Serum From Diabetic BB/W Rats Enhances Calcium Currents in Primary Sensory Neurons

HELEN RISTIC, 1 SHANTHI SRINIVASAN, 1 KAREN E. HALL, 1 ANDERS A. F. SIMA, 2 AND JOHN W. WILEY 1

1 Department of Internal Medicine and 2 Department of Pathology, University of Michigan Medical Center and the Ann Arbor Veterans Affairs Medical Center, Ann Arbor, Michigan 48105

Ristic, Helen, Shanthi Srinivasan, Karen E. Hall, Anders A. F. Sima, and John W. Wiley. Serum from diabetic BB/W rats enhances calcium currents in primary sensory neurons. J. Neurophysiol. 80: 1236–1244, 1998. We examined the hypothesis that exposure of nondiabetic rat dorsal root ganglion (DRG) neurons to sera from diabetic BB/W rats would produce an increase in calcium currents associated with impaired regulation of the inhibitory G protein–calcium channel complex. Acutely dissociated rat DRGs were incubated for 18–24 h in medium supplemented with sera (10% vol/vol) from either diabetic rats with neuropathy or age-matched, nondiabetic controls. Exposure of DRG neurons to sera from diabetic BB/W rats resulted in a surface membrane immunofluorescence pattern when treated with an anti-rat light-chain antibody that was not observed in neurons exposed to control sera.

Calcium current density (I_{Ca}) was assessed with the use of the whole cell variation of the patch-clamp technique. I_{Ca} in neurons exposed to diabetic sera was significantly increased compared with neurons exposed to control sera. Guanine nucleotide-binding (G) protein regulation of calcium channel function was examined with the use of a two-pulse “facilitation” or I_{Ca} enhancement protocol in the presence of activators [guanosine 5′-O-(3-thiotriphosphate) (GTPyS)] or antagonists [guanosine 5′-O-(2-thiodiphosphate) (GDPβS)] and pertussis toxin (PTX) of G protein function. Facilitation was significantly decreased in neurons exposed to diabetic sera. Intracellular diffusion of neurons with GDPβS blocked facilitation, whereas dialysis with GTPyS increased facilitation to a similar magnitude in neurons exposed to either diabetic or control sera. Treatment with PTX resulted in a significant increase in I_{Ca} and ~50% decrease in facilitation in neurons treated with control sera but no significant changes in neurons exposed to diabetic sera. We conclude that serum from diabetic BB/W rats with neuropathy contains an autoimmune immunoglobulin that impairs regulation of the inhibitory G protein–calcium channel complex, resulting in enhanced calcium influx. Regulation of the inhibitory G protein–calcium channel complex involves PTX-sensitive and -insensitive G proteins.

**METHODS**

**Animal model**

Previous approval for these experiments was obtained from the University of Michigan Committee on Use and Care of Animals according to National Institutes of Health (NIH) guidelines. Plasma was obtained from two groups of 9-mo-old male BB/W-rats, nondiabetic controls and diabetic rats treated with small daily injections of insulin. Control and prediabetic BB/W rats were obtained from the NIH-sponsored colony at the University of Massachusetts (Worcester, MA). After the onset of diabetes, rats were injected daily with protamine–zinc insulin (0.2–2.5 IU/dl, Eli Lilly). Diabetic rats were maintained at hyperglycemic blood glucose levels between 16 and 25 mM/dl (300–450 mg/dl). Body weight, urinary glucose, and ketone bodies were monitored daily, and the insulin dose was titrated accordingly. Sural nerve conduction velocity was monitored monthly as described previously (Sima et al. 1990). Neuropathy was confirmed in diabetic rats as a decrease in nerve conduction velocity compared with controls [43.8 ± 0.6 vs. 59.1 ± 0.7 (SE)/ms, *P* < 0.001]. Blood was drawn from the rats at the time of their killing. Plasma was separated...
from cells by centrifugation, heated for 30 min at 56°C to inactivate complement, and frozen in aliquots at −70°C.

**Cell dissociation**

Isolated, acutely dissociated DRG neurons were aseptically prepared from 3- to 6-wk-old Sprague-Dawley rats according to methods described previously (Hall et al. 1995b). After killing by inhalation of 100% CO₂, the spinal column was removed, and thoracic DRGs were extracted. DRGs were trimmed, minced, incubated with 0.3% collagenase and 0.1% trypsin, and then triturated and centrifuged. Enzymes and incubating media were composed of minimal essential medium (MEM) supplemented with 16 mM NaHCO₃ and 28 mM D-glucose and filtered (0.2 μm; Millipore, Gelman, Ann Arbor, MI). All reagents were obtained from Sigma (Sigma Chemical, St. Louis, MO) unless stated otherwise. Isolated DRGs were resuspended in MEM supplemented with 10% (vol/vol) diabetic or control sera, obtained from the rats described above, and plated on collagen-coated tissue culture dishes. No other sera or growth factors were added to the culture media. Cells were incubated in 93% O₂-7% CO₂ at 37°C for 24 h.

**Drug preparation**

Dynorphin A (Dyn A) was made up in external recording solution containing 0.1% bovine serum albumin to a final concentration of 1 μM and kept at 4°C. Guanosine 5’-O-(2-thiodiphosphate) (GDPβS; 500 μM) and guanosine 5’-O-(3-thiotriphosphate) (GTPγS; 500 μM) were made up in internal recording solution. Pertussis toxin (PTX; 150 ng/ml) was added to DRG incubating media 24 h before electrophysiological recording and was also added to the external recording solution during recording.

**Statistical analysis**

Pixel densities obtained by analysis of individual neurons were compared between diabetic and control sera exposure by using unpaired, two-tailed Student’s t-test, with significance defined as \( P < 0.05 \). Results are presented as means ± SE.

### Table 1. Nerve conduction velocity was significantly decreased in diabetic BB/W rats

<table>
<thead>
<tr>
<th></th>
<th>Body Weight</th>
<th>Age</th>
<th>Duration DM</th>
<th>Blood Glucose</th>
<th>Glycated Hb</th>
<th>NCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic (n = 12)</td>
<td>343 ± 24*</td>
<td>9.5 ± 0.3</td>
<td>7.0 ± 0.2</td>
<td>352 ± 58†</td>
<td>12 ± 0.9*</td>
<td>44 ± 2.1*</td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>480 ± 35</td>
<td>9.2 ± 0.2</td>
<td>0</td>
<td>91 ± 6.3</td>
<td>5.1 ± 0.7</td>
<td>59 ± 2.2</td>
</tr>
</tbody>
</table>

Body weight (g), age (months), duration of diabetes (DM, months), blood glucose (mg dl⁻¹), glycated hemoglobin (mg dl⁻¹), and nerve conduction velocity (NCV, ms⁻¹) in nondiabetic control and diabetic BB/W rats. * \( P < 0.05 \), † \( P < 0.01 \) by ANOVA.
Whole cell voltage-clamp recordings

Isolated, phase-bright DRG cells 20–40 μm in diameter were identified, and whole cell voltage-clamp recordings were made at room temperature with the whole cell variant of the patch clamp technique (Hamill et al. 1981). Glass recording patch pipettes (Microhematocrit tubes; Fisher Scientific, Pittsburgh, PA) with electrode resistance 1–2 MΩ and seal resistances over 1 GΩ were used. Recordings were performed in culture dishes containing non-perfused external bath solution consisting of 5 mM CaCl₂, 67 mM choline Cl, 100 mM tetraethylammonium Cl, 5.6 mM glucose, 5.3 mM KCl, 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), and 0.8 mM Mg²⁺ Cl (pH 7.4, 320–330 mosmol). Recording electrodes were filled with 140 mM CsCl, 10 mM HEPES, 10 mM ethylene glycol-bis(β-aminoethyl ether)-N,N’,N’’,N’’-tetraacetic acid, 5 mM Mg²⁺ adenosine triphosphate, and 0.1 mM Li guanosine 5’-triphosphate (pH 7.3–7.4, 280–290 mosmol). Sodium and potassium currents were negligible under these recording conditions. Some studies were performed substituting barium chloride (5 mM) for calcium chloride as the charge carrier. DRGs were depolarized with voltage steps generated by the program Clampex (pClamp, Axon Instruments, Foster City, CA). Calcium currents were recorded with an Axopatch 200A amplifier with an input resistance of 1–3 MΩ, filtered with a Bessel filter 10 kHz, sampled at 20 kHz, and stored on hard disk. High-threshold currents were evoked by 100-ms duration depolarizations from a holding potential (V_h) of −80 mV to a clamp potential (V_c) of +10 mV every 30 s. The current-voltage (I/V) relationship was evaluated by depolarizing neurons from a V_c of −80 mV in 10-mV steps to V_c of −110 to +60 mV for 100 ms every 5 s to examine the full range of activation for calcium currents (from low to high threshold). Voltage-dependent steady-state inactivation was also evaluated. Currents were elicited by 100-ms depolarizations to a V_c of +10 mV from progressively more positive V_h (−100 to 0 mV in +10-mV increments every 15 s). Peak current amplitude at each V_h was plotted as a function of V_h and fitted with a Boltzmann curve generated with the following formula

\[ I_{\text{amp}}/I_{\text{max}} = 1/1 + \exp((V_h - V_{1/2})/k) \]

where \( I_{\text{amp}} \) is peak inward current at holding potential \( V_h \), \( I_{\text{max}} \) is the maximum peak inward current elicited, \( V_{1/2} \) is holding potential in millivolts, \( V_h \) is the voltage at which half-maximal inactivation occurred, and \( k \) is the slope.

A two-step (prepulse/test-pulse) facilitation protocol was employed to modulate G-protein activity (Ikeda 1991). The current elicited with a test-pulse depolarization from \( V_h = -80 \) mV to \( V_c = +10 \) mV was enhanced (facilitated) when preceded by a strong depolarizing prepulse from \( V_h = -80 \) mV to \( V_c = +80 \) mV. We examined the effect of GDP/βS, GTPγS, and PTX on the facilitation response. This facilitation protocol represents the optimal parameters (e.g., interpulse duration and magnitude and duration of prepulse depolarization and test pulse) that maximized the facilitation response.

Drugs were prepared in either external bath solution (PTX), diffused into cells via internal recording solution (PTX, GDP/βS, and GTPγS), or applied acutely (Dyn A) for 2–3 s with pressure ejection from a glass micropipette (10- to 40-μm tip diameter). The micropipette was positioned one to two cell diameters away from the neuron. Dyn A was applied once calcium currents reached a stable amplitude, which typically occurred 3–6 min after patch rupture. After application of drug, Ca currents were evoked within 2–3 s and then every 30 s until recovery to preapplication current amplitudes was observed.

Analysis of current components

Whole cell calcium currents were normalized to control for differences in the size of the cell body by dividing the current amplitude by whole cell capacitance. Capacitance traces were elicited by small depolarizing voltage pulses (+5 mV for 12 s) from \( V_h = -80 \) mV. Whole cell capacitance (proportional to surface area) was calculated from the formula \( C = A/V \), where \( C \) = capacitance (pF), \( A \) = area under the capacitance curve from the peak inward current to the point at which \( DCa = 0 \) (pA/mV), and \( V \) = calibration voltage step (mV). Peak inward currents were divided by cell capacitance, and the normalized calcium current density (\( I_{DCa} \)) was expressed in units of pA/pF.

Statistical analysis

Peak calcium current densities obtained by analysis of individual recordings were compared after exposure to diabetic and control sera with the use of the unpaired, two-tailed Student’s t-test and significance defined as \( P < 0.05 \). The effect of Dyn A was de-
scribed as the percentage change in the peak calcium current after application of Dyn A compared with the peak current before peptide application. Results are presented as means ± SE.

RESULTS

Diabetic rats demonstrated alterations in functional and physiological parameters

Diabetic BB/W rats had elevated blood glucose levels (352 ± 58 mg/dl) and percent glycated hemoglobin (12.0 ± 0.9%) compared with age-matched control BB/W rats (91 ± 6.3 mg/dl and 5.1 ± 0.7%, respectively; \( P < 0.01 \), \( n = 24 \) neurons; Table 1). At the time of killing duration of diabetes was 7.0 ± 0.2 mo. Diabetic rats significantly reduced body weights (343 ± 24 g) compared with control rats (480 ± 35 g, \( P < 0.01 \)). Nerve conduction velocity was significantly impaired in diabetic rats (44 ± 2.1 ms\(^{-1}\)) compared with control rats (59 ± 2.2 ms\(^{-1}\), \( P < 0.05 \)).

Sera from diabetic BB/W rats contained an autoimmune immunoglobulin directed against an antigenic site on the surface membrane of DRG neurons

We screened for the presence of autoimmune immunoglobulin(s) in sera from diabetic BB/W rats with a fluorescein-tagged anti-rat light-chain antibody. This antiserum recognizes all immunoglobulins. Sera obtained from six controls and six diabetic rats were evaluated. Neurons exposed to sera from diabetic rats demonstrated a characteristic surface membrane immunofluorescence pattern (Fig. 1B, pixel density 275 ± 110, \( n = 28 \)) that was markedly less intense in neurons exposed to control sera (Fig. 1A, pixel density 93 ± 52, \( n = 28 \), \( P < 0.05 \)).

DRG neurons demonstrated enhanced calcium current density when exposed to sera from diabetic rats

We did not observe any significant difference in resting membrane conductance after exposure to diabetic or control

![Graphs showing calcium current density](http://jn.physiology.org)
sera under our recording conditions. Preliminary studies examining the effect of short-duration (5–20 min) exposure of neurons to sera from control and diabetic rats on calcium currents demonstrated inconsistent results. There was a trend toward enhancement of calcium currents in neurons exposed to sera from diabetic rats compared with control sera. Therefore we focused on examining calcium currents in neurons after exposure to control and diabetic sera for 18–24 h. Calcium currents were elicited in neurons exposed to culture medium supplemented with complement-inactivated sera (10% vol/vol) from either diabetic or control BB/W rats as described previously. Representative current tracings are depicted in Fig. 2A. Calcium current ‘run-down’ during the recording period was similar in neurons treated with sera from either control or diabetic animals. Calcium current density \( I_{DCa} \) was significantly greater in DRG neurons exposed to diabetic sera \((141.1 \pm 13.4 \text{ pA/pF}, n = 15)\) compared with neurons exposed to control sera \((70.2 \pm 8.6 \text{ pA/pF}, n = 20; P < 0.05, \text{Fig. 2B})\). Therefore the increase in calcium currents observed after exposure to diabetic serum could not be explained on the basis of differences in the size of the neurons.

**Low- and high-threshold calcium currents were enhanced after exposure to sera from diabetic BB/W rats**

Both low- and high-threshold calcium currents were enhanced by exposure to diabetic sera (Fig. 3). The low-threshold current component was activated at clamp potentials more positive than \(-60 \text{ mV}\) and peaked at clamp potentials between \(-30\) and \(-20 \text{ mV}\) (Fig. 3A). The peak \( I_{DCa} \) for low-threshold currents was significantly larger in neurons treated with diabetic sera \((108 \pm 24 \text{ pA/pF}, n = 6)\) compared with neurons treated with control sera \((28 \pm 10 \text{ pA/pF}, n = 4; P = 0.013, \text{Fig. 3B})\). The high-threshold components of the calcium currents activated at membrane potentials more positive than \(-20 \text{ mV}\) and peaked at membrane potentials between \(0\) and \(+10 \text{ mV}\). The low-threshold calcium currents were inactivated at clamp potentials between 0 and \(+10 \text{ mV}\) and therefore did not contribute to the peak of the high-threshold calcium currents. The voltage range for activation of calcium currents and the voltage at which the peak amplitude in the current-voltage relationship were observed were the same for DRG neurons exposed to either diabetic or control sera (Fig. 3C). Therefore exposure to serum from diabetic BB/W rats increased low- and high-threshold calcium currents via a pathway that did not involve shifts in the peak of the current-voltage relationships.

Voltage-dependent steady-state inactivation of high-threshold calcium currents was the same for DRG neurons exposed to either diabetic or control sera (Fig. 3D). Steady-state inactivation curves were fitted with a Boltzmann equation as described in METHODS, with values for \( V_{1/2} \) and \( k \) of \(-42.2\) and \(14.20 \text{ mV}\) for the control curve \((n = 7)\) and \(-41.4\) and \(13.65 \text{ mV}\) for the diabetic curve \((n = 7)\). Therefore diabetic serum-mediated increase in calcium currents was not associated with a significant shift in either the peak of the current-voltage relationship or voltage dependence of steady-state inactivation.

**G protein–dependent calcium current facilitation was decreased in DRG neurons exposed to diabetic sera**

High-threshold calcium currents were augmented (facilitated) when activation was preceded by a large depolarizing prepulse \((V_{h} = -80 \text{ mV at } V_{c} = +80 \text{ mV for 50 ms})\). 15 ms before the test pulse \((V_{c} = -80 \text{ mV at } V_{t} = +10 \text{ mV, Fig. 4A})\). When neurons were exposed to diabetic sera, calcium current facilitation was significantly decreased \((18.8 \pm 3.3\%, n = 14)\) compared with the facilitation response after exposure to control sera \((28.3 \pm 2.9\%, n = 17, P < 0.05)\), as shown in Fig. 4B. Facilitation of calcium currents was increased or facilitated test pulse \((-41.4\) and \(13.65 \text{ mV}\) for the diabetic curve \((\text{Fig. 4A})\). Therefore exposure to sera from diabetic rats.
abolished in neurons exposed to either control or diabetic sera ≤20 min after patch rupture when the internal (recording electrode) buffer was supplemented with GDPβS (500 μM, n = 6). In contrast, the facilitation response increased to 43 ± 8% (control sera) and 46 ± 6% (diabetic sera) (not significantly different) after 20 min of dialysis with internal buffer containing GTPγS (500 μM, n = 8). Therefore exposure of nondiabetic DRG neurons to diabetic sera for 18–24 h produced a significant increase in calcium but not diabetic sera. Therefore exposure of nondiabetic DRG neurons to diabetic sera produced a significant increase in calcium currents (Fig. 5A). In contrast, we found no significant difference in calcium currents with those exposed to diabetic sera and vehicle [PTX (n = 7) and vehicle (n = 15)].

Treatment with PTX enhanced calcium currents and decreased facilitation in DRG neurons exposed to normal but not diabetic sera

Incubation with PTX (150 ng/ml) for 18–24 h enhanced calcium current density in DRG neurons exposed to control sera from 70 ± 9 pA/pF (vehicle treated, n = 20) to 101 ± 10 pA/pF (PTX treated, n = 8, P < 0.05) and decreased facilitation (from 28 ± 3% to 13.5 ± 4%, P < 0.05, Fig. 5). Of interest, PTX treatment had no significant effect on neurons exposed to diabetic sera on either calcium current amplitude, increased from 141 ± 13 pA/pF (vehicle treated, n = 15) to 154 ± 21 pA/pF (PTX treated, n = 7, not significant), or facilitation response (decreased from 19.8 ± 4% to 17.2 ± 3%, n = 7, not significant). To confirm that PTX-sensitive G proteins were maximally inhibited, we evaluated the ability of the κ-opioid receptor agonist Dyn A to inhibit calcium currents after treatment with PTX. Dyn A inhibits calcium currents by activation of PTX-sensitive (Gt-type) G proteins in DRG neurons (Wiley et al. 1997). In untreated neurons, Dyn A (1 μM) decreased the peak of high-threshold calcium currents evoked from V0.1 = −80 mV at Vh = +10 mV by 38 ± 5% (n = 8). Dyn A-mediated reduction of calcium currents decreased to 5 ± 2.8% in neurons treated with PTX (150 ng/ml) for 18–24 h (n = 6). Therefore PTX-mediated inhibition of Gt-type G proteins was maximal under these conditions. In summary, PTX-sensitive and -insensitive G proteins contribute to tonic inhibitory regulation of voltage-activated calcium channels in DRG neurons and PTX had a differential effect on neurons treated with either control sera or diabetic sera.

**DISCUSSION**

These studies demonstrate that serum from diabetic BB/W rats with neuropathy contains an autoimmune immunoglobulin that enhances calcium influx in nondiabetic primary sensory (DRG) neurons. The molecular basis for the observed increase in calcium current density may involve impaired regulation of the inhibitory G protein–calcium channel complex. The enhancement in calcium current density involved low- and high-threshold currents. This finding was similar to that observed with acutely dissociated neurons from diabetic BB/W rats in which N-, L-, and T-type calcium currents were increased in comparison with nondiabetic controls (Hall et al. 1995b). Enhancement of multiple calcium currents in diabetes provides an explanation for the limited improvement in nerve conduction velocity observed in diabetic rats with neuropathy after treatment with selective L-type calcium channel blockers (Kappelle et al. 1992; Ristic et al. 1996).

We employed a novel approach, i.e., prepulse facilitation, to determine if the serum-mediated increase in calcium current density was associated with impaired regulation of the inhibitory G protein–calcium channel complex. When large amplitude depolarizing prepulses are delivered within milli-
increase in This observation, along with the absence of a significant decrease in facilitation observed after treating nondiabetic cellular diffusion with GTP-g exposed to control sera supplemented with PTX (Fig. 5A). Similar enhancement of facilitation (Ewald et al. 1988; Moises et al. 1994) occurs observed after exposure to diabetic sera. The results demonstrate abolition of facilitation. This result suggests that the facilitation response involves reversal of calcium-dependent inactivation of calcium channels. However, if reversal of calcium-dependent inactivation of calcium channels was the sole mechanism involved in the facilitation response, we would expect that the increase in calcium current density observed after treatment of neurons with diabetic sera would be associated with greater calcium-dependent inactivation and therefore a larger facilitation response. In fact, the facilitation response was smaller in neurons treated with diabetic sera compared with control sera. We believe that this suggests additional mechanisms (besides reversal of calcium-dependent inactivation of calcium channels) are involved in modulating the facilitation response. We propose that the mechanism(s) underlying the increase in calcium influx and decrease in facilitation observed in neurons treated with diabetic sera involves impaired tonic inhibitory G protein regulation of calcium channels. Our study also suggests that both PTX-sensitive and PTX-insensitive G proteins appear to be involved in tonic inhibition of voltage-activated calcium channels in DRGs, in contrast to PTX-sensitive inhibition of calcium currents by opioid receptor agonists (Gross et al. 1990; Moises et al. 1994; Wiley et al. 1997). We observed a significant increase in I_{DCa} in PTX-treated neurons exposed to control sera, presumably through blocking activation of PTX-sensitive inhibitory G proteins coupled to calcium channels. In neurons exposed to diabetic serum supplemented with PTX, there was no significant increase in I_{DCa}, compared with that observed after exposure to diabetic serum alone. However, the increase in I_{DCa} observed after exposure to diabetic serum alone was significantly greater than the I_{DCa} observed in neurons exposed to control sera supplemented with PTX (Fig. 5A). This observation, along with the absence of a significant increase in I_{DCa} in neurons exposed to diabetic sera supplemented with PTX, suggests that exposure to diabetic serum affected both the PTX-sensitive and -insensitive populations of inhibitory G proteins regulating calcium channels in DRGs. The G protein species mediating tonic inhibition of neuronal calcium channels remain to be characterized, although G_i is a likely candidate for the PTX-sensitive component (Ewald et al. 1988; Moises et al. 1994). DRG neurons exposed to either control or diabetic sera demonstrated similar enhancement of facilitation (~50%) in response to intracellular diffusion with GTP-yS which promotes activation of G proteins. This result suggests that exposure to diabetic serum did not increase the percentage of potentially activatable G protein-coupled calcium channels.

Previous studies examining the regulation of calcium channels suggested that alterations in calcium current amplitude correlated with shifts in the voltage dependence of activation (Bean 1989) or inactivation (Doupnik and Pun 1992; Gross and Macdonald 1989). Either voltage-dependent phosphorylation or dephosphorylation of calcium channels may induce a hyperpolarizing shift in channel gating. However, we observed no significant shifts in either the voltages at which peak calcium currents were observed or the voltage dependence of steady-state inactivation in neurons exposed to diabetic serum compared with control sera. In the absence of data supporting an increase in the number of functional calcium channels in neurons exposed to diabetic sera, it is likely that the increase in calcium current density reflects altered single channel kinetics, such as increased open duration or open frequency, or a change in the unitary conductance. Resolution of these issues will require single channel recordings.

The underlying mechanism for facilitation may involve transient uncoupling of the G protein βγ subunit from the calcium channel (DeWaal et al. 1997; Reuveny et al. 1994; Stanley and Mirotznik 1997). In some systems the facilitation response also appears to involve phosphorylation of either the G protein or calcium channel (Sculptoreanu et al. 1995). Intracellular dialysis with constitutively active protein kinase C (PKC) catalytic subunit enhances calcium currents in DRG neurons (Hall et al. 1995a). Either the calcium channel or the G protein may be phosphorylated. Consensus sites for phosphorylation by PKC are present on the α subunit of several G protein subtypes (Katada et al. 1985). Furthermore, hepatocytes from streptozotocin-induced diabetic rats demonstrated increased protein kinase C but not protein kinase A-mediated phosphorylation of the α subunit of Gβ (Morris et al. 1996), resulting in decreased dissociation of the heterotrimeric G protein complex. Therefore, one explanation for the increased calcium current density observed in diabetes mellitus may involve abnormal phosphorylation of inhibitory G proteins, resulting in impaired dissociation and decreased modulation of calcium channels, culminating in increased calcium currents.

Altered calcium homeostasis resulting in increased basal and/or stimulated cytosolic calcium was documented in several models of diabetes (Biessels and Gispen 1996; Levy et al. 1994) and elevated intracellular calcium was implicated in the pathogenesis of neuropathy (Johnson et al. 1992; Nocentini et al. 1992). The increase in calcium current and decrease in facilitation observed after treating nondiabetic neurons with diabetic serum was similar to what we observed in acutely dissociated DRG neurons from diabetic BB/W rats. Of interest, long-term administration of an aldose reductase inhibitor to diabetic animals prevented the slowing in nerve conduction velocity as well as the increase in calcium current density (Hall et al. 1995b) despite persistent hyperglycemia. It is unknown whether treatment with aldose reductase inhibitors will prevent the increase in calcium currents observed after exposure to diabetic sera. The results of this study comparing the effect of sera (1:10 dilution) from diabetic and nondiabetic rats on neuronal calcium currents provide additional support that hyperglycemia or hy-
permosmolar conditions per se did not directly cause the increase in calcium influx.

Juntti-Berggren et al. (1993) demonstrated enhanced calcium currents (L-type) in pancreatic β-cells exposed to sera from newly diagnosed type 1 diabetic patients. They postulated that the substance in diabetic sera may be an autoimmune immunoglobulin. Pittenger et al. (1993, 1995, 1997) reported that exposure of neuroblastoma cultures to complement-containing sera from human type I diabetics was associated with neuronal injury and apoptosis. The authors hypothesized that a complement-fixing autoimmune immunoglobulin participates in the pathway mediating the injury. We utilized complement-inactivated sera from control and diabetic BB/W rats and observed a surface membrane immunofluorescence pattern in non diabetic DRGs exposed to sera from diabetic BB/W rats that was not observed in DRGs exposed to control sera. Several autoantigens have been proposed as possible sites for the targeting of autoimmune immunoglobulins, including glycolipids (Pittenger 1995), gangliosides (Maeda et al. 1991), and glutamic acid decarboxylase (Baekkeskov et al. 1990; Kaufman et al. 1992). Another potential target for serum factors found in diabetic sera may be cell adhesion molecules of the immunoglobulin superfamily (Seilheimer et al. 1989; Williams and Barclay 1988).

In summary, autoimmune immunoglobulin(s) present in the serum of diabetics may contribute to the altered calcium signaling reported in a variety of cell types (Biessels and Gispen 1996; Juntti-Berggren et al. 1993; Levy et al. 1994; Pittenger et al. 1997). Previous studies reported reduced calcium influx in neurons exposed to serum from patients with the paraneoplastic Lambert-Eaton syndrome (Lennon et al. 1995). This study is the first that we are aware of supporting the theory that in a disease state, serum-mediated impaired regulation of the inhibitory G protein–calcium channel complex results in enhanced calcium influx.

We thank B. Dzwonek, H. Sheng, and A. Merry for expert technical assistance.

These studies were supported by a Michigan Peptide Research Center Pilot Feasibility Award and Juvenile Diabetes International Research Award 195036 to K. E. Hall, National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-43884 to A.A.F. Sima and DK-45820 to J. W. Wiley, and a Veterans Affairs Merit Award and University of Michigan Diabetes Research and Training Center Pilot Feasibility Award to J. W. Wiley.

Present address for A.A.F. Sima: Dept. of Pathology and Neurology, Wayne State University Medical School, Detroit, MI 48202.

Address for reprint requests: J. W. Wiley, Veterans Affairs Medical Center, 2215 Fuller Rd., Rm. B501a, Box 111D, Ann Arbor, MI 48105.

Received 28 October 1997; accepted in final form 3 June 1998.

REFERENCES


