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Hakozaki A[1], Imoto K[1], Hayashi Y[2], Sasaki E[2], Kawatani M[3], Furue H[1]
1. *Dept. Info. Physiol., NIPS,* ***2.*** *TAIHO pharm. co., ltd.,* ***3.*** *Dept. Neurophysiol., Akita Univ Grad Sch Med*

SYNAPTIC RESPONSES EVOKED IN PARASYMPATHETIC PREGANGLIONIC NEURONS BY STIMULATION OF PRIMARY AFFERENT FIBERS INNERVATING THE BLADDER.

Hypothesis / aims of study

Parasympathetic preganglionic neurons (PGN) are located in the intermediolateral grey matter (laminae V-VII) of lumbosacral spinal cord and play an important role on control of pelvic visceral organ's activates including urinary bladder function. Previous anatomical studies have suggested that primary afferents innervating the bladder make a mono- or polysynaptic connection to the PGN neurons in the lumbosacral spinal cord (1). Little is known, however, how primary afferents from the bladder excite PGN neurons through the synaptic connection from the bladder. In this study, we investigated synaptic responses evoked in the PGN using whole-cell patch-clamp recording techniques (2).

Study design, materials and methods

Cystometry and immunohistochemistry: Female rats were anesthetized with intraperitoneal injection of urethane (1.2-1.5 g/kg). The urinary bladder was catheterized through the urethra to infuse fluids and to measure the bladder pressure under the conditions that the urethral outlet remained open. Intravesical pressure was measured using a pressure transducer. Saline or acetic acid was infused (3 mL/hr) to the bladder via urethral catheter. After the irritant was applied for two hours, the animals were immediately fixed via intracardiac perfusion by 4% PFA/0.1 M PB. Alternate sections (50 μm) of the spinal cord (L3-S1) were processed for immunohistochemistry to c-fos protein.

Patch-clamp recordings: Two weeks before electrophysiological experiments FAST DiI, a retrograde neuronal tracer, was slowly injected into the ischiorectal fossa bilaterally and between the anus and the external urethral orifice under 2-2.5% isoflurane anesthesia. Then the rats were deeply anesthetized with urethane. The spinal cold at the spinal level L1-S3 was removed and immediately put into a cold Krebs solution equilibrated with 95% oxygen-5% carbon dioxide, and a transverse slice of the L6 spinal cord was made. DiI-positive neurons were visualized with a fluorescence microscopy equipped with the Nomarski differential interference contrast. Whole-cell patch-clamp recordings were made from DiI-positive neurons in the PGN. A blind whole-cell patch-clamp recording was also made from PGN neurons to record synaptic responses evoke by primary afferent stimulation.

Results

To identify which laminae in the spinal cord is responsible for bladder activity, we first detected spinal c-fos protein expressions after bladder constriction in response to acetic acid. Acetic acid instillation into the bladder decreased the intercontraction interval to 94.3 sec from control interval 975.4 sec. The large number of c-fos positive cells in lamina I-II, V-VII included PGN and X of L6-S1 spinal cord was detected. The neurons positive to DiI injected near the bladder were also observed in lamina V-VII and IX including Onuf's nucleus. These neurons were expressed a choline acetyltransferase. Then we recorded from DiI positive cells that presumably innervate the pelvic neuron in the intermediolateral grey matter (n = 22 cells). In response to prolonged depolarizing current pulse (1 s), the neurons exhibited two types of firings, tonic or phasic firing patterns. Electrical stimulation of primary afferents fibers attached spinal cord slices evoked excitatory postsynaptic currents (EPSCs) in PGN neurons. PGN neurons received monosynaptic Aδ and C fiber-evoked EPSCs. The evoked EPSCs were supressed by CNQX (10 μM), an ionotropic glutamate receptor antagonist.

Interpretation of results

The present results suggest that PGN neurons receive monosynaptic and/or polysynaptic glutamatergic inputs through Aδ and C fibers and exhibit tonic and phasic firing properties..

Concluding message

The monosynaptic excitatory synaptic responses evoked by Aδ and C fibers may be responsible for the urinary frequency and urgency urinary incontinence in overactive bladder patients.

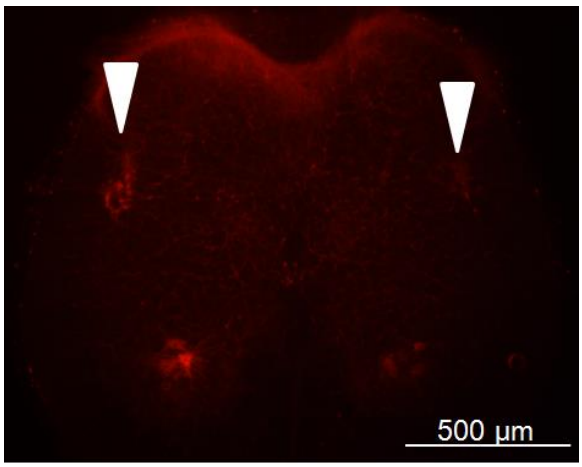

Fig. parasympathetic preganglionic neurons stained with DiI injected near the bladder (arrow heads)

References
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