DGC stimulation results in urethral relaxation

Chapter 4

Electrical Stimulation of the Sacral Dorsal Gray Commissure evokes Relaxation of the External Urethral Sphincter in the Cat

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ABSTRACT
Stimulation of the pontine micturition center (PMC) results in micturition, i.e. an immediate relaxation of the urethral sphincter and a contraction of the detrusor muscle of the bladder. The PMC generates the bladder contraction by way of a direct excitatory pathway to the parasympathetic bladder motoneurons in the sacral cord. The idea is that the PMC produces the relaxation of the urethral sphincter via direct projections to GABA-ergic neurons in the dorsal gray commissure (DGC), which, in turn, inhibit the urethral sphincter motoneurons. According to this hypothesis electrical stimulation in the dorsal gray commissure in three cats should result in a relaxation of the urethral sphincter. The results were in total agreement with this concept. During DGC stimulation a sharp decrease of the urethral pressure was found, the strength of which depended completely on the amplitude of the electrical stimulation.

INTRODUCTION
Micturition depends on a coordinated action of the smooth detrusor muscle of the bladder and its striated external urethral sphincter (EUS). During urine storage, the bladder is relaxed and the EUS is contracted. During micturition a reversed pattern is observed; the bladder contracts and the EUS is relaxed.

The bladder and the EUS are innervated by respectively sacral preganglionic parasympathetic motoneurons (Morgan et al., 1979) and motoneurons in the ventrolateral part of the nucleus of Onuf (Sato. During micturition the activity of these two motoneuronal cell groups is coordinated by the pontine micturition center (PMC) in the brainstem (Holstege et al., 1986; Blok et al., 1997; Blok et al., 1998). Tracing studies in the rat (Loewy et al., 1979) and cat (Holstege et al., 1979; Holstege et al., 1986; Blok and Holstege, 1997; Blok et al., 1997) have shown that the PMC projects to the parasympathetic bladder motoneurons in the sacral intermediolateral cell group (IML), as well as to the dorsal gray commissure (DGC) or intermediomedial cell column (IMM). However, the PMC does not project to the nucleus of Onuf (Holstege et al., 1979; Holstege et al., 1986). Ultrastructural studies have shown that the PMC-fibers to the IML terminate directly on the preganglionic bladder motoneurons. This projection is excitatory, not only because PMC stimulation results in bladder contraction (Holstege et al., 1986), but also because the PMC terminals on bladder motoneurons contain asymmetric clefts and round vesicles (Blok and Holstege, 1997).

Similar excitatory projections were found to the GABA-ergic interneurons in the dorsal gray commissure (DGC) (Blok et al., 1997). Many of the local neurons projecting to Onuf’s nucleus are located in the DGC (Koschang et al., 1985; Nadelhaft and Vera, 1996) and Onuf’s nucleus receives a substantial afferent input from GABA-neurons.
in the lumbosacral cord (Ramirez-Léon et al., 1994), most of which are located in the DGC (Alvarez et al., 1996). Combining these anatomical findings with the observation that stimulation of the PMC not only produces a steep rise in the intravesical pressure, but also a decrease in the intraurethral pressure (Holstege et al., 1986), leads to the following concept of micturition control. The bladder contraction during micturition is the result of direct PMC projections to the parasympathetic bladder motoneurons, and the decrease of intraurethral pressure during micturition is the result of the PMC projections to the GABA-neurons in the DGC. If this concept is true, electrical stimulation of the DGC would result in a decrease of the urethral sphincter pressure as during micturition or stimulation of the PMC. Therefore, the DGC was electrically stimulated and the bladder and urethral sphincter pressures were measured.

MATERIALS AND METHODS
The surgery procedures, pre- and postoperative care, and handling and housing of the cats were in accordance with protocols approved by the Committee on Animal Experiments of the Faculty of Medicine of the University of Groningen. During surgery, heart rate and body temperature were monitored. The animals (weight 4.0 and 4.4 kg) were initially sedated with ketamin (30 mg/kg) intramuscular and anaesthetized with a mixture of halothane, nitric oxide and oxygen. A laminectomy of the lumbar vertebrae was made and the lumbosacral enlargement with its caudal extent was exposed. The intravesical and intraurethral pressures were measured with two microtip transducers.
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mounted on a single 6 French catheter with a lumen (Raumedic, Germany). The distance between the two transducers was 3.5 cm. The transducer at the tip of the catheter was placed intravesically, the second transducer was placed at the point where the intraurethral pressure was at its highest. The bladder was emptied and subsequently filled with 30 ml of saline at body temperature. The spinal cord was electrically stimulated with a monopolar stainless steel electrode with an exposed tip of 0.25 mm. The stimulus consisted of a 2 sec long pulse train of negative going 80 µA pulses with pulse width 25 ms and pulse rate 20 pps. The dura was opened and the caudal lumbar and sacral segments of the spinal cord were exposed. The stimulation was started dorsally just through the pia in the gracile column and the electrode advanced in successive steps of 0.3 mm. At each location the tissue was stimulated with one or more pulse trains. Computer recordings of the intravesical and intraurethral pressures were made during a time period beginning 5 sec before the first stimulation until 15 sec after the last stimulation. The output of the pressure transducers was amplified with Neurolog pressure amplifiers (NL108, Digitimer Ltd., England), low-pass filtered (third order Bessel filter with cut-off frequency at 7.9 Hz) and digitized at a rate of 40 samples/channel/s with a CED 1401plus (Cambridge Electronic Design Ltd, England). Data acquisition and off-line analysis were performed with Spike 2 software (Cambridge Electronic Design Ltd, England) on a Macintosh computer. At the location at which a strong decrease of the intraurethral pressure could be evoked 50 nl of 2% pontamine sky blue was injected with a picopomp. Immediately after the experiments, the animals were deeply anaesthetized with 6% sodium pentobarbital, perfused intracardially with 1.5 l heparinized phosphate buffered saline (PBS) (pH 7.4) at room temperature, followed by 1.51 fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4, room temperature). The lumbosacral spinal cords were removed and placed in the same fixative for 2-3 hours at 4°C. The tissue was dehydrated overnight in 25% sucrose in 0.1 M phosphate buffer (pH 7.4), and the next day the tissue was frozen to -55°C in a isopentane bath and cut in 40 µm sections on a cryostat. Sections of 40 µm thick were made of the spinal segments L7, S1, S2 and S3, and the sections were studied light microscopically with a Zeiss axioplan.

RESULTS

Electrical stimulation in the medial part of the fasciculus gracilis almost invariably resulted in a strong increase of the intraurethral pressure without a significant effect on the bladder pressure (Fig. 1). This effect was not only obtained at sacral levels but also at lower lumbar levels (L5, L6 and L7). When the electrode was advanced further ventrally and reached the DGC, a completely reverse effect was observed. A strong and immediate decrease of the intraurethral pressure was observed without a significant effect on the bladder pressure (Fig. 1). This effect was observed in the caudal L7, and in S1, S2 and S3 segments, but not at levels rostral to caudal L7. In S1 the decrease in urethral pressure was observed over a dorsoventral distance of 1.2 mm. In the S2 and S3 segments this effect was observed at a wider range corresponding with a dorsoventral distance of 1.7 mm. The intraurethral pressure immediately decreased, at the beginning of the stimulus, remained decreased during stimulation, independent of the length of stimulation, but increased sharply to resting levels when the stimulus was stopped. Occasionally, stimulation of the ventral funiculus ventral to the DGC resulted in an increased intraurethral pressure. The halothane anesthesia was of great influence on the stimulation responses. When the animal was deeply anesthetized the intraure-
thral pressure was low (1-2 kPa) and did not show pressure variations. Under such circumstances a decrease of the intraurethral pressure could not be observed during stimulation in the DGC.

**DISCUSSION**
The results are in agreement with the concept that the PMC produces micturition via a direct excitatory connection both with the bladder detrusor muscle motoneurons and with the GABA-ergic inhibitory interneurons in the DGC. Previous investigations on sacral microstimulation did not report a locally evoked decrease in intraurethral pressure (Jonas et al., 1975), possibly because the stimulation was aimed at the IML and not at the DGC.

Theoretically, it is possible that the GABA-ergic DGC cells, which receive afferents from the PMC do not project to Onuf’s nucleus. However, this seems extremely unlikely, because almost all local premotor interneurons, independent of whether they are GABA-ergic, projecting to Onuf’s nucleus are located in the DGC, and a few in the IML. Other sources of GABA-ergic innervation of the Onuf’s nucleus do not exist, because it is not affected by spinal cord transection rostral to the sacral cord or dorsal rhizotomy (Ramirez-Léon et al., 1994). In conclusion, the present results give further support to the concept that the PMC excites the bladder via a direct pathway to their parasympathetic preganglionic motoneurons, and inhibits the bladder sphincter via an excitatory projection to the GABA-ergic premotor interneurons of the urethral sphincter motoneurons in Onuf’s nucleus (Fig. 2).

Fig. 2. Schematic overview of the pathways involved in the control of the bladder and bladder sphincter motoneurons during micturition. Pathways are indicated on one side only.