Models of Visceral Nociception

T. J. Ness

Background

Pains arising from the viscera (for example, angina, colic, dyspepsia, and dysmenorrhea) constitute a large portion of clinically treated pains. Unlike pains arising from the surface of the body, which are well localized and evoke localized motor responses such as withdrawal reflexes, visceral pains are characterized by poor localization, strong emotional responses, immobility coupled with tonic increases in muscle tone, and vigorous but nonspecific autonomic responses such as changes in respiration, heart rate, and blood pressure. Stimuli that produce tissue damage or predict potential tissue damage (such as cutting, burning, and pinching) universally produce reports of pain when applied to the skin but unreliably evoke reports of pain when applied to visceral structures. Stimuli that may produce pain from visceral structures (such as distension of the gallbladder) are also stimuli that occur naturally and daily (albeit at lower intensities) but fail to evoke any sensations at all.

This nonspecificity of both stimuli and responses related to visceral sensation has led to difficulty in the development of valid models of visceral pain. The usefulness of any visceral pain model is a function of three features: validity of the model’s visceral stimulus as a “noxious” visceral stimulus, reliability and reproducibility of responses to that stimulus, and specificity of those responses to noxious (as opposed to nonnoxious) intensities of the same stimulus. Because the scope of this article is limited, the reader is referred to additional reviews related to visceral pain (Cervero 1994 1995; Gebhart 1995; McMahon and others 1995; Ness and Gebhart 1990). Unless otherwise cited, general statements will be referenced to those reviews.

Noxious Visceral Stimuli

Stimuli that have been employed in studies of visceral pain can be categorized into four general groups: electrical stimuli, mechanical stimuli, chemical stimuli, and ischemia. Electrical stimuli have utilized implanted electrodes placed on nerves that innervate visceral structures or have used direct visceral contact to evoke responses. This stimulus is easily quantified and easily controlled. Although electrical stimulation produces reports of pain in humans (for example, Arendt-Nielsen and others 1997) and has some utility in pharmacological studies (for example, Hu and others 1994), the lack of specificity restricts its use. Mechanical stimuli have included probing and stretch, but the most commonly employed mechanical stimulus is distension of hollow organs using fluids or foreign bodies (such as balloons). Mechanical stimuli are also easily quantified and controlled, can be isolated, and are related to a natural stimulus. Chemical stimuli have been applied topically, intravascularly, or via “physiological” pathways (such as systemic cyclophosphamide-induced cystitis). Quantification, control, isolation, and modality-specificity of chemical stimuli are very preparation dependent. Ischemia has been produced by occlusion of vasculature, which also produces a mechanical stimulus. The effects of such occlusion are dependent on collateral blood flow and metabolic activity of the selected organ.

By the traditionally employed research definition of Sherrington (1906), a noxious stimulus produces or predicts tissue damage. As noted before, damage to visceral tissues does not invariably lead to pain, and stimuli that are not tissue damaging (or even predictive of tissue damage) can and do lead to visceral pain. For this reason, terms such as “algogenic” have been proposed as more appropriate than noxious for studies of deep tissues (Cervero and Merskey 1996), although this issue is still in debate (for example, Collins 1996; Gebhart 1996.) For purposes of this article, a visceral stimulus must produce particular results to be termed noxious. These results include (1) pain in humans, (2) aversive behaviors in the studied species, and (3) responses that are modified by analgesic manipulations known to reduce visceral pain in humans (such as morphine).

Visceral Nociceptive Responses

Humans use tissue damage-related descriptors (such as rending) when describing their visceral pain. Hence, the term “nociception,” defined as the perception of damaged tissue, is valid in relation to studies of the viscera. A perception of tissue damage is present even when none is occurring. It is presumed that nonhuman animals have similar perceptions. Reflex responses to noxious stimuli have been termed by Sherrington (1906) to be pseudoadfective responses. In decerebrate animals, these responses included growling, grimacing, muscle contractions (visceromotor responses), pupillary dilation, respiratory changes, and alterations in heart rate and...
blood pressure. Pseudoadverse responses have been proven to be reliable but nonspecific in that numerous alterations unrelated to pain can produce similar autonomic and motor reflexes. These responses appear to be profoundly affected by the presence of anesthesia, as is evident in Figure 1, wherein the visceral stimulus (colorectal distension) is the same in all preparations but the responses are opposite in some cases from those in other preparations. This effect of anesthesia on responses to visceral stimuli has significant ethical ramifications since it suggests that all studies should perhaps be performed in unanesthetized animals.

Many of the visceral stimuli described below utilize invasive surgical procedures to place stimulation equipment, and many use stimuli that are neither of short duration nor escapable. Hence, validity of specific preparations as models of visceral pain is of paramount importance if one is to justify the use of such stimuli in the absence of anesthetics or analgesics. Neurophysiological responses to visceral stimuli have proved to be reliable, but due to the invasive surgery necessary to perform neurophysiological experiments, animals must be anesthetized, spinal transected, and/or decerebrated. Other responses include neuronal early-intermediate gene induction (for example, c-fos), but this response lacks specificity since virtually all stimuli (such as hair brushing) produce the response.

Due to the nonspecificity of most measured responses to visceral stimuli, a response to a noxious visceral stimulus as a model of visceral nociception is considered valid if it is (1) reliable (and ideally reproducible), (2) not inhibited by known nonanalgesics (excluding obvious interactions such as the effect of paralytics on motor responses), and (3) inhibited by manipulations known to produce visceral analgesia (the same as result 3 in the previous section). These criteria establish that models of visceral pain must correlate with known human conditions before they are used to assess novel hypotheses related to visceral nociception. Individual models are discussed below.

**Writhing Test**

A “standard” pharmaceutical screening tool since its initial description in the 1950s (Carroll and Lim 1958; Koster and others 1959; Siegmund and others 1957; Vander Wende and Margolin 1956), the writhing test consists of intraperitoneal injection of a chemical irritant followed by subsequent counting of “writhes”—characteristic contraction of abdominal muscles accompanied by a hind limb extensor motion. Described also as the abdominal stretch test, the abdominal constriction test, or the abdominal contortion test, the writhing test has variations that have been described in primates, cats, dogs, and guinea pigs but predominantly in rats and mice. Methodology has varied with the use of endothelin, bradykinin, adenosine 5'-triphosphate, acetylcholine, magnesium sulfate, hypertonic saline, and iodinated radio-contrast agents as intraperitoneal irritants (for example, Gyires and Torma 1984); however, the most commonly employed agents for the writhing test have been phenylquinone and acetic acid. The writhing test is typically carried out in unanesthetized rodents using an intraperitoneal injection of either a fixed dose (0.2 mL/mouse or 0.5 to 2.5 mL/rat) or weight-adjusted dose (for example, 10 mL/kg) of dilute acetic acid (0.6 to 9% V/V) or phenylquinone (0.1 to 0.3%) solutions. Responses have been quantified as all-or-none responses, but more commonly the number of writhes is counted in 5-min intervals for 30 to 60 min. Schmauss and Yaksh (1984) reported improved reliability and reproducibility in their measurement of behavioral responses to intraperitoneal acetic acid when a 0 to 3 scale was employed: 0 = normal body position; 1 = a leaning position favoring one body side; 2 = stretching of hind limbs and dorsiflexion of hind paws frequently with the pelvis rotated sideward; and 3 = contraction of abdominal muscles followed by stretching of the body and extension of the hind limbs (the classic writhing response).

The stimulus of intraperitoneal irritant application has also been used to evoke neuronal responses, to study counterirritation-related phenomena (for example, Calvino and others 1984), and to produce fos protein induction in the central nervous system (for example, Hammond and others 1992). However, the intraabdominal spread of the irritant in anesthetized preparations has been proven to differ signifi-

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**Figure 1** Example of effect of anesthetics/preparation on pseudoadverse responses to visceral stimuli. The presence and vigor of cardiovascular and visceromotor responses to colorectal distension (80 mmHg for 20 sec) in the rat were dependent on the choice of preparation. A visceromotor response (abdominal/hind limb contraction) was noted in those preparations indicated by an asterisk (*). Pressor responses (ΔMAP = change in mean arterial pressure) occurred in some preparations, and depressor responses in others, as indicated. Data adapted from Ness and Gebhart (1988) and Ness (unpublished observations).
cantly from that observed in awake animals (Calvino and others 1984). Hence, interpretation of "parallel" data from behavioral and neurophysiological experiments is difficult.

This model of visceral pain has proven predictive value as a screening tool for analgesic actions (for example, Porreca and others 1987), but methodological and ethical concerns have presented significant constraints to use of the model. Multiple nonanalgesics such as atropine and naloxone have been demonstrated to have profound inhibitory effects in the writhing test (Chernov and others 1967; Hendershot and Forsaith 1959; Taber and others 1969), and so the specificity of the model has been called into question. The reliability of the response has also been problematic in that 28% of animals may demonstrate no evidence of any writhing response (for example, Hendershot and Forsaith 1959). Within-animal reproducibility of responses has not been demonstrated because animals are typically sacrificed at the end of experiments. A significant ethical concern in this model is that the basic paradigm presents an inescapable noxious stimulus that may be of a 30- or 60-min duration. Unanesthetized animals appear distressed by the stimulus, and it is accepted that this stimulus is aversive. The only human study related to the model is that of Lim and others (1967), who used intraperitoneal bradykinin to produce diffuse abdominal discomfort but failed to evoke painful sensations in some subjects. Although widely used, the writhing test fails to meet the proposed criteria of a valid model of visceral pain.

Focal Application of Algesic Agents

Whereas the writhing test administers algesic agents throughout the peritoneal space, numerous researchers have chosen instead to focally apply algesic substances such as bradykinin to exposed surfaces (such as epicardium, Staszewska-Barczak and others 1976; gallbladder, Longhurst 1995) or by injection (such as intraarterial, Holzer-Petsche 1992). In these studies, the attempt has been to determine the chemical substrates needed for activation of the sensory transducers signaling visceral nociception. A notable rat model described by Euchner-Wamser and others (1994) utilized a looped silicone sleeve sutured to the dermis. On the day of testing, the duodenal balloon was inflated with five pulses of 0.5 to 0.7 mL of saline administered over 30 sec followed by a sustained distension for 1 min. Behavioral responses scored on a scale of 0 to 4 were measured as 0 = normal behavior defined as exploration, escape attempts, and resting; 1 = slightly modified behavior defined as cessation of exploration, focusing, wet-dog shake, excessive facial grooming, teeth chattering, and deep breathing; 2 = mildly to moderately modified behavior defined as wretching-like activity, hunching, abdominal grooming or nipping, and immobility of hind limbs; 3 = severely modified behavior defined as stretching of the hind limbs, arching and dorsiflexion of the hind paws; and 4 = intensive visceral motor activity defined as repetitive stretching of the body, extension of the hind limbs and pelvis, and frequent rotation sideways (similar to the writhing response). Although formal testing has not been performed, the actions of the rats indicate that the stimulus is aversive. Duodenal distension in humans produces reports of pain.

Other investigators have used distension of the duodenum (Diop and others 1994; Moss and Sanger 1990), jejunum (Lembeck and Skofitsch 1982), and proximal ileum (Clark and Smith 1985; Clark and others 1988) to evoke decreases in blood pressure in anesthetized rat preparations. Rats were deeply anesthetized with pentobarbital, urethane, and/or α-chloralose; vascular and tracheal cannulae were placed, and the intestines were exposed by laparotomy. Segments of bowel measuring 3 to 10 cm were then isolated by ligation and intraluminally cannulated, leaving neurovascular connections intact. Fluid was used to distend the bowel to intraluminal pressures of 10 to 100 mmHg. Vigorous and reliable depressor responses of 15 to 40 mmHg have been noted, which were inhibited by morphine, codeine, kappa-opioids, 5-HT₃ receptor antagonists, bilateral vagotomy, spinal analgesia, and prostaglandin E₂ in awake rats led to the rapid acquisition of passive avoidance behavior consistent with the stimulus being aversive. It also produced pressor responses and tachycardia. In anesthetized preparations, a similar pericardial administration of such a mixture produced activation of spinal cord neurons, all of which were also excited by esophageal distension. McDermott and others (1995), who used a similar preparation to administer pericardial bradykinin in anesthetized rats in an effort to evoke cardiovascular reflexes, saw no effect of indomethacin. No other analgesic manipulations apart from denervation have been used in this model.

Small Bowel Distension

Colburn and others (1989) characterized writhing responses that could be evoked in unanesthetized rats chronically implanted with a duodenal balloon. These investigators demonstrated adequate reliability of the responses and so were able to examine pharmacological effects of morphine (Colburn and others 1989), nonsteroidal antiinflammatory drugs (NSAIDs) (DeLeo and others 1989), and spinal corticosteroids (Winfree and others 1992). They also evoked fosprotein expression in the spinal cord (DeLeo and others 1991). These investigators used a chronic duodenal catheter that consisted of a 7.5- to 11-cm-long distensible latex rubber balloon placed during deep anesthesia 4 to 5 days before testing via a surgical gastrostomy. The balloon/cannula assembly was placed in the first portion of the duodenum, and upon exiting the stomach, the catheter was tunneled subcutaneously to become externalized at the base of the skull using a silicone sleeve sutured to the dermis. On the day of testing, the duodenal balloon was inflated with five pulses of 0.5 to 0.7 mL of saline administered over 30 sec followed by a sustained distension for 1 min. Behavioral responses scored on a scale of 0 to 4 were measured as 0 = normal behavior defined as exploration, escape attempts, and resting; 1 = slightly modified behavior defined as cessation of exploration, focusing, wet-dog shake, excessive facial grooming, teeth chattering, and deep breathing; 2 = mildly to moderately modified behavior defined as wretching-like activity, hunching, abdominal grooming or nipping, and immobility of hind limbs; 3 = severely modified behavior defined as stretching of the hind limbs, arching and dorsiflexion of the hind paws; and 4 = intensive visceral motor activity defined as repetitive stretching of the body, extension of the hind limbs and pelvis, and frequent rotation sideways (similar to the writhing response). Although formal testing has not been performed, the actions of the rats indicate that the stimulus is aversive. Duodenal distension in humans produces reports of pain.

Abbreviations used in this article: CP, cyclophosphamide; CRD, colonic-rectal distention; NSAID, nonsteroidal antiinflammatory drug.
transection, neonatal capsaicin treatment, and topical local anesthetics. No effect of NSAIDs was noted by Clark and others (1988). Interestingly, McLean and others (1997) observed that previous infection of the intestines with a nematode caused increased sensitivity to intestinal distension as measured by the depressor responses.

These models of visceral pain that use small bowel distension to evoke effective responses fulfill the suggested criteria for valid pain models because they are related to a stimulus that produces pain in humans, appear aversive to rats, and produce reliable responses attenuated by known analgesics. Non-specific effects of nonanalgesics have not been noted. As used, the stimulus is of relatively short duration (1 min) and could be made escapable. Reliable cardiovascular responses were possible in anesthetized preparations.

**Colonic-Rectal Distension**

The stimulus of distension of the distal gastrointestinal tract (cecum, colon, and rectum) has been used extensively to evoke respiratory, cardiovascular, visceromotor, behavioral, and neurophysiological responses in multiple species including horses, dogs, cats, rabbits, and rats. Studies have been performed in many laboratories in the United States, Europe, and Asia with consistent findings. Responses have been demonstrated to be reliable and reproducible in both awake and anesthetized preparations, although responses may have been altered significantly by the choice of anesthetic/anaesthesia (as in Figure 1). The stimulus does produce pain in humans (for example, Ness and others 1990).

In rats, a flexible balloon wrapped around a pliable catheter has been placed into the descending colon and/or rectum transanally with the catheter secured to the tail. In rabbits (for example, Jensen and others 1988) and dogs (Houghton and others 1991; Sawyer and others 1991), similar assemblies and placement have been described. In horses (Kohn and Muir 1988; Muir and Robertson 1985), cecal distension has been produced by passage of a similar rubber distending balloon through a cecal fistula placed 1 mo before the study. In these different species, cardiovascular responses have been measured via vascular cannulae, and visceromotor responses have been observed directly or more objectively by use of an abdominal strain gauge (for example, Briggs and others 1995; Houghton and others 1991) or by measuring electromyographic activity of selected musculature via surgically implanted electrodes (for example, Julia and others 1995). Other behavioral responses have been shown to be species dependent, with horses demonstrating responses such as sweating, kicking, pawing, and head movement and with dogs demonstrating head lift, altered posture, hind-limb extension, or alterations in ventilation.

In rats, cardiovascular responses to phasic, constant-pressure colonic-rectal distention (CRD) (20 to 100 mmHg for 20 sec) have been demonstrated to be reliable and reproducible (Ness and Gebhart 1988). The visceromotor response to CRD has been quantified as an all-or-none response to a phasic constant-pressure or constant-volume CRD, or as the “visceromotor threshold,” defined as the minimal intraluminal distending pressure (or volume) that produces a visceromotor response (abdominal/hind limb contraction). In rats, the visceromotor threshold is determined by slowly filling the CRD balloon until a measurable response is observed. In rabbits, a similar slow filling of the CRD balloon has been utilized, and the intraluminal pressure at which the rabbit performs a sudden withdrawal of the pelvis has been recorded as the “response.” In dogs, a similar threshold for response was determined with numeric scores related to the observer’s certainty that a response occurred. In horses, balloons have been inflated to a preselected pressure for approximately 3 min, with behavior observed and graded.

Responses to CRD have been used for the study of opioids (for example, Briggs and others 1995; Ness and Follett 1998; Traub and others 1995), NSAIDs (for example, Björkman and others 1990), adrenoceptor agonists (for example, Harada and others 1995), serotonin agonists (for example, Banner and others 1995), inflammatory agents (for example, Julia and others 1995), benzodiazepines (Crawford and others 1993), “analogical” surgical manipulations (for example, Al-Chaer and others 1996), counterstimulus effects (for example, Zhuo and Gebhart 1992), and glutamate-related hypersensitivity phenomena (for example, Kolhekar and Gebhart 1994).

To date, effects of nonanalgesics such as atropine have been formally examined, and nonspecific effects have not been identified (Briggs and others 1995; Ness and Gebhart 1988). CRD produces passive avoidance behavior in rats, which is attenuated by the administration of spinal morphine or neonatal capsaicin treatments (Ness and others 1991).

Using modified versions of the rat preparations, investigators have demonstrated a sensitizing effect of inflammation-inducing compounds on CRD-evoked responses. Trinitrobenzene sulfonic acid (80 mg/kg in 0.3 mL of 50% ethanol; for example, Julia and others 1995), turpentine (25% concentration; for example, Ide and others 1997; Ness and others 1991), acetic acid (5% concentration; Burton and Gebhart 1995), and zymosan (25 mg per rat; Coutinho and others 1996) have been administered to the colorectal mucosa by transanal injection. All administrations have led to histological evidence of inflammation, decreased visceromotor thresholds, increased vigor of visceromotor and cardiovascular responses to colorectal distension, and an increase in the rate of acquisition of avoidance behavior.

CRD-related responses have proven utility in multiple species as a model of visceral pain. Because the stimulus produces pain in humans, aversive responses in nonhuman animals, and reliable and reproducible responses inhibited by analgesics (but not by nonanalgesics), it fulfills the stated criteria for a valid model of visceral pain. The stimulus also employs a brief, potentially escapable stimulus. Interpretation of CRD-evoked activity must be tempered by the realization that this model uses an exclusively mechanical stimulus that induces sensations ranging from painless to painful and stimulating an organ with complex motor activity.
Biliary System Distension

Distension of the gallbladder and associated biliary system, which produces pathological pain when the gallbladder is inflated and/or associated ducts are obstructed, has been experimentally reproduced in humans (for example, Zollinger 1933). In cats, ferrets, and primates, the common bile ducts or cystic ducts have been cannulated or ligated, and distension has been used as a stimulus to evoke neuronal responses of primary afferent (sensory) axons, spinal cord neurons, medullary neurons, thalamic neurons, and cortical neurons (for example, Cervero 1982a,b). Vigorous cardiovascular reflexes can also be evoked by biliary system distension (for example, Ammons and Foreman 1984), and these responses have been used by Cervero (1982a,b) to discriminate nociceptive from nonnociceptive neuronal responses. “Escape” behaviors in cats and dogs with gallbladder distension have been noted (for example, Stulrajter and others 1978). At the time of this writing, reliability and reproducibility of the pseudoadaptive responses to gallbladder distension have not been investigated, and no animal studies have examined effects of analgesics on these responses. Although this stimulus is likely related to visceral pain, such additional characterization of responses may be necessary to fully ascertain the utility of this model.

Artificial Kidney Stones

Giamberardino and others (1995) have described a model of artificial ureteral calculus in female rats. After surgical exposure, an artificial stone was placed into the upper third of the ureter by injecting 0.02 mL of dental resin cement (while still liquid) through a fine needle. Rats were allowed to recover from surgical anesthesia and then continuously observed for typically 4 (up to 14) days for “visceral episodes” demonstrating behaviors similar to those observed in the writhing test (such as abdominal contractions and hind limb extensions with a total of six movements scored). The number of visceral episodes (0 to >60 per rat) varied from rat to rat, with each episode lasting from a few to 45 min. The complexity of each visceral episode was graded on a four-point scale. Ipsilateral lumbar muscular hyperalgesia has been demonstrated using vocalization to electrical stimulation of implanted electrodes as an endpoint (Giamberardino and others 1990). Neurophysiological studies have also included reports of altered sensations in the excitability of spinal neurons receiving such ureteric input (Roza and others 1998). This model has a particularly high level of clinical relevance because it correlates well with the human experience of pain due to kidney stones, muscular hyperalgesia has been noted to occur (Vecchiet and others 1989), and the pain itself appears to be episodic in nature.

Similar to the writhing test, the stimulus appears to be aversive to the rats as demonstrated by an extensive characterization of their activities; however, formal testing of “aversion” has not been performed. Daily injections of morphine produced a dose-dependent reduction of the number of visceral episodes in this model. Methodologically, this model of visceral pain is fairly reliable but quantitatively variable from animal to animal, requiring significant skill in the execution of the experiments. This model also entails ethical concern due to its utilization of a noxious visceral stimulus that is inescapable and of long (multiple days) duration. Reliable responses to artificial kidney stones have not been described in anesthetized or reduced preparations.

Urinary Tract Distension

Using a stimulus related to kidney stones, Brasch and Zetler (1982) characterized hemodynamic (depressor) responses to renal pelvis distension in the pentobarbital-anesthetized rat. They demonstrated inhibitory effects of subcutaneous injections of morphine and the peptide caerulein on these depressor responses. Roza and Laird (1995) demonstrated vigorous pressor responses to ureteric distension in pentobarbital-anesthetized rats. In their preparation, the ureter was cannulated near the bladder and could be ligated at the ureteric-pelvic junction. Their pressor responses were reliable, reproducible, and dose-dependently inhibited by morphine. Few studies have utilized the stimulus of urinary bladder distension in models of pain despite the demonstration of vigorous cardiovascular and visceromotor responses in numerous species. Rather, studies have focused on the evocation of micturition reflexes and their modulation by pharmacological manipulations (for example, Dray and Nunn 1985). Neurophysiological studies have examined the substrates of sensation (for example, McMahon and Morrison 1982) related to the urinary bladder, and various studies have investigated the effects of inflammation of the urinary bladder (see below).

Urinary Bladder Irritants

Inflammation of the bladder commonly produces reports of pain and urgency in patients who suffer from a urinary tract infection or receive bladder irritants as part of other medical treatments. Experiments in animals have artificially inflamed the bladder with the intravesical administration of irritants such as turpentine, mustard oil, croton oil, acetic acid, acetone, xylenes, capsaicin and its analogues, and nerve growth factor. Irritation of the bladder leads directly to fos-protein induction in the spinal cord (for example, Birder and DeGroat 1992), alterations in the spontaneous activity of primary afferent neurons (for example, Dmitrieva and McMahon 1996), and spontaneous behavioral responses; however, most studies examining the effects of bladder inflammation have also used bladder filling as an evocative stimulus. Most studies have been performed in rats, although behavioral and neurophysiological studies have been performed in primates (for example, Ghoneim and others 1995) and cats (for example, Habler and others 1990).
McMahon and Abel (1987) performed an extensive characterization of visceromotor and micturition reflexes in chronically decerebrated rats after inflammation of the bladder with 25% turpentine, 2.5% mustard oil, or 2% croton oil administered directly into the bladder via a urethral cannula. Slow filling of the bladder was effected through a urethral cannula, and intravesical pressures were measured, thereby generating a cystometrogram. After administration of the irritant, increased responses to urinary bladder filling correlated with measures of inflammation such as tissue edema, plasma extravasation, and leukocyte infiltration. Rats became hypersensitive to noxious stimuli applied to the lower abdomen, perineum, and tail as measured by the number of “kicks” evoked by a given stimulus.

Use of a chronic decerebrate preparation, as reported by McMahon and Abel (1987), minimizes ethical concerns related to a potentially painful stimulus that may be presented continuously for hours to days but requires a complicated preparation for the experimenter, thereby limiting its use. Subsequently, this model has been modified for use in anesthetized preparations that have examined novel mechanisms related to visceral hyperflexia. Modulatory effects of glutamate-receptor antagonists (Rice and McMahon 1994), nitric oxide synthase inhibitors (Rice 1995), and bradykinin receptor antagonists (Jagger and others 1998) have all been noted, but the effects of traditional analgesics such as morphine have not been investigated. Formal behavioral testing to determine the aversive nature of the stimulus has also not been performed, and none of the substances employed by McMahon and Abel have been tested in humans as bladder irritants.

A second model using bladder irritant administration is that described by Abelli and others (1989). These investigators performed a midline laparotomy 24 hr before testing and inserted a 1-mm-diameter polyethylene cannula through the dome of the bladder. The cannula was held in place by a purse string suture while the opposite end was tunneled subcutaneously to the midline upper back where it was externalized and anchored by a skin button. The next day rats received an injection through the cannula of 0.3 mL of xylene (30% in silicone oil vehicle). Immediate behavioral responses consisted of abdominal/perineal licking, head turns, hind limb hyperextension, head grooming, biting, vocalization, defection, scratching, and salivation. The time to onset, incidence, and number of individual behavioral responses were recorded for 15 min after xylene administration. Morphine and baclofen produced dose-dependent inhibition of these behavioral responses (Abelli and others 1989). Pretreatment of rats as adults or as neonates with systemic capsaicin abolished the licking of the lower abdomen/perineum but did not block hind limb hyperextension. Bladder denervation abolished all behavioral responses (Abelli and others 1988.) Craft and others (1993), who modified the method of Abelli and others by administering 0.3 mL of capsaicin or its related compound resiniferatoxin (0.1 or 3.0 nmol) in place of xylene, observed similar behavioral responses. These investigators went on to demonstrate that pretreatment with intravesical tetracaine blocked behavioral responses evoked by resiniferatoxin (Craft and Porreca 1994) and to show that systemic mu, kappa, and delta opioid receptor agonists all dose-dependently inhibited the same behavioral responses (Craft and others 1995).

These models of urinary bladder pain are methodologically simple, requiring a minimally invasive surgical preparation 1 day before testing. The noxious stimulus is intermediate in length (15 min); however, it is not escapable. Behavioral actions of the rats suggest that the stimulus is aversive in that species, but formal behavior-modifying paradigms have not been employed. Although no parallel literature related to human intravesical xylene application exists, there is an increasing anecdotal literature related to the painful nature of intravesical capsaicin. Numerous analgesic manipulations have been demonstrated to be inhibitory in this model, and no nonspecific effects of nonanalgesics have been noted. These points all argue for the validity of this model for the study of visceral pain.

A third, distinctly different, model of urinary bladder pain mimics clinically noted pain due to cystitis that is secondary to antineoplastic treatments. Lanteri-Minet and others (1995) administered the cancer chemotherapeutic agent cyclophosphamide (CP) (100 mg/kg intraperitoneally), which is metabolized and excreted as urinary acrolein, a potent irritant of the bladder. Beginning approximately 1 hr after systemic administration and continuing for approximately 4 hr, unanesthetized rats demonstrated alterations in normal behavior. Every 20 min, these alterations were scored on a six-point scale for each second of a 300-sec (5-min) period. A cumulative sum for each period was thereby assigned. The scale was defined as follows: 1 = normal behavior; 2 = lacrimation; 3 = piloerection; 4 = rounded-back posture with alertness; 5 = rounded-back posture with immobility; and 6 = transient “crises.” Presumably the highest applicable score was utilized. Hence, 300 sec of normal behavior resulted in a behavioral score of 300; 300 sec of piloerection with no other alterations resulted in a score of 900, and so forth. Measures of bladder inflammation that were obtained correlated with the induction of c-fos and Krox-24 proteins in the spinal cord. No correlation of behavioral responses and degree of inflammation was performed as part of the original study, but a subsequent study that examined effects of hormonal changes (estrous cycle) on behavioral responses related to CP-cystitis revealed the paradoxical result that rats in the estrus stage of their cycle developed the most severe inflammation but failed to develop the severe behavioral alterations shown by all the other rats (Bon and others 1997). No studies assessing the effect of analgesics or nonanalgesics have been performed using a CP-cystitis model. Data related to the reliability of the behavioral responses are not available, although Bon and others (1997) mention a subset of animals that failed to develop the behaviors characteristic of CP-cystitis. The use of a linear six-point grading scale in these studies might be criticized because these responses, although ordinal, are likely not parametric. An evocative stimulus such as bladder irritation...
Reproductive Organs

Numerous electrophysiological studies of primary afferent neurons, spinal neurons, and higher order neurons (for example, Berkley and others 1995a) have characterized the substrates of sensation due to stimulation of female reproductive organs. Such stimulation produces variable reflex responses that are dependent on the particular sites stimulated and the hormonal state of the animal. Vaginal probing with subsequent pressure applied to the uterine cervix can produce analgesic responses (for example, Komisaruk and Whipple 1986) or evoke reproductive behaviors such as lordosis in young, hormonally cycling female animals; however, it can also produce aversive (escape) behaviors if administered at high intensities (Berkley and others 1995b) or if administered to anestrous animals. Strong cardiovascular and motor responses can be elicited from animals with vaginal and/or uterine stimulation, but similar responses have been evoked in humans without producing reports of pain (Martinez-Gomez and others 1988). Berkley and others (1995b), who implanted balloons into the rat uterus and administered distending stimuli sufficient to produce local ischemia, noted that behavioral responses were unreliable (26% of animals did not respond) even at high intensities. When detected, uterine stimuli produced escape behaviors confirming the aversive nature of the stimulus. These same investigators examined vaginal distension as a stimulus for behavior and found reliable detection of the distending stimulus occurring at intensities less than those required to evoke reliable escape behaviors. Due to the variability of responses and mixed nociceptive/antinociceptive effects of female reproductive organ stimulation, which are hormonally dependent, it is difficult to define a valid animal “pain” model in this system. Chemical stimulation of the uterus (for example, Wesselmann and Lai 1997) has also been employed as a stimulus, but specificity as a pain-related stimulus has yet to be proven.

Studies of pain sensation arising from male reproductive organs, specifically pain due to testicular compression, have been limited despite relatively informative psychophysical work in humans (Woodard and Carmichael 1933). Spinal neuron responses have been noted in rats and primates. An extensive characterization of primary afferent fibers from the testicle have been performed by Kumazawa (1986). Cardiovascular and respiratory responses to the activation of testicular afferents have been noted (Mizumura and others 1988); however, apart from neurophysiological studies, little research has evaluated the utility of this stimulus as a model of visceral pain.

Ischemic Stimuli (Coronary Artery Occlusion)

Cessation of blood supply to most viscera leads to pathological pain and is of profound clinical significance due to its associated morbidity and mortality. Coronary artery insufficiency leads to pathological pain (angina) in humans; and interruption of the blood supply to the myocardium has been utilized as a visceral stimulus to evoke primary afferent, spinal, and medullary neuron responses (for example, Foreman and Ohata 1980). Unfortunately, of the visceral stimuli, ischemia produces responses that are the least reliable. This lack of reliability is reflected in the clinical phenomenon known as silent ischemia, wherein electromyographic evidence of severe cardiac ischemia can occur in the absence of reports of pain. Cardiovascular responses to coronary artery occlusion have been demonstrated and appear to consist of alterations (normally increases) in sympathetic tone. Responses have been highly variable even within studies. In the example of one study (Weaver and others 1981), left anterior descending coronary artery occlusion in the anesthetized cat led to decreases in mean arterial pressure of 60 mmHg in one preparation and increases of 85 mmHg in an identical preparation. Neurophysiological experiments have demonstrated similar variability even in repeated measures of the same neurons (Gutterman and others 1998). Studies of behavioral responses in unanesthetized dogs have demonstrated responses to coronary artery occlusion (Sutton and Lueth 1930), but subsequent studies using the same preparation suggested that the noxious stimulus in those studies was mechanical distortion of perivascular structures rather than ischemia (Katz and others 1935; Martin and Gordham 1938). Longhurst and colleagues have performed extensive studies of abdominal visceral afferent fibers excited by ischemia and have characterized the cardiovascular responses to the same stimuli (for example, Longhurst 1995; Rendig and others 1997). Factors related to the activation of primary afferent fibers and the evocation of cardiovascular reflexes include hypoxia, tissue acidosis, oxygen-free radicals, prostanoids, leukotrienes, kinins, and numerous other metabolic products. Recent studies have presented evidence that ischemia-related activation of primary afferent fibers is not secondary to mechanical changes (Pan and others 1997).

The variability of responses to ischemic visceral stimuli has been proposed to be related to intrinsic neural circuitry within visceral structures and to interactions of vagal, brainstem, and spinal sensory components. The delineation of the mechanisms of this variability may serve to define the overall variability of responses to all visceral stimuli. Given the profound clinical importance of ischemia-related pain, there is a need to further develop models of visceral ischemia with precise definition of its neurophysiological substrates and evoked responses and identification of the mechanisms of response variability.
Miscellaneous

One model of visceral inflammation that deserves brief mention is that of Miamplam and others (1994, 1996), who injected formalin into the colonic wall of briefly anesthetized rats and then observed the rats over 3 hr for four behaviors: (1) abdominal licking and nibbling, (2) body stretching, (3) contraction of the flanks, and (4) whole body contractions. These responses were inhibited by intraperitoneal morphine and oral clonidine. There is no direct human correlate to this model that uses a prolonged stimulus that is inescapable. Another model of visceral pain that has not been extensively investigated but may have significant clinical relevance is a model of pancreatitis used by Houghton and others (1997) to examine the effects of partial cordotomy. Validity of the model is yet to be determined, but it represents another model that utilizes a prolonged, inescapable stimulus to mimic what is considered to be one of the most severe human pains.

Summary

There are many viscera and many models that have been put forward in an attempt to describe visceral pain of different origins. Clinical visceral pains are often prolonged and "relatively" inescapable, as are some of the models examined here. This article has examined models that are in use at the time of this writing, with a focus on their validity as defined by proposed criteria. It is hoped that this focus will assist readers with decisions related to the value of the knowledge to be gained versus the cost of that knowledge and its ethical ramifications.

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References

Crawford ME, Jensen FM, Tofdahl DB, Madsen JB. 1993. Direct spinal


