The neurobiology of pain

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Understanding the plasticity of pain and analgesia exhibited in different pain states may improve therapies for the two major types of pain, neuropathic and inflammatory pain, in which nerve and tissue damage leads to alterations at both peripheral and central levels. At the level of the peripheral nerve, drugs that act on particular sodium channels may target only pain-related activity. Agents that act on some of the peripheral mediators of pain may control peripheral nerve activity. A new generation of non-steroidal anti-inflammatory drugs, cyclo-oxygenase 2 inhibitors, that lack gastric actions are becoming available. In the spinal cord, the release of peptides and glutamate causes activation of multiple receptors, particularly, the N-methyl-D-aspartate receptor for glutamate, which, in concert with other spinal systems, generates spinal hypersensitivity. Blocking the generation of excitability is one approach, but increasing inhibitions may also provide analgesia. Opioid actions are via presynaptic and post-synaptic inhibitory effects on central and peripheral C fibre terminals, spinal neurones, and supraspinal mechanisms. Our knowledge of brain mechanisms of pain is still, however, limited. Other new targets have been revealed by molecular biology and animal models of clinical pain, but the possibility of a "magic bullet" is doubtful. Thus, another approach could be single molecules with dual drug actions, that encompass targets where additive or synergistic effects of different mechanisms may enable pain relief without major adverse effects.

The gate control theory of pain proposed by Melzack and Wall[1] lead to much research in the field of pain. Clinicians welcomed this theory because, from a functional point of view, it explained, or attempted to explain, certain clinical findings that could not be accommodated by previous theories of pain which were far too simplistic.

Since the publication of this theory in 1965, our knowledge of the neurobiology of pain continues to grow, while discoveries in electrophysiology and molecular biology offer glimpses of therapeutic breakthroughs. However, I believe that the gaps between the clinical and basic sciences are becoming wider. To put it simply, basic research is fascinating and flourishes in the public eye, yet too often takes a naive approach to the difficult issues that clinicians are confronted with in terms of providing therapy for certain types of pain. With few exceptions, clinicians have only "old molecules" available with which to treat pain. The partial explanation is that research is difficult and takes a long time, for example, the opioid receptors were formally identified in 1973, but we are still waiting for the development of an opioid with the efficacy of morphine without its side-effects. The best research groups in molecular biology lead the race to clone the three main receptors: μ, δ, and κ.[2] Nevertheless, many questions are unresolved: the classic pharmacological techniques that have been applied to this field suggest that subtypes of the receptors exist, whereas molecular biology has yet to come up with any evidence to support this premise.

Substantial difficulties arise in basic research, and before I sketch out an overview of the neurobiology of pain, I will consider some of these difficulties. Scientists engaged in research need to take a more realistic approach to their results so that clinicians are not lead to believe that many useful treatments for pain are just around the corner.

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Laboratory models

The relevance or not of the major behavioural tests for clinical pain states has been widely debated. Tests used to assess antinociceptive activity in the laboratory include noxious heat, pressure to the tail or paw, colorectal distension, intraperitoneal chemical irritants, and subcutaneous administration of formalin. In most cases, these stimuli are applied to healthy animals in the absence of disorders that commonly occur in patients who experience pain such as hyperalgesia (extreme sensitiveness to painful stimuli), allodynia (pain in response to a non-noxious mechanical stimulus), and hyperesthesia (abnormal sensitivity to sensory stimulus). Some of these tests depend on spinal mechanisms, whereas others involve supraspinal structures. Some tests have good sensitivity for a particular class of analgesics, but other tests frequently produce false-positive results. In addition, many behavioural experiments use only one nociceptive test, and the exact method can vary from investigator to investigator. This situation means that controversy surrounds the pharmacology of pain.

Various genetic approaches have been used in pain research, but the most popular is a laboratory model: the production of transgenic mice. For example, substance P has actions in the periphery and centrally, and mice without substance P (after knockout of the preprotachykinin gene),[3,4] or the neurokinin-1 receptor[5] have been produced. These models are difficult to compare since the first model includes mice without the ligand, substance P, and in the second model the receptor has been knocked out. Nevertheless, in both cases, neurogenic inflammation is substantially diminished, although somewhat surprisingly there is no change in the mechanical hypersensitivity induced by the inflammation.

Woolf and colleagues[6] compared in detail various models of transgenic mice and identified three general factors that are important in terms of the interpretation of these techniques: the genetic background of the animal; the developmental changes that could be encountered; and the redundancy of certain functions of sensory systems. Although there is no doubt that the deletion of receptors, channels, and transmitters by genetic

manipulations produces a powerful tool for the dissection of their roles in complex neuronal systems, the results of genetic approaches need to be interpreted with caution and large-scale studies are needed to complete pharmacological research.

These laboratory and behavioural models are limited because they do not mimic chronic pain states. Chronic pain differs substantially from acute pain in terms of the persistence of the pain and adaptive changes such as neuroplasticity that has been described at various levels of the nervous system. Such limitations have led to the use and development of more appropriate models of chronic pain in the past 10 years. These models include inflammatory pain and neuropathic pain. Although they are not perfect, the development of such experimental models is essential, not only for the detection of new analgesics, but also for a better understanding of pain syndromes that are difficult to manage clinically. Behavioural tests are limited and can be remarkably difficult to carry out properly. Clinicians need to realise, for example, how hard it can be for a researcher, to quantify allodynia by approaching an awake freely moving rat or mouse with a calibrated von Frey hair.

Other difficulties encountered in the development of safe analgesics arise from the complexity of the central nervous system. Some of the transmitters and receptors that may be involved in the transmission or modulation of pain are widely distributed throughout the nervous system, especially in the case of peptides and excitatory or inhibitory aminoacids. Most of these neuroactive substances are involved in multiple physiological functions, and so agents developed to target these systems could produce widespread side-effects. Additional difficulties result from the multiplicity of receptors and the co-localisation of more than one neurotransmitter in a single neuron. A further complexity of the network is that some peptides or excitatory aminoacids, for example substance P and glutamate, are localised not only in primary afferent neurons but also in intrinsic spinal neurons and descending fibers. Thus, caution is needed in interpreting the data and to avoid the temptation of becoming infatuated with the molecule of the moment.

In this paper, I review current knowledge of the different stages in the transmission of noxious messages from the periphery to the brain. Later in this series, Fernando Cervero and Jennifer Laird[7] will examine visceral pain and Clifford Woolf and Richard Martin[8] will explore neuropathic pain.

The peripheral jungle

A widely held assumption is that there is no specific histological structure that acts as a nociceptive receptor and that noxious messages arise from the activation of free unmyelinated terminal arborisations found in cutaneous, muscular, joint tissues, and in certain visceral structures. The nociceptive messages are then transmitted by thin myelinated (Aδ) or non-myelinated (C) fibres, although not all of the fibres are necessarily nociceptors. Studies in animals and human beings have identified various types of nociceptors.[9] Various classification systems have been proposed, for example, in cutaneous tissue in man the existence of unmyelinated polymodal nociceptors, which are responsive to thermal, mechanical, and chemical stimuli (with a slow conduction velocity of <2 m/s) have been established. Similarly, Meyer and colleagues[10] identified Aδ mechanothermal nociceptors and high threshold Aδ mechanoreceptors. Aδ and C nociceptors have been clearly identified in fibres, innervating joints and muscles, but not in viscerae where the situation is much more complicated. Thus, although certain fibres are undoubtedly nociceptors, others are activated by non-noxious stimuli but then increase their activity as the intensity of the stimulus increases.

Receptors localised on primary afferent fibres and their ligands from neuronal and non-neuronal origins

Recptors associated with nociceptors
ATP, neurokinin-1, $GABA_A$, $CABA_B$, neuropeptide Y, acetylcholine, somatostatin, prostaglandin E, cholecystokinin, adrenergic, 5 hydroxytryptamine $(5HT)_{2A}$ receptor, glutamine, bradykinin, noradrenaline, capsaicin, opioid, angiotensin II, adenosine

Ligands with non-neuronal sources
Acetylcholine, ATP, prostaglandin E, opioids, adenosine, glutamate, bradykinin, noradrenaline, serotonin

Ligands in nociceptors
Substance P, opioid, ATP, adenosine, neuropeptide Y, glutamate, cholecystokinin, somatostatin, bombesin

GABA=γ-aminobutyric acid

Adapted with permission from Carlton and Coggeshall.[13]

Sensitivity

When a stimulus is repeated nociceptors exhibit sensitisation in that there can be a reduction in the threshold for activation, an increase in the response to a given stimulus, or the appearance of spontaneous activity. This sensitisation of nociceptors results from the actions of second messenger systems activated by the release of several inflammatory mediators (bradykinin, prostaglandins, serotonin, histamine).[11] These effects, which seem to be specific to the different groups of nociceptors, cause some of the features of the hyperalgesia produced by pathological processes. Indeed, primary hyperalgesia, which by definition occurs at the site of tissue damage and can also be produced by mechanical and thermal stimuli, accounts for much of the peripheral sensitisation of nociceptors, although some sensitisation seems be due to central mechanisms of hyperexcitability.

Sleeping nociceptors

Another important finding is that many nociceptors cannot normally be activated and become excitable only under pathological conditions such as inflammation. These are the silent or sleeping nociceptors, first described by Schaible and Grubb[12] in joint tissue. These nociceptors have subsequently been found in visceral and cutaneous tissue. This simple example illustrates how classification can be too rigid. The terminals of nociceptors and their microenvironment have been described as a jungle through which a scientist has difficulty in forging a route to find the secrets contained within.

In 1997 Carlton and Coggeshall,[13] summarised the receptors found on afferent fibres (panel), which they described by anatomical, electrophysiological, and pharmacological approaches. The panel includes ligands of neuronal origins contained and released into the periphery by nociceptive fibres and ligands with non-neuronal origins. This long list is in fact even more complex since many receptors can also be separated into subtypes.

Pharmacology

I provide only a brief review of the pharmacological features of peripheral nociception; several in-depth reviews have been published.[13–17] Various chemicals (bradykinin, histamine, serotonin, prostaglandins, potassium, protons) are released into damaged tissue cells of vascular origins (platelets, neutrophils, lymphocytes, and macrophages) and also by mast cells. When injected by the intradermal route, some of these chemicals induce nociceptive reactions and can modify the activity of nociceptors either by direct activation or by sensitisation to different types of stimuli, such as thermal, mechanical, and chemical. Bradykinin, for example, a powerful algogenic substance released from kininogens in the circulation activates nociceptors in a way that is dependent on protein kinase C and calcium and sensitises nociceptors by means of the activation of postganglionic sympathetic neurones which then produce prostaglandin E2.

Several peptides are contained within primary afferent fibres and their profile can be altered by sustained stimuli or by damage to the nerve.[16,18] Although the roles of several of these peptides are unclear (galanin, somatostatin, cholecystokinin, vasoactive intestinal peptide), others such as substance P and calcitonin gene related peptide can be released into the periphery via the classic axon reflex. The role of substance P in neurogenic inflammation has been clearly shown. The peptide causes a degranulation of mast cells and thus the release of histamine, vasodilatation, and plasma extravasation with the subsequent release of other algogens (bradykinin, serotonin) and the activation of other inflammatory cells (macrophages, monocytes, and lymphocytes). Furthermore, substance P is able to induce production of nitric oxide, another vasodilator from the endothelial layer of blood vessels.

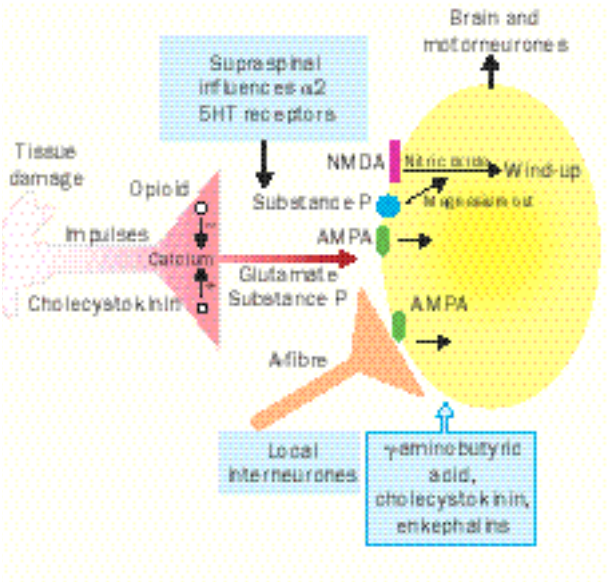
Apart from these substances which, in broad terms, are liberated soon after tissue damage, other factors such as the cytokines (interleukins, interferon, and tumour necrosis factor), are released by phagocyte cells and cells of the immune system and have an important role in the inflammatory process. The role of bradykinin in the sequence of events that lead to the production of the cytokines is well established.[11] Some of these agents are powerful inflammatory mediators that can activate sensory neurones through different mechanisms, some of which include the sympathetic nervous system.[19]

Nerve growth factor has a key role not only in the development of sensory and autonomic neurones, but also in the processes of nociception.[20] This factor, which is upregulated by the process of inflammation, is produced in the periphery by fibroblasts and Schwann cells and then increases the excitability of nociceptors which leads to hyperalgesia. Various central and peripheral mechanisms have been postulated as a basis for these actions of nerve growth factor.[8] The production of antagonists for the receptors—the tyrosine kinase family—has the potential to provide a pharmacological target for the production of new analgesics to reduce the effects of nerve growth factor.

The prostaglandins and probably also the leukotrienes are weak algogens but play a major part in the sensitisation of receptors to other substances. The basis for the analgesic actions of the non-steroidal anti-inflammatory drugs (NSAIDS) is their ability to prevent the production of the prostaglandins. Activation of phospholipase A2 leads to the production of arachidonic acid from membrane phospholipids, which results in the subsequent transformation to thromboxane, the prostacyclins, and the prostaglandins. The main action of NSAIDs is to inhibit the activity of cyclo-oxygenase, the enzyme responsible for the synthesis of the prostaglandins, but this action leads to the production of side-effects. Great hope has been inspired by the characterisation of two isoforms of the enzyme cyclo-oxygenase 1 and 2 (COX-1 and COX-2),[21–23] produced by different genes but with a structural homology of about 60% of the aminoacid residues. However, both the location and regulation of the two isoforms are different. COX-1 is a constitutive enzyme found in endothelial cells, platelets, the mucosa of the stomach, and in the kidney; it is involved in the processes of vascular homeostasis and the regulation of gastric acid and the kidney. Under normal conditions, COX-2 is not found in tissues such as prostatic and lung tissue but can be produced by different signals from hormones, growth factors, mitogens, inflammatory mediators (cytokines), and endotoxins (lipopolysaccharides). Thus, the expression of COX-2 will link prostaglandin synthesis to inflammatory processes. The synthesis of selective inhibitors of COX-2 is an important pharmacological goal in terms of the production of NSAIDs without the side-effects of the present agents. Some laboratories have produced inhibitors of COX-2 the first of which are already available for use in human beings. The anti-inflammatory and antinociceptive effects of these agents seem to be equivalent to those of mixed inhibitors, but the main advantage of the new inhibitors will be the absence of gastric side-effects in patients with chronic pain of inflammatory origins.

Molecular and genetic approaches have lead to a revolution in physiological and pharmacological research in pain, especially at the peripheral level. The cloning of various receptors have advanced our understanding of the mechanisms of transduction and sensitisation. Major breakthroughs include the first cloning of a receptor for capsaicin, the active ingredient of chilli peppers,[24,25] and the receptors for the purines, notably the P2X3 (a ligand-gated ion channel triggered by ATP) which is selectively expressed by small-diameter sensory neurons.[26] Another is the acid-sensing ion channel that is rapidly activated by conditions of acidity below pH 6·5[27] and the tetrodotoxin-resistant sodium channel.[28] Inflammatory mediators, such as prostaglandin E2, adenosine, and serotonin facilitate transmission of action potentials by modification of the voltage threshold of several ion channels, including the tetrodotoxin-resistant sodium channel. Research is in progress for the production of sodium-channel blockers with greater specificity than existing agents so that they would not have the cardiac and central-nervous-system depressant effects that limit the use of present agents.

Thus, it is clear that there are many encouraging approaches that could lead to the production of peripherally acting analgesic drugs that do not pass the blood brain barrier and so lack central side-effects. Another encouraging possibility is that the biological prediction of the structure of macromolecules will allow the three-dimensional structures of receptors to be elucidated, which in turn could lead to the rational development of agonists and antagonists with great specificity and few side-effects. Many substances with neuronal and non-neuronal origins act at the peripheral



Interactions between different excitatory and inhibitory systems in the spinal cord
Adapted with permission from Dickenson[31]

level to modulate the activity of nociceptors and various interactions can occur between these mediators. So would the modulation of only one of these substances sufficient to alter the level of pain in the periphery—could there be a magic bullet with peripheral actions only? This option is unlikely on the basis of current pharmacological information. Only an in-depth analysis of the physiopathology of the different syndromes that originate from peripheral processes can guide a clinician in prescribing the most effective substance. An alternative approach that seems more likely is the production of an analgesic with mixed peripheral actions, so that it acts on different receptor types, or perhaps a move towards a systematic analysis of the effects of administration of several agents.

From spinal cord to brain

The spinal mechanisms of nociception have been studied extensively.[29,30] The detailed characteristics of the neurones of the spinal cord implicated in the transmission of painful messages have been described as the segmental and supraspinal mechanisms that can modulate the information transferred to the brain. But yet again, it is the pharmacological characteristics that attract the attention of research groups. Unfortunately, as with the periphery, the dorsal horn of the spinal cord contains many transmitters and receptors both identified and putative including: several peptides (substance P, calcitonin gene related peptide, somatostatin, neuropeptide Y, and galanin); excitatory aminoacids (aspartate, glutamate); inhibitory aminoacids (γ-aminobytyric acid [GABA] and glycine); nitric oxide; the arachidonic acid metabolites; the endogenous opioids; adenosine; and the monoamines (serotonin and noradrenaline).[11,31,32] This list indicates that there are diverse therapeutic possibilities for the pharmacological control of the transmission of nocicepetive information to the brain. I will address the options related to substance P and glutamate. Since release of substance P is blocked by morphine at the trigeminal level,[33] one would expect this peptide to be one of the principal neurotransmitters released by primary afferent nociceptive fibres at the level of the spinal cord. Although some, although not all, studies show antinociceptive effects of antagonists of the receptor for substance P, the neurokinin-1 receptor in animals, the clinical studies have been disappointing. Furthermore, Mantyh and colleagues' finding[34,35] of stimulus-evoked internalisation of the neurokinin-1 receptor in the spinal cord raises a number of questions. If there is a mismatch at several sites between the localisation of substance P and the receptor, why is it that after the internalisation caused by a noxious stimulus, the receptors do not return to the neuronal membrane until 1 h later? Why does morphine not alter the internalisation, whereas agonists at the GABA B receptor do?

Further questions emerge when one considers the conflicting findings of studies based on different experimental approaches. Thus, Mantyh and colleagues[35] found that selective destruction of the neurones in the superficial spinal cord that express the neurokinin-1 receptor lead to a substantial reduction in allodynia and hyperalgesia induced by inflammation and nerve injury. These findings do not accord with the genetic studies that showed knockout of the preprotachykinin gene or the neurokinin-1 gene lead to only minor changes in the mice. Thus, whether substance P is indeed an important factor in spinal transmission is not known. Perhaps it is not surprising that clinical studies with antagonists of substance P in migraine, pain after dental surgery, in rheumatoid arthritis, and posthepatic neuralgia have been unsuccessful.[36]

The excitatory aminoacids (notably glutamate) are not only the major class of excitatory transmitter in the central nervous system, but are released by primary afferent fibres and have an important role in the spinal mechanisms of pain transmission. Various receptors and subtypes are involved at the spinal level (AMPA, metabotrophic, kainate), but it is the N-methyl-D-asparate (NMDA) receptor that has attracted most attention.

The NMDA receptor is important in the synaptic events that lead to central sensitivity and hyperalgesia.[31,32] The release of peptides such as substance P into the spinal cord on afferent stimulation removes the magnesium block of the channel of the NMDA receptor and thus allow glutamate to activate the NMDA receptor in a range of persistent pain states. This process, unlike other spinal changes, leads to the generation of spinal hypersensitivity and amplification of peripheral inputs. Furthermore, activation of the NMDA receptor leads to an entry of calcium into the neurone which can then produce other mediators from spinal neurons by increasing the activity of enzymes. For example, nitric-oxide synthase generates a gas, nitric oxide, that acts as a freely diffusible transmitter and in a complex way exacerbates the noxious transmission.[37] The entry of calcium can also activate phospholipases and lead to the spinal production of prostanoids, an effect that may be the basis for the central actions of NSAIDs.[38]

The figure shows some of the other possible targets at the spinal level for the control of the transmission of nociceptive messages. These include GABAergic systems, antagonists of cholecystokinin, the inhibitors of the enzymes that degrade the endogenous opioids, and agonists that act at the opioid receptors.[11,39] Morphine exerts a powerful depressive action directly in the spinal cord,[40,41] which is the basis for the clinical applications of

spinal routes of opioid analgesia. Morphine also acts at the brainstem and midbrain levels to alter the activity of descending control systems that are projected from these sites to the spinal cord. Studies on the direct and indirect spinal actions of morphine started 30 years ago and have emphasised this important site in the production of analgesia. However, few studies have examined supraspinal mechanisms in vivo. The importance of the spinal mechanism (direct or indirect) compared with the actions mediated by supraspinal structures is not known. Numerous regions of the brain are rich in opioid peptides and the mRNAs for the opioid receptors.[42] The supraspinal actions of opioids are commonly underestimated and may have a key role in the analgesic effects of systemic morphine. Finally, for the sake of completeness, Stein's[43] finding of a peripheral antinociceptive site of action of opioids in hyperalgesic inflammatory conditions in mice indicates that the local application of opioid agonists or the systemic administration of agonists that do not cross the blood brain barrier could provide analgesia in certain clinical situations. Some clinical studies lend support to this premise, but it is too early to state definitively that this technique has therapeutic value.

The figure also shows the involvement of descending pathways that use serotonin and noradrenaline to control nociception.[44] Many experimental studies have shown that serotonin is important in pain, yet apart from in headache, the production of many analgesics acting on serotonin (5HT) receptors has been confounded by the number of different types and subtypes of the receptors. By contrast, the pharmacology of the systems that use noradrenaline as a transmitter are much simpler and, so, agonists at the α2 adrenoceptors, such as clonidine, have substantial analgesic effects in animals and lesser but still obvious effects in man. However, the agonists at the α2 receptor possess important side-effects and so adrenergic receptor agonists which have improved potency and selectivity are the focus of research based on subtypes of the receptors.

The myriad substances implicated at the spinal level in the transmission and modulation of pain messages leads to the same question that arises at the peripheral level. Is it realistic to expect the development of a single magic bullets or would it be possible to produce one molecule with dual pharmacological actions or use a combination of drugs (multimodal analgesia) to elicit synergistic or additive actions of the combination? Many examples exist of this approach, such as the association of morphine with agonists at the α2 receptor or with antagonists at the cholecystokinin and the NMDA receptor. This type of approach has the dual advantages of improved effects and fewer side-effects through use of lower doses of each agent. This example is only one among many combinations other than with morphine. Although this approach is less spectacular than the magic bullet, it could be more beneficial to the patient and could be used as general principle in this research.

Multipe ascending pathways to the brain

Combinations of electrophysiological and anatomical techniques are increasingly used to identify neurones at the origins of the main ascending pathways and also their termination zomes at higher centres of the brain.[30,45] These neurones at the origins of the ascending pathways are located in superficial and deep laminae of the dorsal horn. Although some still hold to the idea of specificity of pain pathways,[46] this concept is highly controversial. There are multiple pain pathways, including the classic routes (spinothalamic tract, the different components of the spinoreticular tract), the spinocervicothalamic tract, the postsynaptic dorsal column fibres, and the visceral nociceptive tracts that run in the posterieur columns. Villanueva and Bernard[47] have described how the several ascending pathways, quite different from each other, project to the mesencephalon and the diencephalon.[45] In addition, the activation of long and short propriospinal circuits cannot be excluded, and it must be underlined that in these studies of ascending pain pathways, mechanisms of chronic pain are frequently explained on the basis of studies on nociceptive in acute pain, without taking into account spinal-cord readjustment (plasticity) after the lesions. Systematic studies of patients with different spinal lesions and disorders that can be undertaken with current imaging techniques will provide new information and a better understanding of the physiopathological features of these ascending pathways.

On the basis of this multiplicity of pain pathways, it is not surprising that positron emission tomography or functional magnetic resonance imaging have revealed activation of various brain regions during acute pain.[48] Although the results are fairly consistent in healthy patients, controversies have arisen from studies in patients with chronic pains. These data tend to support the idea that pain is not a unique consequence of impulses in specific, unidirectional hardwired lines that originate in the periphery and terminate in the central nervous system. We are still in the early stages of the exploration of the human brain, but controlled studies will allow the identification of the regions of the brain that are involved in the different components of pain. At these levels in the brain the pharmacological approaches falter, which is the main reason for the major thrust to target new analgesics at the spinal and peripheral levels.

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