Hyperosmolar Solutions Selectively Block Action Potentials in Rat Myelinated Sensory Fibers: Implications for Diabetic Neuropathy

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Matsuka, Yoshizo and Igor Spigelman. Hyperosmolar solutions selectively block action potentials in rat myelinated sensory fibers: implications for diabetic neuropathy. J Neurophysiol 91: 48–56, 2004. First published September 17, 2003; 10.1152/jn.00689.2003. Diabetic neuropathy is a common complication of diabetes mellitus patients. It is a wide range of abnormalities affecting proximal and distal peripheral sensory and motor nerves. Although plasma hyperosmolality is a common finding in diabetes mellitus, the effects of hyperosmolality on conduction of various sensory signal components have not been addressed in detail. Here we show that in rat dorsal root ganglion (DRG) preparations from normal rats, hyperosmolar solutions (360 mmol/kg, containing increased glucose, sucrose, NaCl, or mannitol) produce a selective block of signal propagation in myelinated sensory A-fibers. In compound action potential (CAP) recordings with suction electrodes, peak A-fiber CAP amplitude was selectively decreased (20%), while the C-fiber peak remained intact or was slightly increased. Hyperosmolar solutions had smaller effects on conduction velocity (CV) of both A- and C-fibers (approximately 5% decrease). Hyperosmolality-induced CAP changes could not be observed during recordings from isolated spinal nerves but were evident during recordings from desheathed spinal nerves. In intracellular recordings, hyperosmolar solutions produced a block of spinal nerve-evoked action potential invasion into the somata of some A-fiber neurons. Removal of extracellular calcium completely prevented the hyperosmolality-induced CAP decreases. Based on these data, we propose that the decreased CAP amplitudes recorded in human patients and in animal models of diabetes are in part due to the effects of hyperosmolality and would depend on the extracellular osmolality at the time of sensory testing. We also hypothesize that hyperosmolality may contribute to both the sensory abnormalities (paresthesias) and the chronic pain symptoms of diabetic neuropathy.

INTRODUCTION

Diabetic neuropathy is a common finding in diabetes mellitus patients. It is a wide range of abnormalities affecting proximal and distal peripheral sensory and motor nerves, although the exact prevalence varies according to the diagnostic criteria used. Some studies report high prevalence levels of diabetic neuropathy (Benbow et al. 1999; Vinik et al. 2000). In addition, the pain component of the diabetes is a very common complaint (11–32% of patients with diabetes) (Boullon et al. 1985; Partanen et al. 1995; Ziegler et al. 1993), prompting many patients to seek medical intervention. The major morbidity associated with somatic neuropathy is foot ulceration, and the neuropathy increases the risk of amputation (Vinik et al. 2000).

Although plasma hyperosmolality is one of the common signs of diabetes mellitus (serum osmolalities in 80% of the diabetes mellitus patients are >320 mmol/kg (Wachtel et al. 1991), range 320–380 mmol/kg (Arieff and Carroll 1972; Fulop et al. 1973; Gerich et al. 1971), the effects of hyperosmolality on conduction of various sensory signal components have not been addressed in detail. Also, many patients with renal failure present with plasma hyperosmolality (Mehney and Arieff 1983; Muravitskai et al. 1990). Peripheral neuropathy is a frequent complication of chronic renal failure patients (Imam et al. 2003; Murphy and Carmichael 2000). Furthermore, itch (localized and generalized uremic pruritis) is a common complaint in these patients, and is often exacerbated during or immediately after renal dialysis with hyperosmotic solutions (Mettang et al. 2002; Murphy and Carmichael 2000).

Decreases in the peak amplitude and conduction velocity (CV) of myelinated fibers have been demonstrated previously in human diabetic patients (Downie and Newell 1961; Gilliatt and Willison 1962; Mulder et al. 1961), in a rat model of streptozotocin-induced diabetes (Patel and Tomlinson 1999), and in transgenic diabetic mice (Elias et al. 1998). In human diabetic patients, sensory nerve CV decreased by 9% over the 10-year follow-up period while sensory amplitudes diminished by approximately 50% (Partanen et al. 1995). Moreover, acute high blood glucose reduces sensory CV by <5% in diabetic patients (Celiker et al. 1996; Hyllienmark et al. 1995, 1997), and myelinated nerve CV decreases gradually (0.5–0.7 m/s per year) (Arezzo 1999; Partanen et al. 1995). In rat models of diabetes, the reduction of CV also develops slowly (days to weeks) (Patel and Tomlinson 1999) and remains diminished during survival times ≤1 yr (Moore et al. 1980; Sharma and Thomas 1974).

We hypothesized that hyperosmolality may be responsible, at least in part, for the signal conduction deficits seen in diabetes. In this study, we attempted to dissociate the possible effects of hyperosmolality from the metabolic changes of diabetes by studying the effects of hyperosmolar solutions on the extracellular and intracellular properties of sensory neurons in whole-mount dorsal root ganglion (DRG) preparations from normal rats.
METHODS

The Institutional Animal Care and Use Committee approved all animal experiments.

Surgery and DRG preparation

Male Sprague-Dawley rats (200–390 g) were anesthetized with sodium pentobarbital (50 mg/kg) and L4 and L5 DRGs were excised with their dorsal roots, the ventral roots, the spinal nerves, and a variable length of attached sciatic nerves. In some experiments, only dorsal roots, ventral roots, or spinal nerves with attached sciatic nerves were dissected without the ganglia. Prior to recording, the preparations were further trimmed at 0°C in the low- Na⁺ artificial cerebrospinal fluid (ACSF) composed of (in mM) 62 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 2 MgCl₂, 26 NaHCO₃, 10 glucose, and 124 sucrose, and transferred to normal ACSF (20–23°C) composed of (in mM) 124 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 2 MgCl₂, 26 NaHCO₃, and 10 glucose. The ACSF was continuously bubbled with 95% O₂, 5% CO₂ to ensure adequate oxygenation of DRGs and pH 7.4. For recording, preparations were transferred to a custom recording chamber (0.3 ml volume) that was perfused (2.7 ml/min) with oxygenated ACSF at 34.5°C.

Compound action potential and intracellular recording

The compound action potentials (CAPs) were recorded from the dorsal roots or ventral roots of the spinal ganglia using a suction recording electrode according to previously established methods (Spigelman et al. 2001; Stys et al. 1991). In some experiments, the CAPs were recorded from isolated dorsal roots or spinal nerves attached to sciatic nerves. Signals from the recording electrode and the artificial suppression electrode positioned nearby were fed into a differential amplifier (Dam 50, WPI) in AC mode. A calibration pulse (±2 V across a 100-MΩ series resistor) was always included in the CAP recordings to estimate the resistance at the nerve/recording electrode junction (Spigelman et al. 2001; Stys et al. 1991). Since CAP amplitude/area varies linearly with changes in this resistance (Spigelman et al. 2001; Stys et al. 1991), we adjusted the CAP signal based on the calibration pulse amplitude changes during analysis of any given recording.

Intracellular recordings were obtained with borosilicate glass microelectrodes (40–60 MΩ) filled with 3 M K-acetate. The electrode holder/probe was connected to an amplifier (Axoclamp 2A, Axon Instruments). A bipolar suction electrode was used to stimulate the peripheral nerve stumps and to elicit spikes in the soma of an intracellularly recorded neuron. Single current pulses were applied to the bipolar electrode via a stimulator (S88, Grass instruments) and a stimulus isolation unit (PSIU6, Grass Instruments). A bipolar suction electrode was used to stimulate the peripheral nerve stumps and to elicit spikes in the soma of an intracellularly recorded neuron. Single current pulses were applied to the bipolar electrode via a stimulator (S88, Grass instruments) and a stimulus isolation unit (PSIU6, Grass Instruments).

Data from intracellular recordings were further amplified (model 440, Brownlee Precision Instruments). Amplified signals were digitized at 10–20 kHz via the Digidata 1200B interface (Axon Instruments) and displayed on a computer screen using the pCLAMP8 software package (Axon Instruments). Voltage and current recordings were also monitored continuously with a chart recorder (Brush 220, Gould). Conduction velocity was calculated from the length of sciatic nerves (measured from the center of the ganglion after the experiment) and divided by the latencies of the action potentials (Villiére and McLachlan 1996). The latencies were measured between the starting point of stimulation artifacts and starting point of action potentials.

Solutions

All solutions were applied by bath perfusion. Control ACSF was around 300 mmol/kg, and hyperosmolar solutions were adjusted to 360 mmol/kg by increasing glucose, sucrose, NaCl, or mannitol. In some experiments, CaCl₂ was omitted, and 1 mM EGTA was added to the ACSF. Osmolality of each solution was tested with a vapor pressure osmometer (Vapro 5520, Wescor) prior to each experiment.

Data analysis

Data were analyzed off-line using the pCLAMP8 software package (Axon Instruments). All data are presented as mean ± SE. Statistical analyses (paired t-test, 3-way ANOVA, and Friedman repeated measures ANOVA on ranks) were used to compare between responses in control and various hyperosmolar solutions.

RESULTS

Hyperosmolar solutions produce a selective decrease in the myelinated A-fiber CAP

Perfusion of hyperosmolar ACSF containing increased glucose produced a reversible decrease in the A-fiber CAP amplitude of intact DRG preparations (Fig. 1, A and B). On entry of hyperosmolar solution into the recording chamber, the CAP amplitude decreased gradually, reaching its minimum (range, 10–50% decrease) and remaining stable after 6 min of application (Fig. 1A). Therefore all subsequent measurements of the effects of hyperosmolar solutions were obtained 6–9 min after switching bath solutions. In contrast to A-fiber CAP decreases, peak amplitude of the C-fiber CAP was little affected by the hyperosmolar solution (Fig. 1C). The decreases in A-fiber CAP amplitude were seen over a range of stimulation strengths (Fig. 2A). Similarly, the lack of effect on C-fiber CAP ampli-

FIG. 1. Hyperosmolality-induced selective decrease in the myelinated A-fiber compound action potential (CAP). A: time course of changes in the peak A-fiber CAP after a 10-min application of a hyperosmolar solution containing glucose. B: example of a reversible decrease in A-fiber CAP amplitude after application of a hyperosmolar solution containing increased glucose. CAP amplitude recovered after wash with control solution. C: in another recording, peak C-fiber CAP amplitude was unaffected by the hyperosmolar solution. Note the 10-fold difference in the voltage scale between B and C.

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tude was consistent across the entire range of stimulation strengths (Fig. 2B).

Different hyperosmolar solutions were perfused to test whether the A-fiber CAP decrease was specific to the hyperosmolar glucose or whether the effect was independent of the composition of the hyperosmolar solution. Different hyperosmolar solutions containing increased sucrose, NaCl, or mannitol also decreased the A-fiber CAP amplitude at different stimulation strengths (Fig. 3A). Summed positive and negative peaks of the C-fiber CAP at different stimulation strengths showed that hyperosmolar solutions with glucose or NaCl produced a slight but statistically significant increase in the peak CAP amplitude compared with control ACSF, while sucrose- and mannitol-based solutions did not (Fig. 3B).

Hyperosmolar solutions have a small effect on CAP conduction velocity

The average CV of A- and C-fiber peaks was 25.4 ± 1.16 (range, 20.0–33.7 m/s) and 0.94 ± 0.05 m/s (range, 0.85–1.22 m/s), respectively. The CV of both A- and C-fiber CAP peaks were slightly decreased by the hyperosmolar glucose solution (Fig. 4A–D). However, the decreases (range, 0–10%) were much smaller than the decreases in the amplitude of the A-fiber CAP.

Hyperosmolar solutions have no effect on epineurium-protected spinal nerves

Next, we performed experiments designed to determine whether hyperosmolar solutions produce similar effects along the entire length of the DRG preparation, including dorsal and ventral roots and associated spinal nerves. To this end, we tested the effect of several different hyperosmolar solutions during CAP recordings from the ventral root (intact DRG), as well as stimulating and recording from the isolated dorsal root only, or from the isolated spinal nerve (Fig. 5). Hyperosmolar solutions reduced the amplitude of the A-fiber CAP in ventral root recordings, as well as in recordings from isolated dorsal roots, similar to the intact DRG preparations. However, recordings from isolated spinal nerves were unaffected by the hyperosmolar solutions. We mechanically removed the epineurium from the isolated spinal nerves and found that the A-fiber CAP recorded from desheathed spinal nerves was reduced by hyperosmolar glucose similar to the intact DRG preparations (Fig. 5, A and B). The amplitude of the C-Fiber CAP was

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**FIG. 2.** Hyperosmolality effects on the CAP stimulus–response relationship. A: summary graph of 10 experiments measuring A-fiber CAP peak at different stimulus strengths. Data are shown as mean percent of control A-fiber CAP ± SE at a 0.2-mA stimulus, before and after application of a hyperosmolar solution containing increased glucose. B: summary graph of C-fiber CAP peak at different stimulus strengths. Data are shown as mean percent of control C-fiber CAP ± SE at a 10-mA stimulus before and after application of a glucose-based hyperosmolar solution. Control solution, approximately 300 mmol/kg; hyperosmolar solution, approximately 360 mmol/kg. Note the different range of stimulus strengths used to evoke A- and C-fiber responses.

**FIG. 3.** Selective reduction in the A-fiber CAP by different hyperosmolar solutions. A: summary of 10 experiments measuring A-fiber CAP peak at different stimulation strengths. Data are shown as mean percent of control ± SE for each measured stimulus strength, after switching from control solution (approximately 300 mmol/kg) to different hyperosmolar solutions containing increased glucose, sucrose, NaCl, or mannitol (approximately 360 mmol/kg). Control solution vs. hyperosmolar solutions: *P* < 0.001 (3-way ANOVA), between hyperosmolar solutions: not significant. B: summary graph of summed positive and negative parts of C-fiber CAP peak at different stimulation strengths. Control solution vs. glucose-containing hyperosmolar solution: *P* < 0.001 (3-way ANOVA), control solution vs. NaCl containing hyperosmolar solution: *P* < 0.001 (3-way ANOVA).
FIG. 4. Hyperosmolar solutions have a minor effect on CAP conduction velocity. A and B: conduction velocity (CV) of A- and C-fiber CAP were slightly decreased by a hyperosmolar glucose solution. C: summary of 10 experiments measuring peak A-fiber CAP CV at different stimulus strengths. Data are shown as means ± SE percent of control CV after application of hyperosmolar solutions containing either increased glucose, sucrose, NaCl, or mannitol. Control solution vs. sucrose-containing hyperosmolar solution, $P = 0.002$ (3-way ANOVA); control vs. NaCl, $P < 0.001$; and control vs. mannitol, $P < 0.001$ (3-way ANOVA). D: summary graph of peak positive part of C-fiber CAP CV at different stimulus strengths. Data are shown as means ± SE percent of control CV after application of hyperosmolar solutions containing either increased glucose, sucrose, NaCl, or mannitol. Control solution vs. glucose-containing hyperosmolar solution, $P = 0.002$ (3-way ANOVA); control vs. sucrose, $P < 0.001$; and control vs. mannitol, $P < 0.001$ (3-way ANOVA).

...significantly increased only by hyperosmolar glucose during recordings from dorsal roots with intact DRGs and in isolated spinal nerve recordings (Fig. 5, A and C). As expected, no detectable C-fiber CAP could be evoked even at maximal stimulation during ventral root recordings.

Hyperosmolar solutions block somatic invasion of action potentials in some A-fiber neurons

Next we studied the effect of hyperosmolar solutions during intracellular recordings from individual neurons. We concentrated on the A-type neurons since the A-fiber CAP was decreased by the hyperosmolar solutions. A-type neurons were identified on the basis of their membrane properties, action potential shape, and CV (Harper and Lawson 1985a, b; Villière and McLachlan 1996). Once a stable recording was achieved, we determined the threshold for stimulation of the spinal nerve necessary to generate a somatic action potential. We then set the stimulus at 10–15% above threshold (0.05 Hz) and tested for the effects of hyperosmolar solutions. Application of hyperosmolar solutions resulted in the block of somatic invasion of action potentials in more than half of A-fiber neurons (Fig. 6; Table 1). The CV was significantly decreased ($P < 0.001$) in each neuron where the action potential block was observed and did not decrease in those neurons that did not exhibit action potential block. This CV reduction was similar for all hyperosmolar solutions tested (Table 1). We were able to relieve the spike block by a 20% increase in stimulus intensity ($n = 2$). Different hyperosmolar solutions produced different effects on the resting membrane potential. Thus hyperosmolar NaCl produced a reversible depolarization of the resting membrane potential in eight of nine neurons tested, whereas hyperosmolar glucose or sucrose produced a reversible hyperpolarization in one-half of the neurons tested (Table 1).

Removal of extracellular calcium prevents CAP reduction by hyperosmolar solutions

We suspected that calcium influx may play a role in the hyperosmolality-induced decreases of the CAP amplitude, because hyperosmolar solutions produce increases in intracellular calcium (Ogura et al. 1997). Therefore we compared the effects of hyperosmolar glucose on CAP amplitude before and after removal of extracellular calcium. As before, application of hyperosmolar glucose reversibly decreased A-fiber CAP amplitude (Fig. 7A) and increased the C-fiber peak (Fig. 7B). Subsequent application of calcium-free, EGTA-containing normosmol ACSF (300 mmol/kg) increased both A-fiber (110.0 ± 5.7% at 0.2-mA stimulus) and C-fiber (138.7 ± 13.2% at 10-mA stimulus) CAP peaks. The CAP peak increases were significant across the entire range of stimulation strengths (A-fiber, $P = 0.002$; C-fiber, $P < 0.001$). When a 0 Ca/EGTA/glucose-containing hyperosmolar solution was next applied, it did not change the amplitude of either A or C-fiber peaks (Fig. 7, A and B). Examination of the entire stimulus–response relationship revealed that removal of extracellular calcium prevented both the hyperosmolar glucose-induced A-fiber CAP decreases and the C-fiber CAP increases (Fig. 8, A and B).

After switching to 0 Ca/EGTA normosmolar solution, the CV of both A-fiber and C-fiber CAP peaks increased by 5% ($P < 0.001$ and $P = 0.016$, respectively). Interestingly, the CV of A- and C-fiber CAP peaks were slightly decreased (approximately 3%) after switching to the 0 Ca/EGTA glucose hyperosmolar solution ($P = 0.001$ and $P = 0.017$, respectively).

DISCUSSION

The main findings of this study were as follows: 1) hyperosmolar solutions containing increased glucose, sucrose, NaCl, or mannitol consistently decreased the amplitude of the myelinated A-fiber CAP peak (15–25%) but either increased or had no effect on the amplitude of the C-fiber CAP; 2) the conduction velocity of A- and C-fiber CAP was only marginally decreased by some hyperosmolar solutions; 3) intact epineurium prevented hyperosmolar effects on the spinal nerve; 4) hyperosmolar solutions delayed and blocked action potential propagation in some A-type neurons; and 5) removal of extracellular calcium dramatically decreased the effects of a glucose-based hyperosmolar solution on CAP amplitude and CV.

Decreases in the peak amplitude and CV of myelinated fibers have been demonstrated previously in human diabetic patients, in a rat model of streptozotocin-induced diabetes, and...
in transgenic diabetic mice. Our data from normal rats suggest that hyperosmolality per se acutely diminishes the A-fiber CAP amplitude and CV in diabetes, while the long-term changes in CV observed in the previous studies are likely due to the metabolic consequences of diabetes. Metabolic consequences of diabetes (e.g., degeneration of DRG neurons with unmyelinated axons; Horowitz 1993; Schmeichel et al. 2003; Srinivasan et al. 2000) may also explain the long-term decreases in C-fiber amplitudes, which were not decreased by acute hyperglycemic solutions in our experiments.

Voltage-clamp studies at the nodal membrane of sciatic nerve fibers have demonstrated reduced Na\(^+\)/H\(^+\) current in the spontaneously diabetic BB-Wistar rat (Brismar 1983; Brismar and Sima 1981). This Na\(^+\) current decrease was proposed to be due to increased [Na\(^+\)], (Brismar 1993; Greene et al. 1984), an increase in the number of inactive Na\(^+\) channels (Brismar et al. 1987; Cherian et al. 1996), and a shift of the reversal potential for Na\(^+\) current to more negative values (Quasthoff 1998). Na\(^+\), K\(^+\)-ATPase activity that is required for Na\(^+\) extrusion is also decreased in sciatic nerve of streptozotocin diabetic rats (Das et al. 1976).

Hyperosmolar solutions pull water out of cells, thereby causing cell shrinkage. If the seal resistance formed by the tight fit of the glass of the suction electrodes around the nerves were to decrease as a result of tissue shrinkage in hyperosmolar solutions, this could result in decreased stimulus current experienced by the nerve at one end and reduced CAP amplitude at the recording electrode end. However, the likelihood of such resistance changes is small, since we did not observe significant decreases in the calibration pulse responses during application of hyperosmolar solutions. Furthermore, CAP amplitude was compensated for changes in seal resistance between the recording electrode and the nerve during analysis (see METHODS).

Hyperosmolality-induced shrinkage of the axonal diameter would be expected to increase the resistance to axoplasmic current flow and to increase intracellular ion concentrations. Elevation of intracellular [Ca\(^{2+}\)], [Na\(^+\)], and [K\(^+\)] by hyperosmolar solutions have been demonstrated in ventricular myocytes (Ogura et al. 1997). Elevated intracellular [Na\(^+\)] should decrease the Na\(^+\)/H\(^+\) gradient, thereby decreasing Na\(^+\)/H\(^+\) currents. The initiation and propagation of action potentials depend both on the intensity of the Na\(^+\) current as well as the resistance to axoplasmic current flow (Koester 1991a,b). In our study, the somatic invasion of action potentials was blocked by hyperosmolar solutions in almost 50% of the A-fiber neurons, and a significant CV decrease was seen before the block in these neurons. The fact that increasing stimulus intensity could relieve the spike block suggests that this block may occur at the spike initiation site. However, the data do not allow us to determine with certainty that the block did not occur at sites along the axon remote from the site of spike generation.

**Fig. 5.** Hyperosmolar solutions have no effect on epineurium-protected spinal nerves. A: schematic drawings of various recording paradigms. S, stimulating electrode; SN, spinal nerve; DRG, dorsal root ganglion; DR, dorsal root; VR, ventral root; R, recording electrode. B: summary of 8 experiments measuring A-fiber CAP peak at 0.2-mA stimulation strengths. Data are shown as means ± SE percent of control CAP after application of hyperosmolar solutions containing increased glucose, sucrose, or NaCl. C: summary graph of summed positive and negative peaks of C-fiber CAP at 10-mA stimulation strengths (from the same experiments as in A). ***P < 0.001, **P < 0.01, *P < 0.05 by paired t-test comparing control solutions with hyperosmolar solutions.
HYPEROSMOLALITY-INDUCED SPIKE BLOCK

The effect of hyperosmolar solutions on the membrane potential, suggesting that the effect on the membrane potential does not explain the phenomenon of selective CAP decrease. Both sucrose and glucose produced a small membrane hyperpolarization, whereas increasing NaCl produced a consistent membrane depolarization (Table 1). Hyperosmolar sucrose superfusion on rabbit ventricular muscles caused a hyperpolarization that was attributed to increased intracellular $[K^+]$, measured with $K^+$-selective microelectrodes (Fozzard and Lee 1976). Both sucrose and glucose do not permeate through voltage-dependent ion channels and thus their hyperpolarizing membrane effects are expected to be due to changes in intracellular ion concentrations. Interestingly, some types of rat hypothalamic neurons are sensitive to small changes in local concentrations of glucose (Oomura et al. 1974; Silver and Erecinska 1994). DRG neurons can also be considered relatively insensitive since the glucose-induced membrane potential changes are quite small ($\approx 2$ mV for a 60-mM increase in glucose, Table 1) compared with hypothalamic neurons (10–20 mV for a 2-mM decrease in glucose (Silver and Erecinska 1998)).

Hyperosmolar glucose or sucrose-induced decreases in the Na$^+$ current may have also contributed to the small hyperpolarization observed. However, increasing extracellular Na$^+$ in hyperosmolar solutions should increase the transmembrane Na$^+$ gradient, thereby increasing flux through voltage-dependent Na$^+$ channels. This could account for the consistent depolarization observed with increased external NaCl. In this

tion failure by affecting nodal Na$^+$ currents in A-fibers than its effect in C-fibers. Second, action potentials in C-fibers are mediated to a large extent by different species of Na$^+$ channels than those in A-fibers (Blair and Bean 2002). These TTX-resistant (TTX$_R$) Na$^+$ channels have markedly slower kinetics and somewhat different voltage dependence than the TTX-sensitive (TTX$_S$) Na$^+$ channels (Elliott and Elliott 1993; Kostyuk et al. 1981). When hyperosmolar solutions reduce the axonal Na$^+$ gradient or increase the resistance to axoplasmic current flow, the long-duration currents mediated by the TTX$_R$ channels would be expected to allow for continued propagation of the action potential, whereas the fast-inactivating TTX$_S$ currents in A-fibers make them more susceptible to spike block.


![Image](image79x421to278x721)

**TABLE 1. Membrane effects of hyperosmolar solutions on A-type DRG neurons**

<table>
<thead>
<tr>
<th>Membrane Effect</th>
<th>Control</th>
<th>Glucose</th>
<th>$n$</th>
<th>Control</th>
<th>NaCl</th>
<th>$n$</th>
<th>Control</th>
<th>Sucrose</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting membrane potential (mV)</td>
<td>$-63.5 \pm 2.8$</td>
<td>$-65.5 \pm 2.7^*$</td>
<td>10</td>
<td>$-65.0 \pm 1.6$</td>
<td>$-62.2 \pm 1.5^\dagger$</td>
<td>9</td>
<td>$-63.2 \pm 1.7$</td>
<td>$-64.3 \pm 1.5^*$</td>
<td>11</td>
</tr>
<tr>
<td>Membrane input resistance (M$\Omega$)</td>
<td>15.5 $\pm 2.8$</td>
<td>15.2 $\pm 2.9$</td>
<td>10</td>
<td>15.5 $\pm 3.5$</td>
<td>16.6 $\pm 4.4$</td>
<td>9</td>
<td>21.9 $\pm 5.4$</td>
<td>21.7 $\pm 6.7$</td>
<td>10</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>88.8 $\pm 2.8$</td>
<td>87.1 $\pm 3.5$</td>
<td>8</td>
<td>89.7 $\pm 2.0$</td>
<td>91.0 $\pm 1.9$</td>
<td>6</td>
<td>92.3 $\pm 1.6$</td>
<td>91.3 $\pm 1.6$</td>
<td>11</td>
</tr>
<tr>
<td>Spike 10–90% rise time (ms)</td>
<td>0.06 $\pm 0.01$</td>
<td>0.06 $\pm 0.01$</td>
<td>8</td>
<td>0.06 $\pm 0.01$</td>
<td>0.06 $\pm 0.01$</td>
<td>6</td>
<td>0.06 $\pm 0.01$</td>
<td>0.07 $\pm 0.01$</td>
<td>11</td>
</tr>
<tr>
<td>Spike half-width (ms)</td>
<td>0.15 $\pm 0.03$</td>
<td>0.14 $\pm 0.03$</td>
<td>8</td>
<td>0.14 $\pm 0.02$</td>
<td>0.15 $\pm 0.02$</td>
<td>6</td>
<td>0.15 $\pm 0.03$</td>
<td>0.15 $\pm 0.02$</td>
<td>11</td>
</tr>
<tr>
<td>Conduction velocity (m/s)</td>
<td>16.4 $\pm 1.54$</td>
<td>14.3 $\pm 1.51^*$</td>
<td>8</td>
<td>17.8 $\pm 1.65$</td>
<td>16.3 $\pm 1.83^*$</td>
<td>6</td>
<td>17.8 $\pm 1.92$</td>
<td>16.1 $\pm 1.74^*$</td>
<td>11</td>
</tr>
<tr>
<td>Spike block ($n$)</td>
<td>—</td>
<td>4</td>
<td>8</td>
<td>—</td>
<td>5</td>
<td>6</td>
<td>—</td>
<td>6</td>
<td>11</td>
</tr>
</tbody>
</table>

Values are mean $\pm$ SE. * $P < 0.05$, † $P < 0.001$ compared with control solution by ANOVA with posthoc test. Half-width (ms) is the time at ½ spike amplitude.
context, it is important to note that the selective block of action potential transmission in myelinated fibers was similar in different hyperosmolar solutions, including the one with increased extracellular [Na\(^+\)] (Table 1). Therefore it is possible that increased resistance to axoplasmic current flow plays a more important role in A-fiber conduction block by hyperosmolar solutions than decreases in the Na\(^+/\)H\(^+\) gradients.

In our study, removal of extracellular [Ca\(^{2+}\)] increased CAP peak in isosmolar conditions and prevented the hyperosmolar glucose-induced changes in A- and C-fiber CAP peaks. Na\(^+\) currents may increase after extracellular Ca\(^{2+}\) removal, because Ca\(^{2+}\) ions may act as plugs (gating particles) in Na\(^+\) channels (Moore and Cox 1976). Ca\(^{2+}\) may also alter surface charge density (Hille 1968). Brismar (1973) and others have suggested that Ca\(^{2+}\) ions form a diffuse double layer outside the nerve membrane, which can affect the transmembrane electrical field. Increasing extracellular [Ca\(^{2+}\)] would result in increased transmembrane field strength such that a greater depolarization is needed to reduce the field to evoke an action potential (Junge 1992). Conversely, removing extracellular [Ca\(^{2+}\)] (as in our experiments) would decrease the extracellular field strength thus reducing the amount of current needed to evoke an action potential.

In our attempt to determine the site of action of hyperosmolar solutions, we studied their effects on different isolated components of sensory nerves. Only the isolated spinal nerve with intact sheath was unaffected by the hyperosmolar solutions. The whole-mount DRG preparations, the isolated dorsal roots, and ventral roots showed comparable changes in CAP amplitude. Peripheral nerves are known to possess an epithelial layer with tight junctions, but dorsal roots lack this perineurial epithelium, which also serves as a blood–nerve barrier (Gamble 1964; Shanthaveerappa and Bourne 1966). Somata of DRG neurons also lack a blood-nerve barrier (Shinder and Devor 1994). We found that removal of the epineurium made spinal nerves susceptible to the effects of hyperosmolar glucose solution, thus confirming the protective effects of the epithelial layer. Interestingly, the effects of locally perfusing hyperglycemic solutions to sciatic nerve in vivo occur with a greater delay than after similar local perfusion of the DRG (Dobretsov et al. 2003). Taken together, these data suggest that the spinal nerves are acutely protected from the effects of osmolality in the intact organism by the
epineurium, which delays the equilibration of osmolar differences.

The selective blockade of action potential transmission by hyperosmolar solutions in myelinated fibers has important implications for both signal transmission and signal processing. One consequence of the selective conduction block would be a decrement in innocuous sensation transmitted by large diameter A-fibers. Diabetic patients exhibit considerable defects in innocuous sensation (Winkler et al. 2000). We also observed that hyperosmolar solutions decreased the CAP amplitude recorded from the ventral root (Fig. 5). Since the ventral root CAP represents the evoked discharge of motoneuron axons, it appears that hyperosmolality has similar effects on conduction in different subtypes of myelinated nerve fibers (Gilliatt and Willison 1962; Hylleinmark et al. 1995).

The selective block of conduction in sensory myelinated fibers by hyperosmolar solutions also has implications for nociception. Activation of low-threshold A-fiber afferents is known to produce inhibition of nociceptive dorsal horn neurones and decrease the magnitude of pain (Arezzo 1999; De Koninck and Henry 1992; LaMotte et al. 1992). Conversely, A-fiber conduction block that eliminates cold sensibility produces a sensation of burning pain (Ochoa and Yarnitsky 1994; Wahren et al. 1989).

Based on this evidence, we hypothesize that the changes in the osmolality of plasma and extracellular fluids may contribute to the chronic pain symptoms of diabetic neuropathy. Our contention is supported by the recent finding that chronic in vivo perfusion of the L5 rat DRG with a hyperglycemic solution produces mechanical hyperalgesia in transgenic diabetic mice: restoration of C-fiber function with human recombinant nerve growth factor. Diabetes 47: 1637–1642, 1998.


