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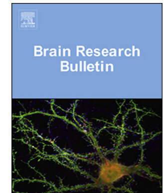

Research report

Electrophysiological responses of the ventrolateral periaqueductal gray matter neurons towards peripheral bladder stimulation

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ABSTRACT

Introduction: Many of the currently available therapies for urinary incontinence target the peripheral autonomic system, despite many etiologies residing in the central nervous system. Following previous experiments that determined the ventrolateral column of the periaqueductal gray matter (vlPAG), to be the main afferent station of bladder sensory signals, we aimed for electrophysiological characterization of vlPAG neurons using single unit recording.
Methods: 15 rats were anesthetized and underwent implantation with electrodes at the dome and the neck of the bladder, to electrically stimulate the detrusor. After craniotomy, a glass micropipette was inserted in vlPAG to record neuronal action potentials. The detrusor was stimulated by a series of 20 Hz pulses, for a total duration of 50 s at an intensity of 2 mA, for each vlPAG neuron selected. Single unit recordings were performed on a total of 26 neurons. Confirmation of electrode position was made by iontophoretic ejection of Pontamine sky blue.
Results: The firing rate of vlPAG neurons decreased significantly during the stimulation period. Peristimulus time histogram (PSTH) analysis showed 24 out of 26 neurons to be unresponsive to stimulation. All recorded vlPAG neurons showed irregular firing patterns.
Conclusions: The change in firing rate may point to an overall inhibitory influence of bladder stimulation on vlPAG neurons. These data suggest an inhibitory relay station at the vlPAG, before sensory bladder signals would affect pontine micturition center. The lack of the inhibitory effect on PSTH may be due to a longer interval between neuronal response and the stimulation.

1. Introduction

Even though there is evidence that the central nervous system (CNS) plays a critical role in the causality of urinary incontinence, most therapeutic approaches focus on peripheral (nervous) targets (i.e. the muscarinic acetylcholine receptor) (Emily et al., 2018). Several structures within the CNS have been identified to play a role in the regulation of urine storage and voiding. Examples are the pontine micturition center, parts of the limbic system such as the anterior cingulate gyrus, and parts of the spinal cord such as the sacral nucleus of Onuf. These structures form a complex network, which receive afferent sensory information and provide efferent motor signals to the bladder.

One important structure in bladder control is the periaqueductal gray matter (PAG). The PAG is a midbrain structure, which is involved in the sensori-motor regulation of bladder function. Besides, it plays a role in autonomic (Rathner and Morrison, 2006; Farkas et al., 1998) and emotional (Mouton and Holstege, 1994) control and nociception (Budai et al., 1998). The PAG is composed of separate columns (Bandler and Shipley, 1994; Parvizi et al., 2000). Various studies mentioned the vlPAG as the column associated with bladder function (Mitsui et al., 2003; Taniguchi et al., 2002; Duong et al., 1999; Matsuura et al., 1998). This has been proven by methods probing both ascending and descending tracts. Noxious stimuli at the level of the bladder increased c-Fos expression in vlPAG in rats (Mitsui et al., 2003). On the other hand,

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electrical or chemical stimulation of the vlPAG in rats resulted in contraction of the bladder or increased micturition frequency (Taniguchi et al., 2002; Stone et al., 2015). Likewise, injection of inhibitory mediators into the vlPAG attenuated bladder contractions and external urethral sphincter electromyographic activity in rats (Matsuura et al., 1998, 2000). Nevertheless, these experiments which stimulate the PAG and record the response in the bladder, have a major shortcoming. Stimulation of PAG has a broad spectrum of effects over autonomic and behavioral systems, which may mask true bladder-specific functionality (Nashold et al., 1969; Jenck et al., 1995). PAG activation has also been shown by fMRI studies in both storage and voiding phases of micturition (Tai et al., 2009). It can be stated, that there is a reciprocal communication pathway between the PAG and the bladder.

There is evidence for the efficacy of sacral neuromodulation for the treatment of functional pelvic disorders, like voiding dysfunction (Sukhu et al., 2016; Rosen et al., 2015; Pettit et al., 2002). In order to explore novel targets for central neuromodulatory approaches and optimize stimulation parameters to restore normal function, it is essential to unravel the functional connectivity of brain structures such as the PAG with the bladder. In our previous studies, we specified particular PAG columns which were activated after bladder stimulation, and defined specific bladder stimulation parameters (Meriaux et al., 2018). We selected stimulation parameters which would induce the micturition reflex, i.e. increase intravesical pressure, and would not arouse pain in the animal (Meriaux et al., 2018). Immunohistochemistry showed that, by using these stimulation parameters, sensory neurons were activated in the spinal cord (Meriaux et al., 2018). In the next step, we identified the cell types of the activated neurons within vlPAG (Zare et al., 2018).

After the identification of a sensory vlPAG-bladder relationship in the previous experiments we aimed at identifying the functional relationship between these two. We did so by evaluating the neurophysiological response of the vlPAG by means of extracellular single unit recordings in anesthetized rats. We hypothesized that vlPAG neurons would react to bladder stimulation, by changing their firing rate.

2. Materials and methods

2.1. Surgical preparation

Fifteen male Sprague Dawley rats (350 g) were anesthetized by intraperitoneal administration of urethane (7.5 ml/kg loading dose and 0.3 ml/repetitive dose for maintenance) from a 20% of weight urethane solution (Sigma Aldrich). Body temperature was controlled and maintained at 37 °C by means of a heating pad. A midline suprapubic incision was made and the bladder was exposed. Two unipolar ventricular pacing electrodes intended for human use (Medtronic®, Minneapolis, USA, STREAMLINE™) were fixed by means of a suture at the dome and the neck of the bladder (Fig. 1). The electrodes were then connected to a stimulator (DLS100, World Precision Instruments, Sarasota, Florida) attached to a pulse generator (DS8000, World Precision Instruments). The rats were then mounted on a standard rat stereotaxic apparatus (Stoelting Co, Illinois, USA). A craniotomy was performed on top of the vlPAG region according to the coordinates relative to Bregma as described in a standard rat brain atlas (−6.96 mm upto −8.16 mm rostrocaudal relative to Bregma, and a maximum of 1.2 mm mediolaterally and bilateral) (Paxinos and Watson, 2007). Considerable care was taken to avoid hemorrhage from the sagittal sinus during the durotomy. To achieve this, a fine needle tip (30 G) was used to lacerate the edges of craniotomy window away from the sinus. Paw withdrawal and corneal reflexes were frequently checked throughout the experiment and additional urethane was injected if required.

2.2. Recording

We used Kwik-Fil™ filamented borosilicate glass capillaries (outer diameter: 1.5 mm, inner diameter: 0.84 mm)(World precision instruments, Sarasota, Florida) to record extracellular single units. The glass micropipette was pulled by a pipette puller (Narashige, Japan). Consequently, the pulled tip of the pipette was broken to a diameter of 1 to 2 μm under microscopic vision. The micropipette was backfilled with 0.5 M NaCl solution and inserted into the vlPAG to record extracellular single units. The glass pipette was descended slowly from the cortical surface as a reference point in the ventral direction by means of a hydraulic drum Microdrive (FHC, Bowdoin, ME USA) until the coordinates of the PAG in the dorsoventral direction were reached. Adequate grounding and referencing was implemented in order to reduce (environmental) noise. The micropipette was subsequently connected to an electrophysiology workstation (Alpha Omega Engineering, Nazareth, Israel). Recordings were performed on 26 neurons in total, in 15 rats, within the area of the vlPAG. A baseline recording took place after the neuron had stabilized. Subsequently recording took place until 600 action potentials had been recorded to correct for variance in firing frequency of individuals neurons. Then the detrusor was stimulated by a biphasic pulse of 20 Hz (a pulse period of 50 ms) and a pulse width of 0.5 ms (a duty cycle of 0.01) for 50 s, at an intensity of 2 mA for each neuron. These stimulation parameters were based on our previous experiments (Meriaux et al., 2018) and aimed specifically at inducing the micturition reflex. Neurons, that were lost during this period were excluded from the analysis.

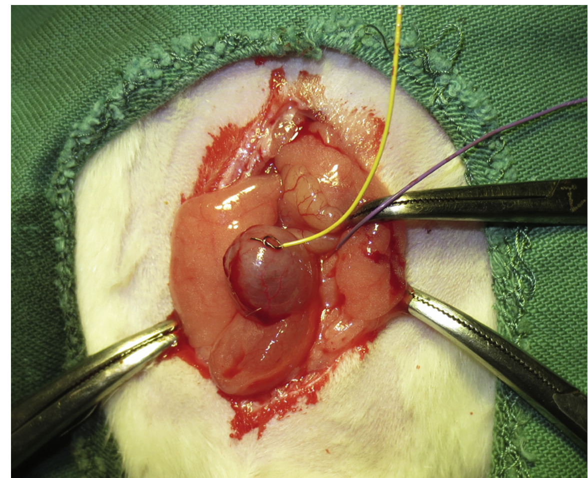

Fig. 1. Electrode implantation on rat urinary bladder. One is implanted at dome and one at the neck of the bladder to give a bipolar electric field spanning the detrusor.

2.3. Iontophoresis

After the final recording, iontophoretic injection of 4% Pontamine sky blue (SIGMA-ALDRICH) diluted in 0.5 M of NaCl was performed with the following parameters: direct current, −20 μA, with a pulse period of 14 s and a pulse width of 7 s (50% duty cycle). The current was set for 15 min using the pipette as anode (DS8000 stimulator, A360 isolator, World Precision Instruments, Sarasota, Florida) in order to verify the recording area in all animals. To give bipolar stimulation, the anode consisted of tungsten electrode inserted into the capillary glass filled with Pontamine sky blue solution, and the cathode as a stainless steel screw secured on top of the skull (1 × 2 mm, GHW Modellbau, Germany).

2.4. Histological confirmation of the electrode location

Finally, rats were decapitated, the brain was quickly removed and was frozen in -40 °C 2-methyl-butane (isopentane) for further histochemical confirmation of electrode position. Brains were serially cut by

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