SEARCH FOR MEDICINAL PLANTS AS A SOURCE OF ANALGESIC AGENTS - A REVIEW

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Summary

From the first recorded accounts, over 7000 years ago, various forms of natural products have been utilized to treat pain disorders. Prototypical examples of such natural products are the opium poppy (*Papaver soniferum*) and the bark of the willow tree (Salix spp.). It was not until the 19th century when individual compounds were isolated from these substances and were determined to posses the desired effects. The known sources of these substances have been thoroughly investigated. Over the last several decades, more analgesic substances have been purified from natural products resulting in novel structural classes and mechanisms of actions. Plants and other natural products described in historical ethnobotanical and ethnopharmacological literature have become of more recent interest in drug discovery efforts. These manuscripts and reports are being utilized to aid in the identification of natural products that have been historically employed in the alleviation of pain. A large factor that has highlighted the importance of discovering novel compounds to treat pain has been in the fundamental understanding of the complex mechanisms of pain transmission in the nervous system. Nociceptive processing involves many receptor classes, enzymes and signaling pathways. The identification of novel classes of compounds from natural sources may lead to advancing the understanding of these underlying pharmacological mechanisms. With the potential of uncovering new compounds with idealistic pharmacological profiles (i.e., no side effects, no addictive potential), natural products still hold great promise for the future of drug discovery especially in the treatment of pain disorders and potentially drug addictions.

Keywords: Analgesics; Natural products; Nociceptive.

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Introduction

Substances derived from natural products have been utilized since the beginning of time for various purposes including the treatment of pain. Opium, for example, has been used since the earliest records of time, some 7000 years ago. Not until the 19th century was individual components of different natural product remedies identified and purified. Today, drug discovery has become a complex field far beyond the use of only natural products. However, natural products have dominated the drug industry for many years and several marketed drugs are based on isolates from such. There has been a recent resurgence in the study of natural products, especially from the dietary supplement industry. The pharmaceutical industry has begun to revitalize programs on the screening of natural products. Academic research has continued to be a strong leader in the field of natural products, especially with respect to newly discovered chemical entities. Research in the area of pain management and drug addiction originally focused on natural products exclusively. More recently, analogs have been made from natural substances and completely synthetic compounds based on natural pharmacophores have been introduced into the market. The research and medical fields still struggle with side-effect profiles from these analgesic substances that are undesirable. Apart from rational drug design and completely novel synthetic efforts, natural products are still being investigated for novel chemical structures that may interact with known analgesic targets. In addition, orphan targets are being investigated for their potential roles in the management of pain. The pharmacology of pain has become a complex field and as more systems approaches are explored, more potential drug targets are being identified. This review will highlight some of the more recent reports of novel, naturally derived compounds that possess analgesic properties.

Pain mechanisms and control

Pain can be simply defined as undesirable physical or emotional experiences. Pain is the most common reason that individuals seek medical attention. It can be divided into two types, acute pain and chronic pain. Acute pain serves as a warning system to remove oneself from particular pain stimuli. Chronic pain can exist for undefined times and undefined reasons and seems to serve no clear purpose. Treatment of chronic pain is a major problem due to the use of available medications and their undesirable side-effect profiles. The side effects of currently used pain medications vary based on the class of agent used however, most medical personnel are concerned with addiction, tolerance, gastrointestinal effects, and abuse. Most recent clinical studies[1] suggest that proper use of pain treatment has low risk of producing addicts and because of this prescribing effort seem to be changing. Regardless, we can separate physical pain into at least four stimuli groups: mechanical, thermal, chemical, or electrical. The stimulation of nociceptive nerve endings of C-fibers or activation of A-fibers carries the painful stimuli[2]. It should be mentioned that endogenous inflammatory pain-producing substances can act in a synergistic way to increase pain levels. Nociceptors (or pain receptors) can be found in the skin but also in other areas. Signals transmitted through these receptors are interpreted as pain in the cognitive centers of the brain. Pain may also arise in the absence of stimuli; this is known as phantom pain.

The complex relationship of pain and injury indeed makes the perception of pain an important research issue. The brain and spinal cord play a major role in central pain mechanisms as previously mentioned. However, the knowledge of brain mechanisms is still relatively limited. The dorsal horn of the spinal cord is endowed with several neurotransmitters and receptors including: substance P, somatostatin, neuropeptide Y, excitatory amino acids, inhibitory amino acids, nitric oxide, endogenous opioids, adenosine, and the monoamines, among others. It is clear that pain transmission to the brain is under diverse physiological control. Undoubtedly, this makes for a difficult challenge in the discovery of ways to inhibit pain sensations without causing side-effects.

Aspirin

Aspirin or acetylsalicylic acid derived from salicylic acid, extracted from the bark of the Willow tree (*Salix alba*), is one of the most widely used and available compounds for the management of mild pain. Aspirin served as the first nonsteroidal anti-inflammatory drug (NSAID) and inhibits the arachidonic acid pathway that eventually leads to the synthesis of eicosanoids, potent mediators of pain[3]. The use of aspirin, that specifically inhibits the cyclooxygenase (COX) enzymes, led to the discovery of other synthetic nonsteroidal anti-inflammatory drugs (NSAIDs). In fact, the study of the biochemical cascade of the COX system led to the discovery of the COX-2 enzyme inhibitors once praised as having safer profiles than other NSAIDS (which inhibit the COX-1 enzyme). Compounds selectively targeting the COX-2 enzyme have recently come under much scrutiny because of the voluntary withdrawal of Vioxx® (rofecoxib) by Merck and Co., Inc. on September 30, 2004 from the prescription drug market due to an increased risk of cardiovascular events.

Opioids

Opioid is the common name for all compounds that have the same mechanism of action as the constituents of opium. The use and abuse of opium juice from *Papaver somniferum*, has been know before history was recorded. All opioids interact with the endogenous opioid receptor system that presently includes four receptor subtypes[4] designated as mu, delta, kappa, and ORL-1 (opioid receptor like receptor). These receptors are widely distributed in the mammalian system and have been found in all vertebrates. There is a relatively high density in the brain and spinal cord but they are also found in the gastrointestinal system and the cells of the immune system. Each subtype seems to play a slightly different role and a good review can be found in any medical text. The research on opioid systems has focused around three groups of modulators. The first are the natural products, such as morphine, codeine, and thebaine. The second and third are out of the scope of this review but are the synthetic compounds that have been designed based on the knowledge of the natural product pharmacophores and the peptides, respectively.

More recently, some novel chemical structures have appeared in the literatures that interact either directly with opioid receptors, or through some other mechanism of controlling opioid receptor signaling. These compounds are interesting from a drug design perspective as most of them do not contain nitrogen. For many years, the extraction and fractionation methods used in natural products research either discarded lipid like molecules or were unable to partition them using acid/base extraction methods as they are neutral compounds.

The first reported[5] non-nitrogenous selective kappa opioid receptor ligand, salvinorin A, has recently attracted much research attention. Salvinorin A, the active component of extracts from *Salvia divinorium*, is one of the most potent hallucinogens known to date[6]. For many years the molecular target for salvinorin A was unknown since researchers believed that it should interact with known targets for hallucinogens, namely the serotonin receptor system. Salvinorin A was initially screened against a battery of receptors that did not include opioid receptors[6]. Roth et al. followed up this screen in the National Institute of Mental Health's Psychoactive Drug Screening program that showed salvinorin A to selectively interact with the kappa opioid receptor. Interestingly, this made intuitive sense due to the psychoactive nature of the kappa opioid receptor system. Kappa opioid receptor agonist has not met with much favor as analgesic agents due to the dysphoric side-effects they produce. Salvinorin A has been reported to produce a dysphoric hallucination in most human subjects[6]. However, it still is listed as a chemical of concern with the United States Drug Enforcement Agency as it is currently marketed as a legal alternative to other illicit hallucinogens. *Salvia divinorium* extracts can be purchased with ease on the Internet and only a few states have limited sales to those over 18 years of age. Legislation has been introduced in the United States House of Representatives, on more than one occasion, to move salvinorin A, *S. divinorium*, and extracts from the plant into scheduled drug status. To date, this legislation has not been acted on. However, several other countries have outlawed its use including Australia and some member countries of the European Union.

There have been reports in the ethnopharmacological literature of *S. divinorium* extracts or pairs of leaves being used in small doses to achieve relief from headaches, as a sedative, and for the treatment of some gastrointestinal disorders[7]. Due to the unique structure of salvinorin A, it could be hypothesized that it is interacting in a novel fashion with the kappa opioid receptor and serves as a suitable lead compound for the development of novel opioid receptor ligands. In this regard, our research has focused on the potential analgesic properties of salvinorin A, with a goal of understanding the mode of interaction with kappa opioid receptors and to potentially develop a useful ligand from these studies. There is great interest in the pharmaceutical industry for kappa opioid receptor antagonists and salvinorin A serves as a highly selective template for development of such.

Salvinorin A, although the most studied and well-characterized non-nitrogenous kappa opioid receptor agonist at this point, is not the only non-nitrogenous compound to be reported to interact with opioid receptors. Recently, pawhuskin A, a stilbene derivative from *Dalea purpurea* was isolated and reported to have low affinity for opioid receptors[8]. This plant has been used by Native Americans to ward of disease and for unspecified ailments. The organic extracts of this plant exhibited moderate opioid activity and therefore lead to the isolation of compounds in this extract. Pawhuskin A, with an opioid receptor affinity of 290 nM demonstrated the highest affinity of any of the isolated compounds from *D. purpurea* to date. The investigators used a non-selective radioligand to perform the receptor binding assays and therefore it is not known which opioid receptor(s) may be responsible for activity. Additionally, no functional activity has been reported for pawhuskin A so it remains to be seen if it is an agonist of opioid receptors and if it can truly serve as a lead compound for the development of novel analgesics based on the stilbene scaffold.

Extracts from *Diclea grandiflora*, a vine found in Northeastern Brazil have been reported to have analgesic effects[9]. Several non-nitrogenous compounds were isolated from these extracts and a minor component, dioflorin, was discovered to be a potent analgesic in a mouse model of tail-flick[10]. The analgesic activity was reversed by naloxone indicating a potential mode of interaction with opioid receptors. However, no receptor binding data has appeared in the literature to date and it is still unclear if this molecule is producing antinociception selectively through direct interaction with opioid receptors.

Menthol, isolated from peppermint (*Mentha piperita*) has been utilized for a number of centuries usually in topical preparations as an antipruritic, antiseptic, and a coolant. It is known to interact and activate cold receptors[11]. More recently, it was evaluated in the hot-plate and acetic acid abdominal constriction assays where it demonstrated potent activity[12]. Moreover, the effects were reversed by naloxone and by the kappa opioid receptor selective antagonist, norBNI, suggesting an interaction with opioid receptors, and more selectively, kappa opioid receptors. It was noted that menthol did not impair motor activity. However, no opioid receptor binding data has appeared in the literature. Given the role of opioid receptors and their interaction with ion channels, where menthol is known to act, it could be possible that a more complex inhibition of opioid receptor signaling pathways are involved. Regardless of the precise mechanism of action, further research is warranted.

Other recently studied, uniquely structured, nitrogen containing compounds isolated from the traditional Thai herb, *Mitragyna speciosa*, have appeared in the literature as opioid receptor ligands[13]. The herb has been used for many years in Thailand as a replacement for opium. More recently, Takayama[14] has reviewed the individual components of the active extracts. They have identified at least two compounds that have opioid receptor activity. The first and one of the major alkaloidal components has been called mitragynine. Mitragynine is a corynanthe based compound that acts as a partial agonist at opioid receptors having about 26% the activity of morphine[14]. The second and possibly more interesting compound, 7-hydroxymitragynine has activity of greater than 1000 times the potency of morphine[14]. It however, is a minor component of the plant but has been demonstrated to be orally active[15]. These compounds have also been shown to interact with other receptor systems including the descending noradrenergic and serotonergic receptor systems[16]. These systems are also known to play a role in centrally mediated nociceptive responses. Interestingly, the herb is not under any control in the United States or on the radar screen of the Drug Enforcement Agency, yet it is readily available on the Internet for purchase by anyone. Indeed, mitragynine and 7-hydroxymitragynine serve as interesting lead compounds for the development of novel chemicals to treat pain but also as potential treatments for drug addiction.

As more chemical components of traditionally used plants for the treatment of pain are elucidated, there is great potential for the development of novel drug treatments acting through opioid receptors.

Voltage-gated ion channels

Many natural products have been found to interact with voltage-gated ion channels. Probably the most well known ligand that blocks sodium channels is cocaine isolated from *Erythroxylon coca*[17].

Cocaine is mostly known and studied for its ability to block the dopamine transporter due to its ability to create a euphoric state[18]. However, its utilization as a local anesthetic is known by its interactions through sodium channel blockage.

Some more recent natural products to be studied at the sodium channels all come from different animals as a natural defense mechanism. These compounds cause their effects through several mechanisms of action.

Tetrodotoxin isolated from the puffer fish, blocks sodium channels and causes great harm to those that ingest it. It does produce numbness in the lip and tongue within 20 min of ingestion but quickly leads to paralysis and in some cases death. This is a major concern in Japan where the puffer fish liver dish, fugu, is a delicacy. Other species have been reported to contain tetrodotoxin, but they seem to not be desirable for food sources. The use of this as a lead compound for analgesic development has been limited by this toxic nature.

Batrachotoxin isolated from the poison dart frog, *Phyllobates terribilis*, has been shown to activate sodium channels[19]. It is apparent that the frog does not produce this compound and it is believed that it comes from a dietary insect source. This toxin is particularly lethal and the estimated lethal dose in humans is between 2 and 200 mg[20]. Obviously, this compound, like other toxins has not been pursued as a potential analgesic. Brevetoxins, a family of marine neurotoxins, isolated from the red algae *Ptychodiscus brevis* or *Gymnodinium breve*, are known to cause repeated firing of sodium channels[21]. Such repeated firing leads to cell death. Brevetoxins have been associated with "red tide" (a massive natural bloom of the algae in saltwater) and has been responsible for large fish kills. Brevetoxin B has been synthesized[22] and is known to bind to sodium channels. Again, brevetoxins are compounds that can help researchers understand the pharmacology and toxicology of sodium channel activation but most likely will not serve as potential analgesic lead compounds.

Potassium channels have also been shown to be involved in pain processes[23]. Activation of potassium channels leads to membrane hyperpolarization that inhibits cell excitability. Depending on the location of these channels they may either act directly or indirectly with transmission of pain signals. Several anesthetics work through interactions with potassium channels that are used clinically today[24]. Certain peptides from natural sources have been identified as well.

Tertiapin, a 21-residue peptide isolated from honey bee venom, has been shown to block inward-rectifier potassium channels[25]. Further research in this area may produce a greater understanding of the modulation of pain responses and may lead to new compounds to treat pain or drug addiction.

Calcium channels have been well studied for their involvement in the contraction of smooth muscles[26]. In particular, calcium channel blockers have been successful on the prescription drug market for the treatment of hypertension. However, calcium channels are also attractive targets for analgesia and neuroprotection. They appear to be able to inhibit or promote opioid function. In this regard, L-type calcium channels may block opioid tolerance[27] and clinical trials with these agents should produce interesting results.

The cone snails (Conus spp.) have been a rich source of a library of novel peptides with several pharmacological actions[28]. Cone snails are hunters and they subdue their prey by injecting them with venom that contains a wide variety of related peptides ultimately paralyzing their prey for ingestion[28].

One of these peptides, known as N-conotoxin has been identified to interact with N-type calcium channels[28]. This compound has been reported to be 100–1000 times more potent than morphine in analgesia measures and also appears to be non-addictive[29] making N-type calcium channels attractive targets for the development of new analgesics.

The American funnel-web spider, *Agelenopsis aperta*, produces N-agatoxin, a peptide that inhibits P/Q-type calcium channels[30]. P/Q-type calcium channels have been reported to play a role in migraine headaches[31, 32]. A study conducted by Knight et al.[33] demonstrated that microinjections, in the rat, of N-agatoxin in the periaqueductal gray region of the brainstem, an area thought to play a role in migraines, produced significant increases in response to dual stimulation as well as spontaneous background activity. Further research in the functional role of P/Q-type calcium channels may prove to provide an additional target for the modulation of pain responses.

Acetylcholine receptors

Acetylcholine receptors are divided into two classes, the muscarinic acetylcholine receptors and the nicotinic acetylcholine receptors. These classes were identified through the utilization of the natural products, muscarine and nicotine, respectively. There has been a well documented role in the modulation of central nociception through these receptors however, they have not met with clinical success to date.

The muscarinic acetylcholine receptors have many known natural product ligands including: hyoscyamine, atropine, scopolamine, and Mamba snake toxins. These have been well reported in the literature and will not be reviewed here.

The nicotinic acetylcholine receptors have demonstrated a more likely role in analgesia. In fact, nicotine was reported as an antinociceptive as early as 1932[34]. This area of research remained relatively unexplored until the discovery of the naturally occurring alkaloid, epibatidine[35]. Epibatidine was isolated from the skin of the Ecuadorian dart-frog, *Epipedobates tricolor*, has been reported to be a potent analgesic compound that could be antagonized by mecamylamine, a nicotinic receptor antagonist, but not by opioid antagonists[35]. This work solidified the role of nicotinic acetylcholine receptors in analgesia and created a great interest in the development of novel compounds related to epibatidine. To date, none of the studied compounds have reached the prescription drug market. However, the ability to produce a non-opioid analgesic that could have equal or better potency than morphine remains a great challenge and research in this area continues.

Cannabinoid receptors

Another well known system involved in nociceptive processes is the cannabinoid receptors. The prototypical ligand, D9-tetrahydrocannabinol isolated from *Cannabis sativa*, is a non-nitrogenous lipophilic molecule that interacts with the cannabinoid G-protein coupled receptors[36]. This was most likely; the first non-nitrogenous ligand discovered that was found to interact with a GPCR. It is currently marketed in the United States for the treatment of nausea and vomiting associated with cancer chemotherapy and also as an appetite stimulant for patients suffering from AIDS wasting syndrome[37]. It has also been shown to produce antinociception in humans and animals[38].

The endogenous families of ligands that interact with these receptors are known as the anandamides. They were recently discovered and are lipid in nature. Anandamide itself, is antinociceptive but not as potent as THC[39]. It has been found to be rapidly degraded by the enzyme, fatty acid amide hydrolase (FAAH)[40]. Studies with FAAH knockout mice have demonstrated 15-fold higher endogenous levels of anandamide than in wild-type mice[41]. Furthermore, these animals seem to have a higher threshold for pain in models of nociception. This work demonstrates the potential for targeting the FAAH enzyme for antinociceptive treatment. As research progresses in the area of cannabinoid systems it is highly probable that a ligand will reach the market as a new way to combat pain.

Vanilloid receptors

Recently vanilloid receptors (VR) have been the focus of many in the pharmaceutical and academic research groups as a potential target for new analgesics. The vanilloid receptors are ion channels and have been shown to be involved in nociceptive processes[42]. However, their clinical potential remains to be proven. Nonetheless, several natural products have been identified as modulators of these receptors.

Capsaicin isolated from red hot chili peppers, is a VR1 receptor agonist[43] and is marketed in the United States in topical preparations for the treatment of arthritis and inflammatory joint pain. There are drawbacks with these preparations as they can cause severe irritation to mucus membranes and the eyes. However, they have a successful market and lead researchers to believe that VR1 receptor agonists can be good pharmacological candidates. Resiniferatoxin isolated from the succulent plant *Euphorbia resinifera*, is another natural product that has been identified as a VR1 receptor agonist[44]. There are several studies in the literature demonstrating resiniferatoxin's use as an analgesic agent. Most recently, Karai et al.[45] have demonstrated that administration of resiniferatoxin selectively ablates nociceptive neurons. Therefore, the potential for the development of compounds interacting with VR1 receptors remains interesting.

Other naturally compounds that act at VR1 receptors have been found in mushrooms. Scutigeral, isolated from the non-pungent mushroom *Albatrellus ovinus*, has been shown to stimulate rat dorsal root ganglion neurons by activation of vanilloid receptors[46]. The compound is a triprenyl phenol and is a novel structural class of vanilloid receptor ligands. Isovelleral, a fungal metabolite of pungent mushrooms, is an unsaturated 1,4-dialdehyde containing molecule and another distinct chemical class[47]. It has been demonstrated that these types of compounds cause irritant effects by activation of vanilloid receptors that are capsaicin-sensitive[47]. Isovelleral is listed in the Sigma-Aldrich cell signaling catalog as an inhibitor of the VR1 receptor and has been reported as both an agonist[47] and antagonist[48] of VR1 receptors.

Conclusions

It is very evident that natural products have been and continue to be a valuable source of novel compounds and peptides that have the potential to serve as analgesic agents or as lead molecules for the development of such. As more research is conducted on natural products it is inevitable that more diverse compounds will be discovered and

new mechanisms of action will be elucidated. With the advances made in analytical chemistry and isolation chemistry, more compounds of a purely lipid nature will be discovered and will advance the recently coined field of lipidomics. There is much reason for excitement for the future of natural products research, particularly with regard to the development of novel agents that interact with nociceptive pathways. The fields of pharmacognosy and medicinal chemistry will work closely to ensure that novel compounds of natural origin are explored for their potential in the development of novel drug candidates.

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