Synaptic transmission at parasympathetic neurons of the major pelvic ganglion from normal and diabetic male mice

John D. Tompkins, Margaret A. Vizzard, and Rodney L. Parsons
Department of Neurological Sciences, University of Vermont College of Medicine, Burlington, Vermont

Submitted 27 April 2012; accepted in final form 27 November 2012

Tompkins JD, Vizzard MA, Parsons RL. Synaptic transmission at parasympathetic neurons of the major pelvic ganglion from normal and diabetic male mice. J Neurophysiol 109: 988–995, 2013. First published November 28, 2012; doi:10.1152/jn.00354.2012.—Bladder and erectile dysfunction are common urologic complications of diabetes and are associated with reduced parasympathetic autonomic control. To determine whether disruption of ganglionic neurotransmission contributes to the loss of function, we investigated synaptic transmission at parasympathetic, major pelvic ganglion (MPG) neurons in control and chronically (20 wk) diabetic mice. In contrast to what has been reported for sympathetic neurons, diabetes did not cause an interruption of synaptic transmission at parasympathetic MPG neurons from streptozotocin-treated C57BL/6J (STZ) or db/db mice. Cholinergically mediated excitatory postsynaptic potentials (EPSPs) were suprathreshold during 5-s trains of 5-, 10-, and 20-Hz stimuli. Asynchronous neurotransmitter release, observed as miniature EPSPs (mEPSPs) during and after stimulation, permitted quantitative assessment of postganglionic, cholinergic receptor sensitivity. mEPSP amplitude following tetanic stimulation (recorded at −60 mV) was reduced in STZ (4.95 ± 0.4 vs. 3.71 ± 0.3 mV, P = 0.03), but not db/db mice. The number of posttetanic mEPSPs was significantly greater in db/db mice at all frequencies tested. Assessment of basic electrophysiological properties revealed that parasympathetic MPG neurons from db/db mice had less negative membrane potentials, lower input resistances, and shorter afterhyperpolarizations relative to their control. MPG neurons from STZ had longer afterhyperpolarizations but were otherwise similar to controls. Membrane excitability, measured by the membrane responsiveness to long-duration (1 s), suprathreshold depolarizing pulses, was unchanged in either model. The present study indicates that, while parasympathetic neurotransmission at the MPG is intact in chronically diabetic mice, obese, type 2 diabetic animals exhibit an altered presynaptic regulation of neurotransmitter release.

diabetes mellitus; experimental; diabetic autonomic neuropathy; autonomic ganglia; synaptic transmission; electrophysiology

DERANGEMENTS IN METABOLIC function associated with type 1 and type 2 diabetes mellitus cause progressive damage to sympathetic and parasympathetic nerves of the autonomic nervous system. The resulting diabetic autonomic neuropathy (DAN) compromises autonomic regulation of visceral function and contributes to the greater morbidity and mortality with diabetes (Freeman 2005; Tesfaye et al. 2010; Vinik et al. 2003). Cardiovascular, genitourinary, gastrointestinal, and sudomotor systems can be compromised and, among many symptoms, may cause arrhythmias, sudden cardiac death, bladder dysfunction, sexual dysfunction, constipation, gastroparesis, and anhidrosis (Freeman 2005; Tesfaye et al. 2010; Vinik et al. 2003).

Bladder and erectile dysfunction (ED) are highly prevalent in the diabetic population, with more than one-half of all patients developing symptoms over time (Brown et al. 2005; Chitaley et al. 2009; Ellenberg 1982; Sasaki et al. 2003). DAN is thought to be a key pathological mechanism associated with both disorders (Freeman 2005; Tesfaye et al. 2010; Vinik et al. 2003). Patients with diabetic bladder dysfunction typically present with an enlarged, hypoesensate bladder showing impaired contractility and elevated postvoid residual volumes which contributes to incontinence and urinary tract infections (Danesghari et al. 2009; Frimodt-Moller 1978; Nanigian et al. 2007). With ED, patients lose the ability to develop or maintain an erection necessary for sexual intercourse. A loss in parasympathetic motor function is associated with both disorders (Chitaley 2009; Danesghari et al. 2009; Frimodt-Moller 1978; Sasaki et al. 2003). Activation of the cholinergic parasympathetic postganglionic fibers causes contraction of the detrusor smooth muscle in the urinary bladder and vasodilatation of the corpus cavernosum of the penis.

While the clinical correlates of DAN are commonly observed, the pathophysiological underpinnings are poorly understood. A growing body of evidence suggests that disruption of ganglionic function contributes to dysautonomia with diabetes. Dystrophic nerve terminals are observed in paravertebral sympathetic ganglia from diabetic human cadaveric tissue (Schmidt et al. 1993), and synaptic transmission through the superior cervical ganglion (SCG), a sympathetic ganglion, is depressed in type 1 and type 2 diabetic mice (Campanucci et al. 2010). The depression in synaptic activity is associated with a reduction of transmitter sensitivity at postsynaptic nicotinic acetylcholine receptors, an alteration suggested to result from oxidative stress caused by hyperglycemia (Campanucci et al. 2010). Whether diabetes causes similar dysfunction at parasympathetic ganglia has not been studied extensively.

In rats and mice, the major pelvic ganglion (MPG) supply both sympathetic and parasympathetic postganglionic innervation to the urogenital organs and lower bowel (Jobling and Lim 2008; Keast 1999; Wanigasekara et al. 2003). The preganglionic parasympathetic fibers originate from sacral segments of the intermediolateral column and project to postganglionic MPG neurons via the pelvic nerve (Keast 2006). Preganglionic fibers synapse directly on the cell bodies of the ganglion neurons (Jobling and Lim 2008; Rogers et al. 1990; Wanigasekara et al. 2003). To determine whether a depression in ganglionic neurotransmission contributes to the loss of parasympathetic tone to the urogenital organs with diabetes, we evaluated the electrophysiological properties of parasympa-
thetic neurons of the MPG in control and chronically diabetic adult male mice. Models for type 1 [streptozotocin (STZ)-treated C57BL/6J] and type 2 (db/db mice) diabetes were used. Several alterations in both the active and passive membrane properties of the parasympathetic MPG neurons were observed; however, synthetically evoked potentials were virtually always suprathermal and generated an action potential. In the obese, type 2 diabetic mice, a significant increase in asynchronous neurotransmitter release during and after nerve stimulation was observed. This is the first report of the analysis of synaptic transmission at parasympathetic postganglionic neurons in the MPG from control and diabetic male mice. Two important conclusions are reached: 1) parasympathetic neurotransmission is not depressed at parasympathetic postganglionic neurons in MPG from chronically diabetic mice; and 2) there is a dysregulation of presynaptic neurotransmitter release in db/db mice.

MATERIALS AND METHODS

All experiments were done in vitro using MPG taken from control and diabetic male mice at 26–28 wk of age. Mice were housed (3 per cage) in the University’s animal care facility (12:12-h light-dark cycles) with free access to food (Prolab Isopro RMH 3000, PMI Nutrition International) and water. All experimental procedures were approved by the University of Vermont Institutional Animal Care and Use Committee and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Diabetic models. Male mouse models of both type 1 and type 2 diabetes were studied. Type 1 diabetes was induced in male C57BL/6J mice at 6 wk using the low-dose STZ induction protocol published by the Animal Models of Diabetes Complications Consortium (Brosius 2011). Mice were obtained from Jackson Laboratories (Bar Harbor, ME) at 5 wk of age and received intraperitoneal injections of STZ (50 mg/kg; Sigma-Aldrich, St. Louis, MO) once a day for 5 days. STZ was prepared daily in Na-citrate buffer (4.5 pH). Only animals with persistently elevated blood glucose levels greater than 500 mg/dl after 2 wk before the MPG were used as controls. Sham-treated animals received injections of buffer alone. Data obtained from each control group were similar, and, therefore, the reported control data for STZ-treated mice has been combined and is referred to as “BL6” throughout the text.

For a model of type 2 diabetes, mice homozygous for the diabetes spontaneous mutation (Lepr<sup>db</sup>) were used (db/db) (BKS.Cg-Dock7<sup>m</sup> <i>+/+</i> Lepr<sup>db</sup>/J, Jackson Laboratories, Bar Harbor, ME). Db/db mice were identifiable obese and had significantly elevated blood glucose levels (≥600 mg/dl) at 5 wk of age. For comparison, littermate BKS mice homozygous for the “misty” allele (Dock7<sup>m</sup> <i>++/+</i>, Jackson Laboratories) were used as controls (referred to throughout text as “BKS”).

All animals were observed regularly to track the development of hyperglycemia and to monitor the animal’s weight and health. Blood glucose concentrations were measured with an over-the-counter blood glucose meter (Onetouch Ultra; Johnson and Johnson, New Jersey) and were measured at the same time each day.

Intracellular recording. MPG were isolated from male mice following euthanasia with isoﬂurane (4%) and thoracotomy. The isolated ganglia were pinned to a Sylgard-coated (Dow Corning, Midland, MI) floor of a glass-bottom recording chamber. Ganglia were superfused (2–3 ml/min) with a physiological salt solution containing the following (in mM): 121 NaCl, 5.9 KCl, 2.5 CaCl<sub>2</sub>, 1.2 MgCl<sub>2</sub>, 8 glucose, and 26 HEPES, pH = 7.35 with continuous carbogen (95% O<sub>2</sub>/5% CO<sub>2</sub>) aeration. The temperature of the superfusate was maintained between 30 and 32°C at the chamber with a thermostatically controlled inline heater (Warner Instruments, Hamden, CT). Neurons were visualized with an upright microscope and impaled with high-impedance borosilicate glass microelectrodes (2 M KCl-filled; 80–120 MΩ). Active and passive membrane properties as well as the synaptic response to pelvic nerve stimulation were recorded from impaled neurons using an Axoclamp-2A amplifier coupled with a Digidata 1322A data acquisition system and pCLAMP 8 software (Molecular Devices, Sunnyvale, CA).

Analysis of pelvic nerve-evoked responses. Responses of postganglionic neurons to pelvic nerve stimulation were elicited by focal stimulation of the pelvic nerve with concentric bipolar electrodes (FHC, Bowdoin, ME). Stimulus currents (1–3 ms duration, 10–500 mA) were delivered with a PSI6 constant-current stimulus isolation unit (Astro-Med, West Warwick, RI) controlled by a Grass S88 stimulator. Synaptic responses to single and repetitive stimuli were measured. Following tetanic stimulation of the pelvic nerve, hyperpolarizing current was injected through the recording electrode to electrotonically maintain the resting membrane potential (RMP) of the MPG neurons at ~−60 mV. This allowed miniature excitatory post-synaptic potential (mEPSP) amplitudes to be compared at the same membrane potential in different cells. The amplitude and frequency of the mEPSPs were analyzed with Mini Analysis software (Synaptosoft, Decatur, GA).

Analysis of active and passive membrane properties. The active and passive cell membrane properties of the parasympathetic postganglionic MPG neurons were characterized using previously published techniques (Tomkins et al. 2010). Only cells with a stable RMP, an input resistance greater than 60 MΩ, and an overshooting action potential were included in the analysis. Afterhyperpolarization (AHP) amplitude was measured as the difference between the RMP and the nadir of the AHP. AHP duration was measured at two-thirds peak amplitude. Input resistance and capacitance measurements were made from hyperpolarizing current pulses (~100 pA).

Neuron excitability phenotype was characterized based on the number of action potentials generated during a series of 1,000-ms-duration depolarizing current pulses of increasing amplitude (0.1–0.4 nA). Cells only firing one or two action potentials were classified as “tonic”, whereas MPG neurons firing three or more action potentials over the course of the depolarizing step were classified as “phasic”.

Statistics. Data are reported as means ± SE. Pairwise comparisons between diabetic animals and their respective controls were made by Student’s t-test. Means of three or more unmatched groups were compared with a one-way ANOVA. The proportion of neuronal phenotypes (phasic vs. tonic) was compared using Fisher’s exact test. P values ≤0.05 were considered significant.

RESULTS

Blood glucose and body weight values in mice with prolonged diabetes. Blood glucose levels and body weight were monitored as diabetes progressed in both animal models. STZ-treated and db/db mice were hyperglycemic (blood glucose ≥300 mg/dl) for a period of ~20 wk before the MPG were isolated for electrophysiological experimentation. Db/db mice had persistently elevated blood glucose levels greater than 500 mg/dl from 6 to 26 wk of age. Blood glucose concentrations in STZ-treated mice rose to above 300 mg/dl, 3 wk after treatments were initiated. At the time when the electrophysiological measurements were made, 26 wk of age, blood glucose values in STZ-treated mice were significantly greater than that of BL6 mice (580 ± 16 vs. 207 ± 29 mg/dl, < 0.0001), and the values for db/db mice were significantly greater than that of the BKS control (597 ± 3 vs. 338 ± 22 mg/dl, < 0.0001).
Body weight of the BL6 mice increased steadily from age 6 to 26 wk. The STZ-treated mice showed moderate weight gain, but were significantly lighter than their controls at 26 wk of age (25 ± 1 vs. 30 ± 1 g, p = 0.002). The db/db mice were significantly heavier than BKS controls at 26 wk of age (37 ± 2 vs. 29 ± 1 g, P = 0.004).

Synaptic responses evoked by single and repetitive pelvic nerve stimulation. Results from earlier studies suggested that synaptic transmission within the mouse SCG was depressed in the diabetic animals (Campanucci et al. 2010). Consequently, we tested whether synaptic transmission might also be depressed at parasympathetic MPG neurons. Two different series of pelvic nerve stimulation protocols were used. Initially, we compared the response to single suprathreshold stimuli and then tested whether there was a change in synaptic response when trains of repetitive stimuli, also supramaximal in strength, were applied to the pelvic nerve.

A suprathreshold fast excitatory postsynaptic potential (fEPSP), which initiated an action potential in the postganglionic neuron, was consistently recorded in MPG neurons from control and diabetic animals following single stimuli to the pelvic nerve. However, the size and shape of the fEPSP, along with the superposed action potential, varied greatly between MPG neurons. The traces in Fig. 1, recorded from four different MPG neurons in BL6 mice, illustrate the different pattern of postsynaptic responses evoked by single suprathreshold stimuli applied to the pelvic nerve. Similar responses were recorded in MPG neurons from both diabetic animals and their respective controls. With single stimuli applied to the pelvic nerve, subthreshold fEPSPs were not recorded in any MPG parasympathetic neurons from the different groups of mice, suggesting that synaptic transmission was not depressed in the MPG of diabetic animals at low-frequency stimulation.

To confirm that the fast ganglionic neurotransmission at parasympathetic neurons in the MPG remained only cholinergically-mediated in diabetic animals, we tested the efficacy of pharmacological blockade with hexamethonium (1 mM; Sigma, St. Louis, MO), a neuronal nicotinic receptor antagonist. A progressive, total block of fast synaptic transmission was produced following addition of hexamethonium to the bathing solution in MPG neurons from BL6 control (5 MPG, 5 cells) and diabetic animals (STZ 4 MPG, 6 cells; db/db 1 MPG, 2 cells).

In subsequent experiments, we tested whether synaptic transmission to MPG parasympathetic neurons was altered in diabetic animals during tetanic stimulation (5, 10, 20 Hz) of the pelvic nerve. Generally, at all frequencies of repetitive stimulation, an action potential was recorded in parasympathetic neurons in MPG from control and diabetic animals (Figs. 2 and 3). Occasionally, especially at the higher frequencies of stimulation, a few subthreshold fEPSPs were recorded, but the occurrence of subthreshold fEPSPs was not noticeably different in parasympathetic MPG neurons from the control and diabetic animals.

In parasympathetic MPG neurons from both control and diabetic animals, asynchronous mEPSPs were recorded following termination of the pelvic nerve stimulation, with the number of poststimulation mEPSPs increasing with increasing frequency of stimulation (Figs. 2 and 3). The number of poststimulation asynchronous mEPSPs at the different stimulation frequencies was similar for both the BL6- and STZ-treated animals (Fig. 4A). In contrast, at all frequencies of stimulation, the number of posttetanic mEPSPs was significantly greater in MPG parasympathetic neurons from db/db mice, relative to MPG parasympathetic neurons in BKS mice (Figs. 3 and 4B). Also, in db/db MPG neurons, mEPSPs commonly were noted during the period of stimulation, as well as after the pelvic nerve stimulation was terminated. During the higher frequencies of stimulation, the cell often was depolarized (Fig. 3). Occasionally, in db/db MPG neurons, summed mEPSPs which occurred during the period of nerve stimulation were sufficiently large to elicit extra action potentials. Poststimulation mEPSPs also summed to elicit action potentials (Fig. 3).

In Fig. 4 we have quantified the averaged number and amplitude of mEPSPs recorded in MPG neurons from the different animals following tetanic stimulation of the pelvic nerve. mEPSP number increased with stimulus frequency in all cases (Fig. 4A and B). The greatest number of events recorded posttetanic stimulation occurred in the db/db MPG neurons. There was a time-dependent decay of the posttetanic mEPSP activity. mEPSPs occurred more frequently immediately after nerve stimulation, but then mEPSP activity decayed exponentially. This is illustrated for mEPSP activity recorded in BKS and db/db MPG neurons in Fig. 4C.

Given that a decrease in postsynaptic response of diabetic SCG neurons has been reported, we analyzed the amplitude of the spontaneous mEPSPs recorded following posttetanic stimulation (Campanucci et al. 2010). For these measurements, the RMP was maintained at −60 mV. As shown in Fig. 4D, the amplitude of mEPSPs in MPG neurons from the STZ-treated mice was significantly smaller compared with mEPSPs re-
corded from BL6 MPG neurons. However, the averaged amplitude of mEPSPs recorded from MPG neurons of STZ-treated animals was ~4 mV, a value above baseline noise. In contrast, no difference in mEPSP amplitude was noted between db/db mice relative to BKS controls.

Measurement of membrane properties and excitability of parasympathetic MPG neurons from control and diabetic mice. In addition to analyzing synaptic transmission, we determined whether the basic membrane properties of the MPG neurons innervated by the pelvic nerve were altered in either model after prolonged diabetes. RMP and input resistance of BL6 and STZ neurons were not different at 26 wk of age (Fig. 5, A and B). At this time, the MPG neurons from db/db mice had less negative resting potentials and lower membrane input resistance relative to BKS neurons (Fig. 5, A and B). Capacitance values, which correlate with cell size, were similar across all groups (Fig. 5C).

We next measured the amplitude and duration of the AHP that follows a single action potential. Action potentials were elicited by direct stimulation through the recording electrode.

Fig. 2. MPG neuron responsiveness to preganglionic stimulation in BL6 control and streptozotocin (STZ)-treated mice. High-frequency stimulation of the pelvic nerve elicited synchronous action potential (AP) firing in the postganglionic neuron. Recordings (5, 10, 20 Hz) were obtained in the same cell from a control (BL6) or a STZ-treated (STZ) mouse. The recordings are representative of responses observed for each group. Few or no failures were seen in either group during stimulation. An asynchronous release of neurotransmitter was occasionally observed during and after stimulation. The number of miniature EPSPs (mEPSPs) increased with the increased frequency of stimulation.

Fig. 3. MPG neurons from db/db exhibited enhanced asynchronous mEPSP activity with tetanic stimulation. Responses were obtained in a control (BKS) and a db/db MPG neuron. mEPSPs are evident both during and following nerve stimulation. The number of mEPSPs increased with the increased frequency of stimulation. There was also a greater number of mEPSPs observed in db/db MPG neurons relative to controls (BKS). The increased mEPSP frequency during stimulation produced a slight depolarization of the membrane in many db/db neurons. Following 20-Hz stimulation, the summated mEPSPs were sufficiently large to elicit APs.
with brief (5 ms) depolarizing current pulses. The amplitude of the AHP was similar in MPG neurons from all groups (Fig. 5D). However, the duration of the AHP was significantly longer ($P = 0.0001$) in neurons from STZ-treated mice relative to BL6 neurons. In contrast, the AHP was significantly shorter ($P = 0.001$) in duration in MPG neurons from db/db mice compared with BKS neurons (Fig. 5E).

We also characterized the response of the parasympathetic MPG neurons to long, suprathreshold depolarizing current injections. The recordings in Fig. 6A, obtained from two different MPG neurons from a BL6 mouse, illustrate an example of a phasic (left traces) vs. a tonic (right traces) firing pattern produced by these depolarizing current steps. The percentage of tonic neurons was not different in ganglia from control or diabetic mice (Fig. 6B).

**DISCUSSION**

A goal of the present study was to determine whether interruption of ganglionic neurotransmission at parasympathetic MPG neurons contributed to urologic dysfunction with diabetes. Both diabetic bladder dysfunction and ED are common urologic complications of diabetes that potentially involve a loss of efferent parasympathetic neurochemical input to the target organ (Chitaley 2009; Daneshgari et al. 2009; Frimodt-Moller 1978; Sasaki et al. 2003). Depressed ganglionic neurotransmission has been indicated as a potential mechanism contributing to autonomic dysfunction with diabetes (Campanucci et al. 2010). However, no extensive analysis has been completed assessing whether synaptic transmission is altered by either type 1 or type 2 diabetes at parasympathetic neurons within the MPG. Using chronic type 1 and type 2 diabetic mice, we determined that pelvic nerve stimulation essentially always evoked a suprathreshold synaptic potential at parasympathetic MPG neurons, an observation indicating that synaptic transmission at this site is not as susceptible to depressed postsynaptic sensitivity as proposed for that occurring at the SCG (Campanucci et al. 2010).

At the SCG of STZ-treated BL6 and db/db BKS mice, 1–12 wk after onset of diabetes, a progressive impairment of ganglionic neurotransmission is observed (Campanucci et al. 2010). A 50–90% decrease in the evoked potential was reported. It was proposed that the synaptic depression resulted from the oxidation of a cysteine residue in the cytoplasmic loop of $\alpha 3\beta 4$- and $\alpha 4\beta 2$-containing nicotinic acetylcholine receptors (Campanucci et al. 2010). A major finding of the present study was that no measurable interruption in ganglionic neurotransmission was noted at parasympathetic MPG neurons after 20 wk of hyperglycemia in either model. However, asynchronous mEPSPs, measured at $-60 \text{ mV}$, in STZ-treated mice were smaller than their nondiabetic controls, an observation indicating that postsynaptic sensitivity to acetylcholine was decreased (Campanucci et al. 2010). However, mEPSP amplitudes were not different in db/db MPG neurons, suggesting that postsynaptic sensitivity to acetylcholine was not altered in parasympathetic MPG neurons in these animals. Therefore, we suggest that significant depression of cholinergic neurotransmission does not necessarily occur at all autonomic ganglion neurons with diabetes. Consistent with our conclusion, preliminary data reported by Krishnaswamy et al. (2012) indicate that synaptic transmission may be depressed by only 10% at the submandibular ganglion, a parasympathetic ganglion, after 3–4 mo of hyperglycemia.

We can only speculate why parasympathetic MPG neurons might be less susceptible to a diabetic disruption of synaptic transmission than sympathetic SCG neurons. Krishnaswamy et al. (2012) suggested that with diabetes there might be an increased generation of reactive oxygen species (ROS) within sympathetic neurons. Sympathetic neurons are catecholaminergic, and a rise in ROS may be more detrimental in these neurons than in the cholinergic parasympathetic neurons. Alternatively, we suggest that the difference in sensitivity might be due to the different morphology of the presynaptic innervation of the two types of autonomic neurons. All MPG neurons we have studied with dye injection, both choline
acetyltransferase-immunoreactive and tyrosine hydroxylase-immunoreactive, lack dendrites (data not shown). Also, in preliminary electron microscopic studies, we have noted that the preganglionic fibers innervating all the MPG neurons form pericellular baskets directly around the soma. This is in support of previous evidence suggesting MPG neurons lack dendritic processes (Jobling and Lim 2008; Rogers et al. 1990). In contrast, sympathetic neurons in other ganglia have complex dendritic arbors where multiple preganglionic inputs converge (Gibbins and Morris 2006). We propose that an increased production of ROS is likely to be more effective in the relatively confined space of the dendritic arbor, as opposed to the neuronal soma, where it may more freely diffuse or be exposed to cellular antioxidants.

This is the first report of the electrophysiological properties of male mouse parasympathetic MPG neurons, although a prior study has characterized properties of MPG neurons from juvenile female BL6 mice (Jobling and Lim 2008). The majority of electrophysiological studies of rodent MPG have been done on rat (Huang et al. 2011; Lee et al. 2002; Park et al. 2001, 2006a, 2006b, 2010; Tan et al. 2007; Won et al. 2006; Zhu et al. 1995). Comparison of the results from the study by Jobling and Lim (2008) and the present study is confounded because of differences in age of the mice and because, in the former study, neurons were randomly sampled without identifying them as either sympathetic or parasympathetic postganglionic neurons. However, with these limitations in mind, the adult male BL6 MPG neurons had a more negative RMP, but similar input

![Fig. 5. Membrane properties of parasympathetic MPG neurons from control and diabetic male mice. A: scatter plot of the resting membrane potential values for MPG neurons for each group. *Db/db neuron membrane potentials were significantly less negative than those of BKS MPG neurons. For all graphs, each dot is an individual measurement. Measurements were made from individual cells of multiple ganglion preparations. Lines with error bars indicate means ± SE. B: input resistance measurements for MPG neurons from each group of animals. *Input resistance of db/db neurons was significantly (P = 0.0345) less than that of BKS neurons. C: membrane capacitance measurements for MPG neurons from each group of animals. Diabetes did not have an effect on membrane capacitance for either group. D and E: the amplitude and duration of the afterhyperpolarization (AHP) following intracellular evoked APs. AHP amplitudes were not different between groups. However, AHP duration was altered in MPG neurons from both STZ and db/db mice. *Significance relative to BL6 controls. **Significance relative to BKS controls.](http://jn.physiology.org/)

![Fig. 6. Parasympathetic MPG neuron excitability in control and diabetic mice. A: recordings from two different BL6 MPG neurons that illustrate a “phasic” firing pattern (left) and a “tonic” firing pattern (right). Excitability was determined for diabetic and control MPG neurons by the number of APs generated by 1 s, depolarizing current pulses of increasing magnitude (0.1–0.4 nA). Neurons firing fewer than 1 or 2 APs were classified as phasic, and those firing 3 or more were classified as tonic. B: the presence of diabetes did not alter the proportion of tonic neurons.](http://jn.physiology.org/)
resistance relative to that of MPG neurons in female juvenile BL6 mice. In addition, adult male MPG parasympathetic
nerve fibers have a greater capacitance and show an increased proportion of tonic neurons (Jobling and Lim 2008).

Diabetes caused several alterations to the basic membrane properties of the parasympathetic MPG neurons of adult male
mice. In db/db mice, MPG neurons had less negative RMPs, lower input resistance, and shorter AHPs relative to the BKS
control. In the STZ-treated mice, the AHP was prolonged relative to the BL6 control. We have previously determined
that AHP duration in MPG neurons is greatly dependent on the activation of apamin-sensitive, small-conductance potassium
channels (Tompkins et al. 2010). A differential effect on the activity of the calcium-activated small-conductance potassium
channels may underlie the difference in AHP properties in the parasympathetic neurons from type 1 or type 2 diabetic ani-
mals.

The most novel finding in the present study is the enhanced asynchronous neurotransmitter release with tetanus stimulation
in MPG from the type 2 diabetic mice. The increased asynchronous activity was observed both during and following
tetanic stimulation. Summed mEPSPs occasionally were superthreshold and initiated action potentials. The frequency of
asynchronous activity increased with stimulation frequency and decreased exponentially after stimulation. Asynchronous
release is believed to be calcium mediated, although the mechanisms underlying this release process are poorly defined (Pang
and Sudhof 2010). Our present data do not elucidate the mechanism(s) underlying the change in asynchronous release.
However, we speculate that the observed enhanced number of asynchronous mEPSPs results from an altered calcium homeo-
stasy in the preganglionic nerve terminals innervating parasympathetic MPG neurons in the db/db mice. Neuronal cal-
cium dysregulation is postulated to be a central mechanism contributing to nerve degeneration within both sensory and
motor nerves of the autonomic nervous system (Biessels et al. 2002; Fernyhough et al. 2010; Kostyuk et al. 1999; Verkhratsky
and Fernyhough 2008). Nerve terminal mitochondria buffer the transient rise in intracellular calcium during high-frequency
nerve activity and diabetes can impair mitochondrial function (Brownlee 2005; David and Barrett 2003). Diabetes can also
disrupt the activity of voltage-gated calcium channels (Jagodic et al. 2007; Shankarappa et al. 2011). These mechanisms might
alter calcium kinetics within the nerve terminal during activity. It is unknown to what extent other factors, including hyperlip-
idemia, hyperinsulinaemia, or disrupted leptin signaling in db/db mice, might also contribute to the asynchronous neurotrans-
mmitter release. The increase in asynchronous activity does not appear to be solely associated with hyperglycemia because
similar increases were not observed in the STZ-treated mice. Elucidating the mechanisms involved in the disruption of the
presynaptic regulation of neurotransmitter release in db/db mice will require additional study.

GRANTS

This project was supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant K01DK081444 to J. D. Tompkins, a component of the National Institutes of Health (NIH).

DISCLAIMER

Contents are solely the responsibility of the authors and do not necessarily represent the official view of NIDDK or NIH.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.D.T. conception and design of research; J.D.T. performed experiments; J.D.T. analyzed data; J.D.T., M.A.V., and R.L.P. interpreted results of experiments; J.D.T. prepared figures; J.D.T. and R.L.P. drafted manuscript; J.D.T., M.A.V., and R.L.P. edited and revised manuscript; J.D.T., M.A.V., and R.L.P. approved final version of manuscript.

REFERENCES

Biessels GJ, ter Laak MP, Hamers FP, Gispens WH. Neuronal Ca2+
Brown JS, Wassell H, Chancellor MB, Howards SS, Stamm WE, Staple-
Brownlee M. The pathobiology of diabetic complications: a unifying mech-
Campanucci V, Krishnaswamy A, Cooper E. Diabetes depresses synaptic
transmission in sympathetic ganglia by inactivating nAChRs through a
Chitaley K. Type 1 and Type 2 diabetic-erectile dysfunction: same diagnosis
Chitaley K, Kupelian V, Subak L, Wessells H. Diabetes, obesity and erectile
dysfunction: field overview and research priorities. J Urol 182: S45–S50,
2009.
bladder dysfunction: current translational knowledge. J Urol 182: S18–S26,
2009.
David G, Barrett E. Mitochondrial Ca2+ uptake prevents desynchronization
of quantal release and minimizes depletion during repetitive stimulation of
Ellenberg M. Diabetes and sexual function. N Y State J Med 82: 927–930,
1982.
Fernyhough P, Roy Chowdhury SK, Schmidt RE. Mitochondrial stress and
the pathogenesis of diabetic neuropathy. Expert Rev Endocri
Frimodt-Moller C. Diabetic cysotrophy. A review of the urodynamic and
clinical features of neurogenic bladder dysfunction in diabetes mellitus. Dan
Gibbins I, Morris J. Structure of peripheral synapses: autonomic ganglia. Cell
Huang XZ, Park JT, Kim HG, Lee CK, Won YJ, Park BG, Jeong SW.
Phenotype-specific down-regulation of nicotinic acetylcholine receptors in
the pelvic ganglia of castrated rats: implications for neurogenic erectile
E, Bayliss D, Jevtovic-Todorovic V, Todorovic S. Cell-specific alterations
of T-type calcium current in painful diabetic neuropathy enhance excitabil-
Jobling P, Lim R. Anatomical and physiological properties of pelvic ganglion
Keast JR. Plasticity of pelvic autonomic ganglia and urogenital innervation.
Keast JR. Unusual autonomic ganglia: connections, chemistry, and plasticity
Kostyuk E, Svichar N, Shishkin V, Kostyuk P. Role of mitochondrial
dysfunction in calcium signalling alterations in dorsal root ganglion neurons
of mice with experimentally-induced diabetes. Neuroscience 90: 535–541,
1999.
Krishnaswamy A, Cooper E. Reactive oxygen species inactivate neuronal
nicotinic acetylcholine receptors through a highly conserved cysteine near

J Neurophysiol • doi:10.1152/jn.00354.2012 • www.jn.org