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Analgesia and Antinociceptive Tolerance to NSAIDs

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ABSTRACT

Pain is a complex, unpleasant sensory experience that typically serves as a protective response to harmful stimuli. The transition from acute sensation to chronic pathology originates with early pathogenic events, defined by the activation of peripheral nociceptors following tissue injury. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used and the most commonly prescribed classes of medication in non-severe pain for their analgesic, antipyretic, and anti-inflammatory effects. They are effective for various pain conditions, including muscle pain, mild trauma, headaches, and various inflammatory conditions such as arthritis and fever-related diseases. Their primary mechanism of action is to block the synthesis of certain prostaglandins by inhibiting cyclooxygenase enzymes. However, long-term and frequent use of non-selective cyclooxygenase inhibitors is believed to be associated with a higher risk of gastrointestinal side effects, including gastrointestinal bleeding, obstruction, ulcers, and perforations. On the other hand, like opioids, repeated, chronic administration of NSAIDs leads to a progressive decrease in pain-relieving effectiveness, developing tolerance.

Our extensive studies over almost twenty years manifest that this tolerance to NSAIDs (diclofenac, ketorolac, ketoprofen, and lornoxicam) developed over four to five days, involving the endogenous opioid and cannabinoid systems and showing cross-tolerance with morphine, suggesting shared pathways in long-term pain management. While NSAIDs primarily work by inhibiting cyclooxygenase and prostaglandin production, their tolerance is linked to the endogenous opioid and cannabinoid systems, as opioid antagonist (e.g., naloxone) and cannabinoid receptor antagonist (AM-251) block this tolerance. Thus, commonly used for acute and chronic pain, this evidence highlights that NSAIDs may have reduced efficacy over time due to adaptive changes in pain-control pathways, including limbic brain structures, central amygdala, and cingulate and insular cortices. This mechanism mediates the descending axis from the midbrain periaqueductal grey matter, rostral ventromedial medulla, and dorsal horn of the spinal cord.

Keywords: allodynia, antinociception, cannabinoids, hyperalgesia, opioids, pain matrix.

Introduction

From a medical point of view, pain is one of the most common complaints, and its character of presentation helps give clues to the underlying disease process. Therefore, understanding the pathophysiology of pain is critical for assessing its significance in a patient's health and for identifying novel treatment options. Surgical patients are a critically important group that must be addressed in the search for safer, more effective analgesic methods, and nanotechnology shows significant potential to meet the postoperative needs of this population[1].

In this regard, postoperative pain remains one of the most common and challenging complications in a patient's recovery. Although prevalence varies by surgery type, systematic analyses suggest a high global burden, with estimates indicating that most surgical patients experience acute pain and more than half report moderate to severe intensity. Inadequate postoperative analgesia is consistently associated with numerous adverse outcomes, including delayed recovery, impaired wound healing, increased cardiopulmonary complications, prolonged hospitalization, and the development of persistent postsurgical pain. The substantial adverse effects associated with opioid-based analgesia and its contribution to postoperative dependence have prompted a shift toward multimodal, opioid-sparing perioperative strategies. Non-opioid analgesics now form the cornerstone of contemporary perioperative management and enhanced recovery after surgery[2,3].

Growing evidence supports the effectiveness of numerous classes of pharmacological agents, such as local anesthetics (e.g., lidocaine, bupivacaine) and nonsteroidal anti-inflammatory drugs (NSAIDs) (ketorolac, diclofenac, lornoxicam, etc.), cyclooxygenase (COX-2) inhibitors (e.g., celecoxib), acetaminophen, gabapentinoids, glucocorticoids, adrenergic α2-receptor agonists (clonidine, dexmedetomidine), and glutamate N-methyl-D-aspartate (NMDA) receptor antagonists (ketamine, magnesium). They have demonstrated significant analgesic and opioid-sparing benefits in a variety of surgical contexts, which prioritize nonopioid therapies to mitigate the harms associated with opioid overuse[2]. On the other hand, one alternative approach to developing new drugs is studying the analgesic effects of animal toxins and venoms. The latter is a complex mixture of several molecules that should be better separated, cleaned, and studied in pain medicine[4].

Finally, modern nanotechnology has emerged as a promising means of achieving effective long-term analgesia while avoiding the adverse side effects associated with conventional pharmacological agents. Nanotechnology-based treatments, including liposomes, other polymeric nanoparticles, and carbon-based polymers, can help mitigate adverse side effects. These nanomaterials can serve as drug delivery systems that facilitate controlled release and enhance drug stability by encapsulating free molecules and protein-based drugs, thereby leading to longer-lasting analgesia and minimizing side effects[1].

Physiology of Pain

Pain is a fundamental, complex topic in animal and human biomedicine, deeply rooted in the evolutionary timeline as an essential function for survival and environmental adaptation. Pain sensation is always considered a subjective experience that encompasses not only physical stimuli but also emotional and cognitive factors, and it varies from one species to another and from individual to individual[4].

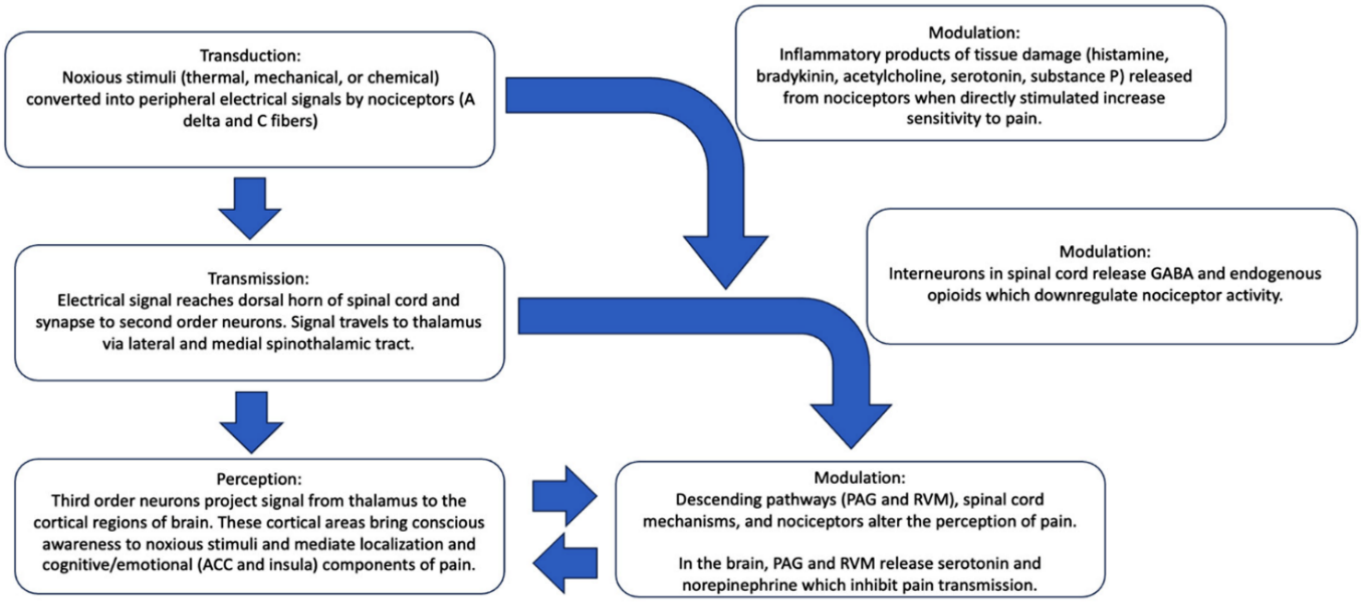

Figure 1. Pain physiology and the four phases of nociception, transduction, transmission, perception, and modulation. Modulations are realized at the periphery (transduction processes) and the central (spinal cord, brainstem) levels (transmission and perception processes). Abbreviations: ACC, anterior cingulate cortex; GABA, gamma-aminobutyric acid; PAG, periaqueductal gray (matter); RVM, rostro-ventral medial (medulla). Adapted from Torpey et al., 2024 [1].

Figure 1 shows how pain signals reveal an intricate network of four principal physiological processes, such as transduction, transmission, perception, and modulation, that involve many different systems within the body. When pain becomes part of the conscious experience, it is realized by special pain receptors (nociceptors). Nociceptors come in two primary forms, each with distinct characteristics: fast myelinated A-delta and slow unmyelinated C fibers.

There are many peripheral nerve terminals in the skin, muscles, and visceral organs that detect pain sensations throughout the body. Noxious stimuli in the form of thermal, mechanical, or chemical stimuli are transduced by nociceptors, in local slow potentials, and then converted into peripheral electrical nervous impulses that are transmitted centrally to the spinal cord and brainstem, and via the thalamus to the cortex (Figure 2).

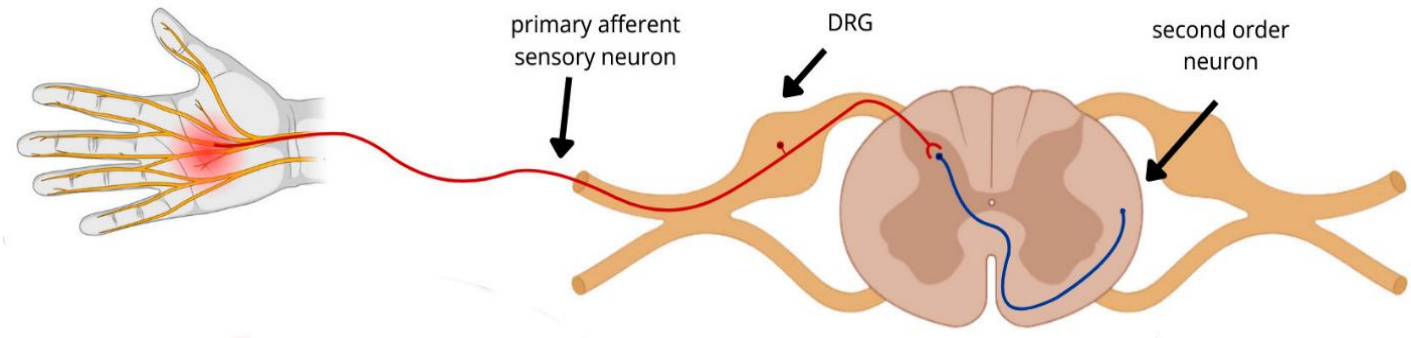

Figure 2. Neuronal pathway of pain processing. Nociceptor afferents transmit signals from the periphery through the dorsal root ganglia (DRG) to the spinal dorsal horn, and then continue through second-order neurons via the spinothalamic tract to the thalamus and brain. Adapted from Angstmam et al., 2026 [4].

Many distinct specialized, e.g., voltage-gated sodium, calcium, potassium channels, transient receptor potential (TRP) channels, proton-gated acid-sensing (ASIC) channels, G protein-coupled receptor (GPCR) channels to opioids, cannabinoids, adenosine, adenosine triphosphate (ATP), as well as bradykinin, serotonin, norepinephrine channels on nerve endings open in response to injury, causing ion influx and membrane depolarization, which generates actionable neural signals (Figure 3). In addition, peripheral tissue injury or pathogen invasion recruits immune cells that release proinflammatory cytokines, leading to heightened nociceptor excitability, which in turn drives neuropeptide release and amplifies inflammation.

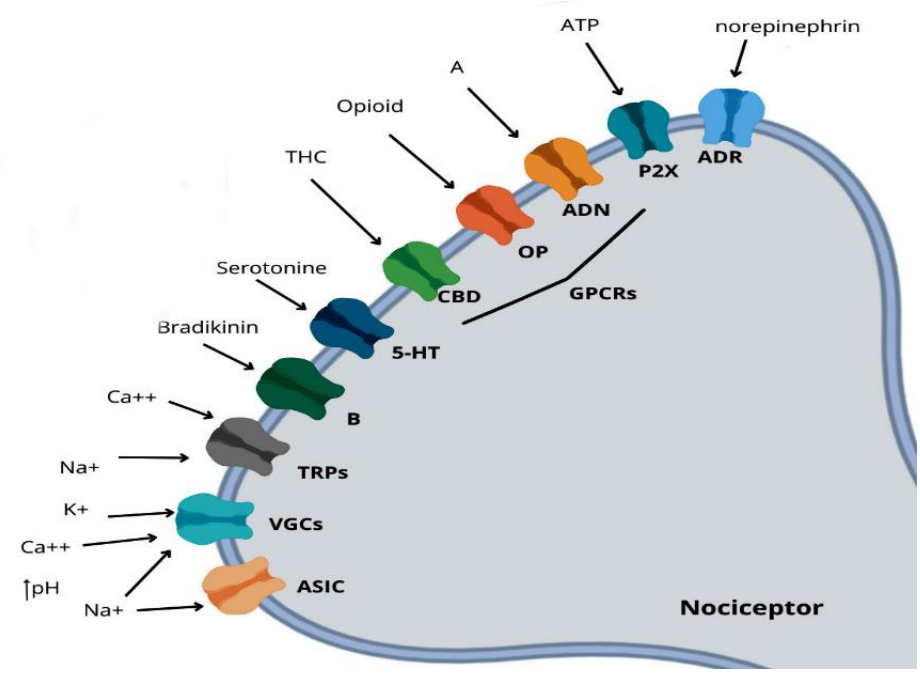

Figure 3. The mechanism of action of pain from a tissue injury involves many receptors and ion channels that are associated with primary afferent sensory neurons. Among them are ASICs (acid-sensitive ion channels), VGCs (voltage-gated channels), TRPs (transient receptor potential), B (bradykinin receptor), 5-HT (serotonin receptor), CBD (cannabinoid receptor), OR (opioid receptor), ADN (adenosine receptor), P2X (purinergic receptor), and ADR (adrenergic receptor). THC, tetrahydrocannabinol. Adapted from Angstmam et al., 2026 [4].

Pain transmission means the process by which noxious stimuli are converted into electrical signals by peripheral nociceptors and conveyed to the central nervous system via A-delta (fast, sharp) and C fibers (slow, dull). These signals travel to the spinal cord's dorsal horn, ascend through the spinothalamic tract to the thalamus and somatosensory cortex, and limbic cortical areas.

Perception, the third pain process, is the brain's cortical interpretation of these signals, bringing conscious awareness to pain. Interpretation of pain is a highly individual and variable process, influenced by factors such as genetics, physical and mental health, past pain experiences, age, culture, and gender as well. The emotional and cognitive aspects of pain are processed in the brain's limbic areas, such as the anterior cingulate cortex (ACC), insula, hypothalamus, central amygdala, and prefrontal cortex. These regions contribute to the affective-motivational and cognitive-evaluative aspects of pain, influencing how it is experienced[1].

Finally, pain modulation refers to the CNS's ability to alter the perception of pain. This process involves both descending pathways from the brain to the spinal cord and local spinal cord mechanisms. The descending pathways originate in areas such as the periaqueductal gray matter (PAG) and the rostroventral medulla (RVM). These areas release neurotransmitters

such as enkephalin, serotonin, and norepinephrine, which inhibit pain transmission at the spinal cord level. The spinal cord features a host of inhibitory interneurons that can downregulate pain signals. These interneurons release neurotransmitters such as the endogenous opioid enkephalin and gamma-aminobutyric acid (GABA), which decrease the activity of pain-transmitting neurons. The balance between excitatory and inhibitory signals determines the final modulation of pain (Figure 4). Central sensitization can also have a modulatory role in persistent, pathological pain conditions that result in pain chronification. These modulatory mechanisms and the intercommunications between the central and peripheral nervous systems play a crucial role in pain modulation and how it should be treated in medical practice[1].

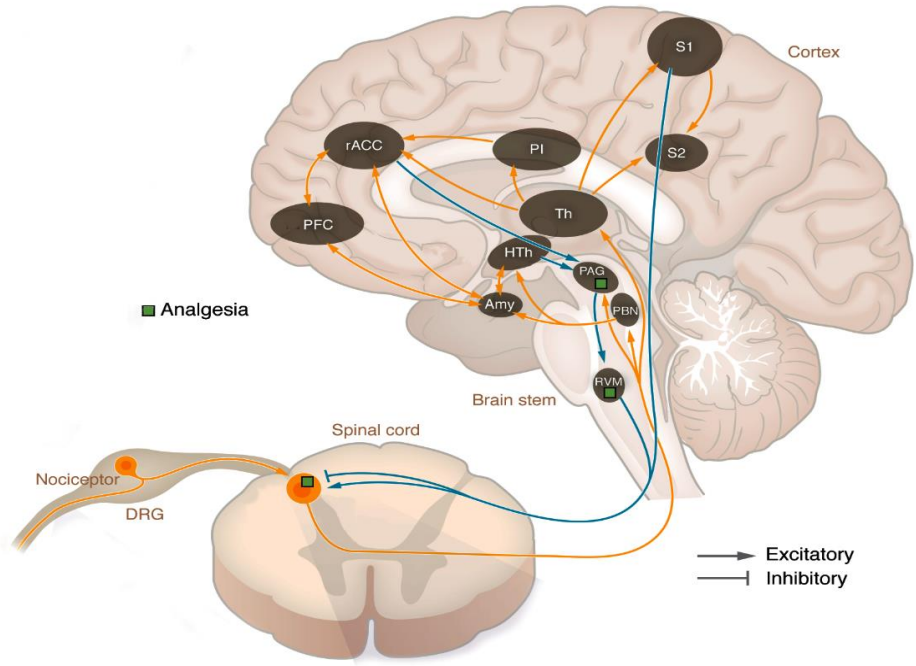

Figure 4. Ascending and descending pathways of pain processing. Ascending structures (yellow arrows) include the parabrachial nucleus (PBN), periaqueductal gray (PAG), hypothalamus (HTh), thalamus (Th), prefrontal cortex (PFC), rostral anterior cingulate cortex (rACC), posterior insular cortex (PI), amygdala (Amy), and primary and secondary somatosensory cortices (S1, S2). Descending pathways (blue) from the rACC, PAG, and rostral ventromedial medulla (RVM) modulate pain transmission. The PAG, RVM, and spinal dorsal horn are primary sites for opioid-induced analgesia (green). Adapted from Zeng et al. (2025) [3].

Modern advances in pain research continue to enhance understanding of pain physiology and improve pain management strategies. Researching pain mechanisms using optogenetic, molecular, and nanotechnology approaches holds promise for developing targeted therapies for both acute and chronic pain.

Short Review of Common Non-Opioid Analgesics

The most commonly used approach to treat pain is the administration of analgesics. However, the majority of medications marketed for pain treatment, especially opioid drugs, come with a series of adverse effects, including dependence, abuse, tolerance, and constipation. Therefore, these conditions often require increasing the drug dose over time due to reduced efficacy and the return of clinical symptoms. The recent opioid crisis has had a devastating impact, with millions of people globally affected by opioid addiction and opioid overdose deaths. Therefore, these limitations underscore the urgent need for safer, more effective analgesics with minimal side effects[5].

NSAIDS

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used and the most commonly prescribed classes of medication for their analgesic, antipyretic, and anti-inflammatory effects. They are effective for various pain conditions, including muscle pain, mild trauma, headaches, and various inflammatory conditions such as arthritis and fever-related diseases. As a result, NSAIDs have found their applications in therapeutic areas for young athletes, the general population, and the elderly. Their primary mechanism of action is via the inhibition of cyclooxygenases (COXs), which are rate-determining enzymes for prostaglandins and other prostanoids synthesis, such as thromboxanes. COX is thought to be involved in the production of prostaglandins (PGs) from the precursor, arachidonic acid (Figure 5). In turn, PGs mediate vasodilation and pain sensation[6-9].

The primary therapeutic action of NSAIDs is to block the synthesis of certain PGs by inhibiting cyclooxygenase enzymes (COX-1 and COX-2). Some NSAIDs (ibuprofen and naproxen) are non-selective COX inhibitors, affecting both COX-1 and COX-2, while others (celecoxib) selectively inhibit COX-2. NSAIDs have known gastrointestinal, cardiovascular, and renal side effects. Long-term, frequent (several months) use of non-selective COX inhibitors is believed to be associated with a higher risk of gastrointestinal side effects, including gastrointestinal bleeding, obstruction, ulcers, and perforations. Cardiovascular side effects include an increased risk of adverse cardiovascular events such as myocardial infarction or stroke, and are more common with selective COX-2 inhibitors. Regarding renal side effects, they are more likely to occur in individuals with underlying renal dysfunction and include electrolyte imbalances and nephrotic syndrome[1,6-9].

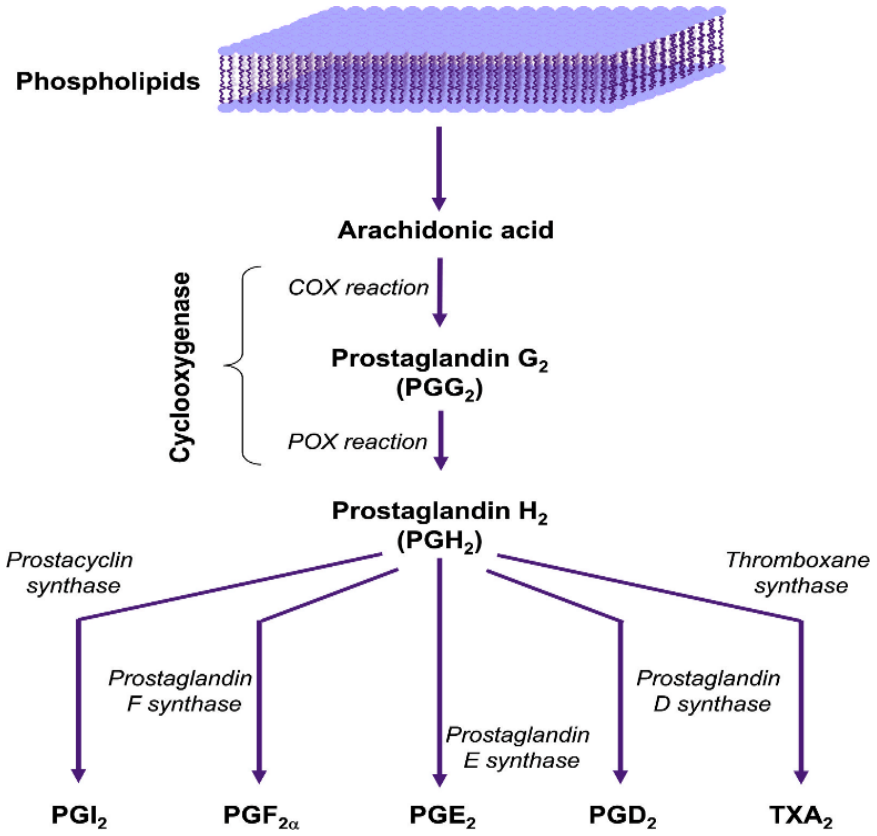

Figure 4. Role of cyclooxygenase in the biosynthesis of prostanoids from phospholipid arachidonic acid. Reproduced from Tena-Garitaonaindia et al., 2025 [9].

ACETAMINOPHEN

Alongside NSAIDs, acetaminophen (paracetamol) is another non-opioid analgesic used for its analgesic and antipyretic effects. Consequently, acetaminophen gained popularity to replace phenacetin and became the most widely used over-the-counter medication. To this day, it remains one of the analgesics commonly used as a prescribed drug to reduce fever and acute and chronic pain in the general population[10].

Acetaminophen is thought to act by being metabolized to p-aminophenol, which can cross the blood–brain barrier and modulate the activity of receptors involved in nociception. Hence, the pain modulation mechanism directly affects the brain[11]. When used short-term and within recommended dosages, acetaminophen is generally well-tolerated; however, liver toxicity may occur with high dosages. Other side effects may include rash, nephrotoxicity, electrolyte abnormalities, and skin reactions with Stevens-Johnson syndrome[11].

ANTICONVULSANTS AND GABAPENTINOIDS

Anticonvulsants like carbamazepine, phenytoin, and lamotrigine have been used to treat trigeminal neuralgia and other neuropathic pain disorders for a long time. However, sleepiness, vertigo, nausea, double vision, and reduced neuromuscular control, as well as cognition issues, are the most frequent patient-reported side effects of these drugs. Lamotrigine acts on voltage-gated sodium channels, inhibiting them and preventing the release of presynaptic glutamate[12,13].

Gabapentinoids (gabapentin, pregabalin) were first introduced as anticonvulsants in the 1990 s and later approved to treat various chronic neuropathic pain disorders. They have found widespread use in perioperative care, particularly for managing acute postoperative pain and postherpetic neuralgia[14].

Gabapentin is an analog of the inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), though it does not act directly on GABA receptors in the CNS. Gabapentin and pregabalin act through a variety of pharmacological pathways, such as blocking calcium transmission through high-voltage-gated channels and competing with L-amino acid transporters. Gabapentinoids reduce neurotransmitter release and synaptic excitability, ultimately leading to neuronal suppression and decreased pain transmission[15-17].

ANTIDEPPRESANTS

Tricyclic antidepressants, namely amitriptyline, doxepin, and imipramine, are a class of medications used for the treatment of major depressive disorders by inhibiting the reuptake of various neurotransmitters, including serotonin and norepinephrine. These mechanisms are thought to be responsible for their use in managing chronic pain. Serotonin and norepinephrine reuptake inhibitors (SNRIs) and selective serotonin reuptake inhibitors (SSRIs) (e.g., fluoxetine) also work by inhibiting the reuptake of various neurotransmitters. SNRIs, including duloxetine and venlafaxine, are effective at reducing neuropathic pain, specifically diabetic neuropathy[1].

Recently, there has been progress in using ketamine during the perioperative treatment. Compared with racemic ketamine, esketamine demonstrates approximately twice the potency, along with a rapid onset of action, mild respiratory depression, and fewer adverse psychiatric effects. As a potent glutamate N-methyl-D-aspartate (NMDA) receptor antagonist with high affinity, it exhibits sedative, analgesic, and antidepressant properties, among others. It is indicated in combination with SNRI or SSRI for the treatment of treatment-resistant depression and major depressive disorder accompanied by suicidal ideation in adult patients[18].

On the other hand, adjunctive analgesics to opioids can reduce opioid consumption while simultaneously providing pain relief through an alternative, complementary mechanism. While tricyclic antidepressants as a drug class have not completely proven to be overtly efficacious for this indication, desipramine, a secondary amine tricyclic antidepressant, may have utility as an adjunctive analgesic in nociceptive and neuropathic pain due to its ability to potentially prolong the analgesic effects of opioids[19,20].

Antinociceptive tolerance to NSAIDs

In general pharmacological terms, tolerance is a decrease in the response to a medication or substance after repeated use. In pain medicine, we consider that, like opioids, antinociceptive tolerance to NSAIDs is a phenomenon where repeated, chronic administration leads to a progressive decrease in pain-relieving effectiveness, requiring higher doses to achieve the same result. Our studies manifest that this tolerance develops over 4–5 days to NSAIDs like diclofenac, ketorolac, ketoprofen, and lornoxicam, involving the endogenous opioid and cannabinoid systems and showing cross-tolerance with morphine, suggesting shared pathways in long-term pain management. While NSAIDs primarily work by inhibiting cyclooxygenase (COX) and prostaglandin production, their tolerance is linked to the endogenous opioid system, as opioid antagonists (e.g., naloxone) can block this tolerance. Thus, commonly used for acute and chronic pain, this evidence highlights that NSAIDs may have reduced efficacy over time due to adaptive changes in pain-control pathways[21-29].

Tolerance to NSAIDs has been demonstrated via repeated injections into pain-modulating brain structures, including the central nucleus of the amygdala (CeA), periaqueductal gray (PAG), rostral ventromedial medulla (RVM), and the dorsal hippocampus. We recently found similar effects of antinociceptive tolerance in limbic brain areas, such as the anterior cingulate and agranular insular cortices[30].

ANTINOCICEPTIVE TOLERANCE TO NSAIDs IN ACC: OPIOID MECHANISM

Studies of the emotional and motivational basis of pain reveal a diverse and complex set of processes by which the affective experience of pain is realized. In particular, the perception of both pain intensity and aversiveness is the complex process by which the brain constructs the sensory and emotional sensation of pain and challenges any standard "perception-action" model[31,32].

The first anatomical, physiological, and behavioral investigations have demonstrated the important role of the brain's limbic system in the affective-motivational component of pain. Some animal studies and clinical evidence have shown the importance of the anterior cingulate cortex (ACC) in affective aspects of pain[33]. It is well known that the ACC is involved in pain perception, primarily receiving extensive projections from the medio-dorsal thalamic nucleus and broadly connecting with relevant regions of the descending pain modulation system[34]. A projection from the spinal dorsal horn through the medial/intra-laminar thalamic nuclei to the ACC has been proposed to process information on pain-related unpleasantness. In addition to contributing to the immediate affective consequences of noxious stimulation, the "ACC system" may contribute to the avoidance learning that sometimes follows as a secondary reaction to pain[35]. However, the mechanisms of the ACC's involvement in pain have yet to be elaborated[34].

In a series of experiments, we found that microinjection of NSAIDs (diclofenac, ketorolac, and lornoxicam into the rostral part of ACC (rACC) produced antinociception as detected by an increase in latency of tail flick (TF) and hot plate (HP) withdrawal tests compared to the baseline control of intact rats and a control group with saline microinjected into the same site as well. However, subsequent NSAIDs microinjections resulted in progressively less antinociception, so by day 4, there was no effect, similar to saline microinjections for both the TF and the HP tests, i.e., induced tolerance and cross-tolerance to morphine[21,22]. In the next set of experiments, we revealed that pre- and post-treatment with a non-selective opioid receptor antagonist, naloxone, and a selective mu-opioid receptor antagonist CTOP prevent antinociception induced by NSAIDs microinjected into the ACC in the TF and HP tests. CTOP is a short octapeptide (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2) that selectively antagonizes the mu-opioid receptor (MOR)[23,30].

These data demonstrated that administration of diclofenac, ketorolac, and lornoxicam, widely used non-opioid, NSAID analgesics, into the rostral part of the ACC, induces antinociception in rats. When administered repeatedly, tolerance developed to the antinociceptive effects of these drugs. The present findings support the concept that the development of tolerance to the antinociceptive effects of NSAIDs is mediated via an endogenous opioid system, possibly involving descending pain modulatory systems, via structures of the PAG and RVM.

ANTINOCICEPTIVE TOLERANCE TO NSAIDs IN ACC: CANNABINOID MECHANISM

Apart from opioid mechanisms, the second neuromodulatory system involved in the pathophysiology of pain, which has recently been recruited with particular interest for the development of new therapeutic strategies, is the endocannabinoid system (ECBS), which plays a key role in pain control. This system is integrated with the cannabinoid receptors, their endogenous ligands, and the enzymes involved in the synthesis and degradation of these ligands[36,37]. Recent research highlights the interaction between ECBS and NSAIDs, paracetamol, and pyrazalone. These agents may enhance endogenous endocannabinoid levels or influence ECBS signaling pathways, providing a multifaceted approach to pain relief[38].

In the series of experiments presented here, we investigated the brain mechanisms of NSAIDs' antinociception in the formalin test for the development of tolerance. To study the relation between these antinociceptive effects and endocannabinoids, we treated experimental rats with CB1 receptor antagonist AM-251 in the rACC following injections with diclofenac, ketoprofen, ketorolac, and lornoxicam.

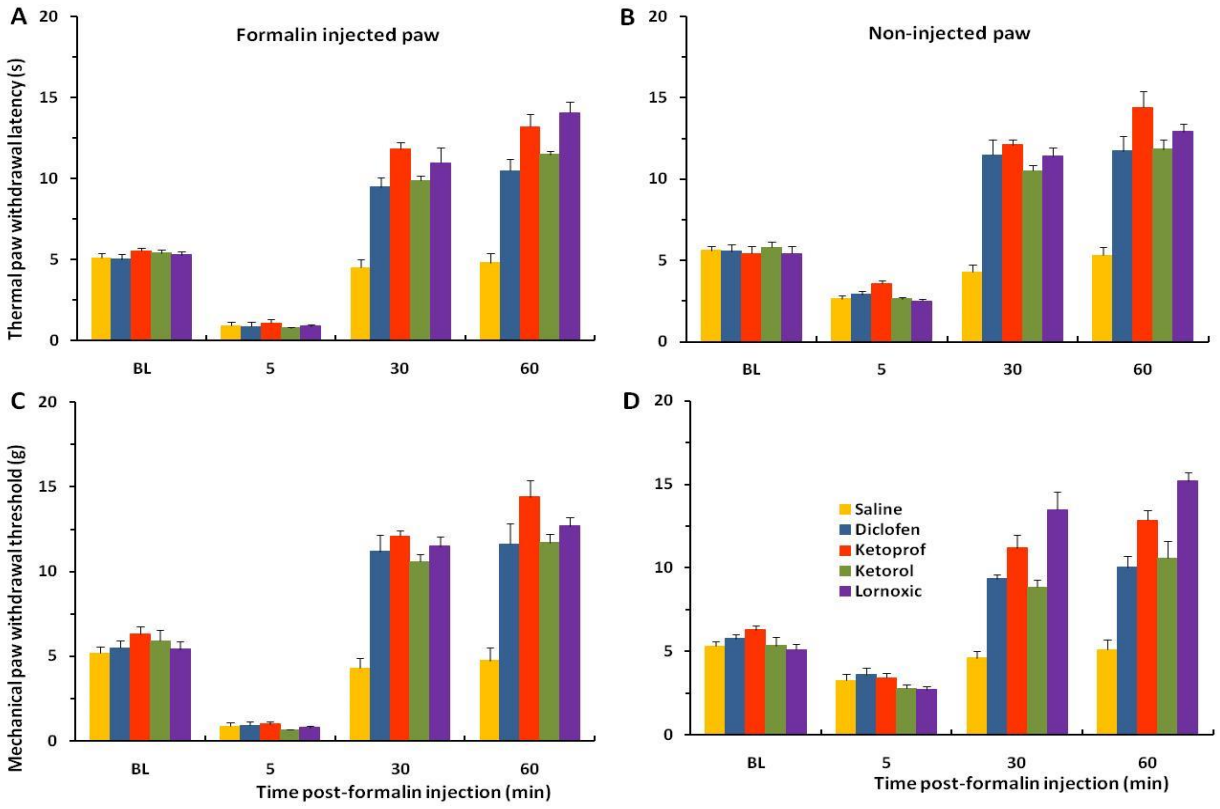

Figure 5. Latencies of the thermal paw withdrawal reflex (s) (A, B) and thresholds of the mechanical paw withdrawal reflex (g) (C, D) after intraplantar formalin injection to one (right) paw on the first day of the experiment. Note analgesics result in a significant increase in latencies and thresholds compared to the saline control for post-formalin phase II (at 30 min and 60 min), in formalin-injected (A, C) and non-injected (B, D) paws. BL – pre-formalin baseline

On the first day of this trial, all four NSAIDs resulted in significant antinociception in the formalin test (Figure 5). However, in four consecutive days, NSAIDs microinjections into the rACC resulted in progressively less antinociception, so by the 4th day, there was no effect, which was similar to saline microinjections for both behavioral tests, i.e., induced tolerance. At the last, the fourth day, post-treatment with AM-251 did not change the latency of thermal and threshold of mechanical withdrawal reflexes (Figure 6). This means that the CB1 receptor antagonist does not affect behavioral withdrawal responses in the tolerant rats' group, unlike the rats on the first experimental day (Figure 5).

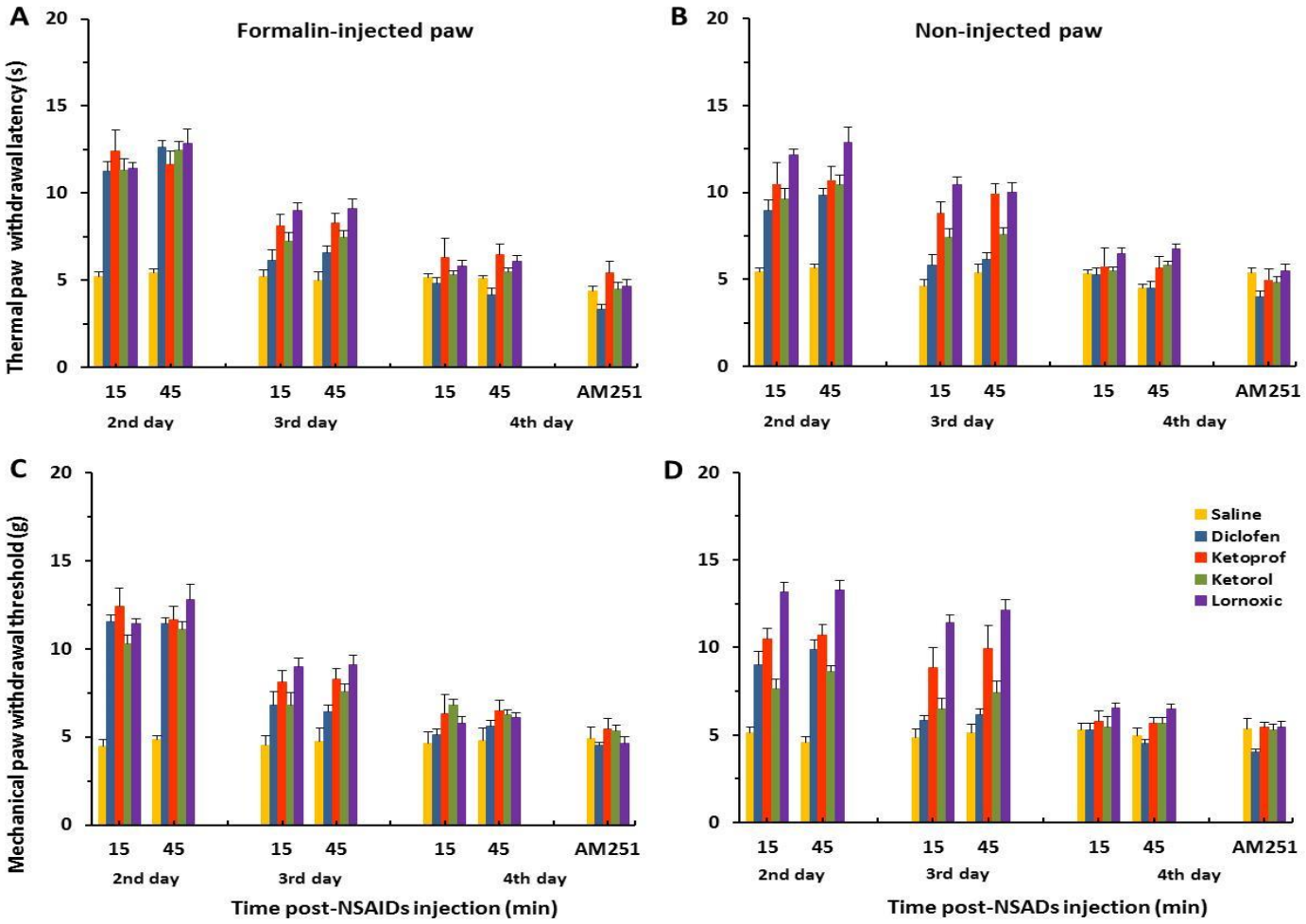

Figure 6. Latencies of the thermal paw withdrawal reflex (s) (A, B) and thresholds of the mechanical paw withdrawal reflex (g) (C, D) after NSAIDs administration into the rACC for three consecutive days. Note that in the subsequent 2–4 days, antinociception decreased gradually for formalin-injected (A, C) and non-injected (B, D) paws, respectively, i.e., tolerance is developed. On the fourth day, post-treatment with AM-251 does not change the latency of thermal and threshold of mechanical withdrawal reflexes.

In the next trials of this study, we tested if pretreatment with AM-251 would prevent NSAIDs-induced antinociception in the rACC in post-formalin phase II. Ten minutes after unilateral intraplantar injection of formalin, rats received AM-251, followed 15 min later by microinjection of one of the NSAIDs or saline. Pretreatment with AM-251 completely prevented any thermal or mechanical antinociceptive or anti-hyperalgesic effect of all four NSAIDs during phase II in the formalin-injected paw (Figure 7A, C). In the non-injected paw, we observed the same reduction in anti-nociceptive effects of all NSAIDs in the rACC during phase II for thermal and mechanical paw withdrawal reflexes (Figure 7B, D).

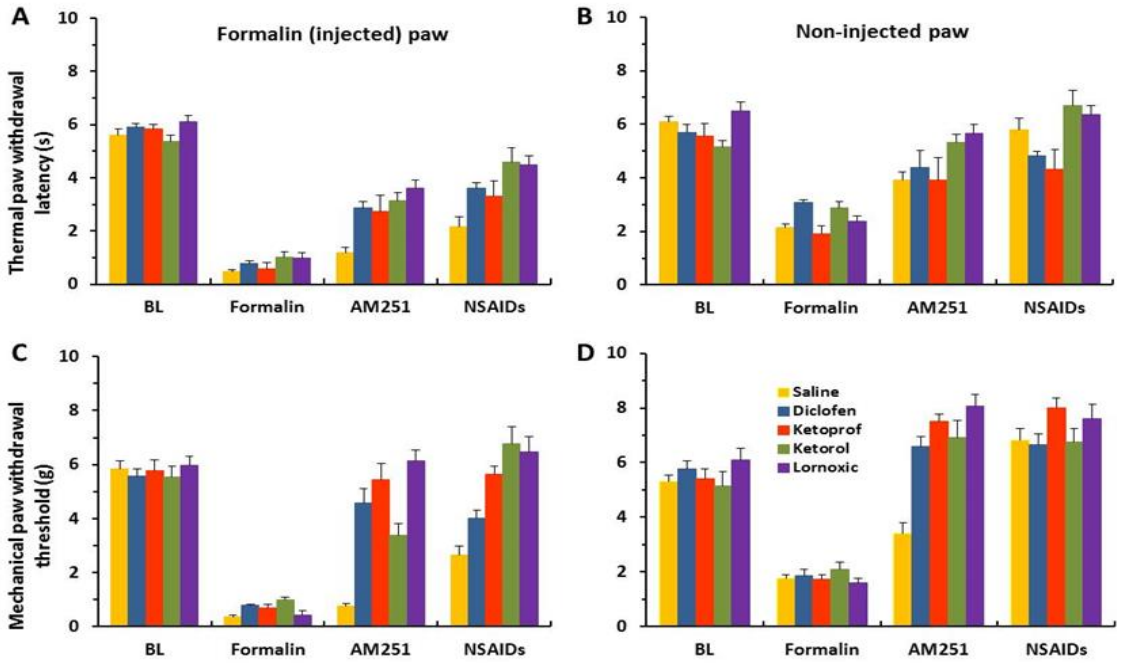

Figure 7. Pretreatment with CB1 receptor antagonist AM-251 completely prevents analgesic effects of NSAIDs in ipsilateral (formalin injected) paw (A, C) and contralateral (non-injected) paw (B, D) in latencies of the thermal paw withdrawal reflex (s) (A, B) and thresholds of the mechanical paw withdrawal reflex (g) (C, D) for post-formalin phase II (30 min), respectively.

In the last set of these experiments, post-treatment with AM-251, following NSAIDs, almost completely abolished analgesia produced by diclofenac, ketoprofen, ketorolac, and lornoxicam injected into the rACC (Figure 8A, C). For the formalin non-injected paw, we observed the same reduction of antinociceptive effects of these NSAIDs (Figure 8B, D). As we mentioned above, such effects of abolishing by the CB1 receptor antagonist AM-251, we did not find in NSAIDs-tolerant rats (Figure 6).

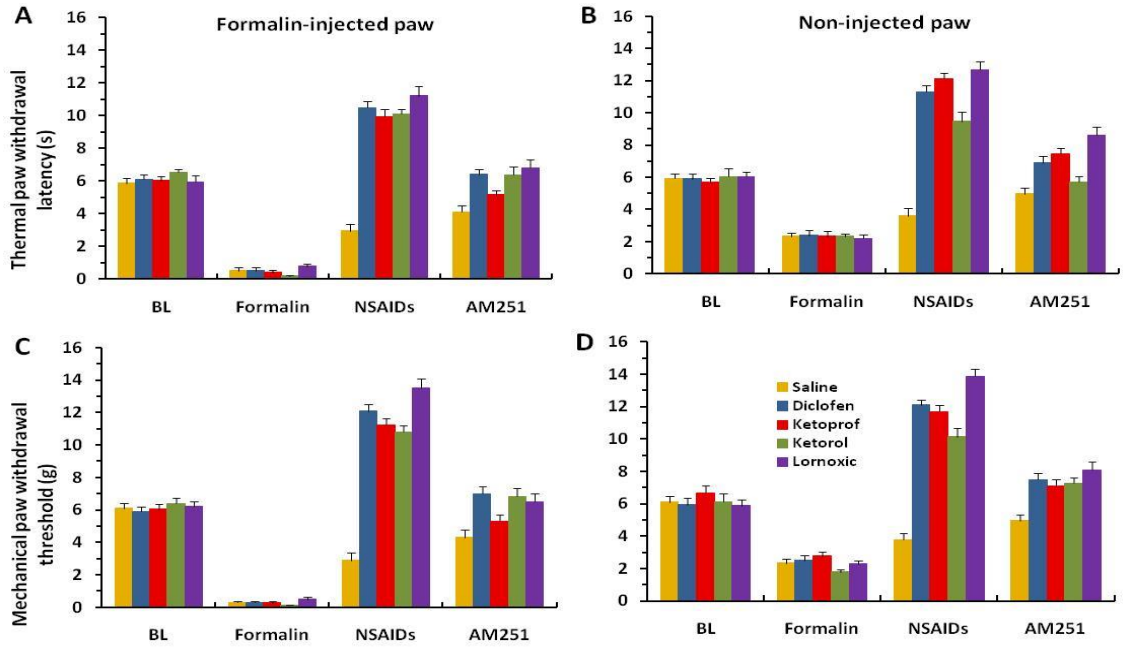

Figure 8. Post-treatment with CB1 receptor antagonist AM-251 completely abolishes analgesic effects of NSAIDs in ipsilateral (formalin injected) paw (A, C) and contralateral (non-injected) paw (B, D) in latencies of the thermal paw withdrawal reflex (s) (A, B) and thresholds of the mechanical paw withdrawal reflex (g) (C, D) for post-formalin phase II (30 min), respectively.

Similar effects were revealed in the agranular insular cortex (AIC) and central amygdala. In particular, microinjection of the same commonly used NSAIDs (diclofenac, ketoprofen, ketorolac, and lornoxicam) in the AIC and central nucleus of amygdala (CeA) produced antinociception in an inflammatory pain model induced by intraplantar injection of formalin into one hindpaw of rats[22,23]. These results confirmed our evidence in an acute pain model with TF and HP tests. These data also confirmed our previous findings obtained in other pain matrix structures, such as the nucleus raphe magnus, periaqueductal grey matter, and dorsal hippocampus[22,24-28,30].

Some spreading hyperalgesic or analgesic effects from the ipsilateral into the contralateral paw that we observed in this study are explained as due to central sensitization or desensitization at the spinal cord level. We suppose that such sensitization/ desensitization can activate or inhibit the system of commissural interneurons, which are present in the spinal cord and brainstem, and which can develop some formalin-induced hyperalgesia or NSAIDs-induced analgesia. This phenomenon is well-documented in pain medicine (see review[39]).

As pain is a complex perception in which sensation, emotion, and cognition interweave, conscious perception and the unpleasantness of pain are mirrored in behavioral reactions (e.g., withdrawal reflex, escape, or sweating due to suffering), which require activity in wide brain regions. Our study clearly showed that antinociceptive tolerance effects in pain matrix structures are mediated by opioid and cannabinoid mechanisms, and pain experience in the formalin test arouses behavioral responses and activates the descending pain modulatory system.

Conclusions

Pain relief by cannabinoids and opioids produces antinociceptive synergy. Cannabinoids such as delta-9-tetrahydrocannabinol (THC) and endocannabinoids such as anandamide (N-arachidonoyl ethanolamine, AEA) also alter endogenous opioid analgesia. Opioids and cannabinoids bind distinct receptors that co-localize in brain areas involved in the processing of pain signals. Therefore, it is logical to look at interactions of these two systems in the modulation of both acute and chronic pain. These drugs are often co-abused. In addition, the lack of continued effectiveness of opioids due to tolerance development limits the use of such drugs. The data indicate that with cannabinoid/opioid therapy, one may be able to produce long-term antinociceptive effects at doses devoid of substantial side effects, while preventing the neuronal biochemical changes that accompany tolerance. The clinical utility of modulators of the endocannabinoid system as a potential mimic for THC-like drugs in analgesia and tolerance-sparing effects of opioids is a critical future direction, i.e., effective antinociception with delayed development of tolerance.

Finally, tolerance to NSAIDs (diclofenac, ketorolac, ketoprofen, and lornoxicam) developed over four to five days, involving the endogenous opioid and cannabinoid systems and showing cross-tolerance with morphine, suggesting shared pathways in long-term pain management. While NSAIDs primarily work by inhibiting cyclooxygenase and prostaglandin production, their tolerance is linked to the endogenous opioid and cannabinoid systems, as opioid and cannabinoid receptor antagonists block this tolerance. Thus, commonly used for acute and chronic pain, these data highlight that NSAIDs may have reduced efficacy over time due to adaptive changes in pain-control pathways, including limbic brain structures, central amygdala, and cingulate and insular cortices. This mechanism mediates the descending axis from the midbrain PAG, RVM, and spinal dorsal horn.

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