An Overview of Antinociceptive Tolerance to Non-steroidal Anti-inflammatory Drugs

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ABSTRACT

Purpose of Review: One of the vital functions of the nervous system is to provide information about the threat of injury. The sensation of pain, by its inherent aversive nature, contributes to this function. The mainstay of medical pain therapy remains drugs that have been around for decades, like opiates and non-opioid drugs. However, adverse effects of opiates, particularly tolerance, limit their clinical use. Several lines of investigations have shown that systemic administration of non-opioid, non-steroidal anti-inflammatory drugs (NSAIDs) induces antinociception with some effects of tolerance. In this review, we report that repeated microinjections of NSAIDs analgin, clodifen, ketorolac and xefocam into the central nucleus of amygdala, the midbrain periaqueductal grey matter and nucleus raphe magnus in the following 4 days result in progressively less antinociception compared to the saline control testing in the tail-flick reflex and hot plate latency tests. Hence, tolerance develops to these drugs and cross-tolerance to morphine in male rats.

Conclusions: Presented data show that repeated microinjections of NSAIDs into the central nucleus of amygdala, the midbrain periaqueductal grey matter and nucleus raphe magnus induce antinociception and then exhibit tolerance. These findings strongly support the suggestion of endogenous opioid system involvement in NSAIDs antinociception, as it is blocked by an opioid antagonist naloxone. Moreover, the descending pain-control system, the periaqueductal grey – rostral ventro-medial part of medulla circuit should be viewed as a pain-modulation system.

Keywords: Analgesia; antinociception; descending pain modulation; hot plate test; non-opioid tolerance; tail-flick reflex.

1. INTRODUCTION

Pain is a response of the body to the action of injuring stimuli. Notwithstanding an unpleasant experience, it appears to be an important component of the defense system of the organism and a permanent regulator of homeostatic reaction. However when the pain signals remain over long periods they generate chronic pain. Despite the investment of significant resources by the pharmaceutical industry to identify novel analgesic drugs, chronic pain, which is most commonly defined as pain lasting longer than 3 months (i.e., outlasting the usual healing process), still represents a difficult treatment challenge in a large sector of the population.

The role of opioids in the treatment of pain has been long known for the humankind for thousands of years [1]. Opioid analgesics are widely used to relieve dull, poorly localized (usually, visceral) pain. Repeated doses may cause tolerance to these drugs and dependence so that a sudden termination of opioid analgesics may precipitate a withdrawal syndrome. Apart from the opioid drugs, non-opioid, non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesics in the treatment of pain. These drugs have analgesic, antipyretic, and at higher doses, anti-inflammatory actions. Aspirin (acetylsalicylic acid) was the first NSAID but has been largely replaced by drugs that are less toxic to gastro-intestinal tract, e.g. paracetamol, ibuprofen, ketorolac, naproxen, xefocam. NSAIDs produce their effects by inhibiting cyclo-oxygenase (COX), a key enzyme in the production of prostaglandins. The latter are one of the mediators released at sites of inflammation. They do not themselves cause pain but they potentiate the pain caused by other mediators, e.g. histamine, serotonin, bradykinin [2].
2. SHORT METHODIC NOTE

Generally, a number of simple animal tests are available for assessing analgesic drugs. We used two simplest tests on unconditioned behaviors to measure the effects of drugs in acute pain models. The first, the tail flick test, a light beam is focused on a small segment of the rat's tail. The initial low intensity of the light beam is increased incrementally until a temperature about 50°C and the normal pain threshold is reached and the tail is reflexively flicked out of the beam. Analgesics, usually, increase the latency to respond to that level of pain. The second, hot plate test is an equally reliable test using thermal heat. A metal plate at the base of a cylinder is maintained at a temperature between 55 and 70°C. When placed on the hot plate, the rat is observed and the latency to kick the hind paws and attempt to escape from the cylinder is recorded. By these two tests we studied tolerance effects induced by several NSAIDs in male rats.

3. NON-OPIOID ANALGESICS INDUCED TOLERANCE

The analgesic effect of opioids is mainly induced by their action in the midbrain periaqueductal grey matter (PAG) [3,4] and this structure probably plays a crucial role in the development of tolerance too [5-7].

The interaction of opioids and NSAIDs is of utmost interest. NSAIDs such as dipyrone (metamizol) and aspirin (lysine-acetylsalicylate (LASA), when microinjected to PAG, cause analgesia, whereas their repeated administrations result in vivid tolerance [8-11]. This antinociception at least in part is realized by endogenous opioid peptides, since microinjections induced analgesia by NSAIDs in PAG and the rostral ventro-medial part of medulla (RVM) is impaired by opioid antagonists [10,11]. NSAIDs repeated microinjection to PAG alongside with tolerance causes cross-tolerance to morphine and drug withdrawal syndrome [8,9,11,14]. Morphine microinjection to PAG inhibits nociception on spinal level through those RVM neurons, which have direct projections to the spinal dorsal roots [14,15].

3.1 The central nucleus of amygdala is involved in tolerance to the antinociceptive effect of NSAIDs

The central nucleus of amygdala (Ce) is considered an integral component of the endogenous pain-modulatory circuit and is critical for systemic morphine-induced suppression of spinal nociceptive reflexes [16]. We have recently discovered for the first time that microinjection of NSAIDs analgine, ketorolac, and xefocam into the Ce of rats both unilaterally and bilaterally elicits antinociception with the development of tolerance [17,18]. This data confirmed our previous results with systemic, intra-peritoneal (i.p.) administration of NSAIDs [18-21], and results of others using microinjection of the same NSAIDs into the PAG [8,11,22]. Importantly, repeated microinjections of NSAIDs into the Ce resulted in a progressive decrease in antinociceptive effectiveness (tolerance) similar to that observed with intra-PAG injections [8,11,22] and reminiscent of the effect of opiates.

A major involvement of opiodergeric mechanisms in tolerance to the analgesic effect of NSAIDs was surprising, because traditionally the cellular and molecular actions of opioids were thought to differ from those of non-opioid analgesics. One interesting aspect of NSAIDs administration, namely tolerance, emphasizes their similarity to opioid analgesics. Indeed, microinjection of metamizol [11,12,22] or LASA [8,12] into PAG or into Ce [17,18], progressively led to a loss of their antinociceptive effects, i.e. produced tolerance. Furthermore, tolerance to metamizol or LASA was accompanied by cross-tolerance to morphine [8,11,22] as if opioid analgesics had been repeatedly administered. Interestingly, tolerance to the effect of PAG-microinjected metamizol can, like tolerance to morphine, be reversed by microinjection of progumide, a cholecystokinin antagonist, at the same PAG site [22]. The latter finding constituted additional evidence that the PAG effects of non-opioid analgesics are similar to those of morphine. In addition, the data suggested that Ce should be incorporated into current models of endogenous pain control circuitry [23] and Ce integrates inputs from the limbic forebrain and diencephalon with ascending nociceptive input from dorsal horn. There are direct projections to the PAG from a number of medial prefrontal areas including anterior cingulate and insular cortices [3,7].

It is well known that morphine injection after administration of NSAIDs or in combination, morphine plus NSAIDs usually potentiates their own analgesic effects [24]. We have recently tested each of NSAIDs for
cross-tolerance to morphine given over a 5-day period in two age groups of rats. There was a significant
difference between adult and juvenile rat groups for the degree of morphine analgesia, which was most
marked on the first and second experimental days. Furthermore, morphine-tolerant rats exhibited cross-
tolerance to analgine, ketorolac, and xefocam for both tail-flick (TF) and hot plate (HP) tests, respectively
[25].

In conclusion, our data confirmed previous studies indicating that the antinociceptive action of NSAIDs
may be closely related to that of endogenous opioids, including the development of tolerance. In addition,
the Ce along with PAG and RVM represents an important component of the endogenous antinociceptive
system.

3.2 Antinociceptive tolerance induced by non-opioid analgesics microinjected into
periaqueductal grey matter

We have already noted that several lines of investigations have shown that in PAG, the microinjection of
non-opioid analgesics, NSAIDs dipyrone (metamizol) and lysine-acetylsalicylate (LASA) induces
antinociception with some effects of tolerance [8,9]. We have just recently examined that microinjectition of
another type of NSAIDs clodifen, ketorolac and xefocam into the PAG leads to the development of
tolerance in male rats. Our obtained data add evidence to the hypothesis that, like opioids, non-opioid
analgesics, particularly NSAIDs analgin, clodifen, ketorolac and xefocam induce tolerance, and also by
mechanisms strongly concern to the PAG [9-11].

It has been shown that other non-opioid analgesic LASA also induces tolerance after repeated
microinjections into the PAG [8,12]. The PAG and its descending projections to the RVM and the nucleus
raphe magnus (NRM) are principal components of the descending antinociceptive pain-control system
[3,4,6,13,27]. In naive rats, microinjection of morphine and dipyrone into the PAG decreases the activity
of pain-facilitating “on-cells” and increases the activity of pain inhibiting “off-cells” thus giving rise to
antinociception [10,28]. The PAG is thus crucial for tolerance to morphine as well as to non-opioid
NSAIDs such as metamizol, clodifen, ketorolac, LASA and xefocam [9,10,12,26]. Our findings confirm the
results of other investigators that microinjection of dipyrone and LASA, and systemically of dipyrone are
abolished by systemic injections and/or microinjections of selective µ-opioid antagonists, naloxone and
CTOP (D-phe-Cys-Tyr-D-trp-Orn-thr-Pen-thr-NH₂) [8,10,24]. The latter is a cyclic analog of the
neuropeptide somatostatin and is known to block the analgesic effect of morphine [10].

Taken together the results presented here and other authors’ findings underscore the strong convergence
of antinociceptive mechanisms of opioids and non-opioids, particularly NSAIDs in the PAG in the acute
effect of and the development of tolerance to both types of analgesics. On the other hand, our data
confirm the results of other authors that NSAIDs are in close relation with endogenous opioids and the
tolerance to these non-opioid drugs probably depends on opioid tolerance.

3.3 Nucleus raphe magnus is opioid sensitive to analgesic effects of NSAIDs

More than 35 years ago, in classic studies carried out in the laboratory of J.-M. Besson [29], electrical
stimulation of the nucleus raphe magnus (NRM) completely suppressed the behavioral responses to
noxious pinch of the skin and modified the jaw-opening reflex threshold to tooth pulp stimulation in the
cat. Furthermore, electrical stimulation of the NRM or opioid microinjection inhibits activity of dorsal horn
nociceptive neurons [30-32]. Subsequently, it has been shown that microinjections of dipyrone
(metamizol) or morphine into the RVM of lightly pentobarbital-anesthetized rats dose-dependently inhibit
the nocifensive TF reflex and produce tolerance [33-35]. We have recently found that repeated for five
days microinjections of analgin, clodifen, ketorolac and xefocam in the NRM produce tolerance to these
drugs [36].

It is interesting that although similar effects of antinociception can be produced by direct action of
morphine upon the PAG and RVM, tolerance to morphine was not remarkably obtained by repeated
microinjection into the RVM [34]. Furthermore, inactivation of the RVM did not prevent the development of
tolerance to repeated morphine microinjections into the PAG, and tolerance to systemic morphine did not
develop if opioid receptors were blocked in the PAG even if the RVM remained intact [35].
To examine into details an opioid sensitivity of NSAIDs action we tested on whether post-treatment with 
\( \mu \)-opioid antagonist naloxone in NRM diminishes NSAID-induced antinociception on the first and second 
experimental days. Thirty minutes after NSAIDs testing, microinjection of naloxone in NRM significantly 
decreased antinociceptive effects of NSAIDs at the first day in the TF for analgin, clodifen, ketorolac, and 
xefoacam, respectively [37].

We discovered the same effects in the HP test. At the second day, naloxone generally had trend effects 
in both TF and HP tests [37]. These results strongly support the suggestion on endogenous opioid 
involvement in NSAIDs antinociception and tolerance [9,24,26,36,37].

The obtained data thus confirm evidence that, like opioids, non-opioid analgesics, particularly NSAIDs 
analgin, clodifen, ketorolac and xefocam induce tolerance. The latter should be realized by endogenous 
opioids, endorphins [9,24,26,38]. Our findings affirm the results of other investigators that microinjection 
of dipyrone and LASA into PAG, and systemically of dipyrone are abolished by systemically injected 
and/or microinjections of selective \( \mu \)-opioid antagonists, naloxone and CTOP [8,10,24]. Moreover, 
endogenous opioids are involved in the analgesic potentiation observed with the combination of morphine 
plus dipyrone (metamizol). The release of endogenous opioids by dipyrone could enhance exogenous 
opiate effects, explaining the need for more amount of naloxone to counteract the antinociception 
produced by morphine plus dipyrone [24].

As stated above, the action of either opioid or non-opioid analgesics in the PAG leads to an excitation of 
PAG output neurons and this causes an activation of RVM off-cells and an inhibition of RVM on-cells, 
thus leading to antinociception (analgesia). When tolerance develops, PAG microinjections of morphine 
[38], or metamizol [10] are no longer capable of affecting RVM neurons and inducing analgesia. These 
results show further neuronal relationships between opioid and non-opioid analgesics as regards the 
descending pain-control and modulation system [39].

From a clinical point of view, the present study and evidence of other colleagues show that NSAIDs exert 
upon the PAG a powerful action, which could delay and depress the establishment of inflammation-
duced spinal hyperexcitability. Upon systemic administration of NSAIDs prior to persistent peripheral 
damage such as inflammation, the patient probably benefits from a central action at both PAG and spinal 
cord. When inflammation and central sensitization are fully developed, the action of NSAIDs upon PAG is 
sufficient to reverse spinal hyperexcitability and presumably therefore hyperalgesia and allodynia. By 
acting upon the PAG, NSAIDs activate the descending pain-control system, whose inhibitory function 
upon the spinal cord is exerted by way of the RVM including the NRM [25,26,37,39].

On the other hand, the present results together with previous data using metamizol and LASA [8] are of 
general relevance to human medicine. The antinociceptive effect of NSAIDs in the PAG is related to 
derogenous opioids and implies that patients under repeated and prolonged treatment with non-opioid 
algesics are, like those under opiates, at risk of tolerance to NSAIDs and a withdrawal syndrome. 
Finally, cross-tolerance between non-opioid and opioid analgesics and an association between systemic 
NSAIDs and endogenous opioids may have undesirable clinical consequences and should be important 
in the clinical setting [8,10,25,26].

4. BRAINSTEM MODULATION OF NOCICEPTIVE PROCESSING

This was an important advance in documenting that the brain itself could regulate the processing of 
nociceptive information. Inspired by stimulation-produced analgesia, a significant research effort led to the 
definition of a brainstem pain modulatory network with critical links in the RVM as well as the PAG. The 
antinociception resulting from stimulation in these structures is in large part due to regulation of 
nociceptive processing at the level of the spinal cord (Fig. 1). The PAG–RVM system mediates the 
algesic actions of opioids, and it is recruited by internal and environmental challenges [6]. 
Accumulating evidence from neuroimaging studies supports a role for this system in top-down modulation 
of pain in humans, such as that produced by placebo or shifts in attention [23].
Fig. 1. Functional organization of brainstem pain modulating system with links in midbrain PAG and RVM. The PAG projects to the RVM. The RVM in turn regulates spinal nociceptive circuitry to the dorsal horn. This system exerts bidirectional control, and separate populations of RVM neurons mediate descending inhibition and descending facilitation. The PAG is reciprocally linked with forebrain structures including prefrontal cortex, amygdala, and hypothalamus. These substantial interconnections provide an anatomical substrate through which emotional and cognitive variables could influence nociception via the PAG–RVM system. (Modified from Heinricher, Ingram, 2009 [6]).

Along with the evidence that this system mediates the analgesic actions of exogenous and endogenous opioids, the fact that the net behavioral effect of nonselective experimental activation of the PAG or RVM is antinociception led to a general view of the PAG–RVM system as an analgesia system. This system is indeed activated by acute stress and opioid and non-opioid (NSAIDs) analgesics to inhibit spinal nociceptive processing. However, there is now increasing evidence that the system, especially the RVM, also facilitates nociception. The RVM has been implicated in hyperalgesia and allodynia associated with inflammation, nerve injury, acute opioid withdrawal, chronic opioid administration, and the sickness response. The PAG–RVM circuit should therefore be viewed not specifically as an analgesia system, but more generally, as a pain-modulation system [4,6,40,41]. From this perspective, the system has the potential for graded enhancement or inhibition of nociception under different external and internal conditions.

4.1 Neurotransmitters and endogenous opioids in the periaqueductal gray

All the three opioid receptors, μ-, δ-, and κ-opioid receptors, are moderately to densely expressed in the PAG [42-45]. The PAG is also rich in endogenous opioids. Particularly, enkephalin-containing terminals are found apposed to both GABA-ergic and non-GABA-ergic dendrites, including those of a small percentage of PAG–RVM neurons. Endomorphin-2 (Tyr-Pro-Phe-Phe-NH2), an endogenous peptide with high selectivity for the μ-opioid receptor is concentrated in the PAG as well as the dorsal horn of the spinal cord [46-48]. Another endogenous opioid found in the PAG is β-endorphin. Discrete populations of β-endorphin-containing neurons in the ventromedial hypothalamus project to the PAG and have been implicated in analgesia produced by electrical stimulation and stress [49].

The μ-opioid receptor agonists produce potent antinociception when applied directly to the PAG. The behavioral antinociception produced by these agents is mediated by activation of output neurons projecting to the RVM μ-opioid receptor agonists are thought to act presynaptically to block GABA-ergic inhibition of PAG output neurons. Consistent with this hypothesis, blockade of GABA transmission within the PAG by microinjection of a GABA_A receptor antagonist produces antinociception [6,50].
Postsynaptic effects of µ-opioid receptor agonists on PAG neurons include hyper-polarization via activation of a postsynaptic G-protein-activated inwardly rectifying potassium conductance (GIRK) and inhibition of calcium channels [51] (Fig. 2).

Fig. 2. Cellular mechanisms of opioid action within the PAG. Enkephalin-containing synapses are apposed to cell bodies as well as to GABA- and glutamate-containing terminals. The postsynaptic µ-opioid receptor activates GIRKs and inhibits voltage-gated Ca\(^{2+}\) channels to hyperpolarize cells and decrease cell activity. Presynaptic µ-opioid receptors (MOR) inhibit both GABA and glutamate release on ventrolateral PAG neurons, and apparently use different signal transduction pathways in the terminals. MORs localized to GABA terminals are coupled to voltage-gated potassium channels via activation of the arachidonic acid/12-lipoxygenase (12-LOX) second messenger pathway. Hyperpolarization of the terminal decreases GABA release. The signal transduction pathway for MOR inhibition of glutamate release is currently unknown. MORs are found on GABA containing interneurons in the PAG, as well as on PAG output neurons projecting to the RVM. Activation of the descending antinociceptive pathway by opioids occurs via disinhibition. 12-HETE, 12-hydroxyeicosenoic acid; Kv, voltage-gated potassium; PLA2, phospholipase A2. (Modified from Heinricher, Ingram, 2009 [6]).

The µ-opioid receptor agonists also have presynaptic effects, inhibiting GABA and glutamate release from terminals within the ventrolateral PAG [52-54]. Presynaptic inhibition of GABA-ergic neurotransmission is through activation of the arachidonic acid–phospholipase A2 second messenger pathway. Stimulation of this pathway results in activation of voltage-gated potassium channels (Kv channels) by metabolites of 12-lipoxygenase [53,54]. This pathway is independent of adenyl cyclase, protein kinase A or protein kinase C activity [54]. Further research is needed to determine the relevance of the various presynaptic versus postsynaptic opioid actions to the nociceptive modulatory function of the PAG.

4.2 Opioid and Non-Opioid (NSAIDs) Interactions

Administrations of NSAIDs systemically or microinjections into the PAG produce analgesia at the behavioral level in awake rats [8-10,21,25,26]. This antinociception is apparently mediated at least in part by an endogenous opioid peptide, as the analgesia produced by NSAIDs microinjected into the PAG and RVM is attenuated by the opioid antagonist naloxone [12,24,26,36,37]. In addition to stimulating release of endogenous opioids, recent evidence at the cellular level suggests that NSAIDs may also augment the signaling pathway used by opiates, potentiating the actions of exogenous opioids [6] (Fig. 3).
Fig. 3. µ-opioid receptor (MOR) coupling in presynaptic GABA terminals changes with chronic morphine administration. Acute administration of MOR agonists activates MORs coupled to phospholipase A2 (PLA2). Activation of PLA2 increases production of arachidonic acid, which is further metabolized by 12-lipoxygenase (12-LOX). Lipoxygenase metabolites such as 12-HETE activate voltage-gated potassium channels (Kv channels) to hyperpolarize and decrease GABA release from the terminals. NSAIDs potentiate this action of opioids by inhibiting cyclooxygenase (COX) mediated arachidonic acid metabolism, thereby shunting arachidonic acid to the 12-LOX pathway. Activation of MORs presumably also acutely inhibits adenylyl cyclase (AC) activity in these terminals. Chronic morphine administration upregulates AC and protein kinase A (PKA) activity. After chronic, but not acute, opioid treatment, GABA release is enhanced by increased PKA activity. MOR agonists are more potent inhibitors of this PKA-dependent release, so that opioid removal or blockade of MORs by antagonists results in a rebound increase in GABA release. This increased GABA release may contribute to withdrawal behaviors mediated by the PAG. (Modified from Heinricher, Ingram, 2009 [6]).

Coapplication of NSAIDs potentiates the inhibition of GABA release by the µ-opioid receptor partial agonist morphine, although NSAIDs have no effect on GABA release in the absence of morphine [54-57]. NSAIDs primarily inhibit cyclooxygenases (COX-1 and COX-2), one of three types of enzymes (cyclooxygenases, 5-lipoxygenases, and 12-lipoxygenases) that metabolize arachidonic acid. One hypothesis proposed to explain the mechanism of increased analgesia with coapplication of opioids and NSAIDs is that blockade of COX-1 shunts arachidonic acid metabolism through the lipoxygenase pathways to increase the potency of opioid receptor agonists [54-56,58] (Fig. 4).
Fig. 4. Proposed model for the interaction of NSAIDs, opioids and cannabinoids in the descending pain control system to induce analgesia. Inhibition of the cyclooxygenases (COX) by NSAIDs reduces the synthesis of prostaglandins (PG) and thromboxanes (TX) and thus increases the availability of arachidonic acid (AA). Opioids also increase the availability of AA by activating the phospholipase A2 via the µ-opioid receptor. Via the 12-lipoxygenases (12-LOX) pathway AA is transformed into hepoxilins, which indirectly inhibit GABA release. By inhibiting COX and fatty acid amide hydrolase (FAAH) the NSAIDs spare arachidonoyl ethanolamide (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG), which bind to the cannabinoid receptor, CB1 and thus inhibit GABA release. Removal of inhibition by GABA enhances the activity of output neurons that inhibit pain. Note, minus symbols indicate inhibition. (Modified from Vanegas et al., 2010 [40]).

As with opioids, one possible mechanism for the analgesic action of NSAIDs could be their induction of endocannabinoid release. A plausible link between NSAIDs and endocannabinoids may be related to the fact that NSAIDs inhibit the cyclooxygenases and the fatty acid amide hydrolase (FAAH) and that these enzymes metabolize the endocannabinoids (Fig.4). By inhibiting the cyclooxygenases and the FAAH, NSAIDs may therefore prevent enzymatic removal of endocannabinoids which, through the cannabinoid receptors, CB1 and CB2 induce analgesia [40]. In the ventrolateral PAG and in the RVM, cannabinoids via the CB1 receptor inhibit the presynaptic release of GABA and thus enhance the activity of postsynaptic neurons, like opioids do. Therefore, by preventing the removal of endocannabinoids, NSAIDs may facilitate the activity of descending neurons in charge of attenuating the transmission of pain signals [40].

The fact that inhibitors of 5-lipoxygenases also appear to potentiate the effects of opioid agonists in the PAG adds further weight to this proposal [55-58]. These results are important in that the combined administration of NSAIDs and opiates may allow lower doses of morphine to be used to provide adequate analgesia while reducing the probability of the development of tolerance and side effects (such as respiratory depression) associated with high doses of opiates. Functional studies of NSAID/ opioid interactions in the PAG would therefore be of great interest [6].

The precise molecular mechanism of NSAIDs tolerance has not yet been known. The term tolerance is sometimes used rather loosely to refer either to very short or long-term loss of agonist efficacy. In our experimental animals, ‘acute’ tolerance can be observed rapidly (minutes to days) during the course of a single episode of opioid intoxication. This type of tolerance may be more closely related to processes of rapid µ-opioid receptor desensitization and internalization that should be distinguished from the more substantial tolerance that emerges after days to weeks of opioid administration [59].

According to our and other colleagues’ data, NSAIDs tolerance mimics opioid tolerance. At present we cannot precisely determine the cellular and molecular mechanisms of such similarities. It is well established that organization of opioid adaptations in the nervous system including: (a) receptor tolerance at the µ-opioid receptor itself showing loss in the coupling of µ-opioid receptor to major cellular effectors, such as the G-protein-regulated inwardly rectifying potassium channel. Several potential mechanisms could account for tolerance at this level of organization, but changes to coupling and perhaps surface expression appear to be most important. (b) Cellular tolerance and withdrawal in opioid-sensitive neurons is due to multiple adaptations to intracellular signaling cascades, but hypertrophy of cAMP signalling is the best established. (c) Systems feedback adaptations in opioid-sensitive nerve and neuroglial networks can develop and contribute to tolerance and withdrawal. (d) Synaptic plasticity and learning in opioid-sensitive nerve networks may involve changes in synaptic plasticity driven by changes in presynaptic release probability, which are well established at many opioid-sensitive GABAergic synapses, but more importantly, mechanisms resembling long-term potentiation and/or long-term depression probably involving AMPA receptor insertion in synapses may produce long-term changes in synaptic strength [60,61].

Future scientific efforts will be directed at deepening our understanding of how adaptive responses by multiple neural systems and molecular mechanisms work together to counteract the analgesic efficacy of commonly used opioids and non-opioids. Future pharmaceutical development will focus on blocking the facilitatory mechanisms that produce these adaptive changes, in the endogenous nociceptive and antinociceptive systems, in response to continual exposure to an opioid analgesic [52-64].

5. SUMMARY AND CONCLUSIONS
The present review reports that microinjection of a nalgin (metamizol), ketorolac, and xefocam into the central nucleus of amygdala of rats elicits antinociception with the development of tolerance. Furthermore, microinjections of these NSAIDs plus clodifen into the periaqueductal grey produced antinociception as revealed by a latency increase in tail-flick and hot plate tests compared to the baseline control with saline microinjected into the same nucleus. However, subsequent testing also took place in the following days, shows strong tolerance to these drugs. Finally, repeated administrations of these NSAIDs into the nucleus raphe magnus in the following 4 days result in progressively less antinociception compared to the saline control, i.e., tolerance develops to these medications. Special control experiments showed that post-treatment with the \( \mu \)-opioid antagonist naloxone into the nucleus raphe magnus significantly decreased antinociceptive effects of NSAIDs on the first day of testing. Thus, the mechanism producing tolerance to NSAIDs can be due to the participation of endogenous opioids, endorphins. Therefore, these findings strongly support the suggestion of endogenous opioid involvement in NSAIDs antinociception and tolerance in the descending pain-control and modulation system.

Concerning the effort to understand the neural basis of nociceptive modulation by the periaqueductal grey–rostral ventro-medial part of medulla system, it highlights the importance of studying functionally identified neurons. The rostral ventro-medial medulla and nucleus raphe magnus can both facilitate and inhibit nociception. Furthermore, this region is also implicated in a number of functions other than nociceptive modulation, including reproductive behaviors, cardiovascular and respiratory control, sleep–wakefulness and arousal, thermoregulation, and behavioral suppression. A meaningful analysis of how the rostral ventro-medial medulla contributes to enhanced pain states therefore requires functional identification of the neurons under study, so that mechanisms contributing to nociceptive facilitation can be distinguished from those involved in nociceptive inhibition or other functions [2,5,7].

**CONFLICT OF INTEREST**

Authors have declared that no conflicting interests exist.

**References**


Abbreviations

AA: arachidonic acid;

AEA: arachidonoyl ethanoamide (anandamide);
CB: cannabinoid receptor;
Ce: central nucleus of amygdala;
COX: cyclooxygenase;
GABA: gamma amino butyric acid;
GIRK: G-protein-activated inwardly rectifying potassium conductance;
HP: hot plate;
i.p.: intraperitoneal;
LASA: lysine-acetylsalicylate;
NRM: nucleus raphe magnus;
NSAIDs: non-steroidal anti-inflammatory drugs;
PAG: periaqueductal gray matter;
RVM: rostral ventro-medial medulla;
TF: tail-flick