

New Methods for the Study of Acute and Chronic Visceral Pain in Experimental Animals

Jennifer M.A. Laird, Ph.D.,

Carolina Roza, B.Sc.

and

Fernando Cervero, M.D., D.Sc.

Department of Physiology & Pharmacology

Faculty of Medicine

University of Alcalá de Henares

Alcalá de Henares, E-28871

Madrid, Spain

Jenny Laird received a PhD in Physiology from the University of Bristol (UK) in 1989 and has worked as a Research Scientist at NIH (Bethesda, USA) and at the Neuroscience Research Centre of Merck Sharp and Dohme (Harlow, UK). She now works at the Department of Physiology and Pharmacology of the University of Alcalá de Henares (Madrid) where she runs a research program on the neurophysiology and neuropharmacology of spinal nociceptive systems. She supervises Carolina Roza, a graduate student of the Department, and both are members of the pain research group run by Fernando Cervero, Professor of Physiology. Correspondence should be sent to Dr. Laird and she can be reached at (e-mail) jjlaird@alcala.es or by phone at +34 1 885 4516 or FAX at +341 1 885 4525.

INTRODUCTION

The word "pain" describes a wide range of unpleasant sensory experiences. However, different types of pain cannot be ascribed to a single neurological mechanism, but are likely to be due to the activation of different mechanisms and different transmitter systems. Thus, it is important to select appropriate experimental models of pain when attempting to study basic mechanisms, or when testing novel therapies or novel analgesics for use in the clinic. The range of pain states has previously been

discussed (Cervero and Laird 1991) in terms of three components, which are not necessarily exclusive, but may coexist, as follows:

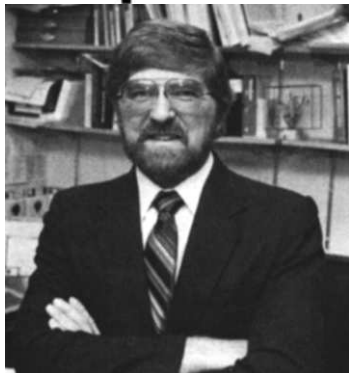
Phase 1: The response of the system to noxious but minor stimuli, such as the sensations produced by pin prick or heat stimuli that are sustained during the course of every-day life.

Phase 2: The response of the system to repeated, sustained and/or damaging stimuli. The afferent input to the CNS under these conditions will be from sensitised peripheral receptors and therefore changed, and in addition, there is evidence that central neurones alter their response properties, becoming more excitable. Different classes of receptors and transmitter systems are also probably activated during this type of stimulation (e.g. NMDA, neuropeptides including the tachykinins, etc.).

Phase 3: The response of the system to very prolonged and severe noxious inputs or to damage to the nociceptive system itself. Chronic conditions, especially nerve injuries, produce qualitative changes in nociceptive systems, such as changes in the properties and proportions of neuronal populations and the formation of new anatomical connections between neurones.

It is clear from the above brief summary of pain states that the most commonly used nociceptive behavioural tests (e.g. tail-Hick, hot-plate, paw pressure) fall into the category of Phase 1 pain, whereas clinically important pain states are of the Phase 2 and 3 types. This is particularly important since the results obtained in studies of acute pain may well not be directly applicable to the problems of more chronic pain. Furthermore, although visceral pain can broadly be included in the Phase 2 group, it has a number of special characteristics which distinguish it from pain of somatic origin. In humans the only sensation evoked by stimulation of most viscera is pain. Visceral pain is also poorly localised, and one of its most distinctive aspects is that the pain appears to originate from regions of the body surface distant from the damaged area. It is a common clinical observation that the area to which the pain is referred also shows signs of tenderness and increased sensitivity to applied stimuli, a phenomenon known as referred hyperalgesia. Whilst evidence from experiments in

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Editor's Column

A gray and cloudy day in Kansas City with a few show flakes in the air. Winter is not over yet. March came in like a sheepish lion, so I suspect that the weather will be as

unpredictable as usual for this time of year. I am always in the minority in wishing for a big storm; it just seems more exciting.

The article in this issue of the Carrier is a first. Its authors are not from the United States, but from Spain. Dr. Cervero and his group are internationally known for their work on the neuro-physiology of pain. The quality of the article demonstrates why. I hope that the article will stimulate others from outside the U.S. to consider writing for the Carrier. Many thanks to Drs. Laird and Cervero and Ms Roza for the fine contribution.

The recent series of bombings in England and Israel after what seemed like a short but very welcome time without such acts of violence in those places comes like a bucket of ice water in the face. While not the only terrible acts of violence of man against man in the news, the scenes of innocent people blown apart by a desperate terrorist must make one pause. Why do people think that killing in cold blood will help their cause, there certainly is enough suffering and horror to go around without making more. Perhaps our science cannot bear on this problem much, but our humanity can. Whether it is deploring all such acts in other countries or being opposed to the violence of poverty, ignorance, illiteracy, sickness, abuse, racism or helplessness in our own neighborhoods, each of us can make a difference. For those of us who are Christians, Lent and Easter are special times to rededicate to forgiving and helping, and I imagine other faiths have no problem agreeing with that. Do It.

Michael M. Patterson, Ph.D.

Science Editor

College of Osteopathic Medicine

The University of Health Sciences

2105 Independence Blvd.

Kansas City, MO 64124-2395

816-283-2308

FAX 816-283-2303

normal animals demonstrates that referred pain is the result of convergence of input from deep and superficial body structures onto common spinal inter-neurons, very little is known about the mechanisms underlying referred hyperalgesia.

We therefore believe it is important to study visceral nociceptive mechanisms since we cannot assume that they are the same as those which process pain from the skin. Further, the majority of the studies of the visceral nociceptive pathway have been carried out in normal animals, and we wanted to study the mechanisms underlying more chronic, Phase 2 type pain of visceral origin.

Noxious stimulation of viscera, particularly in awake animals, is technically much more demanding than stimulation of the body surface, and thus there are only two widely used behavioural models of noxious visceral stimulation, the "writhing test", in which algogenic chemicals (e.g. bradykinin, acetylcholine) are injected i.p. provoking a characteristic "writhing" or "abdominal stretching", and colorectal distension in the rat, a conditioned-avoidance test in which colorectal distension alters or inhibits the normal step-down behaviour of rats when placed on a raised platform in an open field (see Ness & Gebhart 1990 for references)

Methods for noxious stimulation of the ureter

We are particularly interested in ureteric pain as it has obvious clinical relevance and is a characteristic example of pain of internal origin. We therefore decided to investigate methods of increasing the ureteric pressure, and have developed a technique for the stimulation of the ureter and renal pelvis in anaesthetised rats by increasing the intraluminal pressure via a fine cannula inserted into one ureter close to the bladder (Laird and Roza 1995). We characterised the effects of these stimuli using cardiovascular changes as a measure of the nociceptive response since this approach is relatively simple and has been successful for the study of nociceptive reactions evoked by the stimulation of other hollow viscera (for review see Ness & Gebhart 1990).

Experiments were conducted on adult female Wistar rats anaesthetised with pentobarbital. A laparotomy was performed and the renal end of the left ureter cannulated near the bladder with a fine catheter (0.61 mm external diameter). A double barrelled cannula connected the ureteric catheter to a pressure transducer and to a reservoir: The system was initially filled with saline, and left open to allow natural urine drainage except when stimuli (pressure increases) were applied by raising the reservoir above the level of the

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preparation. The pressure applied to the ureter was measured in the connecting cannula. In a separate series of control experiments, we found that when the ureteric pressure was measured directly by placing a second catheter connected to a pressure transducer in the renal end of the ureter, the applied pressure was the same as the directly measured pressure.

In each animal, a series of stimuli between 5 and 90 mmHg were delivered by raising the reservoir above the preparation for 30 s. When the ureteric pressure was increased above the response threshold, all animals showed pressor responses proportional to the intensity of the stimulus applied. The responses were transient, began before the end of the stimulus and outlasted the stimulus (Figure 1). Responses to stimuli of less than 25 mmHg were never observed, and in some rats there was no response to stimuli between 25 and 35 mmHg. The minimum stimulus intensity required to evoke a response ranged from 27 to 58 mmHg in different animals. Simple regression analysis showed that the change in mean arterial blood pressure, the time to peak, the duration of the response and the area under the curve were all significantly correlated with the intensity of the stimulus. The threshold ureteric pressure for inducing a blood pressure response was -25 mmHg. In a separate group of rats stimuli were also applied in the presence of and after the removal of a tight ligature tied around the ureteric-pelvic junction which prevented the increases in ureteric pressure from reaching the kidney. In these animals, there was a reduction in the responses compared to baseline, presumably due to damage to a portion of the innervation, but the difference in the responses in the presence of or after removal of the ligature was less than 10%.

Effects of analgesics on the responses to increased ureter pressure in normal rats

We further tested the hypothesis that these responses were nociceptive reactions by examining the effects on the reflex pressor responses of two different analgesics known to relieve the pain of ureteric colic in man. The μ opiate receptor agonist, morphine, and the non-opiate analgesic, metamizol, which inhibits cyclo-oxygenase, were of experiments. Morphine produced a dose dependent decrease in the pressor response to ureteric distensions which was naloxone-reversible (Laird and Roza 1995). Intravenous administration of metamizol had no statistically significant effect on the baseline blood pressure measured 10 minutes after administered intravenously in separate series each dose but the pressor responses to ureter

distension were dose-dependently inhibited (Laird and Cervero 1996; Figure 2). The fact that two analgesic drugs with different mechanisms of action both dose-dependently inhibit the pressor responses to distension of the ureter thus reinforce the idea that these responses are nociceptive.

Responses of spinal cord dorsal horn neurones to noxious ureter distension in normal rats

In separate experiments, the responses to noxious distension of the ureter of dorsal horn were tested (Laird et al 1995). Female Wistar rats were prepared as described above, with the addition of a laminectomy which exposed the T13-L1 spinal segments (the area receiving the majority of afferents from the ureter). The animal was mounted in a rigid frame, and single-unit extracellular activity recorded with glass microelectrodes. The somatic response properties (cutaneous and/or muscle receptive field) of each unit isolated were tested, and also its response to one or more 30s distensions of the ureter. The neurones excited by ureter distension {35% of those tested} had large nociceptive cutaneous receptive fields on the flanks, which were often bilateral, and increased in size with ureter stimulation. Their responses to ureter distension encoded stimulus intensity, often greatly outlasted the stimulus, and were dose-dependently inhibited by a μ opiate receptor agonist (fentanyl), and by metamizol (Figure 3).

Chronic model of visceral pain: experimental ureteric calculus

This model was originally developed and behaviourally characterised by Giambardino and colleagues (1990, 1995a). They have shown that rats with ureteric stones show "crises" of abdominal stretching, licking of the abdomen and contraction of the ipsilateral musculature indicative of spontaneous visceral pain episodes (for a complete description see Giambardino et al. 1995a). These crises can be dose-dependently diminished by treatment with morphine. They have also shown that rats with ureteric stones develop a lowered response threshold to stimulation of the ipsilateral obliquus externus muscle of the flank, i.e. referred hyperalgesia, which can be decreased by treatment with ketaprofen or with a spasmolytic (Giambardino et al 1990, 1995b).

In our laboratory we use adult female Wistar rats. In sterile conditions the upper third of the left ureter is exposed via a laparotomy, taking care not to disturb the connective tissue around the ureter itself. A small volume (approx. 0.01 ml) of dental cement is injected whilst still fluid using a 0.5 mm (25 gauge) needle. The incision is sutured in layers, and the animal allowed to recover. The rats are individually

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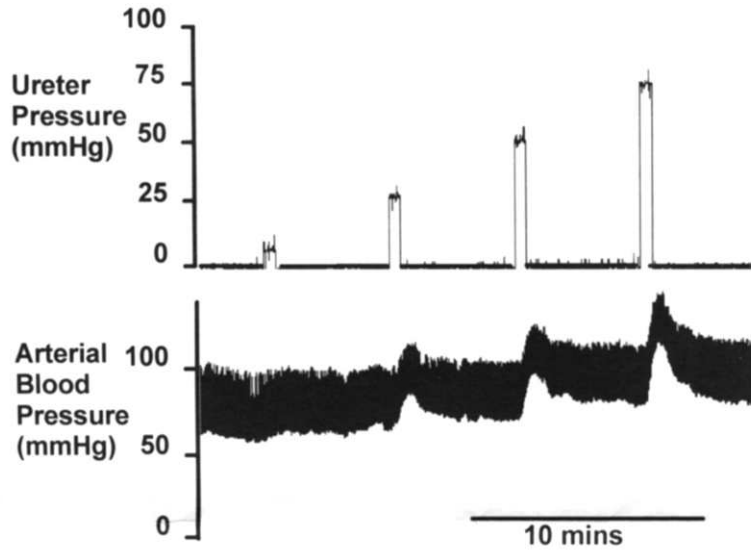


Figure 1. Changes in systemic arterial blood pressure evoked by distension of the ureter. Example of data from an individual experiment. The upper trace shows the pressure applied to the ureter, including 4 stimuli of 30 s duration and increasing intensities. The lower trace shows the arterial blood pressure and the responses evoked by the applied stimuli (data taken from Roza and Laird 1995).

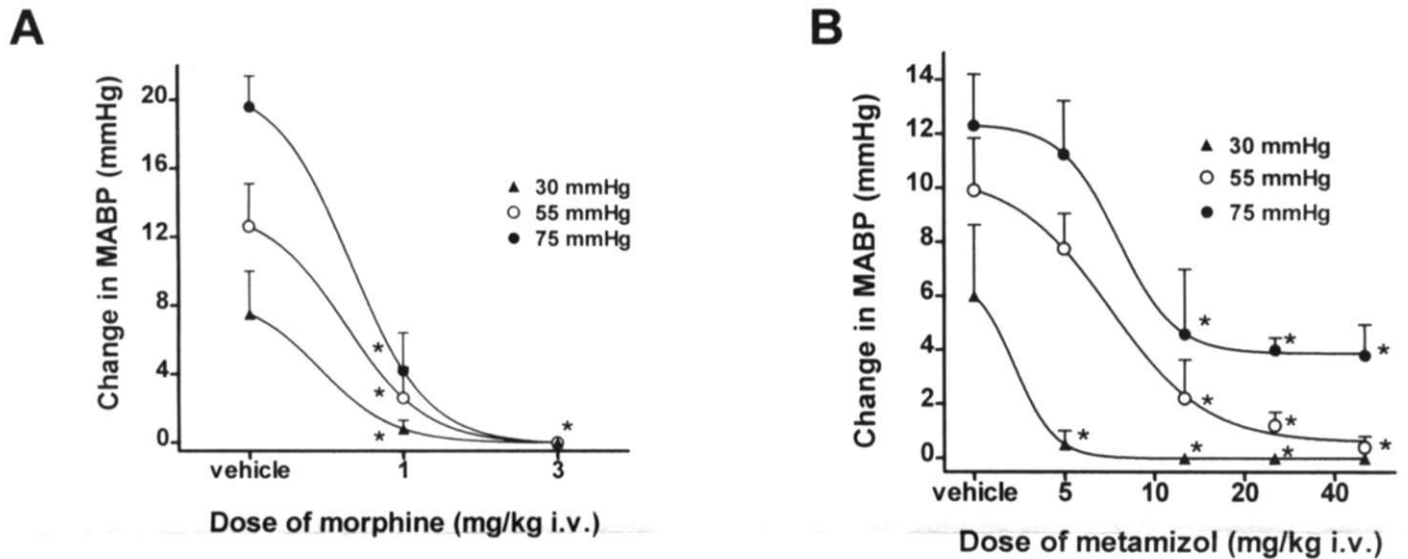


Figure 2. Graph showing the effects of morphine (panel A) and of metamizol (panel B) on the pressor responses evoked by increasing ureter pressure to 30, 55 or 75 mmHg for 30 s, n = 5 for each dose (data taken from Roza & Laird 1995 and Laird & Cervero 1996). Error bars are sem. * denote those doses after which the response was significantly different from control (p < 0.05).

housed and video-taped post-operatively to record their spontaneous behaviour, using a conventional video camera attached to a time-lapse video-recorder which records 1 frame/sec. As originally described, rats with ureteric stones show "crises" of abdominal stretching, licking of the abdomen and contraction of the ipsilateral musculature

indicative of spontaneous visceral pain episodes.

Post-mortem examinations of each rat are performed in which the presence, position and size of the stone is noted. We have found that approximately 20% of rats maintained for 4 days after stone implantation have expelled the stone during that time. In the 80% of rats in which the stone is still

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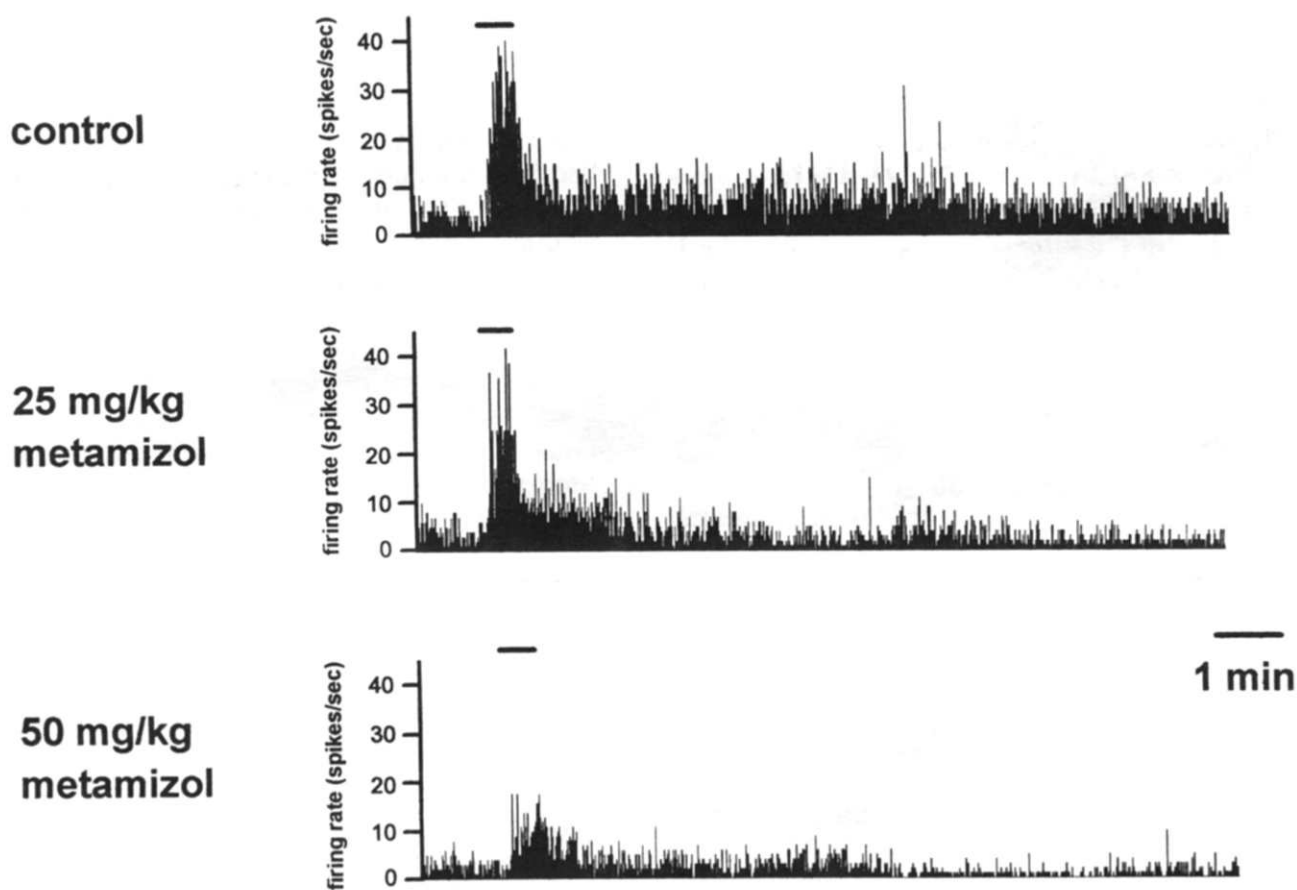


Figure 3. Effect of metamizol on neuronal responses evoked by distension of the ureter. Example of data from an individual class 2 (WDR) neurone located in the deep dorsal horn. The upper trace shows the response to a stimulus of 30 s duration applied to the ureter in control conditions. The centre trace shows the response to the same stimulus after 25 mg/kg metamizol i.v., and the lower trace shows the response to the same stimulus after 50 mg/kg metamizol.

within the lumen of the ureter, one third show signs that the stone has produced occlusion of the ureter (the affected kidney is more than 20% heavier than the contralateral kidney).

Responses of spinal cord dorsal horn neurones to noxious distension of the ureter in rats with ureteric stones

Experiments similar to those performed in normal rats were conducted in rats with implanted stones. Dorsal horn neurones responding to distension of the ureter in stone rats had larger receptive fields and higher background activity than neurones responding to distension of the ureter in normal rats. In rats with stones 44% of neurones with ureteric input were class 1 (low threshold only) however in some cases they revealed nociceptive cutaneous inputs after noxious stimulation of the ureter. The remainder were class 2 (Wide Dynamic Range). In contrast, none of the neurones with ureteric input in control rats were class 1, and none showed changes in input properties after ureter distension. The spinal neurones processing ureteric input

thus appear to be more excitable during and after the passage of a stone (Roza et. al. 1996).

Comments

We have achieved our aim of finding a method of natural noxious stimulation of the ureter, which we have used successfully in normal rats and in rats with experimental kidney stones to evoke both nociceptive cardiovascular reflexes and responses of spinal dorsal horn neurones. We found the technique for implantation of stones in the ureter was fairly simple, but required some practice at first to inject the cement before it hardens too much to pass through the needle, and also to be sure that the tip of the needle was inside the ureter lumen. However, after about 20 rats, our success rate continues to be close to 100%. Both techniques also lend themselves to pharmacological studies of visceral pain.

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