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The Electrophysiologic Effects of Bupivacaine on Adult, Neonatal, and Fetal Guinea Pig Papillary Muscles

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The authors used standard microelectrode techniques to study developmental changes in the effects of bupivacaine on the transmembrane potentials of adult, neonatal, and fetal guinea pig papillary muscles. Bupivacaine hyperpolarized membrane potential in the adult and neonatal muscles but not the fetal muscles. In all three age groups, action potential overshoot and the maximum rate of increase of phase 0 ($\dot{V}_{max}$) were significantly reduced by bupivacaine ≥ 1.0 μg/ml. Bupivacaine 1.5 μg/ml reduced action potential duration at both 50% and 100% repolarization in the fetal tissues, but not in adult or neonatal tissues. Tonic block was not induced by bupivacaine in any of the three groups. Use-dependent block was variable at bupivacaine 0.2 μg/ml in all three groups and was consistent and equivalent at higher concentrations. The onset and offset of use-dependent block were the same in all three groups, with onset occurring between 6.0 and 6.7 beats and the time constant for recovery being 1.9–2.3 s. The authors conclude there is an age-related bupivacaine effect on action potential duration but no age-related change in bupivacaine-induced use-dependent block. (Key words: Anesthetics, local: bupivacaine. Heart, papillary muscles: cellular electrophysiology. Species, guinea pig: adult; fetal; neonatal.)

BUPIVACAINE is a commonly used local anesthetic. Like other local anesthetics, it acts by blockade of the fast inward sodium current in neural tissues.[1,2] Local anesthetics such as lidocaine have a similar action on cardiac tissues and are used as antiarrhythmic agents. However, bupivacaine's actions on cardiac tissues have become important only as adverse side effects.[3] Reports of death resulting from the accidental intravascular injection of bupivacaine during obstetric anesthesia have been attributed to cardiotoxicity.‡ Kotelko *et al.*[4] studied the cardiac effects of equivalent intravenous doses of bupivacaine and lidocaine in sheep. Both drugs produced similar central nervous system toxicity, but only bupivacaine induced serious cardiac arrhythmias. These included ventricular tachycardias, multiform premature ventricular contractions, and ventricular fibrillation. Kasten and Martin[5] showed that bupivacaine induced Torsade de Pointes and other dysrhythmias in a dog model. Clarkson and Hondeghem[6] compared the cellular electrophysiologic effects of lidocaine and bupivacaine in the papillary muscles of guinea pigs. They found the effects on sodium channel block differed for these two local anesthetics. Lidocaine-induced block had a fast on and off rate, whereas the bupivacaine block was fast on but slow off. Bupivacaine, therefore, causes frequency-dependent blockade of sodium channels at lower heart rates than does lidocaine, resulting in slowing or blockade of conduction at lower rates.

Pharmacokinetic studies in pediatric patients have suggested that young individuals might tolerate a higher free fraction of bupivacaine than adults during regional anesthesia.[7] Studies of developmental changes in the effects of various local anesthetics on cardiac Purkinje fibers have demonstrated that, for lidocaine and a quaternary derivative, there is a greater local anesthetic depressant action in adults than neonates,[8] whereas for quinidine,[9] phenytoin,[10] and benzocaine,[11] actions are equivalent in young people and adults. The goal of the current study was to use similar cellular electrophysiologic techniques to determine whether there was a developmental change in the effects of bupivacaine on cardiac tissues. This was considered important in light of the use of bupivacaine in obstetric anesthesia.

Materials and Methods

Approval from our institutional Animal Care Committee was obtained. Guinea pigs of three different ages were studied: adults weighing 400–500 g, neonates from 1 to 10 days of postnatal age, and fetuses between 62 and 66 days gestational age.

Neonates and pregnant and nonpregnant adults were anesthetized with pentobarbital (30 mg/kg intraperitoneally) and ketamine (1 mg/kg intramuscularly). Hysterotomies were performed on pregnant animals, and fetuses were removed individually. Median sternotomies were performed in all animals, and hearts were quickly excised and placed in Tyrode's solution at 4° C containing the following (millimolar): NaCl, 131; $NaHCO_3$, 18; KCl, 5.4; NaH_2PO_4, 1.8; $MgCl_2$, 0.5; $CaCl_2$, 2.7; and dextrose 5.5, bubbled with 95% O_2/5% CO_2.

Papillary muscles from either ventricle were dissected and mounted in a Lucite® chamber perfused with Tyrode's solution at a flow rate of 12 ml/min with temperature maintained at 36 ± 1° C and *p*H kept at 7.35 ± 0.05

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‡ Food and Drug Administration: Drug Bulletin 13:23, 1983.

(measured with a Corning blood gas analyzer [Corning, NY]). The papillary muscles were stimulated with Teflon®-coated bipolar silver wire electrodes. Stimuli were 1–2.5 ms in duration and twice the threshold in amplitude.[12] Transmembrane potentials were recorded through glass capillary microelectrodes filled with 3 M KCl and having resistances of 10–30 Mohm. The microelectrodes were connected through Ag–AgCl junctions to amplifiers with high-input impedance and input capacitance neutralization. The signals were displayed on one channel of an oscilloscope, and the maximum rate of increase of phase 0 ($\dot{V}_{max}$) obtained by electronic differentiation with an operational amplifier was displayed on the second channel.[13] A saw-tooth calibrator generating a pulse of 100 mV amplitude and a rising slope of 200 V/s injected between the tissue chamber and the ground permitted calibration of the system. The saw-tooth calibrator was linear between 100 and 900 V/s. Recordings of transmembrane potentials and $\dot{V}_{max}$ were made with a strip chart recorder and a peak hold unit.[13] Polaroid photographs were also taken.

The following protocol was used for each experiment. After a stable recording after impalement was obtained, each preparation was allowed to equilibrate for at least 45 min before control measurements were recorded. We used $\dot{V}_{max}$ to estimate the tonic and use-dependent block of the sodium channel. Tonic block is defined as the reduction in $\dot{V}_{max}$ at a basic drive cycle length (BCL) of 20 s and use-dependent block as the reduction in $\dot{V}_{max}$ at a BCL of 300 ms.[11] Tonic block was determined by superfusion with control Tyrode's solution for at least 45 min and bupivacaine at concentrations of 0.2 μg/ml, 1.0 μg/ml, and 1.5 μg/ml for 30 min each at a BCL of 20 s. The BCL was then abruptly changed to 300 ms to determine use-dependent block during control and at each bupivacaine concentration. An extrastimulus (S_2) was introduced with increasing delay after a train of 30 beats at BCL of 300 ms to determine the recovery from use-dependent block as previously described.[11]

Data Analysis

Only data obtained from impalements of a single cell throughout the experiment were included in the analysis. The effect of the drug within each age group was analyzed by one-way analysis of variance (ANOVA).[14] Where indicated, the effect was further tested by Scheffe's test.[14] Among age groups, comparisons were made with a nested ANOVA.[14]

The onset of use-dependent block was obtained by using Scheffe's test to compare $\dot{V}_{max}$ of each beat with the $\dot{V}_{max}$ in the last beat of the train. The beat number at which steady state was reached was taken to be that for which no significant change in $\dot{V}_{max}$ was shown. Least-squares linear regression analysis was used to calculate the time constant for recovery from use-dependent block.[11] Recovery of $\dot{V}_{max}$ is defined as $1 - \dot{V}_{max}$ (test)/$\dot{V}_{max}$ (conditioning), where $\dot{V}_{max}$ (test) = $\dot{V}_{max}$ of S_2, and $\dot{V}_{max}$ (conditioning) = $\dot{V}_{max}$ at BCL = 20 s. Diastolic interval was measured from the end of the last beat in a train to the onset of S_2.

Because membrane depolarization was sometimes present during a rapid train, all of the kinetic data were normalized to the measurements obtained during control. This normalization was performed only if the extent of membrane depolarization was the same during control and during drug superfusion. If this was not the case, the preparation could not be used for data analysis and was excluded.

Data are presented as mean ± SE; significance was set at $P < 0.05$.

Results

The transmembrane potential characteristics during control for each age group are summarized in table 1. The only notable difference among the three age groups was in the action potential duration (APD), measured at both 50% (APD_{50}) and 100% (APD_{100}) repolarization. APD_{50} and APD_{100} were prolonged in the fetuses compared with those of the adults and neonates. This was observed at both BCLs. A consistent but similar degree of depolarization in the maximum diastolic potential was seen in all three age groups when the BCL was abruptly changed from 20 s to 300 ms. Representative transmembrane potentials of adult, neonatal, and fetal tissues are illustrated in figure 1.

Effects of Bupivacaine on Action Potential Characteristics

Bupivacaine, 1.5 μg/ml, hyperpolarized maximum diastolic potential (MDP) in adult and neonatal guinea pig papillary muscles but had no effect on fetal papillary muscles (fig. 2). Comparable results were seen at both cycle lengths, but only the 300-ms cycle length is depicted. Bupivacaine did not significantly alter overshoot in any of the three age groups at the BCL of 20 s (data not shown). However, at a BCL of 300 ms, bupivacaine induced a concentration-dependent reduction in overshoot in adult, neonatal, and fetal guinea pig papillary muscles (fig. 3).

Bupivacaine did not change APD at either drive rate in the adult and neonatal tissues. In the fetal tissues, bupivacaine shortened APD_{50} at the two higher concentrations (fig. 4). Comparable results were found for APD_{100} (data not shown). Although control APDs were much

TABLE 1. Control Transmembrane Potential Characteristics

	BCL	Adults (n = 6)	Neonates (n = 8)	Fetuses (n = 8)
MDP (mv)	20 s	−78.0 ± 1.0	−80.5 ± 1.5	−80.5 ± 0.5
	300 ms	−74.0 ± 1.0*	−74.5 ± 2.0*	−74.5 ± 0.5*
Overshoot (mv)	20 s	50.0 ± 2.0	46.0 ± 2.0	51.0 ± 1.0
	300 ms	43.0 ± 2.0	44.0 ± 2.0	47.0 ± 1.0
$\dot{V}_{max}$ (v/s)	20 s	227 ± 15	228 ± 16	220 ± 21
	300 ms	210 ± 14	205 ± 11	199 ± 21
APD_{50} (ms)	20 s	108 ± 16	69 ± 11	166 ± 18†
	300 ms	94 ± 12	69 ± 12	139 ± 13†
APD_{100} (ms)	20 s	148 ± 17	110 ± 13	202 ± 18†
	30 ms	141 ± 13	109 ± 13	177 ± 12†

Results are expressed as mean ± SEM.
MDP = maximum diastolic potential; $\dot{V}_{max}$ = maximum rate of rise of phase 0; APD_{50} and APD_{100} = action potential duration to 50% and full repolarization, respectively. BCL = basic drive cycle length.

* $P < 0.05$ *versus* BCL = 20 s.
† $P < 0.05$ *versus* adults and neonates.

more prolonged in the fetus, when adult and fetal papillary muscles with similar APDs were compared, bupivacaine effects were observed only in the fetus and not the adult (fig. 5).

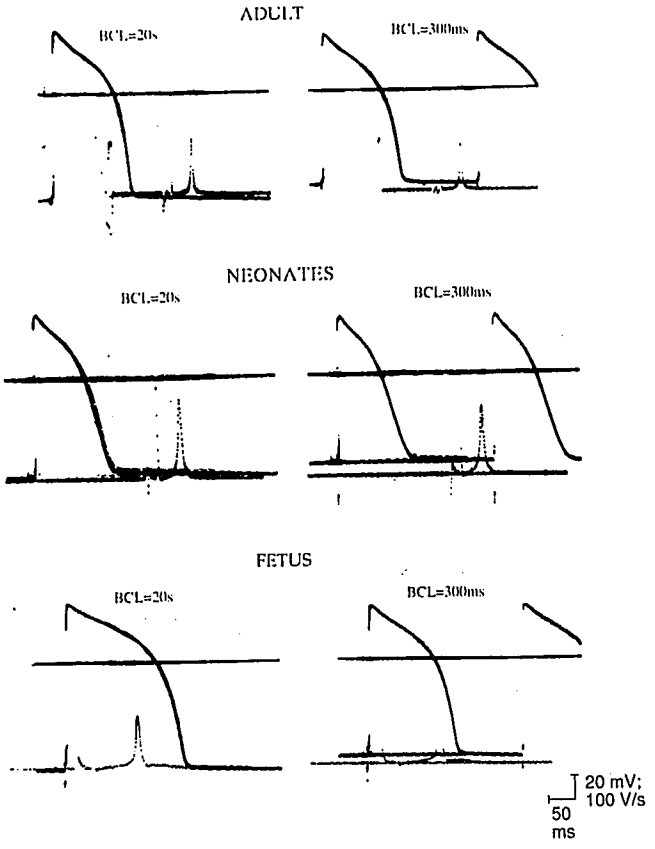

FIG. 1. Control transmembrane potentials recorded in adult (*A*), neonatal (*B*), and fetal (*C*) myocardium at BCL = 20 s and 300 ms. In all panels, the top traces show a "0" line and the transmembrane action potential; the lower traces show the electronically differentiated $\dot{V}_{max}$ of phase 0.

At BCLs of 20 s, the three bupivacaine concentrations studied had no effect on $\dot{V}_{max}$ in any of the three age groups (data not shown). A dose-dependent decrease in $\dot{V}_{max}$ was seen in the adult, neonatal, and fetal guinea pigs at BCLs of 300 ms (fig. 6).

ONSET AND OFFSET OF BUPIVACAINE-INDUCED USE-DEPENDENT BLOCK

We studied the time course of bupivacaine-induced use-dependent block by examining the onset of steady-state block and recovery from use-dependent block. In the adults, neonates, and fetuses, not all tissues showed use-dependent block at the lowest drug concentration (0.2 μg/ml). (Four of six adult, one of seven neonatal, and two of seven fetal tissues showed use-dependent block at this concentration.) At 1.0 μg/ml, bupivacaine-induced use-dependent block was present in all preparations in all three age groups. The onset of steady-state use-dependent

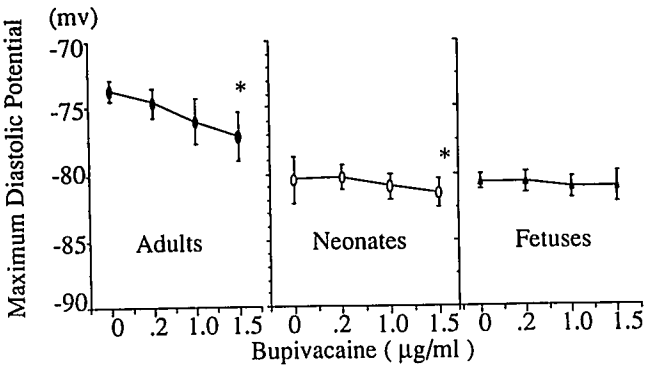

FIG. 2. The effects of bupivacaine on maximum diastolic potential (MDP) at BCL of 300 ms in each of the three age groups. The ordinate shows the MDP. The abscissa indicates superfusion with control solution or bupivacaine. *$P < 0.05$ compared to control.

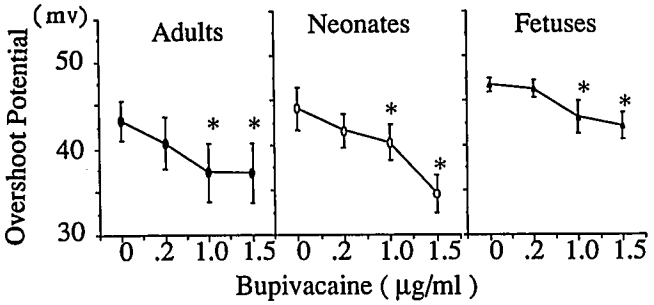

FIG. 3. The effects of bupivacaine on overshoot in the three age groups at BCL = 300 ms. The ordinate is overshoot in millivolts. The abscissa indicates superfusion with control solution or bupivacaine. *P < 0.05 compared to control.

block was 6.7 ± 0.6 beats in the adults, 6.3 ± 0.3 beats in the neonates, and 6.3 ± 0.3 beats in the fetuses. At the highest concentration of bupivacaine, one preparation in each age group developed such severe conduction block that analysis of use-dependence could not be done. In the remaining preparations, onset of block occurred by 6.0 ± 0 beats in the adults, 6.0 ± 0.2 beats in the neonates, and 6.4 ± 0.2 beats in the fetuses. At neither of the two higher concentrations was there any difference in the onset rates among the three age groups (fig. 7). Information on the recovery from use-dependent block is summarized in table 2. The recovery time constants were similar in all three age groups, and they were independent of drug concentration. The recovery rates at 1.0 μg/ml bupivacaine for the adult, neonatal, and fetal tissues are shown in figure 8.

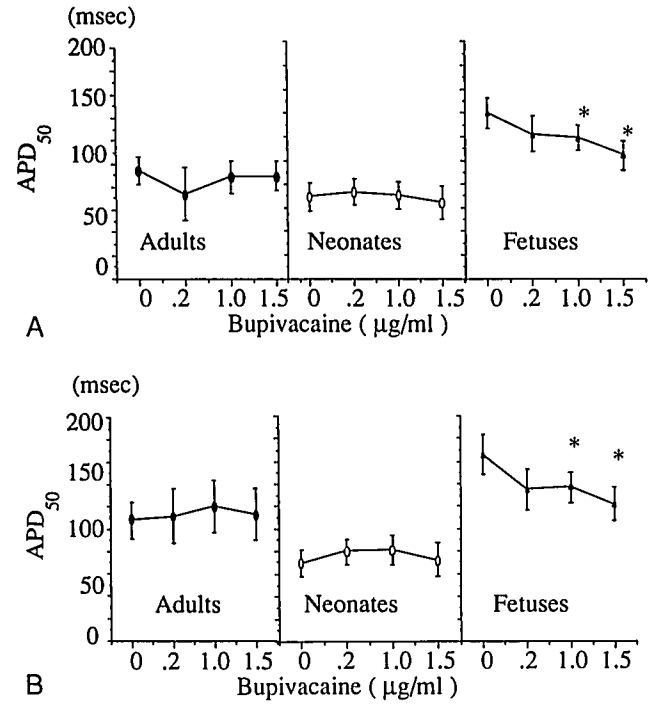

FIG. 4. The effects of bupivacaine on action potential duration at 50% repolarization (APD_{50}) at BCL = 20 s (*A*) and at BCL = 300 ms (*B*) in adult, neonatal, and fetal tissues. The ordinate is APD_{50} in milliseconds. The abscissa shows superfusion during control period and superfusion with different concentrations of bupivacaine. *P < 0.05 compared to control.

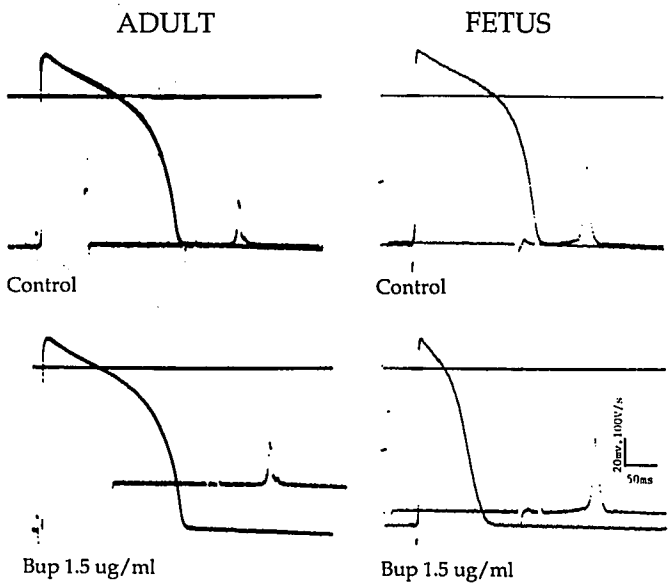

FIG. 5. The effects of bupivacaine on APD in adult and fetal tissues with similar control APDs. In all panels, the top traces show a "0" line and the transmembrane action potential; the lower traces are the differentiated $\dot{V}_{max}$. Adult and fetal action potential durations are similar during control (*top left and top right*). After superfusion with bupivacaine 1.5 μg/ml (at BCL = 20 s), a noticeable reduction in APD is seen in the fetus (*bottom right*), and little effect is observed in the adult (*bottom left*). Bupivacaine 1.5 μg/ml (*bottom left and bottom right*) did not reduce APD from control (*top left and top right*) at BCL = 20 s.

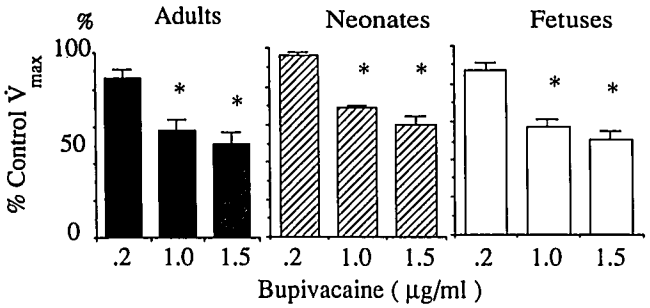

FIG. 6. The effects of bupivacaine on use-dependent block. The ordinate shows the percent change of the steady state value of $\dot{V}_{max}$ from its initial control value (represented as 100%). The abscissa shows superfusion with bupivacaine. Use-dependent block is defined as percent $\dot{V}_{max}$ depression at BCL = 300 ms. *P < 0.05 compared to control.

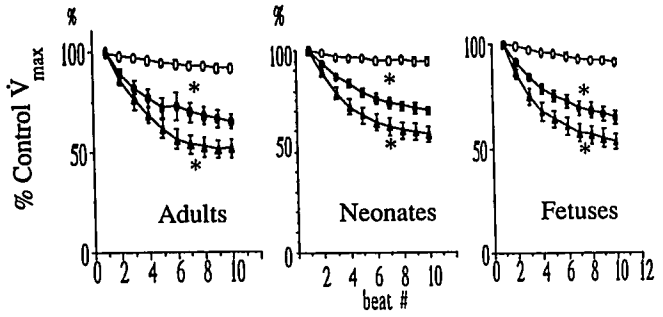

FIG. 7. The effects of bupivacaine on the onset of use-dependent block. The ordinate shows percent change of $\dot{V}_{max}$ from its control value at BCL = 20 s (represented at 100%). The abscissa shows the number of beats at BCL = 300 ms. The asterisks indicate the beat for which $P < 0.05$ compared to the tenth beat at BCL = 300 ms (*i.e.*, all beats to the left of this also differed by $P < 0.05$; all beats to the right did not). Data from adults (n = 5–6), neonates (n = 6–7) and fetuses (n = 6–7) are presented. 0 = bupivacaine 0.2 μg/ml; circles = bupivacaine 1.0 μg/ml; triangles = bupivacaine 1.5 μg/ml.

Discussion

In our study, the control transmembrane potential characteristics were similar in all three age groups, except for the APD, which was longer in the fetal tissues. This was the case when APD was measured at either 50% or 100% repolarization. APD may be more prolonged because of either an increase in the slow inward calcium current during the plateau or a decrease in the outward potassium currents that accelerate repolarization.[15] There is no evidence whether any age-related changes in these currents could account for the developmental differences in APD that we observed.

Bupivacaine reduced APD in the fetal tissues but not in the adult or neonatal tissues. This effect appeared to be age-related rather than secondary to a more prolonged control APD in the fetus, as shown by comparing the effect of bupivacaine on adult and fetal papillary muscles that had similar APDs during control (fig. 5). It is interesting that Clarkson and Hondeghem[6] reported a shortening of the APD in the adults at much higher concentrations of bupivacaine (10–20 μg/ml) than we used. Taken together, these results suggest that the fetus is more sensitive than the adult to the effects of bupivacaine on APD. The mechanism for the greater sensitivity of fetal tissues to the effect of bupivacaine compared with the adult tissues remains undefined. Of note is an analogous developmental differential sensitivity to the effect of tetrodotoxin on neonatal and adult canine Purkinje fiber APD. This was postulated to be related to a possible age-related change in the sodium "window" current.[16] Fujii *et al.*[17] reported maturational changes in sodium channel density in chick embryo heart cells. A very recent report

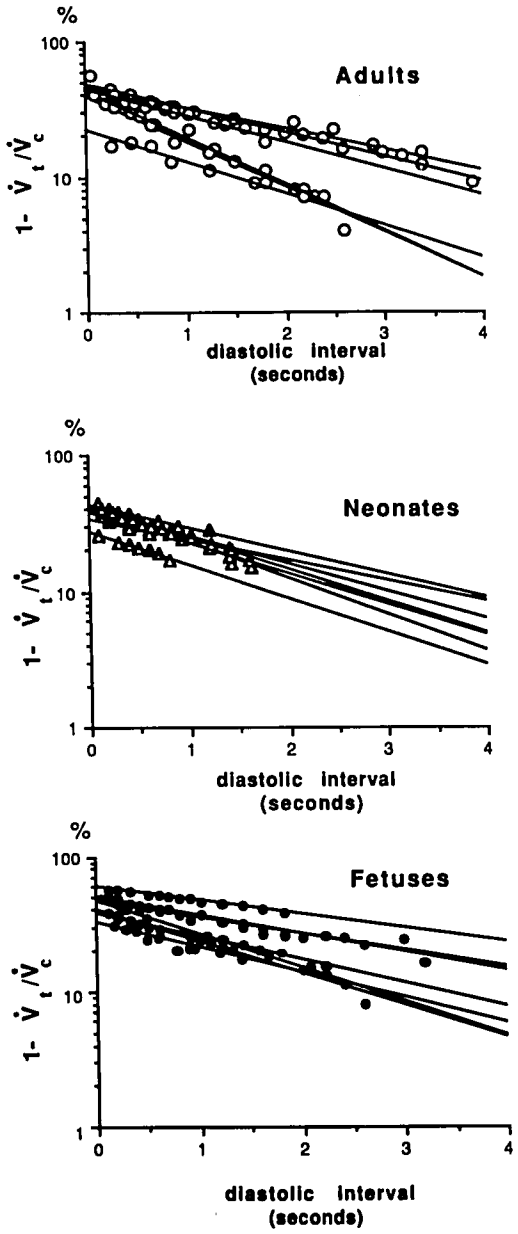

FIG. 8. The recovery kinetics from bupivacaine 1.0 μg/ml. Data are from adults (0, n = 5), neonates (triangles, n = 7), fetuses (circles, n = 7). The recovery time course is plotted on a semilogarithmic scale as the ratio of $(1 - \dot{V}_{max}[test]/\dot{V}_{max}[conditioning]) \times 100$ against the diastolic interval (seconds).

TABLE 2. Time Constants for Recovery from Use-Dependent Block (τ_{off}) by Bupivacaine

	Adults	Neonates	Fetuses
Bupivacaine 1.0 μg/ml	2.1 ± 0.3	1.9 ± 0.2	2.2 ± 0.3
Bupivacaine 1.5 μg/ml	2.3 ± 0.3	2.0 ± 0.2	2.3 ± 0.4

Results are expressed in seconds.

by Zhang *et al.*[18] demonstrated that sodium channel inactivation was significantly modified in neonatal ventricular myocyte cultures with sympathetic innervation. APD is an indication of the duration of refractoriness of cardiac tissues. The greater sensitivity of fetal tissues to bupivacaine's effect in reducing APD would suggest that refractoriness in fetal tissues is more likely to be affected by the drug than that in adult tissues. Any alteration in refractoriness could facilitate the propagation of arrhythmias. In fact, fetal arrhythmias have been observed after paracervical block in obstetric patients.[19] These have been attributed to a direct effect of the drug on the fetal heart secondary to an elevated level of bupivacaine in the fetal circulation.[20]

Although control MDPs were similar in all three age groups, bupivacaine tended to hyperpolarize MDP in both the adult and neonatal tissues but not in the fetal tissues. The hyperpolarization of 2.5 mV was statistically significant but was most probably physiologically unimportant.

The dose-dependent reductions of $\dot{V}_{max}$ and overshoot by bupivacaine are consistent with its action as a blocker of the cardiac sodium channels. Bupivacaine did not reduce $\dot{V}_{max}$ at the slow stimulation rate, and tonic block was not present in any of the three age groups. According to the modulated receptor hypothesis,[2] bupivacaine has a low affinity for sodium channels in the resting state and therefore is not expected to exert much tonic block. In Clarkson and Hondeghem's study,[6] bupivacaine was shown to have its greatest affinity for inactivated sodium channels. Bupivacaine's interaction with the cardiac sodium channels was fast-on but slow-off. In their study, the onset of steady-state use-dependent block was usually reached by the tenth beat, and the time constant for recovery was 1–2 s. Our data are in agreement with these findings.

We chose the three concentrations of bupivacaine in our study to approximate therapeutic and toxic plasma concentrations of bupivacaine; 0.2 μg/ml bupivacaine has been estimated to be the free fraction of drug after epidural administration of bupivacaine in adults.[21] After an intravenous injection, the free fraction of bupivacaine has been estimated to be between 0.5 and 5.0 μg/ml[6]; therefore, 1.0 μg/ml and 1.5 μg/ml might be considered toxic levels after a possible accidental intravascular injection.

At 0.2 μg/ml bupivacaine, there were no significant changes in $\dot{V}_{max}$ or other transmembrane potential characteristics in any of the three age groups studied. Extrapolating these results clinically, we might speculate that there should be no cardiotoxicity related to therapeutic levels of bupivacaine in adults, pediatric patients, or fetuses.

At higher concentrations of bupivacaine, there was a dose-dependent reduction in $\dot{V}_{max}$ in all age groups. In the intact heart, the reduction in $\dot{V}_{max}$ could be manifest as a delayed conduction time with a prolonged PR interval, increased atrioventricular (AV) nodal conduction, AV nodal block, and intraventricular conduction delay. Increased conduction time in the ventricle might predispose the patient to reentrant pathways, which could lead to ventricular arrhythmias. In fact, the above-mentioned ECG characteristics and arrhythmias have been observed in experimental animals subjected to large doses of bupivacaine[4,22–24] and in patients who had received an accidental intravascular injection.[3]

It is interesting to compare the developmental differences in the electrophysiologic effects of lidocaine and bupivacaine. Developmental differences in sensitivity to lidocaine appeared to be a function of age-related differences in APD, in that the more prolonged the APD (as in the neonatal epicardial cells and adult Purkinje fibers), the longer the time constant for recovery and the greater the depressant effect of the drug.[8,25] In the current study, despite the fact that the fetal guinea pigs had a more prolonged APD, there was no demonstrable age-related change in the kinetics of bupivacaine-induced use-dependent block.

One possible explanation for the age-related sensitivity to lidocaine but not to bupivacaine might be related to the fundamental difference that exists in the kinetics of use-dependent blockade of these two local anesthetics. Lidocaine-induced use-dependent block was fast-on and fast-off in both adults and neonates, whereas bupivacaine-induced use-dependent block was fast-on but slow-off in all three age groups. Because recovery from lidocaine block was rapid, the effect of age-related differences in APD would be readily apparent. In contrast, because recovery from bupivacaine block was slow, the additional effect of age-related differences in APD might not be sufficient to significantly alter the course of recovery.

In conclusion, our study showed bupivacaine to have no electrophysiologic effects at the lowest concentration evaluated in all three age groups. Therefore, when properly administered, bupivacaine is probably a safe drug for the adult as well as the neonatal and fetal hearts. At the higher concentrations, bupivacaine has effects in all three age groups. It appears to have a greater effect on APD in the fetal tissues than in the adult or neonatal tissues. As a result of this effect on APD in the fetus, it might be more likely to modify rhythm in the fetal heart.

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