© *Basic & Clinical Pharmacology & Toxicology* 2004, **95**, 97–111.
Printed in Denmark . All rights reserved

Basic & Clinical
Pharmacology & Toxicology
Copyright ©
ISSN 1742-7835

MiniReview

Experimental Human Pain Models: A Review of Standardised Methods for Preclinical Testing of Analgesics

Camilla Staahl[1,2] and Asbjørn Mohr Drewes[1,2]

[1]Centre for Visceral Biomechanics and Pain, Department of Medical Gastroenterology, University Hospital Aalborg, and [2]Laboratory for Experimental Pain Research. Centre for Sensory-Motor Interactions, Department of Health Science and Technology, Aalborg University, Denmark

(Received August 26, 2003; Accepted May 20, 2004)

Abstract: Treatment of pain is one of the major challenges in clinical medicine. However, it is often difficult to evaluate the effect of a treatment, as the many symptoms of the underlying diseases often confound this assessment. Furthermore, as the pain mechanisms in many diseases are poorly understood, the limited successful trial and error approach is most often used in the selection of analgesics. Hence, there is a need for new methods in the characterization and treatment of pain. Human experimental pain models offer the possibility to explore the pain system under controlled settings. The models can also be used to screen the analgesic profiles of drugs targeted to treat pain. This review gives a brief introduction to the methods used to evoke and assess pain in the skin, muscle and viscera. New methods using multimodal stimulation and activation of central pain mechanisms can to a higher degree mimic the clinical situation, and such methods are recommended in the future screening of analgesics. Examples of the use of experimental pain models in the testing of analgesics are given. With these models the therapeutic spectrum may be defined from a differentiated knowledge on the effect of drugs on the pain system. Such information may be used in the future guidelines for trials and clinical use of analgesics.

Pain is probably the most prevalent symptom in clinical practice, and characterisation of pain is of major importance in the diagnosis and choice of treatment (Thumshirn *et al.* 1999). In the treatment of diseases associated with pain, the clinical effects typically guide the selection of the analgesics and titration of the dose. However, in practice, the different symptoms of the underlying diseases confound the characterisation of pain. These confounders may include complaints relating to psychological, cognitive and social aspects of the illness, as well as systemic reactions such as fever and general malaise (Drewes *et al.* 2003). Furthermore, treatment with analgesics often causes sedation and other side effects. This may bias the clinical evaluation, as the patients tend to interpret other effects of the medication – such as an effect on the anxiety and depression relating to the disease – as a relief of pain (Le Bars *et al.* 2001). Because of these confounding factors, *experimental pain models* are often advantageous in preclinical investigations of analgesics. With these models, the investigator can control the experimentally induced pain (including the nature, localisation, intensity, frequency and duration of the stimulus), and provide quantitative measures of the psychophysical, behavioural or the neurophysiological responses (Graven-Nielsen *et al.* 2001; Drewes *et al.* 2003) (fig. 1).

Experimental pain models have been used in *animal studies.* In these experiments, the neuronal nociceptive activity can be recorded or behaviour can be assessed (Sengupta & Gebhart 1994). However neuronal recordings or reactions do not reveal all aspects of pain, since pain is the net effect of complex multidimensional mechanisms that involve most parts of the central nervous system (Le Bars *et al.* 2001). Nociceptive reflexes or electrophysiological recordings from selected pathways in the animal nervous system are important in basic research and screening of analgesics. However, animal experiments typically suppress central pain mechanisms and associated complex reactions seen in man. Furthermore, the neurobiology of nociceptive systems differs between species, and this limits the extrapolation of findings from animal studies to man even further (Le Bars *et al.* 2001). For review of animal studies see (Ness & Gebhart 1990; Le Bars *et al.* 2001).

These limitations stress the need for experimental human pain models in preclinical studies of new analgesics and anaesthetic procedures. Methods related to experimental pain research in man aim at activating different nociceptors, evoking pain from different tissues and activating specific pathways and mechanisms. There are still major problems in exact determination of the activated pathways and pain mechanisms (Woolf & Max 2001), but the experimental hu-

Author for correspondence: Camilla Staahl, Centre for Visceral Biomechanics and Pain, Department of Medical Gastroenterology, Aalborg University Hospital, DK-9000 Aalborg, Denmark (fax +45 99322483, e-mail cst@smi.auc.dk).

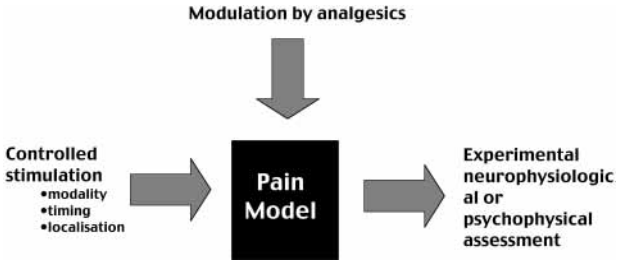

Fig. 1. A schematic view of an experimental pain model. The figure illustrates the context in experimental pain, between the stimulation (input), the analgesic modulation and the pain response (output).

man models provide the possibility to obtain reproducible results in test-retest experiments and hence be useful for drug screening (Handwerker & Kobal 1993). Human experimental models have been refined, and robust models for superficial and deep activation of the nociceptive nervous system now exist. Assessment of the output from these pain models can be based on psychophysical or neurophysiological methods (Gracely 1999). Psychophysical methods are based on the subjective experience of pain, measured on standard scales or as pain thresholds. Examples of neurophysiological methods are measurement of nociceptive withdrawal reflexes or evoked brain potentials.

This review will give a brief introduction to methods for evoking and assessing experimental pain in man. Shortcomings for each pain model are discussed, but some models have not been used extensively and this makes a meaningful discussion difficult. It should be emphasized that this MiniReview is not comprehensive and more in-depth literature is recommended for the reader who is especially interested in the area.

Brief pain taxonomy

Pain is the net effect of peripheral activation and sensitisation of afferent nerves, followed by complex multidimensional mechanisms that involve most parts of the central nervous system (Gracely 1999). Modifications of the central nervous system follow chronic pain, and may result in sensitisation of the nociceptive system (Woolf & Max 2001). Several terminologies are used to describe these processes, and in the following, the terms used in this paper are defined:

Allodynia is a painful response to stimuli that are normally not painful.

Hyperalgesia is an enhanced painful response to stimuli that are normally painful.

Primary hyperalgesia is hyperalgesia in the area of the tissue injury mainly caused by sensitisation of peripheral nerves.

Secondary hyperalgesia is hyperalgesia at tissues outside the area of the original injury. The mechanisms of secondary hyperalgesia are far from clear, but it is mainly believed to be the result of changes in the central processing of sensory input from afferent nerves that normally transmit non-painful sensations.

Temporal summation/central integration. If the stimulus is repeated with a low frequency, the pain remains the same. If the stimulus is repeated at a faster rate (inter-stimulus-interval less than three sec.), the pain increases during the stimulations due to a central amplification of the response. An example is shown in fig. 2.

Spatial summation. Increasing the stimulation area increases the pain intensity via integration of neuronal activity in the central nervous system (CNS). An example is shown in fig. 2.

Referred pain. Pain felt at a site remote from the site of stimulation. This is a phenomenon related to central processing of afferent information typically from nerves innervating deep and superficial tissues respectively.

First pain. The acute, sharp and pricking pain felt immediately after the pain stimulus.

Second pain. The more dull pain felt some time after the first pain.

By evoking different central phenomena like allodynia, hyperalgesia, referred pain or temporal summation in the experimental situation, central pain mechanisms can be studied in humans (Arendt-Nielsen *et al.* 2000). This is of major importance since abnormal central processing of pain characterise many disorders associated with pain (Woolf & Max 2001). The central phenomena can be evoked by stimulation of all tissues, but have most thoroughly been investigated in the skin, where e.g., repeated electrical or thermal stimulation can induce temporal summation mechanisms (Curatolo *et al.* 2000b). However, in the muscles and viscera, central modifications manifested by hyperalgesia/allodynia, can also be induced by application of e.g. strong or long-lasting noxious stimuli (Sarkar *et al.* 2003).

Experimental pain evoked from the skin

Experimental pain models in the skin are highly developed, mainly because of the easy access to the skin. Mechanical, thermal, electrical, and chemical methods make up evaluated methods. For comprehensive review, see Curatolo *et al.* (2000b) and Handwerker & Kobal (1993).

Important experiments related to pathophysiological changes in the pain system are induction of hyperalgesia and allodynia by application or intradermal injection of chemical substances and application of heat or cold (Dirks *et al.* 2002; Hughes *et al.* 2002; Koppert *et al.* 2003). Such procedures may be helpful in the evaluation of various drug effects on peripheral and central mechanisms. Temporal and spatial summation evoked experimentally in the skin also reflects a central modulation of the response, and a number of drugs can block these phenomena (Dirks *et al.* 2002; Hughes *et al.* 2002; Koppert *et al.* 2003).

Mechanical stimulation.

Touch. Applying a light pressure with a finger or using a von Frey hair (a calibrated filament), quantitatively assesses the response to touch. When stimulation with a von Frey hair reaches a certain pressure, the calibrated filaments

bend. Thereby a slight, but exact and reproducible, pressure is applied. Aβ-fibres probably mediate touch sensation evoked by von Frey hair (Le Bars *et al.* 2001).

Major shortcomings. The von Frey hair activates both low threshold mechanoreceptors and nociceptors and is not specific (LeBars *et al*. 2001). Furthermore, touch is not a mean of evoking pain; it is used mainly to explore allodynia evoked by other pain stimuli (Curatolo *et al.* 2000b).

Pinprick. Stimulation of the skin with a needle or a safety pin evokes pinprick stimulation. Pinprick stimulation is believed to activate predominantly Aδ-fibres (Le Bars *et al.* 2001) and is reported as pricking or "first pain" (Curatolo *et al.* 2000b).

Major shortcomings. Conventional techniques do not allow noxious stimuli to be delivered rapidly and briefly enough to produce synchronous excitation of the nerve fibres (Le Bars *et al.* 2001). Furthermore, when mechanical stimuli are truly nociceptive, they are likely to produce changes in the tissue (sensitisation or actual lesions).

Pressure. Pressure algometers apply standardised pressure stimulation. The algometer probe and a pinch handle can be used to test body structures like a toe, a finger or an ear lobe. Both Aδ and C-fibres are believed to mediate pain induced by pressure stimulation (Handwerker & Kobal 1993).

Major shortcomings. The same shortcomings as seen with pinprick are encountered when applying pressure stimuli. In addition contact with the skin also evokes low-threshold mechanoreceptors as well as nociceptors. Consequently, the stimulus is not specific (Handwerker & Kobal 1993; Le Bars *et al.* 2001).

Thermal stimulation.

Cold. Application of ice, a cold gel bag, a wet alcohol sponge, menthol, ether or a Peltier thermode to the skin evokes cold sensation. A Peltier thermode is a device capable of heating or cooling the skin. It consists of semiconductor junctions, which produce a temperature gradient between the upper and lower stimulator surfaces produced by the passage of an electric current, thereby producing a cooling or heating effect. It is assumed that Aδ-fibres mediate cold sensations, and most likely C-fibres mediate cold pain in humans (Fowler *et al.* 1998).

Stimulation with ice water is performed by immersion of a hand or foot into ice-saturated water (0–2°) for one or two min., or as long as the subject endures the pain (Curatolo *et al.* 2000b). Nociceptors of cutaneous veins probably evoke the pain, which is termed cold pressor pain. The

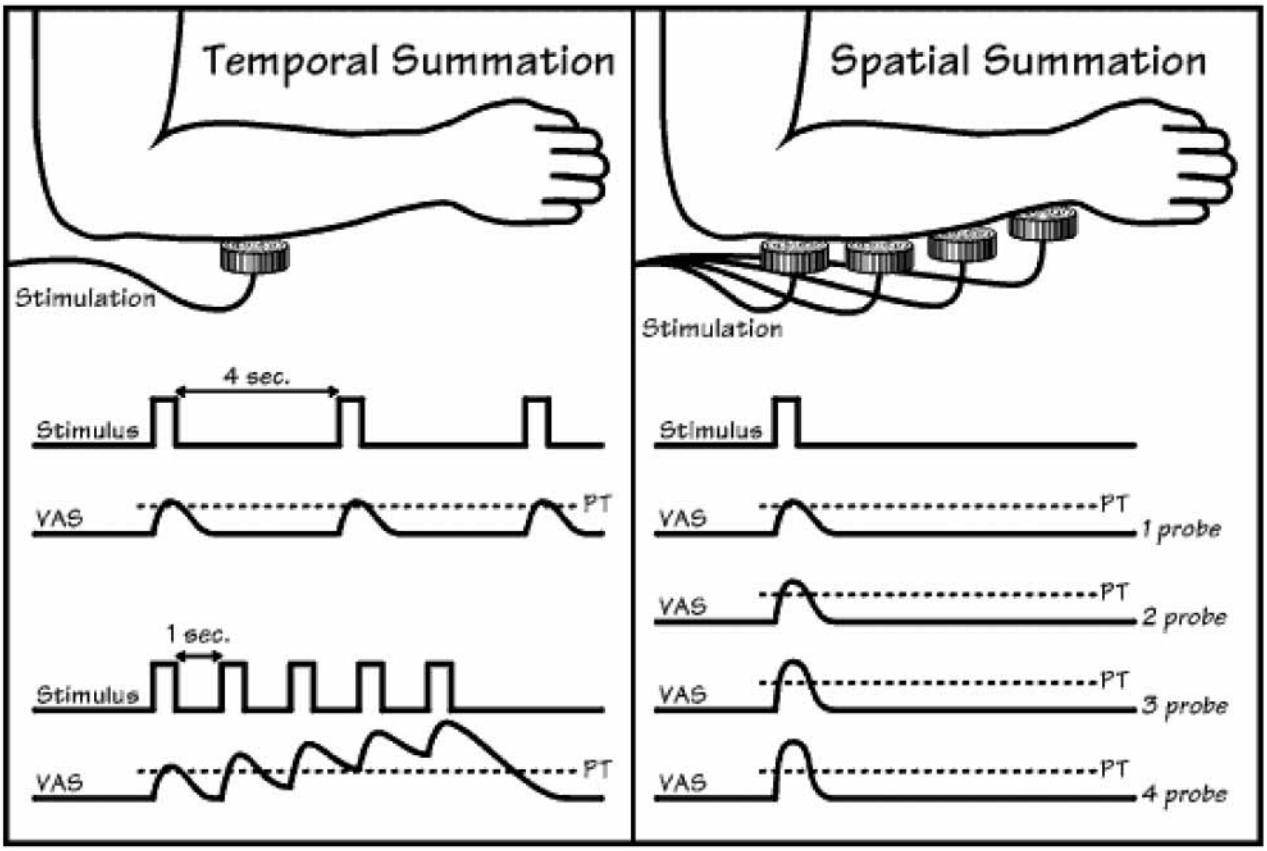

Fig. 2. An illustration of temporal and spatial summation. *Temporal summation:* A stimulus at a fixed intensity repeated with a low frequency (every 4 sec.) gives an unchanged response. The same stimulus repeated with a high frequency (every sec.) gives an increased response. *Spatial summation:* Applying a stimulus at a fixed intensity to a larger number of stimulation sites increases the response.

Fig. 3. A Xenon light stimulator, used for radiant heat stimulation in the skin.

stimulation probably activates both Aδ- and C-fibres (Curatolo *et al.* 2000b).

Major shortcomings. Vascular reactions strongly affect the response and the cold pressor pain has shown contradictionary results in the testing of analgesics (Handwerker & Kobal 1993). Opioids show good effects in this model, whereas non-steroidal anti-inflammatory drugs show variable results. Different explanations for the contradictionary results have been explained by gender differences and different pain scaling (Compton *et al.* 2003). These influencing factors illustrate that great care should be taken when experimental set-ups are designed to reach a design that actually resolves the question of interest.

Freeze lesion. Induction of a freeze lesion provides a model of hyperalgesia, which predominantly is caused by peripheral phenomena. The lesion can be induced by application of cold temperatures (−28°) at a standardised pressure in a certain time interval. The induced hyperalgesia provides stable testing conditions for 1 day (Lötsch & Angst 2003). The testing of oral and topical applied diclofenac shows that a central component most likely also contributes to the hyperalgesia, induced by this model (Burian *et al.* 2003).

Major shortcomings. The testing of oral and topical diclofenac in the freeze lesion shows the complexity of analysing experimental pain. A central component of the analgesia can be explored, even if the method is thought to evoke mainly peripheral mechanisms (Burian *et al.* 2003).

Contact heat. The Peltier thermode or heat foil are used for warmth and heat stimulation. At threshold determinations, rapid skin heating activates first Aδ-fibre, where the evoked sensation corresponds to the "first pain felt within 0.4 sec. after the heat stimulus. The first pain is followed by a C-fibre-mediated second pain, which is less well localized and of longer duration, being described as 'throbbing, burning or swelling' (Hughes *et al.* 2002). Slow heating gives a preferential activation of the C-fibres and the best evaluation of second pain (Handwerker & Kobal 1993). The first and second pain phenomena are also seen in experiments involving rodents validating the human experiments (Le Bars *et al.* 2001).

Major shortcomings. When activating nociceptors, concomitantly low-threshold non-nociceptive nerves can be activated by the contact of the thermode with the skin. This activation can exert an inhibitory influence on pain mechanisms. The rate of thermal transfer depends on the thermode-skin contact and thus on the pressure of application of the thermode. Therefore it is important that the thermode is applied to skin at a standardised fashion. For comprehensive review see (Le Bars *et al.* 2001). Experiments with placebo analgesics have used heat as stimulation, showing a rather big placebo effect at this type of stimuli (Price *et al.* 1999). This probably applies to many types of experimental pain stimuli.

Radiant heat. Laser pulses evoke a distinct pricking pain in the skin. Intensities higher than those evoking pricking pain are avoided, as they may cause superficial burns. Depending on the stimulus intensity, Aδ and C-fibres are believed to mediate pain evoked by laser stimulation (Bromm & Treede 1991). Radiant heat has the advantage that the stimulus can be applied without contact to the skin bypassing the shortcomings for contact heat. The CO_2-laser has the advantage that the radiation is absorbed within the epidermis, independent of skin pigmentation. Reflection thereby becomes irrelevant and the stimulus intensity becomes less variable, even when the stimulus is not applied exactly vertical to the skin. Another intense radiant heat source, the Argon-laser does not share these biophysical properties. Much of the radiant energy is reflected, casing variation, depending on the skin pigmentation and the application angle (Bromm & Treede 1991).

Major shortcomings. A response is evoked, when a certain temperature is reached in the skin. This varies according to numerous factors, such as reflectance, transmission and absorption of the epidermis. This can give variability between individuals (Bromm & Treede 1991; Le Bars *et al.* 2001).

Burn injury. The burn injury model illustrates hyperalgesia. Contact heat and radiant heat can induce burn injury by e.g., application of a constant temperature of 47° for 7 min. This does not evoke spontaneous pain after termination of the stimulus, but produces a burn injury that induces primary and secondary hyperalgesia and thus central responses. Increased sensitivity of A- and C-fibres is responsible for primary hyperalgesia after a burn injury (Curatolo *et al*. 2000b).

Major shortcomings. Animal experiments, activating mainly Aδ-fibres, have shown that C-fibres are more susceptible to sensitization phenomena. Inflammation may also recruit "silent" C-fibres. Thus a threshold for activation of mainly Aδ-fibres may transform into thresholds for activation of C-fibres. As these respond to other stimulus mod-

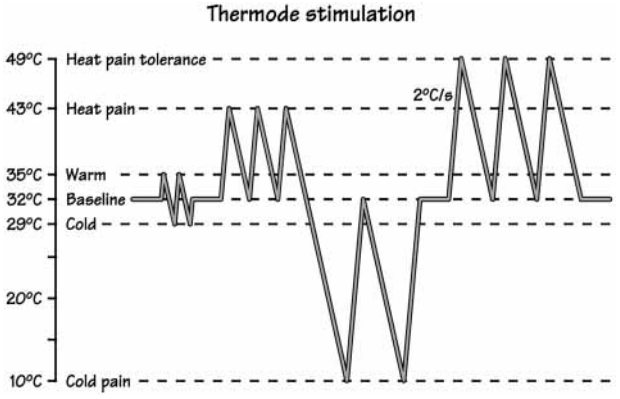

Fig. 4. A schematic illustration of the different pain measures obtainable from thermal stimulation. Typically, the measures are calculated as the average of two or three measurements.

alities it may confuse the testing of an analgesic (Le Bars *et al.* 2001).

Electrical stimulation.

Various electrical stimulator devices connected to electrodes applied to the skin surface evoke electrical stimulation. Stimulator devices can deliver different stimulation patterns, e.g., different waveforms, frequencies, and duration of the stimulus. This activates with some selectivity different afferents and nervous structures, and hence evokes different kinds of pain (Handwerker & Kobal 1993; Le Bars *et al.* 2001). The temporal control of the stimulation eliminates the latency to stimulation of the afferents seen with other methods (Le Bars *et al*. 2001). Summated stimuli can activate central mechanisms (Koppert *et al.* 2003), and the method is very suitable for neurophysiological assessments of the pain (Chen 1993).

Major shortcomings. Electrical stimulation bypasses the receptors and activates the nerve fibres directly, and the method is not a specific activation of the nociceptors. The electrical threshold is related to the fibre diameter and one cannot usually excite small-diameter nerves without additionally exciting others. However, the distance from the surface of the skin and size of terminals are probably more important than axon diameter (Handwerker & Kobal 1993). Differences in impedance between different sites of the body may also influence the results (Le Bars *et al*. 2001). Hence, this model conditions a pain response quite different than the clinical situation, and results from the testing of analgesics should be interpreted with cautiousness (Handwerker & Kobal 1993).

Chemical stimulation.

Capsaicin. Intradermal injection or topical application of capsaicin evokes pain in the skin. The application induces primary and secondary hyperalgesia. Hence, intradermal injection of 100 μg capsaicin evokes a short-lasting burning pain at the site of injection followed by development of secondary hyperalgesia. The same effects can be seen after capsaicin 1% moisturizing cream, applied topically for 30–60 min. Brush and pinprick stimulation of the skin surrounding the injury determines the area of secondary hyperalgesia. Mostly C-fibres are thought to mediate pain induced by capsaicin (Wallace *et al.* 2002).

Major shortcomings. Generally it applies that models producing neurogenic inflammation will activate a larger proportion of C-fibres than Aδ-fibres. This can give some confusion as mentioned under "burn injury" (Le Bars *et al.* 2001). Although capsaicin is believed to mimic pathological changes such as allodynia and hyperalgesia seen in e.g. neuropathic pain, pharmacological testing of lamotrigine and desipramine which are used in the treatment of neuropathic pain failed to show any effects in the model (Wallace *et al.* 2002; Petersen *et al.* 2003). On the other hand gabapentin, which is also used to treat neuropathic pain, suppresses hyperalgesia following heat-capsaicin sensitisation (Dirks *et al.* 2002).

Mustard oil. applied to the skin will induce inflammation and hyperalgesia/allodynia. A compress soaked with mustard oil applied to the skin for 4 min. will evoke burning pain followed by an inflammatory reaction at the site of application, and secondary hyperalgesia. In these models C-fibres are also thought to mediate the burning pain, while Aβ-fibres are believed to mediate allodynia to light mechanical stimuli (Curatolo *et al*. 2000b).

Major shortcomings. The method has not been used much in the testing of analgesics. Precautions as mentioned under the capsaicin model should be taken, when using this or any other model with mimics central phenomena.

Examples of experimental skin pain in the testing of analgesics.

Cutaneous pain models have been used in the testing of a variety of drugs, and have contributed to new insights into the effect of analgesics on differentiated pain mechanisms. This knowledge can be used in the design of subsequent clinical studies. In the treatment of patients, where the pain mechanisms (e.g., peripheral, central) have been unravelled, the data may be used in assessment of the dose-response profile of analgesics etc. Examples of drugs tested with experimental methods include *local anaesthetics, opioids, ketamine, clonidine, neostigmine, desipramine, gabapentin and bupivacaine* (Handwerker & Kobal 1993; Curatolo *et al.* 2000b; Dirks *et al.* 2002; Hughes *et al.* 2002; Lötsch & Angst 2002; Koppert *et al.* 2003; Petersen *et al.* 2003; Burian *et al.* 2003). Local anaesthetics are examples of analgesics, which have been tested in a variety of cutaneous pain models. Multimodal testing procedures have revealed that local anaesthetics differ in their ability to inhibit stimuli of different nature (Curatolo *et al.* 2000b). Because local anaesthetics act in the periphery on nerve conduction, several types of phasic stimulation easily detect their effects. Sensations attenuated by local anaesthetics include touch, pinprick, cold, warmth, pressure pain, and pain induced by laser (Curatolo *et al.* 2000b) (table 1). It should be noted

that some local anaesthetics such as Emla does not significantly affect tactile sensations or itch. A differential effect on the afferents can be obtained by nerve blocks this allows a more differential testing of various fibre types. Most methods block the Aδ-fibres, leaving the C-fibres susceptible for stimulation. Specific block of one fibertype is however not possible and the results must be carefully interpreted (Handwerker & Kobal 1993).

Centrally acting agents also have effect in the cutaneous testing with appropriate models. This is seen with epidural administration of the α-agonist *clonidine*, which modulates the perception of pain evoked by application of capsaicin, pressure pain and electrical pain (Curatolo *et al*. 2000b). Central pain phenomena have been modulated by different analgesics like ketamine and clonidine. These drugs also reduce the hyperalgesia seen after infusion of remifentanil in an electrical model of secondary hyperalgesia (Koppert *et al.* 2003). Ketamine also shows effect in a model of wind-up of second pain induced by heat consistent with its effect on sensitized central pathways (Hughes *et al.* 2002). Diclofenac also shows effects on centrally evoked phenomena, like hyperalgesia induced by freeze lesions (Burian *et al.* 2003).

Different stimulation modalities, such as pain induced by pressure, electrical stimulation, or heat detect the effects of epidural *opioids.* However, the ability of these tests to detect the analgesic effect of drugs may vary with the stimulation pattern applied or the type of response recorded. Both animal and human experiments show that opioids preferentially attenuate nociceptive responses produced by central integration (spinally amplified signals) of tonic C-fibre activation (Gracely 1999; Le Bars *et al.* 2001). Therefore, evaluation of the antinociceptive effect of opioids may be clearer using slow rates of temperature increase in skin or in studies of second pain (Handwerker & Kobal 1993). In the investigation of epidural morphine, modulation of the pain tolerance threshold is also more sensitive than the pain detection thresholds, probably caused by activation of a larger proportion of C-fibres at the supra-threshold level (Curatolo *et al.* 2002b). The opioid remifentanil have shown effect on centrally evoked phenomena, like hyperalgesia induced by freeze lesions (Lötsch & Angst 2003).

Table 1.

Examples of different analgesics, which have been used to modify the pain evoked by different experimental pain models of the skin. The table is not intended as a complete list, but to give examples of different drugs, which attenuate experimental skin pain.

Modality	Stimulus	Analgesic substance
Mechanical	Touch	LA
	Pinprick	LA, clonidine, epinephrine, remifentanil
	Pressure	LA, opioids, clonidine
Thermal	Cold	LA, clonidine
	Ice water	Neostigmine
	Freeze lesion	Diclofenac, remifentanil
	Warm	LA, opioids
	Heat	LA, opioids, lamotrigine, ketamine, dextromorphan
	Laser	LA, opioids
	Burn-injury	Opioids, gabapentin
Electrical	Single stimuli	LA, opioids, clonidine
	Repeated stimuli	Ketamine, remifentanil
Chemical	Capsaicin	Clonidine, gabapentin, lamotrigine
	Mustard oil	Adenosine

LA: Local anaesthetics.

Experimental pain evoked from the muscles

Muscle pain is a cramp-like, diffuse and aching pain. Referred pain in distant somatic structures and trophic changes in the superficial and deep structures are often associated with muscle pain (Mense *et al.* 1997). Usually, the models are divided into methods without (endogenous) and with (exogenous) external stimuli (Graven-Nielsen *et al*. 2001) (table 2). Recent studies have shown that muscular hyperalgesia can be induced experimentally by infusing various algogenic substances into the muscles. This sensitisation mimics the inflammation or lowered pain threshold seen in organic and functional disorders in the musculoskeletal system. This makes the models more relevant from a clinical perspective (Mørk *et al.* 2003). In the following the models to evoke muscle pain are shortly described. For comprehensive review see Graven-Nielsen (2001).

Endogenous methods

The endogenous methods are suited for studying general muscle pain states. They are characterised by a high response rate. However, they have the disadvantage of involving several or all muscle groups within the region investigated and therefore they are difficult to control.

Ischemic stimulation.

The tourniquet model is a classical experimental pain model that induces ischaemic muscle pain. A pneumatic tourniquet is inflated around the thigh after exsanguination of the leg by gravity. The tourniquet is left inflated as long as the subject tolerates the pain, for a maximum of 2 hr. Both pressure at the site of inflation and limb ischaemia are responsible for tourniquet pain.

Since the method is found reliable, it has been used in human analgesic assays. The model is applicable in experimental studies requiring a general tonic pain stimulus (Graven-Nielsen *et al*. 2001).

Major shortcomings. It is a very efficient model to induce pain in the muscles but is non-specific, since skin, periosteum, and other tissues will contribute to the overall pain perception. When activating nociceptors, concomitantly low-threshold non-nociceptive nerves can be activated by the contact of the tourniquet with the skin. This activation can exert an inhibitory influence on pain mechanisms as mentioned under "contact heat".

Pain evoked by exercise.

Various forms of heavy and unaccustomed exercise can evoke exercise-induced pain in specific muscles. Together with overloading and insufficient resting periods, concentric dynamic and isometric contractions will elicit muscle pain, which may share the same pathogenetic mechanisms as ischaemic pain. Eccentric contractions induce a delayed onset (24–48 hr) of muscle pain or soreness. Ultrastructural damage resulting in the release of algesic substances underlies post exercise muscle pain (Graven-Nielsen *et al*. 2001).

Shortcomings. The model may produce an inflammatory reaction, as non-steroidal anti-inflammatory drugs (NSAID's) appear to have an effect on jaw muscle soreness (Svensson *et al.* 1997). However, the outcome of the model seems to depend on the stimulated tissues as Howell *et al.* (1998) was unable to demonstrate an NSAID effects on delayed soreness, caused by eccentric contractions in limb muscles. Hence different results have been obtained when testing in limb and jaw muscles. Animal studies show that a stress-induced analgesia can occur with eccentric exercise (Kehl & Fairbanks 2003). This could also bias the results in analgesic testing.

Exogenous methods

Mechanical stimulation is a typical exogenous experimental pain model. Pressure algometry is the most frequently applied technique for quantification of pain. The method is an experimental parallel to palpation in the clinical practice (Graven-Nielsen *et al.* 2001). The pain threshold and tolerance thresholds are easily measured, but stimulus response functions give additional information on muscle hyperalgesia (and analgesic profiles). The rate of pressure increase and absolute values are monitored when hand-held algometers are used. Standardisation of the technique has been attempted, and normal values for various muscles are published (see Fischer 1998 for review). Several studies have used pressure algometry in evaluating drug efficacy (table 2). Recently, a new cuff algometry technique was developed. Stimulus-response recording of pain response to increasing pressure in a tourniquet (cuff) placed around a limb is recorded. This technique is fully automatised, which increase the reliability and sensitivity (Polianskis *et al.* 2002).

Major shortcomings. The technique is non-specific since receptors in the skin, and probably deeper tissues will be activated. When activating nociceptors, concomitantly low-threshold non-nociceptive nerves can be activated by the contact of the algometer with the skin. This activation can exert an inhibitory influence on pain mechanisms, as mentioned under "contact heat".

Electrical stimulation.

An electrical stimulation result in pain that is only present during the stimulation and the method is adequate for studies requiring muscle pain and referred pain induced in a phasic manner. This provides the possibility to follow a given intervention over time; e.g. analgesic efficacy. Repeated electrical stimulation can induce temporal summation and cause increase in referred pain areas, thus reflecting central changes (Schulte *et al.* 2003).

Major shortcomings. As mentioned under the cutaneous section, electrical stimulation has the disadvantage that it is not nociceptive-specific as it bypasses the receptors. Furthermore, concurrent activated muscle twitches may confound the sensation evoked by intramuscular electrical stimulation (Graven-Nielsen *et al.* 2001).

Chemical stimulation by intramuscular infusion of *hypertonic saline* causes local and referred pain. Hypertonic saline mimics musculoskeletal pain in both subjectively perceived quality as well as its effects on motor performance (Korotkov *et al.* 2002). Since the pain lasts for minutes, a detailed description of sensory and motor effects is obtainable. It is believed that injections of hypertonic saline in the muscle give a direct excitation of the unmyelinated afferents. The reason for this is the drastic elevation of the extracellular sodium concentration. This leads to sodium influx through the cell membrane and depolarisation of the nerve. Stimulation of axotomised afferent nerve fibres has demonstrated an excitatory effect of hypertonic saline on a significant proportion of C-fibres in the muscles (Schulte *et al.* 2003). The dominant sensation following hypertonic saline injections in the muscle is a deep and diffuse pain, corresponding well with a major activation of C-fibres. In earlier studies, manual bolus infusions of hypertonic saline were used.Standardisation of a small bolus volume is easy to accomplish by a computer-controlled infusion pump. This provides a more reproducible method (Graven-Nielsen *et al.* 2001).

Intramuscular injections of algesic substances such as *capsaicin, bradykinin, serotonin, potassium chloride, glutamate, levo-ascorbic acid, and acid phosphate buffer*, are other chemical stimulation methods to evoke muscular pain. In general, these methods elicit mild to moderate intense levels of pain (Graven-Nielsen *et al.* 2001, Mørk *et al.* 2003).

Major shortcomings. Hypertonic saline injections may excite both non-nociceptive and nociceptive nerve fibres. However, the non-sensory manifestations cannot be demonstrated to a detectable degree, and the general opinion is that saline excitation of other receptors than nociceptors do not have a major influence on the sensory manifestation (Korotkov *et al.* 2003). The chemical stimulation methods all have a problematic reproducibility with large inter-individual differences (Mørk *et al.* 2003).

Examples of experimental muscle pain in the testing of analgesics.

The pain system involved in deep pain is different from skin pain, and the data from the cutaneous testing cannot be extrapolated to the deeper tissues. Various analgesics have been tested in muscle pain models, and the data have contributed to new insights into the effect of deep pain. Examples of drugs tested in muscle pain include *opioids,*

ketamine, adenosine, theophylline, and caffeine (Graven-Nielsen *et al.* 2001; Schulte *et al.* 2003) (table 2). These analgesics all demonstrate ability to reduce ischaemic pain in the tourniquet model. In contrast, *dextromethorphan, NSAID's* in monotherapy, *diazepam, and lidocaine* does not reveal any effect in the tourniquet model (Svensson *et al.* 1997; Howell *et al.* 1998).

Intravenous *remifentanil* reduces the slope of the stimulus-response curves for intramuscular electrical stimulation (Curatolo *et al*. 2000a). New data indicate that prolonged electrically induced muscle pain is sensitive to *ketamine* and *alfentanil*, but not to *morphine* (Schulte *et al.* 2003). Moreover, the attenuation of electrically induced muscle pain, by *alfentanil*, shows a dose-response relation (Schulte *et al.* 2003). *Ketamine*, which is an N-methyl-D-aspartate (NMDA) receptor antagonist, also attenuates temporal summation of muscle stimuli (Schulte *et al.* 2003).

Chemically induced muscle pain has also been used to evaluate analgesic potency. Hence, epidural *fentanyl, ketamine* and partly *morphine* reduce saline-induced muscle pain (Eichenberger *et al.* 2003). Moreover, preliminary data indicate that *alfentanil* in a dose-response manner attenuates saline-induced muscle pain (Eichenberger *et al.* 2003; Schulte *et al.* 2003).

Experimental visceral pain

The effect of analgesics on visceral pain is very difficult to evaluate in the clinic, mainly due to the deep and diffuse nature of the pain (Drewes *et al.* 2003). Due to the localisation of the organs, experimental pain studies in the viscera are more difficult than in the skin or muscles. The risk of perforation and the increased autonomic responses to visceral stimuli also limit the possibilities (Ness & Gebhart 1990). However, during recent years, experimental pain has been evoked in most part of the gastrointestinal tract (Drewes *et al.* 2003; Ness & Gebhart 1990), the urinary tract (Maggi 1993) and the uterine cervix (Drewes *et al.* 2003). Recently, new models have been developed where the investigator can use reliable pain stimulation with different modalities and hence stimulation of different groups of afferents. Sensitisation of the nervous system is also possible by e.g., perfusion of the gut with chemical substances. Thus, peripheral and central mechanisms relating to the clinical situation involving chronic pain syndromes can be evoked, and the effect of pharmacological modulation evaluated.

Electrical, mechanical, thermal and chemical stimulation constitutes the different methods for pain stimulation in the human viscera, mainly used in the gastrointestinal tract. In the following these models are shortly described, for a more comprehensive review see Drewes *et al.* (2003).

Mechanical stimulation.

The mechanical properties of the gastrointestinal tract are important for its function as a digestive organ, and the gut contains mechanoreceptors at various locations in the wall, mainly in the muscle layers (Sengupta & Gebhart 1994). Mechanical stimulation in hollow organs is done via distension. To distend organs like the oesophagus, the small intestines or the rectum, a balloon is used. The methods consist of either simple balloon distension or computerised systems such as the "Barostat", where the pressure and volume can be controlled (Van Der Schaar *et al.* 1999). Several protocols and stimulation paradigms are recommended for the Barostat, such as for example "phasic and tonic distensions". The stimulation paradigms have recently been thoroughly discussed (Van Der Schaar *et al.* 1999). The major advantage of the Barostat system and similar pressure-volume based methods are the relatively low costs and reliability making it useful for routine purposes.

Major shortcomings. There are major limitations with these systems relating to e.g., elongation of the balloon during distension. Any bag will tend to elongate in the luminal direction where the resistance is less, rather than distend the gut wall. Hence, recordings of volume (and tension) may suffer from errors due to elongation and deformation of the bag (Gregersen & Christensen 2000). Since many organs such as the rectum and stomach are not spherical, this may cause further bias in the assessment of the degree of distension. These problems may be overcome by calculation of the balloon radius and strain in the tissue. In accordance with recent studies, strain of the gut is probably the most consistently mechanical parameter relating to the sensory response (Drewes *et al.* 2003). Therefore new methods such as impedance planimetry or methods based on ultrasound where the radius of the balloon (and hence strain in the tissue) can be calculated, will probably improve the value of the mechanical methods in future studies.

Table 2.

Examples of different analgesics, which have been used to modify the pain evoked by different experimental pain models of the muscles. The table is not intended as a complete list, but should give examples of different drugs, which attenuate experimental muscle pain.

Type	Modality	Stimulus	Analgesic substance
Endogenous	Ischemia	Tourniquet	Morphine, ketamine, adenosine, theophylline, caffeine, acetaminophen
	Exercise	Eccentric contractions	Ibuprofen
Exogenous	Electrical	Intramuscular	Ketamine, alfentanil, remifentanil
	Mechanical	Pressure	Ketamine, mepivacaine, ibuprofen, morphine, imipramine, clonidine, tramadol, alfentanil, codeine
		Tourniquet	Lidocaine
	Chemical	Hypertonic saline	Alfentanil, fentanyl, ketamine, ketoprofen

Electrical stimulation.

Depolarisation of the nerve afferents by electrical current has been widely used as an experimental stimulus in the human gut (for review see Ness & Gebhart 1990). The electrical stimuli have proved to be safe in all parts of the gastrointestinal tract. Electrical stimuli are easily controlled over time, and central pain mechanisms can be studied by using e.g., repeated electrical stimuli (Drewes *et al.* 2003).

Electrical stimulation of the gut has been widely used to study basic pain mechanisms, pain characteristics, referred pain, and evoked brain potentials to gut stimuli. The electrical stimulus intensity to evoke pain as well as the size of the referred pain area is reliable and reproducible (Drewes *et al.* 2003).

Major shortcomings. The major drawback with older methods was the varying electrode contact with the mucosa, giving inconsistent results. Integrating the electrodes on the biopsy forceps for the endoscopes has improved the methods. This allows stimulation of well-defined areas throughout the gastrointestinal tract. As mentioned earlier, electrical stimuli are not physiological, although this may not have the same impact as in skin models, as the sensation evoked by different stimuli in the gastrointestinal tract is often not related to the stimulus modality (Drewes *et al.* 2003).

Thermal stimulation.

Luminal thermal stimuli are able to activate afferents in the mucosa selectively. This is opposed to mechanical and electrical stimuli, which activate afferents in both the superficial and deeper layers (Sengupta & Gebhart 1994). Although thermal stimuli of the gut were used in animal studies (Ness & Gebhart 1990), only few studies have used temperature stimuli in the human gastrointestinal tract (Sengupta & Gebhart 1994). Humane testing shows a uniform perception of thermal stimuli from the stomach to the jejunum with different reflex responses evoked by the stimuli (Villanova *et al.* 1997). In a recent study, the temperature of re-circulating water was continuously measured inside a balloon positioned in the oesophagus (Drewes *et al.* 2003). The temperature stimuli showed a linear stimulus-response relationship, demonstrating the validity of the activation. Thus, temperature models could provide a valid stimulation modality in future pharmacological research.

Major shortcomings. The methods have not been used in analgesic testing and therefore little is known about the effect in these systems.

Chemical stimulation.

Chemical stimulation approaches the ideal experimental visceral pain stimulus, as this stimulation more closely resembles clinical inflammation (Ness & Gebhart 1990). *Acid* perfusion of the oesophagus is the most used chemical stimulus. The major relevance of this model may be for sensitisation of the peripheral and central pain pathways to subsequent experimental stimulation (Sarkar *et al.* 2000; Drewes *et al.* 2003). Application of *glycerol* to the large intestine evoked pain in patients with the irritable bowel syndrome (Louvel *et al.* 1996). In the colon mucosa injections of 2–6% *hypertonic saline* resulted in deep as well as referred non-painful and painful perceptions (Drewes *et al.* 2003). The injections were non-traumatic and different layers of the gut can be stimulated. *Capsaicin* is another algogenic chemical, which has been widely used to elicit experimental skin pain and secondary hyperalgesia. Studies of the urinary bladder have shown that many of the capsaicin-sensitive afferents run in the submucosa and into the epithelium. These afferents may convey the sensing of inflammatory mediators released during tissue injury and inflammation

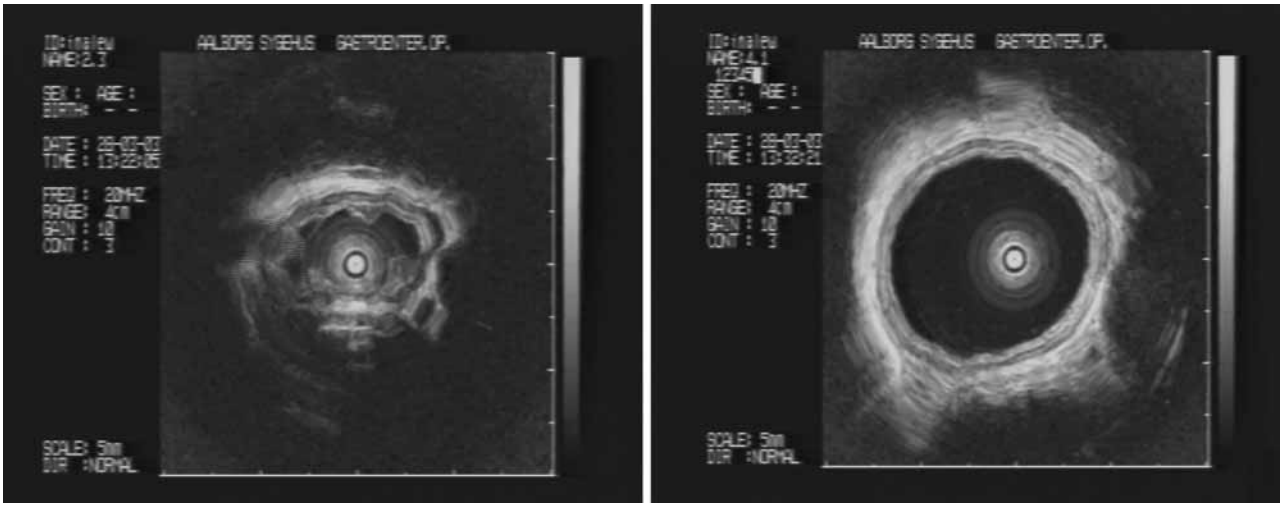

Fig. 5. Distension of the oesophagus with a balloon. The different layers of the oesophagus and the cross sectional areas of the lumen are imaged with ultrasound. The picture to the left illustrates the oesophagus with a nearly deflated balloon, corresponding to a slight, non-painful sensation. The picture to the right illustrates an inflated balloon, corresponding to the pain detection threshold.

(Maggi 1993). Application of capsaicin in the ileum resulted in a dose-dependent pain response and referred pain (Drewes *et al.* 2003). The substance may be a promising new method for evoking chemogenic gut pain in man.

Major shortcomings. The major disadvantage of chemical stimulation is a relatively long latency time to the onset of effects and often responses are not reproducible when repeated (Ness & Gebhart 1990). Human data are sparse and this may limit the use of chemical stimuli in pharmacological research. Application of glycerol into the ileum of healthy volunteers did not evoke pain or hypersensitivity, and the lack of direct pain responses in the healthy gut may limit its use as an experimental pain model in man (Louvel *et al.* 1996).

Examples of experimental visceral pain in the testing of analgesics.

The visceral pain models have only been used in testing of analgesics to a limited extent, and it is mainly by the use of mechanical stimulation. The pain mechanisms relating to the viscera are to some degree different from those in other tissues. Thus, drugs thought to act as analgesics based on somatic studies and/or clinical experience from other tissues will not necessarily have an effect on visceral pain. This may explain why treatment of visceral pain often fails in the clinic. Hence, there is an urgent need for new experimental data relating to visceral pain.

Mechanical pain models have been used in the screening of already existing (e.g., *acetylic salicylic acid, opioids, fedotozine* etc.) and new analgesics (Delvaux *et al.* 1999; Thumshirn *et al.* 1999; Van Der Schaar *et al.* 1999) table 3). Most of the experiments testing analgesics with the Barostat have shown a reduction of the sensation to volume but not pressure (Drewes *et al.* 2003). However, as stated above there are limitations of the simple mechanical methods, and great care should be taken when interpreting these studies. Recently, a prostaglandin E2 receptor-1 antagonist has been tested. This drug shows effect in a pain model using electrical stimulation of the oesophagus, sensitised with hydrochloric acid (Sarkar *et al.* 2003). The experimental data on analgesics and visceral pain are few, and new experiments using controlled mechanical stimulation in combination with other testing modalities should be used to improve our knowledge on the treatment of visceral pain.

Assessment of experimental pain

Psychophysical methods.

Psychophysical methods are based on the volunteer's subjective assessment of the pain. The most used methods are the visual analogue scale (VAS), the McGill Pain Questionnaire (MPQ) and various verbal descriptor scales (VDS) (Gracely 1999). VAS and VDS describe the pain quantitatively, whereas the MPQ and similar instruments also describe the pain quality. The MPQ consists of 20 groups of words, where the volunteers can select those most descriptive for the pain. The different groups of words can give information of the affective, sensory and evaluative dimensions of pain. The VAS consists of a 100 mm line with the endpoints 0 and 100 corresponding to no pain and unbearable pain. The scale is continuous, which gives very detailed information of the scores. Electrical devices for registration of the VAS score make a continuous registration possible. This is useful when stimulus-response curves are needed. A distinction between the pain intensity and pain unpleasantness can be useful in studies of the skin, but is controversial in the assessment of deep pain. Verbal descriptors can be used alone or as a supplement to the VAS scales (Gracely 1999; Drewes *et al.* 2003).

Major shortcomings. The intensity and hence the provided measure of the experienced pain is influenced by many factors, such as pain, anxiety, vigilance etc. that can be difficult to control (Gracely 1999).

Electrophysiological methods.

The electrophysiological methods assess the output in human pain models objectively. The main methods are measurement of the nociceptive withdrawal reflexes and evoked brain potentials after various nociceptive stimuli.

The nociceptive withdrawal reflex.

The reflex is well known in animal experiments as the *withdrawal reflex.* The reflex is the movement associated with flight responses in the three unaffected limbs, in the case of noxious stimuli applied to the forth limb. The nociceptive input followed by secondary processing in the spinal cord initiates the generation of the withdrawal reflex (for review see Schouenborg 2002). The withdrawal reflex is organised as a modular system (Schouenborg 2002), where the differ-

Table 3.

Examples of different analgesics, which have been used to modify the pain evoked by different experimental pain models of the viscera. The table is not intended as a complete list, but to give examples of different drugs, which attenuate experimental pain in the viscera.

Modality	Stimulus	Analgesic substance
Mechanical	Distension controlled by the "Barostat"	Acetylsalicylate, opioids, ondansetron, granisetron, sumatriptan, lidocaine, clonidine, somatostatin, cholecystokinin
Electrical	Electrodes on probe	ZD6416
Chemical	Acid in the oesophagus	ZD6416

ZD6416: A prostaglandin antagonist under development.

ent modules have different properties. General analgesia should therefore be assessed from many reflex modules.

In man, electrical stimulation is frequently used to elicit the reflex. Selection of appropriate time window excludes voluntary contribution during the response analysis. The volunteers are either sitting or are in the supine position and relaxing all muscles of the limb. The reflex is evoked by stimulation of e.g., the sural nerve at the ankle, after which the electromyogram is recorded from the biceps muscle of the thigh during the following withdrawal. In experiments involving supra-threshold stimuli, modulation of the reflex threshold or the reflex size is used as outcome measures. The reproducibility is good for electrical stimulation. Willer (1977) has extensively used the withdrawal reflex in pain research, and one of the main findings was that there are a linear correlation between the subjective pain intensity and the reflex size in the experimental setting. Secondly, a high correlation between the pain intensity stimulus-response curve, and the reflex size stimulus-response curve led to a suggestion of using the reflex as an "objective" measure of experimental pain (Gracely 1999). If the reflex is elicited by a pure nociceptive stimulus, the method provides a useful measure of spinal nociceptive transmission. Activation of either Aδ-fibres or C-fibres allows observation of the influence of different afferent fibre types in eliciting the reflex. The A-fibre mediated reflex responses divide into a component mediated by tactile (Aβ) afferents, and a component mediated by Aδ afferents. *RII* and *RIII* reflex denotes these two reflexes. The term RIII is still used in the literature to denote the Aδ-mediated withdrawal reflex (Graven-Nielsen *et al.* 2001).

Major shortcomings. Animal experiments have shown that the reflex is very much dependent on the position of the stimulated limb, and standardised procedures regarding this are crucial (Le Bars *et al.* 2001). Electrical stimulation can activate the whole spectrum of cutaneous afferents and evoke several reflex components. Among these cutaneous afferents, only a proportion will be nociceptive. This means that the appearance of a flexion reflex does not necessarily means that the stimulus has been nociceptive. Accordingly, since some cutaneous nerves can activate extensor muscles, nociceptive reflexes are not always flexion reflexes (Le Bars *et al.* 2001). Finally, the test merely evaluates a single dimension of the subjective pain experience. Hence, it has no meaning to use the reflex as a measure of a more complex pain experiences and this model does not reveal many of the supraspinal processes (Gracely 1999).

Examples of the withdrawal reflex applied in the testing of analgesics.

In pain research, the nociceptive withdrawal reflex evaluates the spinal nociceptive processing. The reflex is one of the most important tools for screening new analgesics in animal preparations using e.g. paw withdrawal latencies, tail flick latency etc. In man, the reflex has also been used for testing the effect of *opioids* (Poulsen *et al.* 1996), *tricyclic antidepressants* (Poulsen *et al.* 1995), *NMDA antagonists* (Arendt-Nielsen *et al.* 1996) and *local anaesthetics* (Petersen-Felix *et al.* 1995). An analgesic effect mainly gives a reduction in the reflex amplitude (Arendt-Nielsen 2000).

Repetitive electrical stimuli allow testing of central integrative mechanisms (Arendt-Nielsen *et al.* 2000), such as temporal summation. Temporal or spatial summation gives a reflex build-up, with an increase in reflex amplitude. Temporal summation of afferent input evoked by repetitive stimuli is known to involve the NMDA system (Woolf *et al.* 1991), and *NMDA antagonists* suppress the reflex build-up (Arendt-Nielsen *et al.* 1996). Thus, modulation of the nociceptive withdrawal reflex may be important both in the objective assessment of basic pain mechanisms, and in evaluation of analgesic effects.

Evoked brain potentials.

The use of brain-evoked potentials provides another method of electrophysiological assessment of pain. The potentials result from summation of a series of time-locked electroencephalographic (EEG) responses to a stimulus. The early and mid-latency components of the potentials (latency <150 msec.) probably relates to direct thalamo-cortical projections, involved in the pain processing, while the late components (latency >150 msec.) are related to secondary cognitive processes, involved in attention etc., being less specific for pain (Bromm & Treede 1991).

Potentials elicited by mechanical and electrical stimulation are often the result of a cortical response to myelinated fibres. Chemical or laser stimulation mainly elicits potentials resulting from cortical responses to unmyelinated fibres. This has been shown in animals, where nociceptive C-fibre input evoked by CO_2-laser, to the primary sensory cortex has been monitored and modulated by opioids (Kalliomäki *et al.* 1998) (for review see Chen 1993).

Although non-specific, electrical stimulation provides a short-lasting, well-defined stimulus, which is important to give a distinct signal in the measured evoked potentials (Lorenz *et al.* 1997). The amplitude of the human vertex potential evoked by nociceptive stimuli increases with increasing stimulus intensity and hence pain intensity (Chen 1993). Noxious electrical, laser and rapidly increased temperature stimuli provide valid stimuli to be detected by evoked potentials (Chen *et al.* 2001).

Major shortcomings. The peak vertex potential at 300 msec. is often used as a marker for the subjective pain intensity, but the component is relative non-specific and is also seen after non-painful stimulations such as sound and light. Since benzodiazepines can counteract the late vertex peak evoked by laser stimulation, this could reflect measurement of other responses than pain (Zaslansky *et al.* 1996). Large intra- and interindividual variability of the responses may confound the use of vertex potentials, and the many confounding factors necessitate control recordings (Handwerker & Kobal 1993; Gracely 1999). Evoked potentials reflect to a higher degree the intensity of pain, whereas the unpleasantness dimension is not affected to the same degree (Chen 1993; Gracely 1999) Evoked potentials are more dif-

ficult to use in visceral pain models because of the relatively low fibre density in the gut and hence low signal- to noise ratio for the potentials (Drewes *et al.* 2003).

Evoked brain potentials applied in testing of analgesics.

When testing analgesics a reduction in different components of the evoked vertex potentials reveals analgesic effect (Handwerker & Kobal 1993). Evoked potentials have been used in testing of various *opioids,* which reduce the amplitude of the late component (Lorenz *et al.* 1997).

Also peripherally working analgesics can be tested by the means of evoked potentials. *Local anaesthetics* decrease the amplitude of both peripherally recorded action potentials and of centrally recorded evoked potentials (Curatolo *et al.* 2000b). Also *acetylsalicylic acid* and *physostigmine* have shown effects via this method through decrease of various components of the potentials (Chen 1993).

Other methods for pain assessment.

Source analysis of the evoked brain potentials and magnetoencephalography can be used to localise the regional brain activity to pain. Positron emission tomography can be used to measure radioactive labelled glucose, and the glucose utilization reflects changes in regional blood flow in the brain to e.g., painful stimuli (Chen 1993). Receptor functions by ligand binding studies are also important in pharmacological research. Functional magnetic resonance imaging is another method that infers neural activity from changes in blood flow, but does not require ionizing radiation. These measures are able to detect intracerebral activity to pain stimuli, and some studies have also used analgesics to modify the central response (Gracely 1999). These measurements will not be discussed in detail in this review, but there is no doubt that future studies using such methods will increase our insight into pain mechanisms and pharmacological modulation.

Future directions for research

Multi-modal and multi-tissue models.

The differentiated information of the drug effect on many pain modalities/mechanisms/tissues obtained from experimental studies can be used as "proof-of concept", dose-efficacy analysis, and for designing further clinical trials.

However, one of the major limitations of most experimental pain models is that they do not mimic clinical pain. The reason for this is that the stimuli are relative short lasting without the inflammation and subsequent activation of the peripheral and central nervous mechanisms that diseases typically activate. Therefore, the basic neurobiological mechanisms in clinical pain may be different from those relating to an experimental stimulus (Ness & Gebhart 1990). Many experiments have shown that the duration of the painful stimuli is an important factor influencing the outcome of analgesic testing, and selection of tests with relative long duration of the stimulus may be more valid (Gracely 1999). Another approach of mimicking the clinical situation is the use of a *multimodal test*, where different receptor types and mechanisms are activated as in the clinic. The multimodal model has clearly shown its value in somatic pain testing, where single stimuli have been inadequate to test for example pathophysiological changes and effects of specific drugs. Hence, in a recent study a tricyclic antidepressant increased the somatic pain threshold to electrical stimuli, but did not reduce cold pressor pain (Enggaard *et al.* 2001). This is illustrated in fig. 6, where the differentiated effects of various analgesics on different modalities within experimental skin stimulation are illustrated. Hypothetically, these differentiated effects could reflect how the drugs modify different disease mechanisms. Thus, if a major central contribution is expected, drugs with effect on the summation threshold should be preferred in the pain treatment. Furthermore, the clinical effects of a new drug could be compared with older substances given the effects on an experimental pain battery, and the comparison may show the most appropriate drug of choice in a given disease. In the viscera, recent studies of multimodal testing makes differentiated stimulation of the receptors in the superficial and deep layers of the gut possible (Drewes *et al.* 2003). Multimodal testing, with mechanical, electrical and thermal stimuli combined with sensitisation to acid, has for example been applied to the oesophagus (Sarkar *et al.* 2000; Drewes *et al.* 2003). Repetitive or strong stimulation can induce visceral hyperalgesia and evoke central phenomena such as summation, allodynia and referred pain. Hence, these models may increase the knowledge regarding peripheral and central pain mechanisms, and could be suitable for pharmacological testing.

Drugs used in the treatment of muscular and visceral pain are often evaluated in cutaneous pain models. Differences must be expected in the skin and deeper tissues be-

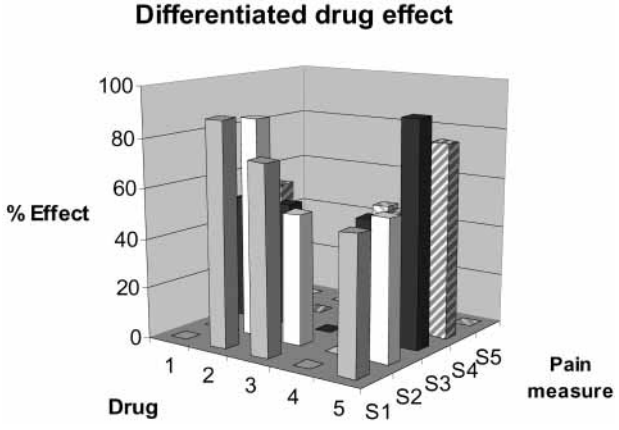

Fig. 6. Differentiated effect of five hypothetical analgesics (drug 1–5) in an experimental pain model of the skin. The pain measures (S1–S4) could be the pain detection threshold (PDT), the pain tolerance threshold (PTT), the temporal summation detection pain threshold and the temporal summation pain tolerance threshold. Theoretically these differentiated effects could give a prediction of how the pain in a well-characterised disease would respond to a given drug.

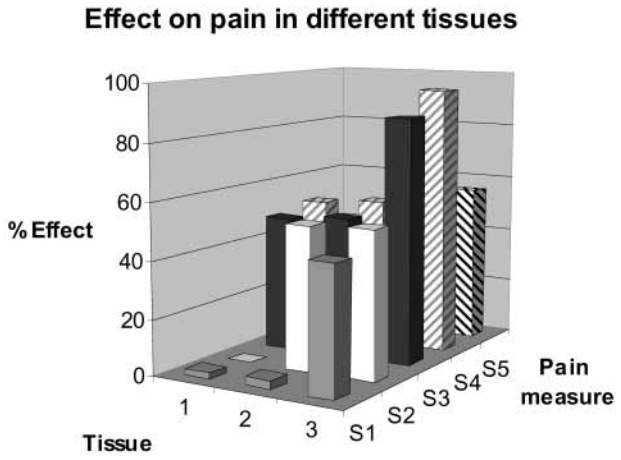

Fig. 7. Differentiated effect of one drug in three different tissues (e.g., skin, muscle and viscera). The pain measures (S1–S5) could be cold and heat pain detection thresholds, mechanical pain detection and tolerance threshold, and electrical pain threshold to summated stimuli.

cause of the different anatomy, physiology and biochemistry of the pain system in these structures (Sengupta & Gebhart 1994; Drewes *et al.* 2003). Therefore, "*multitissue pain models*" used for evaluation of existing and new drugs may be refined to combine skin, muscular, and visceral stimuli to give a more differentiated screening of the effect of analgesics. The use of a certain stimulation modality may not evoke the same response in all tissues. This is seen in e.g., the testing of μ-opioid agonists in the skin and in muscles. Here, a greater increase in the pain tolerance threshold was seen in muscles than in the skin when the μ-opioid agonist remifentanil was given (Curatolo *et al.* 2000a). This shows that testing of an analgesic done in only one tissue can overlook important effects. Wilder-Smith *et al.* (1998) also used the multitissue approach. In this experiment modulation of the pain tolerance threshold to electrical and heat stimuli applied to the hand, and mechanical stimuli in the rectum was investigated under the influence of *dihydrocodeine.* The study discovered a raised pain threshold to heat pain, but not to electrical stimulation on the hand. In the rectum, an increased mechanical pain tolerance threshold was found. Fig. 7 illustrates such a differentiated effect of a hypothetical drug on the multitissue testing battery. Here the drug has the major effects on the visceral pain tolerance and stimulus-response functions. Hence the drug should be expected to act on pain related to diseases of the viscera.

The ability of revealing the various pain mechanisms that can be modified by the analgesics (e.g., involvement of the NMDA receptors in temporal summation) could give a more targeted treatment. Such a *mechanism-based approach* requires detailed knowledge of the basic pain mechanisms underlying the diseases, and an analgesic with well-defined properties for treating the condition (Woolf & Max 2001). Thus, if chronic pain patients are individually tested with a qualitative sensory testing battery to unravel at which levels the pain system is reacting abnormally, the most appropriate treatment could theoretically be selected. This could also be done based on an *a priori* knowledge on the aberrant pain mechanisms in certain well-defined patient groups, together with a knowledge of the effect of the different analgesics on the pain system. This would be beneficial and probably give less variation in the patient's response, when treating various pain conditions. Unfortunately there are still many symptoms for which no pain mechanism has been discovered. Furthermore, only few analgesics have well-defined properties regarding their interaction with various receptors in the pain system. This complicates the logical selection of the appropriate analgesic. On the other hand the current clinical situation uses the trial and error approach, when the analgesic treatment is chosen. A future refinement of the pain models and an increase in the knowledge behind the mechanisms of analgesics is therefore highly warranted in pain medicine.

References

Arendt-Nielsen, L., J. Nielsen, S. Petersen-Felix, T. W. Schnider & A. M. Zbinden: Effect of racemic mixture and the (S+)-isomer of ketamine on temporal and spatial summation of pain. *Brit. J. Anaesth.* 1996, **77**, 625–631.

Arendt-Nielsen, L., F. A. Sonnenborg & O. K. Andersen: Facilitation of the withdrawal reflex by repeated transcutaneous electrical stimulation: an experimental study on central integration in humans. *Eur. J. Appl. Physiol. Occup. Physiol.* 2000, **81**, 165–173.

Bromm, R & R. D. Treede: Laser-evoked cerebral potentials in the assessment of cutaneous pain sensitivity in normal subjects and patients. *Revue Neurologique (Paris)* 1991, **147**, 625–643.

Burian, M., T. Irmgaard, M. Seegel & G. Geisslinger: Peripheral and central antihyperalgesic effects of diclofenac in a model of human inflammatory pain. *Clin. Pharmacol. Therap.* 2003, **74**, 113–120.

Chen, A. C. N.: Human brain measures of clinical pain: a review, I. Topographic mappings. *Pain* 1993, **54**, 115–132.

Chen, A. C. N., D. M. Niddam & L. Arendt-Nielsen: Contact heat evoked potentials as a valid means to study nociceptive pathways in human objects. *Neurosci. Lett.* 2001, **316**, 79–82.

Compton, P., V. C. Charuvastra & W. Ling, W.: Effect of oral ketorolac and gender on human cold pressor pain tolerance. *Clin. Exp. Pharmacol. Physiol.* 2003, **30**, 759–763.

Curatolo, M., S. Petersen-Felix, A. Gerber & L. Arendt-Nielsen: Opioid mu-agonists more strongly inhibit muscular than cutaneous pain in humans. *Brit. J. Anaesth.* 2000a, **85**, 529–532.

Curatolo, M., S. Petersen-Felix & L. Arendt-Nielsen: Sensory assessment of regional analgesia in humans – A review of methods and applications. *Anesthesiology* 2000b, **93**, 1517–1530.

Delvaux, M., D. Louvel, E. Lagier, B. Scherrer, J. L. Abitbol & J. Frexinos: The kappa agonist fedotozine relieves hypersensitivity to colonic distention in patients with irritable bowel syndrome. *Gastroenterology* 1999, **116**, 38–45.

Dirks, J., K. L. Petersen, M. C. Rowbotham & J. B. Dahl: Gabapentin supresses cutaneous hyperalgesia following heat-capsaicin sensitization. *Anesthesiology* 2002, **97**, 102–107.

Drewes, A. M., H. Gregersen & L. Arendt-Nielsen: Experimental pain in gastroenterology – A reappraisal of human studies. *Scand. J. Gastroenterol.* 2003, **38**, 1115–1130.

Eichenberger, U., C. Giani, S. Petersen-Felix, T. Graven-Nielsen, L. Arendt-Nielsen & M. Curatolo: Lumbar epidural fentanyl: Segmental spread and effect on temporal summation and muscle pain. *Br. J. Anaesth.* 2003, **90**, 467–473.

Enggaard, T. P., L. Poulsen, L. Arendt-Nielsen, S. H. Hansen, I.

Bjornsdottir & L. F. Gram: The analgesic effect of codeine as compared to imipramine in different human pain models. *Pain* 2001, **922**, 277–282.

Fischer, A. A.: In: Muscle pain syndromes and fibromyalgia. Pressure algometry for quantification of diagnosis and treatment outcome. *Haworth Medical Press* New York, 1998.

Fowler, C. J., K. Sitzoglou, Z. Ali & P. Halonen: The conduction velocities of peripheral nerve fibres conveying sensations of warming and cooling. *J. Neurol.* 1988, **51**, 1164–1170.

Gracely, R. H.: Pain measurement. *Acta anaesthesiol. Scand.* 1999, **43**, 897–908.

Graven-Nielsen, T., M. Segerdahl, P. Svensson & L. Arendt-Nielsen: Methods for induction and assessment of pain in humans with clinical and pharmacological examples. In: *Methods in pain research*. Ed.: L. Kruger, *CRC Press*, Boca Raton, 2001, pp. 264–304.

Gregersen, H. & J. Christensen: Gastrointestinal tone. *Neurogastroenterol. Motility* 2000, **12**, 501–508.

Handwerker, H. O. & G. Kobal: Physiology of experimentally induced pain. *Physiol. Rev.* 1993, **73**, pp. 639–671.

Howell, J. N., r. R. Conatser, G. S. Chleboun, D. L. Karapondo & A. G. Chila: The effect of nonsteroidal anti-inflammatory drugs on recovery from exercise-induced muscle injury. *J. Musculoskeletal Pain* 1998, **6**, 69–84.

Hughes, A. M., J. Rhodes, G. Fisher, M. Sellers & J. W. Growcott: Assessment of the effect of dextromorphan and ketamine on the acute nociceptive threshold and wind-up of the second pain response in healthy male volunteers. *Brit. J. Pharmacol.* 2002, **53**, 604–612.

Kalliomäki, J., X.-L. Luo, Y.-B. Yu & J. Schouenborg: Intrathecally applied morphine inhibits nociceptive C fiber input to the primary somatosensory cortex (SI) of the rat. *Pain* 1998, **77**, 323–329.

Kehl, L. J. & C. A. Fairbanks: Experimental animal models of muscle pain and analgesia. *Exercise Sport Sci. Rev.* 2003, **31**, 188–194.

Koppert, W., S. Reinhard, K. Scheuber, M. Alsheimer, M. Schmelz & J. Schütter: Differential modulation of remifentanil-induced analgesia and postinfusion hyperalgesia by S-ketamine and clonidine in humans. *Anesthesiology* 2003, **99**, 152–159.

Korotkov, A., M. Ljubisavljevic, J. Thunberg, G. Kataeva, M. Roudas, S. Pakhomov, S. Radovanovic, E. Lyskov, S. Medvedev & H. Johanson: Changes in human regional cerebral blood flow following hypertonic saline induced experimental muscle pain: a positron emission tomography study. *Neurosci. Lett.* 2003, **335**, 119–123.

Le Bars, D., M. Gozario & S. W. Cadden: Animal models of nociception. *Pharmacol. Rev.* 2001, **53**, 597–652.

Lorenz, J., H. Beck & B. Bromm: Differential changes of laser evoked potentials, late auditory evoked potentials and P300 under morphine in chronic pain patients. *Electroencephalography Clin. Neurophysiol.* 1997, **104**, 514–521.

Louvel, D., M. Delvaux, G. Staumont, F. Camman, J. Fioramonti & L. Bueno: Intracolonic injection of glycerol: a model for abdominal pain in irritable bowel syndrome? *Gastroenterology* 1996, **110**, 351–161.

Lötsch, J. & M. Angst: The μ-opioid agonist remifentanil attenuates hyperalgesia evoked by blunt and punctuated stimuli with different potency: a pharmacological evaluation of the freeze lesion in humans. *Pain* 2003, **102**, 151–161.

Maggi, C. A.: The dual, sensory and "efferent" function of the capsaicin-sensitive primary sensory neurons in the urinary bladder and uretra. In: *Nervous control of the urogenital system.* Ed.: C. A. Maggi. Harwood Academic Publishers, 1993, pp. 382–422.

Mense, S., U. Hoheisel, A. Kaske & A. Reinert: Muscle Pain: Basic mechanisms and clinical correlates. In: *Proceedings of the 8th World Congress on Pain* Progress in Pain Research and Management, vol. 8. EdS.: T. S. Jensen, J. A. Turner & Z. Wiesenfield-Hallin. IASP Press, Seattle 1997.

Mørk, H., M. Ashina, L. Bendtsen, J. Olesen & R. Jensen: Experimental pain and tenderness following infusion of endogenous substances in humans. *Eur. J. Pain* 2003, **7**, 145–153.

Ness, T. J. & G. F. Gebhart: Visceral pain: a review of experimental studies. *Pain* 1990, **41**, 167–234.

Petersen, K. L., A. Maloney, F. Hoke, B. J. Dahl, M. C. Rowbotham: A randomised study of the effect of oral lamitrigine and hydromorphone on pain and hyperalgesia following heat/capsaicin sensitization. *J. Pain* 2003, **4**, 400–406.

Petersen-Felix, S., L. Arendt-Nielsen, P. Bak, D. Roth, M. Fischer, P. Bjerring & A. M. Zbinden: Analgesic effect in humnas of subanaesthetic isoflurane concentrations evaluated by experimentally induced pain. *Brit. J. Anaesth.* 1995, **75**, 55–60.

Polianskis, R., T. Graven-Nielsen & L. Arendt-Nielsen: Pressure-pain function in desensitized and hypersensitized muscle and skin assessed by cutt algometry. *J. Pain* 2002, **3**, 28–37.

Poulsen, L., L. Arendt-Nielsen, K. Brøsen, K. K. Nielsen, L. F. Gram & S. H. Sindrup: The hypoalgesic effect of imipramine in different human experimental pain models. *Pain* 1995, **60**, 287–293.

Poulsen, L., L. Arendt-Nielsen, K. Brøsen & S. H. Sindrup: The hypoalgesic effect of tramadol in relation to CYP2D6. *Clin. Pharmacol. Therap.* 1996, **60**, 636–644.

Price, D. D., L. S. Milling, I. Kirsch, A. Duff, G. H. Montgomery & S. S. Nicholls: An analysis of factors that contribute to the magnitude of placebo analgesia in an experimental paradigm. *Pain* 1999, **83**, 147–156.

Sarkar, S., Q. Aziz, C. J. Woolf, A. R. Hobson & D. G. Thompson: Contribution of central sensitisation to the development of non-cardiac chest pain. *Lancet* 2000, **356**, 1154–1159.

Sarkar, S., A. R. Hobson, A. Hughes, J. Growcott, C. J. Woolf, D. G. Thompson & Q. Aziz: The prostaglandin E2 receptor (EP-1) mediates acid-induced visceral pain hypersensitivity in humans. *Gastroenterology* 2003, **124**, 18–25.

Schouenborg, J.: Modular organisation and spinal somatosensory imprinting. *Brain Res. Rev.* 2002, **40**, 80–91.

Schulte, H., T. Graven-Nielsen, A. Sollevi, Y. Jansson, L. Arendt-Nielsen & M. Segerdahl: Pharmacological modulation of experimental phasic and tonic muscle pain by morphine, alfentanil and ketamine in healthy volunteers *Acta anaesthesiol. Scand.* 2003, **47**, 1020–1030.

Sengupta, J. N. & G. F. Gebhart: Gastrointestinal afferent fibers and sensation. In: *Physiology of the gastrointestinal tract.* Ed.: L. Johnson. Raven Press, New York, USA, 1994, pp. 484–519.

Svensson, P., L. Houe & L. Arendt-Nielsen: Effect of systemic versus topical nonsteroidal anti-inflammatory drugs on postexercise jaw-muscle soreness: A placebo-controlled study. *J. Orofacial Pain* 1997, **11**, 353–362.

Thumshirn, M., M. Camilleri, M. G. Choi & A. R. Zinsmeister: Modulation of gastric sensory and motor functions by nitrergic and alpha2-adrenergic agents in humans. *Gastroenterology* 1999, **116**, 573–585.

Van der Schaar, P. J., C. B. Lamers & A. A. Masclee: The role of the barostat in human research and clinical practice. *Scand. J. Gastroenterol.* 1999, **34** *(Suppl. 230)*, 52–63.

Villanova, N., F. Azpiroz & J. R. Malagelada: Perception and gut reflexes induced by stimulation of gastrointestinal thermoreceptors in humans. *J. Physiol* 1997, **502**, 215–222.

Wallace, M. S., D. Barger & G. Schulteis: The effect of chronic oral desipramine on capsaicin-induced allodynia and hyperalgesia: A double-blinden, placebo-controlled, crossover study. *Anaesthesiol. Analg.* 2002, **95**, 973–978.

Wilder-Smith, C., E. Hufschmid & W. Thormann: The visceral and somatic antinociceptive effects of dihydrocodeine and its metabolite dihydromorphine. A cross-over study with extensive and quinidine-induced poor metabolizers. *Brit. J. Clin. Pharmacol.* 1998, **45**, 575–581.

Willer, J. C.: Comparative study of perceived pain and nociceptive flexion reflex in man. *Pain* 1977, **3**, 69–80.

Woolf, C. J. & S. W. N. Thompson: The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation: implications for the treament of post-injury pain hypersensitivity states. *Pain* 1991, **44**, 293–299.

Woolf, C. J. & B. M. Max: Mechanism-based pain diagnosis – Issues for analgesic drug development. *Anaesthesiology* 2001, **95**, 241–249.

Zaslansky, R., E. Sprecher, Y. Katz, B. Rozenberg & J. A. Hemli: Pain-evoked potentials: what do they really measure? *Electroencephalography Clin. Neurophysiol.* 1996, **100**, 384–392.