during atrial pacing were similar to these results for spontaneously beating hearts.

Oxygen Consumption and Extraction Ratio

O_2 measurements were not available for halothane experiments because the agent interacts with the measurement device (Clark electrode) to produce erroneous O_2 tension readings.[17] Control O_2 consumption was significantly greater for infants than for adults (table 1). Control values for paced hearts were virtually identical to those for spontaneously beating hearts. Isoflurane produced a dose-related decrease in O_2 consumption in both infants and adults ($P = 0.0001$) (fig. 10). For spontaneously beating hearts this effect was greater in adults than in infants ($P = 0.0001$). When heart rate was held constant by pacing, the effect of isoflurane was not different between adults and infants ($P = 0.27$). Values for inflow O_2 tensions were: adults, 649 ± 1 mm Hg (mean ± SEM); infants, 649 ± 1 mm Hg. Values

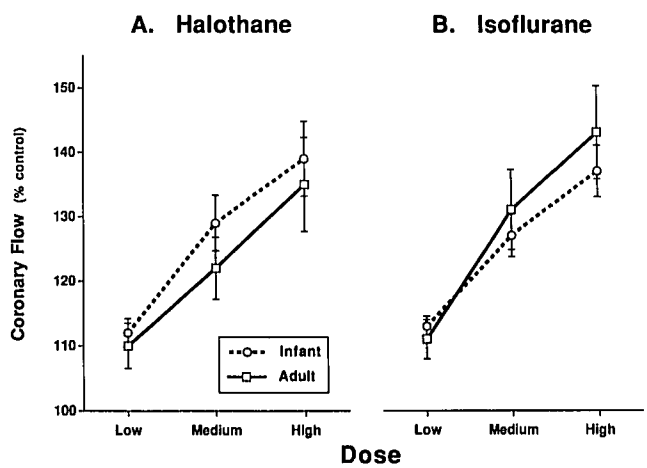

Fig. 9. Effects of (*A*) halothane and (*B*) isoflurane on coronary flow in infant and adult rabbit hearts with constant perfusion pressure. Anesthetic effects were not different between agents for either age group. Anesthetic effects were not different between infants and adults for either agent. (Values are mean ± SEM.)

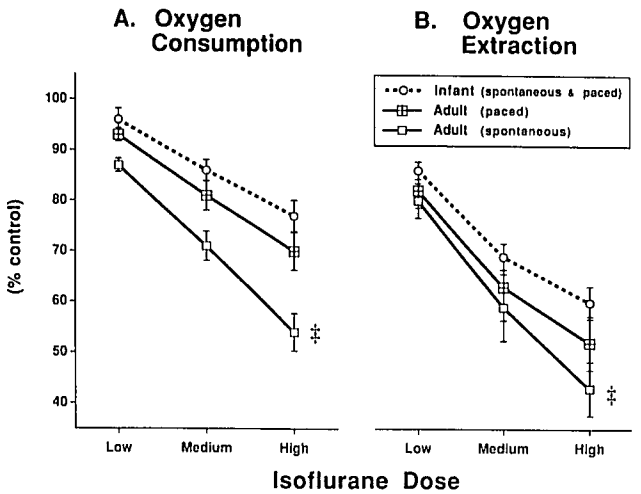

Fig. 10. Effects of isoflurane on (*A*) O_2 consumption and (*B*) O_2 extraction ratio in infant and adult rabbits. ‡For spontaneously beating hearts, isoflurane decreased O_2 consumption and extraction significantly more in adults than in infants. With atrial pacing, the effect of isoflurane was not different between adults and infants. (Infant spontaneous and paced data are combined for graphic representation only because they are virtually identical.) (Values are mean ± SEM.)

for outflow O_2 tensions during pacing were: adults, (control, low, medium, high) 240 ± 19,326 ± 26,394 ± 34,452 ± 33 mm Hg (mean ± SEM); infants, 217 ± 17,291 ± 18,351 ± 17,389 ± 15 mm Hg.

O_2 extraction is the ratio of consumed to delivered O_2. Control O_2 extraction ratio was not different between infants and adults (table 1). Control values for paced hearts were virtually identical to those for spontaneously beating hearts. Isoflurane produced a dose-related decrease in O_2 extraction in both infants and adults ($P = 0.0001$) (fig. 8). For spontaneously beating hearts this effect was greater in adults than in infants ($P = 0.0007$). When heart rate was held constant by pacing, the effect of isoflurane was not different between adults and infants ($P = 0.24$)

Discussion

This study documents developmental changes in the direct myocardial effects of halothane and isoflurane. To determine if developmental changes in myocardial physiology affect the pharmacologic actions of anesthetic agents, it is essential that anesthetic concentrations between age groups are constant. Otherwise, measured differences could be due to physiologic differences or to differences in drug concentrations. MAC is a measure of anesthetic effects in the nervous system and has no relation to direct myocardial effects. Therefore, although MAC multiples are higher in younger animals we used identical concentrations of each agent for both age groups. (If one extrapolates these results to MAC values then depression of myocardial function by the anesthetics will be accentuated in infant hearts.) Developmental differences in anesthetic action were most evident with halothane, which was a more potent depressant of cardiac function than isoflurane. Physiologic changes may explain the differences in sensitivity to volatile anesthetics between infant and adult myocardium.

It is well documented in many species that the intrinsic rate of sinus node automaticity (heart rate) is higher in infants than in adults, but the mechanism for this difference has not been delineated. Sinus cycle length is determined by the diastolic pacemaker potential (spontaneous depolarization between maximum diastolic potential [V_m] and threshold potential where the action potential is initiated [V_{th}]) and the duration of the action potential. There is controversy regarding the ionic events underlying the pacemaker process but several ion channels appear to be involved including the delayed rectifier K^+ current (I_K), the hyperpolarization-activated current (I_f) and both the T and L forms of the slow inward Ca^{2+} current (I_{Ca}).[18–20] Two studies that examined development of sinus node automaticity in rabbits documented an increase in sinus cycle length and action potential duration with age.[21,22] V_m was significantly less negative (closer to threshold) in infants in one study but not in the other. Decreased activity of I_K, which has been described in immature cardiac tissues, may account for a less negative V_m of infant sinus node.[23,24] Developmental changes probably also occur in other pacemaker currents.

In this study infant hearts were relatively resistant (compared with adults) to depression of sinus pacemaker rate by halothane and isoflurane. These agents decrease pacemaker rate by decreasing the rate of diastolic depolarization and increasing action potential duration in adult guinea pig hearts.[25] In adult rabbit hearts 1% halothane decreased the rate of diastolic depolarization and 2% halothane further decreased this rate but also moved V_m closer to V_{th} and these opposing effects resulted in little change of pacemaker rate.[26] In myocytes and Purkinje fibers halothane and isoflurane decrease I_{Ca} and halothane also decreases I_K.[27,28] Depression of these ionic currents may account for the effects of volatile agents on sinus node automaticity,

but it is likely that other ion channels are also involved. Anesthetic depression of heart rate demonstrated in this study more closely resembles that seen when I_f is blocked—maximum slowing of the pacemaker rate by about 15%.[20] Modulation of I_f is important in alteration of pacemaker rate by adrenergic and cholinergic stimuli and by the drug zatebradine, and it may also be important in alteration of pacemaker rate by anesthetic agents.[19] I_f has a greater role in depolarization at more negative V_m,[18] which may explain why the anesthetics have a greater effect in adults than in infants. Future studies should examine the effect of anesthetic agents on I_f.

The shift of pacemaker site from sinus node to AV node that occurred exclusively in adult hearts and significantly more frequently with halothane than isoflurane may be due to depression of sinus node automaticity with escape by a lower pacemaker or due to enhanced automaticity of the lower pacemaker. The relatively fast rate of the lower pacemaker suggests that its automaticity had been enhanced. The occurrence of accelerated junctional rhythms has been reported with halothane and has been attributed to autonomic imbalance.[29] Although halothane and isoflurane do not enhance automaticity in canine subsidiary atrial pacemakers, our results suggest that halothane has a direct effect to enhance AV nodal automaticity in mature animals.[30,31]

Control AV conduction time was significantly longer in adults than in infants. It is well known that just as heart rate decreases with age, AV conduction time increases with age. AV conduction consists of intraatrial, AV nodal, and His bundle-to-ventricle conduction. Studies have demonstrated no age-related difference in conduction velocity or AV nodal conduction time, however, intraatrial and His-ventricle conduction times are significantly longer in young dogs than in adults.[10,32,33] Both anesthetic agents significantly prolonged AV conduction time in this study and the increase was greater with halothane than isoflurane. This is consistent with previous *in vivo* adult animal studies. Atlee *et al.*[34] demonstrated in adult dogs that prolongation of AV conduction time by inhalation anesthetics was not dose-related and they concluded that the mechanism of this effect was indirect through modulation of autonomic tone. Our results indicate that halothane and isoflurane act directly to prolong AV conduction time and this effect is greater in infants than in adults. The effect of volatile anesthetics on AV conduction in infants has not previously been evaluated. With isoflurane the difference between age groups is very small whereas, with halothane the difference is marked and likely represents significant differences in direct actions on AV nodal conduction. Although infant and adult AV nodes are morphologically distinct, their action potential characteristics are similar. AV nodal conduction delay results from several factors, including dependence of depolarization on I_{Ca} and a high coupling resistance between nodal cells. The differential effect of halothane between age groups may be due to an age-related sensitivity to Ca^{2+} channel blockade. Comparison of the effects of other Ca^{2+} channel blockers, such as verapamil, on infant and adult AV conduction time may help to elucidate this.

Control values of left ventricular function were greater in adults than in infants and halothane generally produced more depression than isoflurane. These findings are consistent with what is known about myocardial development and anesthetic potency.[35,36] Also, LVP in the isolated heart is related to perfusion pressure, which is higher in adults than in newborns.[13,37] Immature myocardium generates less tension than adult and the rate of fiber shortening is slower. There are many developmental changes in the cellular mechanisms of contractility that may account for this. Neonatal contractile proteins are isoforms of adult proteins and maximal myofibrillar ATPase activity is reduced.[36,38] More importantly, perhaps, sarcoplasmic reticulum (SR) is sparse and functionally undeveloped.[38,39] The immature cell relies on transsarcolemma Ca^{2+} flux to initiate contraction, but voltage-gated channels, which are largely responsible for this flux in mature cells, are relatively deficient in newborns.[40] The Na^+–Ca^{2+} exchange pump may play a relatively greater role in sarcolemma Ca^{2+} movement in immature cells.[41] Volatile anesthetics depress contractility primarily by limiting Ca^{2+} availability to the contractile apparatus. They alter both sarcolemma and SR Ca^{2+} flux with the net result of depletion of intracellular Ca^{2+} stores.[42–44] Halothane decreases peak intracellular Ca^{2+} concentration more than isoflurane.[2,45] It is not clear whether these agents also alter Ca^{2+} sensitivity of the contractile proteins.[46]

In this study halothane was a more potent depressant of contractile function than isoflurane in both newborns and adults. With isoflurane there were no differential effects between age groups, whereas, with halothane although depression of peak systolic LVP was similar between ages, newborns were more sensitive to depression of developed LVP and $+dP/dt_{max}$. Our re-

sults are consistent with a previous study that found tension development in rabbit RV tissue to be more depressed by halothane in newborns than in adults.[3] Krane and Su[2] found that newborns were less sensitive to halothane depression of SR Ca^{2+} uptake than were adults but more sensitive to halothane-induced Ca^{2+} efflux, potentially resulting in greater depression of contractility. Studies evaluating anesthetic depression of contractile protein Ca^{2+} sensitivity have yielded conflicting results as to whether newborns are more sensitive than adults.[6,7] No studies have directly examined anesthetic effect on sarcolemma Ca^{2+} flux in newborns, although Baum[4] found that halothane and isoflurane decreased the height of the action potential plateau of newborn RV papillary muscle, consistent with an effect on transsarcolemma Ca^{2+} entry. Because other Ca^{2+} channel blockers, such as verapamil, produce greater depression of contractility in newborns than in adults one might predict that volatile anesthetics would also produce greater depression in newborns by action on this channel.[47]

Myocardial relaxation during diastole is an active energy-consuming process of inactivation of contraction. Several extrinsic factors that affect relaxation are not present in this isolated heart model: systolic load, ventricular interaction and the pericardium. Although coronary vascular engorgement may have a small effect late in diastole, diastolic function of the isolated heart is primarily dependent on cellular mechanisms of Ca^{2+} dissociation from troponin C and removal from cytosol. $-dP/dt_{max}$ is a measure of the rate of relaxation very early during diastole, whereas τ, the time constant of isovolumic relaxation, reflects events later in diastole. Higher control values of these variables in adults than in infants may be due to age-related changes in elastic recoil or differences in myocardial size and wall thickness, or to cellular mechanisms of relaxation. $-dP/dt_{max}$ also is dependent on peak LVP, which is higher in adults.

Volatile anesthetics are known to alter diastolic functions of the heart but this has not previously been examined in neonates.[48,49] Anesthetic effects on Ca^{2+} flux that impair systolic function also impair diastolic function. In this isolated heart model in which ventricular volume is constant, minimum diastolic pressure is indicative of the extent of myocardial relaxation. Halothane and isoflurane increased minimum diastolic pressure and therefore reduced the extent of myocardial relaxation. This effect was greater with halothane in infants than with either halothane in adults or isoflurane in either age group. Of note are the two adult hearts in which halothane increased diastolic pressure to very high levels during pacing. This may indicate that relaxation had been substantially slowed so that the duration of diastole was inadequate for full relaxation to occur. Halothane and isoflurane increased τ and this effect was also greater with halothane in infants than for any other group. τ increases with end-diastolic pressure and anesthetic effects on τ are similar to those on diastolic pressure. Our results are different than a previous *in vivo* study that found no effect of isoflurane on τ in swine, but consistent with another *in vivo* study that found similar effects between halothane and isoflurane in spontaneously beating adult canine hearts.[48,50] Differences between studies may be due to differences in species and experimental methods. The greater effect of halothane on diastole in infants may reflect the relatively limited capacity of the immature myocardium to remove Ca^{2+} from the contractile proteins. Sequestration in SR is the principal mechanism for Ca^{2+} removal in mature myocardium, but immature hearts in which SR is relatively undeveloped, are more dependent on sarcolemmal Na^{+}–Ca^{2+} exchange.[51] Developmental changes in contractile proteins and their affinity for Ca^{2+} may also affect anesthetic actions on relaxation. Both anesthetics decreased $-dP/dt_{max}$ but halothane decreased it more than isoflurane and there were no differences between age groups. Differences between agents likely reflect their relative potencies in altering Ca^{2+} flux. The lack of age effects for $-dP/dt_{max}$ may reflect its dependence on other factors, such as peak systolic LVP, which also demonstrate no differential effect with age.

Finally, there are important differences between immature and mature myocardium concerning myocardial O_2 metabolism and coronary flow. In newborn sheep *in vivo* myocardial O_2 consumption is higher than in adults and associated with greater myocardial blood flow.[52,53] The higher O_2 consumption in newborns is commensurate with increased cardiac work (estimated as the rate-pressure product). Our results are consistent with this. O_2 consumption and coronary blood flow were significantly higher in infant than adult hearts, probably because of the infants' higher heart rates and possibly to less efficiency in the intrinsic work of contraction. Matching of coronary flow to myocardial O_2 consumption was similar between age groups as evidenced by similar O_2 extraction ratios. Halothane and isoflurane increased coronary flow in a dose-related manner. Because perfusion pressure was constant, in-

crease in flow indicates a decrease in coronary vascular resistance and, therefore, direct coronary vasodilation by the agents. Changes in coronary flow were not different between agents or age groups. Previous *in vitro* studies have reported an increase in coronary flow by isoflurane and variable effects by halothane in adult animals but anesthetic effects on coronary vessels in newborns have not previously been reported.[12,54,55]

Isoflurane decreased O_2 consumption and O_2 extraction ratio; this change, coupled with increased coronary flow, indicates diminished autoregulation and relative overperfusion of the myocardium. When hearts beat spontaneously, isoflurane decreased O_2 consumption and extraction more in adult hearts than in newborn hearts, probably because of a greater decrease in heart rate in adults. When heart rate was held constant by pacing there were no differences between age groups. Our results are consistent with previous reports in adult animals,[12] but these findings have not previously been reported in newborns.

In summary, this study documents developmental differences in the direct myocardial effects of halothane and isoflurane. Halothane was a more potent depressant of cardiac function than isoflurane and developmental differences were more evident with this agent. Developmental changes in myocardial physiology make the infant heart less sensitive to direct depression of heart rate by halothane and isoflurane, but more sensitive to depression of contraction–relaxation and AV conduction by halothane. There were minimal developmental differences in the myocardial effects of isoflurane.

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