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ACTIVATION OF UNMYELINATED AFFERENT FIBRES BY MECHANICAL STIMULI AND INFLAMMATION OF THE URINARY BLADDER IN THE CAT

BY H.-J. HÄBLER, W. JÄNIG AND M. KOLTZENBURG

From the Physiologisches Institut, Christian-Albrechts-Universität zu Kiel, Olshausenstraße 40–60, D-2300 Kiel, FRG

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SUMMARY

1. We examined the functional properties of unmyelinated primary afferent neurones innervating the pelvic viscera in twenty-five anaesthetized cats. The axons were isolated from the intact dorsal root and the intact or chronically de-efferented ventral root of the segment S2. All units were electrically identified with electrical stimulation of the pelvic nerve.

2. The responses of the neurones were studied with natural stimulation of the urinary bladder using innocuous and noxious increases of intravesical pressure and at the onset of an acute artificial inflammation induced by intraluminal injection of mustard or turpentine oil.

3. Out of 297 unmyelinated afferent units isolated from the dorsal root, seven were excited by an increase of the intravesical pressure during contractions and distension of the urinary bladder. These units were silent when the bladder was empty and had thresholds of 30–50 mmHg which are presumed to be noxious. Further increases of the intravesical pressure were accurately encoded by the discharge rate of the fibres. Out of sixty-eight unmyelinated afferent units isolated from the ventral root none was activated by these stimuli.

4. Intraluminal injection of mustard oil excited mechanosensitive units at short latency. The discharge was not closely related to changes of the intravesical pressure and the units displayed on-going activity after the irritant had been removed. This observation suggests that the units had also chemosensitive properties and that the receptive endings were located in the bladder wall.

5. In sixteen cats ninety-five afferent fibres that were not activated by noxious mechanical stimuli of the urinary bladder were systematically tested with intraluminal injections of mustard oil. This excited 7/67 dorsal root units and 4/28 ventral root units with short latency. Intraluminal application of turpentine oil, tested on twenty-six afferents in four animals, did not produce a rapid excitation.

6. Following the induction of an inflammation some previously non-mechanosensitive units started to respond to changes of intravesical pressure in the biologically relevant pressure range of the urinary bladder.

7. In conclusion, a small subpopulation (2·4%) of unmyelinated visceral afferents responds to high, presumably noxious, intravesical pressure and intraluminal

application of chemical irritants. Acute inflammation excites a larger proportion of afferents (9·5%) that are not activated by acute noxious mechanical stimulation of the normal urinary bladder. In the inflamed bladder some previously non-mechanosensitive units started to respond to increases of intravesical pressure. These novel types of chemosensitive receptors may contribute considerably to the pathogenesis of visceral pain states.

INTRODUCTION

Many forms of long-lasting pain are associated with the appearance of a hypersensitive state. The development of hypersensitivity is a prominent finding of the inflammatory process resulting in the presence of spontaneous discomfort and the exaggerated perception of pain following innocuous stimuli. Although hypersensitivity has been well documented following injury of the skin (Lewis, 1942; Hardy, Wolff & Goodell, 1950) it appears to be particularly important for the generation of deep somatic and visceral pain. Thus, visceral tissues can become the source of pain and on-going tenderness during inflammation, although they are largely insensitive to a wide variety of stimuli under normal non-inflamed conditions (McLellan & Goodell, 1943; Wolf, 1965). Using the urinary bladder as a model, we have studied the properties of visceral primary afferent neurones during acute noxious stimulation of the organ and at the onset of an inflammation.

Pain and non-painful sensations from the pelvic viscera in general and from the urinary bladder in particular are mediated by lumbar and sacral afferent neurones that project through the hypogastric and pelvic nerves respectively (Jänig & Morrison, 1986). As a rule, afferent neurones in both nerves respond to stimulation of one viscus only (Bahns, Ernsberger, Jänig & Nelke, 1986; Jänig & Morrison, 1986; Bahns, Halsband & Jänig, 1987). Afferent fibres innervating the urinary bladder are functionally homogeneous when tested with intravesical pressure stimuli that occur during distentions and isovolumetric contractions (Floyd, Hick & Morrison, 1976; Bahns *et al.* 1986, 1987). They have low thresholds and accurately encode a rise of intraluminal pressure in the innocuous and noxious range by an increase of their discharge frequency. This applies to both myelinated and unmyelinated afferents of the hypogastric nerve and to myelinated sacral fibres of the pelvic nerve (Iggo, 1955; Floyd *et al.* 1976; Bahns *et al.* 1986, 1987). Sacral afferent neurones with unmyelinated fibres in the pelvic nerve have previously been found not to respond to distension and isovolumetric contraction of the urinary bladder (Bahns *et al.* 1987). This is surprising, as a rich supply of unmyelinated pelvic afferent fibres has been histologically detected in the urinary bladder (de Groat, 1987).

In this study we have therefore concentrated on the population of unmyelinated fibres projecting into the pelvic nerve using noxious mechanical and chemical stimulation of the urinary bladder. Our results show that a few unmyelinated afferents can be activated by high, presumably noxious intravesical pressure. A larger population of afferent neurones that does not respond to noxious pressure stimuli in the normal bladder can be recruited at the onset of an acute inflammation. This novel type of visceral receptor may contribute considerably to the development of hypersensitivity and pathogenesis of long-lasting visceral pain states.

METHODS

Anaesthesia and animal maintenance

Twenty-five adult cats of either sex weighing 2·8–5·5 kg were used. Following induction with ketamine (Ketanest^R; 15–20 mg/kg, I.M.) the animals were anaesthetized with α-D-gluco-chloralose (50 mg/kg, I.P.). Supplementary doses of 5–10 mg/kg α-D-gluco-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. Blood pressure and heart rate were continuously recorded after cannulation of the common carotid artery and the mean arterial pressure always exceeded 80 mmHg. Drugs were injected into the external jugular vein. Animals were paralysed by pancuronium bromide (Pancuronium^R; 0·2 mg/kg per bolus, total dose as required, I.V.) and artificially ventilated through a tracheal cannula, keeping the end-expiratory CO_2 concentration at 3–4%. Body core temperature was measured intraoesophageally and maintained close to 38 °C.

Dissection

Following a lumbosacral laminectomy and incision of the dura mater the left dorsal root ganglion S2 was identified. In eighteen animals the dorsal root and in the remaining seven the ventral root of this segment were prepared for recordings. Using a lateral approach, the left pelvic nerve was dissected through the sciatic notch, isolated from surrounding tissue with a plastic sheath and mounted for bipolar electrical stimulation on a pair of platinum electrodes (Fig. 1). To reduce the on-going activity of somatic afferent fibres that otherwise tended to dominate the activity of the filaments, the nerves of the left hindlimb were cut and the tail amputated at its base. Finally, the exposure was covered with warm paraffin oil in a pool made with skin flaps.

The urinary bladder was catheterized transurethrally using catheters with an outer diameter of 2·0 or 2·4 mm for male and female animals respectively. Following a laparotomy a plastic tube (outer diameter 2·0 mm, inner diameter 1·6 mm) was inserted through the apex of the bladder and secured by an atraumatic 5–0 silk suture in the bladder wall. The incision of the laparotomy was sutured and the animal placed in a prone position.

De-efferented ventral roots

The ventral root of three animals had been de-efferented 4 days prior to the terminal experiment to eliminate the population of unmyelinated preganglionic parasympathetic fibres that exit through this root. Following induction with ketamine (Ketanest^R; 15 mg/kg, I.M.) and diazepam (Valium^R; 0·2 mg/kg, I.M.) the animals were anaesthetized with methohexital (Brevimytal^R; 10–20 mg per bolus I.M., total dose as required). With antiseptic precautions a small lumbosacral hemi-laminectomy was performed and the ventral root S2 cut intradurally close to its exit from the spinal cord. The defect in the dura was covered with a plastic sheath and muscle and skin closed in layers. Post-operatively, the animals received subcutaneous doses of pentazocin (Fortral^R; 0·3 mg/kg) as required and recovery was uneventful. In particular, micturition and defaecation were retained in all animals.

Recording and stimulation technique

Neurophysiology. Centrally cut filaments, isolated from either dorsal or ventral root, were repeatedly split with sharpened watchmaker forceps on a Perspex platform until single-unit activity could be recorded. Signals were monopolarly recorded by a platinum electrode with an indifferent electrode positioned nearby. Activity was amplified by a low-noise differential AC preamplifier (input resistance 10 MΩ) and filtered with a bandwidth of 70–120 Hz and 1·2–1·5 kHz. Separate electrodes and signal processing were employed to record from two or three different strands simultaneously. The activity of units was passed through window discriminators and the obtained unitary signals were fed into a laboratory computer to construct peristimulus histograms. To control the lower and upper amplitude of each window discriminator all discriminated spikes were continuously displayed on an oscilloscope after having passed a 5 ms long delay circuit (see inset in Figs 6, 7, 10).

For electrical identification of units, the pelvic nerve was stimulated with square-wave pulses of 0·5 ms duration at 0·2–0·5 Hz and variable intensity up to 30 V. Fibres conducting at less than 2·5 m/s, as estimated over the entire conduction distance, were considered to be unmyelinated.

Urinary bladder. Intravesical pressure could be manipulated by two different protocols. The bladder was either slowly filled transurethrally by an infusion pump or isotonically distended with the help of a pressure reservoir using normal saline at room temperature in both cases (Fig. 1). The rate of filling was 2 ml/min which is slightly above the maximal physiological urine output (Klevmark, 1974). When isotonic pressure stimuli were used the rise time to a pre-selected pressure plateau was 1–3 s. An acute artificial inflammation was induced in twenty animals using 1–2·5% v/v mustard oil ($n = 16$) or 50% v/v turpentine oil ($n = 4$). Both chemicals were dissolved in low-viscosity paraffin oil (Merck) and 10 ml was applied transurethrally for 10 to 30 min, respectively.

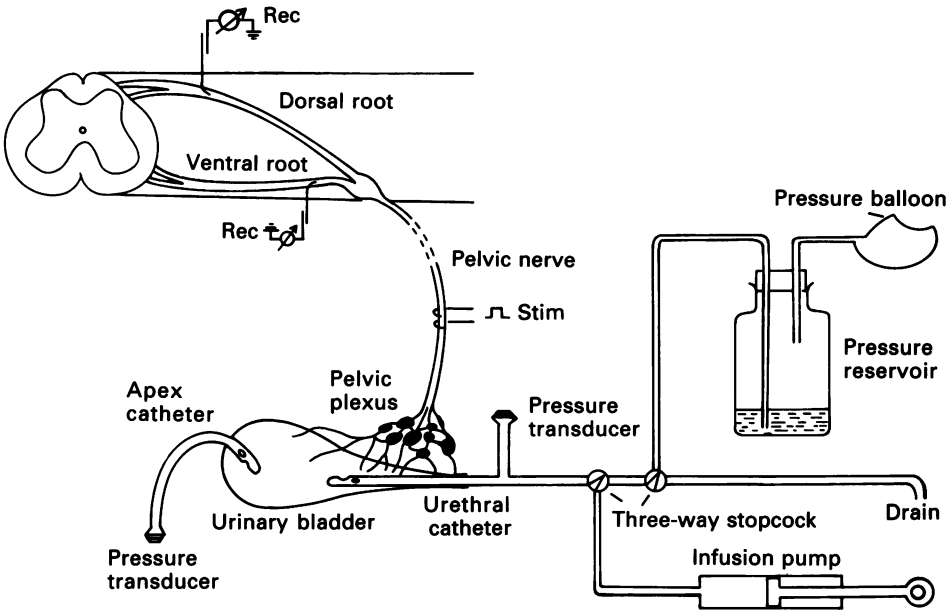

Fig. 1. Centrally cut filaments containing single or few afferent axons were isolated from the dorsal or ventral root S2 for recording (Rec). Neurones projecting into the pelvic nerve were identified by electrical stimulation (Stim). The intravesical pressure and apex was measured with pressure transducers connected to the urethral catheter. The bladder was slowly filled at a rate of 2 ml/min with an infusion pump or isotonically distended using a pressure reservoir.

Post-mortem examinations were carried out at the end of the experiments using standard histopathological techniques. They confirmed – as previously described in the rat (McMahon & Abel, 1987) – that both protocols resulted in oedema and infiltration of polymorphonuclear leucocytes of the mucosa and smooth muscle. These changes were largely absent in animals after 24 h of experimentation when the bladder had not been exposed to irritants.

Throughout the experiment, intravesical pressure was continuously recorded with two separate sets of pressure transducers. One was connected to the apex catheter and the other in parallel to the urethral catheter (Fig. 1). Generally there was a good correlation between two pressure recordings. During slow filling of the urinary bladder virtually identical readings were obtained from both devices. During isotonic distension the readings of the apex catheter had a slight time lag during the rise time to a pre-selected pressure plateau as compared to the measurements through the urethral catheter and this discrepancy can be explained by the elastic properties of the urinary bladder (Bahns *et al.* 1986). Once the pressure plateau had been reached both values were practically identical.

Data acquisition and statistical tests

Intravesical pressure recordings and neural activity were displayed on oscilloscopes for photography and simultaneously read into a laboratory computer (Minc, PDP 11; Digital). All

data were stored on magnetic tape (SE 7000, EMI) for off-line analysis. For statistical calculations Fisher's exact test and the modifications of the two-sided t test for unequal sample size was employed (Sachs, 1984).

RESULTS

Mechanosensitive units activated by an increase of intravesical pressure

Primary afferent neurones projecting into the pelvic nerve innervate various organs such as the urinary bladder, hindgut and internal reproductive tract. To ascertain the target organ for each fibre it was necessary to functionally classify the units. Direct localization of the receptive field with local electrical or mechanical stimulation was not used, as the animal lay in a prone position and manipulation of the viscera would have interfered with the recording conditions. However, previous investigations have demonstrated that sacral afferent neurones innervate one viscus only (Jänig & Morrison, 1986). Therefore all electrically identified fibres were tested with an intravesical pressure stimulus of 75 mmHg lasting 1 min to functionally identify mechanosensitive afferent fibres supplying the urinary bladder.

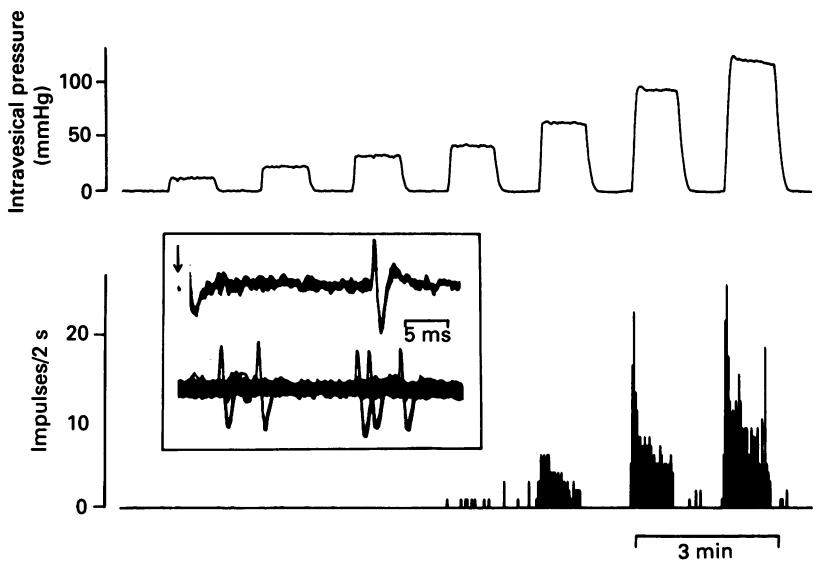

Fig. 2. Activation of an unmyelinated afferent unit by graded distension of the urinary bladder. Inset shows the identification of the unit with electrical stimulation (upper trace, stimulation artifact marked by arrow) of the pelvic nerve (pulse duration 0·5 ms, stimulation intensity 1·5 V; conduction velocity 2·0 m/s) and activation by distension of the urinary bladder (lower trace). Both traces are several times superimposed.

Distension and filling of the urinary bladder

Dorsal root recordings

A total of 297 unmyelinated afferent units responding to electrical stimulation of the pelvic nerve were isolated from the dorsal root S2. The conduction velocity of these units was $1{\cdot}0 \pm 0{\cdot}5$ m/s (mean $\pm$ standard deviation; $n = 210$). In five experiments, only seven afferents were excited by distension of the urinary bladder

and these were investigated in more detail. They had a relatively high conduction velocity of $1{\cdot}4 \pm 0{\cdot}6$ m/s (mean ± standard deviation; $n = 7$) which was significantly different from the total population of electrically identified units ($P < 0{\cdot}05$; t test). They exhibited no spontaneous activity when the bladder was empty and their threshold ranged from 30 to 50 mmHg of the intraluminal pressure. Thus, the threshold of the units is within the range of the intravesical pressure in which humans

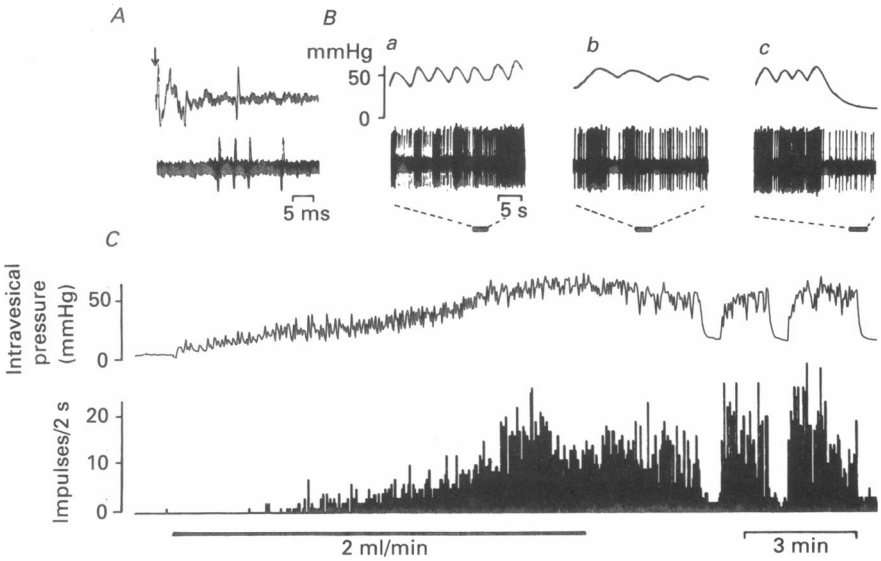

Fig. 3. Response of an unmyelinated afferent unit during slow filling of the urinary bladder. *A*, identification of the unit with electrical stimulation (upper trace, stimulation artifact marked by arrow) of the pelvic nerve (pulse duration 0·5 ms, stimulation intensity 3·0 V; conduction velocity 1·6 m/s) and activation by distension of the urinary bladder (lower trace). Both traces are several times superimposed. *B*, original records of intravesical pressure (upper trace) and neural activity (lower trace). *C*, peristimulus histogram of intravesical pressure and neural activity. The bladder was filled at 2 ml/min (bar).

experience discomfort and pain (Torrens & Morrison, 1987). All units increased their discharge frequency in a graded fashion when the intravesical pressure was raised above the threshold (Fig. 2). To use stimuli that simulate the pressure changes occurring with diuresis rather than stepwise increments of the intravesical pressure, the urinary bladder was slowly filled at a rate of 2 ml/min through the urethral catheter with an infusion pump. This resulted in a gradual increase of the intraluminal baseline pressure with superimposed fluctuations that were caused by contractions and relaxation of the detrusor muscle (Fig. 3). As observed by others (Edvardsen, 1968; Bahns *et al.* 1987) the surgery of the laminectomy, even before incision of the dura mater, causes a somewhat unusual volume–pressure curve. There were relatively few micturition contractions as compared to unoperated anaesthetized cats and consequently the intraluminal pressure increased steadily when the bladder was filled.

Afferents that were excited by distension were also activated during filling of the bladder. When the bladder was slowly filled the afferent units discharged

intermittently at the beginning of this stimulus only when the pressure was sufficiently raised by detrusor contractions. Later, in parallel to the increasing baseline pressure, there was an increase of the neural discharge. Superimposed on the continuous discharge were phasic changes of the neural activity caused by the parallel fluctuations of the intraluminal pressure. Thus, unmyelinated vesical afferents can accurately encode the static and dynamic components of the intravesical pressure changes.

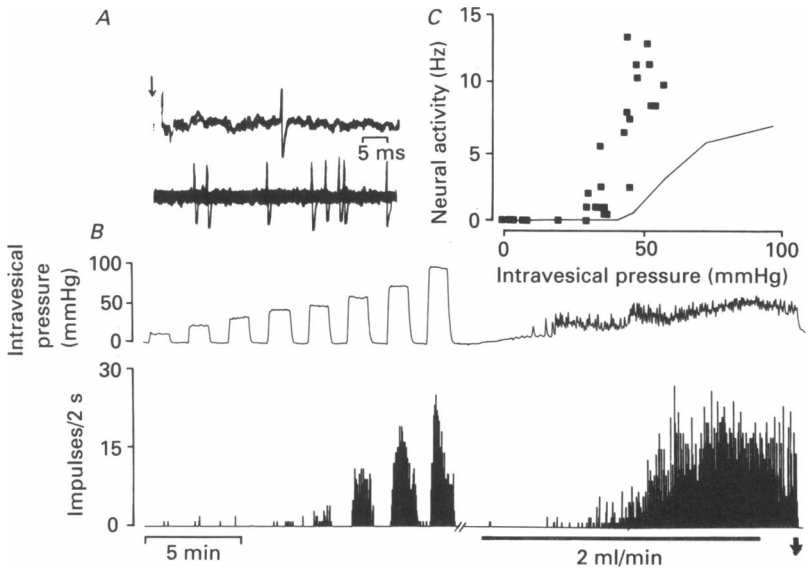

Fig. 4. Response of an unmyelinated unit during distension and slow filling of the urinary bladder. *A*, identification of the unit with electrical stimulation (upper trace, stimulation artifact marked by arrow) of the pelvic nerve (pulse duration 0·5 ms, stimulation intensity 6·2 V; conduction velocity 0·8 m/s) and activation by distension of the urinary bladder (lower trace). Traces are several times superimposed. *B*, histograms of intravesical pressure and neural activity during a series of distension stimuli (left half) and during filling of the bladder (bar) at 2 ml/min. Arrow indicates the emptying of the bladder. *C*, stimulation–response relations for both stimulation procedures: ■, filling; ——, distension.

Stimulation–response curves for both distension and filling of the bladder were obtained for three units. As illustrated in Fig. 4 the units could be activated by both stimuli and stimulation–response curves were separately obtained for either condition. For a series of distension stimuli the mean of each pressure plateau was calculated and plotted against the mean evoked neural response. When the bladder was slowly filled the intravesical pressure was averaged over the periods of 30 s duration and plotted against the averaged afferent activity of that period. The graphs illustrate that unmyelinated afferent units encoded the changes of intravesical pressure in the range between 30 and 100 mmHg by their discharge frequency (Fig. 5). Although the threshold was very similar under both conditions the response curve calculated for the slow filling was slightly steeper than that obtained for the distension series (Figs 4*C* and 5*B*). This was found for all units tested.

Moreover, during filling of the bladder there were typical bursts of activity coinciding with small changes of the intraluminal pressure. Compared with these small pressure increases they represented inappropriately large increases of activity and these were followed by an equally large reduction when the pressure decreased again (Figs 3 and 4). Thus, the afferent response consisted of phasic bursts of activity during small detrusor contractions that were followed by relatively low levels of activity during relaxation of the muscle.

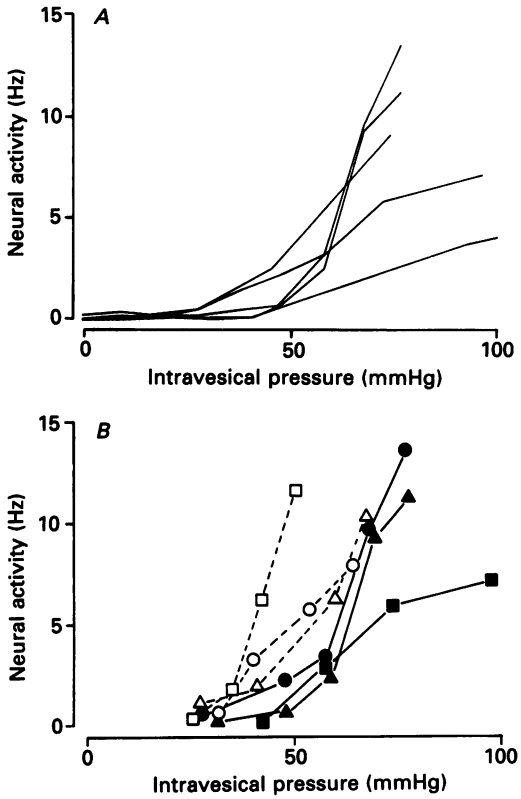

Fig. 5. *A*, stimulation–response curves of five unmyelinated afferents obtained from series of isotonic distentions of the urinary bladder. *B*, for three units, each represented by a different symbol, the stimulation–response curves were constructed when intravesical pressure was increased by both slow filling (□, ○, △) and by isotonic distention (■, ●, ▲).

Ventral root recordings

A total of 135 unmyelinated units were isolated from the ventral root S2 and tested for their responses to distension of the urinary bladder. From these forty-six were isolated from de-efferented roots and can consequently be assumed to represent a pure afferent sample. The remaining eighty-nine fibres were obtained from intact ventral roots and this number would have included unmyelinated preganglionic parasympathetic fibres that make up approximately 75% of the unmyelinated axons in this root (Häbler, Jänig, Koltzenburg & McMahon, 1990). None of these fibres was

activated by intravesical pressure stimuli although the sample would have comprised an estimated sixty-eight afferent neurones. Compared with the occurrence of unmyelinated afferents responding to distension of the bladder in the dorsal root their absence from the ventral root sample is not statistically different ($P > 0{\cdot}2$; Fisher's exact test).

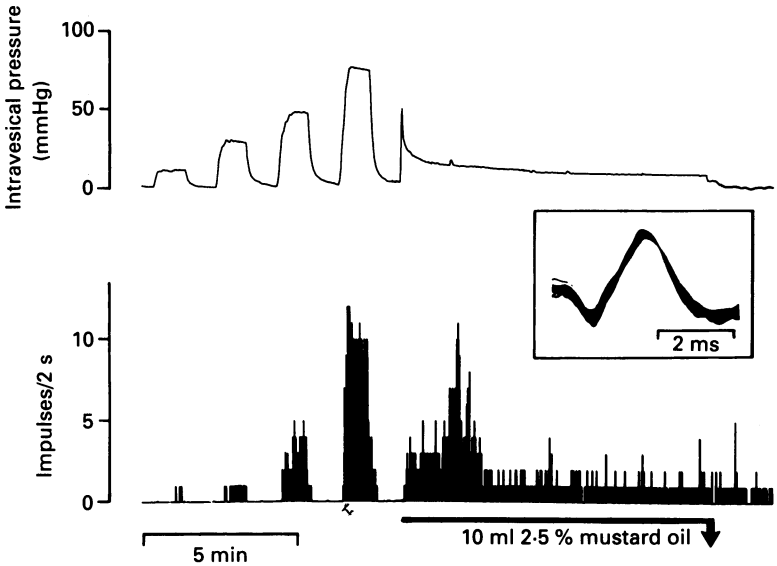

Fig. 6. Activation of an unmyelinated fibre by distension of the urinary bladder and intraluminal injection of mustard oil (bar). Arrow indicates the emptying of the bladder, Inset shows the superimposed action potentials of the unit which had a conduction velocity of 1·5 m/s.

Chemical stimulation of mechanosensitive afferents

Compared with thin myelinated sacral afferents supplying the urinary bladder, the thresholds of the unmyelinated ones are substantially higher. This raises the question whether the receptive endings of the unmyelinated afferents lie in the bladder wall and can therefore be considered to have a nociceptive function or whether the relatively high threshold is a consequence of a distant stimulation of the receptors, that are possibly located in the paravesical tissues. To differentiate between the two possibilities we used intravesical injections of the irritant mustard oil.

Both units that were tested with this stimulus responded rapidly with a short latency (Fig. 6). Although both units were mechanosensitive the neural response to mustard oil was not correlated with the intravesical pressure changes that were associated with the injection of the chemical. Moreover, when the mustard oil was removed and the bladder emptied, the afferents displayed some on-going activity. This shows that unmyelinated mechanosensitive afferents are also chemosensitive. The short time lag for the activation suggests that the receptive terminals lie in the bladder proper, possibly in the mucosa or the luminal layers of the muscularis.

Some non-mechanosensitive afferents are excited by irritant chemicals

Surprisingly few unmyelinated afferents of the pelvic nerve responded to noxious mechanical stimulation of the urinary bladder. This contrasts with the finding for a large number of myelinated afferents which are readily activated by an increase of intravesical pressure (Bahns *et al.* 1987). This could mean that most unmyelinated fibres innervate pelvic viscera other than the urinary bladder or that the appropriate stimuli have not been used for their activation. Since there is evidence that a subpopulation of vesical afferents is activated during the inflammatory process, but not during continence and micturition (Koltzenburg & McMahon, 1986), we have systematically tested non-mechanosensitive pelvic afferents at the onset of an artificial inflammation.

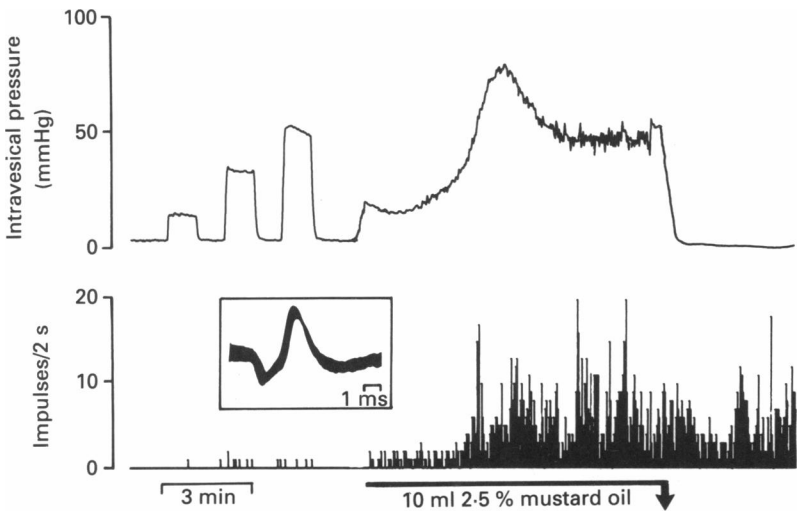

Fig. 7. Activity in an unmyelinated afferent fibre, which was isolated from a de-efferented ventral root. This non-mechanosensitive unit did not encode changes of the intravesical pressure up to 75 mmHg (75 mmHg distension was only applied during the initial identification procedure and is not shown). Injection of mustard oil (bar) excited this unit and the activity was not related to changes of the intravesical pressure. Note the on-going activity after removal of the irritant (indicated by arrow). Inset shows the superimposed action potentials of the unit which had a conduction velocity of 0·5 m/s.

Mustard oil

In sixteen animals 116 units were investigated that had no spontaneous activity and were not excited by noxious distension of the urinary bladder with intravesical pressure stimuli of 75 mmHg. This sample included sixty-eight afferents that were isolated from the dorsal root, twenty-one from chronically de-efferented ventral roots and twenty-eight units from an intact ventral root. Since 75% of the unmyelinated units in intact ventral roots are preganglionic parasympathetic fibres, a total of twenty-eight afferent units can be assumed to be present in the ventral root recordings.

When these fibres were tested with intraluminal injections of mustard oil most units did not respond to this strong irritant. However, eleven units were activated (Figs 7 and 9), of which seven units were dorsal root and four ventral root units. The prevalence of chemosensitive afferents in both ventral and dorsal roots was not statistically different ($P > 0{\cdot}4$; Fisher's exact test). These chemosensitive fibres were activated with a latency of less than 2 min. During the application of mustard oil they developed an irregular activity which was not correlated with the fluctuations of the intravesical pressure. The mean peak frequency within the first 15 min reached 3–7 Hz (Fig 8). Following the removal of the irritant all fibres displayed resting activity that gradually wore off within the next 30–40 min. Some on-going activity, however, persisted in these fibres as long as they were recorded. The conduction velocity of unmyelinated chemosensitive afferents was $0{\cdot}8 \pm 0{\cdot}3$ m/s (mean $\pm$ standard deviation). Although it tended to be lower than the value for unmyelinated mechanosensitive units, this was partially explained by the inclusion of ventral root afferents which generally conduct at a lower velocity than dorsal root afferents (Häbler *et al.* 1990). The conduction velocity of chemosensitive fibres that were isolated from the dorsal root was $1{\cdot}0 \pm 0{\cdot}3$ m/s (mean $\pm$ standard deviation) and this is not significantly different from mechanosensitive units ($P > 0{\cdot}05$; t test).

Turpentine oil

Twenty-six unmyelinated non-mechanosensitive fibres that projected into the pelvic nerve were recorded in the dorsal root and tested with intravesical injection of turpentine oil. None of these units was rapidly activated at short latency with this stimulus.

Development of mechanosensitive properties

After the induction of the inflammation three units were recorded that started to respond to filling and distension of the urinary bladder. These properties appeared in two fibres that were also activated by mustard oil and in one unit after treatment with turpentine oil. When mustard oil had been removed from the bladder, these two units continued to have an on-going activity. When the bladder was filled or distended they were activated by these mechanical stimuli. Although the activity was related to filling or distension of the bladder, the afferents did not accurately encode the intravesical pressure profile as had been the case for the unmyelinated mechanosensitive fibres in the normal bladder (Fig. 9). The unit that had been recorded after turpentine oil injections displayed a very low level of on-going activity during the inflammatory process after the removal of the chemical. When the bladder was filled after induction of the inflammation the unit responded to increases of intravesical pressure both during distension and filling in a graded fashion (Fig. 10).

DISCUSSION

There are two distinct types of unmyelinated vesical afferent fibres

The present study has shown two distinct types of unmyelinated visceral afferent neurones in the sacral segments that innervate the feline urinary bladder. A small population of fibres could accurately encode intravesical pressure changes by their

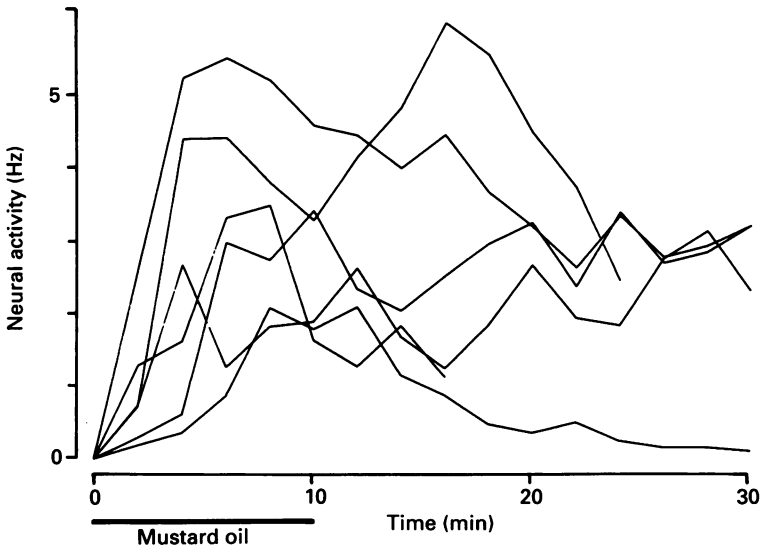

Fig. 8. Time course of the excitation of six single non-mechanosensitive afferent fibres (two ventral root units, four dorsal root units) that were activated by intravesical injection of mustard oil. Mustard oil (1–2·5 % v/v dissolved in paraffin oil) was applied for 10 min. The activity for each individual unit was averaged over 2 min long intervals and is represented by one line.

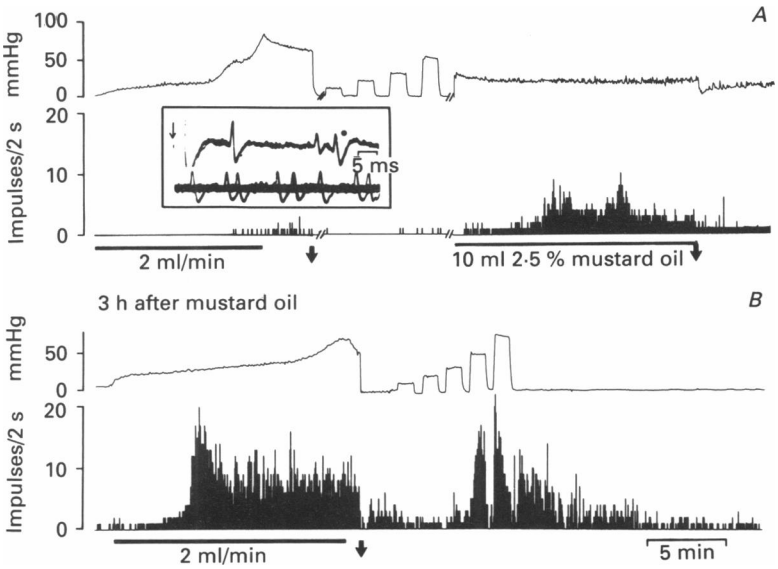

Fig. 9. Record of a single unmyelinated dorsal root unit that did not encode increases of the intravesical pressure during filling of the bladder at a rate of 2 ml/min (bar, left side) or during distension (*A*). Intraluminal injection of mustard oil (bar, right side) excited this fibre. *B*, three hours after injection of mustard oil the unit was excited during filling (bar) and distension of the bladder. Note the increase of the on-going activity after the stimuli. Arrows in the histogram indicate the emptying of the bladder. Inset in *A* shows the identification of the unit (•) at the end of the experiment with electrical stimulation (upper trace, stimulation artifact marked by arrow) of the pelvic nerve (pulse duration 0·5 ms, stimulation intensity 2·4 V; conduction velocity 1·15 m/s) and activation by distension of the urinary bladder (lower trace).

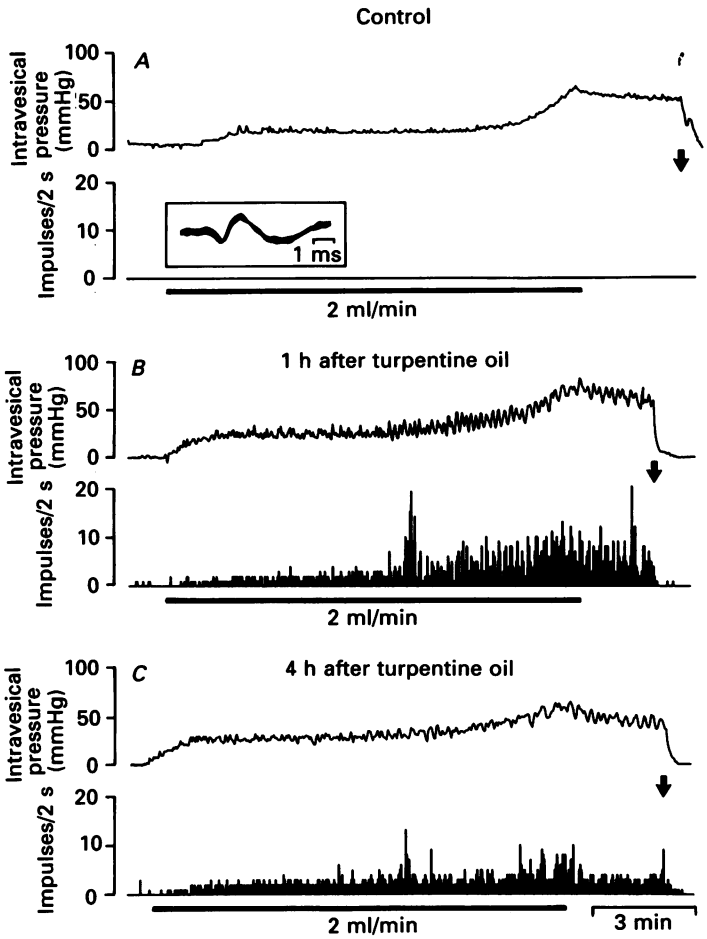

Fig. 10. Unmyelinated afferent unit that was not activated by an increase of intravesical pressure (*A*) during filling of the bladder at a rate of 2 ml/min (bar) or intraluminal injection of turpentine oil (not shown). After the removal of turpentine oil the unit developed some low on-going activity. One hour (*B*) after injection of turpentine oil this unit was activated during filling of the bladder and accurately encoded the intraluminal pressure by its discharge intensity. These new mechanosensitive properties were still present 4 h after injection of turpentine oil (*C*). Arrows indicate emptying of the bladder. Inset in *A* shows the superimposed action potentials of the unit which had a conduction velocity of 1·0 m/s.

discharge intensity. This type of afferent is very similar to the myelinated vesical afferents found in earlier studies (Iggo, 1955; Bahns *et al.* 1987) except for their significantly higher thresholds.

The other principal finding of this study is that there is an entirely new type of chemosensitive visceral receptor. Although distension of the urinary bladder elicited a few action potentials from these fibres they were not capable of encoding the intravesical pressure profile in any biologically relevant pressure range. Thus, the mechanosensitivity of these units is spurious and an adequate stimulus appears to be primarily a chemical one.

Unmyelinated mechanosensitive fibres

As unmyelinated mechanosensitive fibres respond to both distension and contraction it is likely that the receptive terminals were located in the bladder wall rather than in the surrounding paravesical tissues. Further evidence supporting this explanation comes from the observation that all unmyelinated afferents tested were also activated by intraluminally applied irritant chemicals at short latency.

Although we have recorded from a large number of unmyelinated ventral root afferents, our sample did not contain vesical units suggesting an equally low incidence of these afferents in dorsal and ventral root. However, some unmyelinated ventral root afferents have previously been found to respond to manipulation of the urinary bladder (Clifton, Coggeshall, Vance & Willis, 1976). These units were weakly activated by distension, but not by contractions of the bladder. This could indicate that their receptive fields were located in the paravesical tissue rather than in the wall of the bladder and therefore responded to the expansion occurring with isotonic distension, but not to an isovolumetric contraction.

Pelvic afferents supplying the urinary bladder have several functions. They are essential for the co-ordinated micturition reflex, associated conscious sensations and autonomic reflexes that occur during continence and micturition (de Groat, Nadelhaft, Milne, Booth, Morgan & Thor, 1981; Jänig & McLachlan, 1987; de Groat & Steers, 1988). They are also the major pathway for pelvic pain which can be elicited when the intravesical pressure exceeds 20–30 mmHg (Jänig & Morrison, 1986; Torrens & Morrison, 1987).

Role of unmyelinated fibres in sensation

As evidenced by the stimulation–response curves it is clear that the unmyelinated fibres may not be suited to signal the intravesical pressure during continence, and they may only slightly be excited during normal non-painful micturition. This could mean that they have other functions and may be involved in nociception. In agreement with this explanation is the finding that they also respond to irritant chemicals. However, a polymodal sensitivity for mechanical and chemical stimuli is not a unique feature of unmyelinated fibres, but has also been found for low-threshold myelinated fibres (Häbler, Jänig & Koltzenburg, 1988). Although unmyelinated mechanosensitive afferents of the urinary bladder fulfil all criteria of a nociceptor it is unclear whether the relatively small proportion of fibres suffices to mediate painful sensations from the normal non-inflamed bladder.

Role of unmyelinated fibres in the micturition reflex

It is generally thought that micturition in the adult cat is triggered by the activation of myelinated vesical afferents acting in concert with a supraspinal control (de Groat *et al.* 1981; McMahon, 1986; de Groat & Steers, 1988). However, following spinalization the reflex undergoes considerable reorganization and it has been suggested that micturition is initiated by unmyelinated afferents in the paraplegic cat (de Groat *et al.* 1981). This hypothesis is difficult to reconcile with the properties of unmyelinated afferents that are barely suited to elicit the micturition reflex. The reason for this discrepancy could be that the involvement of unmyelinated fibres

in the micturition reflex has mainly been inferred from electrical stimulation experiments and not from adequate natural excitation of the relevant afferents. However, another explanation is that the functional properties of primary afferents might change as a consequence of spinal cord injury. Interestingly, there is a remarkable re-arrangement of the central projection pattern of the unmyelinated vesical afferents after spinal cord transection in the adult cat (Thor, Kawatani & de Groat, 1986). This invites speculation that similar changes could affect the peripheral endings which might eventually lead to altered functional properties of the receptive terminals.

Unmyelinated non-mechanosensitive afferents

Amongst the unmyelinated sacral afferents that cannot encode intravesical pressure in any biologically relevant range there is a subgroup of neurones which is rapidly activated by the intraluminal injection of mustard oil. Intraluminal application of this and related irritant agents has been established as a suitable agent for the induction of a long-lasting visceral pain state in a variety of behavioural tests (McMahon & Abel, 1987; Abeli, Conte, Somma, Maggi, Giuliani, Geppetti, Alessandri, Theodorsson & Meli, 1988). Since we have not systematically tested other chemical stimuli, we obviously cannot exclude for these neurones a chemical sensitivity for non-noxious compounds. However, as the afferents responded particularly well to irritants, they are good candidates for chemosensitive visceral nociceptors. Similar types of non-mechanosensitive fine afferents that are excited by irritant chemicals or at the onset of an artificial inflammation have also been detected in joints (Grigg, Schaible & Schmidt, 1986; Schaible & Schmidt, 1988) and there is some evidence that they are also present in skin (Davis, Meyer, Cohen & Campbell, 1989; Handwerker, Kilo & Reeh, 1989; Simone, Baumann & LaMotte, 1989).

It is obvious that the activation of a previously silent afferent fibre population could precipitate a number of changes that typically present with inflammation of the urinary bladder. Excitation of chemosensitive fibres by intraluminal application of mustard oil (Koltzenburg & McMahon, 1986; McMahon & Abel, 1987) or systemic injection of capsaicin (Lundberg, Brodin, Hua & Saria, 1984) is known to cause plasma extravasation. Importantly, intravesical pressure stimuli that would have activated all myelinated and some unmyelinated mechanosensitive afferents, do not result in an appreciable degree of plasma extravasation (Koltzenburg & McMahon, 1986).

Moreover, several changes within the spinal cord are also related to the activation of chemosensitive unmyelinated afferents. There is an induction of the c-fos proto-oncogene, particularly in neurones of the superficial dorsal horn, the dorsal commissure and lamina X (Birder, Roppolo, Iadarola & de Groat, 1989) where the sacral primary afferents of the urinary bladder are known to terminate (de Groat, 1987). Whilst intravesical injection of chemical irritants appears to be a strong inductor for c-fos, overdistension of the bladder produces only a slight induction (Birder *et al.* 1989). In addition, direct recordings from dorsal horn neurones of the rat sacral spinal cord have demonstrated a class of cells that do not respond to mechanical stimulation of the urinary bladder, but to intraluminal injection of chemicals (McMahon, 1988). Since some of these spinal cord neurones started to respond also to changes of intravesical pressure at a later stage of the inflammation,

it is possible that these new receptive properties of spinal cord neurones are a consequence of the appearance of mechanosensitive properties in unmyelinated primary afferents.

In summary, the excitation of this novel type of visceral afferent fibre bears considerable importance in the pathology of long-lasting visceral pain states and the hypersensitivity of the tissues. Interestingly, pain can be elicited from the human urinary bladder by intraluminal injections of small amounts of irritants (Head, 1893; Nesbit & McLellan, 1939). Moreover, in analogy to the hypersensitivity that can follow injury of the skin, light mechanical stimulation of the inflamed mucosa, which is generally not consciously perceived from normal tissue, has been shown to be painful for a number of viscera including the urinary bladder (McLellan & Goodell, 1943; Wolf, 1965). This suggests that the appearance of mechanosensitive properties in some of the previously non-mechanosensitive fibres observed in this study is an important correlate of such disease states. It also raises the possibility that the transition of a silent afferent neurones into an active state is not an idiosyncratic feature of the viscera, but an example of a general characteristic of hypersensitive states.

Intensity versus specificity coding of visceral pain

There has been some dispute as to whether visceral pain is mediated by a specific subset of nociceptors or the discharge intensity of a homogenous population of low-threshold afferents (Jänig & Morrison, 1986; Cervero, 1988). There are two distinct populations of primary afferent neurones that are both suited to signal noxious stimuli in the normal non-inflamed urinary bladder. Thus, noxious intravesical pressure results in a further increase of activity in low-threshold fibres (Bahns *et al.* 1987) and as shown in the present study there is a recruitment of high-threshold afferents. This condition contrasts with the peripheral mechanisms of cutaneous sensation where there is little overlap between the stimulus intensities that can be encoded by low-threshold mechanosensitive afferents and nociceptors, respectively (Campbell, Raja, Cohen, Manning, Khan & Meyer, 1989). Further, the transition of a previously silent afferent neurone to an active state in the inflamed viscus illustrates that the peripheral mechanisms of nociception critically depend on the state of the tissue. Therefore, the results of the present study could call for the revision of both specificity and intensity theory as a universal explanation for visceral pain states.

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REFERENCES

ABELI, L., CONTE, B., SOMMA, V., MAGGI, C., GIULIANI, S., GEPPETTI, P., ALESSANDRI, M., THEODORSSON, E. & MELI, A. (1988). The contribution of capsaicin-sensitive sensory nerves to xylene-induced visceral pain in conscious, freely moving rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* **337**, 545–551.

BAHNS, E., ERNSBERGER, U., JÄNIG, W. & NELKE, A. (1986). Functional characteristics of lumbar visceral afferent fibres from the urinary bladder and urethra in the cat. *Pflügers Archiv* **407**, 510–518.

BAHNS, E., HALSBAND, U. & JÄNIG, W. (1987). Responses of sacral visceral afferents from the lower urinary tract, colon and anus to mechanical stimulation. *Pflügers Archiv* **410**, 296–303.

BIRDER, L. A., ROPPOLO, J. R., IADAROLA, M. J. & DE GROAT, W. C. (1989). Spinal cord distribution of the c-fos protein in visceral versus somatic models of chemical inflammation. *Society for Neuroscience Abstracts* **15**, 468.

CAMPBELL, J. N., RAJA, S. N., COHEN, R. H., MANNING, D. C., KHAN, A. A. & MEYER, R. A. (1989). Peripheral neural mechanisms of nociception. In *Textbook of Pain*, 2nd edn, ed. WALL, P. D. & MELZACK, R., pp. 22–45. Churchill Livingstone, Edinburgh.

CERVERO, F. (1988). Visceral pain. In *Proceedings of the Vth World Congress on Pain: Pain Research and Clinical Management*, vol 3, ed. DUBNER, R., GEBHART, G. F. & BOND, M. R., pp. 216–226. Elsevier, Amsterdam.

CLIFTON, G. L., COGGESHALL, R. E., VANCE, V. H. & WILLIS, W. D. (1976). Receptive fields of unmyelinated ventral root afferent fibres in the cat. *Journal of Physiology* **256**, 573–600.

DAVIS, K. D., MEYER, R. A., COHEN, R. H. & CAMPBELL, J. N. (1989). Mechanically-insensitive nociceptors in the primate. *Society of Neuroscience Abstracts* **15**, 440.

DE GROAT, W. C. (1987). Neuropeptides in pelvic afferent pathways. *Experienta* **43**, 801–813.

DE GROAT, W. C., NADELHAFT, I., MILNE, R. J., BOOTH, A. M., MORGAN, C. & THOR, K. (1981). Organization of the sacral parasympathetic reflex pathways to the urinary and large intestine. *Journal of Autonomic Nervous System* **7**, 339–350.

DE GROAT, W. C. & STEERS, W. (1988). Neural control of the urinary bladder and sexual organs: experimental studies in animals. In *Autonomic Failure*, 2nd edn, ed. BANNISTER, R., pp. 196–222. Oxford University Press, Oxford.

EDVARDSEN, P. (1986). Nervous control of urinary bladder in cats. I. The collecting phase. *Acta physiologica scandinavica* **72**, 157–171.

FLOYD, K., HICK, V. E. & MORRISON, J. F. B. (1976). Mechanosensitive afferent units in the hypogastric nerve of the cat. *Journal of Physiology* **259**, 457–471.

GRIGG, P., SCHAIBLE, H.-G. & SCHMIDT, R. F. (1986). Mechanical sensitivity of group III and IV afferents from posterior articular nerve in normal and inflamed cat knee. *Journal of Neurophysiology* **55**, 635–643.

HÄBLER, H.-J., JÄNIG, W. & KOLTZENBURG, M. (1988). A novel type of unmyelinated chemosensitive nociceptor in the acutely inflamed urinary bladder. *Agents and Actions* **25**, 219–221.

HÄBLER, H.-J., JÄNIG, W., KOLTZENBURG, M. & MCMAHON, S. B. (1990). A quantitative study of the central projection patterns of unmyelinated ventral root afferents in the cat. *Journal of Physiology* **422**, 265–287.

HANDWERKER, H. O., KILO, S. & REEH, P. W. (1989). Afferent C-fibers from the rat hairy skin not driven by natural stimulation. *Society for Neuroscience Abstracts* **15**, 1265.

HARDY, J. D., WOLFF, H. G. & GOODELL, H. (1950). Experimental evidence on the nature of cutaneous hyperalgesia. *Journal of Clinical Investigations* **29**, 115–140.

HEAD, H. (1893). On the disturbances of sensation with especial reference to the pain of visceral disease. *Brain* **16**, 1–133.

IGGO, A. (1955). Tension receptors in the stomach and the urinary bladder. *Journal of Physiology* **128**, 593–607.

JÄNIG, W. & MCLACHLAN, E. M. (1987). Organization of lumbar spinal outflow to distal colon and pelvic organs. *Physiological Reviews* **67**, 1332–1404.

JÄNIG, W. & MORRISON, J. F. B. (1986). Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception. In *Visceral Sensation. Progress in Brain Research*, vol. 67, ed. CERVERO, F. & MORRISON, J. F. B., pp. 87–114. Elsevier, Amsterdam.

KLEVMARK, B. (1974). Motility of the urinary bladder in cats during filling at physiological rates. I. Intravesical pressure patterns studied by a new method of cystometry. *Acta physiologica scandinavica* **90**, 565–577.

KOLTZENBURG, M. & MCMAHON, S. B. (1986). Plasma extravasation in the rat urinary bladder following mechanical, electrical and chemical stimuli: evidence for a new population of chemosensitive primary sensory afferents. *Neuroscience Letters* **72**, 352–356.

LEWIS, T. (1942). *Pain*. Macmillan, New York.

LUNDBERG, J. M., BRODIN, E., HUA, X. & SARIA, A. (1984). Vascular permeability changes in smooth muscle contraction in relation to capsaicin-sensitive substance P afferents in the guinea-pig. *Acta physiologica scandinavica* **120**, 217–227.

McLellan, A. M. & Goodell, H. (1943). Pain from the bladder, ureter, and kidney pelvis. *Research Publications of the Association of Nervous and Mental Disorders* **23**, 252–262.

McMahon, S. B. (1986). Sensory-motor integration in urinary bladder function. In *Visceral Sensation. Progress in Brain Research*, vol. 67, ed. Cervero, F. & Morisson, J. F. B., pp. 245–253. Elsevier, Amsterdam.

McMahon, S. B. (1988). Neuronal and behavioural consequences of chemical inflammation of rat urinary bladder. *Agents and Actions* **25**, 231–233.

McMahon, S. B. & Abel, C. (1987). A model for the study of visceral pain states: chronic inflammation of the chronic decerebrate rat urinary bladder by irritant chemicals. *Pain* **28**, 109–127.

Nesbit, R. M. & McLellan, F. C. (1939). Sympathectomy for the relief of vesical spasm and pain resulting from intractable pain infection. *Surgery, Gynecology and Obstetrics* **68**, 540–546.

Sachs, L. (1984). *Applied Statistics: A Handbook of Techniques*, 2nd edn. Springer–Verlag, New York.

Schaible, H.-G. & Schmidt, R. F. (1988). Time course of mechanosensitivity changes in articular afferents during a developing experimental arthritis. *Journal of Neurophysiology* **60**, 2180–2195.

Simone, D. A., Baumann, T. K. & LaMotte, R. H. (1989). Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. *Pain* **38**, 99–108.

Thor, K., Kawatani, M. & de Groat, W. C. (1986). Plasticity in the reflex pathways to the lower urinary tract of the cat during postnatal development and following spinal cord injury. In *Development and Plasticity of the Mammalian Spinal Cord. Fida Research Series*, vol. 3, ed. Goldberger, M. E., Gorio, A. & Murray, M. pp. 65–80. Liviana Press, Padova.

Torrens, M. & Morrison, J. F. B. (1987). *The Physiology of the Lower Urinary Tract*. Springer–Verlag, Berlin.

Wolf, S. (1965). *The Stomach*. Oxford University Press, New York.