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Anatomical and physiological properties of pelvic ganglion neurons in female mice

Phillip Jobling * Rebecca Lim

School of Biomedical Sciences, Faculty of Health and Hunter Medical Research Institute, The University of Newcastle, Callaghan, NSW 2308, Australia

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Abstract

Most neurons that regulate motility and blood flow in female pelvic organs are located within pelvic (paracervical) ganglia. In this study we investigated the anatomical and physiological properties of neurons within mouse (C57/Bl/6) paracervical ganglia. Most neurons showed immunoreactivity for choline acetyl transferase (CHAT) and were presumably cholinergic. Few neurons (~5%) were tyrosine hydroxylase (TH) positive. Immunohistochemical labelling for microtubule associated protein 2 showed most neurons had small somata (cross sectional area ~300 μm^2) and lacked dendrites. Action potential (AP) discharge characteristics, determined by depolarising current step injection, revealed most neurons (70%) adapted rapidly to depolarising current injection and were classified as "phasic". The remaining neurons discharged APs throughout the current step and were classified as "tonic". Membrane properties and current–voltage relationships were similar in phasic and tonic neurons, however the afterhyperpolarisation was significantly smaller in tonic neurons. Stimulation of preganglionic axons usually evoked a single strong preganglionic input (21/27 and 9/10 for pelvic and hypogastric nerves, respectively). In 19 preparations where we tested for inputs from both nerves pelvic inputs predominated (23/45 neurons) and inputs via the hypogastric nerve were rarely observed (3/45 neurons). Together, our data indicate that most neurons within mouse paracervical ganglia are cholinergic and parasympathetic. As there is little anatomical or functional evidence for integration of preganglionic inputs we propose that the role of paracervical neurons is restricted to one of spatial amplification or filtering of preganglionic inputs.

Keywords: Female pelvic organs; Synaptic transmission; Ganglia; Afterhyperpolarisation

1. Introduction

The neural control of pelvic organs is characterised by complex spinal reflexes, which are modulated by descending signals from the brainstem and circulating hormones (Giuliano et al., 2002; Hotta et al., 1999; Yan et al., 2007). The final output from the central nervous system to pelvic organs occurs via preganglionic neurons located in lumbar and sacral segments of the spinal cord. These CNS neurons drive their intended target after first traversing a synapse located within autonomic ganglia. The neurons responsible for regulation of motility, vascular resistance, and glandular secretion within pelvic organs such as the bladder, reproductive tract and large bowel are housed in pelvic ganglia. In females, these are termed paracervical ganglia as they lie close to the vagino-cervical junction (Traurig and Papka, 1993). The degree to which autonomic outflow from the spinal cord is modified by neurons in paracervical ganglia is poorly understood.

In a previous study we showed that neurons in paracervical ganglia of guinea-pigs received only one or two strong synaptic inputs. Neurons were equally likely to receive input from sacral or lumbar preganglionic neurons and around 30% of them received convergent inputs from both lumbar and sacral spinal cord segments (Jobling et al., 2003). In rat pelvic ganglia, neurons received synaptic input from either sacral or lumbar preganglionc axons but not both (Tabatai et al., 1986). These data for rats, however, are confounded by the fact that

* Corresponding author. Tel.: +61 2 4921 5126; fax: +61 2 49217903.
E-mail address: phillip.jobling@newcastle.edu.au (P. Jobling).

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both male and female rats were used in this study. Whether this apparent species related difference gives us insight into the role of paracervical neurons, or indeed which of these innervation patterns is more representative remains to be determined.

Many studies have classified postganglionic neurons based on their action potential discharge in response to depolarising current injection (Cassell et al., 1986; Jobling and Gibbins, 1999; Jobling et al., 2003; King and Szurszewski, 1984; Myers, 1998). Typically neurons fall into rapidly adapting (phasic) or slowly adapting (tonic) subclasses. The ionic conductances responsible for these two states are clearly defined in some autonomic ganglia. For example, the major conductances which control action potential discharge in prevertebral sympathetic ganglia are the M-current (I_M), A-current (I_A) and the afterhyperpolarisation (AHP) which follows an action potential (Cassell et al., 1986; Cassell and McLachlan, 1987b; Wang and McKinnon, 1995). However in other cases, including pelvic ganglia, the relation between expression of the above ionic conductances and action potential discharge are more subtle (Jobling et al., 1999; Jobling et al., 2003; Tan et al., 2007). In any case, the functional implications of action potential discharge characteristics in response to current injection are not clear. This is especially so for pathways characterised by strong synaptic inputs and low preganglionic firing frequencies (McLachlan, 2003).

At present, much of our understanding of pelvic ganglia is derived from studies on male animals. While many neurons in male and female pelvic ganglia project to similar targets (lower bowel and bladder), a large proportion of neurons project to the reproductive tract. Consequently, pelvic ganglia in males are developmentally regulated by circulating androgens (Keast, 1999a) whereas those in females are known to express estrogen receptors (Papka and Mowa, 2003). Thus, while we can draw some parallels with studies in male pelvic ganglia, it is important to also establish the functional role of neurons in female paracervical ganglia. Given that little functional cellular data is available for female paracervical ganglia and most data is derived from one species, our aim was to study the sympathetic and parasympathetic inputs to, and the electrical properties of, neurons in paracervical ganglia of female mice.

2. Materials and methods

Paracervical ganglia were obtained from female C57/Bl/6 mice (P21-35) that had been overdosed with sodium pentobarbitone (150 mg/kg i.p) and subsequently exsanguinated. These procedures were approved by the Animal Care and Ethics Committee of the University of Newcastle. The preparation most often consisted of the main ganglion and usually two small accessory ganglia located near the entry point of the hypogastric nerve. The preparation was carefully removed with pelvic and hypogastric nerves and placed in a HEPES-buffered balanced salt solution that contained in (mM): NaCl, 146; KCl, 4.7; MgSO4, 0.6; NaHCO3, 1.6; NaH2PO4, 0.13; CaCl2, 2.5; glucose, 7.8; HEPES, 20, buffered to pH 7.3.

2.1. Immunohistochemistry

Ganglia were processed as wholemounts for multiple-labelling immunofluorescence as described previously (Gibbins and Matthew, 1996; Anderson et al., 2001). Briefly, tissue was fixed by immersion for 24 h in a solution containing 2% formaldehyde and 0.2% picric acid in phosphate buffer (PBS; pH 7.0). After fixation, excess picric acid was washed from the tissue with 80% ethanol (3×). Subsequently the tissue was cleared in three changes of dimethylsulfoxide. Preparations were rinsed in PBS (3×) and placed in primary antisera raised in different species for 48–72 h (all antisera from Chemicon/Millipore, MA). Primary antisera used for double labelling were rabbit anti-tyrosine hydroxylase (TH, 1:100) together with chicken anti-microtubule associated protein 2 (MAP2, 1:1,000), or rabbit anti-TH (1:100) together with goat anti-choline acetlytransferase (CHAT, 1:1000). Following incubation in primary antisera, ganglia were washed in PBS (3×), then incubated for 24 h in secondary antisera. Secondary antisera were all raised in donkey and conjugated to fluorescein isothiocyanate (FITC) or Cy3 (Both obtained from Jackson ImmunoResearch, West Grove, PA). Neurobiotin-filled neurons were visualized by fixing ganglia overnight as described above. Ganglia were washed in 80% ethanol (3×) then DMSO (3×) before being placed in PBS. Ganglia were then incubated in streptavidin conjugated to FITC or Cy3 for 2 h before being washed PBS (3×). Ganglia were mounted on slides in carbonate-buffered glycerol (pH 8.6) and examined under an Olympus BX50 epifluorescence microscope. Images were captured with an Olympus DP70 camera and exported to Adobe Photoshop (V 8.0, Adobe Systems, Mountain View, CA) for construction of figures. Images were only adjusted for brightness and contrast.

2.2. Intracellular electrophysiology

Preparations were dissected free of surrounding fat and connective tissue and tightly pinned to the base of a recording chamber (volume 1 ml) that had been coated with silicone elastomer (Sylgard, Dow Corning, Midland, MI). The preparation was maintained at 35 °C and perfused with oxygenated HEPES-buffered solution (Flow rate = 2.5 ml min^{-1}). Neurons were impaled with glass microelectrodes filled with 0.5 M KCl (resistance 80–200 MΩ). In some cases, Neurobiotin (Vector, Burlingame, CA) 0.5% wt/vol was included in the electrode filling solution to enable post impalement visualization of neurons. Data were recorded using an Axoclamp 2A amplifier (Axon Instruments, Union City, CA). Voltage and current records were digitized at 10 kHz using a Powerlab A:D converter running Chart and Scope software (version 3.6, ADI Instruments, Castle Hill, NSW, Australia). Digitized data were analyzed using Igor Pro (version 3.14, WaveMetrics, Lake Oswego, OR). Membrane