Inflammatory pain: the role of cytokines and its control by drugs which release nitric oxide

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Summary. - We are progressing towards an understanding of the mechanism of peripherally acting analgesics. Knowledge of the participation of the cytokines in inflammatory hyperalgesia and the involvement of the arginine-NO-cGMP pathway in the mechanism of agents which directly down-regulate ongoing hyperalgesia may provide for better therapeutic approaches and permit the development of novel analgesics.

Key words: nitric oxide analgesics, peripheral analgesics, inflammatory pain, analgesics, cyclo-oxygenase inhibitors.

Riassunto (Il dolore connesso con l'inflammazione: ruolo delle citochine e loro controllo con farmaci che liberano ossido d'azoto). - Le ricerche eseguite negli ultimi tempi hanno rivelato notevoli progressi nella comprensione del meccanismo degli analgesici ad azione periferica. La scoperta della partecipazione delle citochine nell'iperalgiesia infiammatoria e il coinvolgimento della via arginina-NO-cGMP nel meccanismo degli agenti che riducono direttamente l'evoluzione dell'iperalgiesia possono fornire migliori approcci terapeutici e permetteranno lo sviluppo di nuovi analgesici.

Parole chiave: analgesici NO, analgesici periferici, dolore infiammatorio, analgesici, inibitori della cicloossigenasi.

Introduction

The idea of controlling pain and distress may be as old as human kind and was in nature that men found the first two prototypes of central and peripheral analgesics, the opium and the salicylates. The explosion of the medicinal chemistry allied to the development of meaningful screening tests lead to the development of current analgesics. Despite the great number of analgesics offered to the modern clinician, he is frequently confronted with the problem of finding the ideal analgesic for his specific case. Although highly effective, central analgesics have proved not to be dissociated from behaviour side effects. On the other hand, the use of peripheral analgesics is usually limited by their inherent and frequently undesirable antipyretic or gastric effects.

The absence of an ideal analgesic is stressed by our difficulties in handling the various types of chronic pain, specially of neurogenic origin. The search for new drugs or analgesic therapies is just starting. In fact, only during the last three decades the basic physiopathological and molecular mechanisms involved in central and peripheral pain started to be unravelled. The aim of this review is to discuss some of the current ideas concerning the physiopathological aspects of inflammatory pain. On the basis of this understanding and on the mode of the action of current used analgesic drugs propose a classification of the peripheral drug control of inflammatory pain. It is hoped that this classification has therapeutic usefulness and points new targets for the development of new drugs.

The presence of foreign material in/or injury to tissue induces an early response that can be envisaged as an alarm reaction in which resident macrophages seem to play a pivotal role in the development of acute inflammation [1]. At the onset of the inflammatory response, the macrophage may act as an alarm cell, signalling the presence of foreign or deleterious stimuli via the release of cytokines or classical inflammatory mediators. With the development of the inflammatory response, migrating cells like polymorphonuclear leucocytes, macrophages, eosinophils and/or lymphotoocytes play an amplifying role. The action of classic, inflammatory mediators specific, cellular receptors triggers local tissue responses, reflected by the cardinal inflammatory symptoms and fever which assure the animal's perception of an ongoing tissue injury and initiate defense mechanisms. Tissue destruction results from the release of lytic enzymes by locally damaged cells or by overactive phagocytes.

Sensitization of the pain receptor is the common denominator of all types of inflammatory pain. C-polymodal, high threshold receptors or receptors connected by fine myelinated fibers have long been associated with inflammatory hyperalgesia. Over recent years, a new "sleeping" nociceptor associated with a small afferent fiber, has been described in deep visceral innervation (colon and bladder) and in joints (see [2]). Sleeping nociceptors cannot be activated in normal tissues. Analogous to a flip-flop switch, the sleeping receptors are switched "on" during inflammation. The functional up-regulation of the pain receptors is clinically referred to as hyperalgesia.
The molecular events associated with hyperalgesia are not yet fully understood. However, there is evidence that an increase in cAMP/Ca^{2+} concentrations is associated with the functional up-regulation of nociceptors. We have described that intraplantar administration of dibutyryl cAMP, Ca^{2+} ionophore or BaCl_2 (which increases the concentration of free Ca^{2+} in the cytosol) to the rat paw causes hyperalgesia. Moreover, the administration of prostaglandins or sympathetic mimetics (noradrenaline or dopamine) known to stimulate neuronal cAMP synthesis also causes hyperalgesia. On the other hand, pretreatment of the paws with a calcium blocker or with lanthanum (which blocks Ca^{2+} influx) prevents the development of hyperalgesia [3]. The hypothesis that hyperalgesia occurs subsequent to an increase in cytosolic cAMP/Ca^{2+} concentrations has received the experimental support of other groups using different hyperalgesic tests [4, 5]. The final biochemical events responsible for the functional up-regulation of the nociceptor are not yet understood. The mechanism may involve the activation of a protein kinase A, with subsequent phosphorylation of an ion channel or the modulation of cytosolic structures that control intracellular calcium levels. There is, however, a system which is able to down-regulate sensitized nociceptors. Direct blockade of ongoing hyperalgesia was observed after local administration of (dibutyryl cGMP) or by substances which stimulate neuronal guanulate cyclase (carbachol or nitric oxide generators). Thus it seems that the functional up- or down-regulation of the nociceptors is dependent on a balance between nociceptor cAMP/cGMP contents.

Thus, there are two distinct groups of inflammatory mediators, those which sensitize and those that activate sensitized pain receptors. Previously ineffective stimuli cause "overt pain" because they are now able to activate the nociceptors.

**Peripheral control of inflammatory pain**

A classification of the therapeutic control of inflammatory pain which takes into account direct activation, as well the functional up/down-regulation of the pain receptors is proposed in Table 1.

**Type 1: inhibition of nociceptor activation**

The prevention of overt pain by inhibition of activation of up-regulated nociceptors obtained either by receptor antagonism or avoidance of excitatory stimuli. In the Type 1 control of inflammatory pain, the only group of antagonists having clinical application is the antihistamines. The H1 antihistamines are effective antagonists of the activation of a special class of

**Table 1. - Peripheral control of inflammatory pain**

<table>
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<tr>
<th>Type 1: Inhibition of nociceptor activation</th>
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<td>Prevention of overt pain by inhibition of activation of sensitized nociceptors by receptor antagonism or avoidance of excitatory stimuli</td>
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A) Anti-histamines  
B) Anti-sympathomimetics  
C) Elimination of mechanical or thermal stimulation  
D) Local anaesthetics

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<th>Type 2: prevention of hyperalgesia</th>
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<tr>
<td>Prevention of functional up-regulation of nociceptors by inhibition of release, or by receptor antagonism, of inflammatory mediators which stimulate the increase of levels of cAMP/Ca^{2+} in nociceptors</td>
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A) Cyclo-oxygenase inhibitors (acidic and non-acidic non-steroidal anti-inflammatory drugs)  
B) Inhibitors of cytokine activity or release (anti-inflammatory steroid drugs, interleukin 1 antagonists, nimesulide)  
C) Sympathomimetics:  
  i. Inhibitors of sympathetic amine release: guanethidine  
  ii. Beta and dopamine 1 antagonists

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<th>Type 3: direct blockade of hyperalgesia</th>
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<td>Functional down-regulation of nociceptors by stimulation of the arginine/NO/cGMP system</td>
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A) Analgesic which directly down-regulate the nociceptors: peripheral opiates, dipyrone, diclofenac  
B) Analgesic which indirectly down-regulate nociceptors through the release of endogenously acting peripheral opiate: clonidine, ST-91, glatomeno.
nociceptors associated with pruritus, i.e., inflammatory itching, [6, 7]. The H₂ antagonists, by blocking the production of acid, eliminate the chemical stimulation of the nociceptors associated with the pain of gastric ulcers and heart burn.

Regional blockade with the α-adrenergic antagonist phentolamine is effective in relieving overt pain, in man. Contribution of the sympathetic system has also been demonstrated in animal models [8, 9]. As the receptors involved, however, seem to depend on the model or on the animal species involved. In fact, afferent fibres innervating rat neurona were excited by the sympathetic amines [10] via α-adrenergic receptors.

Bradykinin became a plausible pain mediator by virtue of its ability when injected in man to produce overt pain. When instilled on the cantharidin blister base [11], or injected in the abdominal cavities [12] or into the cephalic, brachial vein previously sensitized by serotonin [13] BK produced an immediate overt pain which subside after a few minutes. Those observations were supported by numerous behaviour and electrophysiological studies.

However, the prevention of nociceptor stimulation can be achieved without the use of drugs. In many pathophysiopathological conditions, up-regulated nociceptors are frequently activated by mechanical stimulation such as increased tissue tension (excess exudate in a closed cavity) or body movements. The most popular treatment of choice for alleviating inflammatory pain of this origin is drainage of the exudate and rest.

**Type 2: prevention of the development of hyperalgesia: antialgics**

Prevention of hyperalgesia development is achieved via inhibition of release, or receptor antagonism of inflammatory mediators which stimulate the increase nociceptor cAMP/Ca²⁺. Drugs which belong to this type should be referred as "antialgics". In our opinion only two groups of putative hyperalgesic mediators have satisfactorily fulfilled experimental and clinical criteria for direct hyperalgesic mediators: a) the arachidonic acid/cyclo-oxygenase products, prostaglandins (PGE₂, PGI₂) and b) sympathetic amines. Although in experimental models of inflammatory pain both classes of mediator may be involved, their relative contribution depends on the characteristics of the pathological stimulus.

**Cyclo-oxygenase inhibitors**

The ability of prostaglandins to sensitize pain receptors has been demonstrated in man and in animals using both behavioural and electrophysiological techniques (see [14]). Inhibitors of CO is one of the most commonly prescribed groups of analgesics. Our early hypothesis that aspirin-like drugs (non-steroid anti-inflammatory drugs, NSAID), prevent receptor sensitization because they inhibit prostaglandin synthesis is now widely accepted. Blockade of oedema, antipyresis and some of the side effects (gastric irritation) of the acidic NSAID is also correlated with the inhibition of cyclo-oxygenase. The mechanism of action of the non-acidic, non steroidal drugs, acetaminophen (paracetamol) and dipyridamone, is still controversial. They are considered to be more analgesic than anti-oedematogen. It is difficult, however, to comprehend how the general blockade of prostaglandin synthesis can cause analgesia without a concomitant anti-oedematogenic effect. It has been suggested [15, 16] that prostaglandin synthesis by the nervous tissues is specifically sensitive to non-acidic analgesics. This suggestion implies the discrete release of prostaglandins, probably by the nociceptor itself, or from its immediate vicinity. We now have experimental evidence which supports this view since, in the rat test, IL-1B produces strong hyperalgesia without causing oedema, and a tripeptide IL-1B antagonist blocks carrageenin-induced hyperalgesia, without affecting the oedematogenic response [17]. We also found that paracetamol is an effective blocker of interleukin 1 induced hyperalgesia (unpublished observation).

When the nociceptor is functionally up-regulated, i.e., in the presence of ongoing hyperalgesia, drugs which prevent hyperalgesia are not effective analgesics. Thus, the duration of the prostaglandin-induced sensitization of the afferent, sensory fibers of the inflamed tissue limit the readiness and effectiveness of the action of NSAID. A lengthy duration of the effect of a single application of PGE₂ was observed in nociceptive tests of the somatosensory system such as the human skin, the rat paw and dog knee joint. In tests involving visceral pain, such as mouse contortion or potentiation of the bradykinin effect in the dog heart, spleen or knee joints, prostaglandin sensitization was immediate and of short duration (see [18]). PGE₂, the major cyclo-oxygenase metabolite from arachidonic acid generated by endothelial cells, has both a much shorter onset (30 min) and duration (30 min).

We suggested that in physiopathological processes in which the injury is predominantly vascular (headaches for example), the functional up-regulation of the nociceptor is caused by prostacyclin. In this case, the application of NSAID is readily effective. In contrast, when more extensive tissue lesion occurs and there is considerable participation by phagocytes (neutrophils and macrophages), the active cyclo-oxygenase metabolite released is PGE₂. In this case, the administration of a pure cyclo-oxygenase inhibitor analgesic will not cause pain relief. The onset of the analgesic action will depend on the duration of the sensitization of the nociceptors characteristic of the inflamed tissue. The success of the analgesic therapy will also depend on the frequency of application and the half-life of the analgesic. If the
analgesic has a short half-life, frequent administration is necessary to control the ongoing inflammatory process. Because NSAID prevent the development of hyperalgesia, the failure of NSAID analgesic therapy is understandable in some physiopathological events when the therapy is implemented after the patient is already feeling pain. This would also be the case in the persistent hyperalgesia which is observed after repeated hyperalgesia stimulation [19]. Finally we would like to point out that corticoids may prevent the nociceptors sensitization by inducing in the inflamed tissue, the generation of a protein, named lipocortin, which admittedly inhibits phospholipase A₂, thus restricting the formation of arachidonic acid and consequently of cyclooxygenase inhibitors.

Extensive physiological pharmacological and clinical evidence support the existence of a sympathetic modulatory influence on somatosensory input [20]. Our experimental work indicates the involvement of the sympathetic system in the inflammatory reaction. Sympathomimetic amines (noradrenaline, dopamine and 5-hydroxytryptamine) have been shown to cause functional up-regulation of the nociceptors [21]. We have reported that guanethidine depletion of peripheral sympathomimetic amines, treatment with adrenergic antagonists (β-blockers) and a dopamine (DA)-1 antagonist (SCH 23390), significantly reduced carrageen-induced hyperalgesia. These antagonists also abolished the rat paw hyperalgesia induced by several sympathomimetic amines, as well as that induced by a selective DA-1 agonist, SKF 38393. Based on these results, we concluded that there is a sympathetic component, possibly mediated by a DA-1 type receptor, in carrageen-induced hyperalgesia. We believe that sympathetic mechanisms predominate in certain types of inflammatory pain. Contribution of the sympathetic system has also been demonstrated in other animal models [8, 9]; the receptors involved, however, seem to depend on the model or on the animal species involved. In fact, afferent fibres innervating rat neuroma were excited by sympathetic amines [10] via α-adrenergic receptors. In some models, the effect of sympathetic amines seems to be indirect, via cyclo-oxygenase metabolites or even to depend on the previous inflammatory sensitization of the tissue. These differences may result from the type of nociceptor and fibres involved in each model.

**Blockade of the activity or release of cytokines**

It is now becoming clear that in inflammatory response, the release of direct hyperalgesic mediators is secondary to the release of cytokines [17, 22]. In this context, the release of cytokines seems to constitute the link between cellular injury and/or recognition of non-self and the liberation of the “direct acting” mediators responsible for the development of local and systemic inflammatory signs and symptoms. Using specific antisera for IL-1β and IL-8, as well as cyclo-oxygenase inhibitors and sympatholytics, we have demonstrated that these cytokines are responsible for the prostaglandin and sympathomimetic components, respectively, in experimental animal models. In inflammation, the release of these two cytokines is preceded by the liberation of TNF-α which seems to control their release [23].

Among the inflammatory mediators, bradykinin is one of the most potent nociceptor activators known, as shown in experiments with animals and man; however, its contribution to inflammatory pain is not yet clear. The current extensive use of the new family of antihypertensive, angiotensin converting enzyme inhibitors which are strong bradykinin potentiators has not been followed by frequent reports of the worsening of pathological acute or chronic states of pain. Present work of our group indicates that bradykinin, rather than acting as a receptor activator, may contribute to inflammatory hyperalgesia by releasing prostaglandins sympathomimetic amines via the release of hyperalgesic cytokines. We found that bradykinin-induced hyperalgesia is mediated by TNF-α, which stimulates the release of the hyperalgesic cytokines IL-8 and IL-1. Hyperalgesia induced by carrageen and LPS is mediated by the release of bradykinin and TNF-α. With a high concentration of LPS, the importance of bradykinin is overshadowed by the direct release of cytokines. It must be pointed out, however, that following extensive damage, prostaglandins acting as a direct hyperalgesic mediator, may be released by the injured cells without the intervention of cytokines.

It is generally believed that the analgesic action of glucocorticoids results from their “anti-inflammatory activity”. However, as we indicated earlier corticoids stimulate tissue macrophages to secretion lipocortin, which indirectly diminishes the production of prostaglandin in the inflamed tissues. However, it is accepted that glucocorticoids are potent inhibitors of cytokine release by macrophages and other cells. As discussed earlier, IL-1β and IL-8 are respectively associated with the prostaglandin and sympathetic component of inflammatory pain. Furthermore, we found that the release of TNF-α stimulated the release of these hyperalgesic cytokines. This may be the reason for the high efficacy of corticoids in alleviating all classes of inflammatory pain. The success of corticoid treatment on reflex sympathetic dystrophy (see [24]) may indicate the participation of hyperalgesic cytokines like IL-8 released in response to ongoing tissue damage.

**Sympatholytic agents**

The sympathetic nervous system may contribute to abnormal sensory experiences in many types of chronic pain. Sympathomimetic amines mediate some peripheral
pain states in man. In some of these pathological states, there is a striking relationship between the presence of hyperalgesia and the analgesia obtained by local sympathetic blockade with guanethidine [25]. From clinical studies, it is difficult to evaluate whether the efficacy of a drug to block ongoing overt pain is due to an effect on the activation of the nociceptors or to its effect on functional nociceptor up-regulation. The analgesic effect of the beta blockers propranolol and timolol is useful in the management of migraine. We do not know whether their beneficial effect is due to a central or to a peripheral mechanism but penetration of the timolol into the CNS seems to be rather weak. The clinical efficacy of beta blockers still needs to be defined. There are many anecdotal reports of their usefulness in phantom limb pain and cancer pain, and their usefulness deserves controlled, clinical trials, α-receptors seem to be involved in hyperalgesia and pain in damaged fibers. It seems that in this situation, up-regulation of the expression of new α-receptors occurs, thus explaining the effect of the regional phenolamine blockade in reflex sympathetic dystrophy.

Interleukin 1β antagonists are still in the experimental phase. It is expected that antagonists of the hyperalgesic cytokines may be effective in controlling chronic and acute pain in which hyperalgesia is the common denominator. We have determined the region of the IL-1β molecule which seems to contain its hyperalgesic activity (release of prostaglandin by the nociceptor) and we have developed an analog of IL-1β (K(D)P.T.). This tripeptide antagonizes the hyperalgesia induced by carrageen, IL-1β, and TNF-α in the rat. This molecule may constitute a prototype for the development of a new class of analogs.

**Type 3: down-regulation of nociceptor hyperalgesia**

When the nociceptor is up-regulated, i.e., in presence of ongoing hyperalgesia, drugs which prevent nociceptor hyperalgesia, i.e. antialgics do not cause immediate analgesia. In other words, when the accumulation of cAMP/Ca²⁺ is triggered by a hyperalgesic mediator, drugs which prevent Ca²⁺ release or cAMP action do not have analgesic effect. As pointed out above, agents which stimulate the formation of cGMP are able to cause functional nociceptor down-regulation. We have now accumulated evidence that analgesics which cause direct down-regulation of the nociceptors act via stimulation of the arginine-nitric oxide-cGMP pathway [26, 27]. This can be demonstrated by the abolition of their peripheral analgesic action by antagonists of arginine (thus blocking nitric oxide formation) and by inhibitors of guanilate cyclase activation. The analgesic action of these substances is potentiated by specific inhibitors of the phosphodiesterase which inactivates cGMP. The analgesic action of these substances is potentiated by specific inhibitors of phosphodiesterase which inactivate cGMP. The analgesia by nitroglycerin, an NO donor, has been observed after application in thrombophlebitides (Berrazuesta et al., personal communication).

*Analgesic which down-regulate the nociceptors via local release of NO*

We first described that opiates, partial opiate agonists and opiate antagonists can block inflammatory hyperalgesia [28, 29]. The effect of opiates was blocked by their respective receptor antagonists. However, unequivocal evidence for the peripheral analgesic effect of opiates was provided by the use of quaternary agonists and antagonists which, doing to some hindrance, are unable to cross the blood-brain barrier [30, 31]. The opiate blockade of nociceptor up-regulation has also been demonstrated using electrophysiological recording from single neurons in the isolated rabbit ear and in inflamed rat joints [32]. Recently in a well controlled study, the peripheral analgesic action of morphine was demonstrated in post-surgical, knee pain [33]. Peripherally acting opiates are presently undergoing clinical trials.

Type 2 drugs that act by inhibiting prostaglandin formation, show concomitant blockade of oedema and anti-nociception. In experimental models, dipyrrone [34] and diclofenac (personal observation) have little anti-oedematous effect in the presence of significant anti-nociception. Clinically, these substances are considered better analgesics than anti-inflammatory drugs. In fact, dipyrrone is known to inhibit cyclo-oxygenase activity at plasma concentrations higher than those attained during analgesic therapy [35]. Although inhibitors of cyclo-oxygenase, these agents can counteract the ongoing hyperalgesia induced by prostaglandin and cAMP [34].

As analgesics, this group of substances has an advantage over pure cyclo-oxygenase inhibitors because such agents may show early effect by acting upon hyperalgesia, or in hyperalgesia states induced by mediators other than prostaglandins, such as the sympathomimetic amines. Furthermore, this type of agent acts on persistent hyperalgesia induced by repeated hyperalgesia stimulation. We have described that after a long period of stimulation, hyperalgesia persists even after inflammation has disappeared [19]. It is intriguing that after down-regulation of ongoing hyperalgesia by dipyrrone or morphine, very mild stimulation is able to restore the previous, intense, hyperalgesic state. We call this event "peripheral memory of peripheral pain". The "central memory of nociceptor up-regulation" may be one of the missing pieces in the chronic pain puzzle.
Indirect functional down regulation of noiceptor hyperalgesia

Finally, we have examined the possibility that some agents may produce peripheral anti-nociception by releasing endogenous enkephalin. Their analgesic effects are blocked by opiate antagonists. In studying the analgesic action of clonidine, we have found that in addition to its central effect, clonidine induces analgesia by the peripheral release of enkephalin-like substances mediated by an α2-adrenoceptor. ST-91, a clonidine analog which does not cross the blood-brain barrier also promoted significant antinociception [21]. However, the central hypotensive effect of clonidine may obviate its use in man. Glafenine, a widely used analgesic also has a mechanism of action similar to clonidine. The effect of this group of substances is abolished by local or systemic treatment with opiate antagonists.

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