We assessed the effects of epidural anesthesia with bupivacaine in the rat by serial recordings of spinal reflexes. The H wave from plantar muscles after electrical stimulation of the sciatic nerve evaluates a large nerve fiber spinal reflex arch. The extensor reflex response recorded from quadriceps muscle after stimulation of the contralateral tibial nerve assesses a reflex arch with small fiber afferents. After epidural injection of 0.2 mL of bupivacaine (0.25%, 0.5%, and 1.0% solutions) at the L5–L6 vertebral space, nociceptive, H, and extensor reflex responses were abolished within 1–3 min. Duration of complete blockade lasted 20–80 min, increasing with the anesthetic concentration, and complete recovery occurred after an additional period of 30–40 min. The responses recovered to amplitudes similar to preanesthesia controls, indicating that there was no damage to the nervous system. This study shows that electrophysiological recording and quantitation of nerve reflex responses is a useful and accurate method to evaluate the efficacy of local anesthetic agents. © 1996 John Wiley & Sons, Inc.
Key words: electrophysiology • epidural anesthesia • H reflex • nerve conduction • withdrawal reflex

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ELECTROPHYSIOLOGICAL EVALUATION OF SPINAL REFLEXES DURING EPIDURAL ANESTHESIA IN AN EXPERIMENTAL MODEL

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Detailed electrophysiologic studies have shown that it is possible, by using electromyographic techniques, to record most of the reflexes, either proprioceptive or exteroceptive, commonly studied in the clinical examination. These studies have provided insights into underlying physiological mechanisms and also allow for objective and quantitative measurements of the function of neuronal circuits within the central and peripheral nervous system.

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Epidural administration of local anesthetics is frequently used to provide pain relief and anesthesia during surgical procedures and in acute and chronic painful situations. Longer-lasting effects are usually achieved by continuous or repeated epidural administration. Reports of toxicity of local anesthetics on neural tissue[6,14,17,25] and of complications related to epidural catheterization have led to research for new compounds,[1,26] slow-release preparations,[18–20] and synergistic interactions of coadministered drugs.[15]

Quantitative and objective assessment of anesthetic effects in experimental models is necessary to test the efficacy of new drugs and preparations. Different types of testing systems are useful. In vitro testing of conduction properties of isolated nerve preparations is a reliable method, that also provides useful information about the differential sensitivity of motor and sensory nerve fibers.[2,9,28] Peripheral nerve conduction and blockade may be adequately assessed in vivo by motor and sensory nerve conduction studies.[13,14] For evaluation of spinal anesthesia, most reports describe subjective scoring of diminished walking ability and

weight support for motor blockade, and unresponsiveness to painful stimuli applied by pinching, pricking, or heating the skin for sensory blockade.[1,6,7,10,12,15,18,19] In this study we describe the quantitative evaluation of epidural anesthesia in rodents by electrophysiological testing of spinal reflex responses, namely H responses and withdrawal reflexes, that include different types of sensory nerve fibers in the afferent side and alphamotor fibers on the efferent side.

MATERIALS AND METHODS

Female Sprague–Dawley rats, weighing 250–300 g, were distributed in four groups according to the solution administered by epidural route. The animals were maintained under standard conditions, with access to food and water ad libitum. The National Research Council's guide for the care and use of laboratory animals was followed throughout the study.

All procedures were performed under general anesthesia with pentobarbital (40 mg/kg i.p., plus additional doses if required). Once anesthetized, the back and the outer aspect of the right hindlimb were shaved. A plastic cylinder was placed under the abdomen of the rat in order to dorsally arch the lumbar region, and the extremities were held in full extension with tape. Skin temperature was continuously monitored by a surface thermistor (Yellow Springs Instrument Tele-thermometer) and maintained above 32°C by a thermostat controlled flat coil. Epidural injection was performed by transcutaneous punction with a 27-gauge needle at the L5–L6 intervertebral space by inserting the needle along the right side of the spinous apophysis until the rounded tip entered the vertebral foramen. After aspiration for evidence of blood or cerebrospinal fluid proved negative, 0.2 mL of bupivacaine solution (0.25%, 0.5%, or 1.0% in aqueous solution) was slowly injected over 20 s in groups of 10 rats each, and 0.2 mL of saline solution in another 6 rats. In preliminary assays we established the accuracy of the needle location by injecting a methylene blue solution and locating it after careful dissection and laminectomy.

Neurophysiological Methods. Evaluation of the effectiveness of epidural anesthesia was made by neurophysiological techniques designed to quantitatively assess the function of spinal reflex arches through the lumbar segments.

Nociceptive responses were elicited by electrical shocks (pulses of about 100 V, 0.1 ms duration, from a Grass S88 stimulator) applied to the plantar surface of a hindpaw by means of a bipolar metal electrode with conducting gel at the tips.[4] The normal response was a reflex muscle contraction, most evident from the lateral lumbar and abdominal muscles. At each testing interval tested, the response to two consecutive stimuli was subjectively scored on a three-point scale, depending if the response was brisk and bilateral (2), light or unilateral (1), or absent (0).

The sciatic nerve was stimulated by two needle electrodes (Nicolet subdermal needles, 12 mm length, 28 gauge) inserted next to the nerve at the sciatic notch, applying single squared pulses of 0.1 ms and voltage necessary to obtain a supramaximal compound muscle action potential. Muscle action potentials were recorded from plantar muscles with monopolar needles (Fig. 1),[24,30] amplified (Tektronix AM502), and displayed on a digital oscilloscope (Tektronix 2221) under appropriate settings, and printed on a plotter. The amplitude of the negative peak of the M and the H waves and the latencies to the onset of the M wave and the peak of the H wave were measured (Fig. 2).

The withdrawal reflex response was elicited by electrical pulses (supramaximal voltage, 0.5 ms duration) applied via monopolar needles; the cathode placed adjacent to the tibial nerve at the ankle and

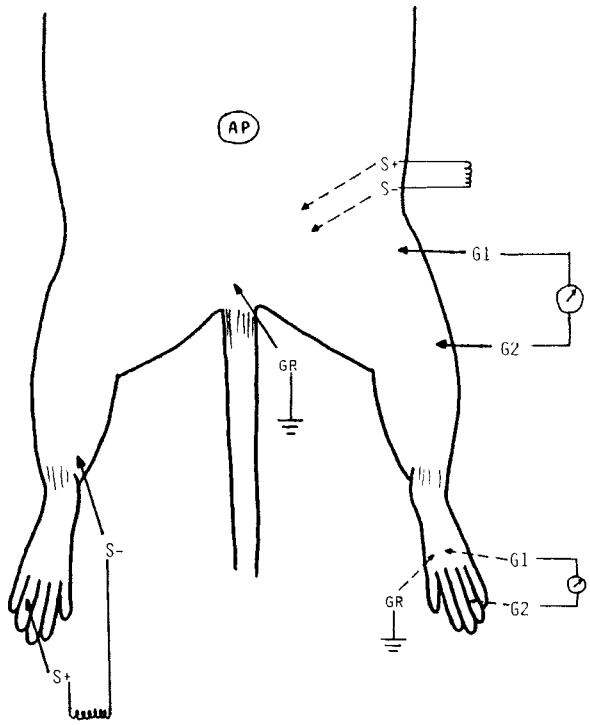

FIGURE 1. Schematic drawing of the electrodes placed in the rat hindlimbs for simultaneous recording of the H wave (interrupted arrows) and the extensor reflex (full arrows).

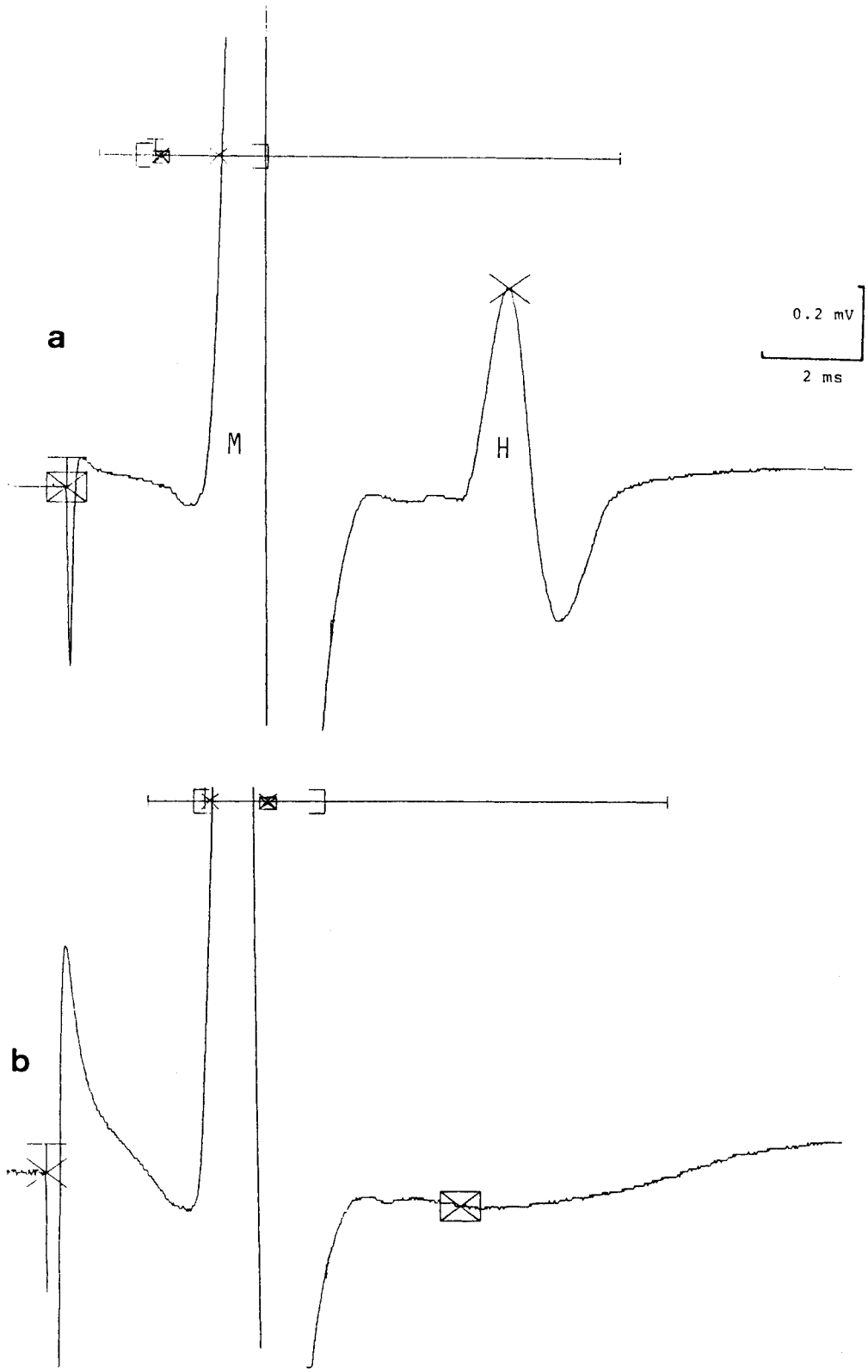

FIGURE 2. Electromyographic recording of the M and the H wave from plantar muscles, before **(a)** and after **(b)** bupivacaine epidural injection in a rat.

the anode inserted in the skin of the dorsum of one paw (Fig. 1). The contralateral extensor reflex was evaluated by recording the motor response from the quadriceps muscle with monopolar needles. The active intramuscular electrode was positioned to record the evoked volley of motor unit action potentials (MUAPs) at their greatest amplitude. We measured maximal amplitude (baseline to peak), latency to onset, and duration of the evoked MUAPs from the oscilloscope screen (Fig. 3).

All tests were applied in duplicate before epidural injection to obtain individual control values, and serially afterwards at intervals of 1, 3, 5, 7, and 10 min and each 5 min thereafter until complete recovery. At all these times the voltage of stimuli was maintained 25% above that which gave a maximal response before injection.

Histological Methods. In order to assess possible damage to the nervous system, 8 rats were studied 1 week after epidural injection, 4 with saline solution and 4 with 0.5% bupivacaine solution. Animals were anesthetized and perfused with 4% paraformaldehyde in 0.1 mol/L cacodilate buffer. Samples of spinal cord dissected at T13, L1–L2, and L3–L4 levels were fixed in the same perfusing solution during 4 h, transferred to phosphate-buffered saline buffer, decalcified, dehydrated, and embedded in paraffin blocks. Eight μm-thick sections were processed for hematoxilin—eosin and Klüver–Barrera stains. Stained sections of spinal cord, lumbosacral roots, dorsal root ganglia, and meningeal membranes were inspected under light microscopy (Leitz diaplan).

Data Analysis. Recorded values for each interval after epidural injection were normalized as the percentage with respect to the control preinjection values for each animal. Results are plotted against time and presented as mean and standard error (SEM). In addition, the following parameters were calculated: onset latency of the anesthetic effect,

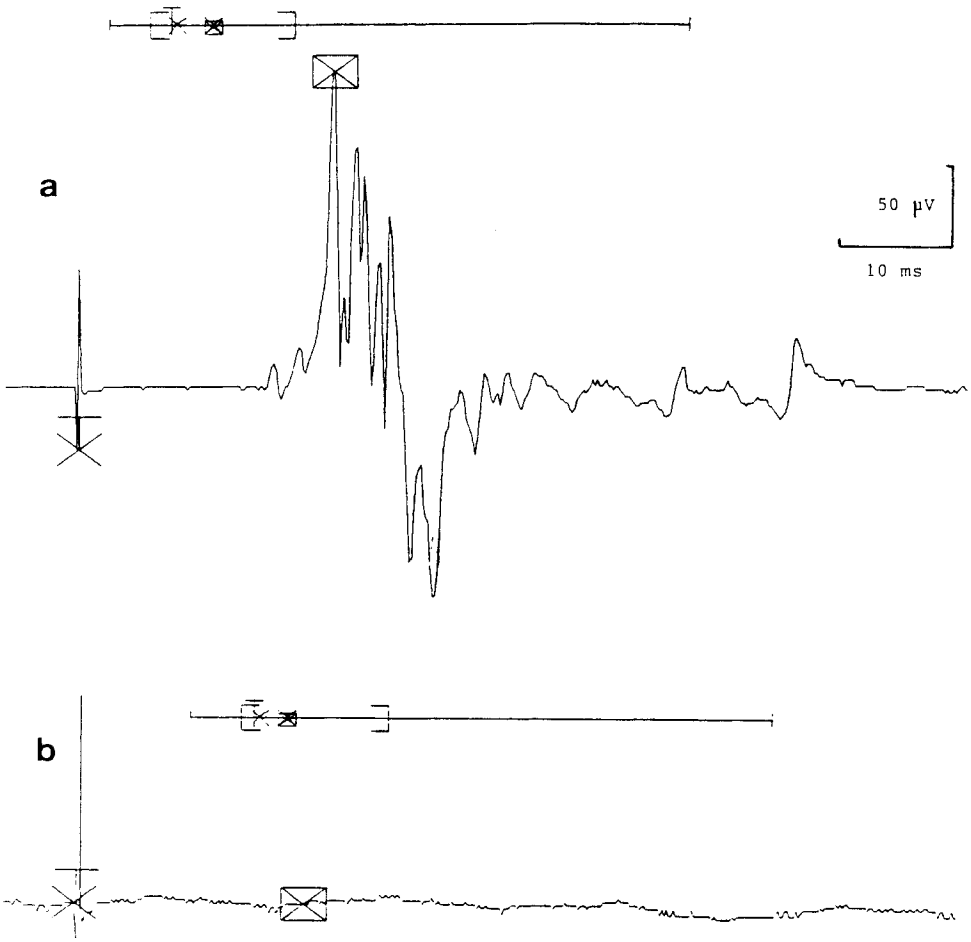

FIGURE 3. Electromyographic recording of the extensor reflex response from the quadriceps muscle, before **(a)** and after **(b)** bupivacaine epidural injection in a rat.

latency, amplitude, and duration of the smallest response (maximal effect), and latency and amplitude of the maximal recovery response. Statistical comparisons between groups were made with the Mann–Whitney U test, and changes over time after injection by analysis of variance for repeated measurements. Differences were considered significant if $P < 0.05$.

RESULTS

Repeated testing before epidural injections showed that electrical pulses given to the hindpaw skin induced consistent nociceptive responses (score 2) without a tendency to habituation. Control values for the muscle responses evoked by sciatic nerve stimulation averaged 7.4 mV (SEM 0.45) in amplitude and 3.1 ms (0.11) in latency for the M wave, and 1.1 mV (0.25) in amplitude and 8.9 ms (0.18) in latency to the peak of the H wave. The extensor reflex response recorded from quadriceps muscles in response to noxious electrical stimulation of the contralateral tibial nerve was polyphasic, integrating several MUAPs. The average maximal baseline to peak amplitude was 224 μV (45), and the latency to the first component was 21.9 ms (1.1). There were no significant differences between the different groups of rats tested.

Epidural injection of saline solution did not induce appreciable changes in the test responses during prolonged follow-up to 45 min in any of the animals tested (Fig. 4), except for a slight decrease in amplitude of the electrophysiological responses at 1 and 3 min, recovered to control levels by 5 min. Variations in amplitude and latency time of the potentials was lower than 10% over later follow-up intervals.

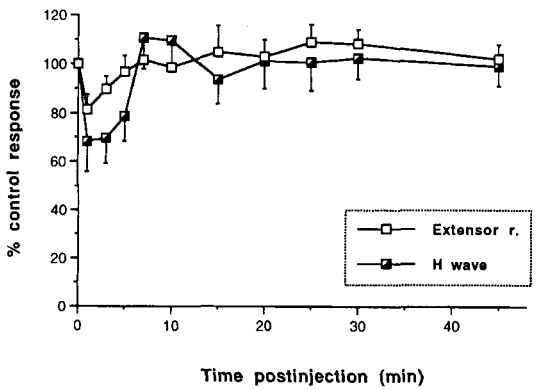

FIGURE 4. Changes over time after saline solution injection in the maximal amplitude of the H wave and the MUAPs of the extensor reflex. Values are percentage of the preinjection control values for each rat.

On the other hand, bupivacaine injection usually caused a complete blockade of sensorimotor functions, expressed as abolition of the nociceptive response, the H-reflex wave, and the contralateral extensor reflex (Fig. 5).

The nociceptive response to electrical noxious stimuli was elicited in only 1 rat after injection of bupivacaine at 0.25% and in 2 at 0.5%, in which a weak response persisted. The extensor reflex and the H-wave responses were completely abolished in all rats except for 3 (including the one with positive nociceptive response) at a concentration of 0.25%, in which the amplitude of evoked potentials decreased to about 5–10% of preinjection values. The anesthetic effects were observed from the first minute after injection until approximately 20, 40, and 80 min thereafter for solutions at 0.25%, 0.50%, and 1.0%, respectively (Table 1). Recovery of responses was gradual over the following 30–40 min to reach responses similar to the control ones in all cases.

During the time of epidural anesthesia the amplitude of the M waves did not change significantly, indicating that there was neither peripheral nerve blocking due to ischemia or compression nor

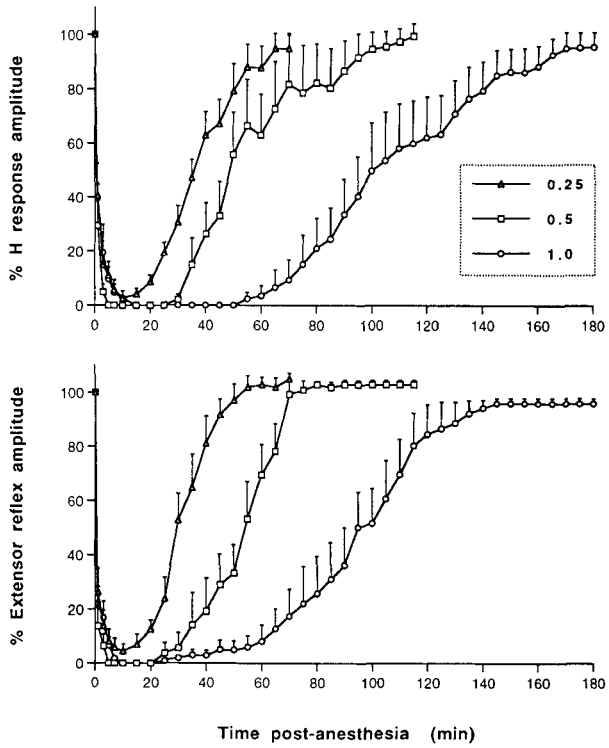

FIGURE 5. Changes over time after epidural injection of 0.25%, 0.5%, and 1.0% bupivacaine solutions on the maximal amplitude of the H wave and of the MUAPs of the extensor reflex. Values are the mean percentage of the preinjection control response for each rat.

Table 1. Comparison of epidural anesthesia with bupivacaine at different concentrations.

	Anesthesia				Recovery	
	Beginning	Minimum	Response	Duration	Maximum	Response
Nociceptive response						
0.25%	1.7 ± 1.0	5.2 ± 4.6	6.2 ± 17.7	22.5 ± 6.6*†	37.5 ± 8.8*†	100.0 ± 0.0
0.50%	3.0 ± 4.9	4.9 ± 5.3	18.0 ± 25.2	43.1 ± 15.6†	56.2 ± 12.7†	100.0 ± 0.0
1.00%	1.7 ± 1.5	5.0 ± 4.3	0.0 ± 0.0	86.9 ± 24.0	104.4 ± 25.4	100.0 ± 0.0
Extensor reflex						
0.25%	1.5 ± 1.4	5.0 ± 3.7	3.3 ± 5.3	16.9 ± 5.9*†	49.4 ± 11.1*†	108.4 ± 8.8†
0.50%	1.0 ± 0.0	2.5 ± 2.1	0.0 ± 0.0	37.5 ± 10.3†	67.5 ± 8.4†	103.2 ± 4.2†
1.00%	1.0 ± 0.0	5.4 ± 3.1	0.0 ± 0.0	73.7 ± 28.4	112.5 ± 22.0	96.0 ± 5.6
H wave						
0.25%	1.0 ± 0.0	5.0 ± 2.1	3.5 ± 5.4	23.1 ± 5.3*†	51.9 ± 10.7*†	94.4 ± 18.0
0.50%	1.2 ± 0.7	3.0 ± 1.5	1.7 ± 4.9	41.9 ± 24.5†	72.5 ± 22.4†	112.5 ± 37.3
1.00%	1.0 ± 0.0	5.6 ± 2.6	0.0 ± 0.0	84.4 ± 22.4	127.5 ± 34.3	91.0 ± 21.3

** = $p < 0.05$ vs 0.5%; † = $p < 0.05$ vs 1.0%*
Response in % of the control response. Beginning, Minimum, Duration and Maximum in minutes after injection.
Values are expressed as mean ± SD.

changes in placement of the recording needles. The rats were allowed to recover from general anesthesia and inspected over 2 weeks thereafter, without any signs of disability or secondary effects. Repeated control testing in animals yielded values of the electrophysiological parameters similar to those recorded the first time.

Microscopic study of the spinal cord sections did not show any differences between animals injected with saline and bupivacaine solutions. In all cases, the neuron bodies of anterior and posterior horns and ganglia did not show nuclear or cytoplasmic abnormalities, the spinal white matter and spinal roots presented a normal axonal pattern and myelin sheath appearance, glial cells showed no abnormalities, and meningeal covers had no remarkable changes.

DISCUSSION

Our study indicates that electrophysiological methods can provide a reliable and quantitative evaluation of epidural anesthesia by comparison of degree of blockade through distinct reflex spinal arches with different types of afferent and efferent nerve fibers. Moreover, H and extensor reflex responses showed similar trends in latency of onset, duration, and recovery of blockade of sensory and motor fibers. These methods provided better evaluation of anesthesia than subjectively assessed responses to painful electrical stimuli. By electromyographic recordings, the H responses and withdrawal reflexes may also be easily applied in a noninvasive manner to human subjects in the clinical setting.[5,8] The techniques are also useful for comparisons of the degree and duration of anesthesia by different drugs and at variable concentrations, as shown in this report for bupivacaine solutions. In fact, quantitative measurements of isometric muscle force and electromyographic recordings have been demonstrated to give more complete information about the time evolution of peridural anesthesia than subjective scales.[23]

Behavioral responses to painful stimuli are widely used to monitor the efficacy of local blockades. In the anesthetized animal, painful sharp stimuli also cause clearly observable reactions, expressed as reflex muscle jerks, as the nociceptive impulses travel through the ascending spinal pathways. The nociceptive responses induced by electrical stimulation on the skin are similar to those seen after pinching or pricking the skin with forceps or needles,[3] but have the advantage that the stimuli are more reproducible and the rats do not show habituation over long periods of repeated testing.[7] Electrical pulses provided by a bipolar electrode with short interelectrode separation have been shown useful to assess loss of sensation after nerve lesions and recovery by regenerating sensory nerve fibers.[4]

The late motor response in rats, which is easily evoked by submaximal or even maximal nerve stimulation, has been demonstrated to be the H wave.[21,30] The H wave is due to impulse transmission through a reflex arch that includes large myelinated afferent fibers that synapse with spinal motoneurons to activate a population of efferent motor fibers to muscle.[8] With proximal epidural anesthesia the H reflex is abolished due to conduction block on the afferent side but a normal M wave is obtained. This monosynaptic reflex response has been utilized by Hersh et al.[11] to mon-

itor intrathecal anesthesia in the rat by recording with electrodes in contact with the nerve.

The withdrawal reflex depends on a complex circuit integrated by nociceptive afferent fibers, polysynaptic connections with ipsi and contralateral spinal motoneurons, and motor efferent fibers to the appropriate muscles. The main components of the withdrawal response are the ipsilateral flexor reflex and the contralateral extensor reflex.[29] The flexor reflex has been widely used to test the efficacy of analgesic drugs to modulate pain responses in experimental animals.[31,32] We chose the extensor reflex to evaluate spinal anesthetic agents because its pathway crosses the spinal cord. The response, therefore, could not be attributed to ephaptic transmission or a late motor response. The usual recorded volley of MUAPs was slightly dispersed in time, and had a latency of 18–26 ms. This implied that conduction was via small-size afferent fibers (as Aδ) and a polysynaptic pathway.

In most previous reports the evaluation of peripheral nerve and spinal blockade induced by injected local anesthetics in experimental animals has been based on qualitative or semiquantitative documentation of variables such as locomotion, posture, and sensory loss to different painful stimuli. The use of quantitative electrophysiological methods, otherwise popular in the evaluation of neuropathic diseases and experimental nerve regeneration,[8,13,16,22,24] will improve discriminative detection and sensitivity of the responses to local anesthetics. Moreover, different types of nerve fibers can be tested simultaneously in the same subject by judicious placement of stimulation and recording electrodes, therefore reducing variability due to the injection procedure or to interindividual differences. The methods described in this report are easily adaptable to other mammals, including humans, without significant discomfort,[5,8] and may open a field of basic and clinical research for neurophysiologists. A further advantage of our experimental model is that delivery of the anesthetic is direct to the site of action, avoiding the adverse effects of chronic catheterization,[6] and the animal remains intact as needed in human studies.

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