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MYELINATED PRIMARY AFFERENTS OF THE SACRAL SPINAL CORD RESPONDING TO SLOW FILLING AND DISTENSION OF THE CAT URINARY BLADDER

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SUMMARY

1. A total of sixty-five sacral afferent neurones with myelinated fibres supplying the urinary bladder was recorded from the sacral roots S2 in anaesthetized cats. All afferent units were identified with electrical stimulation of the pelvic nerve. The discharge properties were quantitatively evaluated using slow filling at rates of 1–2 ml min^{-1} and isotonic distension to preset pressure levels. Eight afferents were studied prior to and after acute sacral de-efferentation of the urinary bladder.

2. All afferent units were silent when the bladder was empty and responded in a graded manner to an increase of intravesical pressure. During slow filling the level of afferent activity correlated closely with the level of the intravesical pressure. All afferents behaved like slowly adapting mechanoreceptors with both a dynamic and static component of their discharge. With the exception of two units the intraluminal pressure threshold was below 25 mmHg. Thus virtually all myelinated afferents respond in the pressure range that is reached during a non-painful micturition cycle.

3. The stimulus–response functions of the afferents were similar regardless of whether intravesical pressure was increased by slow filling or by distension. However, during slow filling stimulation response functions often exhibited steeper slopes between 5 and 25 mmHg indicating that relatively small changes of intravesical pressure result in large changes of afferent activity. Nevertheless, all units displayed monotonically increasing stimulus response functions throughout the innocuous and noxious pressure level.

4. The stimulus–response functions of the afferent neurones did not change after acute de-efferentation of the urinary bladder, although the rapid phasic fluctuations of afferent activity that are produced by small contractions of the urinary bladder under normal conditions largely disappeared. This means that contractions and distension activate the afferent endings by a common mechanism.

5. It is concluded that the myelinated sacral afferents of the urinary bladder form a homogeneous population which encodes all information necessary for the normal regulation of this organ. Furthermore, this set of afferents mediates all sensations which may reach consciousness within a normal micturition cycle.

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INTRODUCTION

The sacral afferent innervation of the urinary bladder is essential for the regulation of storage and evacuation of urine. During continence a low level of afferent activity is thought to evoke sacrolumbar reflexes in lumbar sympathetic neurones resulting in the closure of the internal urethral sphincter and inhibition of the detrusor muscle thereby enhancing bladder capacity (Edvardsen, 1968; de Groat, 1975; de Groat & Booth, 1984; de Groat & Kawatani, 1985; de Groat & Steers, 1990). Activation of sacral bladder afferents beyond micturition threshold powerfully excites parasympathetic neurones of the bladder and consequently elicits micturition contractions of the detrusor muscle (de Groat & Ryall, 1969). Furthermore, the sacral bladder afferents are able to mediate associated non-painful sensations and, exceeding a certain level of intraluminal pressure in the urinary bladder, possibly painful sensations. Human studies have determined that the resting pressure during the storage phase varies typically from 5 to 15 mmHg and it increases under isovolumetric conditions to about 25–30 mmHg when normal, non-painful micturition commences (see Jänig & Morrison, 1986; Jänig & Koltzenburg, 1992). The sacral afferent neurones which are involved in signalling these events have myelinated axons in the cat (Bahns, Halsband & Jänig, 1987).

Sacral afferent neurones with unmyelinated axons are not involved in the physiological regulation of the urinary bladder, but are activated under painful pathophysiological conditions such as inflammatory processes or acute urinary retention (Häbler, Jänig & Koltzenburg, 1990*a*). Investigations after selective lesions of primary afferent pathways indicate that all consciously perceived sensations from the urinary bladder including the pain of acute over-distension can be signalled by primary afferents in the pelvic nerve (Jänig & Morrison, 1986; Jänig & Koltzenburg, 1992).

Few studies have investigated the sacral afferent innervation quantitatively (Iggo, 1955; Clifton, Coggeshall, Vance & Willis, 1976; Floyd & Lawrenson, 1979; Bahns *et al.* 1987). It has been shown that afferent units respond in a graded fashion to short-lasting increases of intravesical pressure produced by bladder contractions and distension. However, little is known about the capacity of myelinated bladder afferents to encode long-lasting changes of intravesical pressure which are the physiologically relevant events of the micturition reflex. In the present study we have therefore quantitatively investigated the responses of the sacral myelinated afferent fibres from the urinary bladder, using two stimulation protocols, namely isotonic distension, as used in most previous studies, and slow filling at low rates (1–2 ml min^{-1}). This is approximately the maximal physiological rate of bladder filling in the cat (Klevmark, 1974) and corresponds when scaled to the speed of filling used in many cystometric investigations in humans. To study the possible modulatory effects of efferent parasympathetic neurones on afferent activity some afferents were also studied prior to and after the acute de-efferentation of the bladder.

Some of the results have been published in preliminary form (Häbler, Jänig & Koltzenburg, 1990*b*).

METHODS

Anaesthesia and animal maintenance

Thirty-one adult cats of either sex weighing 2·8–5·5 kg were used. Following induction with ketamine (Ketanest®; 15–20 mg kg^{-1}, I.M.) the animals were anaesthetized with α-D-glucochloralose (50 mg kg^{-1}, intraperitoneally). Supplementary doses of 5–10 mg kg^{-1} chloralose were given intravenously to maintain deep anaesthesia as judged by the persistence of miotic pupils and the lack of heart rate and blood pressure fluctuations in the absence of visceral stimuli. Further criteria for adequate anaesthesia were the absence of gross blood pressure and heart rate fluctuations and the lack of pupillary reactions during the visceral stimuli. Blood pressure and ECG were continuously monitored after cannulation of the common carotid artery. The mean arterial pressure always exceeded 80 mmHg throughout the experiments. Drugs were injected into the external jugular vein. Animals were paralysed with pancuronium bromide (Pancuronium®; 0·2 mg kg^{-1} per bolus, additional doses as required I.V.) and artificially ventilated through a tracheal cannula, keeping the end-expiratory CO_2 at 3–4 vol. %. Body core temperature was measured intraoesophageally and maintained close to 38 °C.

Dissection

A lumbosacral laminectomy was performed. After incision of the dura mater the left dorsal root ganglion S2 was identified. The left dorsal root (in twenty-four experiments) or the left ventral root (in seven experiments) of this segment was put on a rigidly fixed black Perspex platform and prepared for recording. Using a lateral approach the left pelvic nerve was dissected through the sciatic notch and isolated from surrounding tissue. It was then mounted for bipolar electrical stimulation on a pair of platinum electrodes. In order to reduce the on-going activity of somatic afferent fibres the tail was amputated at its base, and the left sciatic and femoral nerves were cut. The exposure was covered with warm paraffin oil in a pool made with skin flaps.

Recording and stimulation technique

Neurophysiology. Centrally cut filaments were isolated from the dorsal and ventral roots. Signals from single units were monopolarly recorded with a platinum electrode. The indifferent electrode was positioned nearby. Activity was amplified by a low-noise differential AC preamplifier (input resistance 10 MΩ) and band pass filtered (about 90 Hz–1·2 kHz). Action potentials were passed through window discriminators and the obtained standard impulses were fed into a laboratory computer to construct peri-stimulus histograms. In order to control spike discrimination all discriminated action potentials were continuously displayed on an oscilloscope after having passed a 5 ms delay circuit.

For identification of a single unit as being a bladder afferent an isotonic test stimulus was applied to the urinary bladder. For electrical identification of units, the pelvic nerve was electrically stimulated with single pulses (pulse width 0·5 ms, repetition rate 0·2–0·5 Hz, intensity < 30 V). Fibres conducting at more than 2·5 m s^{-1} were considered to be myelinated.

Urinary Bladder. The urinary bladder was catheterized transurethrally (outer catheter diameter 2·0–2·4 mm). After a laparotomy a plastic tube (inner diameter 1·6 mm) was inserted through the apex of the bladder and secured in the bladder wall by an atraumatic 5-0 silk suture. The laparotomy was sutured and the animal placed in a prone position.

Intravesical pressure was varied in two different ways, either by slowly filling transurethrally by means of an infusion pump or by isotonic distension with the help of a pressure reservoir using normal saline at room temperature in both cases. The rate of slow filling was 1–2 ml min^{-1} which is slightly above the maximal rate occurring under physiological conditions (Klevmark, 1974). The rise time to reach a preselected pressure plateau was about 30 s during a stepwise increase of intravesical pressure, the half-rise time being less than 3 s. In most experiments, intravesical pressure was continuously registered with two separate sets of pressure transducers, one being connected to the apex catheter and the other to the urethral catheter. Generally there was a good correlation between the two pressure recordings. During slow filling of the urinary bladder virtually identical readings were obtained from both devices.

Acquisition and quantitative evaluation of data, statistical tests

Intravesical pressure recordings and neural activity were displayed on oscilloscopes for

photography and read into a laboratory computer (Minc, PDP 11; Digital). All data were stored on magnetic tape (SE 7000, EMI) for off-line analysis.

Stimulus–response curves were constructed by plotting the mean neuronal activity during a stimulation period against the corresponding mean intravesical pressure. For this purpose the whole period of slow filling was divided into 30 s intervals. The corresponding pressure values were put in classes of 5 mmHg each. The mean values of neuronal activity were then calculated for each class. In some cases the neuronal activity obtained in each 30 s period was directly plotted against the actual mean pressure prevailing in the bladder (see e.g. Fig. 5*C*). When using isotonic distension the mean neuronal activity was calculated during the whole stimulus lasting 60–90 s. Each series of isotonic distension consisted of four or five different stimuli. Thus, stimulus–response curves obtained from slow bladder filling are constructed from more pairs of values (normally ten) and are therefore more accurate than those obtained from isotonic distension.

For statistical calculations the paired *t* test and the *U* test of Whitney and Mann were used.

RESULTS

A total of sixty-five myelinated afferents (conduction velocity 2·5–15 m s^{-1}) supplying the urinary bladder was investigated. Sixty-two were isolated in the dorsal root, whilst three were recorded in the ventral root S2. All units were reproducibly excited by the search stimulus which consisted of an isotonic distension stimulus of 75 mmHg for 60 s. Thirty-four dorsal root afferents were studied quantitatively with a series of at least four isotonic distension stimuli and twenty-eight dorsal root afferents were studied quantitatively with both slow filling and a series of isotonic distension of the urinary bladder (Fig. 1).

Qualitative responses

In agreement with previous studies all afferents were silent when the bladder was empty (Iggo, 1955; Bahns *et al.* 1987). Furthermore, during the initial phase of slow filling when intravesical pressure increased very slowly the afferent units typically did not discharge (Fig. 1*B*). Before intravesical pressure began to rise more steeply the afferents passed their firing threshold and, from then on, faithfully encoded intravesical pressure by their discharge frequency. Isotonic distension of the bladder by pressure steps (continuous line in Fig. 1*C*) evoked a graded increase of the afferent discharge in all cases, as did slow filling (■ in Fig. 1*C*), but provided less information about the stimulus–response characteristics than slow filling (compare both procedures in Fig. 1*B*).

Quantitative responses

The quantitative evaluation of afferent responses to either stimulation protocol rendered similar results with respect to the shape of the stimulation–response functions and to the firing rates corresponding to a given intravesical pressure (Figs 1*C* and 2). The only difference was that more units showed some saturation in the higher pressure range when slow filling was applied. However, most units encoded the prevailing intravesical pressure including the noxious range with an increase of their discharge frequency. From the normalized responses of the same twenty-eight afferents to slow filling and isotonic distension with pressures not exceeding 50 mmHg (Fig. 3) it is obvious that both procedures evoke a similar slope of the stimulation–response functions and almost identical firing thresholds (which were defined as 10% values of the maximal discharge rate and graphically determined

from the stimulus–response curves). However, slow filling of the bladder shows that many afferents exhibit an increased slope of the stimulus–response function (Fig. 3*A*) between 5 and 25 mmHg, which is the physiological working range of intravesical pressure.

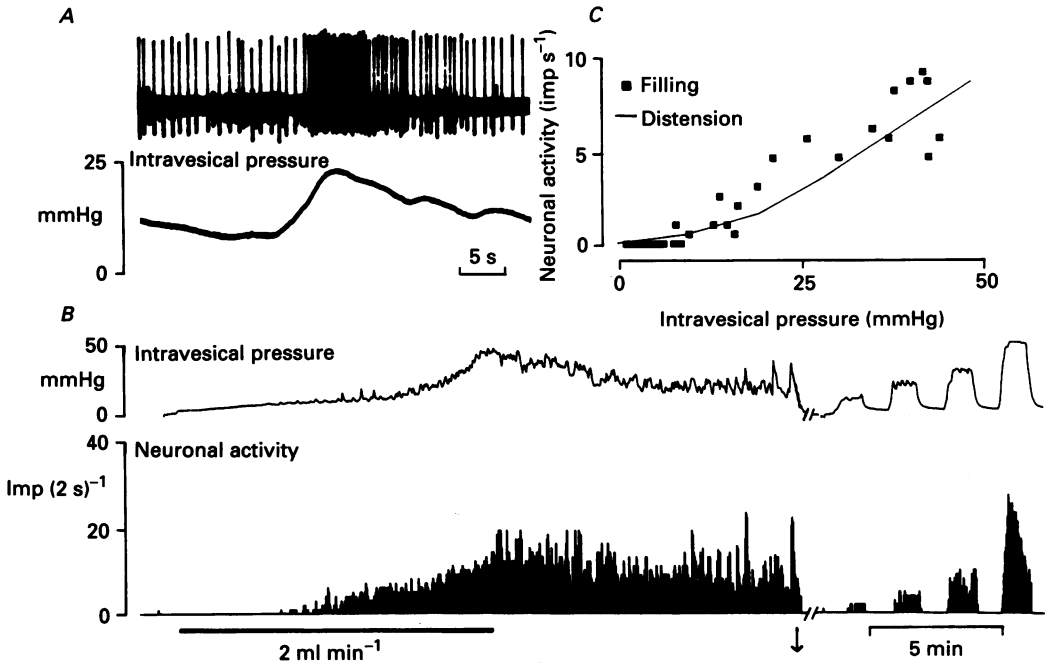

Fig. 1. Responses of an afferent unit during slow filling and distension of the urinary bladder. *A*, specimen record taken during slow filling of the urinary bladder through the urethral catheter at 2 ml min^{-1}. Upper trace, activity of a single afferent fibre; lower trace, intravesical pressure. *B*, histograms of intravesical pressure and neuronal activity during filling of the bladder (bar) at 2 ml min^{-1} and during a series of distension stimuli (right panel). The arrow indicates emptying of the bladder. The afferent unit exhibits no spontaneous activity and does not respond during the first 5 min of slow filling. Thereafter, the increase of intravesical pressure is faithfully encoded. After termination of filling the unit exhibits considerable phasic responses to isovolumetric bladder contractions. *C*, stimulation–response relations of the afferent activity for both stimulation procedures showing a close similarity of the quantitative responses.

Two afferents which were tested to isotonic distension only, one recorded in the dorsal root and the other in the ventral root S2, had a high threshold above 30 mmHg (curves marked by asterisks in Fig. 2*B*). The other units had thresholds which were well below 25 mmHg (Figs 1, 2 and 3).

Dynamic and static firing

All afferents exhibited static as well as dynamic discharge (Figs 1 and 5). When the bladder was slowly filled, they encoded both the level of intravesical pressure and the superimposed rapid contractions (Fig. 1*A*). Isotonic distension evoked a slowly adapting discharge (Figs 1*B* and 4). However, while none of the afferents showed an

exclusive static or dynamic firing pattern, in about half of the units the dynamic discharge behaviour clearly predominated (Fig. 5). This might be due to a particular location of the receptors of these units in the wall of the urinary bladder.

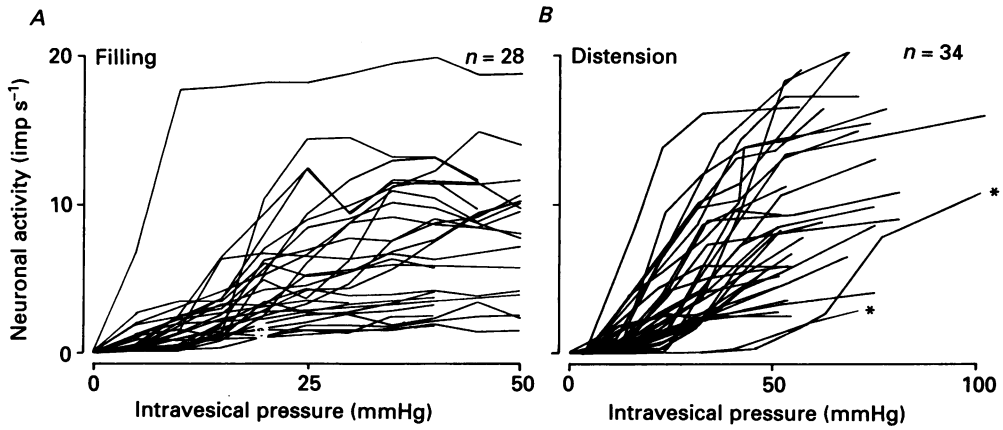

Fig. 2. Stimulation–response curves of activity in single myelinated afferent units to increases of intravesical pressure evoked by slow filling (*A*) and by a series of isotonic distension of the urinary bladder (*B*). The curves marked by asterisks in *B* were obtained from two units which had higher pressure thresholds. The bladder afferents show increasing discharge with rising intravesical pressure. Many reach a plateau during higher pressures, but most units also increase their discharge frequency within the noxious pressure range (> 30 mmHg). Taking into account the different abscissa scales, the response characteristics and the firing rates at a given intravesical pressure are similar in *A* and *B*.

The dynamic and static properties of the thirty-two low-threshold afferents shown in Fig. 2*B* were compared quantitatively (Fig. 4). For this purpose the neural responses to the first 10 s (as a measure for the dynamic component) and the last 10 s (as a measure for the static component) of isotonic distension stimuli lasting 90 s were plotted against intravesical pressure. Whilst the responses are identical in the lower range up to 30 mmHg, there is a significant preponderance of the dynamic responses at higher pressures reaching statistical significance at 40 mmHg (paired *t* test). The lack of a difference in the lower pressure range is probably due in part to the urethral catheter which represents a flow resistance and hence delays isotonic distension of the urinary bladder at lower pressure when the driving force for fluid movement towards the bladder is low.

Sacral rhizotomy

Since passive distension, but also active contractions of the bladder evoked firing in mechanosensitive afferents (see Fig. 1*A*) the question arises as to whether contractions contribute to the excitation of bladder afferents by mechanisms other than increasing intravesical pressure. This was explicitly tested in four experiments in which eight afferents were quantitatively studied before and after complete sacral rhizotomy (Figs 5 and 6).

This procedure abolished the larger, rapidly occurring urinary bladder contractions

leaving only small, presumably myogenic ones which built up slowly. They occurred almost exclusively at the beginning of filling and were absent at higher levels of bladder distension (compare *A* and *B* of Fig. 5). After rhizotomy a comparable pressure to that in the intact animal was only reached with longer filling, as more

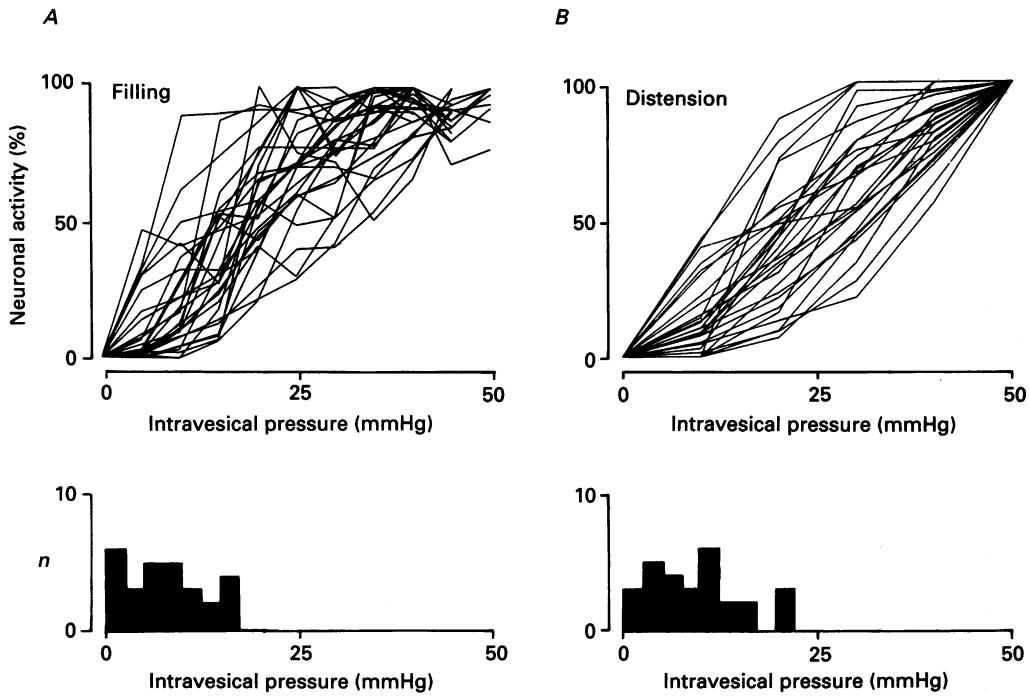

Fig. 3. Comparison of stimulation–response functions constructed from the responses in the same afferent units ($n = 28$) to slow filling (*A*) and to a series of isotonic distension (*B*) of the urinary bladder. Ordinate scale in upper histograms was normalized with respect to the afferent activity at 50 mmHg (100%). Threshold values in lower histograms were defined as 10% activity of maximal response at 50 mmHg and determined graphically from the stimulation–response curves. Note that the gross stimulation–response functions are similar in *A* and *B*, but that slow filling in many cases renders curves with increased slopes between about 5 and 25 mmHg (*A*). The distributions of the thresholds in *A* and *B* do not differ statistically ($P > 0{\cdot}1$, *U* test of Mann and Whitney).

intravesical volume was necessary to build up a corresponding pressure level. The dynamic firing in bursts ensuing from bladder contractions was reduced which resulted also in a reduction of the transient peak frequencies of afferent discharge. By contrast, the static discharge was unaffected (Fig. 5*A* and *B*). The quantitative analysis of the responses before and after rhizotomy shows that the stimulus–response function of the afferent units (Fig. 5*C*) was virtually unchanged after interruption of the reflex pathway. Furthermore, the mean stimulation–response curves of the eight afferent units tested (Fig. 6) also revealed only a slight difference in the higher pressure range which did not reach statistical significance ($P > 0{\cdot}1$, paired *t* test).

DISCUSSION

In the present study sacral myelinated urinary bladder afferents have been recorded while simulating the collection of urine under physiological conditions. This was achieved by slowly filling the bladder at low rates which is comparable to human cystometry. This experimental approach allows a direct comparison between the

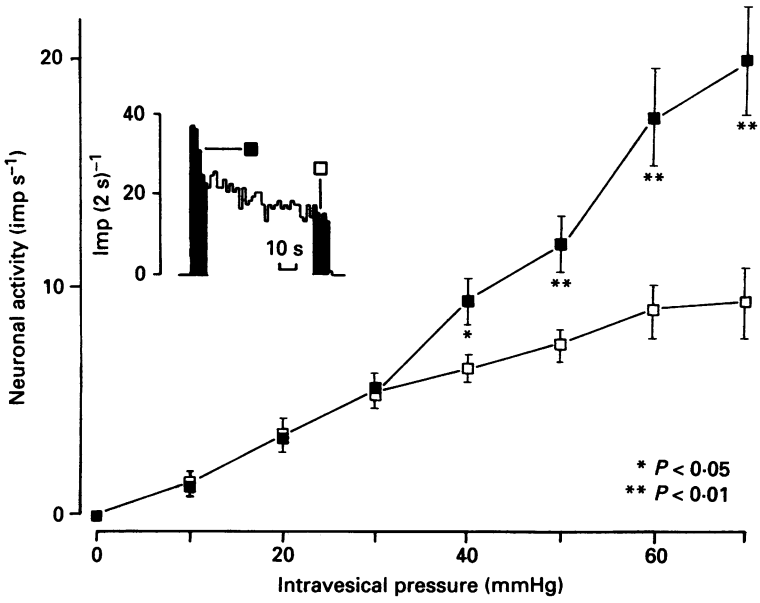

Fig. 4. Quantitative comparison of the phasic responses and the steady-state responses of single afferent units during isotonic distension of the urinary bladder. Stimulation–response functions were constructed from the responses in the first (■) and the last 10 s (□) of distension stimuli of 90 s duration (see inset). The values are expressed as means ± S.E.M. ($n = 32$). The curves are significantly different (paired t test) at pressures exceeding 30 mmHg.

pressure values occurring during the normal regulation of the urinary bladder and the associated sensations that may occur in humans and the discharge characteristics of those primary afferents which are known to be an essential component for the regulation of this organ (Barrington, 1931). Furthermore, the present study demonstrates that slow filling is probably a more accurate way of determining the stimulus–response functions than by stepwise isotonic distension of the bladder. Finally the quantitative responses of thin myelinated bladder afferents in relation to intravesical pressure have been shown to be independent of the efferent parasympathetic innervation.

Three myelinated afferents from the bladder were recorded from the ventral root S2. The existence of afferents in the sacral ventral roots supplying various visceral and somatic tissues, and in particular the urinary bladder, is well established (Clifton *et al.* 1976; Floyd, Koley & Morrison, 1976; Häbler, Jänig, Koltzenburg & McMahon, 1990*c*). It has been suggested that ventral root afferents serve nociceptive functions

(Clifton *et al.* 1976); however, in the present study, only one unit recorded from the ventral root had a threshold in the noxious range while the remaining units were indistinguishable in their receptive properties from the remaining dorsal root afferents.

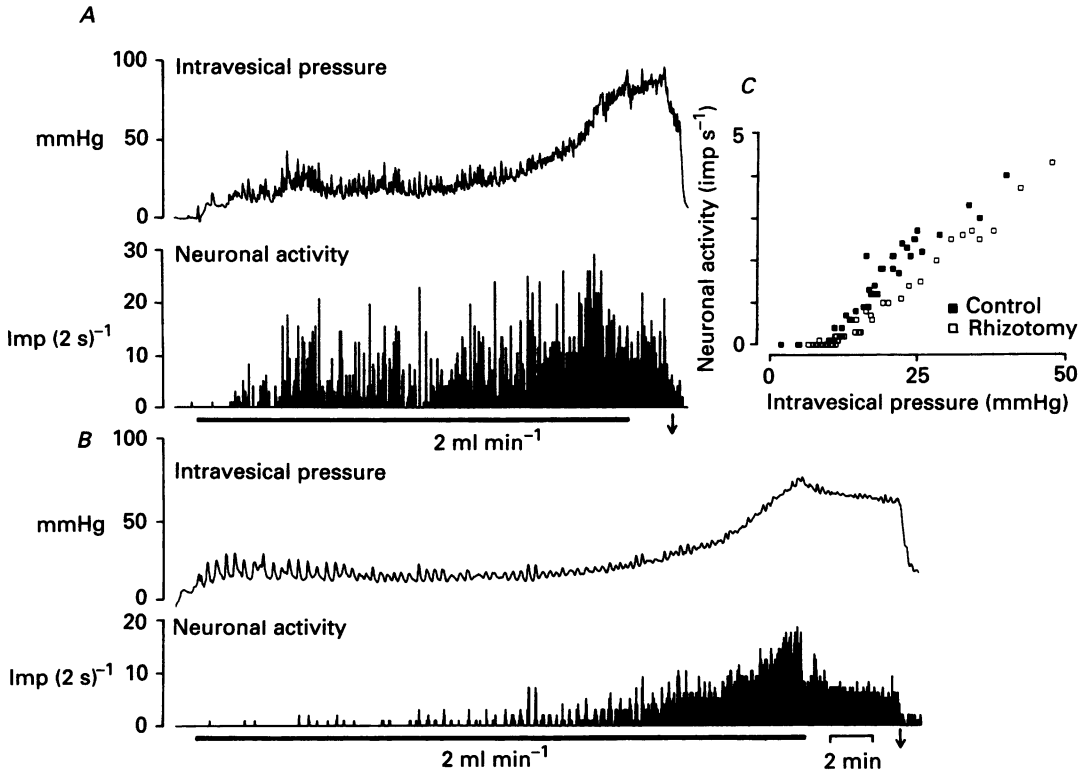

Fig. 5. Response of an afferent unit during slow filling of the urinary bladder before (*A*) and after (*B*) complete sacral rhizotomy. *C* illustrates the stimulation–response functions under both conditions. The dorsal and ventral sacral roots were cut bilaterally between records *A* and *B*. Bars indicate the period of slow bladder filling. Downward arrows indicate emptying of the bladder. Note that the unit shows a pronounced phasic discharge. After rhizotomy the frequency and amplitude of small bladder contractions are reduced (particularly at higher pressure level) whilst the increase of intravesical pressure is delayed resulting in an enhanced volume capacity. The stimulation–response functions are virtually identical before and after rhizotomy (*C*).

The sacral myelinated bladder afferents are known to elicit micturition, once an appropriate volume is collected in the urinary bladder. The tension of the bladder wall necessary to evoke micturition in the cat has been found to correspond to an intravesical pressure of about 10–15 cmH_2O (Edvardsen, 1968) and humans report an urge to micturate at similar pressure levels (Denny-Brown & Robertson, 1933; Lapides & Lovegrove, 1965). In most studies the maximal transmural pressure (i.e. intravesical minus intra-abdominal pressure) which has been measured under isovolumetric conditions, i.e. before the bladder outlet opens for micturition, was generally below 30 mmHg (Claridge & Shuttleworth, 1964; Scott, Quesada & Cardus,

1964; Backmann, Garrelts & Sundblad, 1966; Sundblad, 1971; Frimodt-Möller, 1974; Walter, Oleson, Nordling & Hald, 1979). Within this range of intravesical pressure almost all myelinated bladder afferents have their thresholds and greatest sensitivity. It is also in this pressure range that vesico-sympathetic reflexes are evoked in vasoconstrictor neurones (Häbler, Hilbers, Jänig, Koltzenburg, Kümmel, Lobenberg-Khosravi & Michaelis, 1992) leading to a rise of systemic blood pressure (Lapides & Lovegrove 1965; Häbler *et al.* 1992). Furthermore, the inhibitory responses observed in primate thoracic spinothalamic tract neurones to graded urinary bladder distension also mirror threshold and quantitative pressure–response relations of myelinated bladder afferents (Brennan, Oh, Hobbs, Garrison & Foreman, 1989).

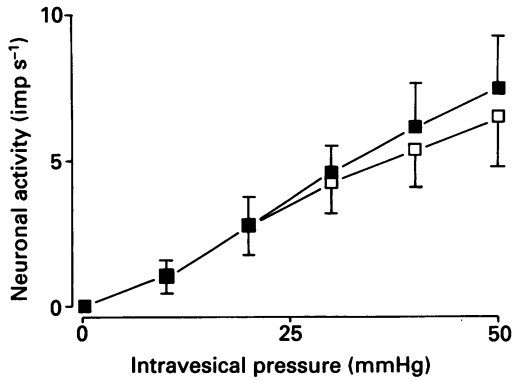

Fig. 6. Stimulation–response functions obtained from the responses of eight single afferent units to slow filling of the urinary bladder before (■) and after (□) cutting the ventral and dorsal sacral roots. Values are expressed as means ± S.E.M. The values before and after rhizotomy do not differ statistically ($P > 0.01$, paired t test).

The myelinated afferents analysed in this study are the appropriate, and only, candidates for eliciting the micturition reflex and the vesico-sympathetic reflexes. They constitute a homogeneous population with graded thresholds below 25 mmHg and greatest sensitivity between 5 and 25 mmHg. It is possible that the two myelinated neurones found in the present study (curves marked with asterisks in Fig. 2*B*) with a threshold exceeding 30 mmHg represent the tail of a functionally homogeneous population.

The finding that myelinated bladder afferents are silent when the urinary bladder is empty and during the initial phase of slow filling corresponds well with the fact that humans do not notice the collected intravesical volume (which is initially collected without a substantial increase in intravesical pressure) before 300 ml or more have accumulated (Denny-Brown & Robertson, 1933; Lapides & Lovegrove, 1965; Torrens & Morrison, 1987).

Those vesical afferents with a particularly pronounced phasic discharge may be located in the bladder neck being activated only at the micturition threshold when the bladder base may be distended passively (Torrens & Morrison, 1987). These afferents may play an important role in triggering micturition contractions, since it is known that an afferent burst pattern facilitates the responses of sacral

preganglionic parasympathetic neurones supplying the urinary bladder (De Groat & Ryall, 1969; de Groat, 1975).

Sacral rhizotomy does not change the stimulation–response functions of myelinated afferents quantitatively. This means that in principle it makes no difference whether the receptive endings are activated by passive distension or active contraction, confirming an assumption already made by Iggo (1955). Nevertheless, the reflex arc involving the parasympathetic pre- and postganglionic neurones to the bladder may serve as a servo-mechanism leading to a positive feedback which is necessary for the strong micturition contractions to build up rapidly (Torrens & Morrison, 1987). On the other hand, muscular tone of the detrusor is able to regulate the sensitivity of the mechanoreceptors and consequently bladder capacity. This can lead to a vicious cycle under pathological conditions.

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