Prolonged Modulation of the Micturition Reflex by Electrical Stimulation

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My home town!

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Dedicated to
My Father and Mother

谨献给
我敬爱的父亲和母亲
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ABSTRACT

Intravesical electrical stimulation (IVES) has been used in treatment of patients with urinary bladder dysfunctions for more than four decades. While some investigators have reported excellent results others have observed less convincing effects or outright failures. The discrepancies may reflect differences in patient selection or stimulation procedure. A better theoretical understanding of the IVES working mechanism might help to improve the success rate of the treatment. The aims of the present study were to provide such information.

Experiments were performed on adult female cats and rats under α-chloralose anesthesia. IVES was delivered by a catheter electrode in the bladder. At proper intensity and frequency, IVES evoked reflex detrusor contractions that were abolished by bilateral rhizotomy of sacral dorsal roots. Stimulation parameters and response characteristics revealed that bladder mechanoreceptor Aδ afferents were activated by the IVES, the same afferents that drive the normal micturition reflex.

Five minutes of continues IVES at 20 Hz induced a prolonged, significant decrease in the micturition threshold volume of anesthetized rats. Similarly, selective bladder Aδ afferent stimulation induced a long-lasting enhancement of micturition reflex discharges in cats. A comparable prolonged inhibitory effect on the micturition reflex was demonstrated after ano-genital afferent stimulation. Both modulatory effects occurred without changes in response sensitivity of stimulated afferents. The IVES induced modulation was prevented by transient exposure of the bladder to a local anesthetic and by systemic administration of a glutamate NMDA receptor antagonist.

In conclusion, IVES induces a prolonged modulation of the micturition reflex by an LTP like enhancement of excitatory synaptic transmission in the central micturition reflex pathway. The findings provide an experimental explanation for the neuronal mechanisms underlying the curative effect of IVES in patients with bladder evacuation problems.
LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


<table>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CPPene</td>
<td>3-(2-carboxypiperazin-4-yl)-1-propenyl-1-phosphonic acid</td>
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<td>IVES</td>
<td>Intravesical electrical stimulation</td>
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<td>LTD</td>
<td>Long term depression</td>
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<td>LTP</td>
<td>Long term potentiation</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartic acid</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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INTRODUCTION

The functions of the lower urinary tract are to store urine for periods of time to allow daily activities and to release the urine at behaviorally suitable times and places. These seemingly simple functions require a rather complicated neuronal control system, which involves sensory receptors in the bladder wall, a supra-spinal reflex loop for the micturition reflex and several segmental and supra-spinal inhibitory pathways (Barrington, 1925; Kuru, 1965; de Groat, 1975; Torrens & Morrison, 1987; Yoshimura & de Groat, 1997). Unfortunately, the complexity of these neuronal circuits makes the system rather vulnerable. Even subtle neuronal lesions or dysfunctions may result in either defective voiding or incontinence. This may explain why micturition disorders have a high prevalence and involve tremendous costs for the society. In USA alone, approximately 13 million people suffer from urinary incontinence at an annual cost of about $16 billion (Payne, 1998). The situation is comparable in Sweden where the cost of incontinence was estimated to about 2 billion SEK in 1992 (about 2% of the total health care budget; Milsum et al., 1992). Voiding disorders are also associated with considerable sufferings for the affected individuals with restricted social life and decreased self-esteem. Clearly, more research is required to develop new, effective treatments for the benefit of both patients and society.

Neuronal control of lower urinary tract

Peripheral innervation of lower urinary tract

There are three sets of peripheral nerves that participate in the micturition control: sacral parasympathetic, thoracolumbar sympathetic and lumbosacral somatic nerves (Fig. 1). These nerves contain both afferent and efferent fibers from and to the lower urinary tract.

The sacral parasympathetic system is responsible for the bladder evacuation. Any disturbance of this system could result in incomplete voiding or urinary retention. The preganglionic motor neurons to the bladder are located in the intermediolateral cell group of the caudal spinal cord. In man, the efferent fibers run in the S2 - S4 ventral roots, while in cats and rats they are found in the S1 - S3 and L6 - S1 ventral roots respectively. The preganglionic efferents, which belong to the thinnest myelinated Aδ fibers, exit from the sacral plexus to form the pelvic nerves (together with parasympathetic fibers to other pelvic organs e.g. rectum, uterus). In the retroperitoneal space the pelvic nerves split into many thin branches to form the pelvic plexus, a neural network located on both sides of the rectum (de Groat, 1993). Here the preganglionic fibers branch several times before terminating on postganglionic neurons, located in small groups (ganglia) at branching points of the nerve network. In man some ganglia are found as distally as in the bladder wall (Elbadawi, 1996).

The postganglionic motor fibers, which are unmyelinated C-fibers, terminate extensively within the smooth muscle bundles that form the detrusor muscle of
the bladder. It follows that most myelinated fibers found in the bladder wall, especially the largest, are afferents (de Groat et al., 1981; Mallory et al., 1989; Vera & Nadelhaft, 1990). Compared to humans and cats, the pelvic plexus of the rat has a simple anatomical structure, with limited ramifications and inter-connections. The pelvic nerve form a single trunk and converge with the hypo-gastric nerve in a single major pelvic ganglion. In the female rat, this ganglion is located close to the dorsolateral surface of the uterine cervix. From the ganglion a few thin nerve branches containing unmyelinated postganglionic effector project towards the base of the bladder. There are no further ganglia close to or within the bladder wall (Purinton et al., 1973; Torrens & Morrison, 1987; Steers, 1994).

The parasympathetic postganglionic nerves release acetylcholine as the main transmitter substance. Acetylcholine binds to specific muscarinic receptors on detrusor cells to elicit muscle contractions (Yoshimura & de Groat, 1997). Adenosine triphosphate, which is released as a co-transmitter, acts on purinergic receptors to enhance the contraction in many species, including cats and rats. This atropine resistant, non-cholinergic component seems to play little role for the normal detrusor function in man and old world monkeys (Craggs et al., 1986; Steers, 1994). Activation of the parasympathetic system also induces a relaxation of the urethral smooth muscle, mainly via the release of nitric oxide (Andersson & Persson, 1994; Bennett et al., 1995; Lundberg, 1996).

The main functions of the sympathetic system to the lower urinary tract are to inhibit bladder contractions, close the bladder neck, and contract the urethra in order to maintain urinary continence. The sympathetic preganglionic pathways arise from the intermediolateral cell group in the lower thoracic and upper lumbar spinal cord (Elbadawi, 1996). After a relay in the sympathetic chain ganglia or the inferior mesenteric ganglion, postganglionic fibers pass to pelvic structures via the hypogastric nerve and sacral sympathetic chain (Fig. 1; Kuo et al., 1984). The latter fibers join the pelvic nerve close to the sacral plexus while the hypogastric nerve projects directly to the pelvic plexus, where the postganglionic parasympathetic neurons are inhibited. Sympathetic fibers also project to the bladder, especially the bladder neck, and the urethra (Yoshimura & de Groat, 1997).

The sympathetic postganglionic terminals release noradrenaline, which has different actions on the smooth muscles in different parts of the lower urinary tract. At the bladder body, the transmitter is inhibitory through \( \beta_2 \)-adrenoceptors, at the bladder neck and urethra it is excitatory via \( \alpha_1 \)-adrenoceptors (de Groat, 1997). The sympathetic inhibitory effect on the excitatory para-sympathetic outflow from pelvic ganglia has been found only in cats, not in rats (de Groat & Booth, 1980; Mallory et al., 1989; Steers, 1994). This effect is mediated by \( \alpha_2 \)-adrenoceptors (de Groat, 1997). Apart from the prevention of retrograde ejaculations in males, the sympathetic pathways are often claimed to be of little importance for the lower urinary tract function in man (Nordling,
Fig. 1. Schematic diagram of peripheral innervation of the cat’s lower urinary tract

1983; Brindley, 1986). No human studies have been performed, however, in situations when the sympathetic system would be expected to be active, e.g. during stress.

The lumbosacral somatic nerves innervate the striated muscles of the pelvic floor. The pathways originate from specific motoneurons in the Onuf’s nucleus of the ventral horn (Sato et al., 1978; Torrens & Morrison, 1987). This nucleus is found at about the same segmental level as the parasympathetic motor neurons to the bladder (S2 - S4 segments in man, S1 – S3 in cats, L6 - S1 rats). The external urethral sphincter (rhabdosphincter) is innervated by the pudendal nerve while the pelvic floor musculature receives its innervation through thin branches directly from the sacral plexus (de Groat, 1997). The motor nerve terminals release acetylcholine, which acts on muscle type nicotinic receptors to elicit a contraction (Yoshimura & de Groat, 1997). The combined activation of the sympathetic and somatic pathways to the urethra elevates bladder outlet resistance and contributes to urinary continence.

Bladder receptors and sensory functions

The afferent nerve fibers from the bladder consists of small myelinated (Aδ) and unmyelinated (C) fibers that travel in both the pelvic and hypogastric nerves. The afferents originate from mechanoreceptors, thermoreceptors, nociceptors and possibly also specific chemoreceptors (Torrens & Morrison, 1987; Häbler et al., 1993a,b; Morrison, 1997). The exact morphology and location of the specific receptors remain to be determined.
Bladder mechanoreceptors are equipped with A\(\delta\) afferents in the cat (and presumably man) while many such receptors seem to have unmyelinated afferents in the rat (Vera & Nadelhaft, 1990; Sengupta & Gebhart, 1994). The mechanoreceptors are responsible for the sensation of bladder fullness, they also trigger and drive the micturition reflex. Most of them are tension receptors “in series” with the detrusor muscle fibers and they respond to both passive bladder distension and active detrusor contractions (Iggo, 1955; Bahns et al., 1987; Häbler et al., 1993a,b; Downie and Armour, 1992). Their responsiveness is approximately described by Laplace’s law \((T \sim P^{\frac{3}{2}})\), indicating that they are more sensitive to changes in bladder pressure than to changes in bladder volume. This fact is important, since the relaxed detrusor can be distended over a large range with little or no increase in bladder pressure. Thus, the bladder wall tension and the mechanoreceptor activity increase very slowly during bladder filling (until an active detrusor contraction is initiated). The mechanoreceptors have, like other sensory receptors, a distinct threshold below which they do not respond during physiological conditions. The term “afferent threshold volume”, which is an important parameter in this thesis work, refers to the volume at which the tension produced by the combined action of the pressure and volume exceeds this critical level.

The bladder mechanoreceptors can be sensitized by inflammatory agents (Häbler et al., 1993a,b) which may explain why urgency is a common symptom in cystitis. In the same situation, many normally silent C-afferents, connected to nociceptors, start to respond. These receptors are believed to be responsible for the bladder pain sensation (Steers, 1994; Wen & Morrison, 1995). A subgroup of these nociceptors may be high threshold mechanoreceptors that respond to overdistension of the bladder (Morrison, 1997). Recently, bladder and urethral cold receptors have been described both in the cat and man (Fall et al., 1990; Lindström & Mazières, 1991; Geirsson, 1993). Also these receptors have unmyelinated C-afferents. They are responsible for the so-called “bladder cooling reflex” which can be evoked in healthy infants and also in adults with certain types of neurogenic voiding disorders (Geirsson et al., 1993, 1994; Mazières et al., 1998). The afferents responsible for the ordinary micturition reflex and the bladder cooling reflex all travel in the pelvic nerve. The functional role of the sympathetic afferents is still obscure.

Central control of micturition

Effective voiding requires good co-ordination between the detrusor muscle and the urethral sphincters. Both autonomic and somatic nerves are involved in this process. Different from many other visceral functions, the normal micturition is under conscious, voluntary control, which requires participation of higher centers in the brain.

Early experiments in the cat by Barrington (1925) revealed that neurons in the pontine brain stem just below the inferior colliculus have an essential role in
Fig. 2. Schematic diagram of the micturition reflex pathway
(PAG - periaqueductal gray)

The coordinated control of the micturition. Transection of the brain stem or spinal cord at any point below this level abolishes the normal voiding while it is facilitated by intercollicular decerebration. The latter effect seems to be due to the interruption of inhibitory inputs from more rostral structures. Bilateral lesions in the rostral pons, medial to the mesencephlic trigeminal tract, abolish micturition, whereas electrical or chemical stimulation at this site triggers bladder contraction and micturition (Kuru, 1965; Holstege, et al., 1986; de Groat, 1993). These observations indicate that the ordinary micturition is mediated by a spinobulbospinal reflex pathway that passes through a center in the rostral pons (Barrington’s pontine micturition center; Fig. 2). The pathway functions as “on-off” switch that is activated at a critical level of activity arising from bladder mechanoreceptors. The function of the pontine center is, in turn, modulated by inhibitory and excitatory influences from areas of the
brain rostral to the pons (e.g. the diencephalon and the cerebral cortex; Gjone & Setekleiv, 1963; Gjone, 1966; Torrens & Morrison, 1987; de Groat, 1997).

Information about bladder filling reaches the pontine center by ascending spinobulbar fibers in the dorsal part of the lateral funiculus. The cells of origin seem to be located in or close to the sacral parasympathetic and dorsal commissural nuclei. The organization of the pathway at the brain stem level is not yet clear. While Holstege and collaborators (Blok & Holstege, 1994, 1998; Blok et al., 1995) maintain that the information in the cat reaches the Barrington’s nucleus indirectly via a relay in the ventrolateral periaqueductal gray, Ding and colleagues (Ding et al., 1997) found a substantial direct spinal input to the Barrington’s nucleus in the rat. They believe that the periaqueductal gray may have a modulatory influence on the micturition. The descending fibers from the Barrington’s nucleus project via the middle lateral funiculus to terminate in the sacral spinal cord on interneurons and preganglionic parasympathetic neurons to the bladder (Blok & Holstege, 1997). The same fibers also terminate dorsally to the central canal on inhibitory interneurons which project to the Onuf’s nucleus (Blok et al., 1997a). It is believed that this projection is responsible for the sphincter relaxation, which is an integrated part of the coordinated micturition reflex.

Both ascending pathways to and descending from the pontine micturition center are formed by excitatory glutamatergic neurons (Fig. 2; Yoshimura & de Groat, 1997). In experimental animals, excitatory input from the bladder mechanoreceptors to the pontine center and its efferent output are bilaterally organized. Interestingly, recent PET-studies in man seem to indicate that the pontine (and suprapontine) control of the micturition is lateralized to the right side (Blok et al., 1997b). The same seems to be true for a descending excitatory pathway from the pons to the Onuf’s nucleus, believed to be important for continence.

Lindström and collaborators (1984) pointed out that the micturition reflex is organized as a positive feedback mechanism. A reflex detrusor contraction generates an increased tension in the bladder wall, which leads to further activation of the bladder mechanoreceptors and a stronger excitatory drive of the pontine micturition center. This process rapidly escalates until the pelvic motor output to the bladder is maximal. Once urine enters the urethra, the reflex is further facilitated by activation of urethral afferents (Barrington, 1928; Mazières et al., 1997). This all-or-nothing behavior of the parasympathetic motor output to the bladder provides a strong detrusor contraction and an efficient bladder evacuation. When the bladder is empty the pressure will drop, the afferent drive of the micturition reflex will cease and the detrusor contraction stop automatically. So the micturition is, from a neuronal point of view, a self-sustained and self terminated process. It follows that incomplete bladder evacuation with residual urine would mainly occur after failure in some
component of the positive feedback system, either the peripheral afferent or efferent system or the central reflex loop.

**Neuronal control of continence**

Effective storage of urine requires a stable bladder with high compliance (i.e. a relaxed detrusor) and a sealed urethral conduit. A drawback with the positive feedback system of the bladder is that it may easily become unstable. Any stimulus that elicits a small burst of impulses in mechanoreceptor afferents, such as coughing, lifting and jumping may trigger a full blown, involuntary micturition reflex and cause urine leakage. Fortunately, the neuronal control system of the bladder is equipped with several safety circuits at both spinal and supraspinal levels, which can keep the bladder motor neurons silent and the urethra closed.

A typical example is Edvardsen’s reflex, which involves activation of sympa-thetic neurons to the bladder and urethra by bladder mechanoreceptors (Edvardsen, 1968). This reflex is only operative during the bladder filling phase and is suppressed during voidings (de Groat & Lalley, 1972). Likewise, moto-neurons to the external urethral sphincter and pelvic floor are activated by bladder mechanoreceptors as the bladder fills. Thereby, the urethra remains closed during the entire filling phase. As already mentioned, this reflex is also suppressed by descending pontospinal signals during the micturition (Torrens & Morrison, 1987).

Other bladder inhibitory systems originate from afferents in anorectal branches of the pelvic nerves, dorsal clitoral or penile branches of the pudendal nerve and various muscle nerves in the limb (de Groat, 1971; Lindström & Sudsuang, 1989). These afferents activate the inhibitory sympathetic neurons to the bladder and provide central inhibition of the preganglionic bladder motor neurons through a direct route in the sacral cord (Fall & Lindström, 1991). The spinal ascending neurons that form the afferent limb of the spinobulbospinal micturition reflex are also inhibited (Jiang et al., 1991). At the same time, the external urethral sphincter is activated. All these mechanisms would be activated by anal dilatation, by mechanical stimulation of the genital region and by walking, running or jumping. They are not suppressed during voidings, which provide the rationale for the use of electrical stimulation of these systems as treatment of urge incontinence (Fall & Lindström, 1991).

Inhibitory effects on the micturition has been described after electrical stimulation at many supraspinal sites in the brain stem, hypothalamus, basal ganglia and neocortex (Gjone & Setekleiv, 1963, Gjone, 1966, Kuru, 1965). The organization of these controlling systems is poorly understood. However, it is easy to understand that a subnormal function in one or more of the spinal or supraspinal inhibitory systems may lead to imbalance in the excitatory and inhibitory control of the micturition reflex. Such changes may result in various
forms of bladder instability and urge incontinence (Lindström et al., 1984; Fall & Lindström, 1991; de Groat, 1997).

Electrical stimulation as treatment of voiding disorders

Defective voiding, urgency and urge incontinence are common voiding disorders, which lack effective conventional treatment. Anticholinergic drugs with or without additional calcium blocking action are widely used for urgency and urge incontinence, in most cases with rather disappointing results. In effective doses such drugs have frequent, unacceptable side effects and they are hardly ever curative. Attempts to treat bladder evacuation problems with drugs have been even more disappointing. Catheterization is the only realistic treatment alternative in this situation. The procedure presents with a high incidence of bacteriuria, requires a certain degree of dexterity and may be associated with urethral trauma and discomfort. If the procedure fails, more invasive surgical procedures, such as urinary diversion, has to be performed in order to avoid serious complications with upper tract deterioration. With this background, electrical stimulation has been used as an alternative, perhaps more physiological, therapy. Several modes of stimulation have been tried for different voiding dysfunctions.

Ano-genital afferent stimulation

In patient with urgency or urge incontinence, afferents involved in bladder inhibitory control have been activated, with the intention to suppress involuntary detrusor contractions. In most cases vaginal or anal electrode carriers have been used (Fall & Lindström, 1991, 1994). Surface stimulation of limb nerves or direct stimulation of the pudendal nerve with needle electrodes have also been tried (McGuire et al., 1983; Ohlsson et al., 1989). Experimental studies have indicated, as mentioned above, that the stimulation of these afferent systems evokes multiple reflex effects. The sympathetic inhibitory pathway to the bladder is activated together with a spinal inhibitory system to bladder preganglionic motor neurons (Lindström et al., 1983). Spinal ascending cells, involved in the projection of bladder sensory information to the pons and the cortex, are also inhibited. The latter effect may explain the decreased urge sensation with stimulation.

Clinical experimental studies have revealed that phasic bladder contractions are suppressed and bladder capacity increased by ano-genital afferent stimulation (Fall et al., 1977; Vodusek et al., 1988). More interestingly, repeated daily stimulation sessions may lead to restored urinary continence, even for pro-longed periods after termination of stimulation. Several studies have revealed that this so-called “reeducation” may occur in about a third of the treated patients (Godec & Cass, 1978; Plevnik & Janez, 1979; Fall, 1984; Eriksen et al., 1989). So far, there are no experimental studies to explain this long-term effect of stimulation.
Conus and ventral root stimulation

Patients, who lack voluntary control of the bladder due to spinal cord injury can have the sacral parasympathetic nucleus of the spinal cord (Nashold et al., 1982) or the corresponding ventral roots stimulated by implanted electrodes (Brindley, 1977; Brindley et al., 1982). The principle of this treatment is to activate the bladder parasympathetic motor output to evoke detrusor contractions when the patient wants to void. In other words, the stimulation provides a form of artificial voiding. The electrode implantation is often combined with dorsal root rhizotomy to eliminate unwanted reflex contractions of the bladder. A problem with spinal cord or root stimulation is that somatic motor fibers to the external urethral sphincter and pelvic floor muscles are activated concomitantly with the efferents to the bladder. Both types of efferents travel in the same roots but the somatic motor fibers have lower thresholds for electrical stimulation. Therefore, the stimulation induced the micturition would be disturbed by the high resistant of urethral sphincter (detrusor-sphincter dys-synergia). Intermittent stimulation with post-stimulus voiding can be used to minimize the problem. Several evaluations of the clinical results from many patients treated by ventral root stimulation have recently been published (Brindley, 1994; Van Kerrebroeck et al., 1996; Egon et al., 1998).

Sacral root stimulation

During recent years “neuromodulation” with electrodes placed in sacral fora-mina has attracted much interest in treatment of voiding disorders. The procedure was introduced in the late eighties (Schmidt, 1988; Tanago & Schmidt, 1988) and involve a two step process. Initially, a stimulation electrode is introduced percutaneously close to the appropriate sacral root, in most cases S3. If desired effects on bladder function are obtained by stimulation of the selected sacral root, a permanent electrode is implanted together with a remotely controlled stimulator. In most cases the stimulator is used for continuous low intensity stimulation, only interrupted for voidings.

Sacral root stimulation was devised to treat both urge incontinence and retention. The sacral roots are, at the level of stimulation, functionally mixed with somatic and visceral efferent and afferent fibers. Usually, the intensity of stimulation is limited by the induction of strong, unpleasant pelvic floor and toe muscle contractions. At usable intensity only afferents are stimulated. Experimental studies indicate that both ano-genital and bladder afferents can be activated by such stimuli (Schultz-Lampel et al., 1998). The former provides continuous inhibition of unstable detrusor contractions (se above) while the latter may be responsible for a strong rebound facilitation of micturition contractions on termination of stimulation. From a functional point of view, sacral root stimulation may be considered as a hybrid procedure that combines effects of ano-genital and intravesical stimulation. Clinical outcome with this stimulation procedure has been discussed in several recent reports (Vapnek & Schmidt, 1991; Elababy, et al., 1994; Bosch & Groen, 1998; Goodwin et al., 1998; Weil et al., 1998). An extensive in-depth account of the procedure is given in a recent thesis by D. Schultz-Lampel (1997).
Intravesical electrical stimulation (IVES)

In patients with defective voiding and large residual urine or retention, the therapeutic aim would be to restore a proper micturition reflex function. Intravesical electrical stimulation (IVES) with a catheter electrode in the bladder would be an alternative for these patients. The first trials with this procedure can be traced back to 1878 when the Danish surgeon M. H. Saxtorph treated patients with urinary retention by transurethral bladder stimulation (Saxtorph, 1878). The technique was reintroduced in the late fifties by the Hungarian neurosurgeon F. Katona as treatment of neurogenic voiding disorders (Katona, 1973, 1975). In his protocol, the patients received IVES for 60 – 90 minutes per day in combination with biofeedback, often for long periods of time.

Katona (1992) summarized his experience of IVES in a large group of patients (922), most with different types of neurogenic voiding dysfunction secondary to spinal injury, disease or malformation. He reported normalization in 60 % of the cases and improvement in another 27 %. Several other groups have reported regained detrusor activity and increased awareness of bladder filling after IVES in patients with hypocontractile or acontractile bladders (Seifert et al., 1978; Madersbacher et al., 1982, 1987; Kaplan & Richards, 1986, 1988; Primus et al., 1996; Kiss et al., 1998). IVES seems to be particularly effective in children with non-neurogenic bladder dysfunction of “lazy bladder” type (Madersbacher and Ebner, 1992; Gladh et al., 1993). Improvement of continence and bladder compliance has also been reported (Berger et al., 1978; Cheng et al., 1996). These investigators agree that IVES treatment can be given without serious side effects but that it may be rather cumbersome and time consuming. The latter problem may be overcome by patient controlled home stimulation (Gladh et al., 1993).

Despite these promising results, the IVES technique has not been widely accepted as routine treatment of patients with defective voidings. The main reason may be that negative results have also been published from several medical centers (Nicolas & Eckstein, 1975; Petersen, 1987), some quite recently (Boone et al., 1992; Lyne & Bellinger, 1993; Decter et al., 1994). The discrepancy in effect may reflect differences both in procedure and selection of patients.

Published reports on the clinical use of IVES vary considerably in treatment protocols. Intermittent or continuous stimulation at frequencies from 20 to 100 Hz has been used. Stimulation intensity has ranged from 1 to 40 mA with pulse duration from 0.5 to 10 ms. Duration of each IVES session varied from 30 to 90 minutes and total number of IVES sessions from 5 to more than 100. Most investigators have tried to induce detrusor contractions by the IVES and have combined the stimulation with a visual display of the detrusor response for the patient. Others have used treatment without biofeedback and have actively avoided contractions (Gladh et al., 1993). This diversity of procedures reflects
the fact that IVES treatment has been practiced without theoretical understanding of its basic working mechanism.

The present experimental work was motivated by the conviction that IVES could provide a useful means to improve micturition contractions and decrease residual urine in patients with defective voiding – patients which otherwise would be left to life-long catheterization or urinary tract diversion. It was surmised that a better theoretical understanding of the IVES working mechanism might help to improve the selection of patients and optimize the stimulation procedure and thereby increase the success rate of the IVES treatment.
AIMS OF THE STUDY

The overall aims of this experimental study were to identify and characterize the neuronal mechanisms underlying the therapeutic effects of intravesical electrical stimulation (IVES) and to determine optimal stimulation conditions and parameters. The specific aims were as follows:

1. To identify the excitable structures that are activated by IVES.
2. To demonstrate a prolonged excitatory effect on the micturition reflex by IVES.
3. To identify the site and mechanism of neuromodulation induced by IVES.
4. To determine optimal IVES parameters for prolonged modulation of the micturition reflex.
5. To demonstrate a prolonged inhibitory effect on the micturition reflex by stimulation of ano-genital afferents.
6. To verify the specificity of modulation by selective stimulation of bladder A\textsubscript{\delta} afferents combined with direct recordings of efferent reflex discharges in pelvic nerve fibers to the bladder.
MATERIALS AND METHODS

Animals and anesthesia

Cats: Eleven adult female cats were used in the study (papers I and VI). For more than a century, the cat has been the primary animal model for studies of the neuronal control of micturition. Their voiding behavior closely resembles that of humans. They are socially continent and easy to house train (in contrast to non-human primates), indicating that they have a strong voluntary control of the lower urinary tract. There are also good reasons to believe that their neuronal control of the bladder is quite similar to that of humans. Their stress tolerance and relatively large body size makes them quite suitable for electro-physiological studies of micturition reflex functions.

The cats were initially anaesthetized with an alphaxalone-alphadolone mixture (Saffan, Glaxovet, 20 mg/kg i.m.) or with a ketamine-xylazine mixture (Keta-lar, Parke-Davis AS, 15 mg/kg + Rompun ver., Bayer AG, 1 mg/kg i.m.). After surgery, they were transferred to the long-lasting anaesthetic α-chloralose (55 mg/kg i.v., supplemented as necessary to keep a stable level of anesthesia). Alpha-chloralose was used for its long lasting action and limited depressive effect on the micturition reflex (see Discussion).

Rats: One hundred and sixty-two female rats (Sprague-Dawley, 250 - 350 g) were used in the study, most in experiments on long term modulation of the micturition reflex. Awake rats void quite frequently with small urine jets, seemingly with little behavioral concern. However, they are less expensive and easier to handle than cats and were therefore used when groups of animals were required for the analysis.

In rats, induction of anesthesia was achieved with methohexitone (Brietal, Lilly, 70 mg/kg i.p.) followed by α-chloralose (70 mg/kg i.v.). Anesthesia was maintained at an appropriate, stable depth during the entire experiment by a continuous i.v. infusion of α-chloralose (10 - 15 mg/kg.h).

In both cats and rats, the femoral vein and artery were cannulated to allow for fluid injections and blood pressure monitoring and a tracheotomy was performed to ensure safe respiration. Blood pressure was continuously recorded and kept at a mean level about 120 mmHg. Body temperature was maintained at 38° C by a feedback controlled heating lamp. Some animals were paralyzed and artificially ventilated. For cats gallamine triethiodide (Flaxedil, May and Baker Ltd., 20 mg/kg) was used and for rats pancurone bromide (Pavulon, Organon, 2 mg/kg). Subsequently, half the dose was given about every hour to maintain the paralysis. The ventilation was adjusted to give end expiratory CO₂ levels between 3.5 and 4.0 %. Care was taken to ascertain that the paralyzed animals were adequately anaesthetized by regularly controlling that strong paw-pinches failed to induce changes in blood pressure and heart rate. The animals were
sacrificed at the end of the experiment by an overdose of the anesthetics followed by severance of the heart.

The experiments were approved by the Animal Research Ethical Committees of Göteborg and Linköping in accordance with Swedish law.

**Surgical preparations**

*For IVES and cystometry:* The proximal urethra and bladder neck was exposed extraperitoneally by a low midline incision. Intravesical electrical stimulation (IVES) was delivered by a specially designed catheter (8 F for cat, 1.5 mm o.d. for rat) with an electrode inside the tip. The catheter was inserted into the bladder through a slit in the urethra and fixed in place with a ligature. The same catheter was used to fill and empty the bladder and to monitor bladder pressure with an external transducer (cystometry in the rat). In all experiments the abdominal cavity was left open to exclude any influence from intraabdominal pressure changes. As an extra precaution, the rectus abdominis muscles were cut at their insertions into the pubis bone. Thus, the recorded bladder pressure was in reality equal to the detrusor pressure.

*For afferent and efferent recordings:* In five cats (paper I), a laminectomy of the L6 - L7 vertebrae was performed to allow transection of the S1 - S3 dorsal roots and stimulation of the corresponding ventral roots (Fig. 1 in paper I). In the same experiments, afferent recordings were obtained from several thin filaments, split from the S2 dorsal root. The filaments were mounted for mono-phasic recordings on a pair of silver electrodes within a paraffin pool. Six other cats (paper VI) had a small bladder pelvic nerve filament dissected and cut close to the bladder for recordings of efferent reflex discharges. These nerve filaments contained both pre- and postganglionic parasympathetic fibers. The reflex responses were evoked by stimulation of the remaining ipsilateral bladder pelvic nerves. In some cases, urethral or dorsal clitoris branches of the pudendal nerve were exposed for stimulation. One cat had the sympathetic supply to the bladder transected bilaterally.

In some rats (paper III) bladder afferent or efferent activity was recorded during cystometry. A thin nerve branch to the bladder was dissected distal to the major pelvic ganglion and cut close to the ganglion for afferent recordings or close to the bladder for efferent recordings. Multi-unit afferent and efferent activity was collected by hooking the nerve branches onto a bipolar platinum electrode (inter-electrode distance 1 - 2 mm). Due to the anatomical arrangements in the female rat (Purinton et al., 1973), the efferent recordings were exclusively from postganglionic fibers. For both cats and rats, the exposed nerves were covered by body warm paraffin oil in a pool formed by sewn-up skin flaps.
Stimulation procedures

IVES

Electrical stimulation was administered with an isolated constant current stimulator with square wave pulses of adjustable duration, amplitude and frequency. IVES was the major form of stimulation used in paper I - IV. The intravesical electrode was used as cathode and a brass plate wrapped in a saline soaked gauze-pad or a wire electrode placed under the abdominal skin at the level of the bladder as anode. The standard IVES procedure (papers II - IV) involved continuous stimulation for 5 minutes at 20 Hz and the intensity adjusted to give maximal reflex bladder contractions (7 - 11 mA, pulse duration 0.5 ms). During stimulation, the bladder was filled with body-warm saline to a volume below threshold for spontaneous micturition contractions (if not otherwise specified).

In some experiments (paper I, IV), individual parameters were varied to define optimal IVES conditions. In the cat, frequency was varied from 1 to 100 Hz, intensity from 1 to 20 mA and pulse duration from 0.1 to 5 ms (paper I). In the rat (paper IV), frequencies 2, 10, 20, 50, 100 Hz and stimulation periods 1, 5, 20 minutes were tried in separate groups of animals. Other parameters were the same as in the standard procedure.

Other stimulation procedures

IVES like stimulation was applied to the forepaw in a small group of rats (paper II). The stimulation was designed to induce vigorous muscle twitches away from the pelvic region as an unspecific arousal stimulus. The procedure was the same as for the standard IVES except that the cathode was connected to a needle electrode inserted the left forepaw instead of a catheter electrode in the bladder.

Ano-genital afferents were stimulated by a surface electrode in one group of rats (paper V). The electrode was shaped as a small ring from a silver wire and placed in the vagina or the anal canal and used as cathode. Stimulation fre-quency was 10 Hz, other parameters were the same as those of the standard IVES procedure. In one animal, the dorsal clitoris nerves were dissected and mounted on a pair of electrodes for direct stimulation. In this case a much lower stimulation intensity (0.8 mA) was used.

Direct nerve stimulation was exclusively used in cat experiments intended to demonstrate a prolonged enhancement of the micturition reflex by selective stimulation of bladder or urethral Aδ afferents (paper VI). Test reflexes were evoked by a train of three stimuli at 10 ms interval with a stimulus repetition rate of 1 Hz. In most trials, the stimulation intensity was adjusted to give a maximal Aδ reflex response (< 300 µA with 0.2 ms pulses). In two cats, small
test responses could be evoked while the bladder was empty and open, in the others, it was necessary to provide some background facilitation of the reflex from ongoing activity in bladder mechanoreceptor afferents. Such activity was obtained by filling the bladder with saline from a reservoir, elevated to give an isotonic bladder pressure just below threshold for a spontaneous micturition reflex (< 1 kPa).

Tetanic stimulation at 20 Hz for 5 minutes was used to modify the reflex response. The conditioning stimulation was delivered to the Aδ afferents at the same intensity as used for the test reflex. Several trials of conditioning stimulation were performed in each experiment at different intervals (from 20 minutes to 10 hours) and stimulation intensities. In three experiments, a similar conditioning stimulation of afferents in the dorsal clitoris nerve (10 Hz, 5 min) was tried in an attempt to depress the test reflex.

**Recording procedures**

**Cystometry**

Repeated cystometries were performed to evaluate the long-term effects of IVES and ano-genital stimulation (papers II – V). Micturition threshold volume was determined and used as an indicator of micturition reflex modulation. After an initial stabilization period in each animal, cystometries were repeated at about 10 minutes intervals before and after the stimulation. The bladder was filled with body-warm saline by an infusion pump at a rate of 0.1 - 0.2 ml/min, depending on bladder capacity. Once a micturition contraction occurred, the infusion was stopped and the catheter opened in order to minimize adequate activation of bladder mechanoreceptors. The bladder was thoroughly emptied after each cystometry by lowering the catheter outlet. After the stimulation, cystometries were resumed until the micturition threshold volume returned to the control value. If there was no threshold volume change, at least four post-stimulus cystometries were sampled. When several stimulation sessions were attempted in the same animal, a resting period of more than one hour was allowed after full recovery before next trial. There was no serial order effect with repeated IVES sessions in the same animal.

The used infusion speed was rather high compare to the physiological bladder filling rate (Steers, 1994). It was used, however, to achieve a sufficient number of cystometries during a reasonable observation period before and after stimulation. Anyhow, the bladder compliance was not measurably different from that obtained with more physiological filling rates.

The control threshold volume varied considerably between individual animals. Therefore, the values were in some experiments normalized with respect to the control threshold volume. To evaluate the effect of stimulation, the mean
threshold volume of the three cystometries immediately before the stimulation was compared to the mean volume of the first three after the stimulation.

**Detrusor contractions**

Isovolumetric recordings of detrusor contractions were used to identify the excitable structure(s) that would be activated by IVES (paper I). The evoked detrusor response was monitored while the IVES intensity, frequency, pulse duration and polarity were systematically varied. Likewise, the effect of rhizotomy of the S1 - S3 dorsal roots and of direct stimulation of corresponding ventral roots was recorded. The detrusor response was displayed on a chart recorder and analyzed by measuring the peak pressure and the area under the detrusor contraction curve.

**Nerve activity**

*Cat:* Multi-unit afferent discharges were recorded from thin dissected dorsal root filaments during IVES (paper I). The responses were appropriately filtered, amplified and displayed on an oscilloscope and, for further evaluation, stored on photographic film. The latency and threshold intensity with IVES was determined for each recognized afferent unit. To estimate their conduction velocity, the distance between the bladder and the dorsal root recording site was measured at the end of the experiments (90 to 95 mm in different animals). For many units, the response to passive and active changes in bladder pressure was determined qualitatively using an acoustic display of their spike activity.

Evoked Aδ micturition reflex response were recorded from a dissected bladder pelvic nerve filament (paper VI). The reflex discharge was displayed on a digital chart recorder and also full-wave rectified, digitized and averaged (20 or 32 responses) for off-line analysis. The averaged responses were quantified by determining the size of area under the reflex response curve. To allow comparison between experiments, the reflex responses were normalized and expressed as per cent of the mean value of control responses.

*Rat:* Afferent or efferent discharges were recorded from thin bladder pelvic nerve filaments during cystometry before and after IVES (paper III). The objective was to explore the relationship between changes in micturition threshold and nerve activities. During a sequence of cystometries, bladder pressure, nerve activity and full-wave rectified nerve responses were continuously recorded on a digital recorder for subsequent off line analysis with a PC based system. Measured parameters were afferent or efferent threshold volumes, micturition threshold volume and pressure, afferent activity at micturition threshold, peak afferent or efferent activity, bladder pressure at peak activity, afferent sensitivity (see Definitions). In each trial, the mean values of these parameters were determined from three consecutive cystometries before and immediately after the IVES.
Pharmacological tests

Intravesical instillation of lidocaine (Xylocain, Astra) was used in combination with IVES to achieve a transient blockage of bladder nerves (Paper I and II). A small volume of the drug (2% for cats; 0.5% for rats) was left in the bladder for a brief period of time until reflex bladder responses were suppressed. In the rat, the bladder was thoroughly rinsed with saline before the IVES was applied. The intention was to obtain a rapid recovery of bladder nerve function immediately after the stimulation.

Central N-methyl-D-aspartic acid (NMDA) receptors were blocked by administration of the specific NMDA antagonist CPPene (3-(2-carbo-xyperazin-4-yl)-1-propenyl-1-phosphonic acid; Sandoz) in an attempt to interfere with the IVES induced modulation of the micturition reflex (paper III). The drug is a potent, competitive NMDA antagonist that penetrates the blood-brain barrier (Lowe et al., 1994). It was given i.v. in a dose of 1 mg/kg, half an hour before IVES.

Statistical analysis

The non-parametric Wilcoxon signed rank test was mainly used for statistical analysis, for multiple comparisons proceeded by the Kruskal-Wallis ANOVA. Student’s paired and unpaired t-tests were used for analysis of normalized values, one-way ANOVA for multiple comparisons. Normalized values were indicated in figures as means ± SEM except in paper IV where the 95% confidence interval (c.i.) was presented. Differences where considered significant when p < 0.05. More details are found in the original papers. All statistical analyses were done with commercially available software (STATISTICA, StatSoft Inc., Tulsa, OK, USA).
DEFINITIONS

The following parameters were measured in different parts of this thesis work:

Micturition threshold volume = amount of fluid in the bladder at onset of a micturition contraction

Micturition threshold pressure = bladder pressure immediately prior to onset of a micturition contraction.

Basal pressure = lowest bladder pressure during cystometry

Bladder compliance = \frac{\Delta \text{volume}}{\Delta \text{pressure}} = \frac{\text{micturition threshold volume}}{\text{threshold pressure} - \text{basal pressure}}

Afferent (efferent) threshold volume = bladder volume at which the integrated afferent (efferent) response starts to deviate from baseline

Afferent (efferent) peak activity = maximal afferent (efferent) activity during an isovolumetric micturition contraction

Afferent activity at mict. threshold = relative amplitude of integrated afferent activity at micturition threshold (in % of afferent peak activity).

Afferent sensitivity = slope factor of linear regression of integrated afferent activity versus bladder pressure (expressed in % of afferent peak activity per kPa (Fig. 4 in paper III).
RESULTS

Immediate effect of IVES (paper I)

IVES at appropriate intensity and frequency evoked strong reproducible detrusor contractions in both cats and rats. The response was clearly reflexo-genic since it was abolished by bilateral rhizotomy of the S1 - S3 dorsal roots (Fig. 2 in paper I) or by transection of the pelvic nerves. Control stimulation of the corresponding ventral roots evoked equally good detrusor responses as before the deafferentation. The abolished reflex response could not be un-masked by increasing the stimulation intensity up to 3 times that giving a maximal response before the sacral root transection. Thus, pelvic efferent nerve fibers or detrusor smooth muscle cells were not directly activated at the used intensities of IVES. Apparently, these excitable structures had too high thres-hold to be accessible by the IVES procedure. The sympathetic system, which was left intact by the transection of the sacral dorsal roots, was not involved in the IVES effect either.

Bladder mechanoreceptor afferents are known to be responsible for the normal micturition reflex. In this study it was found that they were also involved in the IVES effect. Unitary responses recorded in S2 dorsal root filaments showed that bladder afferents with conduction velocities from 10 to 50 m/s were acti-vated by the IVES. The majority had conduction velocities in the Aδ range (10 - 30 m/s) just like most mechanoreceptor afferents in the cat. In addition, many recorded units showed slowly adapting activity following passive and active increases in bladder pressure, a characteristic behavior of bladder mechano-receptors. The effective IVES intensity for the unitary responses was 3 - 10 mA with 0.5 ms pulses. Within this intensity range, afferent units were recruited in parallel with the increase in the reflex detrusor response (Fig. 4 in paper I).

The IVES induced reflex response was reversibly blocked by intravesical installation of the local anaesthetic lidocaine (2 %) but was unaffected by general paralysis of skeletal muscles. All these observations indicate that the IVES response was bladder specific and resulted from artificial activation of bladder mechanoreceptor afferents, the same afferents that underlie the normal micturition reflex.

With respect to stimulation parameters, intravesical cathodal stimulation was more effective than anodal. The optimal stimulation frequency was 10 - 20 Hz with no major difference in outcome between continuos and intermittent stimulation.

Prolonged effect of IVES (paper II)

To be clinically useful the IVES effect has to outlast the stimulation period. Such an effect was observed in experiments on anaesthetized rats. In these
animals, the IVES induced a prolonged, reversible decrease in the micturition threshold volume (Fig. 2 in paper II). The effect lasted for about one hour after 5 minutes of IVES at 20 Hz and maximal intensity. There was no detectable change in bladder compliance, micturition threshold pressure or peak detrusor pressure associated with the IVES induced modulation of the micturition threshold.

Three types of control experiments were performed to confirm that the IVES induced modulation was specific to activation of bladder mechanoreceptor afferents. First, the bladder nerves were temporarily blocked by local anesthesia during the stimulation period. In this situation the IVES was completely ineffective with respect to changes in the micturition threshold volume. Second, IVES was given to some animals during general muscle paralysis. The reason was that the stimulation often induced obvious contractions in neighboring muscles due to current spread to corresponding somatic motor nerves. However, the micturition threshold volume changed to the same degree by IVES in paralyzed as in nonparalyzed animals. Third, IVES like stimulation was applied to a forepaw to provide an unrelated arousal stimulus. This kind of stimulation had no effect on the micturition threshold volume. Furthermore, there was no alteration in heart rate and blood pressure following a standard IVES. Thus, muscle twitches and unspecific arousal effects could be excluded as source of the micturition reflex modulation.

Site of IVES action (Study III)

Since bladder mechanoreceptor afferents are artificially activated by the IVES it was conceivable that their tension sensitivity was increased after a period of IVES. Such an increase could account for the observed decrease in the micturition threshold volume. No such effect was observed in experiments designed to analyze changes in afferent sensitivity following IVES. During a typical cystometry, afferent activity increased very gradually from the threshold volume up to onset of the micturition contraction (Fig. 2 in paper III). At this point, there was a large, steep increase in afferent activity with the increase in detrusor pressure. Neither the afferent threshold volume nor the afferent pressure sensitivity (slope of pressure - response plot) changed after IVES despite a significant change in the micturition threshold volume. The level of afferent activity at micturition threshold was, however, significantly lower after the stimulation. Such a change would be expected if the afferent sensitivity remained the same after the IVES. Other parameters were unchanged, e.g. micturition threshold pressure, afferent peak activity and bladder pressure at afferent peak.

The postganglionic efferent activity to the bladder appeared in an all or nothing fashion. During bladder filling, there was no detectable discharge until just before onset of the micturition contraction (Fig. 5 in paper III). In all experiments, the efferent threshold volume decreased significantly after IVES to a
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bladder volume at which there was no efferent activity before the stimulation. Other parameters, i.e., peak efferent response, peak pressure or ratio between efferent activity and evoked bladder contraction, were unchanged. Thus, the motor output from the spinal cord rather than the peripheral motor system was modified by the IVES.

The NMDA antagonist CPPene was used to explore the mechanism behind the IVES induced central modulation of the micturition reflex. In control trials, the micturition threshold volume decreased significantly after the IVES, as in previous experiments. One hour after recovery from the control session, CPPene (1 mg/kg) was given intravenously. The drug itself did not affect the micturition threshold volume but it completely blocked the IVES induced modulation. Thus, the modulation seems to involve an NMDA dependent mechanism.

**Optimal IVES parameters (Study IV)**

IVES with standard parameters (7 - 10 mA, 20 Hz, 5 min) induced a prolonged decrease in the micturition threshold volume to about 80% of the control value. There was no difference in effect between IVES trials with the bladder filled and contracting during stimulation or empty and noncontracting. In separate experiments, volume imposed “spontaneous” bladder contractions for 5 minutes did not change the micturition threshold volume.

When different IVES frequencies (2, 10, 20, 50 and 100 Hz) were tried with respect to the prolonged modulation of the micturition reflex, the best effects were obtained at low frequencies (2 - 20 Hz). However, the frequency-effect curve was rather shallow and some changes were observed also after IVES with high frequencies. With respect to IVES duration, three periods (1, 5 and 20 min) were tried. Only the middle IVES duration (5 minutes) gave a significant prolonged decrease in the micturition threshold volume.

**Ano-genital stimulation (Study V)**

The micturition reflex is inhibited by activation of ano-genital afferents. Continuous stimulation of such afferents for 5 minutes resulted in a significant prolonged increase in the micturition threshold volume. This inhibitory modulation of the micturition reflex lasted more than 40 minutes after the stimulation. There was no difference in effect between stimulation of anal or vaginal afferents. The micturition threshold pressure and peak contraction pressures were not changed by the stimulation.

The observed increase in the micturition threshold volume occurred without any tonic after-discharge in the stimulated afferents. There was not any change in the afferent response to perivaginal mechanical stimulation either. Thus,
Prolonged reflex enhancement (Study VI)

Stimulation induced enhancement of the micturition reflex was investigated in six cats. Reflex discharges with a latency of 90 - 120 ms were evoked in bladder efferent fibers by stimulation of ipsilateral bladder pelvic afferents at Aδ intensity. Such test responses were greatly enhanced following repetitive conditioning stimulation of the same afferents for 5 minutes at 20 Hz (Figs. 2 and 3 in Paper VI). This central modulatory effect lasted more than one hour. Equally good reflex enhancement was obtained in one animal with bilateral transection of the sympathetic supply to the bladder. Micturition reflex responses were also evoked by stimulation of urethral afferents in the pudendal nerve (Mazières et al., 1997). These responses were not facilitated by the conditioning stimulation of bladder pelvic afferents or vice versa. These results reflect some degree of pathway specificity for the reflex enhancement. Less consistently, the micturition reflex was suppressed beyond the period of stimulation by conditioning stimulation of afferents in the dorsal clitoris branch of the pudendal nerve (10 Hz, 5 min).
Intravesical electrical stimulation (IVES) has been used clinically for more than four decades as treatment of voiding dysfunctions (Katona, 1992). The rationale for this procedure rests on clinical experience without proper experimental support. The present thesis work is, to my knowledge, the first attempt to find theoretical explanations for the working mechanisms of IVES and to provide suggestions for optimal use of this treatment modality.

Use of anesthesia

Most anesthetics in appropriate doses have a strong depressive influence on the micturition reflex. In the present animal studies, \( \alpha \)-chloralose was chosen since it is known to be minimally depressive with respect to bladder reflexes (Rudy et al, 1991). Another advantage with this anesthetic is that it is slowly metabolized so that long-lasting stable anesthesia could be easily maintained.

Throughout the study, great care was taken to exclude that observed changes in micturition threshold volume and reflex amplitude were due to drifts in the level of anesthesia. Based on pilot experiments, \( \alpha \)-chloralose was given as continuous intravenous infusion in rats, with the infusion rate adjusted individually during a pre-stimulus equilibrium period. Long term experience with the anesthetic showed that such precautions were not necessary for cats. There was no difference in effect on the micturition reflex whether supplementary doses of \( \alpha \)-chloralose were given intermittently or as a continuous infusion. The explanation is the long half-life of the drug in this species (8 - 10 hours). With this background, it is obvious that rapid micturition reflex changes, time-locked to the IVES, with effect duration of about one hour, cannot be due to transient changes in the depth of anesthesia. There was no independent indication of unspecific arousal effects following the conditioning stimulation.

Excitable structures activated by IVES

The physiological working mechanisms of IVES considered by Katona (1975) are somewhat obscure. He speculates that IVES induces depolarization of receptors in the bladder wall, resulting in activation of the intramural motor system. This arouses local muscle contractions and subsequently leads to depolarization of afferents, which evokes sensation and strong centrally induced detrusor contraction. The second part of this idea is consistent with the present finding (paper I) that IVES induces direct activation of bladder mechanoreceptor afferents, the same afferents that initiate and drive the normal micturition reflex. The detrusor response on IVES was mediated by a central reflex pathway and was completely eliminated after bilateral sacral dorsal root transection. IVES induced afferent discharges, recorded in S2 dorsal root filaments, clearly originated from bladder mechanoreceptor \( \Lambda \delta \) afferents. The IVES effect was also blocked by intravesical installation of the local anesthetic.
lidocaine. Finally, it was not possible to activate bladder efferent nerves or the detrusor muscle directly by IVES at clinically relevant intensities.

Technically, IVES is a rather unspecific form of stimulation. It can be viewed as field stimulation of the bladder wall, delivered by the catheter electrode and conducting fluid in the bladder, through the bladder wall and surrounding tissue, to the anode (indifferent electrode). Of available excitable structures, it is well known that nerve fibers have lower threshold for electrical stimulation than smooth muscle cells. Of nerve fibers within the bladder wall, mechanoreceptor afferents have the largest diameter (myelinated Aδ fibers) with lowest threshold for electrical stimulation (Mazières et al., 1998). Visceral nociceptors, including those from the bladder, have unmyelinated (C) afferents with much higher electrical thresholds than the stimulation intensity used in this study (Häbler et al., 1990, 1993a; Morrison, 1997). Postganglionic bladder motor fibers are also unmyelinated with high thresholds, a fact that agrees with our failure to elicit detrusor contractions after bladder deafferentation even at quite high stimulation intensities. The majority of the preganglionic motor fibers apparently terminated in pelvic ganglia too far away from the zone of highest field density to be significantly stimulated.

Due to the small size of the experimental animals, it was inevitable to evoke skeletal muscle twitches with IVES at sufficient intensity to induce a maximal detrusor response. These muscle responses were partly due to current spread to neighboring somatic motor nerves. However, this muscle activity had no bearing on the IVES effect since similar IVES results were obtained in animals with general muscle paralysis.

**Prolonged modulation of micturition threshold volume**

Effective treatment of patients by IVES requires that the detrusor response is reinforced not only during stimulation but also afterwards. This requirement motivated the search for IVES induced modulation of the micturition reflex beyond the period of stimulation.

A prolonged decrease in the micturition threshold volume was regularly demonstrated in anaesthetized rats following a short period of IVES (papers II - IV). This effect was assumed to result from an increased excitability of the micturition reflex. Although highly significant, the observed change in micturition threshold volume was rather small, about 15 - 20 %. The likely explanation for this limited effect is that healthy rats with normal voiding functions were used for the experiments. Nevertheless, the effect was quite consistent with comparable reflex modulations in all subgroups of animals that received standard IVES in different studies.

The IVES induced decrease in micturition threshold was dependent on direct stimulation of bladder afferents i.e. the excitatory input to the micturition
reflex. The modulation did not occur when the bladder nerves were blocked by local anesthesia during the IVES application. The outcome was also the same whether the IVES was allowed to induce bladder contractions or not. Likewise, it was irrelevant whether the animals were paralyzed and non-paralyzed during the stimulation. Intentional induction of muscle twitches by forepaw stimulation did not produce any change in micturition threshold. Thus, it is extremely unlikely that unspecific arousal activity was the cause of the modulation.

A prolonged change in the micturition threshold volume but in the opposite direction to IVES was induced by ano-genital afferent stimulation (paper V). It is well known that such afferents have an inhibitory effect on the micturition reflex (Lindström et al., 1983) and that their activation by vaginal or anal stimulation may suppress unstable detrusor contractions in patients (Fall et al., 1977; Vodusek et al., 1988). Ano-genital afferent stimulation may also have a curative effect in urge incontinence (Fall, 1984; Fall & Lindström, 1991). The present prolonged increase in micturition threshold volume was again due to direct stimulation of the appropriate afferents. The effect cannot be explained by an indirect mechanism, involving reflexes from contracting pelvic floor muscles, since the modulation occurred in paralyzed animals. Such muscle contractions have often, explicitly or implicitly, been inferred as responsible for the therapeutic effect of “pelvic floor stimulation”. A prolonged, stimulus induced increase in the threshold of reflex detrusor responses, similar to that observed here, has recently been demonstrated after dorsal penis nerve stimulation in humans with spinal cord injury (Shah et al., 1998).

Neuronal mechanisms of IVES induced modulation

In principle, the IVES induced decrease in micturition threshold volume could be caused by an increase in tension sensitivity of bladder mechanoreceptors or by an increase in efficacy of motor fibers to the bladder. In both cases, the micturition contraction would start at a lower bladder volume after the stimulation. Alternatively, the excitability in the central micturition reflex pathway might be enhanced. If so, reflex micturition contractions are facilitated by lowering of the central “set-point” for activation of bladder parasympathetic preganglionic neurons.

Direct recordings of bladder afferent activity (paper III) gave no evidence for an increase in afferent sensitivity following IVES, despite a clear decrease in the micturition threshold volume. If the afferents were sensitized by IVES, they should start to respond earlier during the bladder filling or increase their activity more rapidly than before with the increase in volume. This was not the case. Instead, both the afferent threshold volume and the increase in afferent activity with bladder filling remained the same after IVES (as illustrated schematically in Fig. 3). As a result the micturition reflex was evoked at a lower level of afferent activity after the IVES than in the control situation. Furthermore, there was no change in the afferent pressure sensitivity, measured as increment in
afferent activity per unit change in bladder pressure (see Definitions). Both observations imply that the IVES induced decrease in micturition threshold volume was not due to the recruitment of extra afferent activity at lower bladder volumes.

The most important finding with direct recordings from bladder efferents (paper III) was that the nerve response started just a few seconds before onset of micturition contractions. In control recordings, there was no trace of efferent activity at bladder volumes corresponding to the micturition threshold volume after IVES (see diagram in Fig. 3). Furthermore, there was no evidence of peripheral amplification of the motor response. Thus, the IVES induced decrease in micturition threshold volume was clearly due to a lowering of the output level of preganglionic motor neurons to the bladder.

It is well known that activity induced enhancement or depression of excitatory transmission may occur in many central synapses. Some changes are long-lasting and usually referred to as long term potentiation or depression (LTP, LTD; Teyler & Discenna, 1987; Madison et al., 1991; Linden, 1994). Long term potentiation of excitatory synapses with glutamate as transmitter usually requires activation of postsynaptic glutamatergic receptors of NMDA type. Such potentiating effects can be blocked by specific NMDA receptor anta-gonists. Thus, it seemed reasonably to postulate that if LTP like mechanisms
were involved in the present IVES effect it should be possible to prevent the modulation by administration of NMDA receptor antagonists. There were reports that both the ascending and descending pathways to and from Barrington’s pontine micturition center have glutamate as the excitatory transmitter (Fig. 2) and that NMDA receptors are involved in the postsynaptic response (Matsumoto et al., 1995; Kakizaki et al., 1998).

A potent, competitive NMDA antagonist, CPPene, was used to determine if the IVES induced modulation of the micturition reflex depends on activation of NMDA receptors (paper III). This drug abolished the IVES induced modulation although it had no effect by itself on the micturition threshold. Thus, it seems likely that the IVES induced prolonged modulation of the micturition reflex threshold involves an LTP like enhancement of synaptic transmission. In analogy, the increase in micturition threshold volume after ano-genital stimulation (paper V) might be due to an LTD related process. Where in the central reflex pathway these synaptic modulations take place and whether one or several synapses are involved remains to be determined.

**Prolonged reflex enhancement**

To decrease residual urine in patients with defective voidings it is presumably more important to increase the strength (gain) of the micturition reflex than to decrease its threshold volume. It cannot be taken for granted that these two aspects of the micturition reflex are controlled in parallel by the same central regulatory mechanisms.

In the rat experiments the micturition threshold volume was clearly decreased by IVES. However, any gain change could not be revealed by cystometry, presumably because the positive feedback mechanism of the micturition reflex gave a saturated response already in the control situation. This drawback was avoided by direct recordings of stimulation induced reflex discharges in cats (thereby bypassing the positive feedback system; paper VI). Such reflex discharges were much enhanced for at least one hour after IVES like stimulation of bladder Aδ afferents. The opposite effect, a prolonged depression of the micturition reflex discharge, was observed after corresponding stimulation of inhibitory afferents in the dorsal clitoris branch of the pudendal nerve. This, not only the threshold but also the amplitude of the micturition reflex was modified by repetitive afferent stimulation indicating a general modulation of excitability in the central micturition reflex pathway.

Another benefit with the cat experiments was that bladder afferents could be activated selectively in a controlled manner. Hereby, unintentional co-activation of other visceral or somatic afferents (or bladder C-afferents) could be avoided. In the cat, the micturition reflex is driven exclusively by myelinated bladder afferents (Yoshimura & de Groat, 1997), while in the rat, mechanosensitive C-afferents may contribute to the response (Vera & Nadelhaft, 1990;...
Morrison, 1997). In the rat IVES experiments, we attempted to avoid co-activation of bladder C-afferents by keeping the stimulation intensity below threshold for direct activation of unmyelinated bladder postganglionic motor fibers. Even so, it might be difficult to exclude some contribution of C-afferents to the observed modulatory effect. With direct bladder pelvic nerve stimulation, the threshold intensity for C-afferents is more than 10 time higher than that of a maximal Aδ response (Mazière et al., 1998). It follows that the observed micturition reflex enhancement was obtained without the involvement of C-afferents.

**Physiological role of micturition reflex modulation**

Is there a physiological role for the observed modulation of the micturition reflex? Yes, this is probably the case. The micturition reflex has to be adjusted both in terms of threshold volume and gain during normal growth. For instance, the micturition threshold volume increases in man from about 20 ml at birth to 200 - 400 ml in the adult, i.e. 10 - 20 times. In addition, there are large individual variations (by at least a factor of five), which seem to depend on differences in nighttime diuresis (Mattsson & Lindström, 1995). There are also structural differences in urethral size, at least between sexes, which will affect the outflow resistance and the functional demand on the micturition reflex. A dynamic adaptive process seems more suitable to handle such changes than a predetermined growth program.

From an evolutionary perspective, a flawless function of the bladder would certainly be important. Incomplete bladder emptying increases the risk of urinary tract infections with secondary kidney destruction. Therefore, a good bladder evacuation function seems critical for the ability of individuals to reproduce successfully. It is a matter of speculation whether the observed modulation of the micturition reflex is a reflection of such a physiological adaptive process.

It seems likely that bladder mechanoreceptors are involved in the adaptive process. After all, they are activated by bladder filling and drive the normal micturition reflex. To be effective, the duration of a mechanoreceptor driven adaptive process should be consistent with the normal voiding behavior. For instance in the rat, which voids 20 - 30 times a day (Malmgren et al., 1987), the duration should be rather short - maybe about one hour, as found for the modulation in the present study. Since normal rats were used for the experiments their adaptive mechanism was presumably already optimized. As mentioned above, this may explain why the IVES induced change in their micturition threshold volume was rather moderate. The same fact may explain why five minutes of physiological activation of bladder afferents by imposed bladder contractions was not enough to modulate the micturition threshold (paper IV). Perhaps it is not possible to push the system beyond the optimal physiological level unless intensive artificial stimulation is applied, as with IVES.
The cat and man voids less frequently than rats, about 5 - 6 times per day, which means that they would require a more prolonged modulation. In this perspective, it was probably important for the reflex enhancement experiments (paper VI) that the bladders were left empty and resting for at least 5 hours before an effective conditioning stimulation. The lack of reflex enhancement with subsequent stimulation within an hour from the first session agrees with the idea of a longer modulation period in cats. In the future, it would be desirable to develop chronic animal models with defective voidings to determine if a proper micturition function can be restored by IVES on a long-term basis.

**Clinical application of IVES**

Based on the above considerations, it seems likely that the clinical application of IVES capitalizes on a normal physiological adaptive process. Many stimulated patients would begin with a down-regulated synaptic transmission in the central micturition pathway after long term use of catheterization or abdominal straining. Such procedures to empty the bladder would give little activation of bladder mechanoreceptors. The subnormal perception of bladder fullness in many such patients is consistent with down-regulated synaptic transmission in the sensory pathway from the bladder to the cerebral cortex (Katona, 1975). In this scenario, it is easy to understand why IVES treatment may give a lasting improvement of bladder function. The micturition reflex will initially be upgraded by the artificial stimulation of bladder afferents. Once the patients can void normally, an adequate function will be maintained by daily “training sessions” – the normal voidings – just as in healthy individuals.

The optimal IVES frequency (10 to 20 Hz) was the same for prolonged modulation of the micturition reflex threshold in rats (paper IV) as for the induction of reflex detrusor contractions in cats (paper I). This relatively slow rate corresponds closely to the maximal maintained firing rate of bladder mechanoreceptor afferents (Häbler et al, 1993b). As with other forms of neuromodulation (Madison et al., 1991; Linden, 1994), the frequency match presumably reflects the properties of the underlying biological process. A reasonable inference is that low IVES frequencies might be better in patients as well (Madersbacher & Ebner, 1992; Gladh et al., 1993). Much higher frequencies have been used in most other clinical studies.

The IVES duration is another relevant parameter. Optimal prolonged modulation in the rat was obtained by rather short periods of stimulation (5 minutes; paper IV). IVES for one minute was not enough, while 20 minutes seemed to be too long, probably because of some fatiguing process. In current clinical practice, considerably longer IVES sessions (60 to 90 min) are used. Although patients may not behave as rats, these longer stimulation periods have not been derived from clinical trials. In fact, shorter periods of stimulation (20 min) of pudendal afferents are routinely applied for patient with urge incontinence (Godec et al., 1978; Plevnic et al., 1979). Therefore, a shorter IVES period
seems worth trying. The clinical benefits would be a more tolerable procedure at a lower cost.

The IVES intensity is also critical. Several clinical studies with negative results have apparently used stimulation intensities even below those used in rats and cats (Nicolas & Eckstein, 1975; Boone et al., 1992). In such cases, no therapeutic effect can be expected. Due to the larger size of the human bladder, considerably higher stimulation intensities would be required to obtain the same field density in the bladder wall, as in the experimental animals. Since only Aδ afferents need to be stimulated, patients can still be treated adequately by IVES without induction of pain. Visceral nociceptors in the pelvic region have, as already mentioned, unmyelinated (C) afferents (Cervero, 1994) and would not be activated.

Pain sensation may also arise at submaximal IVES intensities by stimulation of cutaneous nociceptors of Aδ type at the site of the anode. This problem can be avoided by the use of skin electrodes with good contact over a large surface area (G. Gladh & S. Lindström, unpublished observations). Thereby, the local intracutaneous current density can be kept quite low, below the threshold of cutaneous Aδ afferents. Note that nerve fibers are stimulated more effectively by the cathode than the anode. This is the reason why IVES is most effective with the intravesical electrode as cathode (paper I).

Muscle twitches, due to unwanted current spread to the sacral plexus, are occasionally encountered with IVES in man (G. Gladh, personal communication). This activity is easily eliminated by a small adjustment of the position of the intravesical electrode and/or reduction in stimulation intensity. An abdominal placement of the anode may help to reduce the current spread to the sacral plexus by biasing the electrical field in the ventral direction. This position for the anode is also preferable for safety reasons since it minimizes the electrical field over the heart.

Most clinical investigators using IVES treatment (Katona, 1975; Madersbacher et al., 1987; Kaplan & Richards, 1988) maintain that the additional facility of biofeedback is an important component of the IVES procedure. Stimulation induced contractions elicit a bladder sensation that is reinforced by the biofeedback. It is believed that this process improves the patient’s awareness of the state of the bladder and thereby their conscious control of the micturition. This notion seems intuitively attractive. However, as shown here, the micturition reflex can be modulated by IVES without bladder contractions in unconscious animals. Thus, it may not be necessary to elicit contractions nor to infer a cognitive process for the IVES effect in man. The treatment has in fact been used with comparably good results at pediatric clinic in Linköping for more than five years without biofeedback (G. Gladh & S. Lindström, personal communication). Apart from the conceptual simplicity in omitting this step, patient managed home stimulation is easier to arrange.
SUMMARY AND CONCLUSIONS

• IVES induced reflex detrusor contractions in both cats and rats. The response was due to field stimulation of mechanoreceptor Aδ afferents in the bladder wall.

• Short periods of IVES at proper intensity and frequency gave a prolonged decrease in micturition threshold volume in anesthetized rats.

• Similar stimulation of ano-genital afferents produced the opposite effect, a prolonged increase in micturition threshold volume.

• The prolonged changes in the micturition reflex threshold were not due to increases in afferent sensitivity or efferent efficacy but to modulation of synaptic transmission in the central micturition reflex pathway.

• The IVES induced modulation of the micturition reflex was prevented by systemic administration of an NMDA antagonist, suggesting that an LTP like mechanism was involved.

• The optimal IVES frequency was 10 to 20 Hz for 5 minutes.

• Bladder contractions were not critical for the modulatory effect.

• IVES like selective stimulation of bladder Aδ afferents evoked a prolonged enhancement of the micturition reflex discharge in nerve fibers to the bladder – a further demonstration that the modulation was specific for bladder Aδ afferents and involved facilitation of central synapses.

• It is proposed that the IVES induced modulation of the micturition reflex reflects a physiological adaptive mechanism, which can be mobilized to restore normal voiding functions in patients.

• The findings may provide the first steps towards a theoretical explanation of the clinical beneficial effect of IVES in patients with defective voidings.
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