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Increased Sensitivity of the Isometric Contraction of the Neonatal Isolated Rat Atria to Halothane, Isoflurane, and Enflurane

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Isolated atria from neonatal (0–5 day old) and adult (50 ± 5 day old) rats were perifused in oxygenated Kreb's Henseleit solution at 30 ± 0.5° C and exposed to four different concentrations of halothane, isoflurane, or enflurane while isometric contractile tension was recorded and compared with control atria. ED_{50} values (mM of anesthetic required to produce 50% reduction in contractile tension) of neonates for halothane (0.18 ± 0.01), isoflurane (0.41 ± 0.05), and enflurane (0.41 ± 0.04) were significantly lower than those of adults (0.35 ± 0.02, 0.80 ± 0.05, and 1.15 ± 0.05, respectively). Furthermore, neonatal ED_{50} calculated as per cent of adult ED_{50} was significantly less for enflurane (35%) than for halothane (54%) or isoflurane (51%). (Key words: Anesthesia: pediatric. Anesthetics, volatile: enflurane; halothane; isoflurane. Heart: atria; contractility; neonatal.)

HALOTHANE,[1] ISOFLURANE,[2] and enflurane[3] produce dose-related arterial hypotension in humans and experimental animals.[4] It has been the clinical impression that the degree of hypotension with these agents is greater in neonates[¶] and infants[5–7] than in older children[8] or adults.[1,2] The hypotensive effect is related to their ability to depress myocardial contractility, to alter the heart rate, or to decrease peripheral vascular resistance.[1–3] The degree of hypotension produced by these agents is the result of an interplay between the depression of myocardial contractility and the reflex compensatory mechanisms.

In an effort to distinguish between possible mechanisms, we examined atrial contractility in an *in vitro* model to determine whether the isolated neonatal rat atrium exhibited a greater sensitivity to the depressant effects of halothane, isoflurane, and enflurane when compared with the isolated adult rat atrium.

Methods

Fifty-two newborn (0–5 days, wt 5–15 g) and 45 adult (50 ± 5 days, wt 180–220 g) Sprague Dawley rats were killed by decapitation. The heart was dissected and transferred into a container filled with ice-cold Krebs-Henseleit solution within 30 s. The solution was oxygenated by bubbling carbogen (mixture of 95% oxygen and 5% carbon dioxide) continuously. The atria were cleared of adjoining tissue and, with the isthmus intact, were separated from the ventricles. One end of the preparation was attached to a thin gold chain, while the other end was attached to a stimulating electrode.

The atria were suspended vertically in an organ bath with the stimulating electrode on the lower and the gold chain on the upper side. The other end of the gold chain was attached to a Statham® strain gauge. The bath had an inlet and an outlet, which were connected to a 400-ml reservoir containing Krebs-Henseleit bicarbonate solution (mM NaCl 120, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4 \cdot 7\ H_2O$ 3.3, KH_2PO_4 1.2, $NaHCO_3$ 25.3, and glucose 5.55). The Krebs-Henseleit solution in the reservoir was oxygenated continuously with carbogen. A rotary pump was used to circulate the Krebs-Henseleit solution from the reservoir to the bath and back to the reservoir continuously throughout the experiment (perifusion). With this arrangement, the fluid in the reservoir could be oxygenated with carbogen with a fritted disc, thus avoiding the problem of bubbling the bath that held the atria and causing interference with the contractile pattern of the tiny neonatal atria. The reservoir was placed in a warm water bath, and the outer lumen of the organ bath was continuously circulated with heated water to maintain the temperature of the perifusate solution at 30 ± 0.5° C. Even though there is a difference in drug effects on atria at different temperatures, we elected to conduct the experiments at 30° C, since isolated atrial function is more stable at this temperature than at 37° C.[9,10] The atria were subjected to isometric contraction by stimulating with a Grass S9® stimulator at a rate of 200 pulses/min with twice the threshold voltage. The output from the strain gauge was amplified and recorded on a Beckman® recorder.

In order to study the effects of drugs on isolated cardiac muscle, two methods often are used to optimize resting tension (length). In one method the length–tension curve is determined for each individual muscle and then the experiment is conducted at the peak of the length–tension curve or at a length below this point. In the alternate method the optimal resting tension (length) is determined in a different group of control muscles. This average optimal resting tension then is used for further experiments. In reproducible preparations both methods are valid and

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¶ Diaz JH, Lockhart CH: Is halothane really safe in infancy? (abstract) ANESTHESIOLOGY 51:S313, 1979.

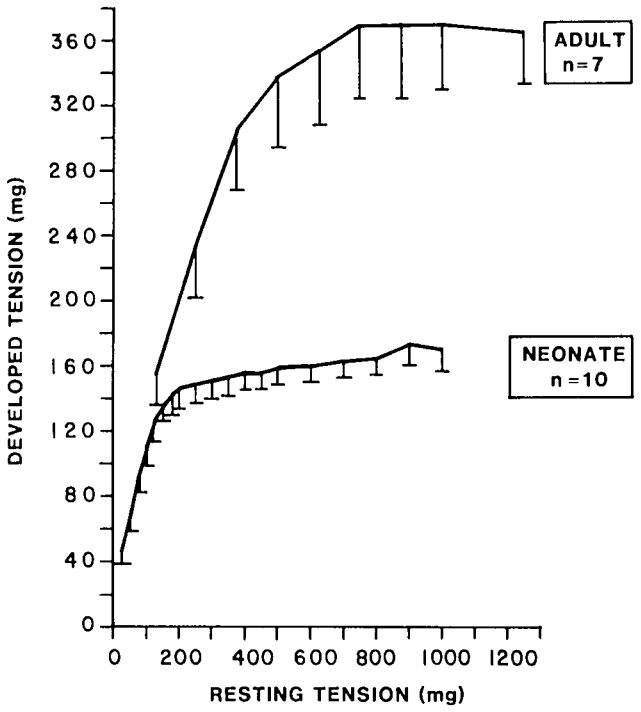

FIG. 1. Resting tension-developed tension curves for the neonatal and adult isolated rat atria.

have been used extensively.[9,10] For our experiments we elected to use the second method, since we believe that determining the length–tension relationship for each individual study muscle could be detrimental to the preparation. The hazards of this procedure include inadvertent overstretching of especially the fragile neonatal study atria and prolongation of the total time of the individual experiment. Both factors would compromise viability of the preparation and could possibly influence the results of drug effects.

The length–tension relationship, although well established for adult rat atria,[11] is unknown for the neonatal rat atria. Therefore, in a series of 10 neonatal preparations, we determined the length–tension relationship. The length was altered by increasing resting tension in 25-mg increments. Following stabilization of the base line after each 25-mg increment in resting tension, the developed tension was recorded. For comparison purposes we also evaluated the length–tension relationship in seven adult rat atria. The resting tension in adult atria was changed in 125-mg increments. The results of these length–tension studies are shown in figure 1. For the neonatal atria we found a positive correlation between resting tension (length) and developed tension up to a resting tension value of 150 mg. The changes in developed tension with resting tensions of greater than 150 mg were minimal. Furthermore, the resting tension–developed tension results for individual experiments were within a very narrow range (*e.g.*, at 150 mg resting tension, the developed tension was 136 mg ± 13 mg). In the adult rat atrial preparations we found a positive correlation between resting tension (length) and developed tension up to a resting tension of 750 mg. Our results of adult rat atrial length–tension relationships are in agreement with previously published studies.[12]

On the basis of these studies we selected a resting tension of 150 mg for neonatal atria and 750 mg for adult atria for further experiments.

Twenty-one neonatal (halothane, nine, isoflurane, six, and enflurane, six) and 19 adult (halothane, seven, isoflurane, six, and enflurane, six) Sprague Dawley rats were used for this study, and an equal number of neonatal and adult atria were used for paired simultaneous controls. An equilibration period of 60 min was allowed before readings were taken. The developed force of contraction at 60 min was expressed as 100%.

The experiments were conducted in sets of either two adult or two neonatal atria. Each set consisted of one control and one anesthetic-exposed atria. Both atria in each set were perifused simultaneously with the same solution and were studied under similar conditions. The contractile force of the atria in each set was recorded simultaneously and expressed as per cent of force developed at the 60-min equilibration period.

The force of contraction of both adult and neonatal control atria not exposed to anesthetic remained relatively stable over the 3-h experimental period, declining less than 15% (fig. 2), thus indicating that the atria were adequately oxygenated for the duration of the experimental procedure. No statistically significant differences in force of contraction were observed among atria serving as controls for each of the anesthetics in either the neonatal or adult groups (analysis of variance), and the controls in each group were therefore pooled (fig. 2).

To study the effects of volatile anesthetics on neonatal or adult atria, the respective anesthetic (halothane, isoflurane, or enflurane) was introduced into the perifusate via a halothane or enflurane vaporizer following the 60-min equilibration period. The effects of four different concentrations of anesthetics were studied. These anesthetic concentrations were chosen on the basis of preliminary dose–response (anesthetic concentration *vs.* force of contraction) experiments on neonatal and adult rat atria. The concentrations of the anesthetics were increased at 30-min intervals. Thus, the atria were exposed to four different incremental concentrations of the anesthetics in a 2-h period. The initial vaporizer setting and the incremental changes were different for the neonatal and adult atria for each of the three anesthetics studied. The developed tensions were recorded at 10-min intervals for the 2-h duration of anesthetic exposure and for an additional hour after discontinuation of the anesthetic to

monitor the recovery of atria from the effects of the anesthetic. The force of contraction data obtained during the anesthetic exposure period was normalized to exclude the effect of natural decay (up to 15% depression in force of contraction over a 3-h period, fig. 2) of atrial preparations. The data were normalized in the following way. At 10-min intervals, the force of contraction for each anesthetic-exposed atria was calculated as a per cent of its preanesthetic value then expressed as a per cent of the mean of control atria not exposed to anesthetic.

The concentrations of the anesthetics in milligrams per deciliter in the organ bath were estimated every 10 min for 2 h with a gas chromatograph and converted to mM and also volumes per cent. In order to convert these concentrations of anesthetics in milligrams per deciliter to volumes per cent in air (analogous to alveolar concentration), perifusate/air partition coefficients were determined at 30° C and were 1.05 ± 0.03 (M ± SD) for halothane, 0.85 ± 0.02 for isoflurane, and 1.00 ± 0.02 for enflurane, respectively. The ideal gas law ($P = n/V \cdot RT$) was used to calculate the partial pressure of the anesthetic in air, from which the volumes per cent of the anesthetic at sea level was calculated.

ED_{50} (concentration of anesthetic required to produce 50% reduction in force of contraction) was determined for each experiment from data taken at 30, 60, 90, and 120 min after starting the anesthetic, *i.e.*, at the end of the 30-min exposure to the same vaporizer setting.

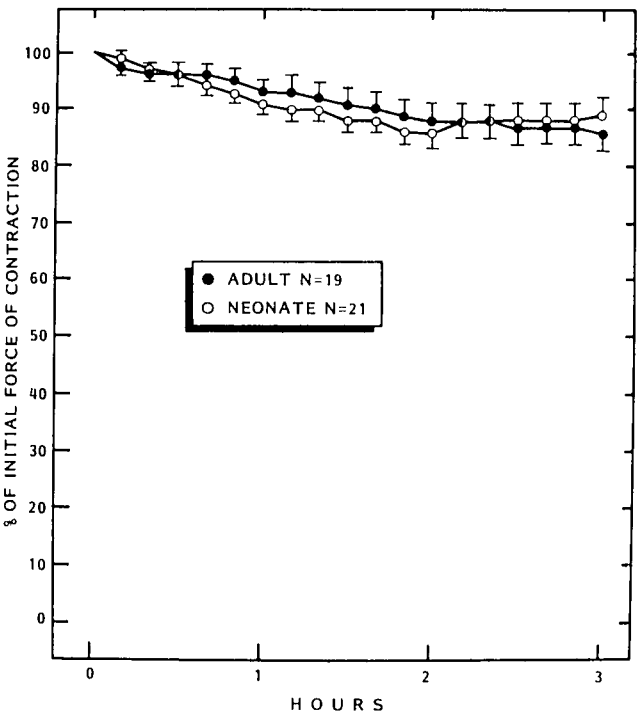

FIG. 2. Per cent of initial force of isometric contraction of control adult and neonatal isolated rat atria. The force of isometric contraction at the end of the first hour of equilibration was considered 100% and was placed at zero time.

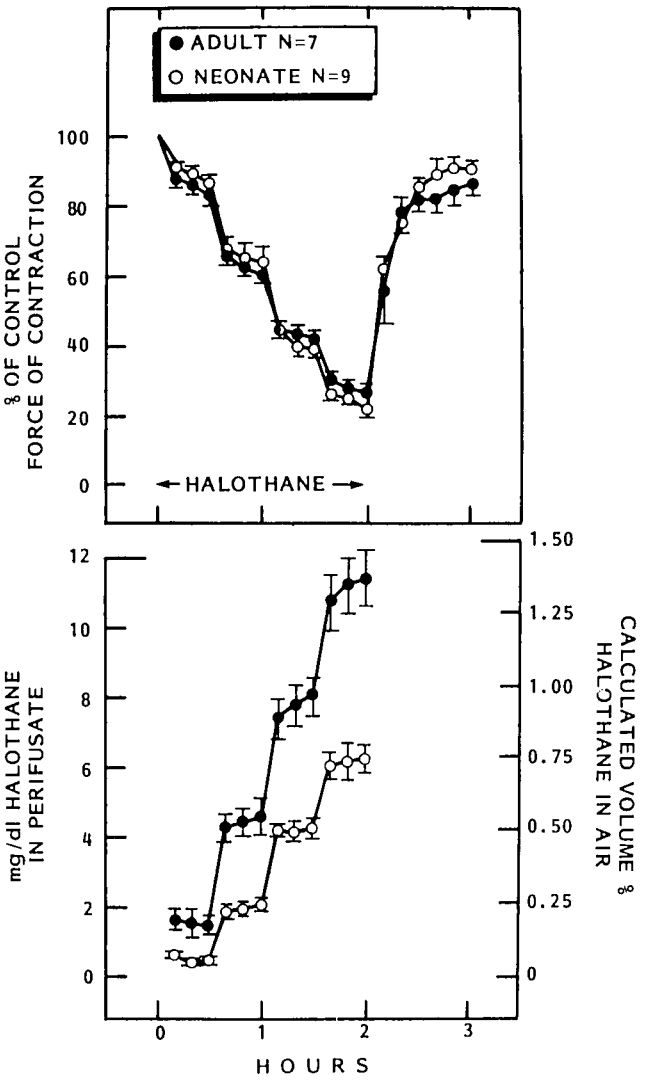

FIG. 3. Effect of halothane on the force of isometric contraction of adult and neonatal rat atria. *A* (*top*) depicts the per cent of control force of isometric contraction as a per cent of control over time. *B* (*bottom*) depicts the concentration of halothane in the perifusate both as milligrams per deciliter and volumes per cent, producing the reduction in isometric force of contraction seen in *A*. Halothane was introduced at zero hour, concentration increased at every half hour, and discontinued at the end of 2 h.

The force of contraction data were subjected to logit transformation.[13] The logit of the force is defined as $y = \ln(f/[100 - f])$, where f = per cent of control force of contraction. From linear regression analysis for each experiment, the concentration of anesthetic producing 50% of the control force (ED_{50}) was calculated. The ED_{50} values for halothane, isoflurane, and enflurane for the neonatal atria were compared with the corresponding ED_{50} values of the adult atria by unpaired Student's *t* tests. The neo-

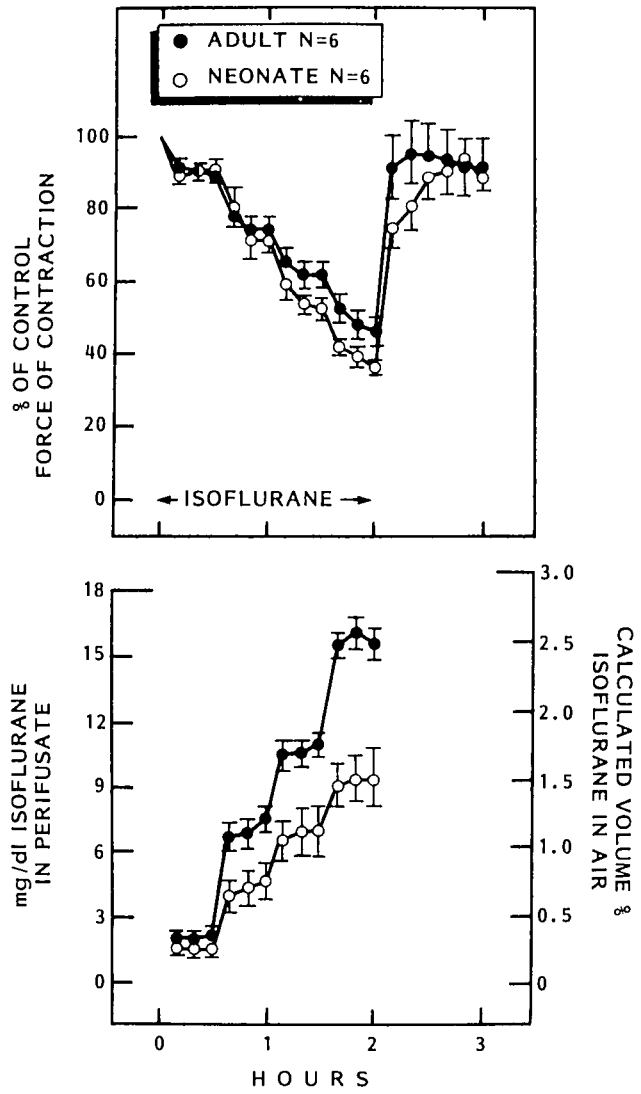

FIG. 4. Effect of isoflurane on the force of isometric contraction of adult and neonatal isolated rat atria. See figure 3 for additional details.

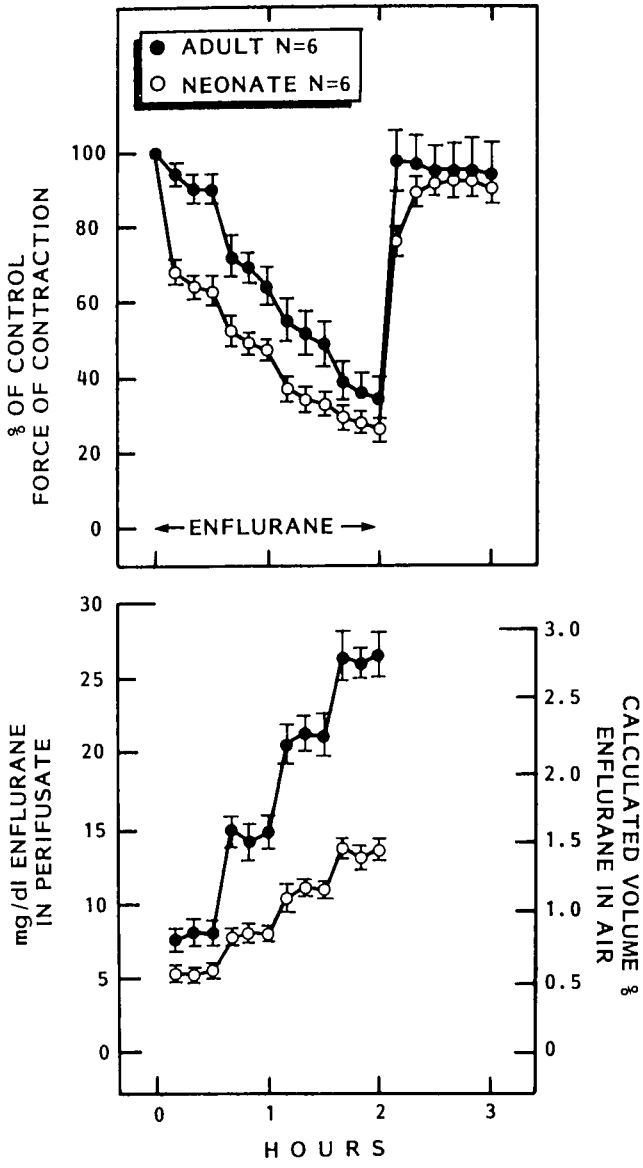

FIG. 5. Effect of enflurane on the force of isometric contraction of adult and neonatal isolated rat atria. See figure 3 for additional details.

natal ED_{50}, calculated as per cent of adult ED_{50} for halothane, isoflurane, and enflurane, were compared with each other by one-way analysis of variance, followed by Student-Newman-Keuls' test.[14]

Results

Atria, exposed to four different vaporizer levels of each anesthetic for 30-min periods, achieved near steady state perifusate concentration and force of contraction within the first 10 min (figs. 3–5). Much smaller concentrations of halothane (fig. 3), isoflurane (fig. 4), and enflurane (fig. 5) were required to depress the force of contraction of neonatal atria to levels equal to or greater than that of adult atria at each 30-min exposure period. Calculated ED_{50} values for neonatal halothane and isoflurane were approximately half those of adult, whereas the enflurane neonatal ED_{50} was approximately one-third that of the adult (table 1).

Discussion

Halothane, isoflurane and enflurane are known to cause dose-dependent cardiovascular depression in humans and intact animals. There is evidence to indicate that the neonate and young infant may have greater sensitivity to the cardiovascular depressant effects of the volatile anesthetics. Neonates** and young infants[5–7] manifest a

** Diaz JH, Lockhart CH: Is halothane really safe in infancy? (abstract) ANESTHESIOLOGY 51:S313, 1979.

greater degree of hypotension in response to halothane and isoflurane at apparently equipotent anesthetic concentrations when compared with older children.[8] Infants up to 16 weeks of age exhibit a significantly greater incidence of hypotension (arterial pressure 30% or more below control) than observed in infants at age 17–24 weeks.** Halothane in newborn lambs causes more frequent reductions in heart rate, mean arterial pressure, and cardiac output than in adult sheep.††

Lichtor *et al.*[7] studied the cardiovascular effects of halothane in infants younger than 6 months of age, using echocardiography, and reported a significant decrease in stroke volume and ejection fraction and increase in end-diastolic volume. They concluded that the effect of halothane on ventricular function was greater in infants than in children and adults.

Cook *et al.*[15] compared the *in vivo* cardiovascular index (the ratio of the halothane concentration in the heart at cardiovascular failure and the halothane concentration in the heart at anesthesia) between 15- and 30-day-old rats and found the concentration of halothane in the heart at cardiovascular failure was less in the younger rats than in the older rats, indicating reduced cardiovascular tolerance of the younger rats to the effects of halothane.

Our study revealed a dose-dependent direct depression of the isometric force of contraction of the isolated neonatal and adult rat atria by all three agents. We found the ED_{50} value for the neonatal atria to be significantly lower ($P < 0.001$) than the ED_{50} value of the adult atria, indicating increased sensitivity of the neonatal isolated atria to the depressant effects of the three anesthetic agents studied. The ED_{50} of halothane and isoflurane for neonatal atria was approximately 50% less than that for adult atria; however, the ED_{50} of enflurane for neonatal atria was approximately 65% less than that of adult atria, indicating that the neonatal atria are extremely sensitive to the depressant effects of enflurane.

In our study we used a preload of 150 mg for the neonatal and 750 mg for the adult atria, based on the resting tension (length) and developed tension curves as shown in figure 1. These preloads are the resting tension values slightly below the peak of the length–tension values for each group of atria. Even though the resting tension values are different for these two groups of atria, we were able to statistically compare the depressant effects of the three anesthetic agents by deriving their concentrations that produced the same amount of depression (*i.e.*, 50% depression) in both groups of atria. The different preload values in these two groups of atria are similar in the sense that they both produced peak developed tensions as seen in the length–tension curves. Thus, we believe, we are justified in comparing the ED_{50} values for the three agents between the neonatal and adult atria. However, the confirmed evidence as to the relative potencies of the three anesthetic agents shown to produce myocardial depression eventually will have to be determined in clinical studies.

TABLE 1. ED_{50} Concentrations* for the Isolated Neonatal and Adult Atria Exposed to Halothane, Isoflurane, and Enflurane

	Adult	Neonate	Neonatal ED_{50} as Per Cent of Adult ED_{50}
Halothane			54
mg/dl	6.87 ± 0.43 (7)†	3.63 ± 0.24 (9)‡	
mM	0.35 ± 0.02	0.18 ± 0.01	
vol %	0.82 ± 0.05	0.44 ± 0.03	
Isoflurane			51
mg/dl	14.8 ± 0.9 (6)	7.63 ± 0.93 (6)‡	
mM	0.80 ± 0.05	0.41 ± 0.05	
vol %	2.36 ± 0.14	1.21 ± 0.15	
Enflurane			35§
mg/dl	21.2 ± 0.9 (6)	7.50 ± 0.71 (6)‡	
mM	1.15 ± 0.05	0.41 ± 0.04	
vol %	2.86 ± 0.13	1.01 ± 0.10	

* Concentrations are expressed as milligrams of anesthetic liquid per deciliter of perifusate, the corresponding mM concentration, and a calculated volumes per cent value (ml of anesthetic gas/100 ml of total gas).
† M ± SE—number of experiments in parentheses.
‡ $P < 0.001$ compared with adult ED_{50}.
§ $P < 0.05$ compared with halothane and isoflurane.

There are minor differences in the structure and electrophysiology of atrial and ventricular myocardial cells.[16,17] Therefore, it is possible that the response of ventricular cells to various drugs may not be identical to that of atrial cells. The atrial cells are smaller in diameter, and their transverse tubules are not as prominent as that of the ventricular cells. This probably decreases the total amount of membrane that must be depolarized by the action potential, and this speeds up the conduction of the action potential across the atrium.[16] The resting potential in the rat atrial cell is less negative than in the rat ventricular cell (−62 *vs.* −90 mV) and the action potential in the atrial cell is lower than that of rat ventricular cell (75.2 *vs.* 120 mV).[17] Ouabain has been shown to produce both "high-dose" (19 to 35 μM) and "low-dose" (0.3 μM) effects in the rat ventricular muscle but only "high-dose" effects on the atrial muscle. The mechanism of "low-dose" effect of ouabain is not well known. The "high-dose" effect of ouabain is related to the inhibition of Na^+-K^+ ATPase (sodium pump) and subsequent mobilization of a pool of calcium via the Na^+-Ca^{++} carrier of the sarcolemmal membrane.[18,19] In guinea pig heart the concentration of Na^+-K^+ ATPase is higher in the ventricular than in the atrial muscle,[20] but it is not known if this is also true of the rat heart. In contrast to ouabain, the effect of halothane on rat atrial and ventricular tissue appears

†† Robinson S, Gregory GA: Circulatory effects of anesthesia in the developing sheep (abstract). I. Halothane. ANESTHESIOLOGY 53:S330, 1980.

to be similar. In a previous study from our laboratory conducted on isolated perfused intact rat heart, the depression of force of contraction was similar to that of the adult atria reported in this study. The concentrations of halothane in the two studies were similar.[21] However, there were several differences in the methods of the two studies, and hence the results of our study on atria may not be directly transferable to ventricular tissue.

There are differences between the adult and the neonatal myocardium that might contribute to a greater depression of neonatal myocardium by these agents. Langer *et al.*[22] reported a significant difference in the Na^+ flux through a slow membrane channel between the neonatal and older rat hearts. He observed a greater increase in dP/dt in neonatal than in older rats when exposed to ouabain and also by accelerating the heart rate from 30 to 90 beats/min. These positive glycoside and staircase responses were virtually eliminated by a 50% reduction in sodium and a 25% reduction in the calcium concentrations in the perfusate while maintaining a constant Ca^{++} to Na^+ ratio. The duration of the ventricular action potential also progressively decreased with age. Their results suggested that Na^+ flux through a slow membrane channel played a significant role in the positive staircase and glycoside responses of the neonatal rat heart presumably by activation of a Na^+-Ca^{++} exchange mechanism, which becomes reduced with age.

We conclude that the neonatal rat atria is more sensitive to the myocardial depressant effects of halothane, isoflurane, and enflurane than adult rat atria. From our data we can speculate that the greater hypotension seen in younger animals could be related to greater direct depression of the force of myocardial contraction in the younger than in the older animals. However, a direct clinical application of this data to humans should be restrained and should be based on future clinical studies in neonates and infants on the cardiac effects of these inhalational agents. The extent to which the direct myocardial depression induced by the anesthetics is modified *in vivo* by neural and hormonal factors in neonatal rats in comparison with those of adult rats is unknown.

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