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# Antinociceptive Actions of Spinal Nonsteroidal Anti-Inflammatory Agents on the Formalin Test in the Rat[1]

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## ABSTRACT

Subcutaneous injection of formalin into the dorsal surface of the hindpaw evoked a two-phased flinching (phase 1: 0–9 min; phase 2: 10–60 min) of the injected paw. Intrathecal administration of the nonsteroidal anti-inflammatory drugs (NSAID) produced minimal effects upon phase 1, but showed a significant, though submaximal, dose-dependent suppression of the phase 2 response. Ordering of i.t. potency was ($ID_{50}$ in nmol): indomethacin (1.9) ≥ flurbiprofen (2.1) > ketorolac (5.2) ≥ zomepirac (5.9) > *S*(+)ibuprofen (16) ≥ ibuprofen(racemic) (19) > acetylsalicylic acid (27) > acetaminophen (250) > *R*(−)ibuprofen (>270) = 0. Intraperitoneal administration also produced a dose-dependent inhibition of phase 2, but only at doses which were 100 to 1000 times higher than those required to produce similar effects after i.t. injection. Intrathecal and i.p. dose-response curves showed similar distinct plateaus of maximum achievable inhibition (intrinsic activity) of the phase 2 behavior, ranging from 20 to 50% of the control response. Varying the time of drug injection reveals that injection 9 min after formalin yielded effects the same as those observed when the agent was given 2 min before formalin. Pretreatment at longer intervals indicated that the duration of the antinociceptive effect was between 3 to 6 hr after the i.t. injection. The i.t. injection of the highest doses of the several NSAID were without significant effect upon the 52.5°C hot plate test. These studies indicate that NSAID have a powerful effect upon spinal nociceptive processing evoked by the s.c. injection of formalin.

It has been widely postulated that the analgesic actions of NSAID result from the inhibition of the peripheral synthesis of prostaglandins formed secondary to tissue injury and inflammation (Guzman *et al.*, 1964; Lim *et al.*, 1964; Lim, 1970; Ferreira *et al.*, 1978). This hypothesis is supported by the observation that 1) the NSAID, though structurally a diverse group, share at least in part the ability to inhibit cyclooxygenase (Vane, 1971); 2) prostanoids applied to the peripheral nerve terminal can facilitate its discharge (Schaible and Schmidt, 1988); and 3) methodologically, demonstration of the antinociceptive effects of NSAID in experimental behavioral models typically depends upon the presence of an inflammatory state and hyperalgesia. Thus, after inducing inflammation in the knee joint (Moncada *et al.*, 1975, 1979), the skin (Ferreira, 1972) or by the injection of agents such as bradykinin, which release prostanoids (Guzman *et al.*, 1964; Lim *et al.*, 1964), the associated algogenic behavior can be significantly diminished by aspirin-like drugs. In this manner, it has been widely appreciated that NSAID, by inhibiting the biosynthesis of prostaglandins, do not, strictly speaking, produce analgesia, but inhibit the hyperalgesic state evoked by the sensitization of the peripheral afferent. Under such conditions, these agents would only be effective when prostaglandins are released (Ferreira, 1972; Ferreira *et al.*, 1973).

In spite of this emphasis on a peripheral action, several lines of evidence have pointed to a potential central action of these families of agents. Intracerebroventricular administration of NSAID have been shown to inhibit the carrageenin evoked hyperalgesia in the rat paw (Ferreira, 1983). More recently, systemically administered NSAID have been found to dose-dependently depress the activity evoked by noxious stimuli in single neurons of the rat thalamus in both normal and arthritic rats (Attal *et al.*, 1988; Carlsson *et al.*, 1988; Groppetti *et al.*, 1988; Jurna and Brune, 1990). Alternatively, indication for a spinal antinociceptive effect of prostaglandin synthesis inhibitors derives from studies with these agents administered in the lumbar intrathecal space (Yaksh, 1982). In this work, i.t. NSAID diminished the pain behavior evoked in rats by i.p. injection of irritant. Given that these effects were observed spinally at doses which were not effective when given systemically, it was argued that spinally released prostaglandins are important for the transmission of nociceptive stimuli in these tests and that NSAID altered nociceptive transmission not only at a peripheral site (diminishing the augmented sensitivity

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**ABBREVIATIONS:** NSAID, nonsteroidal anti-inflammatory drugs; ASA, acetylsalicylic acid; HP, hot plate test; CI, confidence intervals.

of the peripheral terminals), but by a spinal action as well (Yaksh, 1982). Consistent with this hypothesis, it has been shown that: 1) sensory afferent input can elevate the extravascular, extracellular levels of prostanoids (Ramwell *et al.*, 1966; Coderre *et al.*, 1990a; L. S. Sorkin and T. L. Yaksh, unpublished observation); and 2) certain prostanoids can facilitate the firing of central neurons (Coceani and Viti, 1975) and 3) prostanoids can augment the release of neurotransmitters (Chiu and Richardson, 1985; Bloomquist and Kream, 1987), notably from spinal primary sensory afferents by augmenting $Ca^{++}$ influx. (Nicol *et al.*, 1992).

In spite of the potential mechanisms underlying a possible spinal action of NSAID, the earlier literature on the antinociceptive actions only focused on a few NSAID. The possibility of mechanisms of action unique to these compounds and unrelated to prostaglandin synthesis inhibition could not be excluded. We sought in the present experiments to characterize further the spinal pharmacology of these NSAID using representative compounds from several classes and examining the stereospecificity of the effect. We have used the rat paw formalin test. The injection of formalin under the skin on the dorsal surface of the hindpaw causes an immediate and intense increase in the spontaneous activity of C fiber afferents (Heapy *et al.*, 1987) and evokes a distinct quantifiable behavior indicative of pain: flinching/shaking and licking/biting of the injected paw (Dubuisson and Dennis, 1977; Wheeler-Aceto *et al.*, 1990). The behavioral response to subcutaneous formalin is biphasic, with an initial acute phase (phase 1: 0–10 min), followed by a quiescent period and then a prolonged tonic response between 20 and 60 min (phase 2: 10–60 min). It is suggested that the early phase is due to a direct effect of formalin on nociceptors, whereas the late phase involves inflammatory components with release of different pain-mediating substances that possibly can activate small afferents (Hunskaar and Hole, 1987). Additionally, compounds which have shown minimal antinociceptive effect in acute pain models (*e.g.*, HP test) like N-methyl-D-aspartate or neurokinin-1 antagonists have been reported to be active in attenuating the second phase of the formalin test (Murray *et al.*, 1991; Yamamoto and Yaksh, 1991, 1992).

## Methods

### Animal Preparation

Male Sprague-Dawley rats (280–320 g; Harlan Industries, Indianapolis, IN) were housed in group cages and maintained on a 12-hr light/12-hr dark cycle. Animals had free access to food and water at all times. For the spinal administration, chronic i.t. catheters were implanted under halothane anesthesia according to modification of the method described by Yaksh and Rudy (1976). Briefly, through an incision in the atlanto-occipital membrane, a polyethylene (PE-10) catheter was advanced caudally extending to the rostral edge of the lumbar enlargement. After implantation of i.t. catheters, rats were housed in individual stainless steel cages. Intrathecal injection studies were carried out 7 to 10 days after surgery. Only animals with normal motor function were used. Similarly, rats used for i.p. studies were housed in pairs 7 to 10 days before the studies were performed. Experiments were carried out according to a protocol approved by the Institutional Animal Care Committee of the University of California, San Diego.

### Drugs and Injections

The drugs used in the study were chosen on the basis of belonging to several chemical groups (salicylate: ASA, *para*-aminophenol derivative: acetaminophen, acetic acid: indomethacin, propionic acids: ibuprofen and flurbiprofen, and pyrrolo pyrrole: ketorolac), reference compounds (ASA, indomethacin), commonly used drugs (ASA, ibuprofen, acetaminophen) and highly effective in inhibiting prostaglandin synthesis *in vitro* (flurbiprofen) and having clinical analgesic efficacy being reported higher (ketorolac, zomepirac) than expected for this group of compounds.

The i.t. administered drugs were delivered in a total volume of 10 μl followed by 10 μl of saline to flush the catheter. The i.p. injected drugs were dissolved in similar vehicles and administered in a volume of 1 ml/kg. The following drugs were used in this study: ASA (MW = 300.26; Sigma Chemical Co., St. Louis, MO), ketorolac tromethamine (MW = 376,41; Toradol i.m., injection syringe 30 mg/ml, Syntex), zomepirac sodium (MW = 291.74; McNeil), indomethacin (MW = 357.81; Sigma), acetaminophen, (MW = 151.16; Sigma), flurbiprofen (MW = 244.27; courtesy D. P. Bauer, Ethyl Corporation, Orangeburg, SC), ibuprofen (racemic), *S*(+)ibuprofen and *R*(−)ibuprofen (MW = 206.27; courtesy D. P. Bauer and C. W. Matthews, Ethyl Corporation, Baton Rouge, LA). Ketorolac was further diluted in physiological saline (0.9% w/v). Zomepirac, flurbiprofen, ibuprofen, *S*(+)ibuprofen, *R*(−)ibuprofen and ASA were prepared in a 5% solution of 2-hydroxypropyl-β-cyclodextrin (cyclodextrin; Research Biochemicals Inc., Natick, MA). Acetaminophen and indomethacin were dissolved in ethanol with a final concentration of 5% ethanol.

### Formalin Test

To perform the formalin test, the rats were placed in a Plexiglas box connected to a halothane vaporizer and allowed to breathe halothane (3%). After 2 to 3 min, there was a momentary loss of spontaneous movement with preservation of the deep spontaneous respiration, and blink and pinnae reflexes. The animal was then quickly removed, and 50 μl of 5% formalin solution was injected s.c. into the dorsal surface of the right hindpaw with a 30 gauge needle. The rat was then individually placed in an open Plexiglas chamber for observation, and within a maximum interval of 1 to 2 min, the animal displayed recovery from anesthesia with spontaneous activity and normal motor function. A mirror was placed on the opposite side of the Plexiglas chamber for the unhindered observation of the formalin-injected paw. Pain behavior was quantified by periodically counting the incidents of spontaneous flinching/shaking of the injected paw. Animals were observed individually and the flinches counted for 1-min periods at 1- to 2-, 5- to 6- and 5-min intervals during the interval from 10 to 60 min. Two phases of spontaneous flinching behavior were observed as previously described (Wheeler-Aceto *et al.*, 1990): phase 1 started immediately after formalin injection and lasted through the second observation interval (5–6 min), followed by the phase 2, which began after 10 min with a maximum response typically observed at around 25 to 35 min after the formalin injection. As will be described, NSAID were typically most active in reducing the formalin response during the first period of the second phase of the formalin response (*i.e.*, the development and the peak of the second phase). For the purpose of data analysis, the second phase was then further divided into two phases: phase 2a (10–39 min) and phase 2b (40–60 min). After the observation period of 1 hr, animals were immediately sacrificed with an overdose of barbiturate mixture (Beuthanasia, 50 mg/kg, i.p.).

### Behavioral Assessment

In addition to the formalin response, observation of general behavior was carefully carried out for all rats tested. Motor function was tested by examining the placing/stepping reflex (characterized as an upward lifting of the paw from the surface of the table and a plantar placement evoked by drawing the dorsum of the respective hindpaw across the edge of the table), normal ambulation and righting (normally, the rat's immediate coordinated twisting of the body when placed horizontally with its back on the table). Moreover, the presence of allodynia was assessed by looking for agitation (escape, vocalization) evoked by lightly stroking the flank of the rat with a pencil.

To assess the effects of several of these agents on other nociceptive

endpoints, separate animals were also examined on the HP. Rats were placed on the 52.5°C hot plate. The amount of time licking the hindpaw was then measured. Failure to respond within 60 sec was cause to remove the animal from the surface and assign that score.

### Experimental Paradigms

Because all animals could not be run in a single group, drugs and doses were assigned in a randomized fashion to be given over the course of these studies. Control animals (*i.e.*, formalin animals receiving no injection, i.t. and i.p. saline, 5% cyclodextrin or 5% ethanol) were run interspersed concurrently with the drug-treated animals. This prevented all of the controls being run on a single group of animals at one time in the course of the investigation.

**Spinal action of NSAID.** Time course of peak action and the dose dependency of the effect were determined. To study the time course of the agents, i.t. administration of a maximal effective dose was made 2 min, 3 hr, 6 hr and 24 hr before (pretreatment) and 9 min after (post-treatment; before the second phase of the formalin response) formalin injection.

Dose-response curves were carried out with the agents administered i.t. immediately before (2 min) the formalin injection, an interval of maximum effect based on the initial time course studies. The compounds used for i.t. administration are the following: ASA, indomethacin, flurbiprofen, ketorolac, zomepirac, acetaminophen, ibuprofen (racemic), *S*(+)ibuprofen and the other stereoisomer, *R*(−)ibuprofen. To achieve appropriate control groups, rats were treated with i.t. saline, 5% cyclodextrin or 5% ethanol. Control group data were collected throughout the study period. The highest dose examined in the dose effect studies was selected on the basis of two factors: 1) when the dose did not differ in effect by more than 10% of the maximum effect achieved by the immediately lower dose (*i.e.*, the plateau effect of the drug was presumably reached), and 2) when it was impossible to dissolve the drug in a higher concentration.

**HP test.** With several of the more potent agents (*i.e.*, ketorolac, zomepirac, indomethacin, flurbiprofen and ASA), the highest dose of drug which was used in the formalin test was given i.t. and the effect upon the HP (52.5°C) response latency was assessed.

**Systemic action of NSAID.** Intraperitoneal injections were carried out 2 min before the formalin injection in order to determine the dose-response and time *vs.* response of the agents after systemic administration. In this study, the NSAID used were: ASA, indomethacin, ketorolac, flurbiprofen, zomepirac and acetaminophen. These were selected because of their potency or reference compounds. For the appropriate control group, rats were given i.p. 5% cyclodextrin and 5% ethanol. In addition, the vehicle-treated control group was compared to the untreated rats in the formalin test. The highest doses used in this series was determined as described for the i.t. route mentioned above.

### Statistical Analysis

The time-response data are presented as the mean flinches per min and the S.E.M. for the period of 1 to 2 min, 5 to 6 min and at 5-min intervals after that up to 60 min. Dose-response curves are presented as the sum of flinches for that particular observation period [*i.e.*, sum of flinches for phase 1 (0–9 min) and phase 2a (10–39 min), respectively]. The cumulative flinching response was calculated for each rat and the dose-response curve represents the mean of these values and the S.E.M. The data were examined by one-way ANOVA with a Dunnett's test ($P < .05$) for multiple comparisons. The dose-response lines were fitted using least square linear regression, and the $ID_{50}$ (inhibitory dose resulting in a 50% reduction of the control formalin response) and 95% CI were calculated according to Tallarida and Murray (1987). The potency ratio and 95% CI were estimated according to Tallarida and Murray (1987) for the relative potency of the agent administered i.t. *vs.* i.p.

Inspection of the results clearly revealed a plateau of activity for all of the NSAID. In order to compare the efficacy [*i.e.*, the maximal inhibition of the formalin response between the different drugs with similar administration routes (*i.e.*, i.t. or i.p., respectively)], a two-way ANOVA was used. To define the maximum inhibition, dose-response curves were inspected. When two sequential NSAID doses differing by 0.5 log units produced effects differing by 10% or less, the effect produced by the higher of the two doses was defined as the maximum inhibition. For comparison of the maximum suppression achieved by the several drugs given i.t. or i.p., a one-way ANOVA followed by Dunnett's test was used. All statistical significance was considered at a critical value of $P < .05$.

## Results

### General Behavior

The formalin injected s.c. into the dorsal surface of the right hindpaw resulted in a highly reliable flinching response with two distinct phases. This biphasic effect is indicated in the typical results presented in figure 1. The timing or magnitude of the measured behavior did not differ in the control group over the time of study or between the different treatments used as control vehicles for the respective drug groups: i.t. and i.p. saline, 5% cyclodextrin, 5% ethanol or untreated rats (one-way ANOVA, $P > .05$). All control experiments were therefore pooled and used as a common control group.

In general, none of the NSAID at the highest doses used had

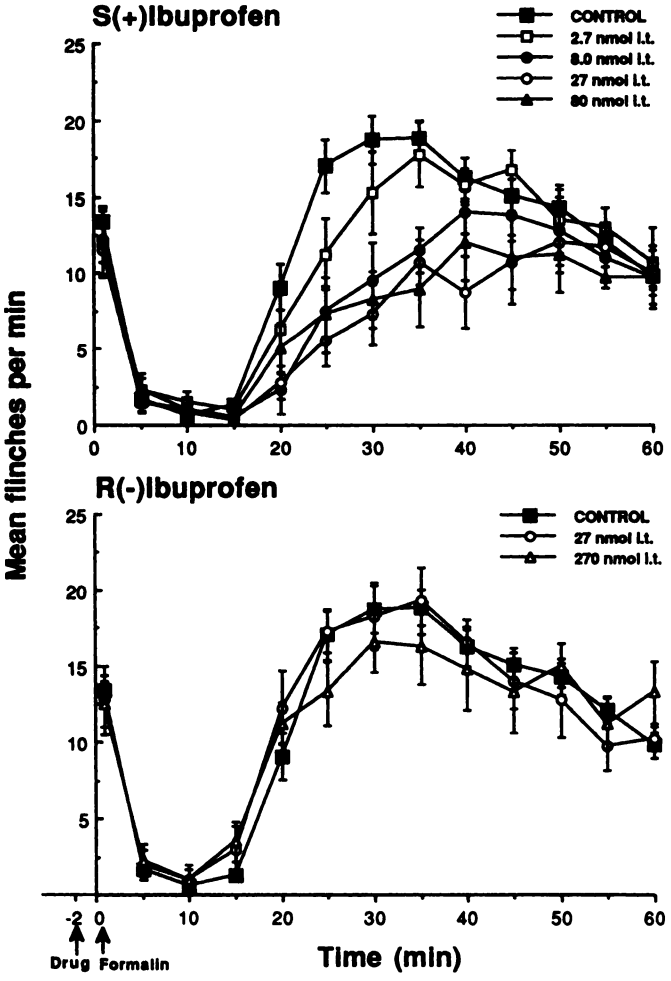


**Fig. 1.** Time effect curve of i.t. *S*(+)ibuprofen (top) and *R*(−)ibuprofen (bottom) administered 2 min before formalin. The number of flinches per min is plotted *vs.* the time after the formalin injection into the dorsal surface of the right hindpaw. The line representing the control group includes 20 animals; otherwise, each line represents the mean and S.E.M. of 4 to 6 animals.

any effect on general behavior or motor function when administered i.t. or i.p. Thus, there were no changes in placing/stepping, righting reflexes or ambulation. In 50% of the animals receiving the highest doses of indomethacin i.t. (28 nmol) and ketorolac i.t. (27 nmol) after 1 and 3 hr, respectively, a transient allodynia with squeaking and agitation evoked by touching of the flank was observed.

Although there was no effect upon motor function, the i.t. injection of the several NSAID resulted in a potent suppression of the flinching behavior observed during phase 2, and to a much lesser extent in the phase 1 portion of the algogenic activity observed after the s.c. injection of formalin. A typical time course of effect, with different doses of *S*(+)ibuprofen given i.t. 2 min before the injection of formalin, is presented in figure 1. In contrast, the stereoisomer *R*(−)ibuprofen was without effect on the flinching behavior in the formalin test (fig. 1).

Typically, the first portion of the second phase (phase 2a; 10–39 min after formalin) was observed to be most sensitive to the inhibitory effects of these agents. In contrast, even at the highest doses, there was no statistically significant effect of *S*(+)ibuprofen at the highest dose on the phase 1 or phase 2 responses.

### Potency and Efficacy of i.t. NSAID

**Formalin test.** As indicated for *S*(+)ibuprofen in figure 1, the suppressant effects of these i.t. agents on the appearance and magnitude of the second phase activity were dose dependent. Figure 2 presents the i.t. dose-response curves for the several NSAID determined on the phase 2a (10–39 min) response evoked by s.c. formalin. The rank order of potency (defined by the $ID_{50}$ in nmol) of i.t. NSAID on the phase 2a flinching response was found to be: indomethacin (1.9) ≥ flurbiprofen (2.1) > ketorolac (5.2) ≥ zomepirac (5.9) > *S*(+)ibuprofen (15.7) ≥ ibuprofen(racemic) (18.9) > acetylsalicylic acid (27) > acetaminophen (257) > *R*(−)ibuprofen (>270) = 0 (table 1). For comparison, the potency of morphine to inhibit the second phase of the formalin response is of the same magnitude as the more potent NSAID [morphine $ID_{50}$ and 95% CI: 3.8 (2.6–5.4) nmol, data not shown].

A very modest reduction of the flinching response was frequently observed in phase 1 (0–9 min), although a statistically significant dose dependency was only found for ketorolac [$ID_{50}$ and 95% CI: 24 (5–130) nmol]. For comparison, morphine is as potent in inhibiting the first phase as the second phase of the formalin response [$ID_{50}$: 3.7 (1.6–8.1) nmol, data not shown].

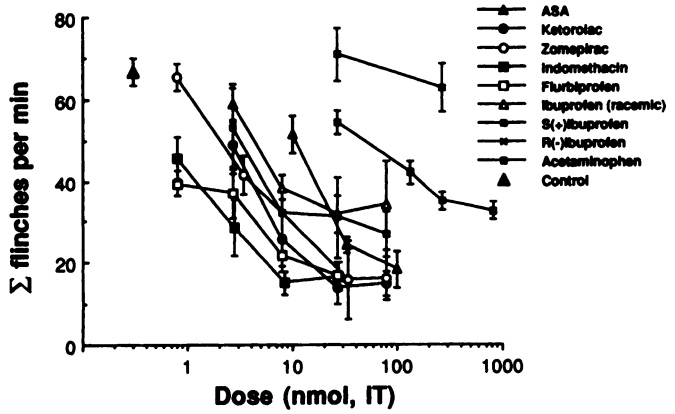


**Fig. 2.** Dose-response curves for i.t. NSAID: ASA, ketorolac, zomepirac, indomethacin, flurbiprofen, ibuprofen(racemic), *S*(+)-ibuprofen, *R*(−)-ibuprofen and acetaminophen, presented as the cumulative number of formalin evoked flinches during the phase 2a interval (10–39 min). Each dose point on the graph represents the mean and S.E.M. from 4 to 6 rats except for the control group, which includes 20 animals.

As indicated in the dose-response curves for i.t. agents, all agents on the phase 1 and phase 2 endpoints showed a limitation in the maximum achievable suppression of the flinch behavior (*e.g.*, efficacy). Intrathecal and i.p. dose-response curves showed similar distinct plateaus with numerically small but statistically significant differences between the agents. As indicated in figure 3 (top), the inhibitory effect obtained with the i.t. administration of indomethacin, flurbiprofen, ketorolac, zomepirac and ASA approached a level which was about 20 to 30% of control. These values were only 40 to 50% of control for ibuprofen, *S*(+)ibuprofen and acetaminophen, and 95% for *R*(−)ibuprofen, which were without effect. Less difference was found between i.p. administered drugs, although acetaminophen resulted in a significantly less maximal possible suppression than ketorolac and flurbiprofen (fig. 3; bottom). However, comparing the i.t. with the i.p. administration route for a single agent did not reveal any difference except for indomethacin, which showed slightly more suppression after i.t. injection. In contrast, spinal morphine (80 nmol) is able to totally diminish both the first and second phases of the formalin response (data not shown).

**HP test.** Even with the highest doses of i.t. ketorolac, zomepirac, indomethacin, flurbiprofen and ASA, these agents did not produce any significant effect on the HP latency (table 2). In comparison, morphine, in a dose similar to the NSAID doses tested (80 nmol), elevates the response latency to 60 sec.

### Time Course of Action of i.t. NSAID

To define the time course of the effects of i.t. NSAID, drugs were delivered at intervals of 9 min after and 2 min, 3 hr, 6 hr or 24 hr before the injection of formalin. As shown in figure 4 for ketorolac, administration of the NSAID at different intervals before and at 9 min after the application of formalin revealed that the maximum inhibition of the phase 2a response did not differ, whether the agent was delivered 2 min before or 9 min after the injection of formalin.

Increasing the pretreatment intervals resulted in a progressive reduction in the suppressive effects upon the phase 1 and phase 2b effect, as compared to the 2-min pretreatment intervals. Thus, as indicated in figure 5 for the several agents examined, the maximum pretreatment intervals during which the effect obtained was not statistically less than the maximally achieved suppression (measured with the 2-min pretreatment interval) were: ASA (100 nmol), 3 hr; flurbiprofen (27 nmol), 3 hr; zomepirac (34 nmol), 3 hr; and ketorolac (27 nmol), 6 hr.

Importantly, postformalin treatment, just before the initiation of the second phase (9 min after formalin injection), decreased the flinch behavior in phase 2a by the same magnitude as the pretreatment of 2 min before the formalin (fig. 2). The lack of a difference between the 2-min pretreatment and the 9-min post-treatment on the phase 2a effects of any of the agents examined emphasizes that these agents after i.t. administration have a very short latency to onset. Moreover, there were no differences between the phase 2a and 2b effects as a function of time of drug pre- or post-treatment, suggesting that the differential effects of i.t. NSAID upon these phases were independent of administration time.

TABLE 1

**Inhibitory effects of i.t. and i.p. administered NSAID on the 2a phase (10–39 min) of the formalin test, presented as percent of control response**

$ID_{50}$ values and 95% CI calculated from regression lines shown in figures 2 and 6.

| Drug: | i.t. $ID_{50}$[a] 95% CI | i.p. $ID_{50}$ 95% CI | i.t. Potency Ratio[b] vs. ASA | i.p. Potency Ratio vs. ASA | Potency Ratio[c] i.p. vs. i.t. |
|---|---|---|---|---|---|
| | *nmol* | *μmol* | | | |
| Acetylsalicylic acid | 27.0 (18–41) | 8.0 (5.4–11.8) | 1.0 | 1.0 | 182 (175–188) |
| Indomethacin | 1.9 (1.2–4.0) | 2.6 (1.3–5.3) | 14 | 3.0 | 807 (759–857) |
| Flurbiprofen | 2.1 (1.0–4.3) | 3.1 (2.3–4.1) | 13 | 2.5 | 930 (902–959) |
| Ketorolac | 5.2 (3.2–8.3) | 3.0 (2.4–3.8) | 5.2 | 2.6 | 216 (209–223) |
| Zomepirac | 5.9 (3.9–8.9) | 5.5 (2.1–14) | 4.5 | 1.4 | 307 (234–400) |
| S(+)ibuprofen | 15.7 (6.7–36) | | 1.7 | | |
| Ibuprofen (racemic) | 18.9 (9.3–38) | | 1.4 | | |
| Acetaminophen | 257 (163–405) | 6.0 (0.8–44) | 0.1 | 1.3 | 23 (22–24) |
| R(−)ibuprofen | >270 | | >0.1 | | |

[a] The $ID_{50}$ value represents the total dose resulting in 50% inhibition of the formalin control response.

[b] Potency ratio showing the relative potency compared to the potency of ASA (*i.e.* indomethacin is 14 times more potent than ASA of inhibiting the formalin response after i.t. administration).

[c] Relative potency with 95% CI of dose (total doses)-response regression lines from i.t. *vs.* i.p. administration.

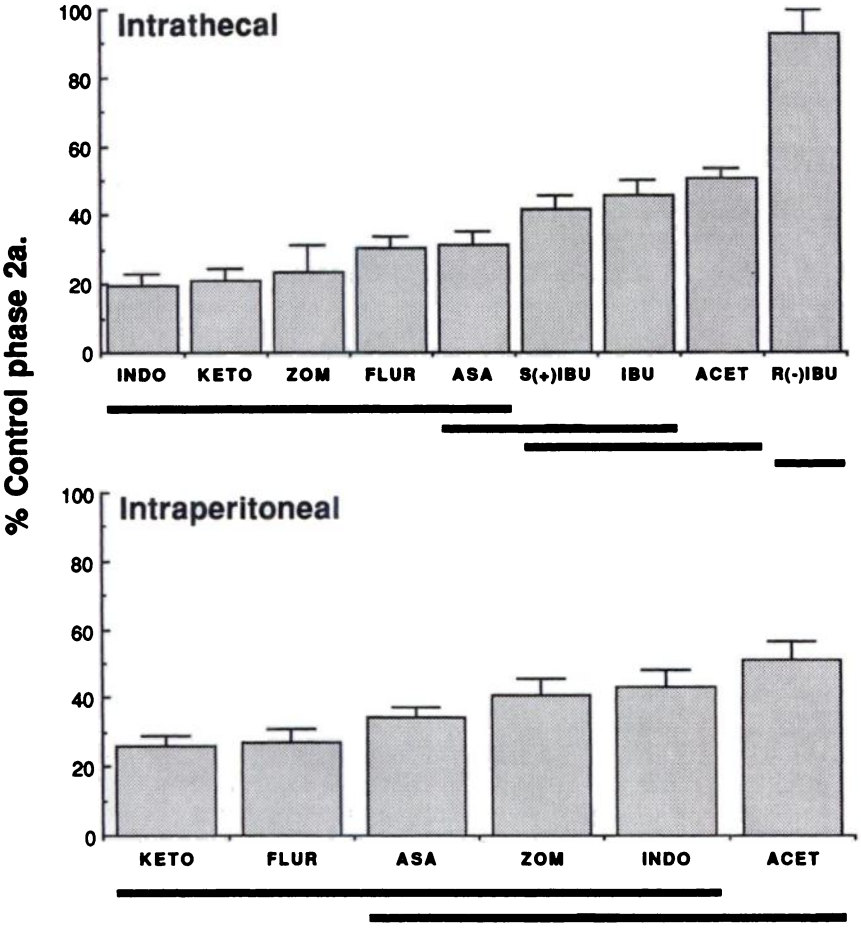


**Fig. 3.** Maximal inhibitory effect obtained by i.t. (top) and i.p. (bottom) injection, presented as percent of the phase 2a control response. The results of the statistical analysis are indicated by the black bars at the bottom. Drug effects not joined by a common or overlapping line are different ($P < .05$). Abbreviations: INDO, indomethacin; KETO, ketorolac; ZOM, zomepirac; FLUR, flurbiprofen; IBU, ibuprofen; ACET, acetaminophen. The bars present the mean and S.E.M. for 4 to 10 rats for the two highest doses that did not differ more than 10% in effect from the immediate previous dose (*i.e.*, plateau effect).

### Behavioral Effects of i.p. NSAID

The i.p. administration of ASA (2.7–83 μmol/kg), ketorolac (2.7–83 μmol/kg), zomepirac (2.7–83 μmol/kg), indomethacin (2.7–27 μmol/kg), flurbiprofen (2.7–46 μmol/kg) and acetaminophen (2.7–27 μmol/kg) had no detectable effect upon motor function or general behavior.

Intraperitoneal injection of these agents did, however, produce a significant suppression of the phase 2a formalin behavior and, to a lesser extent, the phase 1 response, in a manner similar to that described above for i.t. administration.

### Potency and Efficacy of i.p. NSAID

The i.p. administration of the NSAID resulted in a dose-dependent inhibition of the phase 2a formalin response, with the rank of order of potency ($ID_{50}$ in μmol, total dose) being: ketorolac (3.0) ≥ flurbiprofen (3.1) ≥ zomepirac (5.5) ≥ acetaminophen ≥ acetylsalicylic acid (8.0). Dose-response lines are presented in micromoles per kilogram (fig. 6), and the mean value (and S.E.M.) for the weight of the rats used was 309 ± 5 g. Note that the i.p. dose is in micromoles as compared to the i.t. dose in nanomoles.

TABLE 2
**Maximal Possible Effect (%MPE) of i.t. NSAID on the 52.5°C HP**

| Drug | Dose | %MPE ± S.E.M.* |
|---|---|---|
| | *nmol* | |
| ASA | 100 | 14.6 ± 3.0 |
| Ketorolac | 80 | 13.3 ± 2.5 |
| Flurbiprofen | 80 | 10.3 ± 2.1 |
| Indomethacin | 30 | 9.0 ± 5.6 |
| Zomepirac | 80 | 6.5 ± 4.2 |
| Morphine | 30 | 80.0 ± 10 |

* The results represent the mean and S.E.M. of five to eight animals.

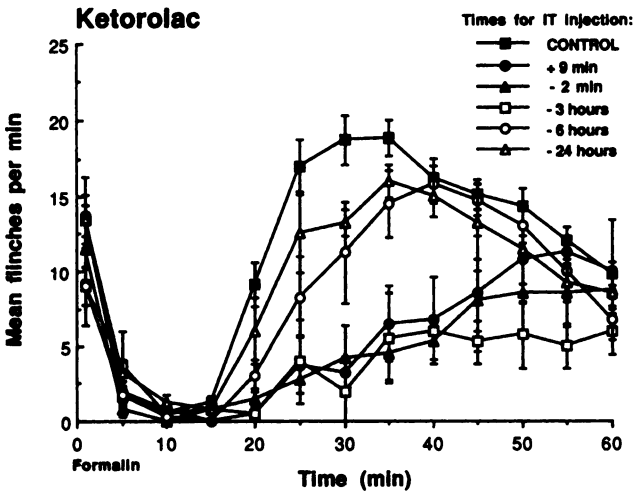


**Fig. 4.** Time effect curve with i.t. ketorolac (27 nmol) administered 2 min, 3, 6 and 24 hr before and 9 min after the formalin. Number of flinches per min is plotted *vs.* the time after the formalin injection into the dorsal surface of the right hindpaw. The line representing the control group includes 20 animals; otherwise, each line represents the mean and S.E.M. of 4 to 6 animals.

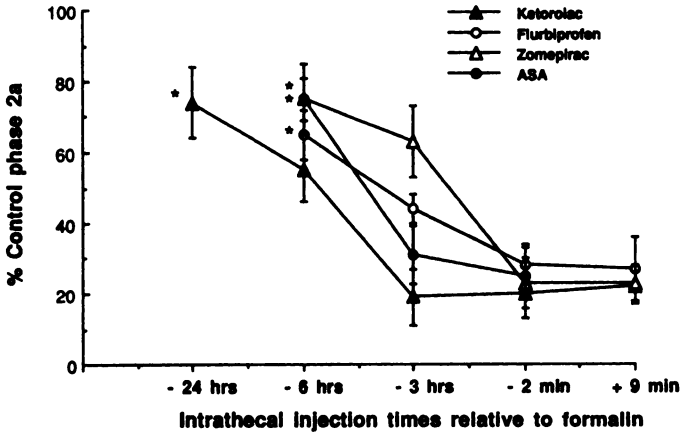


**Fig. 5.** Time course of the effect on the phase 2a formalin response produced by a just maximally effective i.t. dose of ketorolac (27 nmol), flurbiprofen (27 nmol), zomepirac (34 nmol) or ASA (100 nmol). Data are presented as the percent of the formalin control response of phase 2a. Drug injections were made 2 min, 3, 6 and 24 hr before and 9 min after formalin. *P < .05 as compared to the inhibition of the flinching response found with administration immediately before (2 min) formalin. Each point on the graph represents the mean and S.E.M. of four to six animals.

Similar to the i.t. administration paradigm, the major effect of the i.p. NSAID on the formalin response was found in phase 2a. Although the phase 1 was mildly attenuated by the highest doses used (see below), the effect on the first phase was not found to be dose dependent for any of the i.p. administered drugs.

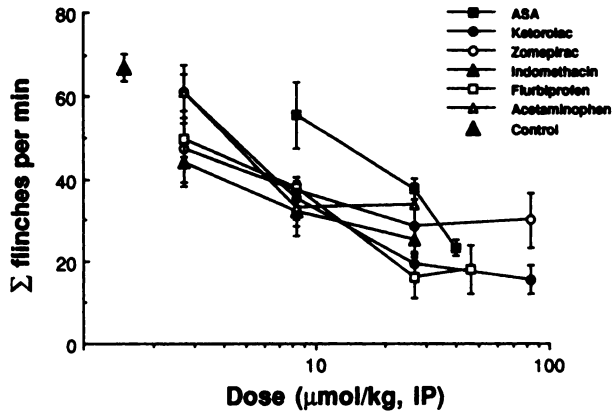


**Fig. 6.** Dose-response curves for i.p. NSAID: ASA, ketorolac, zomepirac, indomethacin, flurbiprofen and acetaminophen, presented as the cumulative number of formalin evoked flinches during phase 2a formalin response. Each dose on the graph represents the mean and S.E.M. of 4 to 6 rats except for the control group, which includes 20 animals.

Typically, there were no differences in the maximal inhibitory effect found between the highest doses of the tested NSAID in phase 2a, with the exception of acetaminophen, for which the maximum suppression was statistically less than that obtained with ketorolac and zomepirac (fig. 3). In phase 1, all agents displayed an inhibition of the flinch response as compared to control (table 2).

## Discussion

In the present study, we sought to define the antinociceptive potency and efficacy of spinally and systemically administered NSAID on the formalin test. As indicated, a variety of structurally diverse agents were examined and found to result in a reliable, dose-dependent, stereospecific suppression of specific phases of the formalin response after both spinal and systemic administration. The principle observations were: 1) agents were approximately 100 to 1000 times more potent after spinal than after systemic administration; 2) in spite of the significant difference in potency, all agents displayed surprisingly similar efficacy (*i.e.*, the same maximal degree of suppression), and the maximal suppression was similar whether the agent was administered systemically or i.t.; and 3) given i.t. at the highest doses, these agents had little effect on the escape response generated by the acute thermal stimulus. Jointly, these observations provide strong support that the structurally diverse class of molecules classified as NSAID can produce a powerful and direct effect upon spinal nociceptive processing and that this effect reflects upon a characteristic of the spinal processing induced by the ongoing afferent barrage generated by the irritant stimulus. Such observations are consistent with previous reports in which it was shown that i.t. injection of ASA or zomepirac would diminish the acetic acid evoked writhing response in rats at doses which were neither systemically active nor altered the response on the HP (Yaksh, 1982).

### Spinal NSAID Action: Cyclooxygenase Inhibition

In the present study, we found a considerable variation in the apparent potency of the NSAID. The relative i.t. potency in attenuating the phase 2 response was: indomethacin (14) > flurbiprofen (13) > ketorolac (5.2) > zomepirac (4.5) >

S(+)ibuprofen (1.7) > ibuprofen (1.4) > ASA (1.0) > acetaminophen (0.1) > R(−)ibuprofen (>0.1). As all drugs were administered i.t. and showed a rapid onset of action, it appears unlikely that the blood-brain barrier, significant acute metabolism or differential protein binding (given the low levels of protein in the cerebrospinal fluid) are variables contributing to this marked differential potency, as would occur after systemic administration.

In spite of their structural diversity, these molecules designated as NSAID share the property of cyclooxygenase inhibition. A positive correlation of their relative potency in blocking this enzyme with their i.t. antinociceptive potency would provide corollary support for the role of cyclooxygenase inhibition in mediating their observed spinal actions. Assessment of their relative potency as cyclooxygenase inhibitors in the commonly used *in vitro* assay of bovine seminal vesicles reveals the relative potency to be: flurbiprofen (200) > zomepirac (110) ≥ indomethacin (100) > S(+)ibuprofen (16) > ibuprofen (10) > ASA (1.0) > acetaminophen (0.3) > R(−)ibuprofen (0.02) (Adams *et al.*, 1975, 1976; Garcia-Rafanell and Forn, 1979; Pruss *et al.*, 1980; Shen, 1979). Comparable potencies have been reported for the brain. Thus, the endogenous biosynthesis of prostaglandins by brain tissue slices *in vitro* showed the relative inhibitory potency to be: indomethacin (400) > ASA (1) > acetaminophen (0.1) (Wolfe *et al.*, 1976). Additionally, *in vivo* suppression of prostaglandin biosynthesis, measured as prostaglandin $E_2$ and prostaglandin $F_{2\alpha}$, after oral administration showed the rank order of potency of inhibition in rat brain to be indomethacin ≥ flurbiprofen > ibuprofen > acetaminophen (Fitzpatrick and Wynalda, 1976); whereas the post mortem increase in prostaglandin $E_2$ synthesis in mouse brain *ex vivo* after inhibition of orally administered NSAID was demonstrated to be indomethacin (280) ≥ zomepirac (115) > ibuprofen (16) > ASA (1.0) ≥ acetaminophen (0.3) (Ferrari *et al.*, 1990). These orders of potency are comparable to the rank order of potency observed in the present studies. In addition, considering those cyclooxygenase inhibition studies where complete dose-response curves have been established, all agents examined show the same maximum suppression, though their relative potencies differed. Similarly, as noted above, examination of the ability of these agents *in vivo* to suppress the phase 2 behavior revealed that the active drugs resulted in a comparable, subtotal reduction in the formalin-evoked flinch behavior. These parallels, although not proving, are consistent with the hypothesis that cyclooxygenase inhibition is an intervening variable in the spinal actions of these agents in modifying phase 2 of the formalin response, and that failure of all of the agents to produce a complete suppression (*i.e.*, demonstrates a plateau effect) does not reflect upon an inability to produce a complete suppression of cyclooxygenase.

The stereospecificity of the effects observed in the present experiments provides further supportive evidence for the specificity of the observed spinal NSAID activity. Ibuprofen is a compound exhibiting optical isomerism. *In vitro* studies show that only the S(+)enantiomers is effective as a prostaglandin synthesis inhibitor (Adams *et al.*, 1976). *In vivo* after oral administration in rats and mice, stereoselectivity was not found because conversion of the inactive R(−)isomer to the active S(+) form can occur (Adams *et al.*, 1976). Importantly, in the present studies, the R-isomer was without effect, emphasizing that unlike the systemic route of administration, conversion did not occur, and suggesting both that the spinal cord does not contain significant quantities of the converting enzyme and that the spinally administered agent does not reach significant systemic concentrations where such conversion could most certainly occur. Acetaminophen appears to block the enzyme only in an environment that is low in peroxides (*e.g.*, hypothalamus), which might explain the poor anti-inflammatory capacity in inflamed tissue peripherally (Marshall *et al.*, 1987). Importantly, however, we found acetaminophen to be active in the formalin test, with significant intrinsic activity after spinal administration.

Though clearance studies have not been accomplished, the duration of action of the several spinally administered NSAID were on the order one would anticipate based on the kinetic clearance of the agents from the spinal space. Thus, agents which are known to produce an irreversible inhibition, such as indomethacin flurbiprofen and ASA (Roth *et al.*, 1975; Kantor and Hampton, 1978; Rome and Lands, 1975), did not show a particularly longer analgesic effect than agents such as ibuprofen, which produce a substrate-competitive, reversible inhibition of cyclooxygenase (Smith and Lands, 1971; Rome and Lands, 1975).

It is interesting to note that detailed reviews of the experimental literature have emphasized the dissociation of the cyclooxygenase-inhibiting activity of these agents and their antinociceptive actions (Brune *et al.*, 1991; Clisshold, 1986; McCormack and Brune, 1991). Comparison in the present studies of the magnitude of the relative potencies reveals a close rank ordering. It should be stressed, however, that the greatest observed dissociation between analgesic and cyclooxygenase activity is often noted in studies with systemically delivered agents, as cited in the reviews noted above. Even in the present studies, although there was a positive correlation between the i.t. *vs.* i.p. potencies, the relative differences in potency observed by each route were consistently greater after i.t. than after i.p. drug delivery. Given the probable spinal actions of these agents in this model and the absence of systematic data on brain levels of the several agents, relative potency estimates after systemic administration might be considered potentially misleading. Such differences in potency are frequently observed between systemic and i.t. or intracerebral dose drug potency (see Herz and Teschemacher, 1971, for example). Nevertheless, it should be emphasized that all of these agents may have a variety of effects which could influence neuronal function. Thus, indomethacin, in low concentrations, will directly inhibit phospholipase $A_2$ in rabbit polymorphonuclear leukocytes (Kaplan *et al.*, 1978). Indomethacin and ASA may also directly inhibit the cyclic AMP second messenger system by a mechanism not related to phospholipase or cyclooxygenase inhibition (Kantor and Hampton, 1978; Dinnendahl *et al.*, 1973).

### Functional Mechanisms Underlying Actions of Spinal NSAID

In spite of the limitation of the effect to the phase 2 of the formalin response with little or no effect upon the phase 1 or the HP test, we do not believe that these results reflect upon a peripheral anti-inflammatory action of the agents. First, doses which were in excess of 100 times those active spinally were required to produce the same effects after spinal injection. If the spinal effects were mediated by a peripheral redistribution, such differences in potency would not be anticipated. Moreover, as noted above, conversion of the inactive isomer of ibuprofen was not observed to occur, emphasizing that the agent did not reach significant systemic concentrations. Second, systematic

examination of the pathophysiology observed after intradermal formalin injection reveals increased paw volume and the appearance of inflammatory cells at intervals considerably later than 2 to 3 hr (Wheeler-Aceto *et al.*, 1990).

If the action of these NSAID correlates with their ability to attenuate cyclooxygenase activity, and this effect is not mediated by a peripheral action, then the explanation for this observation must include several points: 1) Prostaglandin synthesis within the spinal cord must be increased by some property of the particular stimulus state induced by intradermal formalin; and 2) spinal prostaglandins must act to induce a hyperesthetic state. Several issues pertinent to this hypothesis will be considered below.

**Formation of prostaglandins.** It is commonly appreciated that increasing the intracellular $Ca^{++}$ will result in the activation of phospholipase, leading to a subsequent increase in cytosolic arachidonic acid. In the presence of molecular oxygen, membrane-bound cyclooxygenase rapidly converts such free pools of arachidonate to subsequent elements in the prostaglandin cascade (see Leslie and Watkins, 1985). Membrane depolarization leading to an opening of voltage-sensitive $Ca^{++}$ channels (see Janis *et al.*, 1987), as well as the occupancy of a variety of receptor ionophores, such as for the N-methyl-D-aspartate receptor (MacDermott *et al.*, 1986), will lead to marked increases in cytosolic $Ca^{++}$. Although it appears probable that at least some of the release of prostanoids in brain may derive from neurons (Gonzales *et al.*, 1989), release of prostaglandins has been demonstrated in rat spinal cord astrocyte cultures in response to treatment with low (submicromolar) concentrations of substance P (Marriott *et al.*, 1990). Consistent with the effects of excitation on prostaglandin synthesis, increasing neuronal activity has been shown to increase the extracellular levels of prostanoids in brain ventricular superfusion *in vivo* (Romero *et al.*, 1984; Navarro *et al.*, 1988, 1989), and in spinal perfusates after high threshold afferent stimulation (Ramwell *et al.*, 1966), increased potassium (Yaksh, 1982) and noxious thermal stimulation (Coderre *et al.*, 1990a). In recent work, it was found in lumbar spinal dialysates in rats and primates that there was a significant increase in prostanoid release after protracted electrical activation of C fibers or in the presence of peripheral inflammation of the knee joint (L. S. Sorkin, unpublished observation). These characteristics would suggest that prostanoids might be optimally elaborated under conditions associated with ongoing activity in small primary afferents. Importantly, measurement of neuronal outflow from dorsal horn neurons excited by formalin injections into the respective paw reveals two components to the formalin effect: an initial burst lasting several minutes associated with the injection, followed by a second protracted phase (Dickenson and Sullivan, 1987).

As post-treatment with NSAIDs are able to diminish the second phase response, it appears likely that the processes of spinal prostaglandin generation, release and clearance occur at a high rate. The rapid turnover of the active prostanoids may be metabolic (*i.e.*, by conversion of the active form to an inactive product, such as occurs with prostaglandin $I_2$ being converted rapidly to 6-keto-prostaglandin $F_{1\alpha}$, or by bulk diffusion). Because of their lipid solubility, such extraparenchymal clearance will be very rapid. Thus, the estimated half-life of prostaglandin $F_{2\alpha}$, for example, after i.c.v. administration is on the order of 8 min (Hagen *et al.*, 1977).

**Effects of increased extracellular prostaglandins.** Extracellular prostanoids can significantly influence the excitability of neurons. Thus, by a receptor-mediated mechanism, prostaglandins of the E series will stimulate adenylate cyclase and activate protein kinase A *via* an increase in cyclic AMP (see Axelrod *et al.*, 1988). As will be discussed below, the local application of lipidic acids can induce an augmented release of afferent neurotransmitters (Geppetti *et al.*, 1991; Nicol *et al.*, 1992). The behavioral relevance of these membrane events is emphasized by the observation that the i.t. injection of a variety of cyclooxygenase products, such as prostaglandin $E_2$, prostaglandin $F_{2\alpha}$ or prostaglandin $D_2$ results in a dose-dependent hyperalgesia (Ferreira *et al.*, 1978; Ferreira, 1983; Taiwo and Levine, 1986; Uda *et al.*, 1990; Yaksh, 1982). Furthermore, the oral administration of competitive antagonists of prostaglandin E have been reported to be antinociceptive in the formalin test and in the acetic acid-induced writhing test (Drower *et al.*, 1987).

**Mechanisms of facilitatory actions of prostaglandins.** As emphasized above, afferent input can evoke an increase in the extracellular levels of the several prostanoids, and it is apparent that these lipidic acids can exert a direct effect upon neuronal function. The mechanism underlying this augmentation are not understood, but three alternatives appear likely.

First, prostanoids may directly augment, by a receptor-mediated effect, the release of excitatory neurotransmitter. Vasko and colleagues have shown that prostaglandin $E_2$ will augment a voltage-sensitive $Ca^{++}$ current in dorsal root ganglion cell cultures and facilitate the depolarization-evoked release of the C afferent neurotransmitter substance P (Nicol *et al.*, 1992). Arachidonic acid has been shown to evoke calcitonin gene-related peptide (CGRP) release from capsaicin-sensitive primary afferents, and the release was inhibited in the presence of indomethacin (Geppetti *et al.*, 1991). Similarly, infusion of prostaglandin $I_2$ and prostaglandin $E_2$ released CGRP in a dose-dependent manner, suggesting that a prostanoid can mediate sensory neuropeptide release.

Second, prostanoids may serve to diminish an inhibition. Prostaglandins of the E series act presynaptically to inhibit noradrenalin release (Bergström *et al.*, 1973; Hedqvist, 1973). The hyperalgesic effect of i.t. prostaglandin $E_2$ is blocked by i.t. pretreatment of the *alpha* adrenergic antagonist phentolamine or the catecholaminergic neurotoxin 6-hydroxydopamine (Taiwo and Levine, 1988), suggesting that afferent-evoked release of prostanoids may inhibit a reflex-evoked bulbospinal inhibition.

Third, in addition to the evidence for prostaglandin release into the extracellular space to act as neurohormones, these agents play an intracellular role in signal transduction (Axelrod *et al.*, 1988). Prostaglandins may directly (*i.e.*, not through a receptor) mediate GTPase activation not associated with adenylate cyclase activation (Bitonti *et al.*, 1980). Prostaglandin $E_2$, leukotrienes and lipoxine A have been demonstrated to increase cytosolic $Ca^{++}$ independent of inositol phosphate$_3$ formation (Wolf *et al.*, 1986; Halushka and Burch, 1984). For the metabolites produced by the lipoxygenase pathway, it has recently been shown that they can affect the activity of membrane ion channels and protein kinases and are possibly involved in neurotransmitter release (Piomelli and Greengard, 1990). The arachidonic acid metabolites from the 5-lipoxygenase pathway may participate in the modulation of $K^+$ channel activity and thus be involved in hyperpolarization of neurones (Piomelli *et al.*, 1987). The membrane potential could be prevented by

phopholipase $A_2$ inhibitors and with lipoxygenase inhibitors, but not with the cyclooxygenase inhibitor indomethacin (Piomelli *et al.*, 1987). Given the cyclooxygenase selectivity of the agents used in the present study, the role of the central changes on nociception mediated by neuronal lipoxygenase products remains to be assessed.

As outlined above, the current body of data suggests that under conditions of a modestly protracted period of stimulation of small afferents, there will be an increase in the formation of prostaglandins. Extracellular movement of these lipidic acids would then, by specific receptor-mediated mechanisms, act to augment the excitability of the cells to the ongoing afferent input by facilitating the release of neurotransmitter, perhaps from the primary afferent, by a direct postsynaptic action or by intracellular mechanisms wherein changes in ion channel permeability lead to reduced transmembrane voltage stability. Based on these conditions, cyclooxygenase inhibitors would serve to alter the behavioral consequences of a noxious stimulus only under those stimulus conditions which bring the above characteristics into play. The plateau in the antinociceptive effects observed after spinal NSAID administration likely reflects upon the fact that the prostanoids simply serve to upregulate the excitability of the membrane and not absolutely control its sensitivity to the afferent barrage.

### Pharmacology of the Formalin Test

Formalin injected s.c. results in an immediate and intense increase in the spontaneous activity of C fiber afferents, evoking a fast transmitter monosynaptic input to projection neurons in the spinal cord, followed by a second-phased afferent barrage (Heapy *et al.*, 1987). This biphasic excitatory input is mirrored in the activation of wide dynamic range dorsal horn neurons (Dickenson and Sullivan, 1987). Protracted (sec to min) C fiber (but not A fiber) stimulation, aside from its direct excitatory effect, evokes a central facilitated state in which the wide dynamic range cell shows an exaggerated discharge to a given C fiber input ("wind-up"; Mendell, 1966). Of particular significance, i.t. injections of opiates are able to suppress the augmented response only at doses which block the initial C fiber burst. This observation corresponds to the ability of opiates to block the release of primary afferent peptides (see Sabbe and Yaksh, 1990). In contrast, antagonists for the N-methyl-D-aspartate receptor fail to block the initial evoked activity, but prevent the development of the second facilitated state (*i.e.*, wind-up) (Dickenson and Sullivan, 1990). These spinal responses appear to parallel the biphasic behavior observed after similar injections of formalin in the unanesthetized rat. Intrathecal opioids administered before phase 1 can completely suppress in a dose-dependent fashion the first and second phase response. Spinal N-methyl-D-aspartate antagonists at low doses have minimal effect upon phase 1 and produce only a 70 to 80% reduction in the phase 2 response. Interestingly, the administration of opioids after the phase 1 but before the phase 2 response readily blocks the phase 2 response, but such post-treatment with N-methyl-D-aspartate antagonists is without effect (Yamamoto and Yaksh, 1992; T. J. Coderre, personal communication). Identical results have been observed with i.t. antagonists for the neurokinin-1 tachykinin receptor (Yamamoto and Yaksh, 1991). Importantly, in the present study, we demonstrated that NSAID were equally effective in suppressing the second phase after both pre- and post-treatment relative to formalin injection. These studies may be interpreted as supporting the hypothesis that afferent C fiber barrages generated by the formalin stimulus, leading to a spinal release of glutamate (Skilling *et al.*, 1988) and substance P (Kuraishi *et al.*, 1989), among other mediators, may give rise to an increase in intracellular $Ca^{++}$, an increase in cytosolic arachidonate and the subsequent generation of diffusable prostanoids. These prostanoids may then evoke a facilitated excitability of the local neuronal population by mechanisms discussed above. Interestingly, i.t. substance P has been shown to produce mild agitation behavior and a subsequent hyperalgesia (Moochhala and Sawynok, 1984; Yasphal *et al.*, 1982). Hunskaar *et al.* (1985) reported that the agitation behavior of i.t. substance P can be attenuated by systemic ASA and acetaminophen. Such observations provide grounds for considering that at least some of the events brought into play by the local activation of receptors postsynaptic to the primary afferent may involve the generation of a cyclooxygenase-mediated central facilitation. In recent studies, we have shown that the i.t. injection of NSAID in doses which block the phase 2 of the formalin test will also block the thermal hyperesthesia occurring secondary to i.t. N-methyl-D-aspartate and substance P. This effect is stereospecific and achieved by doses which are identical to those which diminish the phase 2 of the formalin test (Malmberg and Yaksh, 1992).

Although it appears certain that the ongoing state of facilitation may be initiated by the stimulatory effects of substance P and glutamate, the continued activity of the respective N-methyl-D-aspartate and neurokinin-1 receptors is not necessary for the maintenance of the spinal facilitated state, as indicated by the failure of post-treatment to effectively blunt the second phase response. We believe it likely that the initial stimulation, however, yields an increase in cytosolic arachidonic acid and the initiation of an ongoing and prolonged generation of cyclooxygenase intermediaries which serve to exert a facilitatory effect upon the excitability within the dorsal horn. It is interesting to note the similarities between synaptic plasticity changes of long-term potentiation in the hippocampus after an afferent barrage (Collingridge and Bliss, 1987), where arachidonic acid and nitric oxide are proposed to serve as intermediary mechanisms induced by the activation of the N-methyl-D-aspartate receptor (Williams *et al.*, 1989; Garthwaite *et al.*, 1988), and spinal nociceptive transmission. N-methyl-D-aspartate activation results in a $Ca^{++}$-dependent increase in cyclic GMP through the production of nitric oxide (Garthwaite *et al.*, 1988). In recent studies, we have found that competitive inhibitors of nitric oxide are able to totally suppress the second phase of the formalin test and the hyperesthesia induced by i.t. N-methyl-D-aspartate in a dose-dependent manner (Malmberg and Yaksh, in preparation).

### Clinical Significance

The developing scenario outlined above offers insights into the probable organization of the pain state which develops in the immediate period after the generation of a C fiber barrage. It has now been demonstrated in behavioral studies of the formalin response that the second late phase depends upon the generation of an initial afferent barrage. Coderre *et al.* (1990b) have shown that local anesthetics given before and shortly after the formalin will prevent the facilitation, reflecting a mechanism perhaps mediated by the acute activation of the N-methyl-D-aspartate and neurokinin-1 receptors. Such observations emphasize the growing appreciation in anesthesiology that aggressive preincision pain control may significantly reduce the post-

operative analgesic requirements (McQuay *et al.*, 1988). Similarly, the observation that cyclooxygenase inhibitors may serve to markedly diminish the second phase component of the pain response appears to reflect upon the growing appreciation of the relative potency of NSAID in managing at least certain types of postoperative pain. Thus, concurrent administration of certain NSAID clearly reduces the amount of opiate required for the complete relief of the postsurgical pain state (for example, Reasbeck *et al.*, 1982; Gillies *et al.*, 1987). Importantly, in recent studies, we have observed, using isobolographic protocols, that concurrent spinal administration of morphine and NSAID in rats will indeed yield a remarkable synergy (Malmberg and Yaksh, unpublished observations). The demonstration that a powerful analgesia can be obtained in humans in the absence of changes in inflammatory signs and that the clinical analgesic potency of NSAID do not covary with their anti-inflammatory effects (McCormack and Brune, 1991) is in accord with the hypothesis that there are important NSAID-sensitive processes relevant to pain processing that are not related to changes in peripheral inflammatory processes. Indeed, limited studies in humans have suggested that, as in the present work, the spinal delivery of ASA may also have analgesic effects (Devoghel, 1983). Further studies emphasizing toxicity and kinetics appear warranted.

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