Naturally Occurring Antinociceptive Substances from Plants

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Despite the progress that has occurred in recent years in the development of therapy, there is still a need for effective and potent analgesics, especially for the treatment of chronic pain. One of the most important analgesic drugs employed in clinical practice today continues to be the alkaloid morphine. In this review, emphasis will be given to the important contribution and the history of *Papaver somniferum*, *Salix species*, *Capsicum species* and *Cannabis sativa* in the development of new analgesics and their importance in the understanding of the complex pathways related to electrophysiological and molecular mechanisms associated with pain transmission. Recently discovered antinociceptive substances include alkaloids, terpenoids and flavonoid. Plant-derived substances have, and will certainly continue to have, a relevant place in the process of drug discovery, particularly in the development of new analgesic drugs.

INTRODUCTION

The use of medicinal plants is a traditional form of providing relief from illness and can be traced back over five millennia in several civilizations. Over the years, natural products have contributed enormously to the development of important therapeutic drugs used currently in modern medicine (Cragg et al., 1997; De Smet, 1997; Shu, 1998). The potential of higher plants as sources for new drugs is still largely unexplored. Among the estimated 250,000 plant species existing world-wide, only a small percentage have been investigated phytochemically, and the fraction submitted to biological or pharmacological screening is even smaller (Hamburger and Hostettmann, 1991). The search for new pharmacologically active agents obtained from plants has led to the discovery of many clinically useful drugs that play a major role in the treatment of human disease. About 25% of all available modern drugs are derived directly or indirectly from higher plants (Farnsworth and Bingel, 1997; reviewed De Smet, 1997). In spite of the progress that has taken place in recent years in the development of therapy, the medical community still urgently needs effective and potent analgesics, especially for chronic pain. Thousand of patients with intense and unrelenting pain, such as that resulting from cancer or injury, have to depend on morphine, despite its well-known side effects (Shu, 1998). This has renewed the interest of the major pharmaceutical companies in higher plant-derived secondary metabolites as part of the search for new clinically useful drugs.

In this review article we will focus on the contribution of higher plants to the development of modern analgesic drugs and to our understanding of the complex mechanisms involved in pain transmission. Emphasis will also be given to the current advance of naturally occurring secondary metabolites derived from plants as a potential source for the development of new clinically relevant analgesic drugs.

BASIC MECHANISMS INVOLVED IN PAIN TRANSMISSION

Pain transmission is a mechanism that involves a very complex interaction of peripheral and central structures from the skin surface to the central cerebral cortex (Fürst, 1999). In accordance with the International Association for the Study of Pain, pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage (Merskey and Bogduk, 1994). In addition, some disorders commonly occur in patients who experience pain such as hyperalgesia (extreme sensitivity to pain stimuli), allodynia (pain in response to a non-noxious mechanical stimuli), and hyperesthesia (abnormal sensitivity to sensory stimuli) (Besson, 1999). There are several pain types, namely ‘nociceptive’, ‘neuro-
genic’, ‘neuropathic’ and ‘psychogenic’, which are associated with a stimulation of nociceptors, damage to neuronal tissue, dysfunction of a nerve, or psychological factors, respectively (Fürst, 1999; Millan, 1999).

In terms of duration, the pain episode might be transient, acute or chronic. In the transient type, the activation of nociceptive transducers is elicited in the absence of any tissue damage. In the acute type, injury and activation of nociceptors at the site of local tissue damage occurs. Chronic pain is commonly triggered by an injury or disease, but may be perpetuated by factors other than the cause of pain (Loeser and Melzack, 1999). The acute pain associated with tissue injury may last for less than 1 month, but sometimes it lasts longer than 6 months. Preclinical studies have shown that neuronal expression of new genes (the basis for neuronal sensitization and remodelling) occurs within 20 min of injury. Chronic pain can initiate long-term behavioural and histological changes within a day or so after interventions such as transient nerve ligation. An emergent clinical literature also suggests that acute pain may rapidly turn into chronic pain (Carr and Goudas, 1999).

Our understanding of the pain processes has progressed dramatically in recent years, in great part due to clarification of the mechanisms underlying the afferent fibre physiology and synaptic processing in the dorsal horn of the spinal cord (reviewed in Dray, 1997; Besson and Chaouch, 1987; Grubb, 1998; Fürst, 1999; Millan, 1999; Wall and Melzack, 1999). This progress has been made possible by the use of multiple experimental approaches, including behavioural studies, in vivo and in vitro electrophysiology, anatomical and mainly molecular biological techniques (Grubb, 1998; Fürst, 1999; Millan, 1999). Notwithstanding these advances, the mechanisms of pain continue to be incompletely understood. Thus, a complete review of the topic is naturally beyond the scope of this article. Several complete reviews on the control of pain processes have been published recently (Kolzenburg, 1997; Grubb, 1998; Besson, 1999; Millan, 1999; Urban and Gebhart, 1999). Here, we will briefly summarize some of the main transmitters and the key mechanisms that can control or modulate pain transmission.

Noiception is a mechanism by which noxious stimuli are transmitted to the central nervous system (CNS) (Fürst, 1999). The nociceptors are pain-sensitive neurones located in the skin, vessels, muscles, fascia, joints and viscera. They are predominantly myelinated (Aδ) or non-myelinated (C) fibres activated by noxious stimuli (mechanical, heat, cold and chemical) that conduct these signals to the CNS (Grubb, 1998; Russo and Brose, 1998; Besson, 1999; Fürst, 1999; Millan, 1999). All tissues, with the exception of the neurophil of the CNS, are innervated by afferent fibres, although their properties differ markedly depending on whether they are somatic afferents (innervating skin, joints, muscles) or visceral afferents (innervating cardiovascular or respiratory tissue, the gastrointestinal tract, or renal and reproductive systems) (Dray and Perkins, 1997). The cell bodies of these nociceptors lie within the dorsal root ganglion adjacent to the spinal cord (see Fig. 1). The primary nociceptors make a synapse in the dorsal horn with second-order neurones, predominantly within Lamina II (substantia gelatinosa) of the spinal cord. The second-order neurones cross the spinal cord to ascend in the spinothalamic tract with their terminal fibres predominantly localized in the thalamus. In the thalamus, third-order neurones send subsequent axons through the internal capsule to the somatosensory cortex where discrete localization of the noxious stimuli occurs, or send axons to the anterior cingulate gyrus where they become involved in the emotional components of pain. The pain pathways described above represent the classical route, but there are other possible pathways involving different nervous structures (see Besson, 1999). In addition, the spinothalamic tract appears to arborize into the midbrain and rostral pons, synapsing on nuclear complexes, including the nucleus raphe magnus (NRM) and the nucleus reticularis gigantocellularis (NRG), both of which seem to be involved in some descending regulation of the activity within the second-order neurones (Russo and Brose, 1998; Fürst, 1999; Besson, 1999; Millan, 1999).

The neurotransmitters involved in this descending noxious inhibition (such as endogenous opioids, serotonin, noradrenaline) all seem to inhibit the firing of the second-order neurones in the presence of noxious stimuli (Russo and Brose, 1998; Fürst, 1999; Millan, 1999). However, noiception is not a uniform sensation, and both the quality of pain and the initiation of protective responses are determined by many factors within the spinal cord and in higher brain structures involved in the integration and modification of nociceptive signals.

The direct or indirect action of chemical mediators, such as arachidonic acid metabolites (prostaglandins and leukotrienes), peptides (enkephalins, enkephalin gene related peptide, galanin, cholecystokinin, vasoactive intestinal peptide), serotonin, acetylcholine, cytokines, nerve growth factor, glutamate, nitric oxide, ATP, ADP, adenosine and protons, among others, which can be produced or released following tissue injury or by exogenous irritants (formalin, acetic acid, capsaicin, some

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Venoms, etc.), are responsible for the multiplicity of events that occur during pain transmission, in both the peripheral and central nervous systems (Collis and Hourani, 1993; Belfrage et al., 1995; Dray, 1997; Sawynok, 1998; Fürst, 1999; Besson, 1999; Millan, 1999).

There are several important sources from which pathophysiologically mediators are generated: damaged tissues, the vasculature, immune cells and surrounding tissues, sensory and sympathetic nerves. These mediators can act via a multiplicity of receptors which are widely distributed through central and peripheral nerves, many of which are coupled to heterotrimeric G-proteins and associated with formation of multiple second messengers, such as protein kinases A, C and G, cAMP, cGMP and mobilization of intracellular calcium. Other neurotransmitters, such as excitatory amino acids, acetylcholine (acting at the nicotinic receptor), directly activate ion channels, and in turn control the membrane ion permeability (Wood and Docherty, 1997; Millan, 1999). Several factors, including physical damage to tissues, exposure to some inflammatory mediators, such as prostaglandin E₂, bradykinin, substance P, histamine, adenosine, serotonin, are known to cause sensitization of nerve ending nociceptors to mechanical and thermal stimuli (Besson, 1999; Millan, 1999).

In view of such a multiplicity of mechanisms known to modulate pain transmission, the occurrence in the literature of so many compounds that can directly or indirectly modulate pain transmission is not surprising. However, few of them reach sufficient selectivity of action or potency and consequently have little clinical interest. Thus, most reported analgesic drugs produce their effects by modulating the release of endogenous analgesic mediators or inhibiting allogenic neurotransmitters, through either pre- or post-synaptic mechanisms at central and peripheral levels (Dray, 1997; Grubb, 1998; Sawynok, 1998; Besson, 1999; Fürst, 1999; Millan, 1999; Urban and Gebhart, 1999).

PLANTS AND PLANT-DERIVED COMPOUNDS THAT HAVE CONTRIBUTED TO THE DEVELOPMENT OF MODERN ANALGESIC DRUGS AND TO THE UNDERSTANDING OF THE MOLECULAR MECHANISM OF PAIN TRANSMISSION

**Papaver somniferum**

There is general agreement that the Sumerians cultivated poppies (*Papaver somniferum* L. (Papaveraceae)) and isolated opioid from their seed capsules at the end of the third millennium BC, and that it may have been employed as an euphoriant in religious rituals (Brownstein, 1993). Opium derives from the latex obtained by incision of the unripe capsules of *P. somniferum*, dried partly by spontaneous evaporation or by artificial heat. Opium contains about 25 alkaloids, including morphine, codeine, thebaine and papaverine (Trease and Evans, 1978). In 1805, the German pharmacist Serttüné isolated the active ingredient in opium and named it ‘morphine’ (codeine was isolated from opium a few years later) (Brownstein, 1993; Benyhe, 1994). Morphine was the first alkaloid to be discovered, and its isolation, therefore, was a breakthrough in organic chemistry (Benyhe, 1994).

Apart from its presence in *P. somniferum*, morphine has been shown to be present in milk, in cerebrospinal fluid, and also in nervous tissue extracts of animals. Evidence has been put forward that suggests that biosynthetic pathways for morphine exist in animal and even human tissues such as liver, blood and brain (Benyhe, 1994). Morphin began to be used in the 1850s for minor surgical procedures, for postoperative and chronic pain, and as an adjunct to general anaesthetics. In 1939, meperidine, the first opiate with a structure altogether different from that of morphine was discovered. Later in 1942, naltorphine the first opioid receptor antagonist was produced. Hughes et al. (1975) isolated, from brain extracts the first endogenous opioids, Met- and Leu-enkephalin (see Scheme 1 for details).

The pharmacological properties of morphine are quite complex and can vary depending upon the dose, site of action and administration route, and wide variation among animal species has been reported (Benyhe, 1994). The most relevant characteristic of morphine is its property of modulating the perception of pain, resulting in an increase in the threshold of noxious stimuli. Antinociception induced by morphine is now known to be mediated via activation of membrane opioid receptors, and therefore it can be inhibited by opioid receptor antagonists, e.g. naloxone. Also, some well-known undesirable side effects of morphine such as euphorogenic properties, inhibition of gastrointestinal transit, constipation, suppression of appetite, hypothermia, bradycardia and urine retention seem to involve receptor-mediated actions (Benyhe, 1994).

There are, so far, three known ‘classical’ types of opioid receptors, namely μ, δ and κ, and the genes encoding these receptors were cloned in the early 1990s (Chen et al., 1993; Evans et al., 1992; Kieffer et al., 1992, Yasuda et al., 1993). Recently, an ‘orphan’ receptor was
identified and has been named ORL₁ (Mollereau et al., 1994). These receptors possess a common general structure, the seven transmembrane domains and when activated by opioids, couple to pertussis toxin-sensitive Gᵢ proteins to inhibit adenylyl cyclase and/or voltage-gated Ca²⁺ channels, or stimulate an inwardly rectifying K⁻ conductance (Dhawan et al., 1996). Endogenous opioid ligands include enkephalins, dynorphins, endorphins, endomorphins and nociceptin, which are derived, in mammals, from three larger precursors: proenkephalin, pro-dynorphin, pro-opiomelanocortin and pro-nociceptin (Dhawan et al., 1996; Meunier, 1997; Stone et al., 1997) (see Scheme 1 for details).

Despite tremendous efforts in the search for safe, efficacious and non-addictive opioids for pain treatment, morphine remains the most valuable painkiller in contemporary medicine (Mathes et al., 1996). The opioid receptors are located at various levels in the pain transmission pathways, for example spinal cord, mid-brain and thalamus, and peripheral sensory-nerves fibres. Thus, the activation of these receptors has been associated with spinal, supraspinal and peripheral analgesia (Fürst, 1999).

Antinociceptive effects of opioids. Animal studies: The analgesia produced by morphine is antagonized by a μ-, but not by a δ-opioid receptor antagonist, suggesting that its effect might be mediated primarily by stimulation of μ-opioid receptor (Suh and Tseng, 1990). These data have been confirmed by a molecular biology approach. In mice lacking the μ-opioid-receptor (MOR) gene the analgesic effect of morphine was abolished, as well as reward effects and withdrawal symptoms, suggesting that MOR gene product is the molecular target of morphine in vivo and that it is a mandatory component of the opioid system for morphine’s action (Mathes et al., 1996).

Human studies and uses: Morphine is not only the oldest, but is still the most effective drug for the management of severe pain in clinical practice (Benyhe, 1994). Opioids are unequivocally indicated in the management of severe acute pain and moderate-to-severe pain associated with cancer. There is increasing acceptance of the role of opioids in the management of recurring acute pain, chronic non-malignant pain of organic origin, and severe neuropathic pain (Cherny, 1996; Portenoy and Lesage, 1999). There is a significant degree of individual variability in the opioid-induced inhibition of pain. Preclinical studies have suggested a significant degree of variability which seems to be influenced by the subject’s genotype, and clinical studies also suggest that some variability could be related to the severity of pain (Elmer et al., 1998). Unfortunately, opioid analgesics drugs produce some undesirable side effects, namely dysphoria, respiratory depression, constipation and nausea. Various strategies can be used to overcome these side effects, such as the use of alternative routes of administration and combination with other agents that increase the opioid effect (Meert, 1996).

Structure-activity relationships. The search for a safe, orally active and non-addictive analgesic compound based on the opiate structure, has led to the investigation of structure-activity relationships that guide new developments. An interesting analogue of morphine is another natural alkaloid: codeine, which is a methyl ether of the phenolic group (Fig. 2). Codeine is only 0.1% as active as morphine, and for this reason it is classified as a weak opioid (Brasseus, 1997). It is widely used as an analgesic and antitussive.

The acetylation of the two hydroxyl groups gives the diacetyl ester or heroin that is two times more active than morphine. As heroin has two masked polar groups, it is more efficient in crossing the blood–brain barrier. It is important to note that heroin, like marijuana, continues to hold a place among the probes used in brain research (Barinaga, 1992).

Important compounds are obtained by varying the substituent group on the nitrogen atom. When the allyl group replaces the methyl group, nalorphine is obtained from morphine and naloxone is obtained from oxy-morphine (Fig. 3). Nalorphine acts as an antagonist at the μ and δ receptors, but it acts as a weak agonist at the κ receptor, and thus gives slight analgesia. However, nalorphine has hallucinogenic side-effects. Naloxone is an antagonist at the three opioid receptors. This compound is used to elucidate the possible roles of opioids in response to stress (Ainsah et al., 1999), to study the role of opioid pathways in the mechanism of action of antidepressant drugs that could be of relevance in the development of novel antidepressants (Burnett et al., 1999), among other types of studies.

The modifications altering the structure of morphine lead to the observation that the aromatic ring and the basic nitrogen atom that interacts with the opioid receptor

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**Figure 2.** Molecular structure of morphine, codeine and heroin.

**Figure 3.** Molecular structure of antagonist opioid, nalorphine and naloxone.
Cannabis sativa

Throughout history, Cannabis sativa L. has been used as a natural therapeutic herb. Usage of marijuana for medical purposes can be traced back 5000 years. In 2737 BC the Chinese Emperor Shen Nung published a monograph describing the use of Cannabis in the treatment of several diseases (Lemberger, 1980). In 1842, O’Shaughnessy, an army physician in India, published an extensive treatise on the use of cannabis in various medical conditions including as an analgesic. As a result, Cannabis was introduced into European medicine and subsequently into other areas of Western medicine, including the United States. Preparations such as tincture and extract of Cannabis were recognized for a long time as official drugs and were listed in the US Pharmacopoeia from 1850 until 1942. However, although Cannabis sativa remained in the Pharmacopoeia until 1942, its medical use was essentially abolished in 1937, when the Marijuana Tax Act was introduced (Lemberger, 1980). The Cannabis plant contains a complex mixture of substances that include at least 60 different cannabinoids, many of which have been shown to present pharmacological activities (Burstein, 1997).

Until 1964, it was generally assumed that the active principles of Cannabis were an unidentified mixture of isomers of tetrahydrocannabinols (THC) (Mechoulam, 1970). In 1964, a crystalline derivative was prepared from which pure (–)-Δ⁹-trans-THC could be obtained, apparently for the first time; its structure and stereochemistry were elucidated, and a partial synthesis was achieved (Gaoni and Mechoulam, 1964; Mechoulam, 1970) (see Scheme 2).

When administered to humans, THC produces a wide range of effects, including increase in pulse rate, decreased blood pressure, muscle weakening, increased appetite, euphoria followed by drowsiness, depersonalization, altered time sense and decreased memory reflection, hearing becoming less discriminative and visual signals becoming sharper but distorted (Hirst et al., 1998). The effects of THC at the biochemical level were virtually unknown until recently. Earlier pharmacological studies have suggested that THC and other active cannabinoids might act at specific receptor sites (see Hirst et al., 1998). The CB₁ and the CB₂ receptors for cannabinoids were discovered by Matsuda et al. (1990) and Munro et al. (1993) respectively, and were confirmed as being G-protein-coupled receptors that inhibit adenylate cyclase activity. The CB₁ receptor is expressed in both the periphery and CNS, while CB₂ is only expressed in the periphery (Hirst et al., 1998). Endogenous ligands for CB₁ (called anandamide) and for CB₂ (palmitoylethanolamine) receptors have been described (see Scheme 2) (Devane et al., 1992; Facci et al., 1995). The low potency and the high lipophilicity of the cannabinoids has led to the search for synthetic compounds with cannabimimetic activity (Hirst et al., 1998) (see below).

Antinociceptive effects of cannabinoids. Animal studies: Marijuana, hashish and other preparations have been used for centuries for the relief of pain. The major active constituent of Cannabis, THC, has been shown to possess antinociceptive properties when assessed in several experimental models, and this effect is attenuated by a CB₁ receptor antagonist (Formukong et al., 1989; Hirst et al., 1998). Its potency seems to depend on the route of administration (Hirst et al., 1998; Martin, 1985). Other constituents of Cannabis have also been shown to exhibit antinociceptive action. The ethanol extract of the herb, and the cannflavones isolated from it, have been shown to possess analgesic action (Formukong et al., 1989). CB₁ agonists suppress formalin-induced c-fos expression in rat spinal cord and their intrathecal injection produces antinociception, indicating that cannabinoids inhibit spinal processing of noxious stimuli (Hirst et al., 1998). The central actions of cannabinoids have been demonstrated in rats after intracerebroventricular injection under conditions in which they were undetectable in the spinal cord (Howlett, 1995; Martin et al., 1993). Seven anatomical sites have been identified through which cannabinoids might produce their pharmacological effects, including periaqueductal grey, the

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with the receptor and are correlated with the inhibition of adenylyl cyclase. Not all of these features are necessarily required. The other review analyses the development of the cannabinoid antagonists (Barth and Rinaldi-Carmona, 1999). The authors show that the first approach to design of \( \Delta^8 \)-tetrahydrocannabinol analogues was to modify the structure of THC but that this research had been disappointing. However, a new family of cannabinoid ligands, 1,5-diphenylpyrazoles, seems to be important to the production of valuable therapeutic agents. Thomas et al. (1998) have studied the SAR data on a series of 1,5-diphenylpyrazoles, proposing a pharmacophoric alignment of the diarylpyazole antagonist with THC. Pop (1999) has also studied natural and synthetic cannabinoids, leading to a better understanding of the role of these compounds in physiological processes and their potential uses for medicinal purposes. Anandamide, an endogenous cannabinoid, has led to important developments in cannabinoid research in recent years.

A recent paper of Khanolkar and Makriyannis (1999) determines some SAR for anandamide for the CB\(_1\) receptor and indicates the structural requirements of the two major molecular fragments: the polar ethanolamido head group and the hydrophobic arachidonyl chain. Barth and Rinaldi-Carmona (1999) have pointed out, in a review article, that ‘millennial therapeutic use of \( C. \ sativa \) may well lead to modern medical science based on \( Cannabis \) antagonist’. We would like to emphasize that this concept could be applied to several medicinal plants.

**Capsicum sp.**

The *Capsicum* species belong to the family of Solanaceae and originated in Central and South America. About 20 *Capsicum* species are included in this family. They are distributed throughout the world, but only five species are widely cultivated: *C. annuum*, *C. frutescens*, *C. chinense*, *C. pendulum* and *C. pubescens*.

The oldest documented use of *Capsicum* is in Mexico, where scientists have found evidence of peoples who consumed peppers as early as 7000 BC. It seems that they are among the oldest cultivated plants in the world (5200–3400 BC) (Szallasi and Blumberg, 1999). The study of pungent principles began in the 1810s utilizing the names “capsicol”, “capsicin”, “capsacutin”, etc., but only much later was the active principle of *Capsicum* species isolated by Thresh (1846) and named capsaicin (Scheme 3). The exact chemical structure of this compound was only determined after half a century by Nelson (1919). Capsaicin was recognized as the major component, constituting about 70% of the total pungent acid amides contained in plants belonging to the *Capsicum* species, while dihydrocapsaicin, an analogue of capsaicin (capsaicinoid), amounted to 30% or less (Suzuki and Iwai, 1984).

Since its discovery, the medicinal use of hot pepper has varied greatly. The native Americans used *Capsicum* to cure cramps, diarrhoea and dyspepsia. Other folk medicinal uses of capsaicin include appetite stimulation, treatment of gastric ulcers, rheumatism and restoration of hair growth (Szallasi, 1995). The antinociceptive properties reported recently (Jancso and Lynn, 1987; Lynn 1990; Szolcsanyi, 1991; Szallasi and Blumberg, 1993) for capsaicin are not novel: toothache was treated with capsaicin in the 19th century (Szallasi, 1995).
In the 1980s, Jancso proposed that capsaicin might act on a ‘pain receptor’ to produce its neural effects. Once its therapeutic properties had been explored, curiosity about this receptor grew. In 1975, resiniferatoxin (RTX), an extremely irritant diterpene, was isolated from the latex of *Euphorbia* by Hergenhahn who proposed its action as an ultrapotent capsaicin analogue (Hergenhahn *et al.*, 1975). Due to the significant differences among the potencies for several biological responses and chemical structures of compounds (capsainoids and RTX), the receptor at which these ligands interact was referred to as the ‘vanilloid’ receptor. Similarly, the compounds which act on this receptor were named ‘vanilloids’. Another piece of evidence for the existence of a vanilloid receptor was the development of capsazepine (Urban and Dray, 1991), a selective competitive antagonist of the effects caused by RTX and capsaicin. Using a [³H]resiniferatoxin autoradiographic method, Szallasi (1995) demonstrated the heterogeneity of vanilloid-sensitive sensory neurones in several species (rat, pig, hamster, guinea-pig and human tissues). Oh *et al.* (1996) showed that capsaicin activates ion channels permeable to mono and divalent cations (Na\(^+\) or Ca\(^{2+}\)) in dorsal root ganglion (DRG) neurons. In addition, as proposed by Jancso in the 1980s, Caterina *et al.* (1997) have recently cloned the vanilloid receptor (VR1) (Caterina *et al.*). In 1995, Szallasi (Szallasi *et al.*, 1995) showed that capsaicin is the only natural irritant that induces desensitization, a rapidly developing refractory process. This phenomenon might contribute to the analgesia produced after prior exposure to capsaicin and its analogues. Apart from this, treatment with capsaicin (vanilloids) causes a depletion of Substance P (SP) and CGRP and somatostatin, and also blocks the intra-axonal transport of macromolecules, such as the neural growth factor (NGF) (Szallasi and Blumberg, 1999).

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Human studies: In spite of the undesirable features reported for the human use of capsaicin and some of the capsaicin-analogues, the cloning of the vanilloid receptor and the possibility of developing capsaicin analogues free of the reported undesirable side effects has, in recent years, attracted the attention of most pharmaceutical companies, since such compounds might present an interesting and novel strategy for the development of a useful therapeutic class of analgesics.

Capsaicin is an ingredient of several commercially available medicinal formulations for muscle pain. As described in the 19th century for toothache, capsaicin is used for atypical odontalgia and ‘burning mouth’ syndrome (Vickers *et al.*, 1998; Huang *et al.*, 1996). In several neuropathic pain states, the topical use of capsaicin has been recommended for the treatment of post-herpetic neuralgia, painful diabetic neuropathy, post-mastectomy pain syndrome, and osteo- and rheumatoid-arthritis (Szallasi and Blumberg, 1999). Other uses include the treatment of detrusor hyperreflexia of spinal origin, bladder hypersensitivity, vasomotor rhinitis, natalgia paresthetica and others (see for review: Szallasi and Blumberg, 1999).
Structure-activity relationships. Walpole et al. (1993a, b, c) studied the structural requirements for capsaicin-like activity in three regions of the molecule: the aromatic ring, the amide bond and the hydrophobic side-chain which provides the basis for the design of compounds with increased potency. The SAR of capsaicin analogues was studied by Klopman and Li (1995) who proposed a three-dimensional pharmacophore model for the capsaicin–receptor interactions. Based on the three regions of the capsaicin molecule necessary for agonist properties, Wrigglesworth et al. (1996) have synthesized two potent, orally active analogues. Recently, the method of back-propagation artificial neural networks was successfully used by Hosseini et al. (1997) to explore the correlation between the activity and molecular structure of capsaicin analogues. All these studies on the correlation between structure and activity of capsaicin analogues will guide, in the future, the design of more selective and potent analogues working by this mechanism of action.

Salix species

The genus Salix (Salicaceae), containing about 500 different species of plants, is known popularly as willow. The species S. alba L., S. fragilis L. and S. purpurea L. are the most regularly used for medicinal purposes. The principal active constituent of Salix sp. is salicin. However, studies have shown that a whole series of phenolic glycosides, such as salicortin, fragilin and tremulacin, are present in the bark of this plant (Trease and Evans, 1978; Robbers and Tyler, 1999).

The earliest descriptions were by a Greek physician, Dioscorides, who, in the first century, prescribed willow bark to patients suffering from rheumatism. The first clinical use of it in fever was reported in 1763 (Norton and Meisinger, 1997). In 1829, Leroux isolated the active ingredient, salicin, from the willow bark, and in 1838 salicylic acid was obtained. Salicylic acid was first used therapeutically as an external antiseptic and antipyretic and also in the treatment of rheumatism and arthritis when Kolbe synthesized it in 1860. The sodium salt of salicylic acid was introduced as an antipyretic in 1875. The first synthetically produced conversion of salicylic acid was acetylsalicylic acid, which Gilb synthesized in 1859. This gained therapeutic importance when Dreser discovered its strong fever-reducing effect in 1899 and named it ‘aspirin’ (Hass, 1983; Walker, 1995) (see Scheme 4).

Acetylsalicylic acid (ASA) is a prototype of a group of drugs called non-steroidal antinfiammatory drugs (NSAID). The NSAID possess both analgesic and antinfiammatory properties, but the analgesic effect is not necessarily secondary to an antinfiammatory response because NSAID have clear analgesic effects in the absence of infiammation. They are used to treat a variety of painful and infiammatory disorders such as postoperative pain, dental surgery, headache and acute and chronic musculoskeletal pain including osteoarthritis and rheumatoid arthritis (Walker, 1995). However, this NSAID and also salicylates, possess several side effects, including gastrointestinal, renal and hepatic disorders, and hypersensitivity (Clissold, 1986).

The precise mechanism of action of NSAIDs remained unknown for almost a century. Only in 1971 did Vane obtain the first pieces of evidence that the analgesic properties of salicylates, more specifically acetylsalicylic acid (aspirin), were the result of prostaglandin synthesis inhibition through inhibition of cyclooxygenase (COX) activity. In the periphery, prostaglandins cause hyperalgesia by sensitizing the afferent nociceptors to various other mediators. NSAIDs are known to produce analgesic effect in most infiammatory states (Ferreira, 1972). In addition to these peripheral actions, direct effects of salicylates within the CNS have also been described. This effect may be the result of interference with the formation of prostaglandins in the CNS, or may be mediated by endogenous opioids or serotonin (Cashman, 1996).

COX was purified from vesicular glands in 1976 and cloned in 1988 by several groups (Miayamoto et al., 1976; DeWitt and Smith, 1988; O’Banion, 1999). However, some studies have shown differences in response to analgesic drugs by COX, suggesting the existence of two COX isoenzymes (Fu et al., 1990; Masferrer et al., 1990). The discovery of COX-2 (Xie et al., 1991; Kujubu et al., 1991) was the explanation for the variable analgesic, antipyretic and antinfiammatory effects observed among the NSAIDs, that originally Vane (1971) ascribed to different cellular pools. Generally, the side effects and therapeutic effects are related to COX-1 (constitutive) and COX-2 (induced) inhibition, respectively. Salicylates non-selectively inhibit both the constitutive and induced COX activity. Studies implicate COX-2 induction as a critical event in infiammation and pain, supported by effective suppression of infiammatory and nociceptive responses in experimental animals by selective COX-2 inhibitors (O’Banion, 1999; Xu et al., 1999). Salicylic acid has virtually no inhibitory effect against purified COX-1 and COX-2, although it inhibits prostaglandin synthesis in

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intact cells. Recently, it has been reported that aspirin and sodium salicylate, equipotent and at therapeutic concentrations, block COX-2 mRNA and protein levels (Xu et al., 1999). This finding suggests that some salicylate actions, including antiinflammatory and analgesic effects, may be mediated by suppressing COX-2 induction.

**Antinociceptive effects.** There is a considerable debate as to whether ASA or salicylate is the most effective analgesic. Experiments have demonstrated that, compared with salicylate, ASA is more potent in inhibiting COX in vitro (Vane and Botting, 1995). This is because ASA is able to acetylate COX-1 and 2, and salicylate, which lacks an acetyl group, is less efficient as an inhibitor of COX (Smith and Dewitt, 1996; Walker, 1995). There is, however, in vivo evidence to suggest that both compounds are equipotent analgesics (McCormack, 1994; Preston et al., 1989).

ASA and other salicylates are most effective in relieving mild to moderate pain such as toothache, headache, arthralgia, dysmenorrhea, and a wide range of muscular aches and pains. Salicylates are also useful in suppressing other forms of pain, such as postoperative, postpartum, and chronic pain of visceral origin in cancer patients. In addition, they are employed to treat pain associated with inflammatory disorders (e.g. rheumatoid arthritis and other rheumatic conditions) (Clissold, 1986).

**Caffeine**

Caffeine (1,3,7-trimethylxanthine), primarily from beverages made from coffee beans, tea leaves and kola nuts, has been used therapeutically in combination with ergotamine for migraine headaches and in combination with non-steroidal antiinflammatory drugs in analgesic formulations. Caffeine alone is used as a stimulant, to treat various headache conditions. It is also used for respiratory depression in neonates and to treat post-prandial hypotension and obesity (Sawynok, 1995). The analgesic adjuvant properties of caffeine in combination with aspirin-like drugs in a variety of other pain states (i.e. postpartum, post-dental extractions) seems to be associated with the blockade of peripheral and central adenosine receptors involved in the regulation of pain transmission (Sawynok, 1995; 1998; Sawynok and Reid, 1996).

**Structure-activity relationships.** Analogs of caffeine and theophylline in which the 1-, 3- and 7-methyl substituents have been replaced by other groups were assessed for potency and selectivity as antagonists at A1 and A2 adenosine receptors in brain tissue by Daly et al. (1986). A similar study was carried out by Shammin et al. (1989) on the effects of 8-phenyl and 8-cycloalkyl substituted alkylxanthines with substitution in the 1, 3- and 7-positions. Kim et al. (1994) have studied the structure-activity relationships of 1,3-dialkylxanthine derivatives in rat A1 adenosine receptors. More recently, Muller et al. (1997) have studied the SAR of 3,7-dimethylxanthine derivatives as A2A-selective adenosine receptors. Montana et al. (1998) studied novel xanthine analogues that are selective phosphodiesterase 4 (PDE4) inhibitors with improved therapeutic potential over theophylline. Finally, Cavallaro et al. (1999) studied a range of caffeine analogues, in which the methyl groups in the 1 and 7 positions were replaced with alkyl chains containing different functional groups in the ryanodine receptor. These studies could possibly provide novel therapeutic agents.

**Alkaloids**

In recent years, a large number of different kinds of naturally occurring alkaloids with antinociceptive activity has been reported. Rios et al. (1989) reviewed the chemical structures and the main pharmacological actions of aporphinoid alkaloids and found that some of them exhibited antinociceptive properties, namely pro-nuciferine, glaucine, nuciferine and pukateine. A crude alkaloid extract from Hunteria zeylanica has been found to exert pronounced antinociception when assessed in several chemical (but not thermal) models of nociception in mice. This activity might be attributed to the presence of (−)-eburnamine and other derivatives and pleiomutinine (Reammondkoll et al., 1994a; 1995). Two matrine-type lupin alkaloids, (+)-allomatrine and (+)-matrine, isolated from Sophora alopecuroides, exhibit antinociceptive effects, which are mediated through the activation of κ-opioid receptors and both μ- and κ-opioid receptors, respectively (Xiao et al., 1999). Mitragynine, the major alkaloidal constituent found in young leaves of Mitragyna speciosa, exerts an opioid-like activity, but its selectivity for the opioid receptor subtypes differs from that of morphine (Matsumoto et al., 1996; Thongpradchote et al., 1998). The alkaloids isolated from Psychotria colorata show a marked naloxone-reversible antinociceptive activity in animals (Elisabetsky et al., 1995). Furthermore, these alkaloids have an inhibitory effect on [3H]-naloxone binding, providing a neurochemical basis for the opioid-like activity in vivo (Amador et al., 1996). Isoretuline, but not O-acetyllisoretuline and N-desacylisorotuline, isolated from Strychnos henningsii Gilg, have antinociceptive and antiinflammatory action in animals (Tits et al., 1991).

**Cis-8, 10-Di-N propyllobelidiol hydrochloride. Sipho-campey campylus verticullatus (Campanulaceae) is a plant native to Brazil and is used traditionally in the management of asthma. We reported previously that the hydroalcoholic extract prepared from the dried stems and leaves was active in several different functional groups in the ryanodine receptor. These studies could possibly provide novel therapeutic agents.**

**RECENT WORK ON NOVEL NATURALLY OCCURRING SUBSTANCES DERIVED FROM PLANTS WITH ANTINOCICEPTIVE PROPERTIES**

**Alkaloids**

In recent years, a large number of different kinds of naturally occurring alkaloids with antinociceptive activity has been reported. Rios et al. (1989) reviewed the chemical structures and the main pharmacological actions of aporphinoid alkaloids and found that some of them exhibited antinociceptive properties, namely pro-nuciferine, glaucine, nuciferine and pukateine. A crude alkaloid extract from Hunteria zeylanica has been found to exert pronounced antinociception when assessed in several chemical (but not thermal) models of nociception in mice. This activity might be attributed to the presence of (−)-eburnamine and other derivatives and pleiomutinine (Reammondkoll et al., 1994a; 1995). Two matrine-type lupin alkaloids, (+)-allomatrine and (+)-matrine, isolated from Sophora alopecuroides, exhibit antinociceptive effects, which are mediated through the activation of κ-opioid receptors and both μ- and κ-opioid receptors, respectively (Xiao et al., 1999). Mitragynine, the major alkaloidal constituent found in young leaves of Mitragyna speciosa, exerts an opioid-like activity, but its selectivity for the opioid receptor subtypes differs from that of morphine (Matsumoto et al., 1996; Thongpradchote et al., 1998). The alkaloids isolated from Psychotria colorata show a marked naloxone-reversible antinociceptive activity in animals (Elisabetsky et al., 1995). Furthermore, these alkaloids have an inhibitory effect on [3H]-naloxone binding, providing a neurochemical basis for the opioid-like activity in vivo (Amador et al., 1996). Isoretuline, but not O-acetyllisoretuline and N-desacylisorotuline, isolated from Strychnos henningsii Gilg, have antinociceptive and antiinflammatory action in animals (Tits et al., 1991).
Terpenoids and steroids

Terpenoid and steroid compounds are widely distributed in the vegetable kingdom and exhibit distinctive pharmacological properties. Among other actions, naturally occurring terpenoids present antiinflammatory and antinociceptive properties, inhibit platelet aggregation, and interfere at the intracellular level with several steps of signal transduction mechanisms (Calixto et al., 1998; Mahato et al., 1992; Safayhi and Saider, 1997).

The monoterpenes, sesquiterpenes, and steroids present in the essential oil of Cymbopogon citratus exhibited antinociception when assessed in different experimental models of pain. Myrcene was the most active component of the oil, having an antinociceptive effect similar to that described for peripheral-acting opiates or dipyrone (Lorenzetti et al., 1991). Lipadin, a bicyclic sesquiterpene from Ferula linkii, trans-dehydrocrotonein, a 19-nor-clerodane diterpene from Croton cajucara, and four triterpenes isolated from dichloromethane extract of Ganoderma lucidum, denoted ganoderic acids A, B, G and H, were effective in inhibiting acetic acid-induced abdominal constrictions in mice (Valencia et al., 1994; Carvalho et al., 1996; Koyama et al., 1997). Bacosina, isolated from aerial parts of Bacopa monnieri, and 1,8-cineole, present in the essential oil of Nepeta italica, exhibited antinociception by interaction with opioidergic pathway (Vohora et al., 1997; Aydin et al., 1999).

The isolation and identification of several terpenes with antinociceptive effects have been demonstrated in preliminary studies. Kaurenolic acid, the major component of Wedelia paludosa (Block et al., 1998a, b), marrubiin, a furanolactone diterpene from Marrubium vulgare (De Jesus et al., 2000), the pholodion and 24-methyleneoctylartan isolated from Epidendrum mossei (Floriani et al., 1998; Ferreira et al., 2000), mornotenone and glutinol isolated from Sebastianiana schottiana (Gaertner et al., 1999), z-amyrin and y-amyrin from Aleurites moluccana (Meyre-Silva et al., 1998), z-amyrin acetate, b-amyrin acetate and glochidone isolated from Ipomoea pes-caprae (Krogh et al., 1999), Ichigoside F1 from Rubus imperialis (Nieto et al., 1999), and 24-hydroxytormentic acid isolated from Ocotea suaveolens (Beirith et al., 1999) exhibited dose-dependent and significant antinociception when assessed in acetic acid, formalin and capsaicin tests. Other structurally similar diterpenes to marrubiin also cause significant inhibition of the abdominal constrictions induced by acetic acid (Brand et al., 1999).

Polygodial. Polygodial sesquiterpene is the main constituent present in the bank of Drymis winteri, a well-known medicinal plant found in Brazil and some South American countries, commonly used in folk medicine as an antiinflammatory and for the treatment of asthma and allergy. The hydroalcoholic extract of D. winteri, given orally to rats, inhibits paw oedema formation caused by several inflammatory agents and ovalbumin (in actively sensitized rats). Moreover, it increases the survival rate when assessed in anaphylactic shock in mice that have been sensitized to ovalbumin (Tratsk et al., 1997). In vitro, the extract of D. winteri and its main sesquiterpene polygodial antagonize the contractile response elicited by most inflammatory mediators and ovalbumin in the guinea-pig trachea (El Sayah et al., 1997; 1998). Polygodial elicits an endothelium-dependent and -independent relaxation in vessels isolated from guinea-pig and rabbit, by a mechanism involving the release of nitric oxide or a nitric oxide-related substance and by activation of soluble guanylate cyclase mechan-
isms (André et al., 1999). Very recently, El Sayah et al., (2000) reported that polygodial antagonized the rat portal vein constriction stimulated by several inflammatory mediators, by a mechanism that involved an interaction of calcium influx through voltage-sensitive channels and interaction with protein kinase C.

The extract of D. winteri and polygodial antagonized paw oedema and contractions mediated by most inflammatory and pain mediators, we investigated whether they would reveal antinociceptive properties in rats and mice. Administered either i.p. or p.o. to mice, the extract of D. winteri and polygodial produced dose-related and long-lasting (at least 8h) inhibition of abdominal constriction caused by acetic acid, kaolin and zymosan, and the neurogenic and inflammatory pain caused by formalin and capsaein (Mendes et al., 1998; 2000). Polygodial, administered either i.t. or i.c.v. to mice, produced marked antinociception against both phases of the formalin response, indicating its spinal and supraspinal site of action. Moreover, administered p.o. to rats, the extract of D. winteri reversed fully the hyperalgesia produced by paw injection of bradykinin and substance P, but not the prostaglandin E2 and carrageenan-induced hyperalgesic responses (Mendes et al., 1998). The antinociceptive caused by the extract of D. winteri and polygodial was largely reversed by previous treatment of animals with naloxone, suggesting an involvement of the opioid system, although it failed to interfere with thermal nociception when assessed in the tail-flick and hot-plate assays (Mendes et al., 1998; 2000). The mechanism of the antinociceptive action of polygodial is probably unrelated to interaction with L-arginine nitric oxide or GABA A and GABA B receptors, or to activation of small or large-conductance calcium or ATP-activated K+ channels. On the other hand, opioid system (namely κ and μ receptor subtypes), serotonin, α1 adrenoceptor pathways, and activation Gia protein sensitive to pertussis toxin all appear to be involved in the mechanism by which polygodial induces antinociception (Mendes et al., 2000). Finally, polygodial action was not cross-tolerant to morphine, nor was it affected by previous adrenalec- tomy of animals. In a preliminary study, Cechinel Filho et al. (1998) demonstrated that the sesquiterpene 1-β-(p-methoxy-cinnamyl) polygodial isolated from the bark of D. winteri also caused significant inhibition of acetic acid-induced nociceptive response in mice, although it was less potent than polygodial. Thus, polygodial or its derivatives might be of great interest in the development of new analgesic drugs for the management of pain. Recently, Sterner and Szallasi (1999) suggested that the novel naturally occurring unsaturated dialdehyde vanillid receptor agonists, including polygodial, may constitute a new and very attractive target for the development of a new class of clinical analgesic drugs.

12-hydroxy-8,9-dehydroshizukanolide. The sesquiterpene lactone 12-hydroxy-8,9-dehydroshizukanolide isolated from the stems and leaves of the Brazilian plant Hedyosmum brasiliense (Chloranthaceae) has been isolated recently from the stems and leaves of the Brazilian plant Hedyosmum brasiliense (Chloranthaceae) which is used in folk medicine for the management of headache and rheumatism (Trentin et al., 1999). The extract and the 12-hydroxy-8,9-dehydroshizukanolide isolated from H. brasiliense caused pronounced antinociception in the acetic acid, formalin and capsaein tests, an effect which was not reversed by naloxone (Trentin et al., 1999). In addition, the sesquiterpene lactone produced spinal and supraspinal antinociception in mice (Trentin et al., 1999). Unpublished results from our group have shown that 12-hydroxy-8,9-dehydroshizukanolide produces concentration-dependent inhibition of [3H]glutamate binding in mouse cortex membrane (about 75% at 500 μM), but fails to block the [3H]gluta- mulate release from mouse brain synaptosomes elicited by KCl. The 12-hydroxy-8,9-dehydroshizukanolide given systemically was found to be effective in blocking the hyperalgesia produced by intrathecal injection of gluta- mate in mice (unpublished results), a model that has been demonstrated to be dependent on activation of the nitric oxide cGMP pathway (Ferreira et al., 1999). As evidence now suggests that the excitatory amino acid glutamate might be involved in pain transmission (Grubb, 1998; Millan, 1999), the sesquiterpene lactone 12-hydroxy-8,9-dehydroshizukanolide may be of interest for the develop- ment of analgesic drugs, particularly for the management of neurogenic and neuropathic pain.

Flavonoids

Preliminary studies have demonstrated that various flavonoids, including rutin and quercetin, two common and abundant flavonoids in nature, luteolin isolated from Wedelia paludosa (Block et al., 1998a, b) and the luteolin derivative, luteolin-4′-O-neohesperidoside, isolated from Caralluma attenuata (Ramesh et al., 1998), quercetin 3-O-glycoside (isoquercitrin) isolated from many plants, taxifolin but not its glycoside derivative, astilbin, isolated from Hymenae marriana (Cechinel Filho et al., 2000), two kaempferol glycoside derivatives isolated from Hedyosmum bonplandianum (Cárdenas et al., 1993), pectolinarin isolated from aerial parts of Cirsi um scoparioideum (Martínez-Vazquez et al., 1998), and gossypin (Viswanathan et al., 1984) all produced significant antinociception in the acetic acid-, formalin- and capsaein-induced nociceptive response. Hesperidin, a citrus flavonoid (Emim et al., 1994), 2′-O-rhamnosylsvertisin, but not swertisin, isolated from Aleurites moluccana (Meyre Silva et al., 1999), and some biflavonoids, such as amontoffavone (Kim et al., 1998), volkensiflavone, GB-2a, fukugetin, fukugeside (Luzzi et al., 1997), and GB-1a (Bittar et al., 2000), which are well-distributed in the families of Clusiaceae and Guttiferae, also exerted pronounced antinociception in mice against the nociception caused by i.p. injection of acetic acid. Quercetin-3-O-galactoside (hyperoside) possesses analgesic effects related to a reduction of calcium influx in afferent nerve endings without anesthetic action (Chen et al., 1989). Ginkgetin, a biflavone isolated from Ginkgo biloba leaves, has been reported as an inhibitor of group II phospholipase A2. This compound strongly reduces arthritic inflammation, confirmed by histological examination of the knee joint. In addition, ginkgetin showed antinociceptive activity in acetic acid-induced writhing, suggesting that this compound may be a potential antiarthritic agent having an analgesic effect (Kim et al., 1999). Moreover, a clinical study showed that Ginkgo biloba extract (standardized to 21.0 mg flavonoglycosids and 3 mg folic acid) treatment improved the nerve function and pain associated with autonomic neuropathy in ten patients (Koltringer et al., 1989).
Phyllanthus. The genus *Phyllanthus* comprises a great number of species widely distributed in most tropical and subtropical countries. The infusion of the leaves, stems and roots of most *Phyllanthus* species is largely used in folk medicine in most countries to treat hepatitis, disturbances of the kidney and urinary bladder, intestinal infections and diarrhea (for review see Calixto et al., 1998). Previous studies carried out by our group have demonstrated for the first time that in addition to the reported medicinal uses, the extracts obtained from leaves, stems and roots of several species of plants belonging to this genus, namely *P. niruri*, *P. urinaria*, *P. carolinensis*, *P. amarus*, *P. tenellus*, *P. fraternus*, *P. sellowianus*, *P. orbiculatus*, *P. stipulatus* and *P. corcovadensis*, administered either by i.p. or p.o. routes to mice, exhibit pronounced antinociception when assessed against chemical pain response caused by acetic acid, formalin or capsaicin, but not in thermal pain models (Gorski et al., 1993; Santos et al., 1995a, b; Cechinel Filho et al., 1996; Calixto et al., 1998). Interestingly, and in contrast with nonsteroidal antiinflammatory drugs, the extract of *Phyllanthus* species caused an important inhibition of the neurogenic pain responses elicited by formalin or capsaicin. From the mechanism involved in the antinociceptive action of extracts of plants of the genus *Phyllanthus*, it is apparent that their effects are unrelated to interaction with opioid, serotonin or L-arginine–nitric oxide pathways. Furthermore, these antinociceptive actions were found to be independent of modulation by endogenous glucocorticoids nor were they associated with non-specific effects such as muscle relaxation or sedation (Santos et al., 1995b).

Phytochemical studies carried out by our group and also by others have demonstrated the presence of a large number of naturally occurring secondary substances in these plants, such as flavonoids, alkaloids, lignans, tannins, steroids, phenols, triterpenes, coumarins, etc. (see for recent review Calixto et al., 1998). We have evaluated a large number of pure compounds isolated from many *Phyllanthus* plants in distinct models of nociception in order identify the active principles responsible for the antinociceptive action present in these plants. At least six naturally occurring substances present in most of the plants, namely gallic acid ethyl ester, furosin, geraniin, quercetin, rutin and stigmasterol, as responsible for the antinociceptive action present in these plants, namely gallic acid ethyl ester, furosin, geraniin, quercetin, rutin and stigmasterol, as demonstrated for the extract of these plants (Santos et al., 1995b), the L-arginine–nitric oxide, serotonin and opioid pathways, or even motor incoordination, have apparently no major participation in the gallic acid ethyl ester-mediated antinociception (Santos et al., 1999b). As these particular types of K⁺ channels are implicated in the regulation of most physiological and pathological processes, including pain transmission (Welch et al., 1995b), such compounds might be of potential therapeutic interest.

We have investigated further the action of rutin, quercetin and geraniin, all compounds present in the *Phyllanthus* species, on the [³H]glutamate binding and on [³H]GMP-PNP, a GTP analogue which binds to extracellular sites modulating glutamatergic transmission in rat brain membranes. Only quercetin was found to be effective in inhibiting the binding for both ligands (Martini et al., 1999). Whether or not such data might account for the antinociception caused by quercetin remains to be seen. Together, the data summarized above indicate that diverse classes of naturally occurring secondary metabolites present in most plants of the genus *Phyllanthus* are probably involved in the antinociceptive action reported for such plants. It is quite possible, although it has not yet been demonstrated experimentally, that the diversity of compounds that are present in low concentration in these plants might act in a synergistic manner in the extract. As clinical trials have demonstrated that some extracts of *Phyllanthus* species are well-tolerated in humans, with no evidence of side effects (Thyagarajan et al., 1988; Blumberg et al., 1989; Wang et al., 1995; Santos 1990), these plants might be of potential clinical interest in the development of well-controlled and standardized phytomedicinals for the management of painful disorders, especially in the treatment of neurogenic pain.

Panax ginseng. The root of *Panax ginseng* C.A. Meyer is a mild oriental folk medicine that is reported to relieve a variety of ailments. A standard extract of *P. ginseng* (200 mg/kg, i.p.) produced analgesia and hypothermia when assessed in the tail flick test. These effects were not reversed by the non-selective opioid receptor antagonist, naltrexone. However, the same extract of *P. ginseng* antagonized (at lower doses) the analgesic, hypothermic, and cataleptic effects of morphine, probably by a non-opioid mechanism (Ramarao and Bhargava, 1990). A study using the whole-cell patch-clamp method demonstrated that the ginseng root extract inhibits calcium channels through a pertussis toxin-sensitive non-opioid mechanism (Nah and McCleskey, 1994). Ginsenosides and ginseng saponins are important pharmacoactive molecules isolated from *P. ginseng*, and there exists evidence that ginsenosides are involved in pain modulation as well as in opioid-induced antinociception and tolerance (Yoon et al., 1998). Systemically applied ginsenosides Re, Rd, Re and Rf, but not Rb1 or Rg1, caused antinociception in writhing and formalin tests, but had no effect in tail-flick and hot plate tests (Mogil et al., 1998; Shin et al., 1999). This antinociceptive effect was not blocked by the opioid receptor antagonist naloxone and seems to be unrelated to non-specific motor dysfunction (Shin et al., 1999). Moreover, an in vitro study has shown that the ginsenoside RF suppresses Ca²⁺ channels in large, but not small, nociceptors (Mogil et al., 1998).

Ginsenosides, when applied by the intrathecal route, have antinociceptive activity in formalin and substance P-induced licking tests, suggesting a possible blockade of
the substance-P postsynaptic sites at the spinal cord by ginsenosides. However, ginsenosides produced analgesia when injected i.c.v., demonstrating that substances might act at the supraspinal level (Yoon et al., 1998). It was also reported that the extract and the pseudoginsenoside-RP1 isolated from *Randia siamensis* exhibited antinociceptive action in writhing and paw pressure tests (Reanmongkol et al., 1999).

### Miscellaneous compounds

Several studies have shown that other classes of naturally occurring substances, including xanthones, tannins and saponins, possess antinociceptive properties. Nepetalactone, a lactone extracted from *Nepeta casearea*, is the main antinociceptive component of this plant, and shows a specific opioid receptor subtype agonistic activity (Aydin et al., 1998). Acteoside, a phenylethanoid glycoside, has been isolated as an antinociceptive principle from *Lipia triphylla*, a Peruvian medicinal plant, by activity-guided separation (Nakamura et al., 1997). 1,7-Dihydroxy-2,3-dimethoxy-xanthone, isolated from *Polygala cyparissias*, produces dose-related inhibition of acetic acid-induced abdominal constriction in mice, being more active than some reference drugs (De Campos et al., 1997; Pinheiro et al., 1998). Furthermore, 1,7-dihydroxy-2,3-dimethoxy-xanthone antagonizes, in a concentration-dependent fashion, several inflammatory mediator-mediated contractions in the guinea-pig trachea (El Sayah et al., 1999).

Recent studies conducted by our research group demonstrated that different components of *Croton urucurana*, such as acetyl aleuritolic acid, catechin, gallocatechin, etc. presented antinociceptive effects, but the potency of the isolated compounds was similar to or lower than those exerted by extracts or fractions, suggesting the existence of other minor active components or the existence of a synergistic effect (Peres et al., 1998). Smilaxin B, a spirastrol glycoside isolated from *Smilax sieboldii* Miq., caused antinociception when administered i.c.v. and analysed in the tail-flick test. This effect was mediated by GABA<sub>A</sub> and N-methyl-D-aspartate (NMDA) receptors, but not GABA<sub>B</sub> or non-NMDA receptors located at the supraspinal level. However, the antinociceptive effect may have been produced by activation of descending noradrenergic systems without affecting opioidergic or serotonergic pathways (Suh et al., 1996). The 2-(4-bromobenzoyl)-3-methyl-4,6-dimethoxy benzofuran, a xantholine derivative revealed interesting antinociceptive spinal and supraspinal actions when assessed in several chemical and thermal models of nociception (Vaz et al., 1996).

### SUMMARY AND CONCLUSIONS

Although significant scientific progress has been made in recent decades on the elucidation of the neurobiology of pain transmission, especially by application of modern techniques of electrophysiology and molecular biology, the need for new and more effective analgesics for clinical use, free as far as possible of undesirable side effects, are still urgently required. One of the most important analgesic drugs employed in clinical practice today continues to be the alkaloid morphine, in spite of its well-known undesirable side-effects. It is also important to point out that the development of analgesic drugs for the treatment of neuropathic pain is also urgently needed. However, from this review it has become clear that there are many possible targets and available strategies that might permit the development of new and effective analgesic drugs from naturally occurring secondary metabolites derived from plants, and which may be expected to have therapeutic benefit in the management of different pain disorders. Although naturally occurring secondary metabolites derived from plants with antinociceptive properties are frequently found in the literature, the great majority of these reported studies are preliminary with few studies on the mechanism of action, toxicology and clinical trials of such substances. Also, plant-derived secondary metabolites have, over the years, greatly contributed to our current understanding of the process of pain transmission, and, especially, have permitted us to characterize the receptor types and endogenous ligands involved in the mechanism of nociception. Morphine, capsaicin and cannabinoids, among others, are good examples. Thus, this field of research has become the focus of intense interest, on the part of both academics and pharmaceutical companies, and efforts towards the identification of effective and safe analgesics, direct from plants or from derivatives, will certainly reap great rewards in the near future. Thus, naturally occurring substances derived from plants currently have and will certainly continue to have a relevant place in the process of drug discovery, particularly in the development of new analgesic drugs.

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