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Effect of Bupivacaine on the Isolated Rabbit Heart

Developmental Aspect on Ventricular Conduction and Contractility

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Background: **Newborns and infants seem to be at greater risk of bupivacaine cardiotoxicity than adults do. Few experiments have studied the effects of local anesthetics on myocardium associated with developmental changes, and their conclusions are conflicting. The authors compared the effects of bupivacaine on an isolated heart preparation in newborn and adult rabbits.**

Methods: **The authors used a constant-flow, nonrecirculating Langendorff preparation paced atrially. Adult and newborn rabbit hearts were exposed to step-increasing concentrations of bupivacaine. For each concentration, heart rate was modified with pacing from 180 to 360 beats/min by increments of 30 beats/min. QRS complex duration (index of ventricular conduction) and the first derivative of left ventricular pressure (index of contractility) were measured. The two groups were compared using an Emax model.**

Results: **In newborn and adult rabbits, QRS complex duration increased with increasing bupivacaine concentration. No difference was observed between neonatal and adult hearts. Contractility decreased with increasing bupivacaine concentration. Newborn rabbits were approximately three times more sensitive than adult rabbits to the effects of bupivacaine. However, the concentration leading to 50% decrease in the first derivative of left ventricular pressure was much higher than the concentration leading to half maximum increase in QRS complex duration.**

Conclusions: **In conclusion, using a whole organ preparation, the authors demonstrated that bupivacaine induces similar impairment in ventricular conduction in newborn and adult rabbits. In particular, the tonic and the phasic blocks were of similar intensity in both groups. Conversely, the effect of bupivacaine on contractility was markedly higher in newborn rabbits than in adult rabbits. Also, contractility was less impaired than ventricular conduction in both groups.**

BUPIVACAINE is a long-acting local anesthetic agent with marked toxic effects on the heart. Newborns and infants seem to be at greater risk of bupivacaine cardiotoxicity than adults.[1,2] Pharmacokinetic and pharmacodynamic causes may be advocated as the origin of this increased toxicity. Increased myocardial sensitivity has been related to pharmacokinetic differences between age groups.[3] Serum protein binding is lower in neonates and in infants than in adults because of a low concentration of α_1-acid glycoprotein.[4,5] This decrease in the binding capacity of serum proteins may lead to a high free bupivacaine concentration. Because the free drug concentration is considered to be the toxic moiety, it is highly plausible that neonates and infants may be more prone to the cardiotoxic effects of bupivacaine than older children and adults. This phenomenon is amplified by the low hepatic clearance observed in younger patients due to the immaturity of some cytochrome P-450 isoforms (3A4 for bupivacaine and ropivacaine, 1A2 for ropivacaine).[5–7]

However, this increased toxicity may be of pharmacodynamic origin because of a greater cardiac sensitivity of young subjects to bupivacaine as compared with adults. The direct effect of bupivacaine on the heart (on ventricular conduction and on contractility) may also change with development. Few experiments have studied the effects of local anesthetics (lidocaine or bupivacaine) on myocardium associated with developmental changes.[8–11] These experiments studied the currents elicited by depolarization of cardiomyocytes, Purkinje fibers, or papillary muscles. However, they yielded divergent results, likely because of the different methodologies used. Therefore, we compared the effects of bupivacaine on a whole isolated rabbit heart preparation in newborn and adult animals.

Materials and Methods

Langendorff Preparation

We used an isolated rabbit heart model as previously described.[12–14] The study was approved by our local institutional animal care committee (Université de Paris-Sud, Le Kremlin Bicêtre, France). This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Helsinki Declaration. Thus, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (J.-X. M.). Adult New Zealand rabbits (weight, 1,650–1,950 g) were anesthetized with 6 mg/kg pentobarbital intraperitoneally. A tracheotomy was performed, and the animals were man-

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ually ventilated. The chest was opened, and after intravenous heparin injection, the heart was removed and quickly mounted on a nonrecirculating Langendorff apparatus, and coronary arteries were perfused *via* the aorta at a constant flow of 40 ml/min with a modified Krebs-Henseleit buffer bubbled with a mixture of 95% O_2 and 5% CO_2. The hearts were paced atrially throughout the study with a bipolar electrode using a Grass S88 stimulator (Astromed, Trappes, France). The same buffer with the following composition was used throughout the study: 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 5.5 mM glucose, and 2.0 mM Na pyruvate. The pH of the perfusate was maintained between 7.37 and 7.42, and the temperature of the preparation was maintained at 37° ± 0.1°C with a water-jacketed container. Two- or 3-day-old New Zealand rabbits (weight, 55–80 g) were anesthetized with 6 mg/kg pentobarbital intraperitoneally. The chest was rapidly opened, the heart was rapidly excised, and the aorta was cannulated in cold buffer and mounted on the Langendorff apparatus. The buffer used for newborn rabbits was similar to the buffer used for adult rabbits. In a pilot study, two different aortic flows were tested in two groups of five hearts each, a normal flow of 6 ml/min[15,16] and a high flow of 12 ml/min. This experiment showed (1) that spontaneous heart rate and ventricular pressures were similar with both flows and (2) that bupivacaine uptake and effect on QRS complex duration was related to the concentration, not to the dose. The newborn rabbits were then infused at an inflow rate of 6 ml/min. The following criteria for validity of the preparation were used: absence of aortic regurgitation and spontaneous sinus rhythm between 120 and 170 beats/min without arrhythmias. When ventricular pressure was measured, a first derivative of left ventricular pressure (P/dt_{max}) above 1,000 mmHg/s (adult rabbits) or above 400 mmHg/s (newborn rabbits) was also required. Six adult hearts and eight newborn hearts (four for QRS duration and four for dP/dt measurement) were studied at random to verify the stability of the preparation.

Experimental Protocols

Because it was not possible to simultaneously study the electrocardiogram and the left ventricular pressure in the newborn rabbits, two different experiments were conducted. In a first series of experiments, the effect of bupivacaine on QRS duration (ventricular conduction) was tested. Eight newborn and eight adult rabbits were studied. After a 10- to 12-min stabilization period, bupivacaine (Sigma, Saint Quentin-Fallavier, France) was infused into the inflow perfusate using 10-min ascending and descending steps at 0, 0.8, 1.7, 2.5, 3.3, 2.5, 1.7, 0.8, and 0 μM. An electrocardiogram was recorded via surface electrodes by a Gould monitor (Gould Electronics, Ballainvilliers, France) connected to a personal computer by a Powerlab/4S analog-to-digital converter (AD Instruments, Les Ulis, France). The data were analyzed using Chart Software version 3.4.6 (AD Instruments, Les Ulis, France). To quantify the rate dependence of QRS duration, the rate of pacing was modified in a predetermined random order from 180 to 360 beats/min using 30-beat/min intervals (180, 210, 240, 270, 300, 330, and 360 beats/min). The measurements were performed during the last minute of each infusion step.

In a second series of experiments, the effect of bupivacaine on ventricular contraction force was measured. In the adult rabbits, the left atrium was incised, and a balloon catheter (Hugo Sachs Electronik, March-Hugstetten, Germany) was inserted into the left ventricle. The balloon was inflated with a constant volume of saline (0.6 ml), which was not modified during the experiment. The same procedure was performed in the newborn rabbits, using a balloon containing 0.12 ml saline. The ventricular pressure was measured, and the maximal value of the first derivative of the left ventricular pressure as a function of time (dP/dt_{max}) was computed. Six newborn and five adult rabbits received the same concentrations using the same paradigm as previously described. In addition, five newborn and six adult rabbits received bupivacaine at 0, 5, 8, and 12 μM using the same increasing and decreasing steps. However, in this group, most hearts escaped from pacing at the highest rates. Thus, pacing was not done at the same predetermined rates for all hearts. Nevertheless, all the contractility data were fitted together using an additional random effect for between-occasion variability.

Pharmacodynamic Modeling

Because QRS duration was not influenced by heart rate in the absence of drug, the increase in QRS duration (E) was fitted to a simple Emax model considering pseudo–steady state:

$$E = E_0 + \frac{Emax\ C^{\gamma}}{C_{50}^{\gamma} + C^{\gamma}}, \quad (1)$$

where E_0 is the basal QRS duration, Emax is the maximum increase in QRS duration, C_{50} is the drug concentration in the perfusate producing half the maximum increase in QRS duration, C is the inflow perfusate bupivacaine concentration, and γ is the Hill term for sigmoidicity. Preliminary fitting showed that dP/dt_{max} was dependent on both heart rate and drug concentration in perfusate. A combined-effect model was then used, considering additivity of the effects of increasing heart rate and increasing concentration:

$$E = E_0\left(1 - \frac{\Sigma\ V_i^{\gamma i}}{1 + \Sigma V_i^{\gamma i}}\right), \quad (2)$$

where $V_i^{\gamma i}$ is the normalized concentration (C/C_{50}) or heart rate (HR/HR_{50}) and γ_i is the Hill coefficient of sigmoidicity. Here, C_{50} is the drug concentration in the

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Table 1. QRS Duration in the Two Groups as Function of Heart Rate and Bupivacaine Concentration

	Heart Rate						
Dose	180 beats/min	210 beats/min	240 beats/min	270 beats/min	300 beats/min	330 beats/min	360 beats/min
Neonatal hearts							
0 μM	26.19 ± 3.5	25.48 ± 2.98	25.73 ± 3.50	26.05 ± 4.01	25.77 ± 3.46	26.28 ± 3.31	26.53 ± 3.74
0.8 μM	26.7 ± 2.13	28.09 ± 2.34	29.53 ± 2.67	30 ± 3.04	30.47 ± 2.89	31.88 ± 3.34	34.64 ± 4.24
1.7 μM	30.98 ± 3.61	33.07 ± 2.70	34.88 ± 4.30	35.7 ± 4.22	39.14 ± 4.34	42.03 ± 6.08	45.36 ± 8.68
2.5 μM	35.63 ± 4.59	39.38 ± 6.81	41.56 ± 6.0	41.81 ± 6.63	51 ± 7.04	56.79 ± 9.38	67.08 ± 9.38
3.3 μM	41.75 ± 8.64	44.17 ± 12.01	48.13 ± 11.93	53.5 ± 16.16	57.81 ± 9.49	64.69 ± 11.83	78.13 ± 15.19
Adult hearts							
0 μM	26.23 ± 3.72	26.08 ± 3.42	26.11 ± 3.83	26.27 ± 3.92	26.7 ± 3.50	26.42 ± 4.05	26.42 ± 4.05
0.8 μM	27.5 ± 4.26	28.64 ± 4.17	29.27 ± 4.81	29.55 ± 4.93	30.64 ± 4.89	30.56 ± 6.20	30.95 ± 6.27
1.7 μM	30.48 ± 5.84	35.44 ± 5.58	36.58 ± 5.92	38.98 ± 6.84	42.31 ± 8.38	41.86 ± 7.5	44.39 ± 9.46
2.5 μM	42.75 ± 11.26	42.91 ± 12.14	45.52 ± 11.83	47.41 ± 12.83	50.75 ± 12.28	54 ± 15.14	60.25 ± 16.26
3.3 μM	42.25 ± 8.86	45.75 ± 17.39	47.68 ± 11.24	50.83 ± 12.44	58.54 ± 16.78	65.83 ± 16.18	83.13 ± 12.31

Data are presented as mean ± SD (in milliseconds). Eight rabbit hearts were studied in each group.

perfusate leading to 50% decrease in dP/dt_{max}, and HR_{50} is the heart rate leading to 50% decrease dP/dt_{max}.

Statistical Analysis

Between-group comparison of QRS duration and of dP/dt_{max} measured before and at the end of infusion (*i.e.*, without any drug in the perfusate) was performed using analysis of variance. Results are expressed as the arithmetic mean ± SD, with $P < 0.05$ as the minimum level of significance. The data were fitted using nonlinear mixed-effect modeling. The significant difference between parameters describing adult and neonatal myocardial properties was tested using the log likelihood ratio test with $P < 0.01$ as the minimum level of significance. An approximate 95% confidence interval was calculated for Emax and C_{50} using log likelihood profiling (a bootstrap analysis was not possible because of prohibitive calculation time).

Results

Preliminary experiments performed in newborn rabbits with two different aortic flows (6 and 12 ml/min, n = 5 each) showed similar QRS duration (26.3 ± 4.2 *vs.* 27.7 ± 4.9 ms, low *vs.* high flow, respectively) and similar dP/dt_{max} at 240 beats/min (780 ± 366 *vs.* 832 ± 311 mmHg/s, low *vs.* high flow, respectively) in the two groups. Moreover, in these two groups, the effect of bupivacaine was related to the drug concentration (amount/volume/time) in the perfusate, not to the amount (amount/time). QRS duration remained constant throughout the study period in control hearts, whereas dP/dt_{max} decreased by less than 10% during the 80-min observation period.

Basal QRS duration was similar in the newborn and adult rabbits (26.2 ± 3.5 *vs.* 26.2 ± 3.7 ms, newborns *vs.* adults, respectively). QRS duration was constant in the range of rates used both for the adult rabbits and for the newborn rabbits (180–360 beats/min). When bupivacaine was added to the perfusate buffer, QRS duration increased, and the widening exhibited marked rate dependence, with a significant increase in Emax (maximum QRS duration) when heart rate was increased (tables 1 and 2). However, no difference between adult and newborn rabbits was observed (fig. 1).

Basal dP/dt_{max} was much lower in the newborn rabbits than in the adult rabbits (830 ± 424 *vs.* 2,244 ± 741 mmHg/s in the low-concentration group and 618 ± 221 *vs.* 1,749 ± 639 mmHg/s in the high-concentration group, newborns *vs.* adults, respectively; table 3). When heart rate was increased by pacing, dP/dt_{max} decreased rapidly both in the adult rabbits and the newborn rabbits. In the high-concentration group, most hearts escaped from pacing at the highest rates, and some hearts exhibited a spontaneous heart rate of less than 180 beats/min. In the absence of bupivacaine, increasing heart rate induced a progressive decrease in dP/dt_{max}, which was more pronounced in the adult rabbits than in the newborn rabbits (tables 3 and 4 and fig. 2). The effect of bupivacaine was greater in the newborn hearts than in the adult hearts (table 3 and fig. 3). The effects of frequency and of bupivacaine were strictly additive: HR_{50} estimated by fitting a subset of data containing only

Table 2. Summary of Fitting (Ventricular Conduction)

Heart Rate, beats/min	Emax*, ms	C_{50}*, μM	γ*
180	75 (50.8–132)	4.03 (3.21–5.93)	2.62 (2.08–3.2)
210	88.4 (59.6–153)	†	†
240	98.5 (71.6–169)	†	†
270	108 (74.3–191)	†	†
300	133 (89.6–234)	†	†
330	153 (105–269)	†	†
360	185 (126–326)	†	†

Data are presented as typical value of estimated parameter and 95% confidence interval obtained by profiling the log likelihood in parentheses.

* Not significantly different between adult and newborn rabbits. † Not significantly different when heart rate increases.

C_{50} = drug concentration in the perfusate producing half Emax; Emax = maximum increase in QRS duration; γ = Hill coefficient of sigmoidicity.

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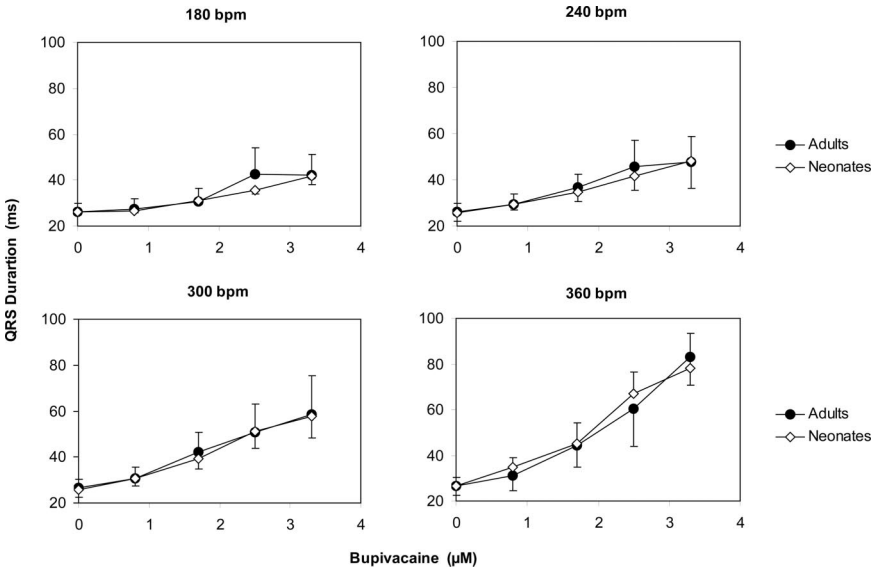

Fig. 1. QRS widening as a function of bupivacaine concentration in the perfusate. The block is rate dependent, but no difference was found between adult and newborn rabbits. bpm = beats/min. Data are presented as mean ± SEM for clarity.

the dP/dt_{max} data with no bupivacaine in the perfusate was not statistically different from HR_{50} obtained when fitting was performed with the whole data set. Because the effect observed is due to the combination of frequency and concentration, we calculated an apparent C_{50} at different heart rates (table 5).

Discussion

Our results show that, within the limits of our methodology, ventricular conduction impairment measured by the QRS widening is similar in newborn and adult rabbit ventricle. However, the decrease in contractility measured by the dP/dt_{max} was significantly less in adult hearts than in neonatal hearts (C_{50} was three times lower in neonatal hearts than in adult hearts at 240 beats/min). The two ventricular functions (conduction and contractility) were sensitive to heart rate, *i.e.*, both tonic and phasic (use-dependent) blocks occurred, but the effect of frequency was strictly identical in neonatal and adult hearts for conduction slowing, whereas the effect of heart rate

Table 3. Maximum Value of the First Derivative of Left Ventricular Pressure as Function of Heart Rate and Bupivacaine Concentration

	Heart Rate						
	180 beats/min	210 beats/min	240 beats/min	270 beats/min	300 beats/min	330 beats/min	360 beats/min
Neonatal hearts							
Low concentration							
0.0 μM	830 ± 121 (n = 6)	819 ± 131 (n = 6)	809 ± 135 (n = 6)	781 ± 128 (n = 6)	751 ± 133 (n = 6)	700 ± 114 (n = 6)	586 ± 118 (n = 5)
0.8 μM	756 ± 74 (n = 6)	763 ± 119 (n = 6)	733 ± 75 (n = 6)	725 ± 136 (n = 6)	739 ± 221 (n = 6)	643 ± 132 (n = 6)	538 ± 93 (n = 4)
1.7 μM	729 ± 107 (n = 6)	730 ± 125 (n = 6)	710 ± 152 (n = 6)	668 ± 199 (n = 6)	668 ± 172 (n = 6)	504 ± 250 (n = 4)	422 ± 177 (n = 4)
2.5 μM	693 ± 115 (n = 6)	711 ± 161 (n = 6)	662 ± 166 (n = 6)	689 ± 134 (n = 5)	641 ± 153 (n = 6)	442 ± 287 (n = 3)	370 ± 203 (n = 4)
3.3 μM	637 ± 140 (n = 6)	630 ± 219 (n = 6)	641 ± 190 (n = 6)	620 ± 202 (n = 4)	566 ± 115 (n = 6)	354 ± 306 (n = 3)	296 ± 216 (n = 3)
High concentration							
0.0 μM	618 ± 126 (n = 5)	621 ± 95 (n = 5)	633 ± 165 (n = 5)	600 ± 104 (n = 5)	562 ± 92 (n = 5)	517 ± 76 (n = 5)	491 ± 81 (n = 5)
5.0 μM	527 ± 90 (n = 5)	542 ± 206 (n = 4)	500 ± 138 (n = 4)	357 ± 217 (n = 4)	345 ± 116 (n = 4)	— (n = 0)	— (n = 0)
8.0 μM	441 ± 84 (n = 5)	375 ± 104 (n = 3)	364 ± 99 (n = 3)	— (n = 0)	— (n = 0)	— (n = 0)	— (n = 0)
12.0 μM	321 ± 95 (n = 3)	— (n = 0)	— (n = 0)	— (n = 0)	— (n = 0)	— (n = 0)	— (n = 0)
Adult hearts							
Low concentration							
0.0 μM	2,244 ± 212 (n = 5)	2,036 ± 174 (n = 5)	1,828 ± 130 (n = 5)	1,729 ± 141 (n = 5)	1,511 ± 144 (n = 5)	1,280 ± 151 (n = 5)	1,142 ± 139 (n = 5)
0.8 μM	1,993 ± 238 (n = 5)	1,825 ± 375 (n = 5)	1,791 ± 263 (n = 5)	1,708 ± 232 (n = 5)	1,532 ± 147 (n = 5)	1,309 ± 260 (n = 5)	1,168 ± 133 (n = 5)
1.7 μM	1,828 ± 311 (n = 5)	1,803 ± 402 (n = 5)	1,753 ± 205 (n = 5)	1,689 ± 205 (n = 5)	1,481 ± 218 (n = 5)	1,258 ± 133 (n = 5)	1,122 ± 68 (n = 5)
2.5 μM	1,719 ± 526 (n = 5)	1,686 ± 388 (n = 5)	1,708 ± 217 (n = 5)	1,645 ± 155 (n = 5)	1,474 ± 184 (n = 5)	1,059 ± 294 (n = 5)	944 ± 150 (n = 3)
3.3 μM	1,697 ± 498 (n = 5)	1,680 ± 343 (n = 5)	1,642 ± 129 (n = 5)	1,582 ± 151 (n = 5)	1,372 ± 168 (n = 5)	944 ± 640 (n = 2)	842 ± 326 (n = 4)
High concentration							
0.0 μM	1,824 ± 183 (n = 6)	1,663 ± 128 (n = 6)	1,539 ± 148 (n = 6)	1,466 ± 139 (n = 6)	1,387 ± 140 (n = 6)	1,170 ± 66 (n = 6)	949 ± 106 (n = 6)
5.0 μM	1,480 ± 378 (n = 6)	1,546 ± 476 (n = 6)	1,421 ± 194 (n = 5)	1,438 ± 231 (n = 6)	1,087 ± 300 (n = 6)	537 ± 402 (n = 4)	638 ± 180 (n = 5)
8.0 μM	1,264 ± 442 (n = 5)	1,392 ± 574 (n = 4)	1,001 ± 378 (n = 3)	768 ± 297 (n = 6)	— (n = 0)	— (n = 0)	— (n = 0)
12.0 μM	956 ± 669 (n = 4)	1,073 ± 562 (n = 2)	— (n = 0)	— (n = 0)	— (n = 0)	— (n = 0)	— (n = 0)

Data are presented as mean ± SD (in mmHg) and number of hearts efficiently paced in parentheses.

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Table 4. Summary of Fitting (Contractility)

	$dP/dt_{max}0$, mmHg/s	HR_{50}, beats/min	C_{50}, μM	γ_{HR}	γ_D
Adult hearts	1780 (1,720–1,805)	386 (364–403)	48.5 (47.2–49.9)	4	1.08
Neonatal hearts	693 (692–694)	469 (464–472)	15.6 (8.95–26.2)	4*	1.08*

Data are presented as typical value of estimated parameter and 95% confidence interval obtained by profiling the log likelihood in parentheses.

* Not significantly different between adult and newborn rabbits.

C_{50} = dose of bupivacaine leading to half decrease in dP/dt_{max} at HR = 0; $dP/dt_{max}0$ = estimated basal dP/dt_{max} at HR = 0 beats/min and bupivacaine dose = 0 μM; γ_D = Hill coefficient of sigmoidicity for the bupivacaine component; γ_{HR} = Hill coefficient of sigmoidicity for the rate component; HR_{50} = heart rate leading to half decrease in dP/dt_{max} at bupivacaine dose = 0 μM.

was higher in adult hearts than in neonatal hearts for dP/dt_{max}.

Models Used

Our Langendorff preparation at constant flow allowed us to adequately measure the effect of drugs on both ventricular conduction (QRS duration) and contractility (left ventricular dP/dt_{max}). QRS duration may be considered to reflect only ventricular conduction,[17,18] not auriculoventricular conduction, which mainly depends on the calcium channels activity.[19] Because the preparation is infused with Krebs solution, the amount of drug in the perfusate corresponds to the free drug concentration in the case of protein-bound drugs such as local anesthetics. However, the preparation has a relatively low oxygen supply, which induces an inverse force–frequency relation from heart rates of approximately 180 beats/min. Finally, we used rabbits because interspecies comparison of ventricular contractile functions show that rabbits are relatively close to humans (the order is usually human, dog, rabbit, rat, mouse[20,21]).

We used a simplification of our previously described pharmacokinetic–pharmacodynamic model.[12–14] In a pilot study, we determined the relation among inflow rate, amount of drug infused, and effect (QRS duration and dP/dt_{max}) in the neonatal hearts. Doubling the inflow perfusion rate did not modify the effect when bupivacaine concentration remained constant in the perfusate. However, when bupivacaine was infused on a mass basis (constant amount per time), the effect was decreased when the inflow rate was doubled. This clearly showed that, in the range of bupivacaine concentration administered and in the range of perfusate flow, uptake was rate limited rather than flow limited. Therefore, we decided (1) to use a simplification of our pharmacokinetic–pharmacodynamic model and (2) to use a normal flow (6 ml/min) rather than a high flow (12 ml/min) design for the neonatal hearts. We have previously showed that QRS widening[12,14] and change in dP/dt_{max}[13] occurred nearly immediately after drug infusion changes and that steady effect was attained in less than 2–3 min. Therefore, we considered that the effects occurred in the central compartment, and the concentration–effect data were directly fitted to a Hill equation. The use of nonlinear mixed-effect modeling allowed us to perform one-stage modeling (for each effect) with the log likelihood ratio test rather than a multistage procedure.

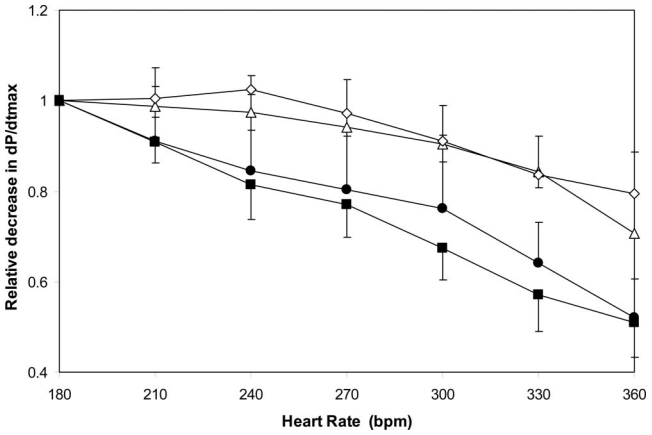

Fig. 2. Relative decrease in the first derivative of left ventricular pressure (dP/dt_{max}) as a function of heart rate. Adult rabbit hearts are significantly more sensitive than newborn rabbit hearts to the increase in heart rate. Neonatal values are depicted by *open triangles* (low-concentration group) and *open diamonds* (high-concentration group). Adult values are depicted by *closed squares* (low-concentration group) and *closed circles* (high-concentration group). bpm = beats/min. Data are presented as mean ± SEM for clarity.

Effect of Bupivacaine on Ventricular Conduction

As usual, bupivacaine increased the QRS duration in a dose-dependent manner. This impairment in ventricular conduction was rate dependent. As we previously showed, basal QRS duration was remarkably constant in the range of heart rates studied, in both newborn and adult rabbits. No difference in block was observed between the two groups in the tonic or the phasic component of the blocks (table 2 and fig. 1). Values of C_{50}, the bupivacaine concentration leading to half maximum effect, were similar at all heart rates in both groups, but Emax increased regularly with increasing stimulation rate, being nearly 2.5 times higher at 360 beats/min than at 180 beats/min. C_{50} was lower in this experiment than in our previous experiments.[14] This is likely due to the difference in the methodology.[13] However, it is important to remember that the inflow perfusate was made of buffer without any protein and that this concentration represents the free drug concentration. Adult and newborn rabbit hearts behaved identically, but the effect of

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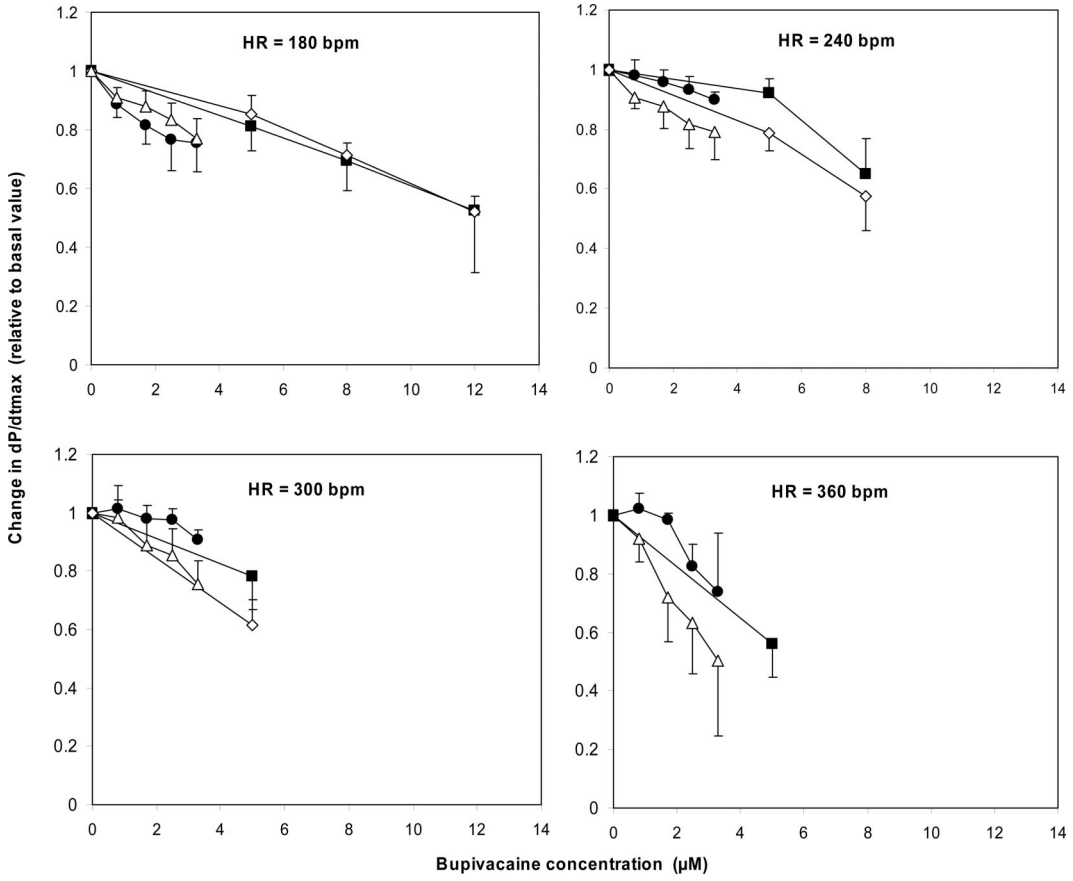

Fig. 3. Relative decrease in the first derivative of left ventricular pressure (dP/dt_{max}) as a function of bupivacaine concentration. At low frequency of stimulation (180 beats/min), no heart escaped from pacing, and the adult and newborn rabbit hearts behaved similarly (newborn rabbits are significantly more sensitive to bupivacaine but significantly less sensitive to frequency than adult rabbits). Progressively, when heart rate increased, some hearts escaped from pacing at the higher rates, and the higher sensitivity of newborn rabbits to bupivacaine became more evident. Neonatal values are depicted by *open triangles* (low-concentration group) and *open diamonds* (high-concentration group). Adult values are depicted by *closed squares* (low-concentration group) and *closed circles* (high-concentration group). bpm = beats/min; HR = heart rate. Data are presented as mean ± SEM for clarity.

bupivacaine on the ventricular conduction may be considered in its clinical context: newborn rabbits have a much higher heart rate than do adult rabbits, and thus, the effect of bupivacaine on conduction is certainly greater in the younger subjects than in the older ones, only because of the difference in heart rate.

Table 5. Calculated C_{50} at Various Heart Rates in Adult and Newborn Rabbit Hearts

	C_{50}, μM	
Heart Rate, beats/min	Adult Hearts	Neonatal Hearts
180	46.5	15.2
210	44.4	15.0
240	41.6	14.7
270	37.6	14.1
300	31.8	13.3
330	23.9	12.1
360	13.0	10.5
0*	48.5	15.6

* C_{50} (dose of bupivacaine leading to half decrease in the first derivative of left ventricular pressure) estimated by the model is calculated for a theoretical heart rate of 0 beats/min.

Effect of Bupivacaine on Ventricular Contractility

Contrary to the lack of effect of tachycardia on conduction in the absence of bupivacaine, increasing heart rate markedly altered contractility in our preparation. When heart rate increased, dP/dt_{max} decreased, at least in the range of frequencies studied, *i.e.*, above 180 beats/min (fig. 2). This effect of heart rate was less in newborn rabbits than in adult rabbits, with an HR_{50} (stimulation rate leading to 50% decrease in the theoretical basal dP/dt_{max} at rate zero) higher in newborn rabbits (469 beats/min) than in adult rabbits (386 beats/min). This is not surprising because newborn rabbits have a natural heart rate higher than adult rabbits do. When bupivacaine was infused, ventricular contractility estimated by the dP/dt_{max} decreased (fig. 3). The block extrapolated at heart rate zero was more than three times greater in newborn rabbits than in adult rabbits (table 4). The decrease in contractility observed when heart rate was increased was similar regardless of whether bupivacaine was added to the perfusate. We may then consider that, contrary to ventricular conduction, contractility impair-

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ment is not influenced by tachycardia, *i.e.*, no phasic block occurs for contractility. However, adult rabbits are more sensitive *per se* to the effects of increasing heart rate (at 180 beats/min, the C_{50} ratio between adults and newborn rabbits was 3.0, and at 360 beats/min, the C_{50} ratio was 1.2; table 5 and fig. 3).

Our results show marked differences between the effects of bupivacaine on conduction and the effect on contractility. First, contractility was less impaired than ventricular conduction: C_{50} was 7–10 times higher for contractility than for conduction in the adult hearts, depending on heart rate. This difference in the effects of bupivacaine on ventricular conduction and contractility was less marked in the neonatal hearts, with only a difference between 2.5 and 3.5, depending on heart rate. We observed similar basal QRS durations between the two groups, likely due to a lower basal conduction velocity in neonatal myocardium than in adult myocardium. However, the increase in QRS duration as a function of bupivacaine concentration was the same in the two groups, and no difference in the phasic block was observed. This lack of age-related changes in the effects of bupivacaine on QRS duration suggests that the blocks (both tonic and phasic) induced by bupivacaine on ionic channels involved in ventricular conduction do not vary with age. Using neonatal and adult cardiomyocytes, Xu *et al.*[10] showed that lidocaine produced both more tonic and more phasic block in neonatal tissue than in adult tissue. However, this study was performed with very negative holding potential (−140 mV). Using a similar preparation, Sun and Rosen[11] did not find any age-related effect of bupivacaine on phasic block. Studying the effect of lidocaine on guinea pig papillary muscle, Jeck and Rosen[8] did not find any difference in phasic block between neonatal and adult tissues. However, in this latter study, the effect of bupivacaine on canine epicardium was different with a marked age-related use-dependent effect. Because all of these studies used different tissues from different species and also because they studied either the transmembrane Vmax or the whole action potential,[9] it is difficult to draw any definite conclusion. Ventricular conduction depends mainly on the activity of cardiac sodium channels, and bupivacaine impairs conduction by blocking cardiac sodium channels.[17] This effect on sodium channels leads to ventricular conduction slowing with a marked phasic block.[14,22,23] We observed similar findings in the current experiment, but no age-related difference was observed.

The effects on contractility are not so simple, and many channels of receptors are potential sites of action. Transduction of the conduction signal by the sodium channels,[24] effect on calcium channels, ryanodine receptors, sodium–calcium exchanger, or potassium channels seem to be intricately associated to induce the effect of agents such as bupivacaine on cardiomyocyte contractility.[25–28] We observed an increased sensitivity of newborn rabbit myocardium as compared with adult rabbit myocardium, which is not surprising because changes in contractility associated with development are well known.[29] It is interesting to note that, studying the effects of halothane and isoflurane on newborn and adult rabbits hearts, Palmisano *et al.*[30] described age-related differences in the depression elicited by halothane on systolic function. We have measured the same higher sensitivity to bupivacaine in neonatal hearts as compared with adult hearts. What is more surprising is the lack of phasic block observed with contractility impairment. Targets different from frequency-dependent ionic channels are likely involved in the effect of bupivacaine on contractility.

In conclusion, using a whole organ preparation, we demonstrated that bupivacaine induces a similar impairment in ventricular conduction in newborn and adult rabbits. In particular, the tonic and phasic blocks were of similar intensity in both groups. Conversely, the effect of bupivacaine on contractility was markedly higher in newborn rabbits than in adult rabbits. Also, contractility was less impaired than was ventricular conduction in both groups. Although these experiments must be confirmed in different species and with different methodologies, we believe that care should be taken in neonates and infants when using long-lasting local anesthetics.

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