Determination of Local Tissue Concentrations of Bupivacaine Released from Biodegradable Microspheres and the Effect of Vasoactive Compounds on Bupivacaine Tissue Clearance Studied by Microdialysis Sampling

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Purpose. Incorporation of bupivacaine, a short acting local analgesic, into injectable microspheres provides a long acting formulation. Co-incorporation of dexamethasone into the microspheres results in extended activity. The purpose of this study is to compare tissue concentrations of bupivacaine resulting from the two types of microspheres and to determine if the observed sustained tissue concentration of bupivacaine is due to changes in its tissue clearance.

Methods. Microdialysis probes were implanted into rat muscle. Following microsphere injection, bupivacaine tissue concentration was monitored (HPLC-UV), and the tissues histologically examined. The effect of vasoactive compounds on the tissue concentration of bupivacaine, not formulated in microspheres, was monitored.

Results. Hind muscle histology showed significant granulomatous reactions around the probe and both types of microspheres. A higher, prolonged bupivacaine concentration was observed from microspheres. A higher, prolonged bupivacaine concentration was observed from microspheres with co-incorporated dexamethasone relative to those without dexamethasone. Addition of vasoconstrictors to the perfusate containing bupivacaine resulted in decreased bupivacaine delivery compared to bupivacaine alone, whereas the addition of a vasodilator increased bupivacaine delivery.

Conclusions. The longer lasting effect of microspheres with co-incorporated dexamethasone results from higher, prolonged tissue concentrations of bupivacaine. Dexamethasone, a vasoconstrictor, decreases the clearance rate of bupivacaine from the tissue resulting in a higher, prolonged tissue concentration of bupivacaine.

KEY WORDS: bupivacaine; microspheres; controlled release; microdialysis sampling; vasoactive compounds.

INTRODUCTION

The use of sustained release local analgesic systems (surgical implants, lipo- and polymer-based microspheres) offer an alternative over catheter applications or repeated injections of short duration neuroblocking agents. Biodegradable microspheres represent an attractive method for the local delivery of anesthetics because they are both injectable and biodegradable, allowing for prolonged therapeutic effects and eliminating the necessity of surgical implantation or removal. Previous work has shown that incorporation of dexamethasone into biodegradable microspheres containing bupivacaine provides prolonged regional effects: prolonging rat sciatic nerve sensory blockade by eight to thirteen times compared with that produced by bupivacaine microspheres alone (1) and prolonging intercostal nerve blockade in sheep using controlled-release of bupivacaine and dexamethasone from polymer microspheres (2).

Microdialysis, originally developed to monitor neurotransmitter concentrations in the brain, has been shown to be a powerful tool for pharmacokinetics applications (3). This is especially true concerning the distribution and metabolism of drugs (4,5). The use of microdialysis sampling to study the pharmacokinetics of drugs in peripheral tissue has been demonstrated in numerous reports (6,7). Microdialysis sampling is suitable for a wide range of matrices and has been applied to virtually every major organ of the body including the liver, kidney, heart, skin and muscle (4).

Microdialysis is a diffusion-controlled process. A solution, termed the perfusate, is slowly pumped through a probe that is implanted in the experimental animal. The perfusate is an aqueous solution that mimics the pH and ionic composition of the surrounding sample matrix. The rate of perfusion is typically in the range of 0.5–5.0 μl min^{-1}. At this flow rate, no net transport of liquid across the dialysis membrane occurs. Therefore, the existing concentration gradient is the driving force for mass transport. A diagram of the microdialysis process is shown in Fig. 1.

Microdialysis is a dynamic technique where the perfusion solution is constantly being pumped through the probe, therefore, equilibrium is not established. Because equilibrium is not established, the concentration of the dialysate is related to the concentration in the external medium by the extraction efficiency (8,9). Key to this work is that the extraction efficiency is a function of the clearance of the compound from the surrounding tissue. As the clearance of bupivacaine from the tissue increases, the delivery of bupivacaine to the tissue also increases.

The extraction efficiency can be determined by conducting a delivery experiment. In a delivery experiment, a known concentration of analyte is included in the perfusate whereas the external medium contains no analyte. The extraction efficiency is the ratio of the loss of the analyte from the perfusate relative to its initial concentration in the perfusate. The extraction efficiency is calculated by:

$$EE_D = (Cp - Cd)/Cp$$

where Cp is the initial concentration in the perfusate and Cd is the concentration in the dialysate.

The objective of the this work is to evaluate the mechanism of action of the microsphere formulations for the prolonged activity of bupivacaine *in vivo*. The two hypotheses are: (1) the release rate of bupivacaine from the microspheres is decreased by the co-incorporation of dexamethasone rela-

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ABBREVIATIONS: LAB, long-acting bupivacaine; LAB-D, long-acting bupivacaine with co-incorporated dexamethasone; PAN, polyacrylonitrile; PLGA, poly-DL-lactide-co-glycolide.

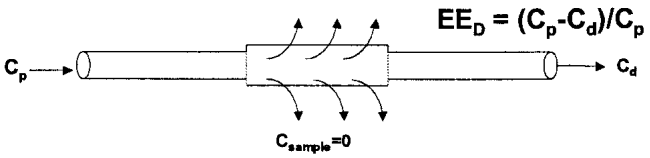

Fig. 1. Diagram of the microdialysis process. EE_D, delivery extraction efficiency; Cp, concentration of analyte in the perfusate; Cd, concentration of analyte in the dialysate.

tive to microspheres containing bupivacaine alone (2) dexamethasone acts as a vasoconstrictor to decrease the blood flow around the microsphere. Decreased blood flow will cause a decrease in the clearance of bupivacaine from the tissue, thus producing higher tissue concentrations that are maintained for a longer period of time. If bupivacaine clearance from the muscle was affected by the vasoactive compound, this would be reflected by a change in bupivacaine delivery.

MATERIALS AND METHODS

Materials

Bupivacaine hydrochloride, dexamethasone, LAB, LAB-D, and the diluent (1% Sodium Carboxymethylchitosan, 0.1% Tween 80) were obtained from Purdue Pharma LP (Yonkers, NY). Ringer's solution consisted of 145 mM NaCl, 2.8 mM KCl, 1.3mM $CaCl_2$, 1.2 mM $MgCl_2$, all purchased from Sigma (St. Louis, MO). Dexamethasone, epinephrine, and norepinephrine were purchased from Sigma (St. Louis, MO). Sumatriptan (Imitrex) was purchased from GlaxoWellcome. Papaverine was purchased from PolyPharm (Russia). HPLC grade acetonitrile and o-phosphoric acid (85%) were purchased from Fisher Scientific. Ketamine was purchased from Fort Dodge Animal Health and xylazine was purchased from Bayer. Sterile Ringer's solution (0.9% sodium chloride) used for post-surgical administration was purchased from Baxter Healthcare Corporation. Roccal D purchased from Pharmacia and Upjohn (Kalamazoo, MI) was used for sterilizing the surgical instruments.

Animals

Female Sprague-Dawley rats weighing 235–360 g were housed in cages in temperature-controlled rooms with free access to food and water. All experiments were in accordance with the *Principles of Laboratory Animal Care* (NIH Publication no. 85-23, revised 1985).

Surgical Procedure

Adult female rats were initially anesthetized by inhalation of isofluorane followed by i.m. injection of a ketamine (100 mg/kg)/ xylazine (20 mg/kg) mixture. The animal's body temperature was maintained until it recovered from surgery by placing the animal on a heating pad. Strict aseptic technique was used during the surgical procedure. All drapes were sterilized by autoclave. All surgical instruments were sterilized in a stock solution of Roccal-D Plus that was diluted 1:100 in water. Roccal-D Plus is a veterinary and animal care disinfectant made of dimethyl ammonium chloride. After shaving the hair and cleaning the skin on the back of the neck and over the leg muscle, a 4-cm long skin incision was made to expose the target tissue for probe implantation. A linear probe (Bioanalytical Systems Inc. (BAS), Inc., West Layfayette, IN) was implanted in the muscle tissue by inserting a 20-gauge needle into the muscle tissue of the left hind leg. One end of the probe tubing was threaded through the needle. The needle was withdrawn leaving the dialysis membrane imbedded in the muscle. The membrane was fixed with small drops of tissue glue (VetBond, 3M, Inc.). The probe was then tunneled under the skin to the nape of the neck using a skin tunneling needle. The animal was placed in a harness for attachment to the animal containment system. The probe tubing was then pulled through a small hole in the harness for attachment to the microdialysis system. The skin incisions were closed using tissue glue and stainless steel clips leaving an exposed probe outlet site for precise injection of microspheres around the active window of the probe. At the end of every surgical procedure, 10-ml sterile Ringer's solution was given subcutaneously to replace fluids that may be lost during surgery.

Dialysis System

Linear microdialysis probes with a 10-mm dialysis membrane made of a biocompatible plastic polymer (240 μm i.d., 320 μm o.d., molecular weight cut off of 6–7 kDa) were used. The inlet of the microdialysis probe was connected with fluorinated ethylenepropylene (FEP) tubing to a Hamilton syringe mounted on a CMA 100-microinjection pump (BAS). The microdialysis probe outlet was connected to a CMA 160-injection valve (BAS) with FEP tubing. The microinjection pump delivered the perfusion medium at a flow rate of 1 μl min^{-1} for the experiments involving vasoconstrictors added to bupivacaine and at a flow rate of 2 μl min^{-1} for the experiment where the vasodilator, papaverine, was added to bupivacaine. On-line injections were made at 10-min intervals for all pharmacokinetic experiments. The animal was housed in a Raturn awake animal containment system (BAS) is shown in Fig. 2.

Chromatographic System

The chromatographic system consisted of an ISCO 2350 pump (ISCO Inc., Lincoln, NE) and a Shimadzu SPD-6AV UV-Vis spectrophotometric detector (Shimadzu Scientific Instruments, Inc., Columbia, MD). Separation of bupivacaine from the vasoconstrictors was achieved using a Zorbax Eclipse XDB column (50 mm × 2.1 mm i.d., 3.5 μm ODS, Agilent Technologies) with a mobile phase of 50 mM phos-

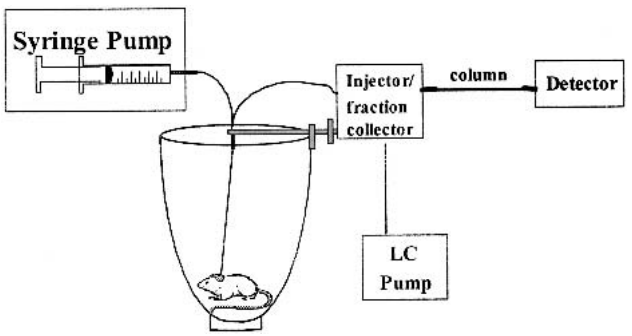

Fig. 2. Awake animal containment system for *in vivo* microdialysis studies.

phate buffer, pH 2.6/acetonitrile (80:20 v/v). Separation of bupivacaine from the papaverine was achieved using a Micra NPS column (33 mm × 4.6 mm i.d., 1.5 μm ODS) with a mobile phase of 50 mM phosphate buffer, pH 2.6/acetonitrile (90:10 v/v). The flow rate was 200 μl/min for all separations. Detection was at a wavelength of 210 nm for all separations. Chromatographic data was acquired using TurboChrom software (Perkin Elmer, San Jose, CA, USA).

Calibration of Microdialysis Probes

The microdialysis probe extraction efficiency (EE) was determined *in vitro* by determining the recovery of bupivacaine from a stirred Ringer's solution at 37°C according to the following:

$$EE_R = Cd/Cs$$

where EE_R is the extraction efficiency using recovery, Cd is the concentration of analyte in the dialysate, and Cs is the concentration of analyte in the sample vial. The microdialysis probe extraction efficiency was also determined *in vitro* by determining the delivery of bupivacaine to a stirred Ringer's solution according to the following:

$$EE_D = (Cp–Cd)/Cp$$

Where EE_D is the extraction efficiency using delivery, Cp is the initial concentration of analyte in the perfusate, and Cd is the concentration of analyte in the dialysate. The delivery of 0.5 μg/ml, 5 μg/ml, and 20 μg/ml bupivacaine solutions was determined. A linear probe was placed in the vial with the ends extending out of the cap. Dialysate samples were collected until at least three consistent samples were obtained.

In vivo extraction efficiency was determined by estimation of the delivery of the same bupivacaine solutions in the muscle tissue after the animal had recovered from surgery. The delivery continued until at least three consistent samples were obtained. Following the delivery, the probe was again perfused with Ringer's solution at a flow rate of 1 μl/min.

Monitoring Bupivacaine Release from Sustained Release Formulations *in Vitro*

A microdialysis probe with previously determined EE was placed into a stirred vial containing Ringer's solution maintained at 37°C. The probe was continuously perfused with Ringer's solution at 1 μl/min and samples were collected over 30–60 min intervals. A known concentration of microspheres (3 mg of bupivacaine·HCl in 1 mL of Ringer's solution) was then added to the vial with constant stirring. Samples were collected for at least 25 h after the addition of microspheres.

Long-Term Monitoring of Bupivacaine Release *in Vivo*

The Raturn (BAS) is an awake animal system, designed to counteract movements of the rat by means of a motorized turntable. The externalized probe inlet and outlet tubing was connected through fluorinated ethylenepropylene (FEP) tubing attached to a CMA\100 microinjection pump and a CMA\140 fraction collector or CMA\160 HPLC injection valve for on-line bupivacaine monitoring. The perfusion flow rate was 1μl/min. The implanted microdialysis probe was then calibrated by delivery. Once the probe was calibrated, the fully recovered, awake rat was locally treated with either bupivacaine hydrochloride (3 mg), LAB (50 mg), or LAB-D (50 mg) injected in close proximity to the microdialysis probe under visual control. Using this system, dialysate samples were continuously collected from awake, freely moving animals for up to 10 days.

Study Design—Vasoactive Compounds

Immediately following the surgical procedure, the probe was perfused with a Ringer's solution to continuously wet the probe. The animals were allowed to recover from surgery for 3–4 h. A stock solution of bupivacaine (2 mg/ml) was prepared by dissolving 20 mg of bupivacaine in 2 ml of acetonitrile and 8 ml of Ringer's solution. The stock solution was then diluted to 40 μg/ml (138 μM) in Ringer's solution and used as the perfusate. This solution was perfused through the probe to determine the steady-state delivery of bupivacaine. A solution of bupivacaine plus the vasoactive compound was then perfused and the bupivacaine delivery again allowed to reach steady state. The concentrations of the vasoactive compounds were as follows. For dexamethasone, a 2mg/ml stock solution was prepared in acetonitrile and diluted to 100 μg/ml (254 μM) in Ringer's solution. This was then diluted 1:50 into the bupivacaine solution for use as the perfusate. For epinephrine and norepinephrine, a 1mg/ml (5.46 mM) stock solution was prepared in Ringer's solution and diluted 1:100 into bupivacaine solution for use as the perfusate. For sumatriptan, a 5mg/ml (16.9 mM) stock solution was prepared in Ringer's solution and diluted 1:100 into bupivacaine solution for use as the perfusate. For papaverine, a 2 mg/ml (5.89 mM) stock solution was prepared in Ringer's solution and diluted 1:100 into bupivacaine solution for use as the perfusate.

Three animals were used in each test group. The difference in the steady-state delivery of bupivacaine alone and in the presence of the vasoactive compound was determined in each experimental animal with a single microdialysis probe, therefore, each animal served as its own control to eliminate interanimal and interprobe variability. Because the concentration of bupivacaine in the perfusate was the same for all experiments, the concentration of bupivacaine in the dialysate can be used to determine the change in delivery. A greater delivery of bupivacaine will result in a smaller peak height for bupivacaine in the dialysate. Differences between the delivery of bupivacaine alone and with the vasoactive compound were evaluated using a paired two sample T-test.

Histologic Examination

Post-mortem histologic evaluations for inflammation, infection and correspondence of the site of probe implantation and microsphere injection were conducted. After completion of an experiment on bupivacaine monitoring or at particular time intervals, the animal was euthanized, and the muscle tissue surrounding the microdialysis probe was excised and placed in a 10% formaldehyde solution for fixation. The tissue was then embedded in paraffin and stained with hematoxylin-eosin. Histologic examination was performed by light microscopy in the morphologic lab of Lawrence Memorial Hospital (Lawrence, KS)

Table I. Extraction Efficiency (EE) of Linear Microdialysis Probe for Bupivacaine Determined by Recovery and Delivery Experiments (n = 5)

Bupivacaine concentration	*In vitro* EE_R	*In vitro* EE_D	*In vivo* EE_D
0.5 μg/ml	25.6 ± 2.2	26.1 ± 3.7[a]	17.4 ± 1.9[b]
5 μg/ml	27.1 ± 2.7	24.9 ± 3.3[a]	17.0 ± 2.4[b]
20 μg/ml	24.4 ± 2.0	25.7 ± 2.4[a]	17.9 ± 3.1[b]

[a] $P < 0.50$.
[b] $P < 0.005$.

RESULTS AND DISCUSSION

Calibration of Microdialysis Probes

The extraction efficiency of 1-cm linear microdialysis probes varied between 24.4% and 27.1% for experiments *in vitro* and between 17.0% and 17.9% for delivery *in vivo* (Table I). The delivery and recovery values showed no concentration dependence. The EE_R is not statistically different than EE_D *in vitro*. However the EE_D *in vivo* is less than the EE_D *in vitro*. These extraction efficiencies are sufficient to monitor the concentration of bupivacaine in muscle dialysate over a 10-day study.

Bupivacaine Release from LAB and LAB-D Formulations *in Vitro*

To evaluate the possible difference in bupivacaine release from LAB and LAB-D, the time course of bupivacaine release from microspheres was compared using the microdialysis sampling technique. Previous results on bupivacaine release kinetics *in vitro* from current long-acting formulations were confirmed using microdialysis sampling. Almost identical release curves were observed *in vitro* for both formulations (Fig. 3). Bupivacaine released from the microspheres reached equilibrium with the surrounding medium 45–48 h after the addition of 5 mg of microspheres to 2 ml of Ringer's solution at 37°C.

Pharmacokinetics of Bupivacaine·HCl in the Rat Hind Muscle after Bolus Intra-Muscular Injection

The time course of bupivacaine in the muscle interstitium around microdialysis probes differs substantially between anesthetized and awake, freely moving rats. In both cases, high levels of bupivacaine were determined in the first dialysate sample collected immediately after injection. However, in anesthetized animals the local concentration of bupivacaine remains higher and longer, beginning to decrease only after general anesthesia begins to subside. In awake animals, elimination of bupivacaine from the hind muscle after local administration occurred within 3 h after the time of injection (Fig. 4).

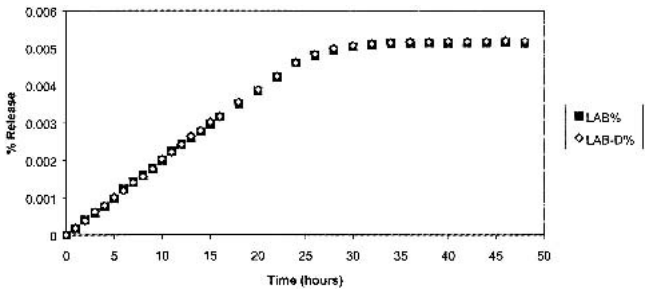

Fig. 3. *In vitro* bupivacaine release from LAB and LAB-D microsphere formulations. LAB% (filled squares), LAB-D% (open diamonds).

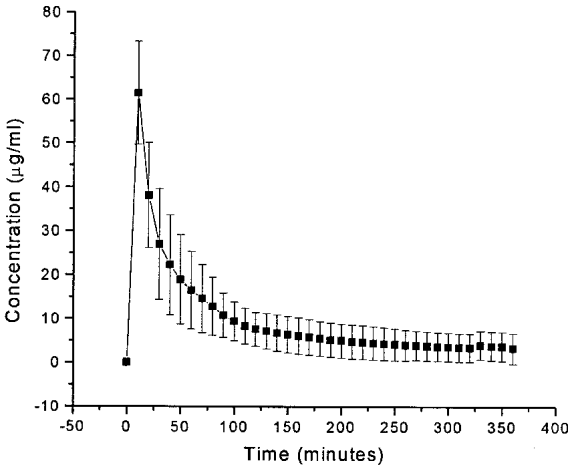

Fig. 4. Elimination of bupivacaine from the hind muscle of an awake rat following i.m. injection of 3 mg of bupivacaine·HCl in 1 mL of Ringer's solution.

Bupivacaine Release from LAB and LAB-D Formulations *in Vivo*

Administration of sustained release formulations into the leg muscle under visual control provided a comparatively homogenous suspension of LAB or LAB-D around the microdialysis probe. The use of the Raturn system for awake, freely moving animals provided long-term monitoring of bupivacaine release from LAB and LAB-D formulations for up to 10 days. Time courses of interstitial bupivacaine following i.m. injection of the LAB and LAB-D formulations are shown in Fig. 5. Due to the mixing time of microsphere suspensions before administration and injection of these sustained release

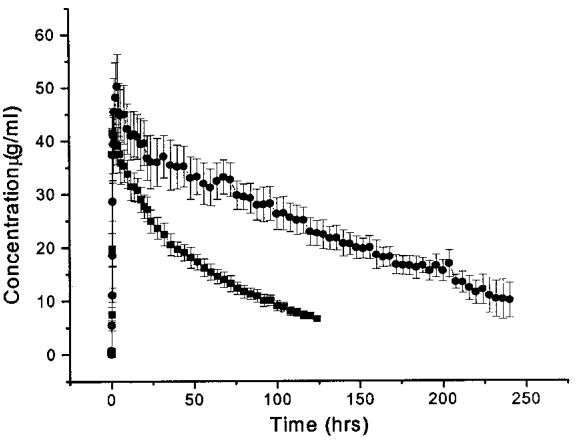

Fig. 5. Time courses of interstitial bupivacaine following i.m. injection (50 mg of microsphere in 1 mL of diluent) of LAB and LAB-D. LAB (closed squares), LAB-D (closed circles).

Table II. Time Course of Bupivacaine in Muscle Dialysate

Bupivacaine formulation	(C_{max}) (μg/mL)	(T_{max}) (hr)	Average concentration of bupivacaine (μg/mL)			
			2 day	4 day	6 day	8 day
Bupivacaine hydrochloride[a]	32.1 ± 10.1	0.2 ± 0.0	ND[d]	ND[d]	ND[d]	ND[d]
LAB[b]	38.8 ± 10.5[e]	1.4 ± 0.9[e]	21.0 ± 3.9[e]	11.1 ± 2.7[e]	7.0 ± 1.4[e]	3.8 ± 0.8[e]
LAB-D[c]	56.4 ± 14.2	4.5 ± 1.4	37.8 ± 9.9	29.2 ± 6.2	23.2 ± 5.1	15.6 ± 4.1

[a] N = 8.
[b] N = 14.
[c] N = 12.
[d] ND = not detected.
[e] P < 0.001.

formulations to still anesthetized animals, the initial part of the time course corresponds to the elimination of free bupivacaine in an anesthetized animal. The highest concentrations of bupivacaine occurred in the muscle dialysate shortly after the administration of LAB or LAB-D microspheres (Table II) and showed a dependence on the accuracy of formulation administration around the microdialysis probe. The maximum interstitial concentrations were in the range of 26–62 μg/ml for LAB and 25–72 μg/ml for LAB-D. Higher concentrations of bupivacaine were observed for LAB-D compared to LAB starting two days after microsphere administration (Table II). The largest difference between LAB and LAB-D was observed at more than 3 days after administration. On day 6, the tissue concentration of bupivacaine from LAB-D was approximately 3 times that from LAB. Negligible concentrations of bupivacaine were detected after day 8 from LAB while significant amounts remained from LAB-D.

Assessment of Tissue Reaction to Experimental Procedures

Because some damage invariably occurs when foreign bodies are implanted or injected into tissues, the reaction to probe implantation and microsphere injection was investigated. Reactions such as local hematoma, areas of necrosis, leukocyte infiltration and scar tissue formation were assessed by histologic examination. The tissue reaction to microsphere injection and microdialysis probe implantation were studied individually and in combination. Fig. 6 (A) shows the reaction of muscle tissue to the injection of a bupivacaine hydrochloride solution. It should be noted that this was a non-sterile solution as solid bupivacaine hydrochloride was dissolved in sterile Ringer's solution. The photomicrographs clearly show the infiltration of leukocytes into the area of injection with the extent of infiltration increasing with time. Variable amounts of inflammatory cells, hemorrhage, myofibroblasts and fibroblasts can also be seen after injection. This is a normal response for wound healing. The extent of cell infiltration was maximal at eight days after injection. Post-mortem macro-examination of the muscle showed no difference in the muscle surface between normal tissue and tissue into which an injection had been made.

The tissue response to the injection of LAB-D is shown in Fig. 6 (B). Again, these injections are not sterile. Although the Ringer's solution diluent was sterile, the microsphere suspension was not sterilized. As can be seen in the photomicrographs, the tissue response was essentially the same as upon injection of soluble bupivacaine. A gradual increase in leukocyte infiltration occurred, transforming to fibroblast migration with formation of scar tissue at the site of injection around eight days after injection. The extent of tissue response was larger for the microspheres than for soluble bupivacaine, probably reflecting the wider area of insult due to the microspheres. In this case, post-mortem visual inspection of the tissue showed slight discoloration at the site of injection. There was no difference in tissue response to LAB-D compared to LAB.

Figure 6 (C) shows the reaction of muscle tissue to the implantation of a microdialysis probe. The microdialysis probe was sterilized in 70% ethanol prior to injection. The tissue response shortly (a few hours) after manipulation was limited to mild myocyte cell damage and hematoma from the 23-gauge needle used for implantation. One to two days after implantation, mild congestion of neutrophils in the tissue occurred and continued for the next several days. The extent of tissue response depended on the degree of damage caused during the implantation procedure.

The tissue response to the combination of microdialysis probe implantation and microsphere injection is shown in Fig 6 (D) No difference occurred in the tissue response to these procedures relative to injection of microspheres alone. This indicates that the response to the injection was greater or equivalent to the response to microdialysis probe implantation. The conclusion is that the probe implantation does not perturb the tissue relative to the normal procedure. Therefore, microdialysis sampling can be used to monitor the re-

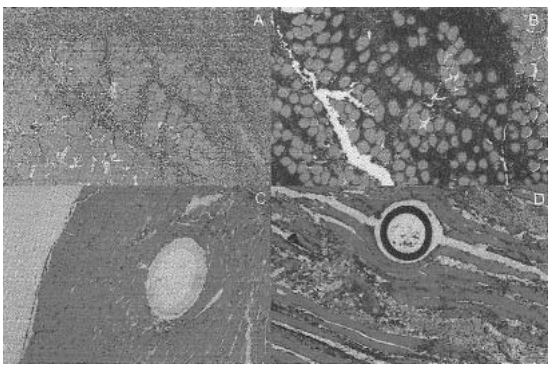

Fig. 6. Assessment of tissue reaction to experimental procedures. (magnification = 40×) (A) Reaction of intact muscle to the injection of bupivacaine hydrochloride. (B) Reaction of intact muscle tissue to the injection of LAB-D. (C) Reaction of intact muscle tissue to the implantation of the microdialysis probe. (D) Reaction of intact muscle tissue to the implantation of the microdialysis probe and injection of LAB.

lease of bupivacaine from the microspheres without affecting that release by inducing a local tissue response.

Effect of Vasoactive Compounds on the Clearance Rate of Bupivacaine

The amount of bupivacaine remaining in the dialysate was determined chromatographically by peak height. The amount of bupivacaine in the dialysate is inversely proportional to the delivery, therefore the change in delivery was determined by the change in bupivacaine in the dialysate. To establish that there were no interferences, Ringer's alone was first perfused for about 45 min. A representative chromatogram of the blank dialysate is shown in Fig. 7 (A). The perfusate was then switched to a bupivacaine solution until a steady-state delivery was established. A representative chromatogram of the perfusion of bupivacaine alone is shown in Fig. 7 (B). To determine the effect of the vasoactive compounds on bupivacaine delivery, the perfusate was switched to contain bupivacaine with the vasoactive compound and a new steady-state delivery of bupivacaine established. The vasoactive compounds did not interfere with the chromatographic determination of bupivacaine. A representative chromatogram of the perfusion of bupivacaine with dexamethasone is shown in Fig. 7 (C). As can be seen, the peak height for bupivacaine increased when dexamethasone was co-perfused, indicating a decrease in the delivery of bupivacaine.

A typical time course for changes in bupivacaine delivery with co-perfusion of dexamethasone is shown in Fig. 8. When changing the perfusion solution a time delay was observed before the change was reflected in the collected dialysate sample. This delay is attributed to the system volume of the tubing between the microdialysis pump and the chromatographic injector. When perfusing bupivacaine alone, a steady-state delivery of bupivacaine was achieved approximately 40 min after the bupivacaine was first observed in the dialysate (Fig. 8). The discontinuity at 120 min is caused by stopping the microinfusion pump to change to the perfusate containing both bupivacaine and dexamethasone. Using a perfusate containing both bupivacaine and dexamethasone, the peak height of bupivacaine in the dialysate increased from that observed when perfusing with bupivacaine alone (Fig. 8). The time course for this increase was similar to that of switching from Ringer's solution to bupivacaine. The time courses of all of the vasoactive compounds were similar. The time course for the vasodilator, papaverine, was similar except that the peak height of bupivacaine decreased instead of increased upon co-perfusion of the vasodilator.

The changes in the bupivacaine delivery on addition of the vasoactive compounds, as determined from the bupivacaine remaining in the dialysate, are given in Table III. In all cases, administration of bupivacaine with a vasoactive compound resulted in statistically significant changes in the delivery of bupivacaine relative to bupivacaine alone. Administration of bupivacaine combined with vasoconstrictors resulted in a significant decrease in the delivery (increase in chromatographic peak height) of bupivacaine vs. the delivery of bupivacaine alone for sumatriptan, dexamethasone, norepinephrine, and epinephrine ($p < 0.01$, 0.02, 0.05, 0.10 respectively). Administration of bupivacaine plus the vasodilator, papaverine, resulted in an increase in the delivery of bupivacaine compared to the delivery of bupivacaine alone. A statistically significant increase was observed ($p < 0.05$).

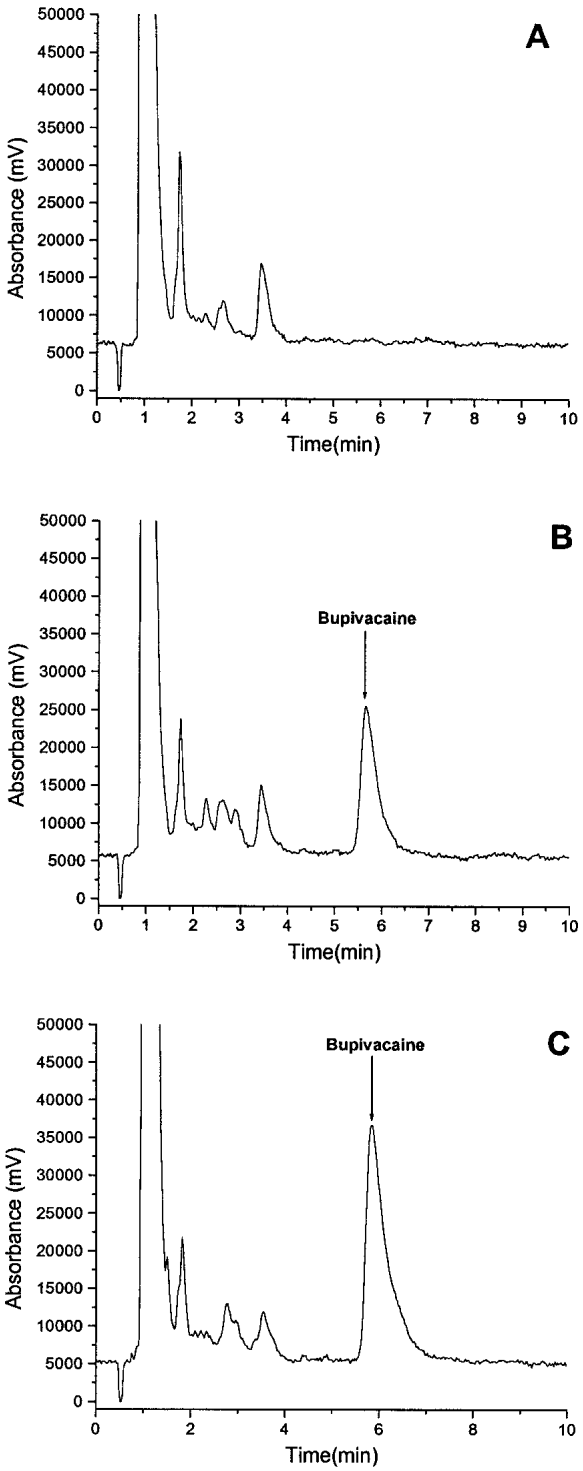

Fig. 7. (A) Chromatogram of an injection of rat dialysate when only Ringer's solution is perfused. (B) Chromatogram of the perfusion of bupivacaine. (C) Chromatogram of the perfusion of bupivacaine with dexamethasone.

A change in the delivery of bupivacaine *in vivo*, reflects a change in the clearance of bupivacaine from the tissue. Adding a vasoconstrictor causes a decrease in blood flow and therefore a decrease in the clearance of bupivacaine. This decrease in clearance is reflected in a decrease in the delivery of bupivacaine from the microdialysis probe. Similarly, addi-

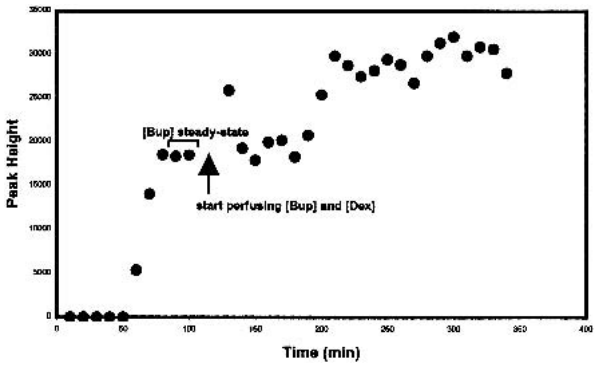

Fig. 8. Example of raw data for determining bupivacaine delivery. Bupivacaine is perfused at time zero. The perfused is switched to contain bupivacaine plus dexamethasone at 120 min.

tion of a vasodilator causes an increase in the blood flow that results in an increase in the clearance of bupivacaine. The increase in clearance is reflected in an increase in the delivery of bupivacaine from the microdialysis probe.

CONCLUSIONS

A higher prolonged concentration of bupivacaine is observed *in vivo* for microspheres containing bupivacaine with incorporated dexamethasone compared to microspheres containing bupivacaine alone. The release rate of bupivacaine from the both LAB and LAB-D is not statistically different. However, significant changes in the delivery of bupivacaine from a microdialysis probe implanted in muscle tissue were observed when a vasoactive compound was added to the perfusate. The change in delivery is due to a change in the clearance of bupivacaine from the tissue surrounding the microdialysis probe. These results are consistent with the observation that dexamethasone co-incorporated into microspheres re-

Table III. Peak Heights of Bupivacaine alone and Combined with Vasoactive Compounds

Dexamethasone			
	PH(B)[a](×1000)	PH(B+D)[b](×1000)	[PH(B)-PH(B+D)] (×1000)
Rat 1	66.6	86.2	–19.6
Rat 2	18.2	34.3	–16.1
Rat 3	66.7	78.6	–11.9
mean			–15.9
st. dev.			3.9
P-value			P < 0.02
Sumatriptan			
	PH(B)	PH(B+I)	PH(B)-PH(B+I)
Rat 1	25.6	35.9	–10.3
Rat 2	18.4	29.8	–11.4
Rat 3	24.6	39.1	–14.5
mean			–12.1
st. dev.			2.2
P-value			P < 0.01
Norepinephrine			
	PH(B)	PH(B+N)	PH(B)-PH(B+N)
Rat 1	48.0	75.7	–27.7
Rat 2	72.2	102.2	–30.0
Rat 3	37.0	90.9	–53.9
mean			–37.2
st. dev.			14.5
P-value			P < 0.05
Epinephrine			
	PH(B)	PH(B+E)	PH(B)-PH(B+E)
Rat 1	20.0	81.6	–61.6
Rat 2	22.9	45.7	–22.8
Rat 3	73.7	115.1	–41.4
mean			–41.9
st. dev.			19.4
P-value			P < 0.10
Papaverine			
	PH(B)	PH(B+P)	PH(B)-PH(B+P)
Rat 1	44.7	33.5	11.2
Rat 2	42.1	28.4	13.7
Rat 3	33.6	25.8	7.8
mean			10.9
st. dev.			3.0
P-value			P < 0.05

[a] Peak height of bupivacaine.
[b] Peak height of bupivacaine + vasoactive cmpd.

sults in a higher and longer lasting concentration of bupivacaine at the site of implantation relative to identical microspheres not containing dexamethasone. The dexamethasone causes local vasoconstriction that results in slower clearance of bupivacaine from the tissue and therefore a higher concentration of bupivacaine.

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