Anesthesiology
65:186–191, 1986

Dose-dependent Effects of Bupivacaine on Rat Muscle Arterioles

Roger A. Johns, M.D., Walter C. Seyde, M.D.,* Cosmo A. DiFazio, M.D., Ph.D.,† David E. Longnecker, M.D.†*

The dose-dependent actions of bupivacaine on the microvasculature were evaluated by television microscopy in an *in vivo* rat cremaster muscle preparation. Animals were anesthetized with chloralose and urethane. Mean arterial pressure was measured *via* a carotid artery cannula; heart rate was calculated from the phasic pressure trace. The cremaster muscle was suffused with a balanced electrolyte solution that was controlled for temperature, *p*H, P_{O_2}, P_{CO_2}, and osmolarity to provide a physiologic environment. Internal diameters of fourth-order arterioles were measured with an electronic vernier displayed on the video monitor. Arteriolar diameters were measured every 30 s during a 10-min control period, a 10-min period of topical application of bupivacaine hydrochloride, and a 30-min recovery period. Bupivacaine 10^{-1}, 10^{0}, 10^{1}, and 10^{2} $\mu g \cdot ml^{-1}$ produced progressive vasoconstriction to 82.7 ± 2.9%, 75.0 ± 5.6%, 71.0 ± 7.0%, and 65.7 ± 9.4% of control ($P < 0.05$ for each), respectively. Bupivacaine, 10^{3} and 2.5×10^{3} $\mu g \cdot ml^{-1}$, did not alter arteriolar diameters significantly, although there was a tendency for vasodilation. In a second group of animals, arteriolar diameters were measured during intravenous bupivacaine infusion that produced stable plasma concentrations of 2.3 ± 0.2 $\mu g \cdot ml^{-1}$. Vasoconstriction of 91.4 ± 2.2%, of control ($P < 0.01$) was observed. These results demonstrate that dose-dependent arteriolar constriction occurs even with blood bupivacaine levels that are at the upper limits of those expected to occur during regional anesthesia. (Key words: Anesthetics, local: bupivacaine; lidocaine. Arteries: arterioles; microcirculation. Microcirculation: arterioles.)

SINCE THE REPORTS of cardiovascular collapse following regional anesthesia with bupivacaine,[1–3] numerous studies have investigated cardiac effects of this drug.[4–8]‡ Very little information is available, however, concerning its actions on the peripheral circulation. Most existing information has been derived from indirect studies of regional or systemic vascular resistance,[9,10] from changes in local blood flow,[11,12] or from *in vitro* studies of isolated vascular smooth muscle.[13,14] The results of these studies are difficult to interpret because they provide evidence for both vasoconstricting and vasodilating actions of bupivacaine.

The aim of this study was to define the microvascular actions of bupivacaine over a wide range of concentrations, including those that occur either locally or systemically during regional anesthesia. We used an *in vivo* microvascular preparation that allowed direct observation of the dose-related effects of bupivacaine on the precapillary arterioles in the rat cremaster muscle.

Methods

These studies employed an experimental protocol similar to that used previously to document the microvascular actions of lidocaine.[15] Male Sprague-Dawley rats (173 ± 9 g) were anesthetized with chloralose, 60 $mg \cdot kg^{-1}$, plus urethane, 800 $mg \cdot kg^{-1}$, intraperitoneally. This combination has been shown to have minimal effects on systemic hemodynamics and microvascular responses in the rat.[16,17] The animals breathed room air spontaneously through a tracheostomy. Mean arterial pressure (MAP) was recorded continuously from a catheter in the left carotid artery. Heart rate (HR), obtained from the phasic arterial trace, was recorded at 5-min intervals. Rectal temperature was measured continuously and maintained at 37° C by a heat lamp. A catheter was placed in the right femoral vein of animals receiving intravenous bupivacaine.

The left cremaster muscle was exposed through a midline scrotal incision and prepared for microvascular observations according to the method of Baez.[18] The rat was placed on a platform and the muscle, with nerves and vessels intact, was spread over a heated, transparent pedestal. The whole preparation was then placed on the stage of a compound video microscope. A warmed, buffered balanced-salt solution (containing in mM: $NaHCO_3$, 25.5; NaCl, 131.9; KCl, 4.7; $CaCl_2$, 2.0; $MgSO_4$, 1.17) with or without bupivacaine, was continuously suffused over the muscle at a constant flow rate of 1.5 $ml \cdot min^{-1}$. The *p*H of all suffusion solutions was controlled at a value of 7.2 in all experiments. The suffusion solution was equilibrated with 95% nitrogen and 5% CO_2 to maintain a constant P_{CO_2} and to provide a surface oxygen tension similar to that within the muscle (oxygen diffuses into the suffusion solution from the atmosphere), because increased oxygen tension has been shown to alter the microvasculature.[19] Cremaster muscle temperature was measured continuously and maintained at 34–35° C, the physiologic temperature of the rat scrotum.[20] The microscopic image was displayed continuously on a video monitor (total magnification, ×625) equipped with an electronic vernier,§ which was used to measure internal vessel diameters at 30-s intervals throughout all experiments. System accuracy was ±0.5%. The preparation was allowed to stabilize for 1 h prior to beginning experiments in order to min-

* Research Fellow in Anesthesiology.

† Professor of Anesthesiology.

Received from the Department of Anesthesiology, University of Virginia Medical Center, Charlottesville, Virginia 22908. Accepted for publication March 12, 1986. Presented in part at the American Society of Anesthesiologists Annual Meeting, San Francisco, California, October 1985.

Address reprint requests to Dr. Johns.

‡ Block A, Covino BG: Effect of local anesthetics on cardiac conduction and contractility. Regional Anesthesia 6:55–61, 1981.

§ Circon Corporation, Santa Barbara, California 93111.

imize the effects of tissue manipulation on microvascular diameters.

Cremaster microvasculature consists of a series of branchings of arterioles and venules from central vessels, and each successive branching was defined as a specific "order" of artery or vein. Fourth-order arterioles were studied because these precapillary arterioles are the major site of vascular resistance in the peripheral circulation and because they are the most responsive to vasoactive stimuli.[21,22]

The protocol consisted of three intervals: a 10-min control period, a 10-min period of topical bupivacaine, and a 30-min recovery period. Bupivacaine hydrochloride monohydrate was applied in one of seven concentrations: 10^{-2}, 10^{-1}, 10^{0}, 10^{1}, 10^{2}, 10^{3}, and 2.5×10^{3} $\mu g \cdot ml^{-1}$ (3.58×10^{-8} to 8.95×10^{3} M, respectively). Each concentration of bupivacaine was applied to the cremaster muscle of seven to ten animals.

A second group of experiments involved the intravenous infusion of bupivacaine. The protocol consisted of two 45-min intervals: a saline infusion followed by a bupivacaine (in saline) infusion. Bupivacaine was administered as a 3.5 $mg \cdot kg^{-1}$ bolus followed by 0.23 $mg \cdot kg^{-1} \cdot min^{-1}$ in each of seven rats. Both control and drug infusions were administered at a flow rate of 20 $\mu l \cdot min^{-1}$. Arteriolar diameters were measured only during the final 15 min of each interval in order to allow time for plasma bupivacaine levels to stabilize. Plasma concentrations of bupivacaine were measured by gas chromatography after 2.5×10^{3} $\mu g \cdot ml^{-1}$ topical bupivacaine applications and after 30 min of bupivacaine infusion.

Arteriolar diameters were normalized as per cent of control by determining the mean diameter for each control period and then dividing all values by the average control value. Average data for arteriolar diameters were determined for each measurement interval, and overall average responses during the final 5 min of topical bupivacaine application (when vascular responses were maximal and stable) were calculated also. HR and MAP were averaged for both the control and drug application intervals. Arteriolar diameters, MAPs, and HRs were compared by paired t tests; $P < 0.05$ was accepted as significant. Values are expressed as the mean ± the standard error of the mean.

Results

MAP and HR were not significantly different from control for topically applied concentrations of bupivacaine from 10^{-2} to 10^{3} $\mu g \cdot ml^{-1}$ (table 1). During bupivacaine, 2.5×10^{3} $\mu g \cdot ml^{-1}$, MAP and HR decreased to 91.2 ± 0.1% and 93.1 ± 1.1% of control, respectively ($P < 0.01$).

TABLE 1. Mean Arterial Pressure (MAP) and Heart Rate (HR) during Control and Drug Application Periods

	MAP (mmHg)		HR (beats · min^{-1})	
Bupivacaine	Control	Drug	Control	Drug
Topical application $\mu g \cdot ml^{-1}$				
10^{-2}	101 ± 4	98 ± 5	398 ± 11	400 ± 22
10^{-1}	97 ± 4	107 ± 2	486 ± 6	480 ± 12
10^{0}	101 ± 4	107 ± 3	390 ± 6	444 ± 36
10^{1}	100 ± 5	99 ± 5	497 ± 29	462 ± 42
10^{2}	97 ± 3	97 ± 5	408 ± 32	396 ± 25
10^{3}	98 ± 3	101 ± 3	437 ± 48	407 ± 60
2.5×10^{3}	96 ± 2	88 ± 2*	434 ± 23	406 ± 22*
Intravenous infusion	97 ± 4	87 ± 3*	436 ± 16	373 ± 11*

Mean ± SEM; n = 7–10 animals at each concentration.
* $P < 0.05$ by paired t test.

Dose-dependent arteriolar constriction was observed during topical bupivacaine (figs. 1 and 3). Bupivacaine, 10^{-2} $\mu g \cdot ml^{-1}$ produced no significant change in arteriolar diameters. Bupivacaine 10^{-1}, 10^{0}, 10^{1}, and 10^{2} produced progressive arteriolar constriction to 82.7 ± 2.9%, 75.0 ± 5.6%, 71.0 ± 7.0%, and 65.7 ± 9.4% of control ($P < 0.05$ for each), respectively. There was a slight tendency for vasodilation at concentrations of 10^{3} and 2.5×10^{3} $\mu g \cdot ml^{-1}$, but these values were not significantly different from control. Mean plasma concentration of bupivacaine after topical bupivacaine, 2.5×10^{3} $\mu g \cdot ml^{-1}$, was 1.0 ± 0.1 $\mu g \cdot ml^{-1}$. The microvascular actions of topical bupivacaine became maximal and stable only after the tissues had been exposed to the drug for approximately 5 min (fig. 1). Similarly, the microvascular effects were slow to recede, requiring 15–30 min of suffusion with drug-free electrolyte solution before arteriolar diameters returned to control values (fig. 1).

Intravenous infusion of bupivacaine resulted in arteriolar constriction to 91.4 ± 2.2% of control ($P < 0.01$; fig. 2). The mean plasma bupivacaine concentration during intravenous infusion was 2.3 ± 0.2 $\mu g \cdot ml^{-1}$. During intravenous bupivacaine, MAP and HR decreased to 90.2 ± 3.4% and 85.5 ± 2.6% of control, respectively ($P < 0.01$ for each).

Discussion

Previous indirect studies of the effects of bupivacaine on peripheral vasculature provided evidence for both vasodilating and vasoconstricting actions of this drug. Jorfeldt *et al.*[9] demonstrated a 20–40% increase in systemic vascular resistance index in humans at mean plasma bupivacaine concentrations of 2.1 $\mu g \cdot ml^{-1}$. Liu *et al.*[10] observed no consistent alterations in total peripheral resistance during the infusion of bupivacaine, 0.3 to 10 $mg \cdot kg^{-1}$, in dogs. However, they observed a marked in-

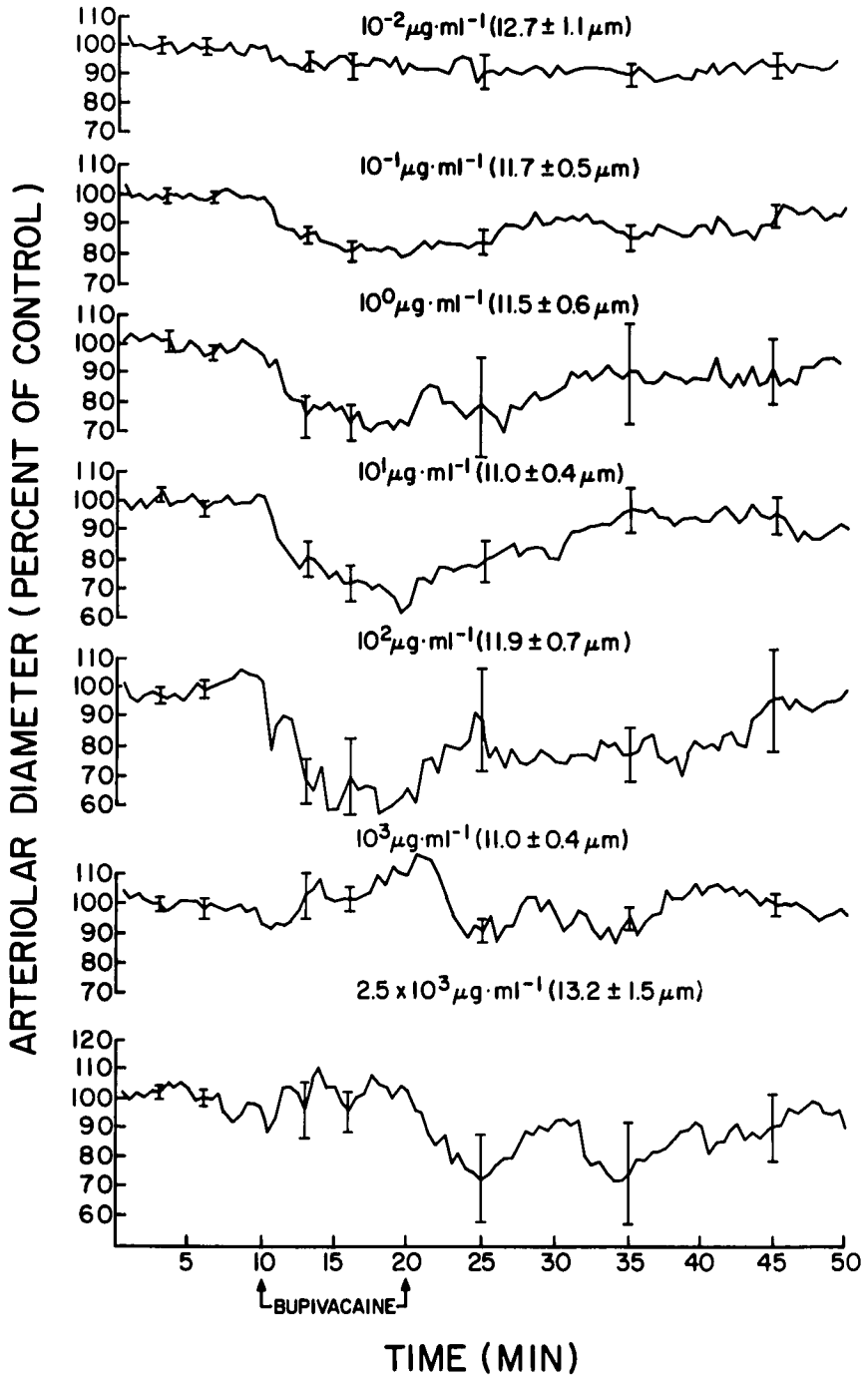

FIG. 1. Time course of arteriolar responses to topical bupivacaine. Values in parentheses represent average control diameters (±SEM). Significant ($P < 0.05$) constriction occurred at 10^{-1}, 10^{0}, 10^{1}, and 10^{2} $\mu g \cdot ml^{-1}$. Arteriolar diameters during bupivacaine 10^{-2}, 10^{3}, and 2.5×10^{3} $\mu g \cdot ml^{-1}$ were not significantly different from control (n = 7–10 animals for each concentration).

crease in pulmonary vascular resistance, suggesting a pulmonary vasoconstrictor action of bupivacaine.

Evidence for vasodilation was provided by Blair,[11] who showed increased blood flow in the hindlimb of dogs following intraarterial injection of 1 mg of bupivacaine in saline. Likewise, Dhunér and Lewis[12] demonstrated increased local clearance of radioactive xenon following the intramuscular injection of 0.1 to 0.4 ml of bupivacaine, 5×10^{3} $\mu g \cdot ml^{-1}$. Aps and Reynolds[23] used blanching or erythema as indices of vasoconstriction or vasodilation after injecting varying concentrations of bupivacaine intradermally in humans. They concluded that lesser concentrations (1.25×10^{3} $\mu g \cdot ml^{-1}$) resulted in vasoconstriction, whereas greater concentrations ($2.5–5 \times 10^{3}$ $\mu g \cdot ml^{-1}$) produced vasodilation.

Tuvemo and Willdeck-Lund[13] studied the cumulative dose–response effects of bupivacaine on isolated strips of human umbilical arteries. In five of ten preparations, a

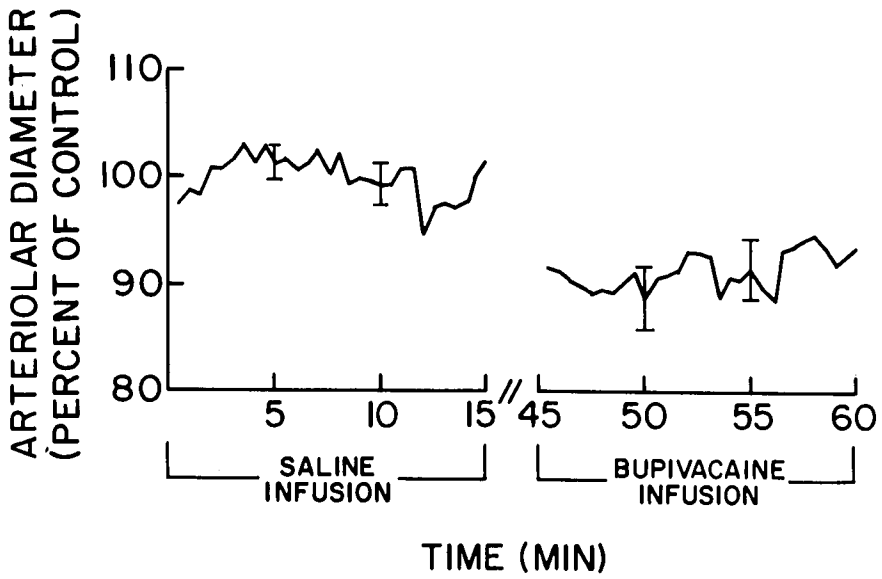

FIG. 2. Vasoconstricting effect (mean ± SEM; n = 7) of intravenous bupivacaine (mean plasma level 2.3 ± 0.2 $\mu g \cdot ml^{-1}$) on fourth-order arterioles ($P < 0.01$). Average control diameter was 9.9 ± 0.9 μm. The gap in the abscissa represents a 30-min period to achieve stable plasma bupivacaine levels.

dose-dependent contraction was observed with concentrations of 5–250 $\mu g \cdot ml^{-1}$. In five other preparations, a biphasic response was noted, with slight relaxation occurring at higher concentrations (100–250 $\mu g \cdot ml^{-1}$). In isolated rat portal veins that were precontracted with norepinephrine, Aberg and Wahlström[14] demonstrated dose-dependent relaxation with bupivacaine concentrations of 10^2 to 1.6×10^3 $\mu g \cdot ml^{-1}$.

There are several limitations associated with the experimental models just described. Results obtained from studies of isolated blood vessels may be altered by trauma, by isolation of the tissue from other vasoactive substances that might be activated by bupivacaine, and by the artificial environment of the tissue. Intravascular administration of bupivacaine may produce changes in systemic arterial pressure by direct or reflex mechanisms such that the peripheral vascular effects of the drug might be obscured by its systemic actions. Many of these problems were avoided in the present study, which employed an *in vivo* model that allowed direct observation of the microvessels during topical or intravenous administration of bupivacaine. By carefully controlling the suffusion solution that bathed the microvascular environment, we prevented changes in local temperature, osmolarity, *p*H,

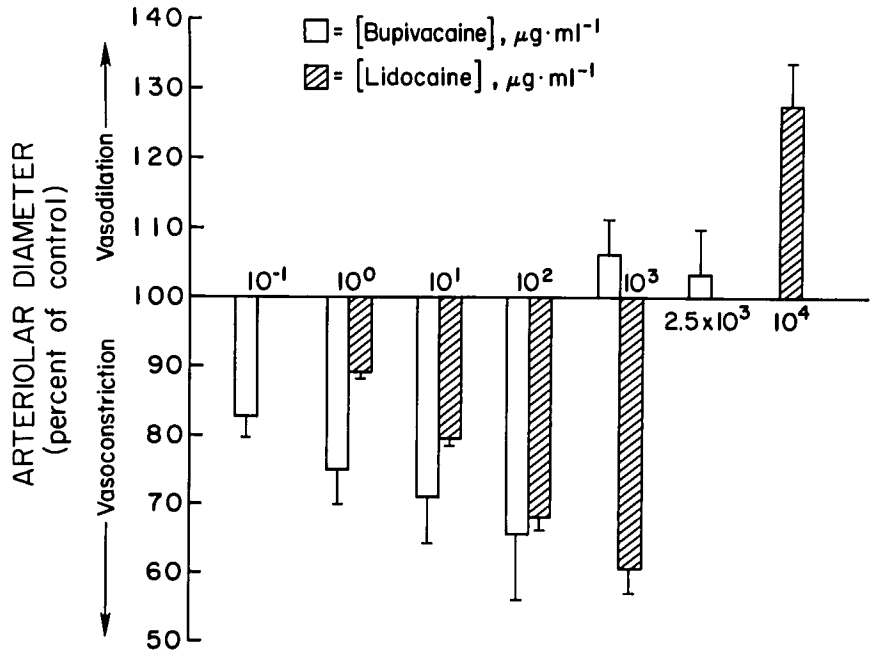

FIG. 3. Average arteriolar diameter (±SEM) during each 10-min period of bupivacaine application, compared with previous data for lidocaine obtained during identical experimental conditions. All values expressed as per cent of the average control value.

P_{O_2}, or P_{CO_2} that might have altered arteriolar diameters. The preparation also allowed us to obtain dose–response data in a noncumulative manner.

Our data demonstrate a dose-dependent response to increasing concentrations of topically applied bupivacaine. Progressive dose-dependent vasoconstriction was observed at lesser concentrations, whereas greater concentrations resulted in unchanged arteriolar diameters.

Figure 3 compares the current data for topically applied bupivacaine with those reported previously for lidocaine.[15] (The protocols were identical in both studies.) Lidocaine caused progressive vasoconstriction in concentrations of 10^0 to 10^3 $\mu g \cdot ml^{-1}$ and vasodilation at 10^4 $\mu g \cdot ml^{-1}$. There are several important differences between the actions of the two drugs: 1) at lesser concentrations, the threshold dose for vasoconstriction with bupivacaine is one-tenth that for lidocaine; 2) at the greatest concentrations, lidocaine causes marked vasodilation, whereas bupivacaine has no significant effect; 3) the time to peak vasoactive effect is much slower for bupivacaine (5 min) than for lidocaine (1–2 min), which may be related to the amount of free base available for each drug; 4) the duration of effect is much longer for bupivacaine, which requires 15–30 min to return to control values, as compared with 8–10 min for lidocaine, most likely related to the greater lipid solubility of bupivacaine; and 5) whereas none of the topically applied concentrations of lidocaine produces systemic cardiovascular effects, bupivacaine, 2.5×10^3 $\mu g \cdot ml^{-1}$, causes decreases in both HR and MAP.

The systemic effects of greater concentrations of bupivacaine might have obscured any direct vasodilating actions of the drug, because decreases in MAP might have activated reflex vasoconstrictor mechanisms, thereby modulating a direct vasodilating action of the drug. We have no experimental evidence to either confirm or deny this possibility, but greater concentrations of topical lidocaine (which did not alter systemic MAP or HR) did produce precapillary arteriolar dilation in our previous study.

The more potent vasodilating effects of lidocaine and the more prolonged vascular effects of bupivacaine are consistent with the observations of Covino.¶ He concluded that there was no correlation between relative anesthetic potencies and peripheral dilating properties of local anesthetics, but that a correlation exists between the duration of action of local anesthetics and the duration of their vascular effects.

Because vasoconstriction occurs with the topical application of bupivacaine concentrations equivalent to those that occur in the plasma after inadvertent intravenous injection or during regional anesthesia, it is unlikely that arteriolar dilation contributes significantly to the cardiovascular collapse associated with the intravenous administration of this drug. This conclusion is also supported by the observations of Liu *et al.*[10]

The molecular mechanisms by which local anesthetics produce their effects on blood vessels are not well defined. Current understanding of the regulation of vascular smooth muscle emphasizes the importance of myoplasmic calcium for the formation of both phosphorylated and nonphosphorylated crossbridges,[24] thus an increased concentration of calcium is necessary for contraction. Blair[11] suggests that local anesthetics could increase cytoplasmic calcium by decreasing the uptake of calcium by internal membrane structures and by preventing efflux of calcium from the cell.

Local anesthetics could also cause vasodilation by limiting the influx of calcium into the myoplasm. At high (mM) concentrations, local anesthetics exhibit calcium channel-blocking properties.[25] Alternatively, vasodilation could be explained by tissue-irritating effects of local anesthetics. Grim *et al.*[26] studied the histochemical effects of local anesthetics on muscle blood vessels and demonstrated a gradual disintegration of a portion of the muscle capillary bed, resulting in ischemic necrosis. This was more prominent with lidocaine, 2×10^4 $\mu g \cdot ml^{-1}$, than bupivacaine, 5×10^3 $\mu g \cdot ml^{-1}$.

In summary, using an *in vivo* microvascular preparation, we demonstrate a dose-dependent response of precapillary arterioles to increasing concentrations of topical bupivacaine. Progressive vasoconstriction was observed with bupivacaine, 10^{-1} to 10^2 $\mu g \cdot ml^{-1}$. At greater concentrations ($1–2.5 \times 10^3$ $\mu g \cdot ml^{-1}$), vasoconstriction is abolished and arteriolar diameters are unchanged from control (although a slight tendency toward vasodilation was observed). Our data provide no evidence that bupivacaine causes significant vasodilation that might contribute to the circulatory changes associated with this drug.

References

1. Albright GA: Cardiac arrest following regional anesthesia with etidocaine or bupivacaine. ANESTHESIOLOGY 51:285–286, 1979
2. Moore DC, Crawford RD, Scurlock JE: Severe hypoxia and acidosis following local anesthetic-induced convulsions. ANESTHESIOLOGY 53:259–260, 1980
3. Marx GF: Cardiotoxicity of local anesthetics—The plot thickens. ANESTHESIOLOGY 60:3–5, 1984
4. Kotelko DM, Shnider SM, Dailey PA, Brizgys RV, Levinson G, Shapiro WA, Koike M, Rosen MA: Bupivacaine-induced cardiac arrhythmias in sheep. ANESTHESIOLOGY 60:10–18, 1984
5. deJong RH, Ronfeld RA, DeRosa RA: Cardiovascular effects of convulsant and supraconvulsant doses of amide local anesthetics. Anesth Analg 61:3–9, 1982
6. Hasselström LJ, Mogensen T, Kehlet H, Christensen NJ: Effects of intravenous bupivacaine on cardiovascular function and

¶ Covino BG: Toxicity of local anesthetic agents. ASA Refresher Course Lecture, 1984.

plasma catecholamine levels in humans. Anesth Analg 63:1053–1058, 1984
7. Tanz RP, Heskett T, Loehning RW, Fairfax CA: Comparative cardiotoxicity of bupivacaine and lidocaine in the isolated perfused mammalian heart. Anesth Analg 63:549–556, 1984
8. Hotvedt R, Refsum H, Helgesen KG: Cardiac electrophysiologic and hemodynamic effects related to plasma levels of bupivacaine in the dog. Anesth Analg 64:388–394, 1985
9. Jorfeldt L, Löfström B, Pernow B, Persson B, Wahren J, Widman B: The effects of local anaesthetics on the central circulation and respiration in man and dog. Acta Anaesthesiol Scand 12: 153–169, 1968
10. Liu P, Feldman HS, Covino BM, Giasi R, Covino BG: Acute cardiovascular toxicity of intravenous amide local anesthetics in anesthetized ventilated dogs. Anesth Analg 61:317–322, 1982
11. Blair MR: Cardiovascular pharmacology of local anaesthetics. Br J Anaesth 47:247–252, 1975
12. Dhunér KG, Lewis DH: Effect of local anaesthetics and vasoconstrictors upon regional blood flow. Acta Anaesthesiol Scand (Suppl)23:347–352, 1966
13. Tuvemo T, Willdeck-Lund G: Smooth muscle effects of lidocaine, prilocaine, bupivacaine, and etidocaine on the human umbilical artery. Acta Anaesthesiol Scand 26:104–107, 1982
14. Aberg G, Wahlström B: Mechanical and electrophysiological effects of some local anaesthetic agents and their isomers on the rat portal vein. Acta Pharmacol Toxicol (Copenh) 31:255–266, 1972
15. Johns RA, DiFazio CA, Longnecker DE: Lidocaine constricts or dilates rat arterioles in a dose-dependent manner. ANESTHESIOLOGY 62:141–144, 1985
16. Delano FA, Zweifach BW: Anesthesia and microvascular dynamics in spontaneously hypertensive rats. Am J Physiol 241(Heart Circ Physiol 10):H821, 1981
17. Wergman DL, Joshua IG, Morff RJ, Harris PD, Miller FN: Microvascular responses to norepinephrine in renovascular and spontaneously hypertensive rats. Am J Physiol (Heart Circ Physiol 4):H545–H548, 1979
18. Baez S: An open cremaster preparation for the study of blood vessels by *in vivo* microscopy. Microvasc Res 5:384–394, 1973
19. Duling BR: Changes in microvascular diameter and oxygen tension induced by carbon dioxide. Circ Res 32:370–396, 1973
20. Hutchins PM, Goldstone J, Wells R: Effects of hemorrhagic shock on the microvasculature of skeletal muscle. Microvasc Res 5: 131, 1973
21. Duling BR, Berne RM: Microiontophoretic applications of vasoactive agents to the microcirculation of the hamster cheek pouch. Microvasc Res 1:158–173, 1968
22. Longnecker DE, Creasy RA, Ross DC: A microvascular site of action of sodium nitroprusside in striated muscle of the rat. ANESTHESIOLOGY 50:111–117, 1979
23. Aps C, Reynolds F: The effect of concentration on vasoactivity of bupivacaine and lignocaine. Br J Anaesth 48:1171–1174, 1976
24. Murphy RA, Mras S: Control of tone in vascular smooth muscle. Arch Intern Med 143:1001–1006, 1983
25. Josephson I, Sperelakis N: Local anesthetic blockade of Ca^{++}-mediated action potentials in cardiac muscle. Eur J Pharmacol 40: 201–208, 1976
26. Grim M, Stingl J, Mrazkova O: The impairment of muscle blood vessels after intramuscular injection of local anesthetics. Histochem J 15:314–316, 1983