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Developmental Changes in Effects of Halothane and Isoflurane on Contractile Properties of Rabbit Cardiac Skinned Fibers

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Immature hearts of various animal species and humans have been demonstrated to be more sensitive than adult hearts to the myocardial depressant effects of volatile anesthetics. To further investigate the mechanisms involved, the calcium sensitivity and maximal activated tension of detergent-treated left ventricular fibers of fetuses (30 days), newborn (1-day-old), immature (3-, 8-, and 17-day-old), and adult rabbits were determined by stepwise exposure to increasing Ca^{2+} concentrations. Responses were measured prior to and after exposure to equianesthetic concentrations of halothane (1%) or isoflurane (1.5%) applied in a random order. In control conditions maximal developed tension was the lowest in fetuses (11.1 ± 0.6 mN · mm^{-2}), intermediate in newborn and immature rabbits, and highest in adults (25.6 ± 2.9 mN · mm^{-2}). There were also age-related changes in calcium sensitivity; pCa ($= -\log_{10}[Ca^{2+}]$) for half-activation (pCa_{50}) was significantly less in 1-, 3-, and 8-day-old rabbits (5.444 ± 0.036, 5.425 ± 0.017, and 5.385 ± 0.019, respectively) than in adults (5.517 ± 0.010), whereas it was not different in fetuses (5.521 ± 0.017). During anesthetic exposure both calcium sensitivity and maximal developed tension decreased significantly in all age groups of animals, with both anesthetics having a similar effect in animals of identical age. However, calcium sensitivity decreased significantly more in newborn animals (0.192 and 0.196 pCa unit for halothane and isoflurane, respectively) compared with adults (0.122 and 0.137 pCa units, respectively). By contrast, fetuses were less sensitive to the myocardial depressant effects of anesthetics than were newborn animals. The results of this study are consistent with the hypothesis of a greater sensitivity of contractile apparatus of neonatal heart to the depressant effects of volatile anesthetics. This could be related to developmental changes that are known to affect the contractile proteins, especially the troponin–tropomyosin system. (Key words: Anesthesia, pediatric: fetus; neonate. Anesthetics, volatile: halothane; isoflurane. Heart: contractile proteins.)

IT IS COMMONLY ACCEPTED that human neonates and infants are more sensitive than adults to the myocardial depressant effects of volatile anesthetics.[1–4] Similar conclusions arise from experimental studies on rat atria,[5] rabbit, and cat papillary muscles[6,7] from newborn animals, but the same does not appear to be true in fetal lambs.[8] Such an increased sensitivity to the negative inotropic effects of inhalational anesthetics may result from age-related changes in anesthetic potency and/or in myocardial structure. At the cellular level the latter involve transsarcolemmal exchanges, uptake and release capacities of the sarcoplasmic reticulum, and structural and biochemical changes in the contractile proteins themselves.[9–13] Some of these changes will mainly affect the left ventricle in response to abrupt changes in loading conditions as those occurring immediately after birth.[14,15]

We previously demonstrated that volatile anesthetics decreased both calcium sensitivity and maximal developed tension of skinned rat cardiac fibers, effects that were dose-dependent, reversible, and similar for halothane, enflurane, and isoflurane at equipotent anesthetic concentrations (ranging from 0.5 to 2.0 MAC).[16] As changes in calcium sensitivity had been observed in developing rat,[17,18] dog,[19] and rabbit hearts,[20,21] the present study was carried out to determine whether the effects of volatile anesthetics on contractile proteins vary with age. For that purpose skinned fibers were obtained from the left ventricle of fetal (30-day gestation), newborn (1-day-old), immature (3-, 8-, and 17-day-old), and young adult rabbits, and the effects of equipotent concentrations of halothane and isoflurane were studied in each age group of animals.

Our results suggest that the higher myocardial sensitivity of newborn animals to volatile anesthetics may be partly related to their effects on myocardial contractile proteins because a more important decrease in myocardial calcium sensitivity was observed in 1-day-old newborn than in adult rabbits during exposure to identical concentrations of halothane or isoflurane.

Methods

Preparation of Skinned Fibers

Hearts were rapidly removed from rabbits of various ages that had been anesthetized with pentobarbital sodium iv or ip according to the recommendations of the Institutional Animal Care Committee (INSERM, Paris). Animals were studied in a random order. Fibers were obtained from fetuses at the 30th day of gestation (24 h before birth), 1-day-old newborn (within the 24 h after birth), immature (3-, 8-, and 17-day old), and young adult (8–11 months) rabbits. Fibers were obtained from at least two different animals in each group. Subendocardial fibers from the left ventricle (140–320 μm in diameter) were excised at room temperature in a zero-calcium Krebs solution. After excision the muscles were skinned for 60

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min in a relaxing solution containing the nonionic detergent octylphenoxy polyethoxyethanol (Triton X-100, 1%). This time is sufficient to destroy all functional membranes including sarcoplasmic reticulum, T-tubules, and mitochondrial and sarcolemmal membranes without changing structural and biochemical properties of cardiac myofibrils.[22]

The mechanical apparatus has been previously described.[16] The fibers were mounted between two stainless steel hooks, one of which was stationary and the other connected to an AE801 transducer (AME, Horten, Norway). The fiber length and diameter were determined optically with a binocular microscope and a micrometer. Ventricular fibers were assumed to be cylindrical. Before experiments each preparation was set at the slack length and then stretched by 20% to allow development of maximal force. The length of the preparation was then kept constant to avoid sarcomere length-dependent changes in Ca^{2+} sensitivity. The muscles were immersed in small chambers (2.5 ml) arranged around a disk positioned on a magnetic stirrer. Each solution was well stirred at high speed (>1,000 rpm). All experiments were performed at 22° C.

Solutions

Two different bathing solutions, a relaxing solution (A) and an activating solution (B), were prepared in sufficient amount for all experiments. Aliquots were kept at −20° C to allow identical experimental conditions in each age group of animals. The composition of the solutions used was calculated using the computer program of Fabiato and Fabiato[23] and the binding constants of Fabiato.[24] Solutions were calculated so that the following composition was maintained constant: free Mg^{2+} 3.16 mM, MgATP 3.16 mM, creatine phosphate 12 mM, Na^+ 30.6 mM, imidazole 30 mM, EGTA 10 mM, and dithiothreitol 0.3 mM. Acetic acid was used to adjust *p*H at 7.1. Ionic strength was adjusted to 0.16 M with K acetate. Two solutions of extreme Ca^{2+} concentration were calculated: the relaxing solution (A) containing $[Ca^{2+}] = 10^{-9}$ M (pCa 9) and the activating solution (B) containing $[Ca^{2+}] = 10^{-4.5}$ M (pCa 4.5) ($pCa = -\log_{10}[Ca^{2+}]$). Solutions of intermediate Ca^{2+} concentrations were obtained by mixing solutions A and B.

Experimental Design

For each muscle tension/pCa curves were obtained in control conditions and during exposure to halothane (1%, v/v) and isoflurane (1.5%, v/v) by stepwise exposing the fibers to solutions with increasing Ca^{2+} concentrations and measuring developed tension. For each fiber a tension/pCa curve was first obtained with solutions of increasing Ca^{2+} concentrations free of anesthetics, and then the fiber was allowed to relax for at least 15 min before a second curve was obtained with solutions previously equilibrated with halothane or isoflurane in a random sequence. After exposure to increasing Ca^{2+} concentrations in the presence of the anesthetic, the fiber was then immersed in the control solution B (pCa 4.5) free of anesthetics to measure the relative decrease in maximal force before relaxation (fig. 1). This procedure was chosen because it permits the measurement of maximal force to become time-independent. A third curve was obtained with the second anesthetic, and thereafter a final tension/pCa curve was obtained with solutions free of anesthetics. The mean values of the two control curves (bracketing the two anesthetic curves) were used to assess the effects of the two anesthetics studied. The time interval necessary to perform a complete set of experiments in one fiber ranged between 120 and 150 min.

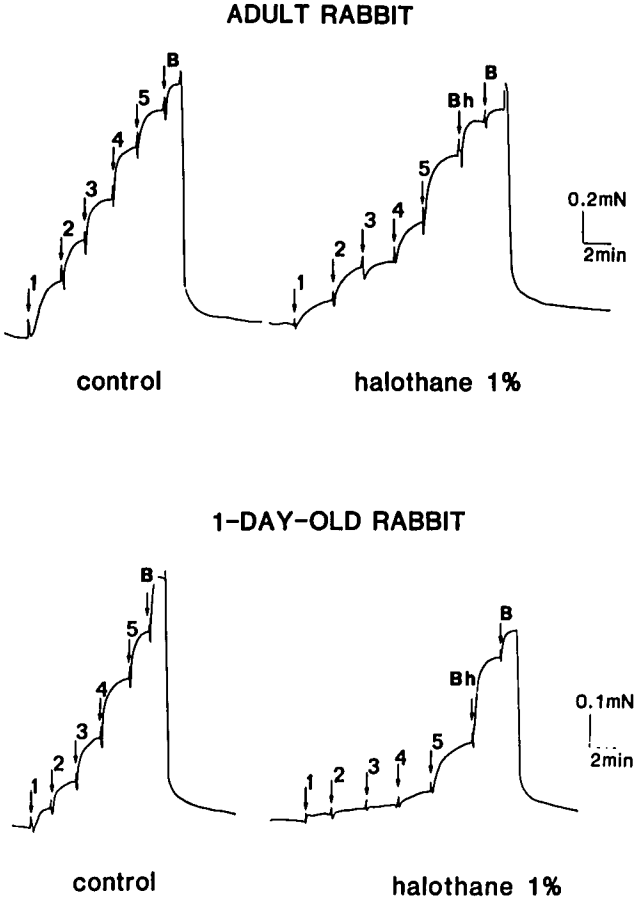

FIG. 1. Experimental tension/pCa curves obtained in one adult rabbit (*upper panel*) and one 1-day-old newborn rabbit (*lower panel*) in control conditions (*left*) and in the presence of 1% halothane (*right*). Arrows indicate changes in solutions. Numbers indicate pCa values: 1 = 5.75; 2 = 5.625; 3 = 5.5; 4 = 5.375; and 5 = 5.25. B = the activating solution (pCa 4.5) free of anesthetics; B_h = the activating solution equilibrated with 1% halothane.

For studying the effects of anesthetics, the test solutions were equilibrated in separate chambers by continuous bubbling for 15 min with the chosen anesthetic. The carrier gas was 100% nitrogen (N_2), which flowed through calibrated vaporizers (Fluotec Mark III and isoflurane Dräeger Vapor 19.1). The anesthetic concentration in the gas phase was monitored with an infrared calibrated analyzer (Normac™, Datex, Finland). The anesthetic concentrations obtained in the solutions were measured by gas liquid chromatography with the head space technique. The anesthetic concentrations measured in the experimental solutions (n = 6) after 15 min of continuous bubbling were 0.63 ± 0.06 mM for halothane and 0.82 ± 0.01 mM for isoflurane.

ATPase Activity

Possible age-related changes in maximal myofibrillar ATPase activity in the presence of 1% halothane were determined in cardiac muscles of all age groups of animals. Four to nine sets of measurements were performed in each age group of rabbits. ATPase activity was recorded fluorimetrically with a method previously described.[25] Muscles were incubated in an activating solution B′, the pCa of which was set at 4.5, free of anesthetics first, and then in a solution previously equilibrated with 1% halothane. The composition of this solution was as follows: Mg^{2+} 3.16 mM, imidazole 30 mM, EGTA 10 mM, and dithiothreitol 0.3 mM. Ionic strength was adjusted to 0.16 M with K acetate and *p*H was adjusted to 7.1. Before each measurement 3 mM ATP, 0.05 mM NADH, 2 mM phosphoenolpyruvate, and 2 IU/ml of both pyruvate kinase and lactate dehydrogenase were added. A decrease in NADH was followed by recording fluorimetric changes on a Jobin Yvon JY3D spectrofluorometer at emission wavelength 460 nm (excitation wavelength 340 nm). The temperature was 22° C.

Data Analysis

Tension/pCa relationships were analyzed using a linearization of the Hill equation where F (relative force) = $[Ca]^{nH}/(K^+[Ca]^{nH})$. The slope coefficient (Hill coefficient, n_H) and the pCa for half-maximal activation ($pCa_{50} = (-\log_{10}K)/n_H$) were computed. Resting tension in relaxing solution A was taken as zero tension and maximal tension at pCa 4.5 (solution B) was taken as 100%. Intermediate tension values were expressed as a percentage of this maximal value.

The effects of halothane (1%, v/v) and isoflurane (1.5%, v/v) were assessed in a randomly designed study. These two concentrations were chosen because they were demonstrated to be equipotent in most animal species, including rabbit,[26] ferret,[27] rat and mouse,[28] and in humans at a temperature of 37° C. One can expect that these two concentrations will remain equipotent at the experimental temperature of 22° C because a similar linear decrease in MAC values with decreasing temperature between 38° C and 27° C was observed for halothane and isoflurane.[29] However, because MAC of volatile anesthetics vary greatly with age, these two concentrations do not represent equivalent anesthetic levels among age groups of animals. To make further comparisons, it was assumed that MAC values of rabbit exhibit similar age-related changes to those observed in humans[30–32]: 25% increase in 1-day-old animal, 60%, 40%, and 30% in 3-, 8-, and 17-day-old rabbits, respectively, compared with the MAC of adult animals. This assumption is probably correct because a 43% increase in MAC was observed in 10- to 14-day-old rabbits compared with adult rabbits.[33] The MAC values used for calculations in the present study are those published by Drummond[26] (1.36% for halothane and 2.05% for isoflurane). In addition, it was assumed that the MAC of fetuses was 50% of that of adult animals, as observed in fetal lambs for both halothane[8] and isoflurane.[34] At a temperature of 37° C, the anesthetic concentrations used in this study would therefore represent 0.72 MAC in adult rabbit, 1.5 MAC in fetuses, and 0.57, 0.45, 0.51, and 0.55 MAC in 1-, 3-, 8-, and 17-day-old rabbits, respectively.

Statistical Analysis

For the parameters calcium sensitivity and Hill coefficient, the control values represent the mean of the initial and the final values obtained in the absence of anesthetics, as these latter did not differ significantly in any age group of animals (paired *t* test; $P > 0.5$). The values of maximal developed tension and ratio of resting over total tension are those obtained at the beginning of each experiment; a regular decrease in maximal tension was observed with time. The possible age-related changes in all these mechanical parameters as well as differences within groups between halothane and isoflurane effects were studied by Kruskal-Wallis analysis. Significant changes were then assessed by *t* test when appropriate. In each age group of animals the effects of anesthetics were studied by repeated analysis of variance (ANOVA). $P < 0.05$ was significant.

TABLE 1. Characteristics of the Rabbits

Age	N	Heart Weight (mg)	Body Weight (g)	Heart Weight/ Body Weight ($\times 10^{-3}$)
Fetus	9	243 ± 10	40.0 ± 4.1	6.09 ± 0.39
1 day	4	291 ± 69	51.7 ± 7.9	5.56 ± 0.61
3 days	4	316 ± 62	61.2 ± 8.5	5.14 ± 0.38
8 days	2	727 ± 87	160 ± 20	4.55 ± 0.20
17 days	3	1,024 ± 117	287 ± 52	3.60 ± 0.24
Adult	4	8,140 ± 1,120	3,710 ± 350	2.18 ± 0.16

Values are mean ± SD.

TABLE 2. Maximal Tension (T_{max}), Resting Tension (RT) Expressed as Per Cent of Total Maximal Tension (TT), Maximal Tension at the End of Experiment Expressed as Per Cent of Control Tension, and Percentages of Decrease in Maximal Tension in the Presence of Anesthetics

Age	Fibers	T_{max} ($mN \cdot mm^{-2}$)	RT/TT	T_{max} End (% of control)	Halothane 1% (% decrease from maximum)	Isoflurane 1.5% (% decrease from maximum)
Fetus	9	11.1 ± 0.6†	8.7 ± 1.8	72.3 ± 1.8†	7.7 ± 1.3	11.5 ± 1.7
1 day	9	16.7 ± 1.5‡	10.6 ± 1.2	50.4 ± 3.8‡	13.4 ± 1.9	17.2 ± 2.9
3 days	5	14.9 ± 1.9‡	9.9 ± 1.2	52.6 ± 4.3	5.5 ± 1.7	10.4 ± 2.6
8 days	5	21.0 ± 1.0	6.6 ± 0.9	55.5 ± 2.4	9.0 ± 2.3	8.9 ± 1.7
17 days	9	16.8 ± 2.4‡	8.3 ± 1.1	58.9 ± 2.9	12.1 ± 2.9	12.3 ± 3.1
Adult	11	25.6 ± 2.9	9.3 ± 1.3	60.8 ± 1.9	7.3 ± 1.1	8.7 ± 1.1
P*	—	<0.001	NS	<0.001	NS	NS

Values are mean ± SEM.
* Results of Kruskal-Wallis analysis.
† Significantly different from all other groups ($P < 0.05$).
‡ Significantly different from adults ($P < 0.05$).

Results

Characteristics of the animals are shown in table 1. As expected, there was a progressive increase in both heart weight and body weight with age, together with a decrease in the ratio of heart weight: body weight.

In the absence of anesthetics, age-related changes in mechanical properties of skinned fibers were observed. The ratio of resting tension over total tension (RT/TT) did not differ between groups (table 2). However, maximal active developed tension was significantly lower in 1-, 3-, and 17-day-old rabbits compared with adults. Maximal tension of fetus skinned fibers was significantly lower than that of all other groups of rabbits, and the decrease in maximal tension with time was significantly less in fetuses than in any other groups. Maximal myofibrillar ATPase activities did not differ between groups (table 3). Calcium sensitivity differed significantly among various age groups of animals (table 4). Compared with adult rabbits, pCa_{50} was found significantly lower in 1-, 3-, and 8-day-old rabbits. A wide range of values were however observed in the group of 1-day-old rabbits; cardiac fibers (n = 2) taken from one of the five animals studied exhibited a high Ca^{2+} sensitivity close to that of fetuses, whereas the other fibers (n = 7) obtained from the four other animals had a much lower Ca^{2+} sensitivity, similar to that of 3- and 8-day-old animals. Mean pCa_{50} values of fetuses were significantly higher than those of 3- and 8-day-old animals but not different from adult values. No changes in the Hill coefficient values were observed (table 5).

In the presence of halothane or isoflurane, a significant decrease in calcium sensitivity (pCa_{50}) was observed in rabbits of all age groups (table 4), together with a decrease in maximal activated tension (table 2). Experimental tension/pCa curves obtained in one newborn animal (1-day-old) and one adult rabbit in the absence and in the presence of 1% halothane are shown in figure 1. Figure 2 shows the mean tension/pCa curves obtained by averaging all the experimental points in the group of 1-day-old (n = 9) and adult rabbits (n = 11). Both absolute curves and the same curves but normalized for maximal tension are represented in figures 2*A* and 2*B*, respectively. In the presence of anesthetics, the tension/pCa curves were shifted to the right, indicating a decrease in calcium sensitivity of cardiac skinned fibers. The effects of the two anesthetics studied, as assessed by changes in calcium sensitivity expressed in pCa units, were identical in a given age group of rabbits. However, there were significant age-related changes in the effects on calcium sensitivity (*P*

TABLE 3. Maximal ATPase Activity ($\mu mol \cdot mg^{-1}$ protein $\cdot min^{-1}$) of Left Ventricular Fibers and Percentages of Control during Exposure to 1% Halothane

Age	Fibers	Control	Halothane 1% (% of control)
Fetus	8	0.0168 ± 0.0015	112.4 ± 9.5
1 day	5	0.0261 ± 0.0035	93.0 ± 6.0
3 days	7	0.0191 ± 0.0018	100.4 ± 6.6
8 days	4	0.0205 ± 0.0025	92.0 ± 3.0
17 days	6	0.0149 ± 0.0021	101.0 ± 7.0
Adult	7	0.0241 ± 0.0038	95.6 ± 2.4

Values are mean ± SEM.

TABLE 4. Calcium Sensitivity (pCa_{50})

Age	Fibers	pCa_{50} Control	pCa_{50} Halothane 1%	pCa_{50} Isoflurane 1.5%
Fetus	9	5.521 ± 0.017	5.418 ± 0.022	5.415 ± 0.029
1 day	9	5.444 ± 0.036†	5.252 ± 0.033	5.248 ± 0.032
3 days	5	5.425 ± 0.017‡	5.278 ± 0.007	5.303 ± 0.017
8 days	5	5.385 ± 0.019§	5.274 ± 0.022	5.235 ± 0.029
17 days	9	5.527 ± 0.005	5.417 ± 0.014	5.399 ± 0.016
Adult	11	5.517 ± 0.010	5.395 ± 0.023	5.380 ± 0.024
P*	—	<0.02	<0.01	<0.001

Decreases in pCa_{50} values were statistically significant in all groups and no differences were observed between halothane and isoflurane effects. Values are mean ± SEM.
* Results of Kruskal-Wallis analysis.
† Significantly different from adults ($P < 0.05$).
‡ Significantly different from adults ($P < 0.01$).
§ Significantly different from adults ($P < 0.001$).

TABLE 5. Hill Coefficients

Age	Fibers	Control	Halothane 1%	Isoflurane 1.5%
Fetus	9	3.14 ± 0.18	3.43 ± 0.26	3.39 ± 0.24
1 day	9	2.62 ± 0.13	3.23 ± 0.30	3.28 ± 0.35
3 days	5	2.57 ± 0.16	2.85 ± 0.24	3.07 ± 0.19
8 days	5	2.56 ± 0.05	3.03 ± 0.07*	2.89 ± 0.09†
17 days	9	2.49 ± 0.13	3.34 ± 0.32*	3.40 ± 0.45†
Adult	11	2.55 ± 0.12	2.84 ± 0.14†	2.91 ± 0.13*

Values are mean ± SEM.
Control values did not differ among age groups.
* Significantly different from control in same age group ($P < 0.01$).
† Significantly different from control in same age group ($P < 0.05$).

< 0.01 for both agents). The decrease in calcium sensitivity in the presence of 1% halothane or 1.5% isoflurane was significantly greater in 1-day-old rabbits than in adults (fig. 3). The magnitude of this effect was similar within the five 1-day-old animals studied despite differences in Ca^{2+} sensitivities already mentioned in this age group. By contrast, this decrease was significantly less in fetuses than in newborn animals with both anesthetics. These age-related changes were even more evident when age-related changes in anesthetic potency were taken into account. The decrease in Ca^{2+} sensitivity observed in 1-day-old newborn rabbits was about twice that of adults, whereas in fetuses this decrease was two times less than that in adults and four times less than that in 1-day-old newborns.

Maximal developed tension decreased significantly in the presence of halothane or isoflurane but to a similar extent among different age groups of animals (table 2). However, when age-related changes in anesthetic potency were taken into account, the decrease in maximal tension was less important in fetuses than in all other age groups of rabbits. Maximal myofibrillar ATPase activity did not change during exposure to 1% halothane (table 3). In all age groups of animals there was a tendency for Hill coefficients to increase during anesthetic exposure, but significant changes were only observed in 8- and 17-day-old animals and in adults (table 5).

Discussion

This study demonstrates that halothane and isoflurane decrease both calcium sensitivity and maximal tension of left ventricular rabbit skinned fibers. The effects of both anesthetics were similar in a given age group of animals, but age-related differences were observed. Indeed, newborn animals were demonstrated to be more sensitive to the effects of anesthetics, whereas near term fetuses were less sensitive.

Developmental changes in mechanical properties of rabbit cardiac skinned fibers were observed. Changes in maximal developed tension are in agreement with previous studies.[35,36] Maximal developed tension was lower in fetuses compared with any other groups of rabbits; intermediate values were observed in newborn and immature rabbits and the highest in adult animals. At 20% above slack length, resting tension and maximal developed tension increased proportionately with animal age such that for any age group the RT/TT ratio was unchanged. This would likely indicate that the higher resting tension observed in intact heart of newborn animals[13] is due to immature excitation–contraction coupling rather than to structural or functional changes in the myofibrils themselves. The lower maximal active tension as observed in

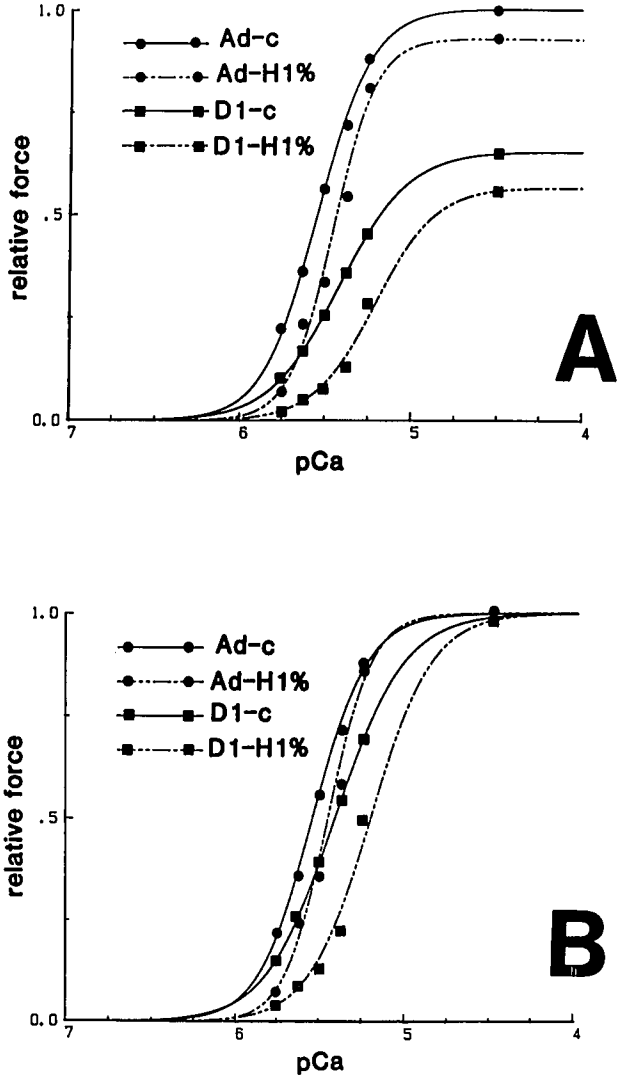

FIG. 2. Mean tension/pCa curves in adult rabbits (*circles*, n = 11) and in 1-day-old rabbits (*squares*, n = 9) in control conditions (*continuous lines*) and during exposure to 1% halothane (*dotted lines*). For reasons of clarity error bars are omitted. *A.* Mean maximal tension in adult rabbit in control conditions represents 100%. *B.* All curves are normalized for tension. A shift to the right (toward lower pCa values) indicates a decrease in calcium sensitivity.

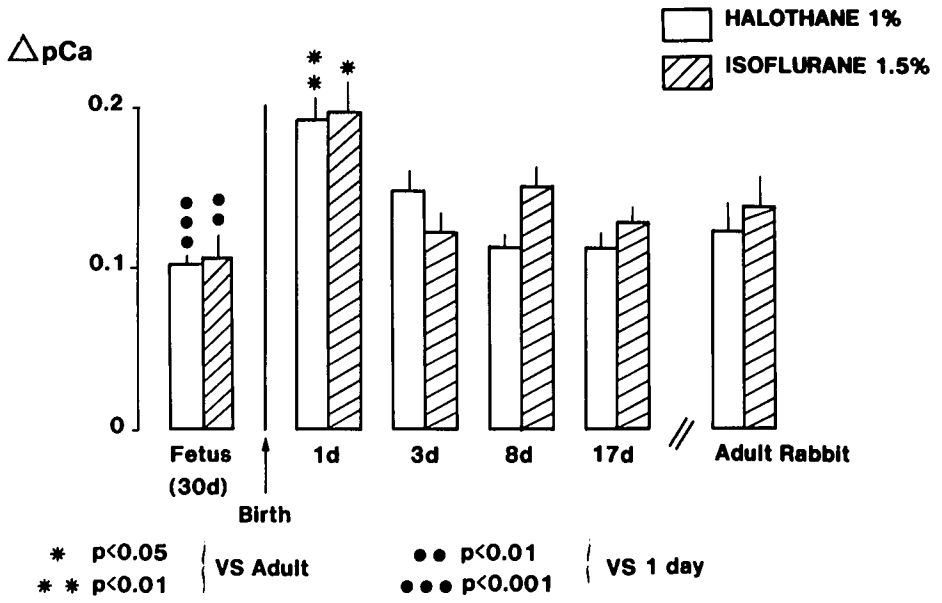

FIG. 3. Decrease in calcium sensitivity (expressed in pCa units) according to age and anesthetic exposure (halothane 1% and isoflurane 1.5%). The decrease in pCa_{50} was significantly more important in 1-day-old rabbits than in adults for both anesthetics. A decrease in calcium sensitivity was significantly less important in fetuses than in newborn rabbits.

immature animals is at least partly related to the decreased contractile mass in these animals compared with adults.[36,37] Maximal myofibrillar ATPase activity did not change with age. This would likely reflect the age-related changes in both myosin composition and properties.[38,39] Indeed, a shift from the rapid V1 to the slow V3 myosin isoform (as observed during the first 2 weeks of life in rabbits) would decrease maximal ATPase activity,[38] whereas maximal myofibrillar ATPase activity was demonstrated to be lower in immature than in mature hearts for a similar proportion of a given myosin isoform.[39] Besides these age-related changes, it should be emphasized that there is no simple relationship between force and ATPase activity.[40,41] Developmental changes in calcium sensitivity had been already reported in rat,[17,18] dog,[19] and rabbit[20,21] hearts. An increased Ca^{2+} sensitivity was first reported by Fabiato in ventricular fibers of 2-day prepartum fetal rats[17] compared with adult rats. Both Solaro *et al.*[18] and Krane and Su[20] observed a slight increase in Ca^{2+} sensitivity in newborn rats (1-day-old) or rabbits (1- to 4-day-old), but only one age group of animals was studied in both reports. A decreased ATPase Ca^{2+} sensitivity was observed in perinatal dog hearts.[19] Our results show that in rabbits an abrupt decrease in calcium sensitivity seems to occur at the time of birth, followed by a slower increase toward adult values within the first 2 weeks of life. Changes in myocardial calcium sensitivity may be related to changes in the composition in the troponin system, especially troponin T.[42-44] Changes in myosin isoforms as observed during development[38] do not play a role in developmental changes in calcium sensitivity, as this latter was demonstrated to be unchanged during experimental cardiac hypertrophy where a shift from the rapid V1 to the slow V3 myosin isoform is known to occur.[45]

The effects of halothane and isoflurane on myocardial Ca^{2+} sensitivity were greater in newborn rabbits than in adults. In a given age group of animals equipotent concentrations of isoflurane and halothane had a similar effect, which is consistent with the results of our previous study.[16] The greater effect of volatile anesthetics in newborn animals differs from the results of Krane and Su[20] who did not find differences in the effects of halothane on mechanically disrupted cardiac skinned fibers of 1- to 4-day-old and adult rabbits. These discrepancies between the two studies may arise from the method used to obtain skinned fibers. The term of "skinned fibers" refers to a number of different preparations in which the surface membrane of a muscle cell is removed or made permeable to small molecules. Both mechanically disrupted and detergent-treated fibers are multicellular preparations. In the first technique large amounts of sarcolemma are retained in the preparations.[22] The other cellular membranes, including sarcoplasmic reticulum, T-tubules, and mitochondria, are presumably intact, and these preparations can be used to study the drug effects on uptake and release capacities of sarcoplasmic reticulum. This latter function can however be blunted by high EGTA concentrations. Nonionic detergent will destroy all cellular membranes, whereas the contractile proteins are basically unaffected by this procedure. The basic problem when using skinned fibers is represented by the ability of the preparation to respond to intracellular phosphorylations as described in hyperpermeable fibers.[46,47] However, our detergent-treated skinned fibers are insensitive to phosphatase treatment (unpublished data), indicating that the

effects of volatile anesthetics reported in our study are not related to changes in the level of intracellular phosphorylations. At least two other differences between the experimental design of Krane and Su[20] and the present one may contribute to explain discrepancies between the two studies. First, they studied rabbit cardiac fibers of the right ventricle, whereas we used fibers from the left ventricle. We chose the left ventricle because most changes during and after birth will mainly affect the left ventricle rather than the right ventricle.[14,15,37,48] To understand the discrepancies between the two studies, we studied left and right ventricular fibers taken from the same heart of 3- and 17-day-old rabbits (n = 10). No differences in calcium sensitivity were observed when the right and the left fibers were compared, and the effects of halothane (1%) on calcium sensitivity were identical. The second possible explanation arises from differences in experimental conditions. The fibers of Krane and Su[20] were studied just above the slack length, whereas our fibers were stretched by 20% above the slack length, which corresponds to a sarcomere length of 2.1–2.2 μm. This length corresponds roughly to the plateau of the force/length relationship, which is reached for a similar sarcomere length in both newborn and adult animals.[36] A moderate amount of stretch reflects more physiologic working conditions than an absence of stretch (which represents the definition of the slack length).[49] It is known that myofibrillar Ca^{2+} sensitivity depends on the sarcomere length and that stretch will result in an increased affinity of myofibrils for Ca^{2+}.[41,50,51] Therefore, the difference in length may alter the functional state of the myofibrils, possibly explaining the discrepancies between the two studies. Indeed, the effects of other drugs that affect myofibrillar calcium sensitivity, such as caffeine, have been shown to be sarcomere length-dependent.[52]

The greater effect of volatile anesthetics in newborn animals may be related to changes in the troponin system that are known to occur around birth.[42–44] The molecular structure of troponin C does not seem to change in the developing heart, but calcium binding to myofibrils from 4-day-old rabbit hearts was found to be lower than that observed in 22-day-old and adult preparations.[53] It was recently suggested that developmental changes in troponin I isoforms may be responsible for the decreased sensitivity of neonatal cardiac muscle to acidosis, whereas developmental changes in troponin T isoforms would better correlate with changes in Ca^{2+} sensitivity.[18,19,42] In addition, the effect of volatile anesthetics on myocardial Ca^{2+} sensitivity seems to be independent of changes in myosin composition because halothane has a similar effect in normal and diabetic rats, whereas in these latter a shift from the normal V1 to the slow V3 myosin isoform is usually observed.[54] Therefore, the greater sensitivity to anesthetics of cardiac contractile proteins from newborn animals may be related to subtle changes in the composition of the troponin–tropomyosin complex. Other drugs, such as sulmazole and milrinone, which are known to exert their positive inotropic effects partly by enhancing calcium sensitivity of the contractile proteins, were also demonstrated to have different effects in developmental myocardium.[55,56] Mechanisms by which volatile anesthetics decrease maximal tension seem to involve processes of attachment and detachment of actomyosin cross-bridges, leading to decrease both the number of cross-bridges involved in force generation and the amount of force developed by individual cross-bridges.[57] Maximal ATPase activity did not change in the presence of 1% halothane. This agrees closely with the data of Merin *et al.*[58] who observed a decrease in maximal ATPase activity only for halothane concentrations higher than 0.9 mM. Thus, for clinical anesthetic concentrations the decrease in maximal force is not correlated with changes in ATPase activity but is probably related to changes in actin–myosin interactions, which alter transduction of energy at the cross-bridge level.[57]

The lesser effect of volatile anesthetics in near-term fetuses compared with 1-day-old newborn animals is an unexpected finding of this study. Few studies have focused on the effects of volatile anesthetics in near-term fetuses. Gregory *et al.*[8] in 1983 reported that fetal anesthetic requirements for halothane were significantly lower than those of both newborn and adult ewes (being about 25% and 50% of the respective neonatal and adult values) and that MAC values increased progressively over the first 12 h of life. Therefore, abrupt changes seem to occur at the time of birth which may be responsible for changes in the sensitivity of young animals to the negative inotropic effects of volatile anesthetics. This may involve structural and/or functional changes related to changes in loading conditions and/or in hormonal secretion, especially in thyroid function.[59]

In conclusion, our study demonstrates that halothane and isoflurane decrease calcium sensitivity of cardiac detergent-treated fibers to a greater extent in newborn rabbits than in adult rabbits. By contrast, fetal rabbits were less sensitive than newborn rabbits. These results may contribute to explain the greater sensitivity of neonatal hearts to the negative inotropic effects of these agents.

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