Roza, Carolina, Jennifer M. A. Laird, and Fernando Cervero.
Spinal mechanisms underlying persistent pain and referred hyperalgesia in rats with an experimental ureteric stone. *J. Neurophysiol.* 79: 1603–1612, 1998. Spinal neurons processing information from the ureter have been characterized in rats 1–4 days after the implantation of an experimental ureteric stone and compared with those of normal rats. The effects of a conditioning noxious stimulation of the ureter in the presence of the hyperalgesia evoked by the calculosis also were examined. Extracellular recordings were performed at the T₁₂–L₁ segments of the spinal cord. In rats with calculosis, more neurons expressed a ureter input (53 vs. 42% in normal rats); such cells being more likely to show background activity, at a higher rate than normals (6.6 ± 1.2 vs. 3.2 ± 0.9 spikes/s; mean ± SE) and increasing with the continuing presence of the stone. The threshold pressure for a ureteric response was higher than in normal rats (79 ± 5 vs. 54 ± 4 mmHg) but the neurons failed to encode increasing intensities of stimulation. Thirty-five percent of the neurons with exclusively innocuous somatic receptive fields had a ureter input in rats with calculosis, whereas none were seen in normal rats. A noxious ureteric distention applied to neurons with ureter input evoked a complex mixture of increases and decreases in somatic receptive field size and/or somatic input properties markedly different from the generalized increases in excitability seen when such a stimulus was applied to normal animals. We conclude that the presence of a ureteric stone evokes excitability changes of spinal neurons (enhanced background activity, greater number of ureter-driven cells, decreased threshold of convergent somatic receptive fields), which likely account for the referred hyperalgesia seen in rats with calculosis. However, further noxious visceral input occurring in the presence of persistent hyperalgesia produces selective changes that cannot be explained by a generalized excitability increase and suggest that the mechanisms underlying maintenance of hyperalgesia include alteration of both central inhibitory and excitatory systems.

**INTRODUCTION**

We previously have reported the functional characteristics of a population of dorsal horn neurons with inputs from the ureter in normal rats (Laird et al. 1996). Their main properties—large somatic receptive fields of neurons driven by noxious stimulation of the ureter—were congruent with the well-known characteristics of ureteric pain: diffuse, poorly localized, and referred to the body wall. However, none of the neurons responding to stimulation of the ureter showed any evidence of a decrease in mechanical threshold of their somatic receptive fields or of other changes in input properties that could account for referred hyperalgesia, another important clinical feature of ureteric pain (Giamberardino et al. 1994). The aim of the present investigation therefore was to identify and characterize the visceral input to the spinal cord in a situation of persistent stimulation of the ureter. Our purpose was to understand the mechanisms involved in the development of the referred visceral hyperalgesia that accompanies pain of ureteric origin. For this study, we have used a model of artificial ureteric calculosis in the rat that has been demonstrated to show signs of spontaneous visceral pain and referred hyperalgesia (Giamberardino et al. 1990, 1995).

We also have examined the effects of acute noxious stimulation of the ureter on the somatic input properties and on the somatic receptive field of spinal cord neurons in rats with an experimental calculosis. The effects of noxious conditioning stimuli on the input properties and on receptive field sizes previously have been examined using electrical stimulation of peripheral nerves (Cervero et al. 1984; Cook et al. 1987) or a variety of natural noxious stimuli applied in or near the somatic receptive field (Hylden et al. 1989; Laird and Cervero 1989; Pozo and Cervero 1993; Woolf and King 1991), to deep somatic tissues (Hoheisel and Mense 1989; Hu et al. 1992), or to the viscera (Cervero et al. 1992; Euchner-Wamser et al. 1993; Laird et al. 1996). All these previous studies were performed in normal animals, and in all cases, the conditioning stimuli produced excitability increases of the neurons such as decreases in threshold, increases in receptive field size, and/or the appearance of novelafferent inputs. These observations have been interpreted as indicating that the central sensitization of spinal cord neurons is important for the induction and maintenance of hyperalgesic states. In the experiments described here, a conditioning noxious visceral stimulus was applied in the presence of a persistent hyperalgesic state induced by the ureteric stone. The effects of a noxious conditioning stimuli in such a situation have not previously been described. This allows us to address the question of which mechanisms may underlie the maintenance of hyperalgesia once it is established.

Some of these results have been published in abstract form (Roza et al. 1996).

**METHODS**

**Stone implantation and behavioral experiments**

Adult female Wistar rats (*n* = 29) with body weights between 220 and 340 g were used. The rats were anesthetized with halothane (4% induction and 2.5% maintenance). Under sterile conditions, a small incision was made in the upper abdomen, and the overlying tissues retracted to expose the upper portion of the left ureter. An experimental stone was produced in the lumen of the upper third of the ureter by injecting a small volume (~0.01 ml) of dental...
cement (Simplex Rapid, Austenal Dental Products, Harrow, UK or Unifast, G-C Dental Industrial, Tokyo, Japan) using a 0.5-mm-diam (25 gauge) needle (adapted from the method of Giamberardino et al. 1990). The wound was sutured in layers, and the animal allowed to recover. After recovery, the animals were housed individually or in small groups in plastic cages with soft bedding in a room kept at 21 ± 1°C with a 12-h light:dark cycle. They had access to food and water ad libitum. In 16 of the animals implanted with an experimental stone, behavioral studies also were performed. Their spontaneous behavior was recorded continuously during the period between surgery and the terminal experiment (i.e., 1–4 days after stone implantation) using a black-and-white TV camera connected to a time-lapse video-recorder, recording 1 frame/s. An infrared light allowed filming during the dark cycle. The spontaneous behavior of the rats typically presents complex behavioral episodes termed “crises,” which are not observed in normal rats or those with a sham operation. A crisis is defined as lasting for ≥2 min and containing at least three of the following behaviors: hump-backed position, licking of the abdomen and/or left flank, contraction of the muscles of the flank associated with inward movements of the hindlimb, stretching of the abdomen against the floor, squashing of the abdomen against the floor, and supine position with the left hindlimb abducted and compressed against the abdomen. For further details and discussion of these criteria, see Giamberardino et al. (1995).

Preparatory surgery for electrophysiology

One to 4 days after the stone implantation the rats were anesthetized with pentobarbital sodium (50 mg/kg ip initial dose, 10 mg·kg⁻¹·h⁻¹ iv maintenance). Body temperature was recorded with a rectal probe and maintained close to 37°C by a feedback-controlled electric blanket. Catheters were placed in the left carotid artery for continuous arterial blood pressure recordings, in the left jugular vein for injection of anesthetic, and in the trachea to allow artificial ventilation. A laparotomy was performed, and the left ureter was cannulated near the bladder (well below the site of dental cement injection) with a fine catheter (0.61 mm OD). A double-barreled cannula connected the ureteric catheter to a pressure transducer and to a reservoir. The system initially was filled with saline and left open to air to allow natural urine drainage except when stimuli (pressure increases) were applied by raising the reservoir above the level of the preparation. The pressures increases were thus applied to the area of the ureter above the site of cannulation, and presumably also to the renal pelvis. (For further details of this arrangement, see Roza and Laird 1995).

A laminectomy (T₉–T₁₂ vertebrae) was performed to expose the T₉–L₁ spinal segments where the recordings were made. This is the area of the spinal cord where the majority of the ureteric afferents (mostly afferents that run in the hypogastric nerve, which innervates the upper two-thirds of the ureter) enter the CNS, as determined by tracing studies (Donovan et al. 1983; Marfurt and Echtenkamp 1991; Su et al. 1986). The animal was mounted on a rigid frame, and a pool was made with skin flaps around the laminectomy. The dura was removed under warm mineral oil. The animal was paralyzed with pancuronium bromide (20 mg/kg ip) and artificially ventilated. End-tidal CO₂ was monitored continuously and kept at ~4% by altering the ventilation parameters. Stability was improved by filling the pool with 4% agar solution and by performing a bilateral pneumothorax. The level of anesthesia was adjusted as necessary such that no precipitate cardiovascular changes were observed on the application of noxious stimuli. Areflexia (when the animals were not paralyzed) and pupillary constriction also were monitored and used as indicators of adequate anesthesia. Experiments were terminated if the mean arterial blood pressure fell <75 mmHg for more than a few minutes.

All experimental procedures were performed in accordance with European Union and Spanish Government animal protection laws and were approved and supervised by the University Animal Care Facility.

Recording techniques

Extracellular single-unit electrical activity was recorded from dorsal horn neurons on the left side (ipsilateral to the stone-implanted and cannulated ureter) of segments T₁₂–L₁. Recordings were made with glass micropipettes filled with 4 M NaCl (impedance measured at 1 kHz was 10–20 MΩ) and displayed on an oscilloscope. The spikes were digitized with a window discriminator. The blood pressure and ureteric pressure signals were collected and digitized on-line with the use of a computer interface (CED 1401, CED) and displayed along with the output of the window discriminator on a computer (running Spike2 software, CED). The data were analyzed off-line. The search stimulus for dorsal horn neurons was electrical stimulation of the skin of the left flank at an intensity supramaximal for Aβ fibers but subthreshold for C fibers (~5 times threshold of the spinal cord field potential). These stimuli were delivered via needle electrodes inserted intracutaneously over the left obliquus externus muscle (where the pain of ureteric origin is referred).

Input properties of the neurons

Neurons encountered between the surface of the spinal cord and the reversal point of the field potential evoked by the electrical stimulation of the skin were examined. Care was taken to exclude recordings from axons (characterized by monophasic spikes with very steep rising phases). Each neuron was tested for its responses to stimulation of its somatic receptive field as follows. The latency and threshold of the response to electrical stimulation of the skin was tested (0.2-ms square pulse). Mechanical stimulation of the receptive field included innocuous stimuli such as brushing, light tapping, and stroking, as well as noxious stimuli such as pin prick, squeezing, and pinching. Neurons were classified on the basis of their responses to mechanical stimulation of the skin as follows. Class 1 included neurons responding only to low-intensity stimuli such as brush and touch; class 2 included neurons responding to both low- and high-intensity stimuli such as pinch of skin folds and pin prick; class 3 included neurons responding only to high intensity stimuli; and class 4 included neurons responding only to stimulation of subcutaneous tissues (Cervero et al. 1976; Menétray et al. 1977). The extent of the high-threshold excitatory receptive field was mapped with small forceps or pin prick, and the extent of the low-threshold receptive field was mapped with small brush or with a von Frey hair. The receptive field of each cell (in the case of class 2 neurons, the high-threshold receptive field) was marked on the skin. The receptive fields thus determined were traced onto transparencies at the end of the experiment and the areas measured with the use of a computerized image analysis system. Cutaneous mechanical thresholds were determined with von Frey hairs made of nylon monofilaments and calibrated in milliNewtons. The whole area of the receptive field was tested with von Frey hairs, and the lowest value at which a response was obtained consistently was taken to be the mechanical threshold of the cell. Subcutaneous inputs were classified only as noxious or nonnoxious and were not taken into account when determining receptive field areas except for class 4 neurons.

Background activity

Once a neuron was properly isolated in the spike discriminator, its somatic inputs were characterized and its receptive field mapped; after this the recording started, avoiding, if present, after-
discharges of the previous somatic stimuli. Before any distentions or other manipulations were applied a period of 2–3 min was recorded to obtain a baseline firing rate. Mean background activity for purposes of comparison between neurons was then measured as the ongoing activity during the baseline period.

**Stimulation of the ureter**

After characterization of the somatic input properties of the neurons, their responses to stimulation of the ureter and renal pelvis were tested. A single 30-s stimulus of 70–80 mmHg pressure was delivered, and if this stimulus evoked an increase or decrease in firing of the neuron, the neuron was classified as having an excitatory or inhibitory ureter input. Neurons that responded to the 80-mmHg stimulus were tested further to characterize their response to stimulation of the ureter with a number of 30-s stimuli with a range of intensities (20–100 mmHg) at intervals of not less than 5 min. If no response was evoked by a particular stimulus, the next stimulus was applied 5 min later. If a stimulus provoked a response, the next stimulus was applied 5 min after the end of the response when the background activity returned to the level originally seen. In the absence of any background activity, responses were considered to have occurred when any firing was seen during or immediately after the ureteric distention was applied. That is to say, stimulus related. In this case, the response was measured as the average of firing rate during the duration of the firing. When background activity was present, a response was considered to have occurred when there was change (increase or decrease) in firing rate ≥15% of the average in background activity recorded 30 s immediately before the stimulus. The mean firing rate (Hz) was measured during the period in which firing remained above this level. The response was expressed as this firing rate minus the background activity in the 30 s immediately preceding the stimulus. After each neuron was characterized completely, the intraluminal ureteric pressure was checked to be sure that the ureter remained intact. Special care was taken with rats in which the terminal experiment was performed at 1 day postimplantation. A fall in ureteric pressure <0 mmhg was an indication that the ureter wall was perforated at the site of the hole produced by the injection of dental cement. In this case, the experiment was terminated. No more than six high-intensity stimuli were applied to the ureter in any experiment, because in previous control experiments, we found that repeated stimuli produced damage of the ureteral wall. The effect of ureter stimulation on the size and response characteristics of the somatic receptive field also was noted; neurons that showed changes also were classified as having been influenced by ureteric stimulation, even if this stimulation did not evoke an immediate change in the background activity of the neuron.

**Histological methods**

At the end of the experiment, the recording electrode was removed and replaced with one containing 4% Pontamine sky blue in 0.5 M sodium acetate. Two marks were made at 500-μm intervals by iontophoretic deposition of dye in the track in which a unit had been recorded to provide a scale that would be subjected to comparisons with the neurons we have previously studied. Neurons that showed any changes in background activity, in somatic receptive properties were verified by iontophoretic deposition of dye in the track in which they were originally seen. In the absence of any background activity, responses were considered to have occurred when any firing was seen during or immediately after the ureteric distention was applied. That is to say, stimulus related. In this case, the response was measured as the average of firing rate during the duration of the firing. When background activity was present, a response was considered to have occurred when there was change (increase or decrease) in firing rate ≥15% of the average in background activity recorded 30 s immediately before the stimulus. The mean firing rate (Hz) was measured during the period in which firing remained above this level. The response was expressed as this firing rate minus the background activity in the 30 s immediately preceding the stimulus. After each neuron was characterized completely, the intraluminal ureteric pressure was checked to be sure that the ureter remained intact. Special care was taken with rats in which the terminal experiment was performed at 1 day postimplantation. A fall in ureteric pressure <0 mmhg was an indication that the ureter wall was perforated at the site of the hole produced by the injection of dental cement. In this case, the experiment was terminated. No more than six high-intensity stimuli were applied to the ureter in any experiment, because in previous control experiments, we found that repeated stimuli produced damage of the ureteral wall. The effect of ureter stimulation on the size and response characteristics of the somatic receptive field also was noted; neurons that showed changes also were classified as having been influenced by ureteric stimulation, even if this stimulation did not evoke an immediate change in the background activity of the neuron.

**Data analysis**

Statistical tests (Mann-Whitney U test for comparisons between 2 groups and χ² tests) were performed on the raw data. Values of P < 0.05 were taken as statistically significant.

**Results**

For the purposes of analysis, the rats were divided as follows: Day postimplantation, in 14 rats, the experiments were conducted 1 day after stone implantation, in 3 rats 2 days after, in 3 rats 3 days after, and in 9 rats 4 days postimplantation. Fate of the stone: in 20 of the 29 rats the stone was still present and in the remaining 9, the stone had been expelled naturally. It is also important to take into account that the stone takes usually >1 day to be expelled completely. In 9 of the 14 rats in which the experiment was performed 1 day after stone implantation, the stone was found below the site of injection, an indication that it had been affected by ureteric motility. Therefore for each animal, two parameters had been considered: the absence of the stone and the day after stone implantation.

All the rats were weighed before the terminal experiment. There was no significant change in their body weights during the survival period.

**Behavior of rats with stones**

Most of the rats whose spontaneous behavior was analyzed showed crises of spontaneous visceral pain (11/16, 69%). Those rats that did not show crises during the survival period (n = 5) did show evidence of subcrises (see Giamberardino et al. 1995) and included two rats in which the terminal experiment was performed after only 1 day postimplantation (it is known that a proportion of rats do not show crises until the 2nd or 3rd day after stone implantation). However, we found no correlation between the number or duration of crises and the fate of the stone. There were no significant differences in the number and duration of crises between groups of rats in which the terminal experiment was performed on different days postimplantation.

**Sample of neurons**

In this study, 101 dorsal horn neurons were recorded of which 86 were fully characterized and tested by raising the ureter and renal pelvis pressure to 80 mmHg for 30 s. Of these 86 neurons, 46 (53%) were influenced by the ureter stimulus as follows: 24 neurons showed a change in firing rate and 22 showed some evidence of being influenced by ureter stimulation, showing changes in the size of their somatic receptive field and/or changes in their somatic input properties. The remaining 40 neurons tested (47%) did not show any changes in background activity, in somatic receptive field size, or in their somatic input properties after ureter stimulation. For the purposes of comparison of neurons responding or not to ureter distention and for subsequent comparisons with the neurons we have previously described in normal animals (Lafrance et al. 1996), in the following description of results all neurons that showed any change after ureter stimulation (n = 46) will be considered as having an input from the ureter.

**Responses to ureter stimulation**

Twenty-four neurons exhibited a clear change in their firing activity after ureteric distention. In 13 of those neurons, more than one ureteric distention was performed, and in
FIG. 1. Neuronal responses to ureter distension at different pressures. Response of the neurons is plotted as the difference between the mean firing rate of the neuron recorded for the duration of the response minus the average of background activity recorded during 30 s preceding the stimulus. See METHODS for further details. A: neurons recorded in normal rats (n = 18) (data taken from Laird et al. 1996). B: neurons recorded in rats with an experimental calculosis (n = 13). ●, neurons that encoded the stimulus intensity; ○, neurons that did not encode the stimulus intensity.

FIG. 2. Example of responses evoked by distension of the ureter in a class 2 neuron. A, top: ureter pressure, including 4 stimuli of 30-s duration and increasing intensities (ureter distensions were given at 15-min intervals). A, bottom: histogram of the responses evoked by the applied stimuli. This neuron did not encode the intensity of the stimulus. B: location of the receptive field of this neuron, which decreased in area as a result of ureter stimulation. ○, larger initial receptive field; ●, final, smaller receptive field. C: recording site of the neuron drawn on a standard transverse section of T15 rat spinal cord.

from 57 to 856 s with a mean of 101 ± 8 s. Therefore in all cases, the responses outlasted the stimulus duration.

The remaining 22 neurons classified as having an input from the ureter did not show an evident change in firing rate compared to their background activity prior to ureter distention but did show changes in their somatic input properties and/or the size of their somatic receptive fields. Further details of their profile of changes are given in following sections.

The mean response threshold to ureter stimulation was 79 ± 5 mmHg (range = 33–96 mmHg). Figure 3 shows the distribution of thresholds for the population of neurons that were either excited or inhibited by ureter distention. For the purposes of discussion, the threshold profile of the neurons with ureter input in normal rats is also included on the figure (data taken from Laird et al. 1996).

The latency of the responses ranged between 2 and 191 s. The mean latency was 40 ± 6 (SE) s; 11 of the neurons responded after the end of the 30-s stimulus. Six of the 13 neurons that responded before the end of the stimulus had very short latencies; their responses began in the first 10 s of the 30-s stimulus. The duration of the responses ranged

FIG. 3. Histogram showing the distribution of threshold ureter pressures for responses to ureter distension of the population of neurons either excited or inhibited by ureter stimulation in normal rats (n = 20, ○) (data taken from Laird et al. 1996) and in rats with experimental calculosis (n = 13, ●).
Somatic input properties

All the neurons examined in this study had somatic receptive fields that included part of the left flank. All but three of the neurons had an excitatory cutaneous input; these three neurons (classified as class 4) could be driven by stimulation of subcutaneous tissues, presumably muscle. Seventeen of the neurons tested were only excited by low-intensity stimulation of the skin (class 1) and 6 (35%) of them had an input from the ureter. Forty-eight (56%) of the neurons tested were class 2 neurons (excited by both low- and high-intensity mechanical stimulation of their cutaneous receptive fields), of which 32 (67%) had an input from the ureter. Eighteen neurons were class 3 (excited by high-intensity stimulation of their cutaneous receptive fields); 7 of them (39%) had an ureteric input. Thus the group of neurons with an input from the ureter included those with somatic receptive fields that responded exclusively to non-noxious stimuli (Fig. 5). However, the mechanical thresholds of the somatic receptive field tested with von Frey hairs was not significantly different between the group of neurons that showed an input from the ureter and the group that did not.

As mentioned above, three of the neurons tested were class 4 (input from deep tissues and muscle but not from the skin). One of these neurons showed a change in its somatic input properties after ureter stimulation, whereas the other two were not influenced (see further). However, a number of neurons (n = 40) had an input from deep somatic tissues in addition to that from the skin. The proportion of neurons with an input from deep somatic tissues was not significantly different between the group of neurons that showed an input from the ureter (46%) and the group that did not (50%).

There were no significant differences in the proportion of different classes of neurons recorded in rats with the stone present or expelled nor when comparing different times postimplantation.

Somatic receptive fields

The somatic receptive fields of neurons recorded in this study were located on the left flank and, in some cases, included the contralateral flank and/or the ipsilateral hind-
Changes in somatic receptive field area and/or somatic input properties

Twenty two of the neurons classified as having an input from the ureter did not show a clear change in firing rate after an acute distention of the ureter, but they showed changes in the size of their somatic receptive field (n = 12), their somatic input properties (n = 6), or both types of change (n = 4); thus they were classified as influenced (U influenced). These kinds of changes were also seen in most of the neurons (17/24) that responded to ureter stimulation (U+) with a clear change in firing rate, eight neurons showed a change in the size of their somatic receptive field, five showed changes in their somatic input properties, and four showed both types of changes. None of the neurons without an input from the ureter (U−) showed any of those changes.

CHANGE IN SOMATIC RECEPTIVE FIELD AREA. A total of 28 neurons with an input from the ureter (61%) showed changes in the size of their somatic receptive fields. Twelve of the neurons were U+, and they showed increases (n = 5, mean increase = 5 ± 2.7 cm²) or decreases (n = 7, mean decrease = 3.7 ± 1.1 cm²) in the size of their somatic receptive fields. All of the neurons that showed these changes were class 2 except one class 3 neuron located in the superficial dorsal horn. There was no relationship between the nature of the responses to ureter distention (excitatory vs. inhibitory) and the direction of the change in area (increase vs. decrease).

The remaining 16 neurons were U influenced; this group of neurons were more likely to show increases (13 of 16) in the size of their somatic receptive fields after an acute ureter distention. The mean increase was 4.2 ± 1 cm², and the mean decrease of the remaining 3 neurons was 2.6 ± 1.2 cm². Neurons showing changes in this group included all classes.

There were no significant differences in the mean change in area when comparing the U+ and U influenced groups of neurons. In both groups, neurons recorded one day after stone implantation showed larger changes but the difference was not significant.

CHANGES IN SOMATIC INPUT PROPERTIES. Ten of the 22 neurons classified as being influenced by the ureter stimulus (U influenced) exhibited changes in their somatic input properties after ureter stimulation. Two neurons showed an increase in their response to noxious pinch and another two a decrease in the responses to noxious pinch and to light brush. One of the neurons showed an increase in its response to light brush, and three neurons showed a long-lasting change in their spontaneous activity (Fig. 7). Interestingly, the remaining two neurons completely changed their somatic input properties after an acute (80 mmHg) ureter distention as follows: one of the neurons with exclusively a deep input (class 4) started to respond to light brush of the skin; after 15 min, this neuron had not recovered its original properties. The other neuron initially classified as class 1 showed a novel response to pinch immediately after ureter distention that lasted for 3 min. This change was produced again when the ureter distention was repeated.

Of the group of neurons that showed a clear change in firing rate as result of ureter distention (U+, n = 24), nine neurons also showed changes in their somatic input properties. Two neurons showed an increase in their response to noxious pinch and three showed a decrease. One of the neurons showed an increase in its response to light brush.
teric calculosis have been described and are compared with previously obtained control data. The effects produced by the ongoing visceral input may underlie the spontaneous pain crises and referred visceral hyperalgesia seen in animals with a calculosis. Second, the dynamic changes produced by a noxious conditioning stimulus (distention of the ureter) applied in the presence of an ongoing hyperalgesic state (produced by the stone) have been examined. Although there are many reports of changes produced by different types of somatic or visceral conditioning stimuli applied in normal animals (for references, see INTRODUCTION), the effects of such stimuli in the presence of hyperalgesia has not previously been described.

Changes in the properties of neurons in rats with a calculosis

VISCERAL INPUTS. Neurons with a ureter input were more likely to have background activity than neurons without an input, and their mean rate of activity was also significantly higher than in control animals. A similar observation has been made by Giamberardino and colleagues (1996) for spinal neurons with an input from the hyperalgesic muscle. We also observed that the excitability of neurons with ureter input increased with the continuing presence of the stone in the ureter; neurons recorded 4 days postimplantation had significantly more activity than those recorded at 1 day postimplantation. The increased background activity likely contributes to the referred hyperalgesia by providing spatial summation; in the presence of ongoing activity, the amount of somatic input required to evoke a response will presumably be less.

Increases in background activity of CNS neurons also have been described after acute inflammation of other hollow viscera. Thus a slow increase in background activity 5–10

Location of recording sites in the spinal cord

The dye marks were recovered and the recording sites were successfully reconstructed for 42 of the neurons recorded (49%). Neurons were recorded throughout the dorsal horn gray matter, as shown in Fig. 9. Neurons recorded in the superficial dorsal horn (n = 7) included class 1–3 cells and one cell that showed novel inputs after an acute ureter distention; all these neurons had small receptive field areas (range = 0.27–7.3 cm²). In deeper laminae, all classes of neurons were found (n = 35); this sample of neurons had a wider range of receptive field sizes (range = 0.66–59.15 cm²); however, there were no significant differences between the sizes of the receptive fields of the neurons located in superficial or deep laminae. Neurons with an input from the ureter were found throughout the dorsal horn.

DISCUSSION

The results from this study may be divided into two parts. First, the properties of dorsal horn neurons in rats with ureteric calculosis have been described and are compared with previously obtained control data. The effects produced by the ongoing visceral input may underlie the spontaneous pain crises and referred visceral hyperalgesia seen in animals with a calculosis. Second, the dynamic changes produced by a noxious conditioning stimulus (distention of the ureter) applied in the presence of an ongoing hyperalgesic state (produced by the stone) have been examined. Although there are many reports of changes produced by different types of somatic or visceral conditioning stimuli applied in normal animals (for references, see INTRODUCTION), the effects of such stimuli in the presence of hyperalgesia has not previously been described.

Changes in the properties of neurons in rats with a calculosis

VISCERAL INPUTS. Neurons with a ureter input were more likely to have background activity than neurons without an input, and their mean rate of activity was also significantly higher than in control animals. A similar observation has been made by Giamberardino and colleagues (1996) for spinal neurons with an input from the hyperalgesic muscle. We also observed that the excitability of neurons with ureter input increased with the continuing presence of the stone in the ureter; neurons recorded 4 days postimplantation had significantly more activity than those recorded at 1 day postimplantation. The increased background activity likely contributes to the referred hyperalgesia by providing spatial summation; in the presence of ongoing activity, the amount of somatic input required to evoke a response will presumably be less.

Increases in background activity of CNS neurons also have been described after acute inflammation of other hollow viscera. Thus a slow increase in background activity 5–10
et al. 1988). However, acute inflammation (Çalı̈k et al. 1992) of dorsal horn neurons previously has been seen enhanced in the presence of an ongoing, neuronal barrage served in these rats. Disruption of the encoding of mechanical (Cervero et al. 1992; Laird and Cervero 1990) controls that are normal, thus contributing to the spontaneous pain crises observed after acute inflammation (Fitzgerald et al. 1996; Laird et al. 1996). The increased background activity seen in neurons with ureter input in the current study may reflect an afferent barrage from sensitized ureteric nociceptors; it is known that ureteric nociceptive afferents can show signs of sensitization after damage (Cervero and Sann 1989). Novel or increased spontaneous activity also has been described in high- and low-threshold visceral afferents in the bladder and the colon after chemical irritation (Häbler et al. 1990, 1993; Sengupta et al. 1996; Su et al. 1997) and also has been described. The increased ureteric motility in rats with an experimental calculosis (Laird et al. 1997) also may contribute to the increased background activity in neurons with ureter input. The increases in motility are such that the amplitude of the contractions reaches the threshold for ureteric nociceptors (Cervero and Sann 1988); levels that are equivalent to those that evoke pseudoaffective responses and excite dorsal horn neurons in normal rats (Laird et al. 1996; Roza and Laird 1995).

An increased number of neurons expressed a ureter input in rats with calculus compared with controls. This change is also an expression of increased central excitability, such that previously subthreshold ureteric inputs provoke action potentials. However, a much greater proportion of neurons expressed a ureter input at 1 day than at 4 days postimplantation, the opposite to what was seen for increases in background activity.

Spinal neurons responding to ureter distention in normal animals encode the intensity of the distentions at pressures >20 mmHg (Laird et al. 1996). However, in rats with calculus, most of the neurons with ureter input did not encode the intensity of the stimuli. Under these conditions, just suprathreshold stimuli will elicit much greater responses than normal, thus contributing to the spontaneous pain crises observed in these rats. Disruption of the encoding of mechanical stimuli by dorsal horn neurons previously has been seen after a series of prolonged pinches of the tail in rats (Cervero et al. 1988). However, acute inflammation (~1 h) of the colon does not appear to affect the encoding of noxious colon distention by spinal or thalamic neurons (Al-Chaer et al. 1996, 1997).

The mean threshold for responses to ureter distention in rats with calculus was much higher than in normal rats. This is contrary to what might be expected, given the previous observations of sensitization of ureteric afferents (Cervero and Sann 1988) and decreased response thresholds in spinal neurons after inflammation of the esophagus (Garrison et al. 1992) or colon (Al-Chaer et al. 1997). However, the increase in threshold seen in the current study may be due to changes in the mechanical properties of the ureter, such that the stimulus applied does not have the same effect on the afferent endings. Rats with calculus have a semiobstructed ureter, and postmortem analyses show both a considerable distention of the area in which the stone was injected and a thickening of the ureteric wall (unpublished observations). Furthermore, immunohistochemical studies on ureters with calculus show a depletion of neuropeptides such as substance P and calcitonin gene-related peptide and also a partial destruction of nerve endings innervating the portion of the ureter above the site of implantation (Sann et al. 1997). These peripheral factors help to explain the necessity for a more intense stimulus of the ureter and also that 28% of the sample of neurons were influenced by ureter distention but did not show a clear response.

**SOMATIC INPUTS.** Dorsal horn neurons responding to noxious stimulation of the ureter in normal animals also have a noxious somatic receptive field (Laird et al. 1996). In rats with calculus, we found a sample of neurons with ureter input with exclusively innocuous somatic receptive fields. These cells may represent sensitized neurons, which, under normal conditions, had nociceptive somatic inputs, or alternatively the novel expression of ureter input in low-threshold neurons. In either case, innocuous stimulation of the somatic area where pain from ureter is referred would presumably now evoke a painful sensation, contributing to referred hyperalgesia.

In normal rats, neurons responding to ureter distention have significantly larger receptive fields than neurons without a ureter input (Laird et al. 1996). In the present study, the mean receptive field sizes of neurons with and without ureter input were not significantly different and were not different from neurons without ureter input in control rats. Furthermore, the areas of the receptive fields of the neurons with ureter input recorded after 4 days tended to be smaller than the areas of those recorded after 1 day. In a situation of chronic noxious stimulation, the contrary would be expected, as described in arthritic rats (Calvino et al. 1987; Grubb et al. 1993; Menétrey and Besson 1982), particularly because increases in receptive field size are produced by acute noxious stimulation of the ureter (Laird et al. 1996) and other hollow viscera (Cervero et al. 1992, Euchner-Wamser et al. 1993). However, no changes in cutaneous receptive field size were observed after acute inflammation of the colon in postsynaptic dorsal column or thalamic neurons (Al-Chaer et al. 1996, 1997).

Receptive field sizes are modulated by supraspinal controls (Cervero et al. 1992; Laird and Cervero 1990) that are enhanced in the presence of an ongoing, neuronal barrage produced by tissue inflammation (Ren and Dubner 1996; Schaible et al. 1990). The presence of an artificial stone likely evokes a similar increase in descending inhibition, explaining the smaller than expected size of receptive fields of neurons with ureter input. The differences compared to arthritic rats may be because the spontaneous pain of calculus is intermittent, but very intense, sufficient to turn on tonic descending inhibition such that it continues between the acute painful crises.

**Dynamic changes in neuronal properties after ureter distention**

Fifteen neurons showed changes in the intensity of their response to noxious and/or nonnoxious stimulation of their...
somatic receptive fields after an acute ureter distention. In three of these neurons, the changes included novel somatic inputs. Twenty-eight neurons showed changes in the area of the somatic receptive field. Changes in input properties and receptive field area of spinal neurons have been described after a variety of noxious conditioning stimuli (Cervero et al. 1984, 1992; Cook et al. 1987; Euchner-Wamsler et al. 1993; Hoheisel and Mense 1989; Hylden et al. 1989; Hu et al. 1992; Laird and Cervero 1989; Laird et al. 1996; Pozo and Cervero 1993; Woolf and King 1991). In all of these studies performed in normal animals, noxious conditioning stimuli produced an increase in the excitability of the neurons studied, as evidenced by decreases in threshold and increases in receptive field size, except for a few neurons strongly inhibited by visceral stimuli, which showed parallel decreases in receptive field area (e.g., Laird et al. 1996).

In the present study, in which the conditioning stimulus was applied in the presence of a lesion sufficient to induce hyperalgesia, a wide variety of effects were seen. These included “mixed” inhibitory and excitatory effects in the same cell such as a simultaneous loss of low-intensity inputs and an increase in nociceptive receptive field area. The changes in somatic properties were not correlated with the inhibitory or excitatory nature of the response to ureter distention. Similarly, after acute inflammation of the colon a population of postsynaptic dorsal column neurons showed increases (2/7 neurons), decreases (4/7 neurons), or no change (1/7 neuron) in responsiveness to cutaneous stimuli, although all seven of the neurons showed increased responses to distention of the colon (Al-Chaer et al. 1997).

Thus a noxious conditioning stimulus applied in the presence of persistent noxious input, or the persistent noxious input itself, does not necessarily result in an increase in excitability, as seen with acute stimuli applied under normal conditions. A sudden intense input may disrupt the balance between the sustained noxious input and the supraspinal and/or segmental controls turned on by it, thus eliciting these atypical responses. We do not know what provokes a crisis of pain-like behavior in rats with calculus or a bout of renal colic in patients, but it is assumed to be stimulation of the ureter by the stone when it moves, or as the ureter contracts around it, because similar behaviors are elicited by electrical stimulation of the ureter (Giamberardino et al. 1988). The effects of the acute ureter distention may thus mimic to some extent those of the input that provokes a crisis.

Conclusions

We conclude that persistent stimulation of the ureter produces an increase of excitability in dorsal horn neurons with ureter inputs, evidenced by an increase in background activity and in the number of neurons expressing ureter inputs and a decrease in threshold of convergent somatic receptive fields, thus accounting for the referred hyperalgesia seen in rats with calculus. Furthermore, these results show that noxious input (such as that evoking crises) occurring in the presence of ongoing hyperalgesia produces a complex mixture of increases and decreases in excitability of individual dorsal horn neurons and thus suggest that the mechanisms underlyng the maintenance of hyperalgesia include alterations of central inhibitory and excitatory systems.

This work was supported by grant SAF 97-0104 from the Comisión Interministerial de Ciencia y Tecnología, Ministry of Education and Science, Spain, by grants AE97-95 and IN94-0151 from the Comunidad Autónoma de Madrid, Spain, and by Boehringer Ingelheim (Eurpharma S.A., España). C. Roza is supported by a postgraduate fellowship from the Ministry of Education and Science, Spain (Formación de Personal Investigador program).

Address for reprint requests: J.M.A. Laird, Dept. of Fisiologia, Facultad de Medicina, Universidad de Alcalá, Alcalá de Henares, E-28871 Madrid, Spain.

Received 18 September 1997; accepted in final form 25 November 1997.

REFERENCES


