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Glucocorticoids Prolong Rat Sciatic Nerve Blockade In Vivo *from Bupivacaine Microspheres*

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***Background*: Previous work showed that incorporation of dexamethasone (0.05 weight/weight percentage) into bupivacaine microspheres prolonged blockade by eight to 13 times compared with that produced by bupivacaine microspheres alone. The determinants of dexamethasone's block-prolonging effect were examined and reported here.**

***Methods*: Polylactic-co-glycolic acid polymer microspheres (65/35) with 75 weight/weight percentage bupivacaine were prepared. Microspheres were injected adjacent to the rat sciatic nerve, and sensory and motor blockade were assessed. A procedure was developed to test drugs for block-prolonging ability *in vivo* by placing test drugs in the injection fluid along with a suspension of bupivacaine microspheres.**

***Results*: Dexamethasone alone in suspension did not produce blockade, nor did it prolong blockade induced by aqueous bupivacaine. Bupivacaine microspheres (150 mg drug/kg rat weight) produced blockade for 6 to 10 h. Dexamethasone in the suspending solution of microspheres prolonged block by up to five times. Glucocorticoids prolonged block in proportion to glucocorticoid/antiinflammatory potency. The corticosteroid antagonist cortexolone inhibited dexamethasone's blockade-prolonging action. Durations of blockade with or without dexamethasone were unaltered by hydroxyurea-induced neutrophil depletion. Microspheres were extracted from rats at time points ranging from 7 h to 7 days, and residual microsphere dry weight and bupivacaine content were similar in groups of rats injected with either bupivacaine microspheres or bupivacaine microspheres containing dexamethasone, respectively.**

***Conclusions*: Glucocorticoids prolong blockade from bupivacaine microspheres. The mechanism appears unrelated to the kinetics of bupivacaine release *in vivo*. (Key words: Anesthesia, regional. Anesthetics, local: bupivacaine; dexamethasone. Pharmacology, drug delivery: sustained release. Glucocorticoid. Measurement techniques, microspheres: biodegradable polyester. Nerves, sciatic: blockade. Animals: rat.)**

PREVIOUS work in our laboratories has characterized implantable sustained-release preparations of local anesthetics in pellets,[1,2] implanted microspheres,[3] and injectable microspheres for prolonged blockade of peripheral nerves.[4] Malinovsky and associates[5] and Estebe and colleagues[6] recently described preparations of bupivacaine microspheres with a much lower percentage of drug loading and more rapid drug release than our preparations contain. They examined epidural and subarachnoid administration of bupivacaine microspheres and found modest prolongation of blockade.

In our previous study,[4] the duration of sciatic blockade obtained from bupivacaine-loaded microspheres containing dexamethasone was as much as 13 times greater than the blockade duration induced by microspheres containing bupivacaine alone. Dexamethasone does not substantially slow the release of bupivacaine from microspheres *in vitro*.[4]

In the current study, we examine some of the features of dexamethasone's novel blockade-prolonging effect.

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To streamline pharmacologic studies, a convenient model was developed to test the ability of other drugs (steroid hormones, nonhormonal sterols, the nonsteroidal antiinflammatory agent ketorolac, and epinephrine) to prolong blockade induced by bupivacaine. In this model, test drugs were added to a suspension of bupivacaine microspheres in the injection fluid, and block duration was assessed. This model allowed testing of several different additives quickly and efficiently and eliminated the need to fabricate different batches of microspheres with each particular test drug incorporated in a range of doses.

Specifically, we tested the following hypotheses:

1. Dexamethasone's block-prolonging effect is shared by other glucocorticoids and is mediated by a glucocorticoid receptor, which can be blocked by the antagonist cortexolone.
2. Other hormonal steroids, nonhormonal sterols, nonsteroidal antiinflammatory drugs, and epinephrine prolong blockade.
3. Dexamethasone's block-prolonging action is a local, not systemic effect.
4. Dexamethasone added to the injection fluid produces conduction blockade and prolongs the blockade from aqueous bupivacaine.
5. Dexamethasone slows bupivacaine release from microspheres *in vivo*.
6. Dexamethasone acts primarily by inhibiting encapsulation.
7. Depletion of circulating neutrophils results in prolonged blockade (more than 24 h) from bupivacaine microspheres without dexamethasone.

Materials and Methods

Materials

The following polymeric microspheres were supplied by Medisorb Technologies International, L.P., Cincinnati, Ohio, and formulated by a solvent extraction method: 65/35 poly-lactic-co-glycolic acid [PLGA]; mean MW, 130,000; loaded with 75 weight/weight percent [w%] bupivacaine and 0.05 w% dexamethasone; 65/35 PLGA loaded with 75 w% bupivacaine and 0.05 w% cholesterol; 65/35 PLGA loaded with 75 w% bupivacaine alone; and 65/35 PLGA containing neither bupivacaine nor dexamethasone (empty microspheres). Estradiol (lot #113H0615), testosterone (lot #11H0756), betamethasone (lot #08544JG), dexamethasone (lot #34H0502), and hydroxyurea (lot #75H0050) were supplied by Sigma Chemical Company, St. Louis, Missouri. Cholesterol (lot #02816DF) and cortexolone (lot #10724BZ) were supplied by Aldrich, Milwaukee, Wisconsin. Sodium hydrocortisone (Solu-Cortef) and methylprednisolone (lot #136KP) were supplied by the Upjohn Company, Kalamzoo, Michigan. Epinephrine (lot #A-1420F) was supplied by Elkins-Sinn, Cherry Hill, New Jersey. Ketorolac tromethamine IM (lot #073218) was supplied by Syntex Laboratories, Palo Alto, California. Bupivacaine hydrochloride (Sensorcaine; lot #312036) was supplied by Astra Pharmaceuticals, Westborough, Massachusetts.

Methods

Technique for Sciatic Blockade. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 200 to 350 mg were used to test the duration of sciatic blockade with each of the preparations described below. The rats were anesthetized briefly (less than 1 min) with 2% to 3% halothane in oxygen using a face mask. Sciatic nerve percutaneous blockade was performed through the posterolateral approach immediately medial and cephalad to the greater trochanter and inferior to the sciatic notch of the pelvis. At least four rats were used in each test. After injection, the halothane anesthetic was discontinued and animals were awakened within 1 min. Sensory blockade was examined using a modified unilateral 56°C hotplate technique developed by Masters and coworkers.[2] A cutoff latency of 12 s was used to prevent development of hyperalgesia or injury. Duration of dense sensory blockade was defined operationally as the time from injection to recovery of hot plate latency to 7 s or less. Each leg was tested three times at each time point, and the latency of the contralateral leg was tested as a within-subject control for systemic analgesic or sedative effects.

In addition, motor tests were performed at each time point to examine the rat's ability to hop and to place weight on its hind leg.[2,7] The motor scale has values of 1 to 4, where 1 indicates a normal paw (*i.e.*, normal dorsiflexion and splaying, or abduction and extension of toes). A value of 2 indicates that the rat has normal dorsiflexion and partially curled (flexed and adducted) toes. A value of 3 represents normal dorsiflexion, but curled toes with inability to spread the toes. A value of 4 denotes a paw exhibiting no dorsiflexion ability and curled toes.[2,7] Duration of motor blockade for purposes of group comparisons and statistics was operationally

defined as the time from injection until recovery of a motor score of 2.

Animals were handled and cared for according to institutional, state, and federal regulations. The animal experimentation protocol was submitted to and subsequently approved by Children Hospital's Animal Care Committee.

Sciatic Blockade Using Bupivacaine–HCl and Dexamethasone in Solution. In separate experiments, dexamethasone (0.14 mg/kg) was injected either simultaneously with 0.6 ml (10 mg/kg) 0.5% w/v bupivacaine-HCl or 3 h after the injection of bupivacaine-HCl when the blockade was waning. Dexamethasone (0.5 mg/kg) was also injected either simultaneously or 3 h after an injection of both 0.6 ml (10 mg/kg) 0.5% w/v bupivacaine-HCl and empty microspheres (those containing neither bupivacaine nor dexamethasone).

Model System to Test Drugs for Blockade-prolonging Ability. PLGA microspheres, 75 w% bupivacaine containing 150 mg bupivacaine/kg body weight (and no dexamethasone) were prepared as described previously.[4] Drugs to be tested for blockade-prolonging effect were placed in 0.6 ml injection fluid (5 g/l low molecular weight carboxymethylcellulose, 1 g/l Tween 80, and 1.8 g/l methylparaben). Similar injection fluids have been used previously to suspend microspheres for clinical use (*e.g.*, Lupron depot, TAP Pharmaceuticals, Deerfield, Illinois).[8] Ketorolac and epinephrine were received in liquid form and were added directly to the injection fluid. All other test drugs were solids dissolved in ethanol before injection. The volume of ethanol used varied from 7 to 70 μL, and the doses of test drugs varied from 0.02 to 20 mg of drug/kilogram rat weight. The microsphere/test drug mixture was vortexed (Vortex Genie®-2, Scientific Industries, Bohemia, New York) at maximum speed for 3 min before injection.

Reversal of the Dexamethasone Blockade-prolonging Effect by the Glucocorticoid Receptor Antagonist Cortexolone. Both cortexolone (1.4 mg/kg) and dexamethasone (0.14 mg/kg) were placed in the injection fluid, which contained suspended bupivacaine microspheres (150 mg bupivacaine/kg rat), and injected into a group of rats (n = 8). The duration of blockade was determined as described previously.

Control to Exclude Systemic Action of Dexamethasone. In separate groups of animals (n = 5 per group), dexamethasone (0.85 mg/kg in 0.6 ml injection vehicle) was injected either into the leg ipsilateral or contralateral to the leg receiving bupivacaine microspheres (150 mg bupivacaine/kg rat).

Table 1. Duration of Latency Obtained Using Liquid Bupivacaine with or without Dexamethasone Dissolved in Vehicle

No. of Rats	Concentration of Liquid Bupivacaine (mg/kg)	Concentration Dissolved Dexamethasone (mg/kg)	Duration of Block (h)
5	3	—	2–3
5	—	0.16	0
5	3	0.16	2–3
5	3	1.3	2–3
4	3	0.14*	2–3
4†	3	0.5	2–4
4†	3	0.5‡	2–4

* Injected 3 h after bupivacaine.

† Injected with PLGA empty microspheres.

‡ Injected 3 h after bupivacaine and PLGA empty microspheres.

Delayed Injection of Dexamethasone to Prolong Blockade from Bupivacaine Microspheres. Two different doses of dexamethasone, either 2.8 mg/kg or 0.5 mg/kg, were injected into two groups of rats (n = 5 and n = 4, respectively) 6 h after an initial injection of bupivacaine microspheres (150 mg bupivacaine/kg rat), and durations of sensory and motor blockade were recorded.

Influence of Neutrophil Depletion on the Dexamethasone Effect. Hydroxyurea (200 mg/kg) was administered on 3 consecutive days *via* tail vein injections to a group of rats (n = 20) to produce neutrophil depletion, as described by Levine and associates.[9] A hemograph including leukocyte and neutrophil counts was determined for each animal by the Hematology Laboratory of Children's Hospital using a Technicon Coulter Counter (Techicon Instruments Corp., Tarrytown, NY) before and after hydroxyurea injections to verify depletion of circulating neutrophils. Either 65/35 PLGA microspheres loaded with 75 w% bupivacaine or 65/35 PLGA microspheres loaded with 75 w% bupivacaine and 0.05 w% dexamethasone were injected into groups of immunosuppressed rats, and the duration of blockade was compared with that observed in parallel control groups of healthy rats that received tail vein injections of saline without hydroxyurea according to a comparable schedule.

Bupivacaine Content in Microspheres Extracted at Different Times. Twenty rats were injected with PLGA microspheres that contained 75 w% bupivacaine

and 0.05 w% dexamethasone, and 20 rats were injected with PLGA microspheres that contained only 75 w% bupivacaine. At 0.3, 1, 3, 5, and 7 days after injection, subgroups of rats were killed and the microspheres were extracted, washed, and lyophilized. The bupivacaine content was determined *via* reversed-phase HPLC using a C18 column with acetonitrile as the mobile phase and an ultraviolet detector. The assay has a lower limit of sensitivity of 5 ng/ml and coefficients of variation less than 5%.

Statistical Analyses. Durations of sensory and motor blockade among groups, hematologic parameters, and residual microsphere bupivacaine contents and dry weights were compared among groups by analysis of variance with Scheffe's or Dunnett's tests for *post hoc* comparisons, as appropriate. A probability value less than 0.05 was considered significant.

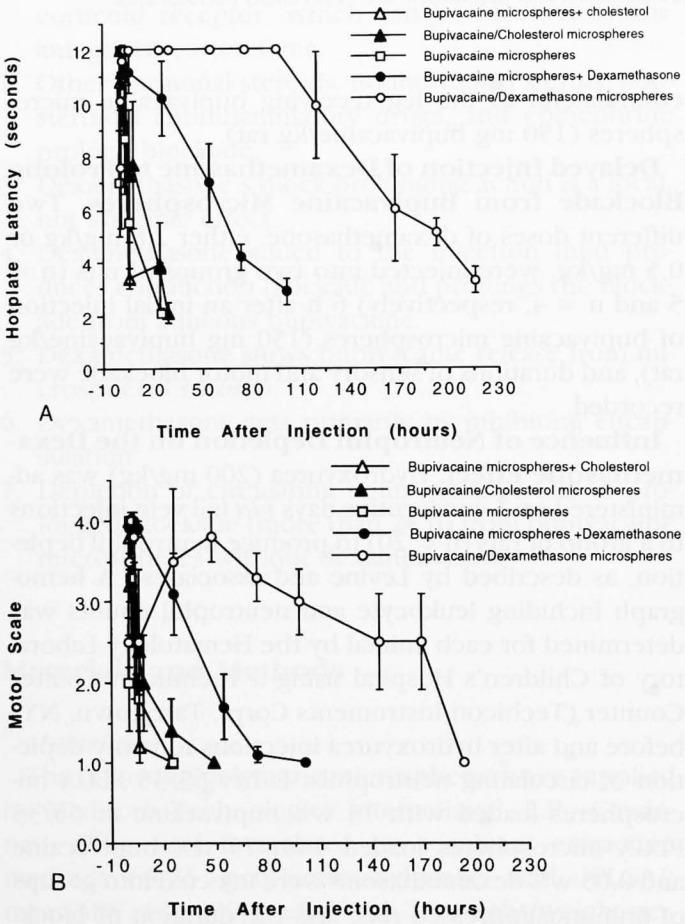

Fig. 1. Hotplate latencies *versus* time as a measure of (*A*) sensory function versus time and (*B*) motor function scores *versus* time for bupivacaine microspheres (B-Ms; n = 4), bupivacaine microspheres with dexamethasone in the injection fluid (n = 7), bupivacaine microspheres with dexamethasone (BD-Ms) incorporated in the microspheres (n = 4), bupivacaine microspheres with cholesterol in the injection fluid (n = 5), and bupivacaine microspheres with cholesterol incorporated in the microspheres (n = 5).

Table 2. The Block Obtained Using 65/35 PLGA Ms Loaded with 75% Bupivacaine and Additives Placed in the Injection Fluid

No. of Rats	Additives (mg/kg rat)	Sensory Block (h)	Motor Block (h)
11	—	6.0 ± 1.0	5.0 ± 0.3
7	Dexamethasone (0.14)	47.0 ± 8.0	38.0 ± 5.0
5	Dexamethasone (0.02)	17.0 ± 11.0	19.0 ± 8.0
5	Dexamethasone (2.0)	36.0 ± 19.0	34.0 ± 12.0
5	Betamethasone (2.0)	44.0 ± 13.0	39.0 ± 11.0
5	Betamethasone (0.8)	46.0 ± 7.0	39.0 ± 5.0
5	Betamethasone (0.25)	36.0 ± 10.0	38.0 ± 11.0
5	Betamethasone (0.032)	19.0 ± 4.0	15.0 ± 4.0
5	Methylprednisolone (20)	34.0 ± 11.0	33.0 ± 9.0
7	Methylprednisolone (2.1)	28.0 ± 6.0	28.0 ± 5.0
5	Methylprednisolone (0.1)	20.0 ± 5.0	13.0 ± 4.0
7	Hydrocortisone (0.1)	10.0 ± 3.0	10.0 ± 3.0
5	Hydrocortisone (1.25)	15.0 ± 5.0	16.0 ± 3.0
5	Hydrocortisone (10)	36.0 ± 10.0	31.0 ± 8.0
5	Ketorolac (2.0)	6.0 ± 0.7	7.0 ± 0.4
5	Ketorolac (6.3)	8.0 ± 2.0	10.0 ± 4.0
4	Estradiol (1.25)	8.0 ± 1.0	9.0 ± 2.0
4	Estradiol (0.125)	11.0 ± 6.0	12.0 ± 6.0
8	Cholesterol (0.1)	4.0 ± 0.4	4.0 ± 1.0
5	Cholesterol (3.1)	8.0 ± 3.0	5.0 ± 1.0
5	Testosterone (1.7)	15.0 ± 5.0	15.0 ± 5.0
5	Testosterone (1.0)	7.0 ± 2.0	6.0 ± 1.0
4	Progesterone (2.0)	8.0 ± 1.0	6.0 ± 1.0
5	Epinephrine (0.01)	12.0 ± 6.0	12.0 ± 4.0
5	Epinephrine (0.1)	14.0 ± 5.0	11.0 ± 3.0

Results

Percutaneous sciatic block injections with dexamethasone alone in the suspending medium did not produce any sensory or motor blockade. Dexamethasone did not prolong blockade when injected either simultaneously with an aqueous solution of bupivacaine-HCl or 3 h after the bupivacaine injection, and the duration of blockade was independent of the presence of empty microspheres (table 1).

Dexamethasone, either incorporated in microspheres or added to the injection fluid containing suspended bupivacaine microspheres, prolonged the blockade ($P < 0.001$ and $P < 0.01$, respectively, for both sensory and motor blockade) compared with bupivacaine microspheres alone (figs. 1a and b). The duration of sensory blockade observed in rats injected with bupiva-

caine microspheres alone was 7 ± 3 h; sensory blockade duration in rats receiving bupivacaine microspheres with dexamethasone (0.14 mg drug/kg rat) in the injection medium was 47 ± 8 h. The prolonged blockade observed when dexamethasone was placed in the injection fluid (0.02 to 2 mg drug/kg rat) was less than that observed when dexamethasone was encapsulated in the microspheres, which produced a blockade lasting

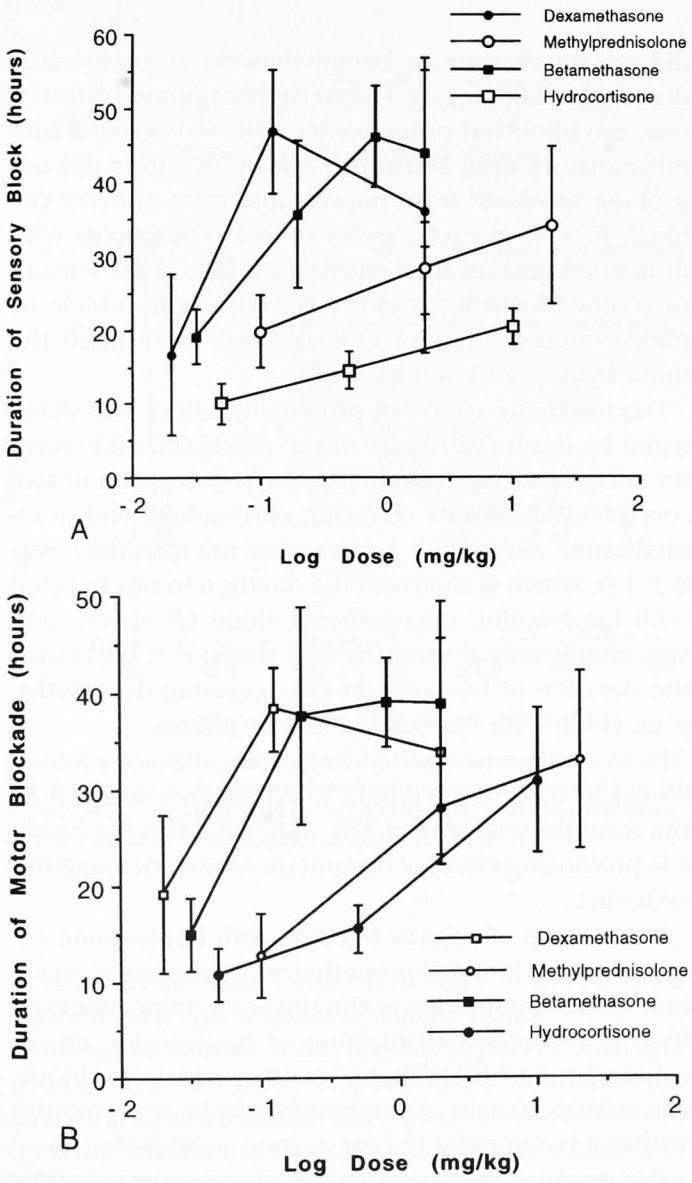

Fig. 2. Duration of (*A*) sensory blockade (y axis; time from injection to recovery of hotplate latency to 7 s) *versus* log dose (x axis; mg/kg), and (*B*) motor blockade (y axis; time from injection to recovery to a motor scale of 2) *versus* log dose (x axis; mg/kg) for glucocorticoid additives placed in the injection fluid along with bupivacaine microspheres (n = 4 to 8 for each point).

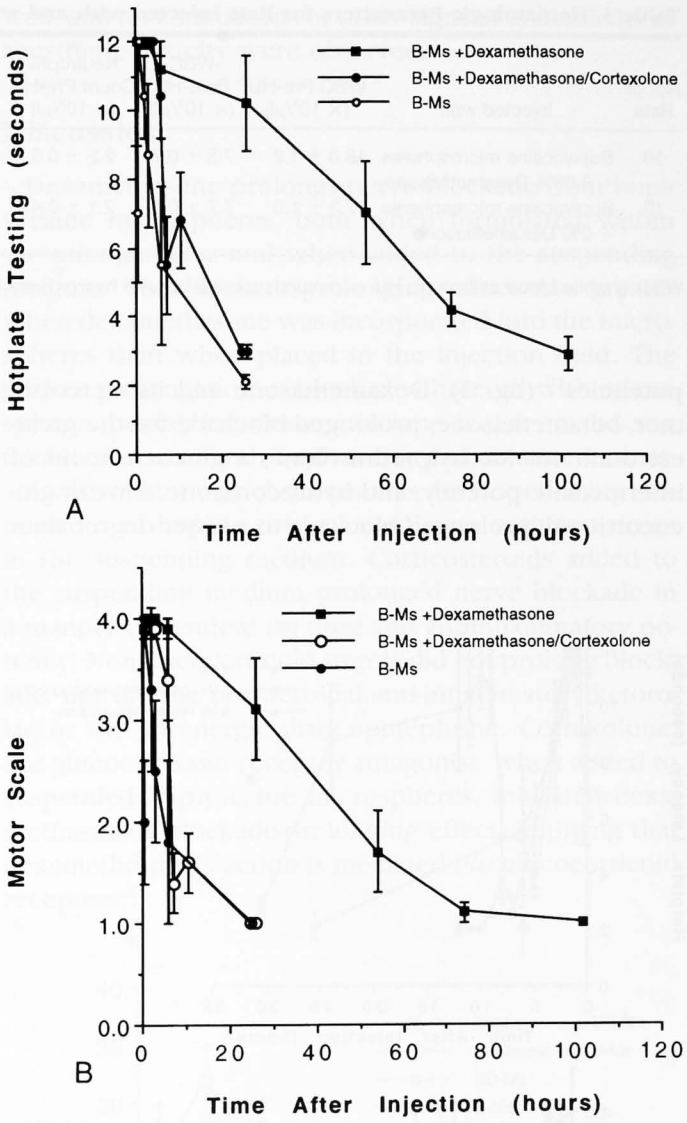

Fig. 3. Hotplate latencies *versus* time as a measure of (*A*) sensory function *versus* time and (*B*) motor function scores *versus* time for the following formulations: bupivacaine microspheres (B-Ms) (n = 4), B-Ms with 0.14 mg/kg dexamethasone in the injection fluid (n = 7), and B-Ms injected with both 1.4 mg/kg cortexolone and 0.14 mg/kg dexamethasone in the injection fluid (n = 8).

134 ± 13 h.[4] Cholesterol, a nonhormonal sterol, did not prolong sciatic nerve blockade, either when added in solution to suspended bupivacaine microspheres or when incorporated into the microspheres (figs. 1a and b).

Among the drugs tested, only glucocorticoids prolonged the blockade obtained from bupivacaine microspheres (table 2). The degree of block prolongation had the same rank order as their relative antiinflammatory

Table 3. Hematologic Parameters for Rats Injected with and without Dexamethasone before and after Immunosuppression

No. of Rats	Injected with	WBC Pre-HU ($\times 10^3/\mu l$)	WBC Post-HU ($\times 10^3/\mu l$)	Neutrophil Count Pre-HU ($\times 10^3/\mu l$)	Neutrophil Count Post-HU ($\times 10^3/\mu l$)	Mean % Neutrophil Depletion	% HCT Pre-HU	% HCT Post-HU	Lymphocyte Pre-HU ($\times 10^3/\mu l$)	Lymphocyte Post-HU ($\times 10^3/\mu l$)
10	Bupivacaine microspheres + 0.05% Dexamethasone	18.0 ± 1.2	7.5 ± 0.7	2.2 ± 0.5	0.2 ± 0.05	88 ± 4	41.1 ± 1.3	34.8 ± 1.8	13.0 ± 1.0	6.8 ± 0.6
10	Bupivacaine microspheres + 0% Dexamethasone	17.3 ± 1.0	7.7 ± 0.6	2.1 ± 0.4	0.2 ± 0.03	90.8 ± 1.3	38.0 ± 1.4	35.6 ± 0.8	12.6 ± 0.8	7.0 ± 0.6

WBC = white blood cell count; HU = immunosuppression with hydroxyurea injection.

potencies[10] (fig. 2). Dexamethasone and its stereoisomer, betamethasone, prolonged blockade for the greatest duration. Methylprednisolone, a glucocorticoid of intermediate potency, and hydrocortisone, a weak glucocorticoid, prolonged blockade to a lesser degree than did dexamethasone or betamethasone at comparable doses ($P < 0.05$; fig. 2). The steroid hormones testosterone, estradiol, and progesterone, the nonsteroidal anti-inflammatory drug ketorolac, and epinephrine did not prolong blockade from bupivacaine microspheres (table 2, P = NS in each case). Control experiments with drug-free injection fluid showed no effects on sensory or motor blockade of either the injection vehicle or trace concentrations of ethanol used to suspend the more hydrophobic drugs.

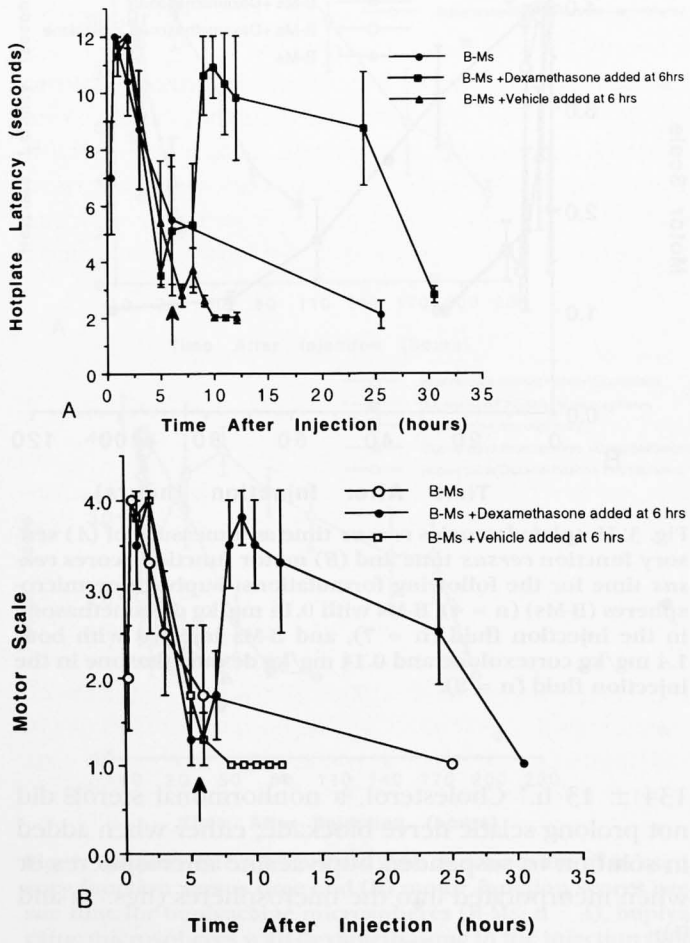

Fig. 4. Hotplate latencies *versus* time as a measure of (*A*) sensory function versus time and (*B*) motor function scores *versus* time for bupivacaine microspheres (B-Ms) (n = 4), B-Ms with 0.5 mg/kg dexamethasone reinjected 6 h later (n = 4), and B-Ms with vehicle reinjected 6 h later (n = 4).

Dexamethasone's block-prolonging effect was eliminated by coadministration of the glucocorticoid receptor antagonist cortexolone (fig. 3). The duration of sensory blockade in rats receiving cortexolone and dexamethasone along with bupivacaine microspheres was 8 ± 1 h, which is similar to the duration in rats injected with bupivacaine microspheres alone (P = NS), and was significantly shorter (by four times; $P < 0.01$) than the duration of blockade in rats receiving dexamethasone along with bupivacaine microspheres.

Dexamethasone injected in one leg did not prolong blockade from bupivacaine microspheres injected in the contralateral leg (P = NS), indicating that the blockade-prolonging effect of dexamethasone is local and not systemic.

For a group of animals injected with bupivacaine microspheres without dexamethasone, subsequent injection of dexamethasone at the time of waning blockade (6 h after the initial injection of bupivacaine microspheres) reestablished partial sensory and motor blockade within 30 min. Complete blockade was restored within 2 h and persisted for as long as 30 h (fig. 4).

Hydroxyurea treatment produced approximately 90% depletion of circulating neutrophils (table 3). Circulating neutropenia had no effect on sciatic blockade durations from bupivacaine microspheres either in the presence or absence of dexamethasone, as shown in figure 5 (P = NS for immunosuppressed or control rats in-

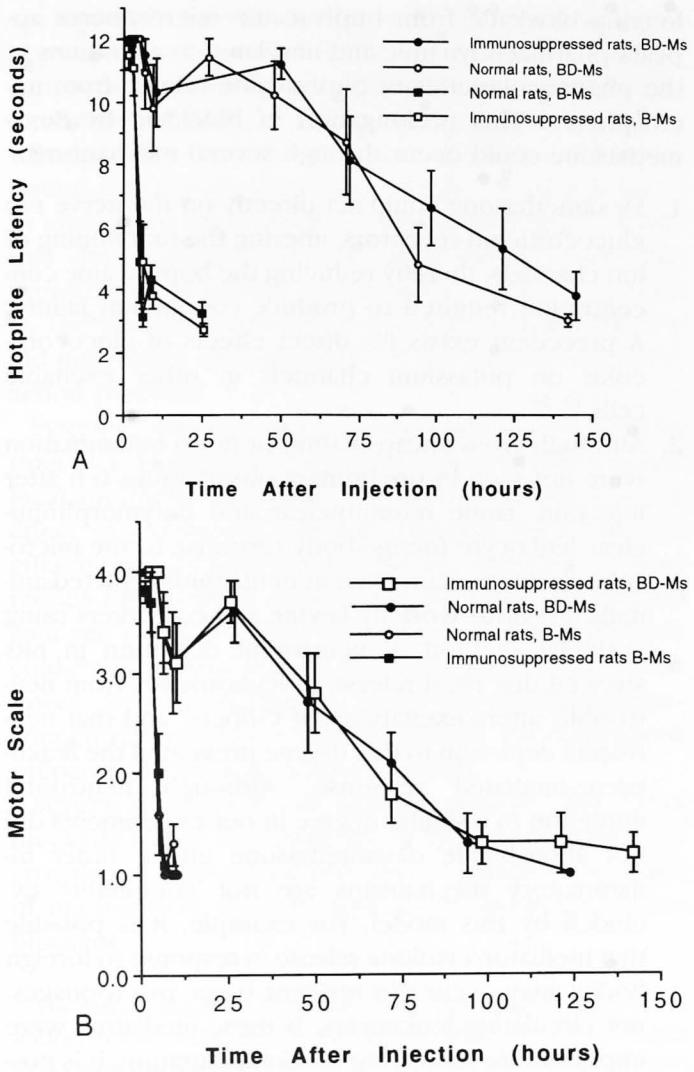

Fig. 5. Comparison of (*A*) sensory blockade *versus* time and (*B*) motor blockade *versus* time for immunosuppressed rats (n = 20) *versus* healthy rats (n = 14) injected with bupivacaine/dexamethasone microspheres (BD-Ms) and bupivacaine microspheres (B-Ms).

jected with the same formulation and $P < 0.01$ for rats injected with and without dexamethasone).

Groups of animals receiving microspheres with and without dexamethasone, respectively, were killed at different times, and residual microspheres were recovered from the injection site and analyzed for dry weight and bupivacaine content, as shown in figure 6. There were no statistical differences in dry weight or bupivacaine content at any time between the groups treated with or without dexamethasone (P = NS for comparisons between groups at each time).

All animals tolerated the experimental conditions well, and no convulsions or other signs of systemic local anesthetic toxicity were observed.

Discussion

Dexamethasone prolongs nerve blockade from bupivacaine microspheres, both when formulated within the microspheres and when added to the suspending medium. The blockade-prolonging effect was greater when dexamethasone was incorporated into the microspheres than when placed in the injection fluid. The greater prolongation of blockade observed with microspheres containing dexamethasone is due to the sustained release of both bupivacaine and dexamethasone.

A convenient model system was developed using suspensions of bupivacaine microspheres with test drugs in the suspending medium. Corticosteroids added to the suspending medium prolonged nerve blockade in a manner dependent on dose and antiinflammatory potency. Nonglucocorticoid sterols did not prolong blockade, nor did the nonsteroidal anti-inflammatory ketorolac or the adrenergic drug epinephrine. Cortexolone, the glucocorticoid receptor antagonist, when added to suspended bupivacaine microspheres, inhibited dexamethasone's blockade-prolonging effect, implying that dexamethasone's action is mediated *via* glucocorticoid receptors.[11]

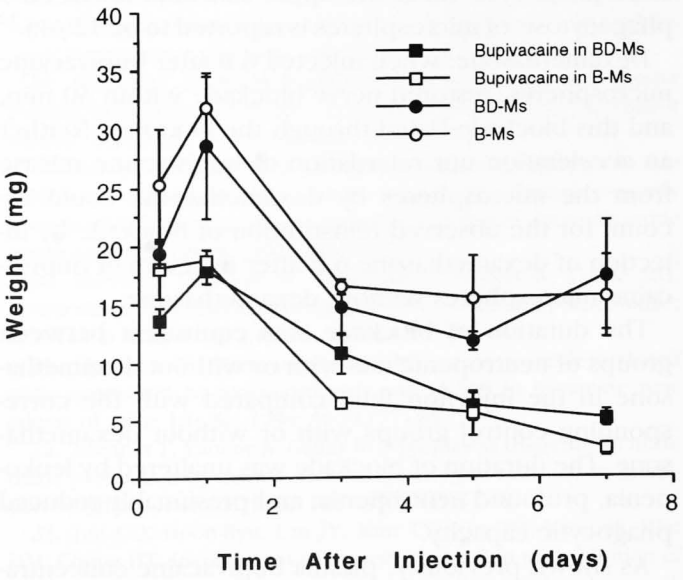

Fig. 6. Weight of either bupivacaine microspheres (B-Ms) or bupivacaine/dexamethasone microspheres (BD-Ms) extracted 0.3, 1, 3, 5, and 7 days after injection and the corresponding bupivacaine content of these extracts (n = 4 for each time point).

Dexamethasone alone produced no measurable blockade, nor did it prolong the short-duration blockade (2 to 4 h) from aqueous bupivacaine-HCl. Coadministration of empty PLGA microspheres along with liquid bupivacaine resulted in short-duration blockade in the presence or absence of dexamethasone. Liquid bupivacaine is cleared from the leg and taken up into circulation rapidly in the presence or absence of dexamethasone, so that at times later than 4 to 6 h there is insufficient bupivacaine remaining in the leg to produce blockade. Thus the dexamethasone effect requires controlled release of bupivacaine from microspheres to prolong the blockade. Dexamethasone's action also appeared local, not systemic, because in control experiments injections of comparable doses of dexamethasone at remote sites did not prolong blockade from bupivacaine microspheres.

Dexamethasone's blockade-prolonging effect cannot be accounted for by an alteration of the kinetics of bupivacaine release from microspheres. Residual bupivacaine contents in the injection site for as long as 1 week after injection were statistically indistinguishable between groups of animals receiving microspheres with or without dexamethasone, respectively. These observations imply that dexamethasone is not altering bupivacaine release from microspheres, such as might occur through inhibited encapsulation. The microspheres we used ranged in size from 20 to 140 μm and are too large to be phagocytosed, as the upper size limit for *in vivo* phagocytosis of microspheres is reported to be 12 μm.[12]

Dexamethasone, when injected 6 h after bupivacaine microspheres, restored nerve blockade within 30 min, and this blockade lasted through the next day. Neither an acceleration nor retardation of bupivacaine release from the microspheres by dexamethasone could account for the observed reinstitution of blockade by injection of dexamethasone 6 h after injection of bupivacaine microspheres without dexamethasone.

The duration of blockade was equivalent between groups of neutropenic rats with or without dexamethasone in the injection fluid compared with the corresponding control groups with or without dexamethasone. The duration of blockade was unaltered by leukopenia, profound neutropenia, and presumably reduced phagocytic capacity.

As shown previously, plasma bupivacaine concentrations were equivalent between groups of animals receiving microspheres with or without dexamethasone.[4] From these observations and previous *in vitro* results,[4] we conclude that dexamethasone's action in prolonging blockade from bupivacaine microspheres appears pharmacodynamic and unrelated to alterations in the pharmacokinetics of bupivacaine release from microspheres. This prolongation of blockade by dexamethasone could occur through several mechanisms.

1. Dexamethasone could act directly on the nerve *via* glucocorticoid receptors, altering the functioning of ion channels, thereby reducing the bupivacaine concentration required to produce conduction failure. A precedent exists for direct effects of glucocorticoids on potassium channels in other excitable cells.[13-16]
2. Although gross encapsulation or florid inflammation were not seen in preliminary observations 6 h after injection, some mononuclear and polymorphonuclear leukocyte foreign-body response to the microspheres does occur, even in neutrophil-depleted animals. Previous work by Levine and coworkers using a similar method of neutrophil depletion in rats showed that local release of leukotrienes from neutrophils alters excitability of C-fibers[9] and that neutrophil depletion to this degree prevented the leukotriene-mediated response. Although neutrophil depletion to a similar degree in our experiments did not abolish the dexamethasone effect, other inflammatory mechanisms are not completely excluded by this model. For example, it is possible that mediator/cytokine release in response to foreign bodies may occur *via* resident tissue macrophages, not circulating leukocytes. If these mediators were important for modifying blockade duration, it is possible that more extreme degrees of immunosuppression could abolish the dexamethasone requirement for prolonged blockade.
3. Dexamethasone could act to inhibit local anesthetic tachyphylaxis. Our previous work implicated hyperalgesia and N- methyl-d-aspartate[17] and nitric oxide mechanisms[18] in tachyphylaxis. Dexamethasone has been shown in other systems to inhibit nitric oxide synthase.[19-23] Experiments are in progress to distinguish these alternatives.

Glucocorticoids are widely administered in the treatment of chronic pain. Their putative analgesic effects are frequently ascribed to antiinflammatory action.[24-27] Previous studies regarding direct analgesic or local anesthetic actions of glucocorticoids have yielded mixed results. McCleane and colleagues[28] found that triamcinolone did not prolong analgesia from ilioinguinal blockade using bupivacaine in patients having her-

nia repair. Devor and associates[29] found that soluble or depot preparations of triamcinolone or dexamethasone suppressed spontaneous discharges in experimental neuromas but had no direct local anesthetic action, in the sense that they did not alter A-fiber or C-fiber compound action potentials from healthy or injured nerve *in vivo*. In contrast, Johannsson and coworkers[30] found that depot preparations of methylprednisolone suppressed the amplitude of the flexion reflex (regarded by the authors as an index of C-fiber conduction) but did not reduce the amplitude of the A-fiber compound action potential.

Systemic accumulation of bupivacaine and delayed toxicity is an important clinical problem with prolonged local anesthetic infusions.[31,32] The blockade-prolonging effect of glucocorticoids may broaden the safety and effectiveness of sustained-release local anesthetic preparations for eventual clinical use. Based on the current data and previous work,[4] it is apparent that glucocorticoids will permit longer blockade with considerably lower dosing and thereby lower plasma bupivacaine concentrations than would be possible for comparable preparations without glucocorticoids.

Several glucocorticoids prolong sensory and motor blockade of the rat sciatic nerve from bupivacaine microspheres. This effect will greatly enhance the utility of microsphere delivery systems for prolonged nerve blockade.

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