Inflammation of the rat urinary bladder is associated with a referred thermal hyperalgesia which is nerve growth factor dependent

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We have assessed whether a referred somatic hyperalgesia to thermal stimulation of the hind limb of rats occurs after inflammation of the urinary bladder. Furthermore, we evaluated whether any such visceral-somatic hyperalgesia (VSH) is dependent on the neurotrophin nerve growth factor (NGF). Limb withdrawal thresholds from thermal stimulation of both fore and hind limbs were assessed simultaneously at baseline and at fixed times for 24 h after various interventions. After plotting curves for the difference in withdrawal time of fore and hind limbs against time, the area under the curve (AUC) was calculated to provide a single measure over the 24-h period. A negative value indicated relative hyperalgesia of the hind limb. With simple catheterization, although there was a trend towards hind limb hyperalgesia, there was no significant difference in this AUC (mean –100.5) compared with naïve control animals (mean AUC +53.6). However, inflammation with 50% turpentine oil was associated with a significant change in AUC (mean –676.8), indicative of relative hyperalgesia of the hind limb. This hyperalgesia was mimicked by intra-vesical instillation of NGF (in place of turpentine) (mean AUC –1418.3 while mean AUC in naïve animals was +439.4). Furthermore, prior administration of an NGF sequestering molecule, trkA–IgG, attenuated turpentine-induced VSH. These findings increase our knowledge of the nature of visceral and referred pain and further implicate NGF in the hyperalgesic response to inflammation of the urinary bladder.

Visceral and somatic pain differ in several clinical and physiological aspects. In particular, a classical feature of visceral pain is that the sufferer perceives the pain as arising from somatic sites distant from the area of visceral injury (referred pain). Several different mechanisms have been put forward in an attempt to explain this phenomenon. These include the axon reflex theory first proposed (but so far with little supportive physiological evidence) by Sinclair, Weddell and Feindel in 1948, which suggests that some axons innervate both visceral and somatic targets. Another suggestion was that of Ruch and Fulton in 1946 who suggested that visceral and somatic afferents converge onto common dorsal horn neurones, with the incoming visceral activity then being misconstrued as arising from a somatic source. Perhaps the most widely accepted current view is that proposed by MacKenzie in 1909, who postulated that the viscera were intrinsically relatively insensitive to pain, but noxious activity arising from the viscera could set up an irritible focus in the spinal cord. Such activity might then facilitate somatosensory inputs to an extent where the threshold for pain perception is crossed. It is interesting to note that this has obvious correlates with the current theory of central sensitization as a mechanism of persistent and chronic pain.

Referral of not only pain but also hyperalgesia to somatic structures after visceral injury has been described in patients suffering from ureteric colic and renal infection. Confirmation of the physiological mechanism of this phenomenon has been hampered by the paucity of animal models. However, in at least one animal model of visceral pain, findings of referred hyperalgesia have been described. Giamberardino, Vecchiet and Albe-Fessard used a rat model of pain associated with a ureteric calculus to assess somatic sensory thresholds in the structures of the body wall. In the initial description, they were able to show that noxious electrical stimulation of the ureter (by electrodes implanted around it under general anaesthesia) led to hyperalgesia of the ipsilateral latissimus dorsi muscles to electrical
stimulation. A similar innocuous stimulation gave rise to no such effect. With a more clinically relevant stimulus of the ureter (implantation of a ureteric stone composed of dental cement) they described a similar hyperalgesia to electrical stimulation of the external oblique muscle of the abdominal wall. This hyperalgesia was ipsilateral to the stimulus, lasted for several days and its duration was increased by the presence of the stone in the lumen of the ureter (thus suggesting the requirement for ongoing inflammation in the prolongation of the effect). Previous work in the turpentine-inflamed urinary bladder model (using decerebrate rats) has shown reduction in tail flick latencies to noxious thermal and mechanical stimuli.9

Nerve growth factor (NGF) is a target-derived secretory protein, which elicits many of its effects after binding to a high affinity tyrosine kinase receptor, trkA. It has an established role in controlling the survival and development of small diameter neurones (both sympathetic and primary sensory afferents).10 However, more recently it has become clear that it is important in the developed organism as a vital link in the neuroimmune interactions resulting in inflammatory hyperalgesia.10–14 It has been shown previously that exogenous NGF administered intra-vesically leads to the production of a viscero-visceral hyper-reflexia (VVH), similar to that seen after instillation of irritant substances (such as turpentine oil) into the urinary bladder.15 Furthermore, sequestration of endogenous NGF by systemic administration of a ligand trap fusion molecule (consisting of two trkA receptors fused to a single IgG molecule) attenuates VVH associated with turpentine inflammation.15 As with the turpentine model, VVH associated with intra-vesical NGF is accompanied by sensitization of both primary afferent neurones in the pelvic nerve16 and neurones in the dorsal horn of the spinal cord.17 In addition, expression of the protein product of the immediate early (IEG) gene c-fos in the dorsal horn is upregulated during bladder inflammation with NGF.18 Furthermore, in the turpentine-inflamed rat urinary bladder, there is increased expression of mRNA for NGF19 and in humans with painful bladder conditions, concentrations of NGF in the bladder mucosa have been shown to be increased.20

Mast cells (known to be present in increased numbers in inflamed and hyperalgesic bladder tissue) express the NGF receptor protein trkA.21 They amplify the pro-hyperalgesic effects of NGF both by further increasing the production of NGF22 and by releasing other pro-hyperalgesic molecules during the degranulation response.21 This NGF-mediated release of mast cell contents has been shown to be associated with hyperalgesia to both thermal and punctate mechanical hyperalgesia.12 The trkA receptor is also known to be expressed on primary sensory neurones, particularly those subserving the viscera.23 In addition, sequestration of endogenous NGF has been shown to be associated with hypoalgesia in the somatic domain,13 supporting the evidence that NGF is an important mediator of hyperalgesic states.

This study was designed to document viscerosomatic referral of hyperalgesia (VSH) to thermal stimuli in an established animal model of persistent visceral pain (the turpentine-inflamed rat urinary bladder)9 and to assess its dependence on NGF. We assessed limb withdrawal thresholds to thermal stimuli in a somatotopically appropriate area (the rat hind paw) in groups of animals before and after various interventions. The hind paw of the rat was chosen as the lateral plantar region is supplied by neurones which enter the cord at the L5 level.24 Noxious stimulation of this area is associated with a lasting, measurable, well documented ‘endpoint’ (limb withdrawal). This is also the spinal level at which it has been shown (by means of immunohistochemical identification of c-fos) that nociceptive neurones innervating the bladder enter the spinal cord.18 25 The interventions assessed were: general anaesthetic alone, simple transurethral catheterization or inflammation of the bladder with 50% turpentine oil or NGF. Furthermore, additional turpentine-inflamed animals were examined after systemic treatment with either trkA–IgG or an IgG-control molecule.

Materials and methods

Animal maintenance and instrumentation

All experiments conformed to British Home Office regulations. Experiments were performed on 57 female Wistar rats, mean weight 217.4 (range 200–230) g, which were housed according to national guidelines, with a 12-h light–dark cycle and free access to food and water. Room temperature and humidity were controlled at a constant level during all experiments, which were performed at similar times in the light–dark cycle. For urethral cannulation and bladder inflation, anaesthesia was induced and maintained either by pentobarbital 40 mg kg–1 i.p. (in the initial experiment assessing the presence or absence of hind paw hyperalgesia after instillation of turpentine) or by inhalation of isoflurane (0–5% for induction followed by 2% maintenance) and nitrous oxide in oxygen (for the experiments assessing the role of NGF). These two separate anaesthetic methods were used as the initial i.p. pentobarbital anaesthetic was possibly associated with a recovery time which complicated assessment at the earliest time of 2 h. This was not the case when the anaesthetic technique was refined to the inhalation method. A sterile, lubricated, nylon catheter with an external diameter of 0.75 mm (Portex, UK, 200/300/020) was introduced into the bladder transurethrally. This was secured in the urinary bladder for the duration of inflation, by application of 3/0 silk ties around the external urethral orifice. An unobstructed flow of clear urine was ensured before instillation of turpentine oil or NGF. Administration of test compounds was achieved via i.v. administration through a 0.6-mm o.d. cannula (Abbocath-T, Abbott Ireland Ltd, Sligo, Republic of Ireland) inserted into a tail vein.
Bladder inflammation

Assessment of referred hyperalgesia after instillation of turpentine

This involved instillation into the urinary bladder of 50% turpentine 0.5 ml in olive oil (first described by McMahon and Abel\(^9\)) which was left in situ for 1 h before removal via the transurethral catheter (which was then itself removed). This is associated with a sterile inflammatory response which commences within 1 h, increases for several further hours and is maintained for at least 48 h.\(^9\) This inflammatory response is consistently associated with a VVH, as evidenced by lowering of the micturition threshold exhibited on the cystometrogram (CMG).\(^9\)\(^26\)\(^\text{--}^29\)

Three groups of animals were assessed (\(n=11\) in each group). Group I were anaesthetized but had no further intervention, group II were catheterized transurethrally while anaesthetized and sterile saline 0.5 ml was instilled for 1 h and in group III, 50% turpentine 0.5 ml was instilled intra-vesically for the same duration.

Assessment of the role of NGF in referred hyperalgesia

Bladder inflammation was induced in four treatment groups (\(n=6\) in each) by one of two methods: animals in group A were anaesthetized but no other intervention was performed; animals in group B were anaesthetized, catheterized and NGF 10 \(\mu\)g (in 0.5 ml of a DMSO/saline carrier, 1:19 dilution) was administered intra-vesically and left in situ for 1 h (this has been shown previously to induce VVH);\(^15\)

group C animals were anaesthetized and control antibody (IgG-control) 1 mg kg\(^\text{--}^1\) was administered i.v. immediately before intra-vesical instillation of 50% turpentine oil 0.5 ml; and the final group of animals (group D) were anaesthetized and the NGF sequestering molecule trkA–IgG 1 mg kg\(^\text{--}^1\) was administered i.v. directly before instillation of the turpentine oil into the urinary bladder.

Thermal threshold assessment

Limb withdrawal thresholds were assessed using Hargreave’s device (Ugo Basile, Italy),\(^30\) which focuses a beam of infrared radiation (IR) at a wavelength of 50 nm onto the skin. The maximum skin temperature delivered was 46°C and the system cuts out automatically after 21.4 s (to prevent thermal tissue damage) or if the animal actively withdraws the overlying limb. After the rats had been acclimatized to the testing environment (a plexiglas box 23×18×14 cm mounted on a dry glass pane which transmits IR radiation with minimal absorption), baseline measurements of the withdrawal threshold of the right fore and hind paws were made. Simultaneous measurement of both fore and hind limb withdrawal thresholds provides an internal control for each animal to allow for any systemic response to inflammation or anaesthesia producing an overall change in limb withdrawal thresholds. The hind paw was chosen as the area for stimulation as its spinal innervation is L4–S3\(^\text{24}\) which converges with the vesical innervation\(^1\text{8}\)\(^\text{25}\) and testing of this area allows the animals to be unrestrained (avoiding any stress response caused by handling) while maintaining a constant distance between light source and test area. On examination, each right paw (fore immediately before hind) was tested three times and the average of these was taken as the withdrawal time. Similar measurements were subsequently made at 2, 4, 6, 8 and 24 h after intervention. All observations in any one experimental procedure were made by a single observer to ensure internal consistency. However, because of the frequency of micturition of the animals with an inflamed bladder, it was not possible to totally blind the observer to the treatment regimen. Although no systematic observation and recording of specific behaviour patterns were made, some animals appeared to show hunching of the back and piloerection after turpentine or NGF inflammation. While behavioural changes may occur, they do not appear to be as severe as those observed previously in the cyclophosphamide-induced cystitis model\(^31\) and the procedure was satisfactorily reviewed by the Home Office after completion of the first six animals. At completion of the final test, animals were killed using an overdose of anaesthetic followed by cervical dislocation.

Materials

NGF and trkA–IgG fusion molecules were kindly donated by Genentech Inc (San Francisco, CA, USA). Control immunoglobulin (human IgG) was purchased from Sigma. NGF was diluted in 10% dimethylsulphoxide (DMSO) to a concentration of 20 \(\mu\)g ml\(^\text{--}^1\) and the immunoglobulins in phosphate-buffered saline 0.01 mol litre\(^\text{--}^1\) to a concentration of 1 mg ml\(^\text{--}^1\).

Statistical analysis

The outcome measure was limb withdrawal time, expressed as a percentage of the baseline value for each animal (to account for inter-animal variation) and as the difference between fore and hind limbs. The measure obtained at each time (\(\Delta\text{Thresh}\)) was therefore calculated by:

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\Delta\text{Thresh} = \left( \frac{H\text{Thresh}}{B\text{HThresh}} \right) \times 100 - \left( \frac{F\text{Thresh}}{B\text{FThresh}} \right) \times 100
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where \(H\text{Thresh} =\) withdrawal threshold of the hind limb (in seconds); \(B\text{HThresh} = \) baseline withdrawal threshold of the hind limb (in seconds); \(F\text{Thresh} = \) withdrawal threshold of the fore limb (in seconds); and \(B\text{FThresh} = \) baseline withdrawal threshold of the fore limb (in seconds). The \(\Delta\text{Thresh}\) value calculated was plotted against time to produce a curve for each animal. A negative deflection of this curve indicates relative hyperalgesia of the hind paw in relation to the fore paw.

To allow assessment across the whole time period of the experiment, the area under the curve (AUC) for each of the \(\Delta\text{Thresh}\) values against time curves was calculated using the trapezium rule (Sigmaplot, Jandel Scientific Software) and
Referred thermal hyperalgesia after inflammation of the rat bladder

Fig 1 $\Delta_{\text{Thresh}}$ vs time for the no catheter control (group I), saline control (group II) and intra-vesical turpentine instillation (group III) groups (mean (SEM)). Turpentine instillation was associated with a marked negative shift in this variable, indicating development of thermal hyperalgesia of the hind paw, relative to the fore paw.

Results

Presence of referred hyperalgesia after turpentine inflammation

Recordings in this group were only possible from 4 h onwards as before this time the residual effects of anaesthesia may have precluded accurate assessment.

There was no significant variation across time from baseline readings in the fore paw withdrawal thresholds in any of the three treatment groups (I, II and III) (ANOVA (Dunnett’s), $P<0.05$). The curve $\Delta_{\text{Thresh}}$ against time is shown in Figure 1. There was minimal deflection in the group I (no catheter control) curve around the zero point. However, groups II (intra-vesical saline) and III (turpentine instillation) showed negative deflection of $\Delta_{\text{Thresh}}$ with time, indicating relative hyperalgesia of the hind limb in relation to the fore limb. Mean AUC values produced from the $\Delta_{\text{Thresh}}$ vs time plot (Fig. 2) were calculated as: AUCcontrol $+53.6$ (SEM 190.0), AUCcatheter $-100.5$ (363.1) and AUCinflamed $-676.8$ (193.2). Statistical analysis ($t$ test) showed that there was no significant difference between AUC values for the no catheter and saline instillation groups. Thus catheterization per se was not associated with significant somatic hyperalgesia to thermal stimulation, but there was a trend in this direction. However, there was a significant difference ($P<0.05$) when the no catheter and turpentine inflammation groups were compared. This indicates that there was significant hyperalgesia of the hind paw in relation to the fore paw in the first 24 h after turpentine inflammation of the bladder.

Role of NGF in referred hyperalgesia

There was no significant variance (ANOVA (Dunnett’s), $P<0.05$) in fore paw withdrawal thresholds across time compared with baseline for any of the four groups (A, B, C and D). Curves plotting $\Delta_{\text{Thresh}}$ against time for the four groups are shown in Figure 3. Curves for both the NGF-treated group (B) and the turpentine–IgG control group (C) showed negative deflection. When assessing the AUC of the $\Delta_{\text{Thresh}}$ vs time curves for the four groups, both the NGF treated and turpentine–IgG control groups were significantly different ($P<0.05$) from the no catheter control group (A), indicating relative hyperalgesia of the hind limb. The trkA–IgG group (D) showed a significant reduction ($P<0.05$) in AUC compared with the IgG-control (C) group, with no significant difference from the no catheter control group (A) (Fig. 4). These data indicate that NGF is capable of producing similar hyperalgesia to turpentine and hyperalge-
Referred pain is a difficult clinical problem and an understanding of the mechanisms underlying its production and modulation may allow for development of new therapeutic strategies to aid its control. The data presented here provide evidence that the referred hyperalgesia observed in patients, pain from the urinary bladder is usually referred to somatotopically appropriate areas,24 these data support the convergence-facilitation theory, with central sensitization induced by peripheral visceral stimulation, enhancing alterations in somatic sensory perception. Not only has sensitization of physiological responses been shown,32 but stimulation of visceral afferent neurones has also been observed to be associated with central sensitization with expansion of the dorsal horn neurone receptive fields33 (possibly via an NMDA- and nitric oxide-dependent process).26 27 Therefore, the potential area over which somatic stimulation might be sensitized is increased. This could explain documentation of referred pain in patients extending beyond the expected S2–4 dermatome distribution.1 32

Previous work has provided evidence that the model used here (which was originally described in chronically decerebrate rats)9 demonstrates several effects seen in the clinical context of pain originating from the lower urinary tract. Specifically, an inflammatory response in the bladder tissue has been shown to be associated with both a bladder hyper-reflexia (VVH) and also a referred hyperalgesia (VSH) to mechanical and thermal stimuli applied to the anterior abdominal wall.9 We have shown development of a referred VSH to the hind limb to mechanical stimulation after vesical inflammation.34 In addition, it has been shown that inflammation provokes sensitization of both primary afferent and dorsal horn neurones35 (involving the glutamatergic27 and nitric oxide26 systems) and activation of previously ‘silent’ afferents.36 37 Induction of the IEG c-fos in dorsal horn neurones of the spinal cord has also been demonstrated after bladder inflammation.18 25 38

Referred pain is a well established phenomenon which often follows a dermatomal pattern.2 Varying explanations for referral of pain to somatotopically appropriate areas have been suggested, the most widely accepted of which are the convergence-projection and convergence-facilitation theories. The convergence-facilitation theory requires that visceral injury is associated with the development of an ‘irritable focus’ in the dorsal horn, a concept which has a striking similarity to the recent discoveries involving injury-induced central sensitization.6 These two theories depend on the fact that there is a high degree of viscerosomatic convergence on dorsal horn cells in the deeper laminae of the spinal cord. In patients, pain from the urinary bladder is usually referred to the perineum and inner thighs, supplied by spinal roots S2–4. However, Erichsen stated that it could also be experienced in the soles of the feet (see Head1) and more recently it has been observed that repeated distension of the uninflamed bladder is associated with a reduction in the noxious threshold and an increase in the area over which discomfort and pain are felt (extending as far as the knees).32 In the rat, sensory innervation of the bladder is predominantly supplied by roots L5, L6 and S1 (as documented by immunohistochemical examination of expression of the IEG c-fos).18 25 The dermatome supplied by the L5 root is at the lateral portion of the hind paw24 which we used to assess hyperalgesia. As the observed VSH referral is to a somatotopically appropriate area,24 these data support the convergence-facilitation theory, with central sensitization induced by peripheral visceral stimulation, enhancing alterations in somatic sensory perception. Not only has sensitization of physiological responses been shown,32 but stimulation of visceral afferent neurones has also been observed to be associated with central sensitization with expansion of the dorsal horn neurone receptive fields33 (possibly via an NMDA- and nitric oxide-dependent process).26 27 Therefore, the potential area over which somatic stimulation might be sensitized is increased. This could explain documentation of referred pain in patients extending beyond the expected S2–4 dermatome distribution.1 32

Our data provide further evidence that in addition to pain, hyperalgesia may be referred to somatic structures when a viscus is inflamed. We have shown that in an established model of visceral pain,9 hyperalgesia develops to thermal stimulation of the sole of the hind paw. In the case of animals with turpentine-induced inflammation of the sole, AUC of the \( \Delta \text{Thresh} \) vs time curve was significantly different from control, with a 13-fold increase in value. A trend towards thermal hyperalgesia was seen after insertion of a urinary catheter in the absence of inflammation (as evidenced by a two-fold change in AUC), but this was not statistically significant. These data suggest that at least in an inflammatory model of visceral pain, hyperalgesia to physiological stimuli may be referred to a site distant from the underlying visceral focus of injury. This hyperalgesia is evident by 4 h after commencement of inflammation and lasts for at least 24 h. The type of anaesthetic used to allow induction of the inflammatory process is probably crucial to the accuracy of the early (2 h) measurement of the limb withdrawal response. When pentobarbital anaesthesia was used, we suspected that the animals had not recovered sufficiently for reliable measurements to be made. In contrast, when the technique was refined to an inhalation one, we did not observe behaviour which would indicate any interference in such measurements.

Referral of pain to somatic structures after visceral injury is a well established phenomenon which often follows a dermatomal pattern.2 Varying explanations for referral of pain to somatotopically appropriate areas have been suggested, the most widely accepted of which are the convergence-projection and convergence-facilitation theories. The

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**Discussion**

Mean (SEM) area under the curve (AUC) of the \( \Delta \text{Thresh} \) vs time plot for the no catheter control (group A), NGF inflamed (group B) and turpentine-inflamed (treated with IgG-control (group C) or trkA–IgG (group D)) groups (n=6 in each group). *P<0.05 compared with the no catheter control group (t test); †P<0.05 compared with the IgG-control group (t test).

**Fig. 4** Mean (SEM) area under the curve (AUC) of the \( \Delta \text{Thresh} \) vs time plot for the no catheter control (group A), NGF inflamed (group B) and turpentine-inflamed (treated with IgG-control (group C) or trkA–IgG (group D)) groups (n=6 in each group). *P<0.05 compared with the no catheter control (group A); †P<0.05 compared with the IgG-control (group C).
after intra-vesical turpentine treatment is dependent on NGF. VSH associated with instillation of turpentine is attenuated by prior administration of an NGF sequestering molecule and is mimicked by administration of exogenous intra-vesical NGF. Mast cells are thought to be important neuroimmune mediators and their concentration is increased in bladder tissue under inflammatory conditions. Mast cells express trkA receptors and are able to release further NGF during the degranulation process. Upregulation of mRNA for NGF has been observed in bladder tissue from both an animal model of bladder inflammation and from women with painful bladder conditions. In animal models of pain, exogenous NGF has been shown to produce hyperalgesia, sensitize both afferent nociceptors and dorsal horn neurones, upregulate the production of sensory neurotransmitters in the dorsal root ganglia and induce expression of c-fos in the spinal cord. In contrast, when endogenous NGF is sequestered by administration of the trkA–IgG fusion molecule, hyperalgesia associated with inflammatory states is prevented. This treatment also prevents upregulation of sensory neurotransmitters and reduces the inflammation associated increase in c-fos expression. Thus sequestration of NGF may be a possible avenue for the treatment of referred pain.

We have shown previously that VVH induced by intravesical turpentine is mediated via bradykinin-dependent mechanisms. Others have shown that NGF-induced thermal hyperalgesia is bradykinin dependent and that the sensitivity of bradykinin binding sites is regulated by NGF. Bradykinin is a potent algogen which (in common with NGF) can activate afferent neurones and evoke the release of neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP). These neuropeptides can amplify nociceptor sensitization both by direct activation and also by further mediator release from inflammatory cells. Additionally, bradykinin can augment the response of afferent neurones to heat stimulation with recruitment of ‘silent afferents’, probably via a prostaglandin-mediated mechanism. Therefore, it seems likely that bradykinin and NGF will prove to have a critical interaction in the production of VSH, and bradykinin antagonists may provide an alternative route for attenuation of referred pain problems.

In addition to the pro-hyper-reflexic interaction between bradykinin and NGF, we have shown previously that the cannabinoid agonists anandamide and palmitoylethanolamide can inhibit the VVH observed in this model of visceral pain. Anandamide probably acts via the neuronally located CB1 receptor which has been identified both in the spinal cord and higher centres of the central nervous system and on NGF-dependent (nociceptive) neurones in the periphery, while palmitoylethanolamide is thought to act via CB2 receptors located on immune tissue (including the trkA-expressing mast cell). Thus NGF-dependent responses, such as VSH demonstrated here, may be modulated not only by sequestration of NGF or inhibition of NGF-enhancing responses, but also by attenuation of NGF amplification systems, such as that achieved by cannabinoid agonists.

In summary, we have documented increased responsiveness to somatic thermal stimulation at a site distant from the underlying visceral injury. Inflammation of the bladder is known to increase the nature of the visceral nociceptor response and these visceral afferents display extensive arborizations within the spinal cord, suggesting that visceral-somatic convergence is the norm, not the exception. Increased responsiveness of dorsal horn neurones is also observed and this peripheral and central sensitization, in association with visceral-somatic convergence, provides a potential mechanism for somatic responses at sites distant from the original visceral stimulation. Modulation of these NGF-dependent responses by a variety of mechanisms may produce new therapeutic modalities.

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