

Actions of Intrathecal Chloroprocaine and Sodium Bisulfite on Rat Spinal Reflex Function Utilizing a Noninvasive Technique

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An *in situ* electrophysiologic technique was developed to study the effects of intrathecal chloroprocaine and sodium bisulfite on spinal reflex activity in the anesthetized rat. By not surgically invading the subarachnoid space, this procedure allows drugs to be administered remotely through an indwelling catheter while monitoring drug effects on the spinal monosynaptic reflex with noninvasive electrophysiologic methods. In this way the normal pharmacokinetic factors are left intact so that time-action relations can be monitored under conditions that may resemble spinal anesthesia in conscious animals. Using this technique it was established that chloroprocaine with and without 0.2% sodium bisulfite blocks the monosynaptic reflex in a fully reversible manner. The reflex block of a higher concentration of bisulfite (0.6%) was poorly reversible in some preparations. These results correlate well with a previous *in vivo* behavioral study on the motor and sensory blocking actions of intrathecal chloroprocaine and bisulfite in the same animal species. The authors feel this technique can be used to study the temporal changes in reflex activity caused by other drugs administered *via* the intrathecal route. (Key words: Anesthetics, local: chloroprocaine. Anesthetic techniques: spinal. Pharmacology: sodium bisulfite. Spinal nerve roots: reflex potentials.)

VARIOUS *in vivo* animal models simulating human spinal anesthesia have recently been used to explore the question of functional neurotoxicity caused by the inadvertent subarachnoid injection of the 2-chloroprocaine anesthetic solution containing the antioxidant sodium bisulfite.¹⁻³ § These studies implicated bisulfite as the cause of the reported human paralyses⁴⁻⁶ ¶ and subsequently led to the complete removal of bisulfite from the chloroprocaine anesthetic solution intended for epidural injection.⁷

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§ Hersh EV, Havelin D, Condouris GA: Evaluation of subarachnoid 2-chloroprocaine and sodium bisulfite in the rat. *Regional Anesthesia* 12:5-9, 1987.

¶ Sheskey MC, Mallampati SR: Prolonged neural blockade following caudal epidural block with 2-chloroprocaine. *Regional Anesthesia* 10: 28-31, 1985.

All of the *in vivo* animal models, including ours, § used behavioral changes as an end point and consequently suffer from a potential lack of sensitivity for the detection of subtle neurologic deficits. We therefore developed a procedure for studying the effects of intrathecal drug administration on a spinal reflex by combining two separate techniques; 1) the delivery of drug *via* an implanted intrathecal catheter;⁸ and 2) the evaluation of spinal reflex actions by recording the monosynaptic reflex outside the vertebral space.⁹ This dual technique enabled us to study the effects of intrathecal chloroprocaine and bisulfite on functional spinal cord electrophysiology in the rat, and provide additional support for the safety of chloroprocaine, itself, and the potential neurotoxicity of sodium bisulfite.

Materials and Methods

SURGERY

Following approval from the Institutional Animal Care Committee male Sprague-Dawley rats weighing 400-500 g were anesthetized initially with i.p. urethane 1.3 g/kg, and were given increments of urethane as needed. Urethane anesthesia was chosen because the surgical procedures and experiments were of relatively long duration (4-5 h) and because anesthetic concentrations of urethane have minimal effects on the release of synaptic neurotransmitter (acetylcholine).^{10,11}

A subarachnoid catheter was first inserted to a length of 100 mm through an incision in the atlanto-occipital membrane as described by Yaksh and Rudy.⁸ The inner aspect of the right hindlimb was shaved and an incision was made from just below the point where the sciatic trunk exits from the vertebral column to just above the ankle. The sciatic nerve and its tibial, greater peroneal, and sural branches were exposed and freed of muscle attachments. The peroneal and sural branches were then cut away from the main trunk leaving 1-2 mm stumps. The distal end of the tibial nerve was tied with thread and cut distal to the tie just before the nerve passed beneath the gastrocnemius muscle. This procedure left the sciatic-tibial trunks as the conduction path. The animal was placed on a heating blanket kept at 37° C and the skin around the surgical site was sutured and tied to metal posts to form a tent so

that the nerve could continuously be bathed in warm (37°C) mineral oil. The nerve was then prepared for electrophysiologic study.

ELECTROPHYSIOLOGIC PROTOCOL

The technique of monitoring the monosynaptic spinal reflex was adapted from that used by Lloyd in the cat.⁹ The distal portion of the tibial nerve was placed on a pair of platinum recording electrodes and the more central sciatic trunk was placed on a pair of stimulating electrodes. A ground electrode was located between the two pairs of electrodes. The conduction distance between the central stimulating electrode (cathode) and central recording electrode ranged from 7–9 mm. The nerve was stimulated with a single square pulse delivered from a Grass S44 stimulator, with a pulse duration of 0.05 ms and voltage adjusted to obtain maximum reflex potential (3–4 V). The direct tibial potential and the reflex potential were amplified through a DC amplifier using a gain of 1,000 or 2,000. The high-frequency filter was set at 3K. The potentials were viewed on a Tetrionix Type 5113 Storage Oscilloscope and photographed with a C59 Oscilloscope Camera.

With appropriate stimulation parameters two potentials were seen with each stimulus pulse (fig. 1 A). One was the "direct" tibial potential which reflected the impulses conducted from the cathode to the first recording electrode. It was almost fused to the shock artifact because of the short conducting distance. The second was a much smaller peak with a delay of about 5 ms which was readily discernible on the negative after potential at higher gains. This elevation could be reversibly reduced by repetitive stimulation that had no effect on the direct compound action potential. Also, cutting the spinal end of the sciatic trunk irreversibly abolished this late potential but had no effect on the direct potential (fig. 1 B). These two observations confirmed that the late potential was indeed of spinal reflex origin,^{9,12} and it was designated as the "reflex" potential. By using this technique it was possible to observe any spinal effect of the subarachnoid injection while simultaneously monitoring the viability of the preparation by noting the status of the direct potential.

DRUG SOLUTIONS

Experimental drug solutions were prepared immediately before use with care being taken to maintain approximately equal osmolarities (302–314 mOsm) by adjusting the NaCl concentration. To mimic the clinical condition, the pH of each solution was adjusted to 3 with 0.1 N HCl. Chloroprocaine HCl powder was donated by Astra Pharmaceuticals (Westborough, MA). Sodium bisulfite (grade 1: 99–100% pure as anhydrous sodium metabisulfite) was purchased from Sigma Chemical Com-

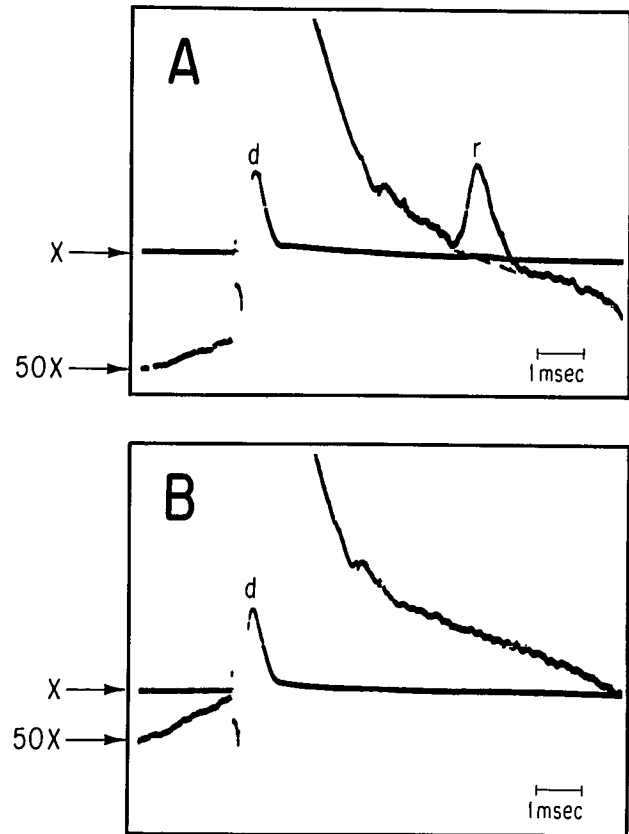


FIG. 1. Evoked potentials recorded from the tibial nerve. Each panel shows the direct (d) and reflex (r) potential at 2 amplifications (X and 50X). A At low amplification (X), the direct potential is readily observed fused to the shock artifact but the reflex potential representing the monosynaptic reflex is almost imperceptible 4.5 ms later. When the amplification is increased by 50-fold, the late spinal reflex potential is readily observed on the descending phase of the expanded direct potential. The dashed line illustrates the extrapolated baseline used in area measurements. B Proof that this late deflection is of spinal origin. When the sciatic nerve is cut just after it exits the vertebral column, the late potential is completely and irreversibly abolished, while the direct potential is unaffected.

pany (St. Louis, MO). All millimolar concentrations of bisulfite were based on the sodium bisulfite species.

EXPERIMENTAL PROCEDURES

Control photographs were first taken of the direct and spinal reflex potentials before drug injections. This was accomplished by first stimulating the nerve with a single pulse to record the direct potential at low amplification. With the direct potential trace on the oscilloscope screen, the nerve was then stimulated by a second pulse of equal intensity 1 min later to record the reflex potential at higher amplifications. Injections into the subarachnoid space were all delivered through the externalized injection port of the subarachnoid catheter in a fixed volume of

40 μ l over 2 min. Following the injection, photographic records were taken using the previously described procedure at 3 and 5 min, and then every 5 min up to 2 h, or less if the spinal reflex potential recovered earlier. A 2-h postinjection observation period was chosen because the preparation began to deteriorate at about this time as evidenced by a reduced amplitude of the direct potential. At the completion of each experiment the animal was killed with a lethal dose of intraperitoneal urethane and laminectomies performed to establish the location of the catheter tip within the subarachnoid space.

DATA ANALYSIS

Drug effects on nerve conduction were determined by measuring the amplitudes of the direct and reflex potentials and for the reflex potential by an alternate procedure in which the area under the potential was estimated by directly weighing the waveform that was cut out from a photocopy of the oscilloscope trace (table 1). The reflex baseline was the extrapolated continuation of the smooth curve of the negative after potential as illustrated by the dashed line in figure 1 A.

The maximum degree of conduction block of both the direct action potential and spinal reflex potential occurring within the first 30 min of drug action was compared between the various groups. The maximum degree of reflex recovery (amplitude and area) within 2 h was calculated for those experimental treatments that induced a block of the spinal reflex potential. Significant differences between treatments were analyzed using a one-way analysis of variance and a Newman-Keuls multiple contrast test ($P < 0.05$).

TABLE 1. Electrophysiologic Parameters for the Various Treatment Groups before the Injection of the Experimental Solutions

Treatment Group	n	Direct Potential Amplitude (mv)	Reflex Potential Amplitude (uv)	Reflex Potential Area (mg)*
0.9% Sodium Chloride	6	3.9 \pm 0.88	64 \pm 17.2	7 \pm 2.14
0.2% Sodium Bisulfite	5	4.1 \pm 0.72	62 \pm 18.5	5.4 \pm 1.40
0.8% Sodium Chloride				
0.6% Sodium Bisulfite	7	4.1 \pm 0.80	59 \pm 14.3	5.9 \pm 1.98
0.55% Sodium Chloride				
3% 2-Chloroprocaine	6	3.8 \pm 0.69	61 \pm 14.4	6.1 \pm 1.20
0.3% Sodium Chloride				
3% 2-Chloroprocaine	5	5.1 \pm 1.26	96 \pm 22.8	8.7 \pm 2.51
0.2% Sodium Bisulfite				
0.2% Sodium Chloride				

Values are mean \pm SEM.

* Mg are the units for area because of the procedure described in the "Data Analysis" section.

TABLE 2. Maximum Degree of Conduction Block within 30 Minutes

Treatment Group	n	Direct Potential Amplitude (% Decrease)	Reflex Potential Amplitude (% Decrease)	Reflex Potential Area (% Decrease)
0.9% Sodium Chloride	6	2 \pm 1.8	5 \pm 2.9	7 \pm 3.3
0.2% Sodium Bisulfite	5	6 \pm 3.1	17 \pm 6.3	12 \pm 4.5
0.8% Sodium Chloride				
0.6% Sodium Bisulfite	7	4 \pm 2.8	83 \pm 14.8*	81 \pm 15.3*
0.55% Sodium Chloride				
3% 2-Chloroprocaine	6	4 \pm 2.7	74 \pm 15.2*	77 \pm 12.9*
0.3% Sodium Chloride				
3% 2-Chloroprocaine	5	3 \pm 2.1	74 \pm 15.2*	67 \pm 17.3*
0.2% Sodium Bisulfite				
0.2% Sodium Chloride				

40 μ l volume; pH = 3.

Values are mean \pm SEM.

* Significantly different than sodium chloride and bisulfite 0.2%. ($P < 0.05$, one-way ANOVA).

Results

The relevant electrophysiologic parameters before the injections were similar for all treatment groups (table 1). The direct potentials were not affected by the drug injection. In contrast, the spinal reflex potential was significantly reduced by solutions containing 0.6% bisulfite or 3% chloroprocaine whether the criterion for blockade was amplitude or area of the potential (table 2). (Area was measured because of the occasional difficulty in measuring amplitude while the negative after potential was declining.) A 100% block occurred in five of seven animals after the injection of 0.6% sodium bisulfite, in three of six animals receiving 3% 2-chloroprocaine, and in two of five animals receiving 3% 2-chloroprocaine with 0.2% sodium bisulfite. All other animals in these three treatment groups exhibited a partial reflex potential blockade except for one animal that received 0.6% sodium bisulfite which did not exhibit any reflex blockade. Representative recordings from the three active treatments are shown in figure 2.

Recovery from the conduction block was excellent for both chloroprocaine-containing solutions but was poor for the 0.6% bisulfite treatment (table 3). It should be noted that all animals receiving chloroprocaine exhibited at least an 80% recovery.

The anatomic location of the catheter tip was established for each animal at a postmortem examination. For all five treatments the left-right-midline distribution of the tips were similar (fig. 3). The intensity of block was dependent on the location of the catheter tip within the subarachnoid space. When it was at the midline or to the

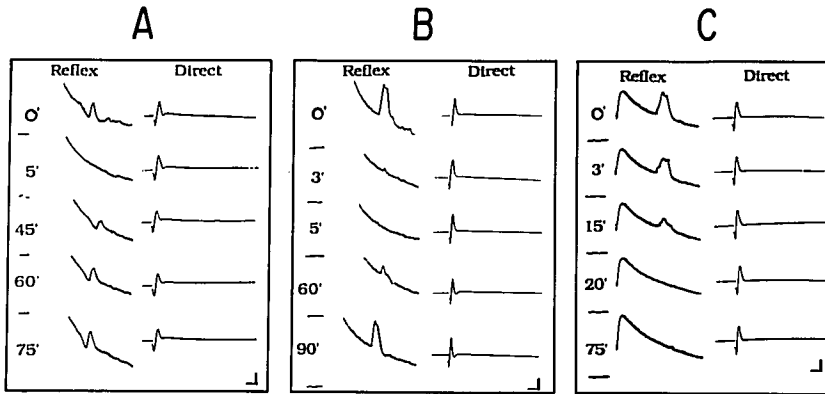


FIG. 2. A Illustrative recordings showing the effects of 3% 2-chloroprocaine, B 3% chloroprocaine with 0.2% sodium bisulfite, and C 0.6% sodium bisulfite, on the spinal reflex potential (Reflex) and on the direct tibial action potential (Direct). Numerical values on the left represent minutes after the subarachnoid injection of the drug; 0 = Control. A blanking of the trace during the rapid rising and falling phases of the direct potential resulted from the high gain used in the reflex recordings. The reflex potential rides on the negative after potential phase of the direct compound action potential. The short horizontal lines found beneath the numerical time points on the left represent the base lines for the reflex records. The horizontal time scale in the lower right

corner is 1 ms; the vertical scale represents 50 μ v for the left column and 2.5 mV for the right columns. Note the recovery of the spinal reflex in panels A and B, and minimal recovery in panel C.

right of the midline, the block was nearly 100% for all three active treatments. Recall that the stimulation and recording was always done on the right nerve; therefore, a left-sided injection would be less effective.

Despite the occasional mismatch of injection site and nerve recording site, the data show that after subarachnoid injection, 3% chloroprocaine, with or without 0.2% bisulfite at pH 3, resulted in a block of spinal reflex activity in the rat that was readily reversible. In contrast, the injection of 0.6% bisulfite alone resulted in a block that was poorly reversible.

Discussion

The present work describes for the first time an *in situ* technique in which the spinal electrophysiologic effects of drugs administered intrathecally are monitored outside the CNS by stimulating and recording from a peripheral

TABLE 3. Maximum Degree of Reflex Recovery within 2 Hours for Treatments that Induced Significant Block of Reflex Potential

Treatment Group	n	Reflex Potential Amplitude (% Control)	Reflex Potential Area (% Control)	Direct Potential Amplitude at Maximum Reflex Recovery (% Control)
3% 2-Chloroprocaine 0.3% Sodium Chloride	6	98 \pm 2.3	92 \pm 2.6	95 \pm 3.4
3% 2-Chloroprocaine 0.2% Sodium Bisulfite	5	94 \pm 2.3	93 \pm 3.3	94 \pm 4.9
0.2% Sodium Chloride 0.6% Sodium Bisulfite 0.55% Sodium Chloride	7	48 \pm 14.9*	49 \pm 14.9*	94 \pm 5.6†

Values are mean \pm SEM.

* Significantly different from other treatments in same column ($P < 0.05$, one-way ANOVA).

† n = 5 (one rat exhibited no recovery and one rat exhibited no block of reflex potential).

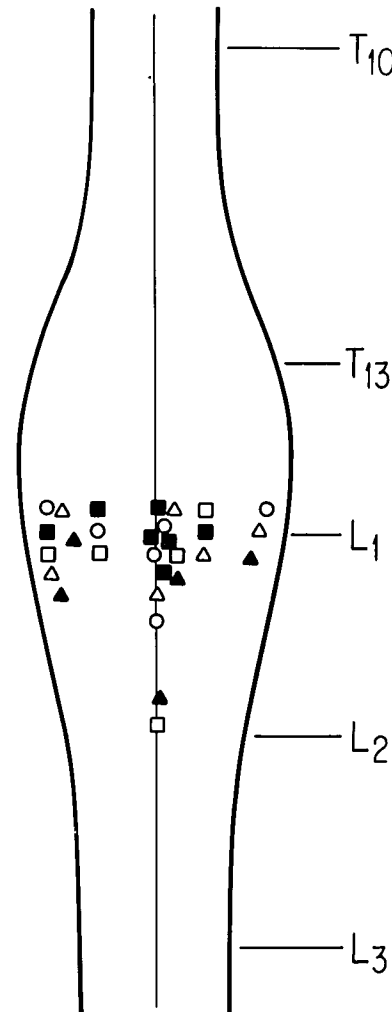


FIG. 3. Location of catheter tips within rat subarachnoid space. Vertebral levels are indicated on the right of the schematic. Various symbols describe drug treatments for individual experiments. 0 = sodium chloride; \square = 0.2% sodium bisulfite; \blacksquare = 0.6% sodium bisulfite; \triangle = 3% 2-chloroprocaine; \blacktriangle = 3% 2-chloroprocaine with 0.2% sodium bisulfite.

nerve, without disturbing the dura mater, and leaving the spinal roots and the cerebrospinal fluid within the subarachnoid space. In this respect, dilution, redistribution, and metabolism of intrathecally administered drugs are left intact. It must be appreciated that CSF distribution and mixing is likely to be somewhat different than the clinical setting because of the small opening of cisternal dura necessary for catheter insertion and because of the mere physical presence of a catheter traversing almost the whole length of the rat spinal cord. However, the vast majority of other techniques for evaluating a drug's effect on spinal reflex potentials involve a laminectomy, incision of the dura at the recording or stimulation site, isolation and cutting of specific dorsal and/or ventral spinal roots, and the administration of drugs topically, iontophoretically, or intravenously.^{13,14} Although a large amount of knowledge has been acquired with respect to transmitter and pharmacologic modulation of spinal synaptic events using these procedures, they eliminate dilution of the drug with CSF and its subsequent redistribution.

The *in situ* effects of intrathecal chloroprocaine and sodium bisulfite on rat spinal electrophysiology correlate well with previously published behavioral results in the same animal species. § Three percent chloroprocaine alone or with 0.2% sodium bisulfite reversibly blocks the monosynaptic reflex potential. Recovery is significantly impaired in rats administered three times this bisulfite concentration (table 3; fig. 2). Therefore, both electrophysiologically and behaviorally, 3% chloroprocaine causes only the expected local anesthetic effects after its injection into the rat subarachnoid space. On the other hand, sodium bisulfite at a concentration of 0.6% can adversely affect spinal cord electrophysiology by blocking reflex activity when injected into the rat subarachnoid space. Behaviorally, this is manifested by a prolonged motor block. § Although one group using a different mode of drug application reported that chloroprocaine caused significantly more axonal edema and axonal dystrophy than amide local anesthetics or sodium bisulfite in an *in vivo* rat sciatic model,^{15,16} the present study clearly demonstrates that chloroprocaine itself does not adversely affect simple reflex activity after intrathecal injection.

It is interesting to note that 40 μ l of bisulfite 0.2% does not block the rat monosynaptic reflex potential. Other investigators using the rabbit and the dog have shown this concentration to cause irreversible hindlimb paralysis when administered intrathecally.^{1,2} However, in both these studies the injection volume exceeded the spinal CSF volume by two-fold or more. In those *in vivo* studies where the injection volume was only 30–40% of the total spinal CSF, concentrations of bisulfite greater than 0.2% were necessary to induce prolonged paralysis.³ § The injection volume used in the current *in situ* study falls into this latter group.

There is one other pharmacokinetic factor that may make the rat more resistant than other animal species (including humans) to the neurotoxic effects of intrathecal sodium bisulfite. The rat possesses 10–20 times greater sulfite oxidase activity than humans and three to five times greater activity than the rabbit.^{17,18} This enzyme catalyses the reaction in which sulfites are oxidized into less toxic sulfates.^{18,19,**} It is noteworthy that Wang demonstrated that unlike sodium bisulfite, subarachnoid injections of sodium sulfate were not neurotoxic in rabbits.² Sulfite oxidase does exist in the rat CNS, although its activity is much less than in liver, heart, and kidney.²⁰ Sulfite oxidase in rat CSF has not been studied.

In summary, we describe a technique developed to study the effects of local anesthetic solution components on rat spinal reflex mechanisms. This methodology is unique because it delivers a drug directly to the site(s) of action and it leaves intact all central connections to and from the spinal cord, as well as the dura and the CSF. In this respect, it is noninvasive and preserves dilution and redistribution of drugs, which is unlike other experimental techniques that have been used to evaluate drug effects on spinal electrophysiology. The present model appears suitable for screening the acute toxicity of novel intraspinal agents.

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