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Hyperalgesia Mediated by Spinal Glutamate or Substance P Receptor Blocked by Spinal Cyclooxygenase Inhibition

A. B. Malmberg and T. L. Yaksh

Inhibition of cyclooxygenase by nonsteroidal anti-inflammatory drugs (NSAIDs) in the periphery is commonly accepted as the primary mechanism by which these agents produce a selective attenuation of pain (analgesia). NSAIDs are now shown to exert a direct spinal action by blocking the excessive sensitivity to pain (hyperalgesia) induced by the activation of spinal glutamate and substance P receptors. These findings demonstrate that the analgesic effects of NSAIDs can be dissociated from their anti-inflammatory actions. Spinal prostanoids are thus critical for the augmented processing of pain information at the spinal level.

NSAIDs have a potent effect on pain behavior induced by inflammatory conditions but little effect on responses evoked by an acute high-intensity stimulus (*1*, *2*). Observations in the 1960s that tissue injury gives rise to the local formation of prostanoids (*3*), that local application of prostanoids facilitates activity of C fiber primary afferents and evokes pain behavior (*4*), and that the structurally diverse class of agents called NSAIDs powerfully inhibits cyclooxygenase that synthesizes prostanoids (*5*) supported the unifying hypothesis that the analgesic effect of NSAIDs reflects their actions in preventing the sensitization of the peripheral afferent terminal.

Subsequent studies, however, emphasize that NSAIDs can also exert an effect within the central nervous system. Direct administration of NSAIDs into the spinal intrathecal space, like systemic administration, significantly diminishes the pain behavior evoked by the intraperitoneal (*1*, *6*) or subcutaneous (*6*, *7*) injection of irritants without affecting the response to acute pain stimuli. Thus, in the formalin test a small volume of irritant (formalin) is injected into the hind paw of the rat. In humans, an equivalent injection induces a sharp stinging sensation, followed by a longer period of discomfort and hyperalgesia (*8*). In rats, this treatment results in an immediate, transient licking and flinching of the injected paw (0 to 5 min, phase 1), with a prolonged recurrence of similar behavior (10 to >60 min, phase 2). The time course corresponds to a pronounced short increase in neural activity, followed by an extended interval of elevated background activity in the primary afferent C fibers that innervate the injected skin (*9*). In this behavioral model, NSAIDs diminish the phase 2 but not the phase 1 response in a dose-dependent and stereospecific fashion (with the active isomer producing cylooxygenase inhibition) (*7*). In the studies noted above the NSAIDs are observed to be 100 to 500 times as active after spinal as after systemic administration.

These observations suggest that the effects of NSAIDs on phase 2 irritant-induced hyperalgesia are likely mediated by inhibition of prostaglandin synthesis within the spinal cord. They also emphasize that the anti-inflammatory component of these

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agents may not be solely responsible for their analgesic actions. Rather, NSAIDs may be acting upon some property of spinal processing peculiar to the afferent drive generated by the irritant stimulus.

Ongoing activation of C fiber afferents due to subcutaneous injection of formalin gives rise to spinal release of the excitatory amino acids (EAA) glutamate and aspartate, as well as several peptides including substance P (SP) (*10, 11*). The role of this release in spinal sensory processing associated with the formalin stimulus has been examined through the spinal intrathecal administration of antagonists to the *N*-methyl-D-aspartate (NMDA) and neurokinin 1 (NK-1) tachykinin receptors. Antagonists for the NMDA and NK-1 receptors produce a dose-dependent reduction in phase 2 but not phase 1 behaviors (*12*). In contrast to "pretreatment" (before phase 1), injection of the antagonists between phase 1 and 2, or "posttreatment," clearly and unexpectedly fails to alter phase 2 behavior. Pretreatment with these agents does not alter the animal's response to acute high-intensity thermal or mechanical stimuli (*12*).

On the basis of these studies it appears that spinal NK-1 and NMDA sites activated by the acute release of SP and EAAs might not serve to transmit the nociceptive information evoked by the acute stimulus but instead to initiate a facilitated state of spinal processing. As suggested by the failure of posttreatment, this facilitated state, once activated, does not require the continued agonist occupancy of the NK-1 and NMDA sites. We directly tested this issue by examining the dose-dependent effects of activating spinal glutamate and tachykinin receptors on the thermal escape latency. The local activation of these spinal sites was achieved by the intrathecal (IT) administration of agonists interacting with the respective EAA and NK-1 sites in rats prepared with chronic spinal catheters (*13*).

Intrathecal administration of NMDA, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxalone), or SP resulted in acute agitation followed by a dose-dependent decrease in the latency of the withdrawal response evoked by a thermal stimulus applied to the hind paw (Fig. 1). At the optimal dose these effects lasted approximately 15 to 45 min. The observed acute agitation and subsequent thermal hyperalgesia induced by NMDA, AMPA, or SP was antagonized in a selective fashion by MK-801, CNQX, and CP96,345, the respective antagonists for NMDA, AMPA, and NK-1 sites (Fig. 2A). The selective antagonism emphasized that the three agonists were each acting at pharmacologically distinctive sites to produce hyperalgesia (*14*). Injection of the antagonists alone did not alter the normal escape latency. Spinal injection of the antagonists 10 min after the respective agonists produced no statistically significant antagonism (Fig. 2B). Thus, the hyperalgesic phase evoked by the agonist persisted after the agonist had interacted at its spinal receptor for 10 min. In other studies we have found such long-lasting augmentation in pain threshold even when the antagonists are administered at intervals as short as 1 to 2 min after the agonists, demonstrating the rapid induction of spinal facilitation.

Formalin injection evokes the spinal release of EAAs and SP. Because these agents participate in producing hyperalgesia, we reasoned that spinal NSAIDs might be effective in blocking EAA- and SP-induced thermal hyperalgesia, just as they block the phase 2 of the formalin test. As previously shown, spinal injection of NSAIDs had no

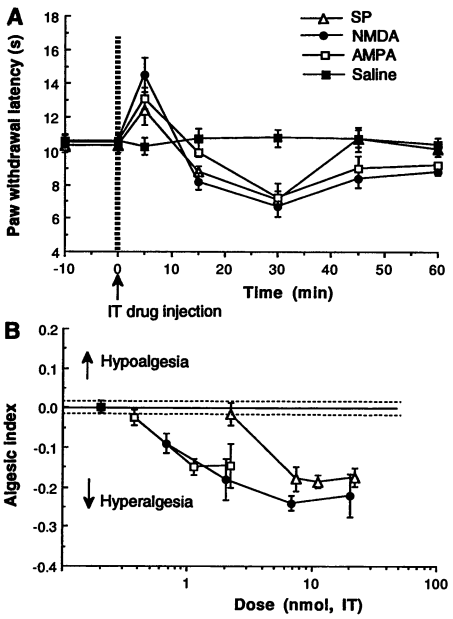

Fig. 1. Intrathecal (IT) injection of NMDA, AMPA, and SP produced a dose-dependent thermal hyperalgesia with a maximal decrease in paw withdrawal latency at 30 min after injection. (**A**) Paw thermal response latency presented as a function of time after the IT injection of SP (7.4 nmol), NMDA (6.8 nmol), AMPA (1.1 nmol), and saline as control. The thermal nociceptive threshold was measured with a radiant heat source beneath a glass floor (*24*). Two-way analysis of variance (ANOVA) indicated a significant treatment effect as compared to saline control for all three agents: NMDA ($F_{1,72}$ = 16.2; $P < 0.001$), AMPA ($F_{1,72}$ = 30.7; $P < 0.001$), and SP ($F_{1,72}$ = 11.9; $P < 0.01$). One-way ANOVA followed by Dunnett's test showed that 5 min after IT administration all the agents produced a small but, as compared to base line, significant increase in latency (hypoalgesia) followed by a significant time-dependent decrease in latency (hyperalgesia) as compared to base line: NMDA (15, 30, 45, and 60 min), AMPA (30, 45, and 60 min), and SP (15 and 30 min). Each point represents the mean of six to eight animals (error bars, ±SEM). (**B**) Algesic index plotted as a function of the log dose of IT SP, NMDA, AMPA, and saline control. For the algesic index [calculated as in (*25*)], positive values indicate an increased threshold (hypoalgesia), negative values indicate decreased thresholds (hyperalgesia), and values near 0 indicate a normal threshold. Each point on the graph represents the mean for four to eight animals (error bars, ±SEM) and the solid line surrounded by dotted lines represents the mean ± SEM for the saline control. One-way ANOVA followed by Dunnett's test ($P < 0.05$) indicated a significant dose dependency for each of the three agents ($P < 0.05$).

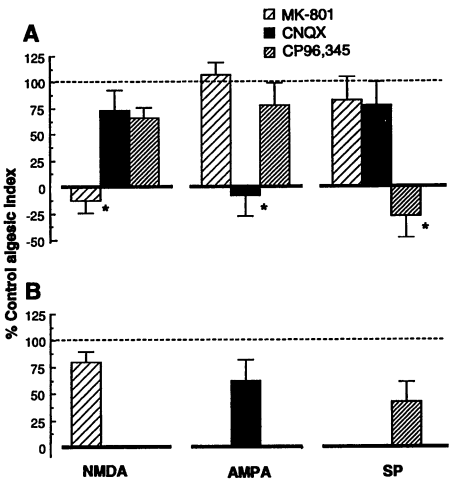

Fig. 2. Effects of the selective antagonists MK-801 {(+)-5-methyl-10,11-dihydro-5*H*-dibenzo-[a,d]cycloheptene-5,10-imine; 29 nmol}, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione; 27 nmol), and CP96,345 {(2S,3S)-*cis*-2-(diphenylmethyl)-*N*-{(2-methoxyphenyl)-methyl-1-azabicyclo-[2.2.2]octan-3-amine; 330 nmol} on the hyperalgesia produced by NMDA (6.8 nmol), AMPA (1.1 nmol), and SP (7.4 nmol). The results are presented as the mean (error bars, SEM) decrease in thermal response latency described as the percent of control algesic index (*25, 26*). A value close to 0 indicates an attenuation of the hyperalgesic effect induced by NMDA, AMPA, or SP, and a result near 100 indicates that the antagonist did not affect the agonist-induced hyperalgesia. One-way ANOVA was used to compare the saline with the drug-treated groups and was followed by Dunnett's test for individual comparisons (asterisks, $P < 0.05$). (**A**) IT injection of MK-801 (29 nmol) administered 10 min before the agonists reversed the NMDA-induced hyperalgesia ($F_{3,17}$ = 13.7; $P < 0.001$). Pretreatment with CNQX (27 nmol) and CP96, 345 (330 nmol) antagonized the thermal hyperalgesia induced by AMPA and SP, respectively ($F_{3,17}$ = 6.5; $P < 0.005$ and $F_{3,17}$ = 9.5; $P < 0.001$). (**B**) IT injection of antagonists 10 min after IT injection of NMDA, AMPA, or SP failed to reverse the hyperalgesic effect by the respective agonist. Each bar presents the mean for four to eight animals (error bars, SEM).

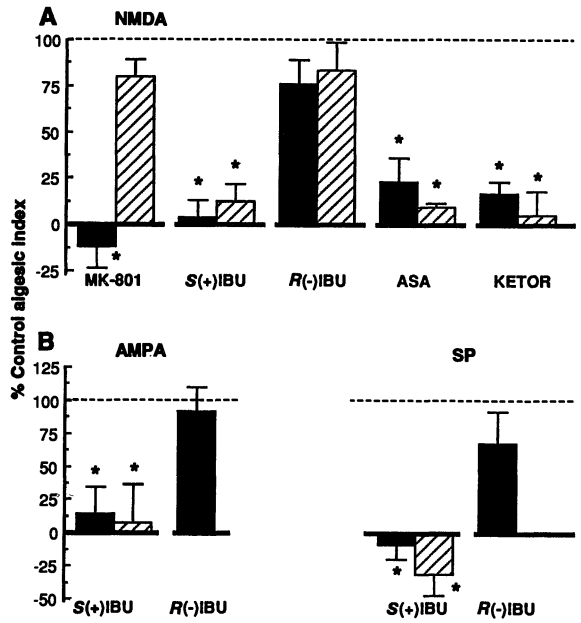

Fig. 3. Cyclooxygenase inhibitors (NSAIDs) administered 10 min before (solid bars) or 10 min (after striped bars) NMDA (6.8 nmol), AMPA (1.1 nmol), or SP (7.4 nmol) were equally effective in attenuating the thermal hyperalgesia produced by NMDA, AMPA, or SP. (**A**) Mean (error bars, SEM) of the percent of control algesic index (each group, four to eight rats) (*15, 16*) for NMDA with pre- or posttreatment with MK-801 (29 nmol), *S*(+)ibuprofen [*S*(+)IBU] (27 nmol), *R*(−)ibuprofen [*R*(−)IBU] (270 nmol), acetylsalicylic acid (ASA) (100 nmol), and ketorolac tromethamine (KETOR) (27 nmol). (**B**) Effects of *S*(+)IBU (27 nmol, injected before and after AMPA or SP) and *R*(–) (270 nmol, injected before AMPA or SP) on AMPA- and SP-induced hyperalgesia, presented as the mean (error bars, SEM) percent of control algesic index. The dotted lines in (A) and (B) indicate NMDA, AMPA, or SP, respectively, treated with saline control, which is defined as 100%. Statistical significance was determined for treatments compared to saline control by using one-way ANOVA followed by Dunnett's test (asterisks, $P < 0.05$).

effect on the thermal response latency when administered alone (*1, 7*). In contrast, these agents completely suppressed the hyperalgesic component induced by the injection of NMDA, AMPA, or SP (Fig. 3), though they had no effect on the acute pain behavior (scratching and vocalization) induced by the IT agonists. In contrast to the several receptor antagonists, NSAIDs displayed a comparable antagonist effect on the thermal hyperalgesia whether administered before or after the several agonists.

The findings that intrathecal NSAIDs block phase 2 of the formalin response at doses considerably lower than those required for systemic effects and that spinal NSAIDs reverse the thermal hyperalgesia mediated by the direct activation of spinal NMDA, AMPA, and NK-1 receptors weigh against a simple peripheral action. These observations argue for the probable spinal action of the NSAIDs, mediated by a mechanism that is independent of the changes in peripheral terminal sensitivity that might follow inflammation.

The likelihood that reversal of thermal hyperalgesia and the second phase of the formalin test reflects cyclooxygenase inhibition by NSAIDs is emphasized by (i) the relative ordered activity of the several NSAIDs (*7*) and (ii) the failure of *R*(−) ibuprofen (the isomer that is inactive as a cyclooxygenase inhibitor) to alter the hyperalgesia at a dose ten times that of *S*(+)ibuprofen (*7*). Importantly, the intrathecal injection of several cyclooxygenase products such as prostaglandin E_2(PGE_2), PGD_2, and $PGF_{2\alpha}$ induces a hyperalgesic state (*1, 15*).

The mechanisms by which prostaglandins may serve an intermediary role in the observed spinal hyperalgesia are not known. Several observations, however, appear relevant. PGE_2 increases calcium influx and the subsequent release of SP from dorsal-root ganglion cells (*16*). Although details of the systems secreting spinal prostanoids are not known, membrane depolarization and occupancy of the NMDA receptor do increase intracellular calcium (*17*) and elevate cytosolic levels of arachidonic acid in neural tissue (*18*). After synthesis the prostanoids appear able to diffuse readily into the extravascular-extracellular space of the spinal parenchyma. Thus, activation of spinal systems by prolonged afferent stimulation (*1*) or the addition of glutamate (*19*) increases the release of several lipidic acids. A neuronal origin for spinal prostanoids is not required. For example, SP can increase the release of prostanoids from glia (*20*) and tachykinins are released widely in the spinal dorsal horn, reaching measurable concentrations in the cerebrospinal fluid (*21*). In light of these findings, it is important that a common characteristic of the behavioral pain models in which NSAIDs show activity is the presence of a protracted afferent C fiber barrage. Physiological studies have shown that such input will evoke a powerful facilitation of spinal nociceptive processing (*22*). It appears that such afferent input is also a prerequisite for generating spinal prostanoids, which augment the animal's response to a noxious stimulus and thereby evoke an NSAID-sensitive hyperalgesia.

The ability of posttreatment with NSAIDs to block hyperalgesia seems at first surprising. However, we interpret this result to mean that once the afferent barrage has generated a threshold effect upon neuronal function, the resulting activation of SP receptors, glutamate receptors, or both, causes the progressive generation of a pool of cytosolic arachidonic acid available for conversion by cyclooxygenase. It appears that the time required to initiate this central facilitation is extremely short, and the ability of posttreatment with NSAIDs to attenuate the effect suggests that the clearance of the active lipid acid or acids must be relatively rapid and subject, for a period, to ongoing replenishment.

From a clinical perspective, our observation that NSAIDs may exert a central action independent of their ability to alter the inflammatory state is consistent with findings that indicate that the analgesic actions of several NSAIDs may be separated from their ability to reduce inflammatory signs (*23*). Thus, although the association of NSAID actions with the presence of inflammation is not totally inappropriate, we believe that the fortuitous effects of NSAIDs on inflammation have in fact served as a logical diversion of our attention from what must be a major role played by prostaglandins in the spinal cord. Thus, NSAIDs function as analgesics in pain models in which there is a spinally mediated hyperalgesic state evoked by a protracted barrage from small afferents. Ironically, the principal pain models in which such a protracted state of small afferent excitation exists appear to be those models in which tissue injury or an inflammatory state has been induced. As a consequence, the potent central actions of the NSAIDs and the role in nociceptive processing of cyclooxygenase products in brain and spinal cord have been generally obscured.

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26. The treatment data are presented as the ratio of the means for the drug-treated group relative to those of the saline-treated group, which we defined as 100% (error bars, SEM). The variation of the means was estimated according to the formulas provided in R. J. Tallarida and R. B. Murray [*Manual of Pharmacologic Calculation with Computer Programs* (Springer-Verlag, New York, ed. 2, 1987), pp. 137–139].
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Modulation of Neural Stereoscopic Processing in Primate Area V1 by the Viewing Distance

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Accurate binocular depth perception requires information about both stereopsis (relative depth) and distance (absolute depth). It is unclear how these two types of information are integrated in the visual system. In alert, behaving monkeys the responsiveness of a large majority of neurons in the primary visual cortex (area V1) was modulated by the viewing distance. This phenomenon affected particularly disparity-related activity and background activity and was not dependent on the pattern of retinal stimulation. Therefore, extraretinal factors, probably related to ocular vergence or accommodation, or both, can affect processing early in the visual pathway. Such modulations could be useful for (i) judging true distance and (ii) scaling retinal disparity to give information about three-dimensional shape.

Stereopsis allows the visual system to determine the position of an object relative to the plane of fixation. It relies on the horizontal retinal disparity between the images of an object projected on the two retinas (*1*), a parameter encoded by neurons in the visual cortex (*2*). However, stereoscopic information alone is not sufficient for accurate binocular depth perception. It has to be combined with information about the viewing distance (derived from cues such as vergence or accommodation, or both). We have addressed the question of what happens to the response properties of individual neurons when the distance of fixation is changed. Three predictions can be made. First, retinal disparity sensitivity could remain the same, despite the distance of fixation (this would entail a purely stereoscopic mechanism). Second, the response properties of cells could be qualitatively modified: Altering the fixation distance could transform a neuron that was in the "far" category to the "near" category; such cells could be directly involved in encoding absolute depth. Finally, the amplitude of the neural discharges could show modulations as a function of the distance of fixation. This could be an intermediate step in encoding absolute depth. Our results show that this last possibility is typical of what actually occurs in neurons of area V1. Changing the viewing distance also produced changes in the amount of the spontaneous firing rate (which is often associated with, but which occurs independently of, the modulation of visual responsiveness).

We have demonstrated these phenomena by recording the responses of individual neurons in cortical area V1 to the presentation of random dot stereogram (RDSs) (Fig. 1). Fifty visually responsive cells were tested at at least two viewing distances in monkeys trained to perform a fixation task (*3*). The visual responses of 41 cells (82%) were modulated by changes in the viewing distance. Changes in the spontaneous activity were also observed in 21 cells (42%).

Two examples of changes in visual responsiveness are shown in Fig. 2. Cell A was weakly visually responsive at 20 and 40 cm but at 80 cm showed a tuned excitatory response. However, at 40 cm there appeared to be a trend toward a mild retinal disparity (the same disparity for which the

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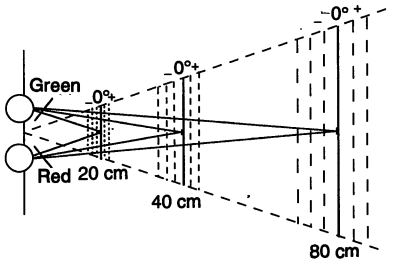

Fig. 1. Schematic representation of visual stimulation. At a given target fixation distance (20, 40, or 80 cm), static RDSs, generated through red-green filters, were presented on a color video monitor in the plane of fixation (0°, solid vertical lines) at two uncrossed horizontal disparities (+) and three crossed disparities (−), indicated by vertical dotted lines. The RDS pattern consisted of a 128 by 128 array of rectangular dots (density 10, 20, or 30%, according to the neuron's best response), which appeared as a large flat surface (width 18°, height 14°, of visual angle) floating in front of or behind a fixation point in the center of the screen. We changed the distance of fixation by physically moving the monitor on rails in a tunnel to fixed distances. The same pattern was used at each fixation distance, but, to keep the angular size of the stimulus constant, the physical size of the image was doubled each time the fixation distance was increased by a factor of 2, which resulted in a fixed angular dot size of 9 by 7 min of arc. The amount of luminance through colored filters was 1 candela per meter squared at the three distances of fixation.