Published in final edited form as:

Auton Neurosci. 2007 July 31; 134(1-2): 26–37.

Electrical properties of neurons in the intact rat major pelvic ganglion

H. Tan¹, G. M. Mawe², and M.A. Vizzard^{1,2,*}

*1*Department of Neurology, University of Vermont College of Medicine, Burlington, VT, 05045

2 Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT, 05045

Abstract

The aim of this investigation was to characterize the electrical properties of neurons in the rat major pelvic ganglia (MPG) using intracellular recording techniques. MPG were dissected from male rats euthanized by isoflurane and thoracotomy. Neurons were classified as “phasic” or “tonic” according to their rate of accommodation during a 500-msec depolarizing current pulse. Phasic cells were further subdivided into rapidly or slowly adapting. The firing pattern of tonic cells was divided into regular high frequency, low frequency or irregular firing. In tonic cells, onset spikes showed TTX resistant discharges; whereas sustained spikes were TTX sensitive. Changing the current pulse amplitude or the stimulation interval could alter the firing pattern in both types of neurons. Subthreshold membrane potential oscillations (SMPOs) were primarily observed when neurons were depolarized. SMPOs were Na⁺ dependent and TTX-sensitive. The majority of tonic and phasic neurons generated rebound spikes, most of which were partially Na⁺ dependent. A small percentage (< 6%) of neurons exhibited spontaneous activity. Taken together these findings are consistent with the concept that neurons in the MPG exhibit heterogeneous electrical properties.

Keywords

firing pattern; TTX; subthreshold membrane potential oscillations; rebound spike; autonomic ganglion

Introduction

Neuronal regulation of the pelvic viscera involves spinal, supraspinal and peripheral mechanisms. Direct neural input to the tissues of the bladder, reproductive organs and distal colon arises from postganglionic neurons residing in major pelvic ganglia (MPG) or intramural ganglia. Pelvic ganglia are unique in that they receive both sympathetic and parasympathetic preganglionic inputs, and contain both sympathetic and parasympathetic postganglionic neurons (Felix et al. 1998; Tabatabai et al. 1986).

Neural output from autonomic ganglia to target organs is largely dependent on the passive and active electrical properties of the postganglionic neurons. Neurons in sympathetic (Cassell et al. 1986) and parasympathetic ganglia (Kajekar et al. 2001) are typically classified as phasic

Correspondence: Dr. Margaret A. Vizzard, Department of Neurology, D415A Given Building, 89 Beaumont Ave, University of Vermont, Burlington, VT 05405, Phone 802-656-3209, Fax: 802-656-8704, Email: Margaret.Vizzard@uvm.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

or tonic on the basis of their discharge patterns in response to a prolonged depolarizing current pulse. MPG neurons respond to prolonged suprathreshold depolarizing current steps with either a single action potential or a brief burst of action potentials at the onset of the stimulus (accommodating or phasic neurons) (Griffith et al. 1980;Tabatabai et al. 1986) or repetitive action potentials throughout the stimulus duration (non-accommodating or tonic neurons) (Griffith et al. 1980;Jobling et al. 2003;Kanjhan et al. 2003). While these descriptors provide a useful indication regarding the relative excitability of these two classes of neurons, additional characteristics provide more precise information about the active properties of the neurons and their propensity to be activated by modulatory inputs. These features include the existence or absence of spontaneous activity, subthreshold membrane potential oscillations (SMPOs), the frequency of action potential generation, and increased responsiveness to a persistent input of a given amplitude (Amir et al. 2002;Bracci et al. 2003).

To date, studies of the electrical properties of pelvic postganglionic neurons are limited to evaluation of enzymatically dissociated cells (Lee et al. 2002;Yoshimura and De Groat 1996) and relatively concise descriptions of their properties in intact ganglia (Felix et al. 1998;Kanjhan et al. 2003;Tabatabai et al. 1986). Recent investigations have reported that both phasic and tonic firing patterns exist in intact (Kanjhan et al. 2003;Tan et al. 2005) or dissociated MPG cells (Lee et al. 2002;Won et al. 2005). In addition, rebound spikes have been demonstrated in tonic neurons in rat MPG (Lee et al. 2002). The objective of this investigation was to undertake a thorough examination of the types of neurons that exist in the intact rat MPG and to provide an extensive analysis of the passive and active electrical properties of these neurons that will be a basis for future studies of these neurons under pathophysiological conditions. Some preliminary data have been reported previously in abstract form (Tan et al. 2005).

Methods and Materials

Animal usage

All experimental procedures involving animals were approved by the University of Vermont Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines. All efforts were made to minimize animal suffering or discomfort and to reduce the number of animals used. Adult male, Wistar rats, weighing 150 to 350 g (≥ 40 days old) were euthanized with isoflurane (4%, inhalation), and thoracotomy. The MPG were removed and used in electrophysiological experiments.

Electrophysiological recordings

The MPG were whole mounted in sylgard-lined dishes with recirculating Krebs solution (in mM: 121 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 25 NaHCO₃, 1.2 NaH₂PO₄ and 8 glucose; all from Sigma, St Louis, MO, USA) that was aerated with 95 % O₂/5% CO₂ and then maintained at a temperature of 34°C. Individual MPG neurons were randomly impaled with glass microelectrodes. Electrodes used for voltage recordings contained 2 M KCl, and had resistances in the range of 50-150 M Ω . An Axoclamp-2A amplifier (Axon Instruments, Foster City, CA, USA) was used in bridge mode to measure transmembrane potential. Electrical signals were saved and analyzed using MacLab Chart or Scope software (AD Instruments, Castle Hills, Australia).

Neuronal electrical properties were determined after the impalements stabilized for 5-min without applying intracellular holding current. During the first 5 min, the ability of the cell to fire an action potential upon intracellular current injection was assessed. Only neurons that were able to fire an action potential with an overshoot above 0 mV and had resting membrane potentials more negative than -35 mV were included in the electrophysiological analysis.

Excitability was measured by injection of 500-msec depolarizing and hyperpolarizing current pulses whose amplitude increased by 10 or 100 pA increments. This protocol revealed input resistance, whether the neuron fired anode break action potentials, the number of action potentials at rheobase and the maximum number of action potentials that each neuron could fire. The response to a suprathreshold 500-msec depolarizing stimulus elicits an accommodating action potential response in phasic neurons and repetitive action potentials in tonic neurons. The majority of neurons were classified as “phasic” or “tonic” according to response pattern to 500-msec current pulses. The “phasic” neurons were further subdivided into rapidly or slowly adapting. A rapidly adapting neuron was one that fired $\leq$ four action potentials in response to prolonged 5 sec and 10 sec current pulses. A slowly adapting neuron was one that fired more than four action potentials in response to prolonged 5 sec and 10 sec current pulses. SMPOs were assessed in response to 500-msec and 5 sec current pulses.

Reagents

N-methyl-D-glucamine (NMG) and tetrodotoxin (TTX) were purchased from Sigma-Aldrich. Drugs were made up as concentrated stock solutions stored in single use vials (to eliminate freeze thaw cycles) at -20°C until usage. All reagents, buffered in bath solution to pH 7.2, were applied directly by pump perfusion with 10 volumes of the recording chamber at a flow rate of 1-2 ml/min.

Statistical Analysis

All data are presented as mean $\pm$ standard error of mean (S.E.M.), where “n” indicates the number of independent experiments. Differences in changes of values between groups were tested using ANOVA, followed by individual post hoc comparisons (Fisher's exact test) or pairwise comparisons (*t* test). In all cases, $p \leq 0.05$ was considered significant. Each macroscopic current trace represents an average of three records in succession.

Results

Intracellular recordings were obtained from 261 neurons in 102 MPG preparations from 62 rats. The cells were classified as phasic or tonic on the basis of their discharge characteristics during 500 msec depolarizing current pulses (Fig. 1 and Fig. 2), as described previously (Cassell et al. 1986; Kanjhan et al. 2003). Phasic neurons were further subdivided into rapidly (≤ 4 APs) or slowly adapting (> 4 APs) based upon the number of action potentials fired in response to a depolarizing current pulse (Table 1). Table 1 provides a summary of the passive and active properties of these neuronal groups. Significant differences between phasic and tonic groups were detected in the means of input resistance, rheobase and time constant ($p \leq 0.05$; Table 1).

Firing pattern during 500 msec and 5 sec-depolarizing current pulses

Rapidly adapting phasic neurons (64%) fired ≤ 4 action potentials at the beginning of depolarizing current steps ranging from 0.1 to 1.2 nA in amplitude (Fig. 1A). In slowly adapting phasic neurons (10.7%), more than 4 action potentials were generated at the onset of the depolarizing current (Fig. 1B). In these cells, action potentials were not generated during the remainder of the depolarizing pulse, even when it lasted as long as 5 sec. The phasic neurons could also be divided into two sub-populations according to the sensitivity of their action potentials to tetrodotoxin (TTX; $1\mu\text{M}$) in current clamp recordings. In 75% of the phasic neurons tested (9 of 12 neurons), action potentials were TTX resistant (Fig. 1C), whereas action potentials were blocked by TTX in the remaining 3 neurons (Fig. 1D).

Neurons that repetitively fired throughout a 500 msec-depolarizing current pulse were classified as tonic cells (25.3%; 66 of 216 neurons). In contrast to the firing pattern of phasic

cells, tonic cells could generate spikes throughout a prolonged depolarizing current pulse lasting up to 5 sec (Fig. 2A1,B1,C1). Incremental increases in the amplitudes of intracellular current pulses increased firing rates, with a correlation between firing rate and current injection (Fig. 2A2,A3,B2,B3,C2,C3). The firing pattern of the tonic neurons during a 500 msec-depolarizing current could be further subdivided into three groups: (1) low frequency with a firing rate less than 20 Hz at up to 10 times rheobase (33.3%, n=22); (2) high frequency regular with a firing rate above 20 Hz (42.4%, n=28); and (3) irregular frequency (24.2%, n=16; Fig. 2D).

In tonic cells, spikes at the onset of depolarizing current pulses were resistant to TTX (0.5-1.0 μ M), whereas TTX terminated the steady state spikes generated by the same intensive depolarizing currents as that of control (Fig. 2E). Substitution of NMG for sodium eliminated the TTX resistant action potentials in both phasic (Fig. 1C) and tonic neurons (not shown).

Subthreshold events and membrane potential oscillations

In many types of neurons, excitability is reflected by the presence or absence of subthreshold fluctuations in the membrane potential, including subthreshold membrane potential oscillations (SMPOs) and rebound subthreshold depolarizations (Fig. 3A1, A2). For example, in primary sensory neurons, burst discharges can be triggered by SMPOs and maintained by depolarizing afterpotentials (Amir et al. 2002). Therefore, we evaluated subthreshold activity at rest and during depolarizing current pulses in MPG neurons. In the current study, most neurons had a stable membrane potential at the resting membrane potential (V_m), with only 5.6% of neurons exhibiting SMPOs at V_m . These neurons were more likely to exhibit spontaneous action potentials. However, during prolonged depolarizing pulses, 22.2% (38/171) of phasic neurons and 86.4% (57/66) of tonic neurons exhibited SMPOs (Table 1, Fig. 3). In phasic cells exhibiting SMPOs, a transient burst of action potentials at the beginning of a 500-msec depolarizing current pulse was followed by SMPOs (Fig. 3). The amplitude of rebound subthreshold depolarizations was dependent upon intensity of the depolarizing pulse (Fig. 3A1, A2). At progressively increasing amplitudes, rebound subthreshold depolarizations could trigger action potentials and maintain sequential action potentials when a series of 500 msec-depolarizing current pulses with 0.1 nA increments were delivered through the intracellular recording electrode (Fig. 3A1). The frequency and amplitude of SMPOs also was increased when the cell was depolarized with progressively increasing current amplitudes.

SMPOs can involve TTX-sensitive Na^+ currents as they are completely blocked by TTX (1-1.5 μ M) in magnocellular neurons of the rat supraoptic nucleus (Boehmer et al. 2000) and rat striatal fast-spiking interneurons (Bracci et al. 2003). In the present study, bath application of 1 μ M TTX reversibly abolished the SMPOs and the remaining spikes appeared similar to steady state spikes tested in ramp depolarization or prolonged 5-10 sec depolarizing pulses (Fig. 4B, $p \leq 0.001$, n=4). The TTX-resistant SMPO activity and related action potentials were reversibly abolished when Na^+ in the bathing solution was replaced by equal molar NMG (Fig. 4A). The effect of Na^+ free Krebs application was similar to that of TTX application. SMPOs were not restored by further depolarization. The magnitude of the SMPO was greatly inhibited by Na^+ substitution ($p \leq 0.001$, n=4) (Fig. 4A).

Changes in excitability patterns in MPG neurons

In some tonic cells, decreasing the interval between depolarizing current pulses resulted in a change in firing pattern in response to successive current pulses that were delivered at the threshold amplitude (Fig. 5A1). Decreasing the interval between depolarizing pulses in this tonic cell shifted the firing properties to that of a slowly adapting phasic cell with a burst of action potentials followed by a quiescent period (Fig. 5A2). Conversely, in slowly adapting phasic cells, a tonic action potential pattern was observed in response to repetitive stimulation

(Figure 6A1, A2). The spike-frequency correlation was relatively stable when the interval of depolarizing pulses was less than 0.1 pulses/s (pps). TTX consistently prevented the augmentation of excitability induced by persistent stimuli at the threshold amplitude (Fig. 6B1). However, the augmentation of excitability was restored when the strength of depolarizing pulses was increased (Fig. 6B2,B3 n=4).

Rebound action potential

Unlike phasic cells in dissociated MPG preparations, which do not exhibit anodal break action potentials (Lee et al. 2002), 59.3% of phasic (Fig. 7B,C) neurons and all of the tonic neurons (Fig. 7A,C) in the intact MPG generated rebound spikes at the end of a hyperpolarizing current injection. Four cells with rebound spikes were resistant to TTX (1 μ M), whereas one was sensitive to TTX (data not shown).

Spontaneous activity

To the best of our knowledge, there are no reports of spontaneous activity in rat MPG neurons (Felix et al. 2001;Kanjhan et al. 2003;Tabatabai et al. 1986); however, in the current study, spontaneous activity was detected in a small number of tonic neurons (Fig. 8A-C). We found that 22.7% of tonic neurons exhibited spontaneous activity. SMPO at V_m exhibited in interburst period or a quiescent period during hyperpolarization following a depolarizing pulse (Fig. 8D). Spontaneous action potentials were blocked by 0.5-1 μ M TTX (n=3; Fig. 8D,E).

Discussion

The urinary bladder and reproductive organs receive direct innervation from neurons that reside within the pelvis. In rodents, these neurons are clustered into MPG that are located on the pelvic floor, near the base of the bladder. The MPG are comprised of heterogeneous populations of neurons, not only with regard to their target tissues, but also because MPG contain both parasympathetic and sympathetic postganglionic neurons. The majority of sympathetic postganglionic neurons are noradrenergic, express tyrosine hydroxylase (TH) and are activated by hypogastric nerve stimulation whereas the parasympathetic postganglionic neurons express the biosynthetic enzyme for acetylcholine, choline acetyltransferase, are TH-negative, vasoactive intestinal polypeptide-positive and they are synaptically activated by stimulation of the pelvic nerve (Dail 1996;Keast 2006,1999). As action potentials originating in the cell body and traveling down the axon are the principal activators of neurotransmitter release from nerve terminals in the target tissue, the electrical properties of MPG neurons have a critical influence on the function of these reflex pathways. The purpose of this investigation was to undertake a thorough examination of the active and passive electrical properties of MPG neurons to provide a foundation for future studies of neuronal plasticity under pathophysiological conditions.

Types of neurons in the MPG

Previous electrophysiological studies of intact MPG have yielded a variety of results regarding the electrical properties of these neurons. In some studies, only phasic neurons were reported (Tabatabai et al. 1986), whereas in other studies, a combination of phasic and tonic neurons was detected (Jobling et al. 2003;Kanjhan et al. 2003). Some investigators have suggested that both phasic and tonic neurons exist in the MPG, with phasic neurons being parasympathetic and tonic neurons being sympathetic postganglionic neurons (Lee et al. 2002). However, a recent investigation by Kanjhan and colleagues (Kanjhan et al. 2003) reported the existence of both phasic and tonic neurons in the MPG, and they found that stimulation of the pelvic or hypogastric nerve leads to activation of both phasic and tonic neurons, but that very few neurons received synaptic input from both nerves (Kanjhan et al. 2003;Tabatabai et al. 1986). On the basis of this information, they concluded that both parasympathetic and sympathetic

postganglionic neurons in the MPG could be either phasic or tonic. Furthermore, they demonstrated that the electrical properties of the MPG neurons were influenced by the age of the animal and also by testosterone (Kanjhan et al. 2003).

In the current study, MPG were harvested from adult male rats, and both phasic and tonic neurons were detected from intact preparations, with the phasic neurons accounting for about 75% of the population. The phasic neurons were subdivided into rapidly or slowly adapting neurons based upon the number of action potentials generated in response to prolonged current pulses. The tonic neurons were divided into three populations on the basis of their firing properties during a prolonged current pulse: neurons that fired high frequency throughout the current pulse, neurons that fired at low frequency throughout the current pulse, and those that generated irregular bursts of action potentials throughout the current pulse. This is the first time that phasic and tonic neurons in the MPG have been divided into subpopulations. Although Kanjhan and colleagues (Kanjhan et al. 2003) reported that they detected neurons that were intermediate between tonic and phasic, they were not studied further. The findings reported here support the concept that MPG contain a heterogeneous population of neurons on the basis of their electrical properties.

Anodal break action potentials are sometimes used as a distinguishing electrical property of autonomic neurons. In a study of dissociated MPG neurons, anodal break APs were only detected in tonic neurons, and tonic neurons were identified as sympathetic neurons due to their TH-immunoreactivity (Lee et al. 2002; Won et al. 2005). The anodal break spikes in dissociated MPG neurons are detected in neurons that express a T-type Ca^{2+} channel in rat MPG (Zhu et al. 1995). In the current study, anodal break action potentials were detected in both tonic and phasic neurons of the intact rat MPG. In our whole mount MPG preparation, 59.3% of phasic neurons generated rebound spikes. Phasic neurons even with a single spike still generated anodal break spikes in our experiment. Therefore, anodal spikes cannot be used as a feature to distinguish between tonic and phasic MPG neurons, but it could be used as a reflection of neuronal excitability (Cooper and Stanford 2000; Lee et al. 2002; Stanford et al. 1998). Furthermore, it appears that excitability of neurons in intact ganglia differs from that of dissociated neurons.

Ionic conductances of MPG neurons

Voltage recording with sharp electrodes is not the optimal approach for characterizing the ionic conductances of a given set of neurons, but it does allow the investigator to obtain recordings from intact preparations, whereas enzymatic dissociation, which can disrupt membrane proteins, is typically necessary for obtaining efficient voltage clamp recordings. Previous studies have demonstrated that there are three major ionic conductances (I_A , I_M , I_H) that contribute to the discharge characteristics in autonomic neurons (de Groat and Booth, 1993). In an effort to gain insight into the ionic conductances that contribute to membrane excitability in rat MPG neurons, channel blockers and ion substitution were used in the current investigation. In previous investigations of dissociated rat MPG neurons, Na^+ conductances were completely blocked by TTX (Felix et al. 2001, 1998; Yoshimura and De Groat 1996). However, in the current study, action potentials persisted in the presence of TTX and were eliminated by Na^+ substitution with the non-permeant monovalent cation, NMG. These results suggest that while TTX-sensitive Na^+ channels are likely to exist in MPG neurons, TTX resistant Na^+ channels also contribute to the action potential in these cells. The presence and proportion of MPG neurons expressing either TTX-sensitive (25%) Na^+ channels or TTX-resistant (75%) Na^+ channels in the present study was very similar to that described in dissociated bladder afferent cells from the lumbosacral DRG using patch clamp recording techniques (Yoshimura and de Groat, 1999; Yoshimura et al., 2001). A recent study in canine cardiac ganglia, a parasympathetic ganglia with ChAT-positive and VIP- and pituitary

adenylate cyclase activating polypeptide (PACAP)-positive postganglionic neurons, has similarly identified TTX resistant Na⁺ channels and TTX-sensitive Na⁺ channels (Scornik et al. 2006). In addition, preliminary studies cited by de Groat and Booth (1993) also reveal TTX-sensitive and TTX-resistant Na⁺ channels in MPG neurons. Thus, both TTX-sensitive Na⁺ and TTX resistant Na⁺ channels are likely to exist in a number of autonomic ganglia. Further studies will be required to resolve the relative contributions of various K channels to the firing properties of rapidly and slowly adapting phasic neurons and tonic neurons in the rat MPG.

Subthreshold oscillatory activity and MPG neuron excitability

SMPOs have been recorded in autonomic ganglia from many mammalian species (Adams and Harper 1995; Amir et al. 2002; Liu et al. 2000; Xing et al. 2003). The SMPOs may contribute to neuropathic pain, and they are augmented by spinal nerve damage and dorsal root ganglion compression (Amir et al. 2002; Liu et al. 2000; Xing et al. 2003). We found that a small proportion of neurons (~6%) exhibited SMPO at V_m, and these neurons often exhibited spontaneous action potentials. As the neurons were depolarized, both phasic (22%) and tonic (86%) neurons exhibited SMPOs. As has been demonstrated previously in primary sensory neurons (Amir et al. 2002; Liu et al. 2000; Xing et al. 2003), the amplitude and frequency of the SMPOs in MPG neurons were voltage dependent, with the amplitude and duration increasing as the neurons were brought to more depolarized potentials. The rising phase of SMPOs in other types of neurons has been shown to be Na⁺-dependent, and they are sensitive to TTX (Amir et al. 2002; Boehmer et al. 2000; Bracci et al. 2003). Similarly, in MPG neurons, the SMPOs were greatly attenuated in the presence of TTX and were eliminated when NMG replaced Na⁺ in the bathing solution. These findings demonstrate the existence of a property of MPG neurons that likely modulates the excitability of these neurons and which may be altered in pathological conditions, leading to alterations in end organ function.

Changes in excitability patterns

In the current study, the vast majority of tonic cells exhibited increased excitability in response to repetitive current pulses of the same amplitude. The mechanism underlying this change is not clear, but it may involve SMPOs. TTX greatly reduced the number of spikes in these cells demonstrating a change in excitability pattern. However, even in the presence of TTX, the change in action potential generation could be elicited when the intensity of the depolarizing current pulses was increased. Data from previous studies suggest that the change in action potential generation to repeated depolarizing current pulses may also involve increased activation of postsynaptic Ca²⁺ channels (Davies et al. 1996; Ireland et al. 1998; Perrier and Hounsgaard 1999). We are not aware of any previous reports of this type of change in action potential firing pattern elicited in autonomic ganglion cells in response to consistent depolarizing current pulses. However, in the enteric nervous system, synaptic activation of myenteric AH neurons over extended periods (1-30 min) at low frequency leads to a sustained increase in neuronal excitability that is associated with a depolarization and increase in input resistance (Clerc et al. 1999).

Concluding remarks

Findings reported here regarding the electrical properties of intact rat MPG neurons have revealed that considerable diversity exists among these neurons, with two types of phasic and three types of tonic neurons being detected. Distinguishing features of these neurons include their responsiveness to depolarizing current pulses, the existence of spontaneous action potentials, anodal break action potentials, SMPOs, and changes in action potential generation in response to repetitive depolarizing current pulses, anodal break and resting condition. Furthermore, differences were detected between the excitability and TTX sensitivity of neurons in intact preparations, as reported here, and those of enzymatically dissociated neurons.

Collectively, these findings are consistent with the concept that MPG contain neurons with diverse electrical properties. Shifts in the electrical properties of these neurons, as have been demonstrated with altered hormone exposure (Felix et al. 2001,1998;Kanjhan et al. 2003), may also occur under pathophysiological conditions, are likely to have significant effects on pelvic organ function.

Acknowledgements

The authors thank O.B. Balemba and E.M. Krauter for technical assistance and discussions related to these studies. The authors also thank Dr. Rodney Parsons for his critical reading of the manuscript. This work was funded by NIH grants DK051369, DK060481, DK065989 to MAV and NS26995 and DK62267 to GMM.

References

- Adams, DJ.; Harper, AA. Electrophysiological properties of autonomic ganglion neurons. In: McLachlan, E., editor. *Autonomic Ganglia*. Luxembourg: Harwood Academic Publishers; 1995. p. 153-212.
- Amir R, Liu CN, Kocsis JD, Devor M. Oscillatory mechanism in primary sensory neurones. *Brain* 2002;125:421–435. [PubMed: 11844741]
- Boehmer G, Greffrath W, Martin E, Hermann S. Subthreshold oscillation of the membrane potential in magnocellular neurones of the rat supraoptic nucleus. *J Physiol* 2000;526(Pt 1):115–128. [PubMed: 10878105]
- Bracci E, Centonze D, Bernardi G, Calabresi P. Voltage-dependent membrane potential oscillations of rat striatal fast-spiking interneurons. *J Physiol* 2003;549:121–130. [PubMed: 12665602]
- Cassell JF, Clark AL, McLachlan EM. Characteristics of phasic and tonic sympathetic ganglion cells of the guinea-pig. *J Physiol* 1986;372:457–483. [PubMed: 2425087]
- Clerc N, Furness JB, Kunze WA, Thomas EA, Bertrand PP. Long-term effects of synaptic activation at low frequency on excitability of myenteric AH neurons. *Neuroscience* 1999;90:279–289. [PubMed: 10188954]
- Cooper AJ, Stanford IM. Electrophysiological and morphological characteristics of three subtypes of rat globus pallidus neurone in vitro. *J Physiol* 2000;527(Pt 2):291–304. [PubMed: 10970430]
- Dail WG. The pelvic plexus: innervation of pelvic and extrapelvic visceral tissues. *Microsc Res Tech* 1996;35:95–106. [PubMed: 8923445]
- Davies PJ, Ireland DR, McLachlan EM. Sources of Ca²⁺ for different Ca(2+)-activated K⁺ conductances in neurones of the rat superior cervical ganglion. *J Physiol* 1996;495(Pt 2):353–366. [PubMed: 8887749]
- de Groat, WC.; Booth, AM. Synaptic transmission in pelvic ganglia. In: Maggi, CA., editor. *Nervous Control of the Urogenital System*. Langhorne: Harwood Academic Publishers; 1993. p. 291-348.
- Felix B, Catalin D, Miolan JP, Niel JP. Effects of testosterone on the electrical properties and nicotinic transmission of the major pelvic and coeliac ganglion neurones. *J Neuroendocrinol* 2001;13:193–198. [PubMed: 11168845]
- Felix B, Catalin D, Miolan JP, Niel JP. Integrative properties of the major pelvic ganglion in the rat. *J Auton Nerv Syst* 1998;69:6–11. [PubMed: 9672118]
- Griffith WH 3rd, Gallagher JP, Shinnick-Gallagher P. An intracellular investigation of cat vesical pelvic ganglia. *J Neurophysiol* 1980;43:343–354. [PubMed: 6247460]
- Ireland DR, Davies PJ, McLachlan EM. The role of N-type Ca²⁺ channels in regulating excitability of guinea-pig sympathetic neurones. *J Auton Nerv Syst* 1998;73:109–114. [PubMed: 9862385]
- Jobling P, Gibbins IL, Morris JL. Functional organization of vasodilator neurons in pelvic ganglia of female guinea pigs: comparison with uterine motor neurons. *J Comp Neurol* 2003;459:223–241. [PubMed: 12655506]
- Kajekar R, Rohde HK, Myers AC. The integrative membrane properties of human bronchial parasympathetic Ganglia neurons. *Am J Respir Crit Care Med* 2001;164:1927–1932. [PubMed: 11734448]
- Kanjhan R, Osborne PB, Ouyang M, Keast JR. Postnatal maturational changes in rat pelvic autonomic ganglion cells: a mixture of steroid-dependent and -independent effects. *J Neurophysiol* 2003;89:315–323. [PubMed: 12522182]

- Keast JR. Plasticity of pelvic autonomic ganglia and urogenital innervation. *Int Rev Cytol* 2006;248:141–208. [PubMed: 16487791]
- Keast JR. Unusual autonomic ganglia: connections, chemistry, and plasticity of pelvic ganglia. *Int Rev Cytol* 1999;193:1–69. [PubMed: 10494620]
- Lee JH, Kim EG, Park BG, Kim KH, Cha SK, Kong ID, Lee JW, Jeong SW. Identification of T-type α_1H Ca^{2+} channels ($Ca_v3.2$) in major pelvic ganglion neurons. *J Neurophysiol* 2002;87:2844–2850. [PubMed: 12037187]
- Liu CN, Michaelis M, Amir R, Devor M. Spinal nerve injury enhances subthreshold membrane potential oscillations in DRG neurons: relation to neuropathic pain. *J Neurophysiol* 2000;84:205–215. [PubMed: 10899197]
- Perrier JF, Hounsgaard J. Ca^{2+} -activated nonselective cationic current ($I(CAN)$) in turtle motoneurons. *J Neurophysiol* 1999;82:730–735. [PubMed: 10444670]
- Scornik FS, Desai M, Brugada R, Guerchicoff A, Pollevick GD, Antzelevitch C, Perez GJ. Functional expression of “cardiac-type” Nav1.5 sodium channel in canine intracardiac ganglia. *Heart Rhythm* 2006;3:842–850. [PubMed: 16818219]
- Stanford IM, Traub RD, Jefferys JG. Limbic gamma rhythms. II. Synaptic and intrinsic mechanisms underlying spike doublets in oscillating subicular neurons. *J Neurophysiol* 1998;80:162–171. [PubMed: 9658038]
- Tabatabai M, Booth AM, de Groat WC. Morphological and electrophysiological properties of pelvic ganglion cells in the rat. *Brain Res* 1986;382:61–70. [PubMed: 3768682]
- Tan, H.; Krauter, EM.; Balemba, OB.; Mawe, GM.; Vizzard, MA. Abstract Viewer/Itinerary Planner 306.3. 2005. Electrical and synaptic properties of neurons in the rat major pelvic ganglia.
- Won, YJ.; Kong, ID.; Lee, JW.; Jeong, SW. Abstract Viewer/Itinerary Planner 845.8. 2005. L-type calcium currents mainly contribute to generation of afterhyperpolarization in pelvic ganglion neurons.
- Xing JL, Hu SJ, Jian Z, Duan JH. Subthreshold membrane potential oscillation mediates the excitatory effect of norepinephrine in chronically compressed dorsal root ganglion neurons in the rat. *Pain* 2003;105:177–183. [PubMed: 14499434]
- Yoshimura N, de Groat WC. Characterization of voltage-sensitive Na^{+} and K^{+} currents recorded from acutely dissociated pelvic ganglion neurons of the adult rat. *J Neurophysiol* 1996;76:2508–2521. [PubMed: 8899623]
- Zhu Y, Zboran EL, Ikeda SR. Phenotype-specific expression of T-type calcium channels in neurons of the major pelvic ganglion of the adult male rat. *J Physiol* 1995;489(Pt 2):363–375. [PubMed: 8847632]

Figure 1.

Phasic neurons generate both TTX-resistant and TTX-sensitive action potentials. A: Rapidly adapting phasic neuron with single spike response to 0.1 nA and 1 nA depolarizing current steps. B: Slowly adapting phasic neuron with a single action potential with a 0.1 nA, 500-msec depolarizing step and a burst of action potentials at onset of the 1 nA, 500-msec depolarizing current pulse. C: 75% of the neurons have TTX-resistant action potentials. Substitution of sodium with NMG eliminated the spikes in these cells. D: 25% of phasic neurons have TTX-sensitive action potentials.

Figure 2.

Three subclasses of tonic neurons can be detected in the rat MPG depending on the action potential firing pattern during a 500-msec current pulse. A1: Consistent high frequency (~ 20 Hz) repetitive firing is detected in 43% of tonic neurons. B1: Consistent, lower frequency (< 20 Hz) repetitive firing is detected in 34% of tonic neurons. C1: Irregular firing of action potentials is detected in 23% of tonic neurons. A2, B2, C2: Numbers of spikes generated with increasing current strength. A3, B3, C3: Firing properties during 5 sec depolarizing pulses by same current intensity as the 500 msec pulses. D: Percentage of the three subgroups of tonic cells according to firing pattern. E: Effect of TTX ($1 \mu\text{M}$) on the tonic firing pattern in the three tonic neuron subtypes.

Figure 3.

Rebound subthreshold depolarizations and SMPOs. A1: In slowly adapting phasic MPG cells, the transient bursts (55.1 ± 1.4 Hz) of action potentials that occurred at the beginning of the 500-msec depolarizing current pulses were followed by SMPOs. Asterisks in inset indicate subthreshold potential oscillations. Lower traces represent current pulses at 3 Hz. The number of repetitive discharges increased with increasing intensity steps and terminated firing in the middle of sustained depolarization. The amplitudes of rebound subthreshold depolarizations (arrow) following spike(s) at the 3rd and 4th pulse increase until successive spikes are generated (5th pulse). The same pattern occurred following pulses with increment of intensive depolarizations. Frequency and amplitude of rebound subthreshold depolarizations were

increased with the cell depolarized by injecting current from 0.1 nA to 0.8 nA. A2: Rebound subthreshold depolarizations and subthreshold potentials vary at same 1 nA-depolarizing currents for same cell as A1. B: Voltage dependent subthreshold membrane potential oscillation with prolonged depolarization in rapidly adapting phasic cell. Insets indicate traces labeled by numbers (1, 2, 3). RMP shows a voltage recording at the resting membrane potential in which no SMPOs are evident. C1: A tonic cell during a 500-msec- depolarizing current pulse and a 10 sec-prolonged pulse. Tonic firing at high frequency lasted for >6 sec. C2: Voltage dependent oscillations from the left recording in C1. SMPOs were not observed at resting membrane potential or with microelectrodes placed in the bath.

Figure 4.

Generation and amplitude of SMPOs was voltage dependent. A: Substitution of Na⁺ in the bath solution with equal molar NMG reversibly abolished the oscillations and the resulting spikes. B: Bath application of 1 μ M TTX reversibly abolished the oscillations and the resulting spikes.

Figure 5.

An alteration in stimulus strength and frequency can cause shifts in action potential firing pattern. A: Variation in tonic firing generated by depolarizing pulses at constant intensity. A1: The number of spikes generated by depolarizing pulses at a longer interval was relatively stable. A2: The variation of firing was increased at a reduced interval of depolarizing pulses. The right two traces with truncated spikes correspond to the numbered dashed boxes. Arrowhead in inset 1 indicates subthreshold potential oscillation.

Figure 6.

Increased excitability of slowly adapting phasic cell in response to repetitive stimulation. A: In this neuron, action potential generation remains similar in response to 0.7 nA current pulses (A1), but increased action potential generation occurred in response to 0.8 nA current pulses (A2). A3. Bar graph showing that peak firing was achieved at the third sequential current pulse. B: TTX (1 μ M) reduced the number of spikes generated during depolarizing current pulses (B1, compare with A1 from the same cell). In the presence of TTX, an increase in action potential generation was still detected, but required higher amplitude current pulses (0.9 nA) and developed more slowly (B2). The plot in B3 shows the number of sequential current pulses necessary to reach peak firing in the absence (0.8 nA) and presence of TTX (0.9 nA).

Figure 7. Rebound spikes are generated in both tonic and phasic neurons. A: Rebound potential and single rebound spike is generated in a tonic neuron. B: Rebound spike and traces in a phasic neuron. C: Bar graph of percentage of tonic or phasic cells demonstrating rebound spikes.

Figure 8.

Some tonic neurons in the MPG exhibit spontaneous activity. A-C: Three different patterns of spontaneous activity are demonstrated: irregular (A), burst (B), and tonic (C). Areas indicated by dashed boxes are demonstrated at a faster time course to the right. D: In this neuron with tonic spontaneous activity, a quiescent period associated with membrane oscillations (dashed box) follows a depolarizing current pulse. In the presence of TTX, the spontaneous action potentials and the oscillatory activity are reduced or abolished, but the action potentials elicited by the current pulse persist. E: In the same neuron, as shown in D, rebound action potentials persist in the presence of TTX, whereas spontaneous action potentials are blocked.

Table 1
Electrophysiological characteristics of neurons in rat MPG

	Rapidly Adapting Phasic (n)	Slowly Adapting Phasic (n)	Tonic (n)
Cell type	64% (167/261)	10.7% (28/261)	25.3% (66/261)
RMP, mV	-52.8 ± 1.7 (54)	-47.8 ± 7.7 (17)	-53.5 ± 2.5 (34)
Input R, MΩ	45.4 ± 3.3 (95)*	47.1 ± 4.2 (17)*	61.1 ± 6.8 (40)
Rheobase, nA	0.21 ± 0.02 (52)*	0.23 ± 0.02 (10)*	0.14 ± 0.02 (18)
AH amplitude, mV	16.1 ± 1.8 (18)	16.8 ± 1.5 (11)	13.9 ± 0.9 (21)
AH duration, ms	155.2 ± 36.8 (23)	147.1 ± 35.5 (9)	159 ± 26.8 (20)
Time constant, ms	4.0 ± 0.2 (78)*	4.33 ± 0.3 (17)	5.1 ± 0.5 (36)
Rebound spikes	48.9% (70/143)	82.1% (23/28)	100% (50/50)
Oscillation	18.2% (26/143)	42.8% (12/28)	86.4% (57/66)
Spontaneous AP	--	--	22.7% (15/66)

*
p ≤ 0.05