Excitatory Synaptic Currents in Lumbosacral Parasympathetic Preganglionic Neurons Elicited From the Lateral Funiculus

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Miura, Akira,Masahito Kawatani, and William C. de Groat. Excitatory synaptic currents in lumbosacral parasympathetic preganglionic neurons elicited from the lateral funiculus. J Neurophysiol 86: 1587–1593, 2001. Excitatory postsynaptic currents (EPSCs) in parasympathetic preganglionic neurons (PGNs) were examined using the whole cell patch-clamp recording technique in L6 and S1 spinal cord slices from neonatal rats (6–16 days old). PGNs were identified by labeling with retrograde axonal transport of a fluorescent dye (Fast Blue) injected into the intraperitoneal space 3–7 days before the experiment. Synaptic responses were evoked in PGNs by field stimulation of the lateral funiculus (LF) in the presence of bicuculline methiodide (10 μM) and strychnine (1 μM). In approximately 40% of the cells (total, 100), single-shock electrical stimulation of the LF elicited short, relatively constant latency (3.0 ± 0.1 (SE) ms) fast EPSCs consistent with a monosynaptic pathway. The remainder of the cells did not respond to stimulation. At low intensities of stimulation, the EPSCs often occurred in an all-or-none manner, indicating that they were mediated by a single axonal input. Most cells (n = 33) exhibited only fast EPSCs (type 1), but some cells (n = 8) had fast EPSCs with longer, more variable latency polysynaptic EPSCs superimposed on a slow inward current (type 2). Type 1 fast synaptic EPSCs were pharmacologically dissected into two components: a transient component that was blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 5 μM), a non-NMDA glutamatergic antagonist, and a slow decay component that was blocked by 2-amino-5-phosphonovalerate (APV, 50 μM), a NMDA antagonist. Type 2 polysynaptic currents were reduced by 5 μM CNQX and completely blocked by combined application of 5 μM CNQX and 50 μM APV. The fast monosynaptic component of type 1 EPSCs had a linear current-voltage relationship and reversed at a membrane potential of 5.9 mV (n = 5), whereas the slow component exhibited a negative slope conductance at holding potentials greater than −20 mV. The type 1, fast synaptic EPSCs had a time of peak 1.4 ± 0.1 ms and exhibited a biexponential decay (time constants, 5.7 ± 0.6 and 38.8 ± 4.0 ms). In the majority of PGNs (n = 11 of 15 cells), EPSCs evoked by electrical stimulation of LF exhibited paired-pulse inhibition (range: 25–33% depression) at interstimulus intervals ranging from 50 to 120 ms. These results indicate that PGNs receive monosynaptic and polysynaptic glutamatergic excitatory inputs from axons in the lateral funiculus.

Introduction

Lumbosacral parasympathetic preganglionic neurons (PGNs) play an important role in regulating pelvic visceral organs including bladder, distal bowel and sex organs (de Groat and Steers 1990; de Groat et al. 1981, 1982). The reflex activation of many of these neurons is controlled in part by axons descending in the lateral funiculus from various nuclei in the brain including the pontine micturition center, locus coeruleus, hypothalamus, and raphe nuclei (de Groat et al. 1993; Kuru 1965; Morrison 1987). Multiple neurotransmitters (glutamate, serotonin, norepinephrine, oxytocin, corticotropin releasing factor) are likely to be involved in the bulbospinal control of the lumbosacral PGNs (de Groat et al. 1993; Espey and Downie 1995; Giuliano et al. 1995; Loewy et al. 1979; Matsumoto et al. 1995a,b; Pavcovich and Valentino 1995; Steers and de Groat 1989; Sutin and Jacobowitz 1988; Suzuki et al. 1990, 1991; Thor et al. 1990; Valentino et al. 1994; Yoshimura et al. 1990).

For example, in the rat glutamic acid acting on N-methyl-D-aspartate (NMDA) and non-NMDA receptors is the major excitatory transmitter in the bulbospinal limb of the micturition reflex (Matsumoto et al. 1995a,b), whereas serotonin and corticotropin releasing factor may function as inhibitory transmitters in pathways controlling micturition. Supraspinal inputs to the lumbosacral parasympathetic nucleus may be mediated via direct monosynaptic connections to the PGNs or via multisynaptic connections through interneurons in the spinal cord (Blok and Holstege 1997; Holstege et al. 1986; Yoshimura et al. 1990).

This issue was examined in the present experiments by studying the excitatory synaptic currents induced in parasympathetic PGNs by electrical stimulation of axons in the lateral funiculus (LF) in the neonatal rat spinal slice preparation. The whole cell patch-clamp recording technique was used to record activity in PGNs identified by retrograde axonal tracing techniques. Previous experiments in neonatal rat pups in vivo (Kruse and de Groat 1990) and in the brain stem-spinal cord-bladder preparation in vitro (Sugaya and de Groat 1994a) revealed that bulbospinal excitatory inputs to bladder parasympathetic pathways are functional in 1- to 2-week-old animals. The present study showed that stimulation of LF axons elicited monosynaptic and polysynaptic glutamatergic excitatory responses in lumbosacral PGN. A preliminary account of some of the observations has been presented in an abstract (de Groat et al. 1998).

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METHODS

Preparation

Sprague-Dawley rats, 6–16 days old, were killed by decapitation, and the spinal cord was rapidly removed. The L₈–S₁ segments of spinal cord were embedded in 2% agar (Sigma) in a physiological salt solution (see composition of external solution in the following text) at 8°C. The spinal cord was sectioned into 150-µm transverse slices using a vibrating slicer (Vibratome, Technical Products International, St. Louis, MO). The slices were incubated at 37°C for 1 h in oxygenated external solution and then transferred to a recording chamber (0.5 ml) on an upright microscope equipped with fluorescent optics (Olympus BH-2). Slices were perfused continuously with the external solution at a rate of 1.5 ml/min. PGNs in lumbarosacral spinal cord slices were identified by retrograde axonal transport of a fluorescent dye (Fast Blue, EMS-Poly-L, GrossUmstadt, Germany) that was injected (5 µl of 4% solution) into the peritoneal space 3–12 days before the experiment. This procedure has been shown to efficiently label autonomic PGNs in the spinal cord (Anderson and Edwards, 1994).

Electrophysiological study

The basic procedures for recording whole cell currents from individual neurons in slice preparations of the cord were identical to those described by Takahashi (1990). Each slice of lumbosacral cord was surveyed for Fast Blue-containing neurons along the intermediolateral border of the gray matter using an upright microscope equipped with fluorescence optics. Motoneurons in the ventral horn were often labeled, but it was easy to distinguish between PGNs and motoneurons by their location. After identification of a cell, it was viewed with Nomarski optics, and its surface was cleaned by a stream of the external solution from a glass pipette that was positioned near the cell. Whole cell currents were recorded from the labeled neurons using an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA). The patch pipettes were made from borosilicate glass capillaries (1B150F-4, World Precision Instruments, Sarasota, FL) and had resistances of 2.5–3.5 MΩ when filled with pipette solution (see following text) and after the tip had been heat polished. Synaptic responses were evoked in PGNs by electrical stimulation with a glass micropipette filled with external solution. The stimulating pipette was placed in the lateral funiculus 100–150 µm lateral or dorsolateral to the labeled PGNs. A voltage pulse (70 µs, 0.2 Hz) of varying intensity (1–12 V) and negative in polarity relative to a reference electrode placed in the recording chamber was applied to the stimulating pipette. The latency of excitatory postsynaptic currents (EPSCs) was measured from the onset of the stimulus artifact to the onset of the synaptic currents. The time to peak was defined as the time between the start of the current inflection and the peak of the EPSCs. The time constants of the rising and decay phase of EPSCs were determined using a nonlinear simplex fit routine based on the least-squares method. Bicuculline methiodide (10 µM) and strychnine (1 µM) were applied in the perfusion solution to block GABA_A and glycine receptor-mediated synaptic inhibitory potentials (Araki and de Groat, 1996). The liquid-junction potentials between the pipette solution and the external solution (see solution in the following text) and after the tip had been heat polished. Synaptic currents. The time to peak was defined as the time between the onset of the stimulus artifact to the onset of the inward synaptic currents were evoked in dye-labeled PGN (Araki and de Groat 1996). Two types of synaptic currents were elicited by LF stimulation (Fig. 1). The most common response (type 1, n = 33 cells) consisted of a short, relatively constant latency (range, 1.4–4.2 ms) large-amplitude (59.0 ± 7.9 pA, n = 33) inward current (fast EPSC) followed by a low-amplitude more prolonged current whose amplitude usually was 10% or less than that of the initial current (Fig. 1A). Averaging of multiple evoked responses yielded traces (Fig. 1Ab) that closely resembled individual recordings (Fig. 1Aa). In the 33 cells, the maximal EPSCs had an average latency of 3.0 ± 0.12 ms and a time to peak of 1.4 ± 0.1 ms. A less common response (type 2, n = 8 cells) consisted of a short, relatively constant latency (2.2–3.8 ms), large-amplitude fast EPSC followed by large-amplitude inward current responses that occurred at variable latency (7.2–30.9 ms; Fig. 1Ba). The average of a large number of EPSCs revealed a very prolonged current (Fig. 1Bb).

The relationship between stimulus intensity and response was evaluated in detail in 11 cells. The responses occurred at threshold stimulus intensities ranging from 1 to 3 V in different PGNs. In some cells (n = 7), low stimulus intensities evoked all-or-none synaptic responses (Fig. 2Ab). In these cells, when the stimulus intensity was gradually increased the EPSCs suddenly appeared with frequent failures. When the stimulus intensity was increased, further the failures

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became infrequent. The amplitudes of individual EPSCs fluctuated but their mean value in individual cells was virtually constant (ranging from 14 to 92 pA) in a limited range of stimulus strengths (1.2 to 2.5 T, T indicates the threshold) above the threshold (Fig. 2B). In some cells (n = 4), the magnitude of the evoked synaptic currents was not all-or-none; instead it gradually increased with increasing stimulus intensities and reached a maximum (34–155 pA) at two to four times (5–10 V) the threshold voltages (Fig. 2, C and D). In three neurons exhibiting all-or-none responses, the EPSC latencies (1.7–2.6, 0.7–1.3, and 2.7–3.1 ms) and time to peak (1.0–1.3, 1.7–2.2, and 0.9–1.2 ms) had unimodal distributions in a narrow range. Data from one neuron, which is illustrated in Fig. 3, shows an average latency of 2.1 ms and time to peak of 1.1 ± 0.02 ms.

**Glutamatergic EPSCs and their time course**

The fast component of LF stimulation-evoked type 1 synaptic currents recorded at a holding potential of −60 mV was attenuated by CNQX (5 μM, n = 7), a specific antagonist of non-NMDA receptors (Fig. 4Ab). The late component of EPSCs remaining after addition of CNQX was completely blocked by APV (50 μM, n = 4), a specific antagonist of NMDA receptors (Fig. 4Ac). The effects of glutamatergic-receptor-antagonists were reversed 15–20 min after washout (Fig. 4Ad). The fast component of LF-evoked type 2 synaptic currents recorded at a holding potential of −60 mV was blocked by CNQX (5 μM, n = 3; Fig. 4Bb). The late component of EPSCs remaining after addition of CNQX was completely blocked by APV (50 μM, n = 3; Fig. 4Bc).

The time course of synaptic currents was measured on averaged responses of 30 individual EPSCs from 33 cells clamped at −60 mV. The fast rising times to peak and decay time constants of the EPSCs mediated by non-NMDA receptors were 2.5 ± 0.2 and 5.7 ± 0.6 ms, respectively. The decay

![FIG. 1. Two types of excitatory postsynaptic currents (EPSCs) were recorded from preganglionic neurons (PGNs) in response to electrical stimulation of the lateral funiculus (LF). The holding potential was −60 mV. A: the most common type of evoked response (type 1) consisted of a short latency, fast component followed by a slow component. Aa: four consecutive responses. Ab: a current trace showing the average of 30 responses to LF stimulation in the same neuron as in Aa. Stimulus intensity, 5 V. B: a less common type of evoked response (Type 2). Ba: four consecutive responses. Bb: a current trace showing the average of 30 responses to LF stimulation consisting of an initial short latency response followed by a slow component with superimposed fast currents in the same neuron as in Ba. Stimulus intensity, 5 V; stimulus duration, 70 μs; stimulus frequency, 0.2 Hz; ••••, baselines.

![FIG. 2. Type 1 EPSCs evoked at different intensities of LF stimulation. The holding potential was −60 mV. A: EPSCs evoked by 1.5 V (Aa), 2 V (Ab), 5 V (Ac), and 7 V (Ad). Four consecutive responses at each stimulus intensity (V) showing unitary EPSCs. B: relationship between stimulus intensity and mean peak amplitude of 30 EPSCs in the same cell as in A. C: four consecutive multiunit EPSCs elicited by different intensities of stimulation (Ca, 1.5 V;Cb, 3 V;Cc, 5 V; and Cd, 7 V). D: relationship between stimulus intensity and mean peak amplitude of 30 EPSCs in the same cell as in C. Vertical bars, means ± SE.

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time constant of the EPSCs mediated by NMDA receptors was 38.8 ± 4.0 ms.

Voltage dependence of EPSCs

The current-voltage relationships of the non-NMDA and NMDA components of evoked EPSCs were examined by measuring the peak amplitude of the EPSCs at 4–8 and 25 ms, respectively, after the stimulus. The early current at 4–8 ms was assumed to reflect mainly the non-NMDA component because the EPSCs mediated by NMDA receptors exhibited a slow time to peak and small amplitude and should make a very small contribution to the EPSCs at this time point. On the other hand, the non-NMDA component would make little contribution to EPSCs at 25 ms after the stimulus (Araki and de Groat 1996). Thus the amplitude of EPSCs at this time point was assumed to reflect the NMDA component (Araki and de Groat 1996; Hestin et al. 1990; Keller et al. 1991). The current-voltage relationship of the non-NMDA component had a linear conductance, whereas that of NMDA component at 25 ms after the onset of response had a negative slope conductance at a hyperpolarized holding potential (more negative than −20 mV) (Fig. 5). The interpolated reversal potentials of non-NMDA and NMDA currents were 5.0 ± 5.9 mV (open circle) and 3.7 ± 3.2 mV (filled circle; n = 5), respectively (Fig. 5B).

Synaptic modulation using paired pulse stimulation

Paired-pulse modulation of type 1 EPSCs was examined in 15 PGN by applying two successive stimuli to the lateral funiculus with interstimulus intervals ranging from 50 to 120 ms at a holding potential of −60 mV. At intervals of 50, 70, 100, and 120 ms, the responses to the second stimulus were considerably smaller (Fig. 6), the percentage of inhibition of the peak amplitude of the second EPSCs being 67.5 ± 7.9%, n = 4; 69.0 ± 8.6%, n = 9; 76.3 ± 7.8%, n = 6 and 63.3 ± 3.2%, n = 11, respectively, excluding four cells of which two showed a small facilitation (mean, 19.5%, n = 2) and two showed no modulation. There were no significant differences in the percentage inhibition at different stimulus intervals.

DISCUSSION

The present experiments revealed that a large percentage (at least 40%) of parasympathetic PGNs in the lumbo-sacral spinal cord of the neonatal rat receive glutamatergic excitatory synaptic inputs from axons of the lateral funiculus. These excitatory inputs activate NMDA and non-NMDA glutamatergic receptors. Latencies of EPSCs revealed two distinct pathways: a pathway evoking relatively short and fixed latency EPSCs probably mediated by monosynaptic or disynaptic projections and a pathway mediating longer and more variable latency EPSCs probably representative of polysynaptic projections. The contribution of bulbospinal and propriospinal axons to these LF-evoked responses are not known but will be evaluated in future experiments.

In some PGNs, LF stimulation evoked what appeared to be all-or-none EPSCs. These responses, which varied in mean amplitudes from 14 to 92 pA (average, 59.0 ± 7.9 pA), presumably represent “unitary EPSCs” evoked by a single LF axon. However, it is also possible that near all-or-none behavior might reflect activation of two axons with very similar electrical thresholds. The fairly large latency fluctuations (e.g., 1.7–2.6 ms) observed with some unitary responses would be consistent with this possibility. Unitary glutamatergic EPSCs evoked by stimulation of single interneurons in the region of the sacral parasympathetic nucleus ranged from 36 to 88 pA (Araki and de Groat 1996).
evoked in PGNs by stimulation of dorsal or medial interneurons (Araki and de Groat 1996) and is comparable with that described in rat sympathetic preganglionic neurons (Krupp and Feltz 1995). The mean of the fast decay time constant of averaged EPSCs, representing the non-NMDA response, was comparable with the value for fast EPSCs evoked in rat sympathetic preganglionic neurons by stimulation of intraspinal axons (Krupp and Feltz 1995) but was longer than the value for the EPSCs evoked in PGNs by stimulation of interneurons (Araki and de Groat 1996). The mean of the slower decay time constant of averaged EPSCs, representing the NMDA receptor-mediated response, was shorter than that reported for the EPSCs evoked in preganglionic neurons by stimulation of axons or interneurons (Araki and de Groat 1996; Krupp and Feltz 1995). However, the current-voltage relationships for LF stimulation-induced glutamatergic EPSCs corresponded to that of other synapses (Jonas et al. 1993) including interneuronal-PGN synapses in the neonatal spinal cord (Araki and de Groat 1996).

One prominent difference between LF and interneuronal inputs to PGN was obvious using paired-pulse stimulation. During electrical stimulation of interneurons in spinal cord slices (Araki and de Groat 1996), paired-pulse facilitation was very prominent (mean, 75% increase in EPSC amplitude) in the dorsal interneuronal pathway and somewhat less prominent (mean 25% increase) in the medial interneuronal pathway. In the present study, paired-pulse inhibition of EPSCs rather than facilitation was obtained during electrical stimulation of lateral funiculus in a large percentage (75%) of PGN. This difference might be related to several factors. First bulbospinal excitatory pathways might be immature in the neonatal rat; therefore synaptic facilitatory mechanisms might be nonfunctional. This is certainly possible in the descending limb of the micturition reflex pathway, which does not become active until the third postnatal week (Araki and de Groat 1997). Development of temporal facilitation in the bulbospinal pathway might be one mechanism which contributes to the emergence of the supraspinal micturition reflex in older rat pups.

A second factor that could contribute to difference between LF and interneuronal paired pulse stimulation is the presence of multiple transmitter systems in the LF. Simultaneous activation of monoaminergic (norepinephrine and 5-HT) or peptidergic axons along with LF glutamatergic axons could produce homosynaptic or heterosynaptic modulation of glutamatergic transmission and thereby elicit paired pulse inhibition. CRF is thought to be an inhibitory co-transmitter in the descending glutamatergic excitatory pathways from pontine micturition center to the lumbosacral parasympathetic nucleus (de Groat et al. 1993; Pavcovich and Valentino 1995; Sawchenko et al. 1993; Suzuki et al. 1990, 1991). Serotonergic mechanisms are also likely to be inhibitory in the micturition reflex pathways (de Groat 1978; Steers and de Groat 1989; Testa et al. 1999) but facilitatory or inhibitory in penile erectile pathways (Giuliano et al. 1995; Steers and de Groat 1989) depending on the type of serotonergic receptor activated (Moreland et al. 2000).

The present study revealed that presumed LF monosynaptic inputs have a longer latency (3 ms) than interneuronal monosynaptic inputs (2.1 ms) (Araki and de Groat 1996). This difference might be due to slower conduction velocity of LF axons or a difference in conduction distance. It might also

![Diagram](image-url)
reflect immaturity of bulbospinal pathways in comparison to interneuronal pathways (see following text).

In the present study, about 60% of PGNs did not respond to electrical stimulation of lateral funiculus. This result might reflect several factors. First, certain populations of PGNs might not receive direct bulbospinal inputs. For example, although the descending pathway from pontine micturition center to PGNs is wired up in the first postnatal week, it does not become functional in controlling the urinary bladder until the second to third postnatal week (Kruse and de Groat 1990; Sugaya and de Groat 1994b). In neonates, micturition and defecation are mediated by a segmental parasympathetic reflex pathway, which is activated by the mother licking perineum of the pup (Beach 1966; de Groat et al. 1975). Starting 3 weeks after birth, this segmental somato-parasympathetic reflex is gradually replaced by a spino-bulbo-spinal micturition reflex pathway, which is essential for voiding in adult animals (Araki and de Groat 1997). Thus many LF inputs to bladder PGNs may be “silent” in the early postnatal period. PGNs involved in other functions might receive excitatory bulbospinal inputs even later in developments (e.g., during sexual maturation), and others such as colorectal PGNs, which are regulated by spinal reflex mechanisms (de Groat et al. 1981) might not receive direct excitatory inputs from bulbospinal or proprio-spinal axons. Another factor contributing to the low percentage of PGNs responding to LF stimulation is that collaterals of rostrocaudal axons in the funiculus might project medially into the PGNs on an angle rather than perpendicularly to the long axis of the cord. Thus many of the medially projecting axons in the 150-μm slice might be transected before entering the PGN. The use of thicker slices could solve this technical problem.

In conclusion, the present experiments provided evidence that PGN in the lumbar sacral parasympathetic nucleus of the neonatal rat receive glutamatergic monosynaptic and polysyn-
aptic excitatory inputs from axons in the lateral funiculus. These inputs are mediated by NMDA and non-NMDA receptors similar to glutamatergic inputs from local interneurons. However, in contrast to interneuronal synapses, LF synapses exhibited paired-pulse inhibition instead of facilitation. This difference might reflect the relative immaturity of bulbospinal autonomic pathways in 1- to 2-week-old rats.

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REFERENCES


