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, pH, P_O, P_CO, and osmolarity to provide a physiologic environment. Internal diameters of fourth-order arterioles were measured with an electronic vernier display that was connected to the video monitor. Arteriolar diameters were measured every 30 s during a 10-min period, a 10-min period of topical application of bupivacaine hydrochloride, and a 30-min recovery period. Bupivacaine 10-4, 10-5, 10-6, and 10-7 µg·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, 10-5 and 2.5 × 10-5 µg·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 µg·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.4-8,10 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,5,10 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·kg⁻¹, plus urethane, 800 mg·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: NaHCO₃, 25.5; NaCl, 131.9; KCl, 4.7; CaCl₂, 2.0; MgSO₄, 1.17) with or without bupivacaine, was continuously suffused over the muscle at a constant flow rate of 1.5 ml·min⁻¹. The pH 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% CO₂ to maintain a constant P_CO₂ 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.
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Address reprint requests to Dr. Johns.

§ 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, 10⁰, 10¹, 10², 10³, and 2.5 × 10⁵ μg·ml⁻¹ (3.58 × 10⁻⁸ to 8.95 × 10⁶ μg, 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·kg⁻¹ bolus followed by 0.25 mg·kg⁻¹·min⁻¹ in each of seven rats. Both control and drug infusions were administered at a flow rate of 20 μl·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 × 10³ μg·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 10³ μg·ml⁻¹ (table 1). During bupivacaine, 2.5 × 10³ μg·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

<table>
<thead>
<tr>
<th>Drug Type</th>
<th>Control</th>
<th>Drug</th>
</tr>
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<tbody>
<tr>
<td>Topical</td>
<td>97 ± 3</td>
<td>97 ± 3</td>
</tr>
<tr>
<td>10⁻³</td>
<td>101 ± 4</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>10⁻²</td>
<td>97 ± 3</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>10⁻¹</td>
<td>101 ± 4</td>
<td>107 ± 2</td>
</tr>
<tr>
<td>10⁰</td>
<td>101 ± 4</td>
<td>107 ± 3</td>
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<tr>
<td>10¹</td>
<td>100 ± 5</td>
<td>99 ± 6</td>
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<tr>
<td>10²</td>
<td>98 ± 5</td>
<td>101 ± 3</td>
</tr>
<tr>
<td>10³</td>
<td>98 ± 5</td>
<td>101 ± 3</td>
</tr>
<tr>
<td>2.5 × 10⁵</td>
<td>97 ± 4</td>
<td>87 ± 3</td>
</tr>
</tbody>
</table>

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 μg·ml⁻¹ produced no significant change in arteriolar diameters. Bupivacaine 10⁻¹, 10⁰, and 10¹ 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 10² and 2.5 × 10³ μg·ml⁻¹, but these values were not significantly different from control. Mean plasma concentration of bupivacaine after topical bupivacaine, 2.5 × 10³ μg·ml⁻¹, was 1.0 ± 0.1 μg·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 sulfonamide infusion 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 μg·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 μg·ml⁻¹. Liu et al.10 observed no consistent alterations in total peripheral resistance during the infusion of bupivacaine, 0.3 to 10 mg·kg⁻¹, in dogs. However, they observed a marked in-
creased in pulmonary vascular resistance, suggesting a pulmonary vasoconstrictor action of bupivacaine.

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

Tuve and Willden-Lund studied the cumulative dose–response effects of bupivacaine on isolated strips of human umbilical arteries. In five of ten preparations, a
dose-dependent contraction was observed with concentrations of 5–250 μg·ml⁻¹. In five other preparations, a biphasic response was noted, with slight relaxation occurring at higher concentrations (100–250 μg·ml⁻¹). In isolated rat portal veins that were precontracted with norepinephrine, Aberg and Wahlström demonstrated dose-dependent relaxation with bupivacaine concentrations of 10⁴ to 1.6 × 10⁵ μg·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, pH,
$P_{O_2}$, 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.\textsuperscript{15} (The protocols were identical in both studies.) Lidocaine caused progressive vasoconstriction in concentrations of $10^8$ to $10^9 \mu g \cdot ml^{-1}$ and vasodilation at $10^6 \mu g \cdot ml^{-1}$. 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, $2.5 \times 10^9 \mu g \cdot ml^{-1}$, 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.\textsuperscript{f} 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.\textsuperscript{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,\textsuperscript{24} thus an increased concentration of calcium is necessary for contraction. Blair\textsuperscript{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.\textsuperscript{25} Alternatively, vasodilation could be explained by tissue-irritating effects of local anesthetics. Grim et al.\textsuperscript{86} 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, $2 \times 10^4 \mu g \cdot ml^{-1}$, than bupivacaine, $5 \times 10^4 \mu g \cdot ml^{-1}$.

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 ($1-2.5 \times 10^5 \mu g \cdot ml^{-1}$), 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
3. Marx GF: Cardiotoxicity of local anesthetics—The plot thickens. ANESTHESIOLOGY 60:3–5, 1984
6. Hasselström LJ, Mogensen T, Kehlet H, Christensen NJ: Effects of intravenous bupivacaine on cardiovascular function and

\textsuperscript{f} Covino BG: Toxicity of local anesthetic agents. ASA Refresher Course Lecture, 1984.
VASCULAR EFFECTS OF BUPIVACAINE