# **Antinociceptive Actions of Spinal Nonsteroidal Anti-Inflammatory Agent Source Copyright C 1992 by The American Society for Pharmacology and Experimental Therapeutics**<br>
Copyright C 1992 by The American Society for Pharmacology and Experimental Therapeutics<br> **Antinociceptive Actions of S**

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## **ABSTRACT**

**And Experimental Therapeutics** 

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The Journal of

Subcutaneous injection of formalin into the dorsal surface of the i.t. injection of formalin into the dorsal surface of the i.t. injection of formalin into the dorsal surface of the i.t. injection were detailed pay a simil ABSTRACT<br>Subcutaneous injection of formalin into the dorsal surface of the<br>hindpaw evoked a two-phased flinching (phase 1: 0–9 min; phase<br>2: 10–60 min) of the injected paw. Intrathecal administration of<br>the nonsteroidal an ABSTRACT<br>Subcutaneous injection of formalin into the dorsal surface of the<br>hindpaw evoked a two-phased flinching (phase 1: 0-9 min; phase sim<br>2: 10-60 min) of the injected paw. Intrathecal administration of sic<br>the nonster hindpaw evoked a two-phased flinching (phase 1: 0–9 min; phase similar distinct plateaus of maximum achievable inhibition (intrin-<br>2: 10–60 min) of the injected paw. Intrathecal administration of sic activity) of the phas the nonsteroidal anti-inflammatory drugs (NSAID) produced minthe nonsteroidal anti-initial<br>imal effects upon phase 1, but showed a significant, though<br>submaximal, dose-dependent suppression of the phase 2 re-<br>sponse. Ordering of i.t. potency was (ID<sub>so</sub> in nmol): indomethacin<br>f(1.9 action and the city of the phase  $\cdot$ , but showed a significant, thought<br>submaximal, dose-dependent suppression of the phase 2 re-<br>sponse. Ordering of i.t. potency was (ID<sub>so</sub> in nmol): indomethacin<br>(1.9)  $\geq$  flurbiprof submaximal, cose-dependent suppression of the phase 2 re-<br>sponse. Ordering of i.t. potency was (ID<sub>so</sub> in nmol): indomethacin Pret<br>(1.9)  $\geq$  flurbiprofen (2.1)  $>$  ketorolac (5.2)  $\geq$  zomepirac (5.9)  $>$  the<br>S(+)ibupr  $S(+)$ ibuprofen (16)  $\geq$  ibuprofen(racemic) (19)  $>$  acetylsalicylic injection. The i.t. injection of the highest doses of the several acid (27)  $>$  acetaminophen (250)  $>$   $R(+)$ ibuprofen ( $>270$ ) = 0. NSAID were without

i.t. injection. Intrathecal and i.p. dose-response curves showed similar distinct plateaus of maximum achievable inhibition (intrini.t. injection. Intrathecal and i.p. dose-response curves showed<br>similar distinct plateaus of maximum achievable inhibition (intrin-<br>sic activity) of the phase 2 behavior, ranging from 20 to 50% of<br>the control response. Va i.t. injection. Intrathecal and i.p. dose-response curves showed<br>similar distinct plateaus of maximum achievable inhibition (intrin-<br>sic activity) of the phase 2 behavior, ranging from 20 to 50% of<br>the control response. Va i.t. injection. Intrathecal and i.p. dose-response curves showed<br>similar distinct plateaus of maximum achievable inhibition (intrin-<br>sic activity) of the phase 2 behavior, ranging from 20 to 50% of<br>the control response. Va similar district plateaus of maximum achievable infibutor (intri-<br>sic activity) of the phase 2 behavior, ranging from 20 to 50% of<br>the control response. Varying the time of drug injection reveals<br>that injection 9 min after sic activity) of the phase z behavior, ranging from 20 to 50% of<br>the control response. Varying the time of drug injection reveals<br>that injection 9 min after formalin yielded effects the same as<br>those observed when the agen the control response. Varying the time of drug injection reveals<br>that injection 9 min after formalin yielded effects the same as<br>those observed when the agent was given 2 min before formalin.<br>Pretreatment at longer interva that injection 9 min after formalin yielded effects the same as<br>those observed when the agent was given 2 min before formalin.<br>Pretreatment at longer intervals indicated that the duration of<br>the antinociceptive effect was those observed when the agent was given 2 min before formally.<br>Pretreatment at longer intervals indicated that the duration of<br>the antinociceptive effect was between 3 to 6 hr after the i.t.<br>injection. The i.t. injection o Pretreatment at longer intervals indicated that the duration of<br>the antinociceptive effect was between 3 to 6 hr after the i.t.<br>injection. The i.t. injection of the highest doses of the several<br>NSAID were without significa

It has been widely postulated that the analgesic actions of hibit the hyperalgesic state evoked by the sensitization of the<br>NSAID result from the inhibition of the peripheral synthesis peripheral afferent. Under such condi It has been widely postulated that the analgesic actions of<br>NSAID result from the inhibition of the peripheral synthesis<br>of prostaglandins formed secondary to tissue injury and inflam-It has been widely postulated that the analgesic actions<br>NSAID result from the inhibition of the peripheral synthe<br>of prostaglandins formed secondary to tissue injury and infla<br>mation (Guzman *et al.*, 1964; Lim *et al.*, 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* It has been widely postulated that the analgesic actions of h<br>NSAID result from the inhibition of the peripheral synthesis pof<br>prostaglandins formed secondary to tissue injury and inflam-<br>mation (Guzman *et al.*, 1964; Lim of prostaglandins formed secondary to tissue injury and inflam-<br>mation (Guzman *et al.*, 1964; Lim *et al.*, 1964; Lim, 1970;<br>Ferreira *et al.*, 1978). This hypothesis is supported by the<br>observation that 1) the NSAID, th mation (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 ab mation (Guzman *et al.*, 1964; Lim *et al.*, 1964; Lim, 1970; 1:<br>Ferreira *et al.*, 1978). This hypothesis is supported by the<br>observation that 1) the NSAID, though structurally a diverse of<br>group, share at least in part 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 a observation that 1) the NSAID, though structurally a dive<br>group, share at least in part the ability to inhibit cyclooxygen.<br>(Vane, 1971); 2) prostanoids applied to the peripheral net<br>terminal can facilitate its discharge ( group, share at least in part the ability to inhibit cyclooxygenase<br>(Vane, 1971); 2) prostanoids applied to the peripheral nerve<br>terminal can facilitate its discharge (Schaible and Schmidt,<br>1988); and 3) methodologically, (Vane, 1971); 2) prostanoids applied to the peripheral nerve<br>terminal can facilitate its discharge (Schaible and Schmidt,<br>1988); and 3) methodologically, demonstration of the antino-<br>ciceptive effects of NSAID in experime terminal can facilitate its discharge (Schaible and Schmidt, 1988); and 3) methodologically, demonstration of the antino-<br>ciceptive effects of NSAID in experimental behavioral models de<br>typically depends upon the presence 1988); and 3) methodologically, demonstration of the antino-<br>ciceptive effects of NSAID in experimental behavioral models<br>typically depends upon the presence of an inflammatory state<br>and hyperalgesia. Thus, after inducing typically depends upon the presence of an inflammatory state<br>and hyperalgesia. Thus, after inducing inflammation in the<br>knee joint (Moncada *et al.*, 1975, 1979), the skin (Ferreira,<br>1972) or by the injection of agents suc 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.*, 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 1972) or by the injection of agents such as bradykinin, wh<br>release prostanoids (Guzman *et al.*, 1964; Lim *et al.*, 1964), if<br>associated algogenic behavior can be significantly diminis<br>by aspirin-like drugs. In this mann 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 inhibi ciated that NSAID, by inhibiting the biosynthesis of prosta-<br>glandins, do not, strictly speaking, produce analgesia, but in-

hibit the hyperalgesic state evoked by the sensitization of the peripheral afferent. Under such conditions, these agents would hibit the hyperalgesic state evoked by the sensitization of the<br>peripheral afferent. Under such conditions, these agents would<br>only be effective when prostaglandins are released (Ferreira, hibit 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). hibit the hyperalgesic state operipheral afferent. Under so<br>only be effective when pros<br>1972; Ferreira *et al.*, 1973).<br>In spite of this emphasis o bit the hyperalgesic state evoked by the sensitization of the ripheral afferent. Under such conditions, these agents would ly be effective when prostaglandins are released (Ferreira, 72; Ferreira *et al.*, 1973). In spite

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Work supported in part by unrestricted funds from Bristol Myers and<br>
NS16541 (TLY)<br> **ABBREVIATIONS:** NSAID, nonsteroidal anti-inflammatory drugs; ASA, acetylsalicylic acid; HP, ciceptive effects of NSAID in experimental behavioral models<br>typically depends upon the presence of an inflammatory state<br>and hyperalgesia. Thus, after inducing inflammation in the rate (Attal *et al.*, 1988; Carlsson *et* IF IN THE INSAID, by inhibiting the biosynthesis of prosta-<br>Injection of irritant. Given that these effects were observed<br>Indins, do not, strictly speaking, produce analgesia, but in-<br>Injection of irritant. Given that thes peripheral afferent. Under such conditions, these agents would<br>only be effective when prostaglandins are released (Ferreira,<br>1972; Ferreira *et al.*, 1973).<br>In spite of this emphasis on a peripheral action, several lines<br>o only be effective when prostaglandins are released (Ferreira, 1972; Ferreira *et al.*, 1973).<br>In spite of this emphasis on a peripheral action, several lines of evidence have pointed to a potential central action of these 1972; Ferreira *et al.*, 1973).<br>In spite of this emphasis on a peripheral action, several lines<br>of evidence have pointed to a potential central action of these<br>families of agents. Intracerebroventricular administration of<br> In spite of this emphasis on a peripheral action, several mess<br>of evidence have pointed to a potential central action of these<br>families of agents. Intracerebroventricular administration of<br>NSAID have been shown to inhibit dependently depress. Intracerebrowentricular administration of<br>NSAID have been shown to inhibit the carrageenin evoked<br>hyperalgesia in the rat paw (Ferreira, 1983). More recently,<br>systemically administered NSAID have been NSAID have been shown to inhibit the carrageenin evoked<br>hyperalgesia in the rat paw (Ferreira, 1983). More recently,<br>systemically administered NSAID have been found to dose-<br>dependently depress the activity evoked by noxi hyperalgesia in the rat paw (Ferreira, 1983). More recently, systemically administered NSAID have been found to dose-<br>dependently depress the activity evoked by noxious stimuli in<br>single neurons of the rat thalamus in both systemically administered NSAID have been found to dose-<br>dependently depress the activity evoked by noxious stimuli in<br>single neurons of the rat thalamus in both normal and arthritic<br>rats (Attal *et al.*, 1988; Carlsson *e* dependently depress the activity evoked by noxious stimuli single neurons of the rat thalamus in both normal and arthricatis (Attal *et al.*, 1988; Carlsson *et al.*, 1988; Groppetti *et* (1988; Jurna and Brune, 1990). Alt single neurons of the rat thalamus in both normal and arthritic<br>rats (Attal *et al.*, 1988; Carlsson *et al.*, 1988; Groppetti *et al.*,<br>1988; Jurna and Brune, 1990). Alternatively, indication for a<br>spinal antinociceptive rats (Attal *et al.*, 1988; Carlsson *et al.*, 1988; Groppetti *et al.*, 1989; Jurna and Brune, 1990). Alternatively, indication for a spinal antinociceptive effect of prostaglandin synthesis inhibitors derives from studie 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, 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 e tors derives from studies with these agents administered in lumbar intrathecal space (Yaksh, 1982). In this work, NSAID diminished the pain behavior evoked in rats by injection of irritant. Given that these effects were ob lumbar intrathecal space (Yaksh, 1982). In this work, i.t.<br>NSAID diminished the pain behavior evoked in rats by i.p.<br>injection of irritant. Given that these effects were observed<br>spinally at doses which were not effective NSAID diminished the pain behavior evoked in rats by i.p.<br>injection of irritant. Given that these effects were observed<br>spinally at doses which were not effective when given systemi-<br>cally, it was argued that spinally rele injection of irritant. Given that these effects were observed<br>spinally at doses which were not effective when given systemi-<br>cally, it was argued that spinally released prostaglandins are<br>important for the transmission of 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 nocice

1992<br>of the peripheral terminals), but by a spinal action as well<br>(Yaksh, 1982). Consistent with this hypothesis, it has been (1992)<br>
(1992) of the peripheral terminals), but by a spinal action as well tive<br>
(Yaksh, 1982). Consistent with this hypothesis, it has been production that: 1) sensory afferent input can elevate the extravas-1992<br>of the peripheral terminals), but by a spinal action as well<br>(Yaksh, 1982). Consistent with this hypothesis, it has been<br>shown that: 1) sensory afferent input can elevate the extravas-<br>cular, extracellular levels of 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 prostano 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 prostano (Yaksh, 1982). Consistent with this hypothesis, it has been<br>shown that: 1) sensory afferent input can elevate the extravas-<br>cular, extracellular levels of prostanoids (Ramwell *et al.*, 1966;<br>Coderre *et al.*, 1990a; L. S shown that: 1) sensory afferent input can elevate the extravas-<br>cular, extracellular levels of prostanoids (Ramwell *et al.*, 1966;<br>Coderre *et al.*, 1990a; L. S. Sorkin and T. L. Yaksh, unpublished<br>observation); and 2) ce cular, extracellular levels of prostanoids (Ramwell *et al.*, 1966)<br>Coderre *et al.*, 1990a; L. S. Sorkin and T. L. Yaksh, unpublishe<br>observation); and 2) certain prostanoids can facilitate the firin<br>of central neurons (Co Coderre *et al.*, 1990a; L. S. Sorkin and T. L. Yaksh, unpublished<br>observation); and 2) certain prostanoids can facilitate the firing<br>of central neurons (Coceani and Viti, 1975) and 3) prostanoids<br>can augment the release observation); and 2) certain prostanoids can facilitate the firing<br>of central neurons (Coceani and Viti, 1975) and 3) prostanoids<br>can augment the release of neurotransmitters (Chiu and Rich-<br>ardson, 1985; Bloomquist and Kr of central neurons ((can augment the relation, 1985; Bloom<br>ardson, 1985; Bloom<br>spinal primary sens<br>(Nicol *et al.*, 1992).<br>In spite of the po In augment the release of neurotransmitters (Chiu and Richdson, 1985; Bloomquist and Kream, 1987), notably from winal primary sensory afferents by augmenting  $Ca^{++}$  influx.<br>  $\frac{1}{3}$  incol *et al.*, 1992).<br>
In spite of t

spinal primary sensory afferents by augmenting  $Ca^{++}$  influx.<br>(Nicol *et al.*, 1992).<br>In spite of the potential mechanisms underlying a possible<br>spinal action of NSAID, the earlier literature on the antinoci-<br>ceptive acti (Nicol *et al.*, 1992).<br>In spite of the potential mechanisms underlying a possible<br>spinal action of NSAID, the earlier literature on the antinoci-<br>ceptive actions only focused on a few NSAID. The possibility<br>of mechanisms 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 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 inhibiti depive actions only notised on a few NSAID. The possibility of mechanisms of action unique to these compounds and u<br>related to prostaglandin synthesis inhibition could not be e<br>cluded. We sought in the present experiments of mechanisms of action unique to these compounds and un-<br>related to prostaglandin synthesis inhibition could not be ex-<br>cluded. We sought in the present experiments to characterize<br>further the spinal pharmacology of these related to prostagiantly synthesis inhibition collid not be ex-<br>cluded. We sought in the present experiments to characterize<br>further the spinal pharmacology of these NSAID using repre-<br>sentative compounds from several clas further the spinal pharmacology of these NSAID using representative compounds from several classes and examining the *H* stereospecificity of the effect. We have used the rat paw formalin test. The injection of formalin u sentative compounds from several classes and examining the Reference oppecificity of the effect. We have used the rat paw for-<br>malin test. The injection of formalin under the skin on the tidorsal surface of the hindpaw ca malin 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 distin dorsal surface of the hindpaw causes an immediate and intense dorsal surface of the hindpaw causes an immediate and intense<br>increase in the spontaneous activity of C fiber afferents (Heapy<br>et al., 1987) and evokes a distinct quantifiable behavior indic-<br>ative of pain: flinching/shaki biphasic, with an initial acute phase (phase 1: 0-10 min), blink and pinnae reflexes. The animal was then quickly removed, and et al., 1987) and evokes a distinct quantifiable behavior indic-<br>ative of pain: flinching/shaking and licking/biting of the in-<br>jected paw (Dubuisson and Dennis, 1977; Wheeler-Aceto et al.,<br>1990). The behavioral response t ative of pain: flinching/shaking and licking/biting of the injected paw (Dubuisson and Dennis, 1977; Wheeler-Aceto *et al.*,  $(3 \text{ H})$ <br>1990). The behavioral response to subcutaneous formalin is  $(3 \text{ h})$ <br>biphasic, with an jected 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), bifollowed by a quiescent period and th 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: 10-6 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 dir followed by a quiescent period and then a prolonged tresponse between 20 and 60 min (phase 2: 10–60 min).<br>suggested that the early phase is due to a direct effectional<br>in on nociceptors, whereas the late phase involvee<br>fla response between 20 and 60 min (phase 2: 10–60 min). It suggested that the early phase is due to a direct effect formalin on nociceptors, whereas the late phase involves is flammatory components with release of different p suggested that the early phase is due to a direct effect of us<br>formalin on nociceptors, whereas the late phase involves in-<br>flammatory components with release of different pain-media-<br>ing substances that possibly can activ flammatory components with release of different pain-media-<br>
intervalse with spontaneous activity and normal motor function. A<br>
ting substances that possibly can activate small afferents (Hun-<br>
skaar and Hole, 1987). Addi ting substances that possibly can activate small afferents (Hunting substances that possibly can activate small afferents (Hunskaar and Hole, 1987). Additionally, compounds which have what shown minimal antinociceptive effect in acute pain models  $(e.g., HP \text{ test})$  like N-methyl-D-aspartate skaar and Hole, 1987). Additionally, compounds which has hown minimal antinociceptive effect in acute pain mod (*e.g.*, HP test) like N-methyl-D-aspartate or neurokinin-1 atagonists have been reported to be active in atten shown minimal antinociceptive<br>(*e.g.*, HP test) like N-methyl-D<br>tagonists have been reported t<br>second phase of the formalin te<br>moto and Yaksh, 1991, 1992). moto and Yaksh, 1991, 1992).<br>**Me**<br>**Animal Preparation** 

## **Methods**

Male Sprague-Dawley rats (280-320 g; Harlan Industries, Indian-<br>apolis, IN) were housed in group cages and maintained on a 12-hr **Animal Preparation**<br> **Animal Preparation**<br>
Male Sprague-Dawley rats (280–320 g; Harlan Industries, Indian-<br>
apolis, IN) were housed in group cages and maintained on a 12-hr<br>
light/12-hr dark cycle. Animals had free access **Animal Preparation**<br>Male Sprague-Dawley rats (280–320 g; Harlan Industries, Indian-<br>apolis, IN) were housed in group cages and maintained on a 12-hr<br>light/12-hr dark cycle. Animals had free access to food and water at all form Male Sprague-Dawley rats (280–320 g; Harlan Industries, Indian-<br>apolis, IN) were housed in group cages and maintained on a 12-hr<br>light/12-hr dark cycle. Animals had free access to food and water at all<br>times. For the light/12-hr dark cycle. Animals had free access to food and water at all permethod under halothane anesthesia according to modification of the armethod described by Yaksh and Rudy (1976). Briefly, through an incision in th planted under halothane anesthesia according to modification of the and method described by Yaksh and Rudy (1976). Briefly, through an wincision in the atlanto-occipital membrane, a polyethylene (PE-10) (Exhibited rats adv planted under halothane anesthesia according to modification of the and phase 2b (40–60 min). After the observation period of 1 hr, animals method described by Yaksh and Rudy (1976). Briefly, through an were immediately sa lumbar enlargement. After implantation of i.t. catheters, rats were<br>housed in individual stainless steel cages. Intrathecal injection studies<br>were carried out 7 to 10 days after surgery. Only animals with normal<br>motor func 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 housed in individual stainless steel cages. Intrathecal injection studies<br>were carried out 7 to 10 days after surgery. Only animals with normal<br>motor function were used. Similarly, rats used for i.p. studies were by<br>housed 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.<br>Experiments were ca motor function were used. Simila<br>housed in pairs 7 to 10 days b<br>Experiments were carried out acconsitents<br>Institutional Animal Care Commi<br>San Diego.<br>**Drugs and Injections**<br>The drugs used in the study we

stitutional Animal Care Committee of the University of California,<br>
n Diego.<br> **ugs and Injections**<br>
The drugs used in the study were chosen on the basis of belonging<br>
several chemical groups (salicylate: ASA, *para*-aminop San Diego.<br>**Drugs and Injections**<br>The drugs used in the study were chosen on the basis of belongi<br>to several chemical groups (salicylate: ASA, *para*-aminophenol deriv

Spinal Action of NSAIDs<br>tive: acetaminophen, acetic acid: indomethacin, propionic acids: ibu-<br>profen and flurbiprofen, and pyrrolo pyrrole: ketorolac), reference **Spinal Action of NSAIDs**<br>
tive: acetaminophen, acetic acid: indomethacin, propionic acids: ibu-<br>
profen and flurbiprofen, and pyrrolo pyrrole: ketorolac), reference<br>
compounds (ASA, indomethacin), commonly used drugs (ASA tive: acetaminophen, acetic acid: indomethacin, propionic acids: ibu-<br>profen and flurbiprofen, and pyrrolo pyrrole: ketorolac), reference<br>compounds (ASA, indomethacin), commonly used drugs (ASA, ibupro-<br>fen, acetaminophen) tive: acetaminophen, acetic acid: indomethacin, propionic acids: ibu-<br>profen and flurbiprofen, and pyrrolo pyrrole: ketorolac), reference<br>compounds (ASA, indomethacin), commonly used drugs (ASA, ibupro-<br>fen, acetaminophen) profen and flurbiprofen, and pyrrolo pyrrole: ketorolac), reference<br>compounds (ASA, indomethacin), commonly used drugs (ASA, ibupro-<br>fen, acetaminophen) and highly effective in inhibiting prostaglandin<br>synthesis *in vitro* in, acetaminophen) and highly effective in inhibiting prostaglandin<br>inthesis *in vitro* (flurbiprofen) and having clinical analgesic efficacy<br>ing reported higher (ketorolac, zomepirac) than expected for this<br>oup of compou

In spite of the potential mechanisms underlying a possible ( $MW = 376,41$ ; Toradol i.m., injection syringe 30 mg/ml, Syntex),<br>spinal action of NSAID, the earlier literature on the antinoci-<br>compirac sodium ( $MW = 291.74$ ; McN synthesis *in vitro* (flurbiprofen) and having clinical analgesic efficacy<br>being reported higher (ketorolac, zomepirac) than expected for this<br>group of compounds.<br>The i.t. administered drugs were delivered in a total volu group of compounds.<br>The i.t. administered drugs were delivered in a total volume of 10  $\mu$ l followed by 10  $\mu$ l of saline to flush the catheter. The i.p. injected drugs were dissolved in similar vehicles and administere The i.t. administered drugs were delivered in a total volume of 10  $\mu$ l followed by 10  $\mu$ l of saline to flush the catheter. The i.p. injected drugs were dissolved in similar vehicles and administered in a volume of 1 m followed by 10  $\mu$ l 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 = 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. 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 = 291.7 (MW = 376,41; Toradol i.m., injection syringe 30 mg/ml, Syntex),<br>zomepirac sodium (MW = 291.74; McNeil), indomethacin (MW =<br>357.81; Sigma), acetaminophen, (MW = 151.16; Sigma), flurbiprofen<br>(MW = 244.27; courtesy D. P. Bau Bathon Rouge, LA). Retaminophen,  $(MW = 151.16$ ; 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. 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). Zom 206.27; courtesy D. P. Bauer and C. W. Matthews, Ethyl Corpora<br>Baton Rouge, LA). Ketorolac was further diluted in physiological solution Rouge, LA). Ketorolac was further diluted in physiological solution of 2-hydroxy-<br> $R$ 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-hydroxy-propyl- $\beta$ -cyclo

(0.9% w/v). Zomepirac, flurbiprofen, ibuprofen,  $S(+)$ ibuprofen,  $S(-)$ ibuprofen,  $P(-)$ ibuprofen,  $R(-)$ ibuprofen,  $P(-)$ ibuprofen,  $P(-)$ ibuprofen,  $P(-)$ ibuprofen,  $P(-)$ ibuprofen,  $P(-)$ ibuprofen,  $P(-)$ cyclodextrin; (Research connected to a halothane vaporizer and allowed to breathe halothane Formalin Test<br>To perform the formalin test, the rats were placed in a Plexiglas box<br>connected to a halothane vaporizer and allowed to breathe halothane<br>(3%). After 2 to 3 min, there was a momentary loss of spontaneous<br>move To perform the formal in test, the rats were placed in a Plexiglas box<br>connected to a halothane vaporizer and allowed to breathe halothane<br>(3%). After 2 to 3 min, there was a momentary loss of spontaneous<br>movement with pr 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 re blink and pinnae reflexes. The animal was then quickly removed, and<br>50  $\mu$  of 5% formalin solution was injected s.c. into the dorsal surface<br>of the right hindpaw with a 30 gauge needle. The rat was then individ-<br>ually pl 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 wit ually 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 a maximum interval of 1 to 2 min, the animal displayed recovery from<br>anesthesia with spontaneous activity and normal motor function. A<br>mirror was placed on the opposite side of the Plexiglas chamber for the<br>unhindered obse 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/shak 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-<br>and 5-min intervals during the interval from 10 to 60 min. Two phases<br>of spontaneou was quantified by periodically counting the incidents of spontaneous<br>flinching/shaking of the injected paw. Animals were observed individ-<br>ually and the flinches counted for 1-min periods at  $1-$  to  $2-$ ,  $5-$  to  $6-$ <br>and 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-<br>and 5-min intervals during the interval from 10 to 60 min. Two phases<br>of spontaneou 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 inje 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), fol formalin injection and lasted through the second observation interval  $(5-6 \text{ min})$ , 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 inj formalin injection and lasted through the second observation interval  $(5-6 \text{ min})$ , 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 inj  $(5-6 \text{ min})$ , 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 redu maximum response typically observed at around 25 to 35 min after the<br>formalin injection. As will be described, NSAID were typically most<br>active in reducing the formalin response during the first period of the<br>second phase 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 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 w **were immediately** sacrificed with an overdose 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 were immediately sacrificed with an overdose of barbiturate mixture

re immediately sacrificed with an overdose of barbiturate mixture<br>euthanasia, 50 mg/kg, i.p.).<br>**havioral Assessment**<br>In addition to the formalin response, observation of general behavior<br>s carefully carried out for all rat (Beuthanasia, 50 mg/kg, i.p.).<br> **Behavioral Assessment**<br>
In addition to the formalin response, observation of general behavior<br>
was carefully carried out for all rats tested. Motor function was tested<br>
by examining the pla **Behavioral Assessment**<br>In addition to the formalin response, observation of general behavior<br>was carefully carried out for all rats tested. Motor function was tested<br>by examining the placing/stepping reflex (characterized **Senavioral Assessment**<br>In addition to the formalin response, observation of general behavior<br>was carefully carried out for all rats tested. Motor function was tested<br>by examining the placing/stepping reflex (characterized In addition to the formalin response, observation of general behavior<br>was carefully carried out for all rats tested. Motor function was tested<br>by examining the placing/stepping reflex (characterized as an upward<br>lifting of by examining the placing/stepping reflex (characterized as an upward<br>lifting of the paw from the surface of the table and a plantar placement<br>evoked by drawing the dorsum of the respective hindpaw across the<br>edge of the ta white back on 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 assesse 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 l immediate coordinated twisting of the body when placed horizontally

**138 Malmberg and Yaksh**<br>endpoints, separate animals were also examined on the HP. Rats were ANC<br>placed on the 52.5<sup>o</sup>C hot plate. The amount of time licking the hindpaw curve<br>was then measured. Failure to respond within endpoints, separate animals were also examined on the HP.<br>placed on the 52.5°C hot plate. The amount of time licking th<br>was then measured. Failure to respond within 60 sec was<br>remove the animal from the surface and assign endpoints, separate animals were also examined on the HP. Rats were<br>placed on the 52.5°C hot plate. The amount of time licking the hindpaw<br>was then measured. Failure to respond within 60 sec was cause to 0.5<br>remove the an

remove the animal from the surface and assign that score. production<br>
inhil<br>
doses were assigned in a randomized fashion to be given over the course<br>
of these studies. Control animals *(i.e.,* formalin animals receiving no Experimental Faradigms<br>Because all animals could not be run in a single group, drugs and<br>doses were assigned in a randomized fashion to be given over the course<br>of these studies. Control animals (*i.e.*, formalin animals r of these studies. Control animals (*i.e.*, formalin animals receiving no<br>
injection, i.t. and i.p. saline, 5% cyclodextrin or 5% ethanol) were run<br>
interspersed concurrently with the drug-treated animals. This pre-<br>
vente

wented all of the controls being run on a single group of animals at one<br>time in the course of the investigation.<br>Spinal action of NSAID. Time course of peak action and the dose<br>dependency of the effect were determined. T **the agents of the investigation.**<br> **the agents, i.t. administration of a maximal effective dose was made** 2<br>
the agents, i.t. administration of a maximal effective dose was made 2<br>
min, 3 hr, 6 hr and 24 hr before (pretre Spinal action of NSAID. Time course of peak action and the dose<br>dependency of the effect were determined. To study the time course of<br>the agents, i.t. administration of a maximal effective dose was made 2<br>min, 3 hr, 6 hr a **injection.** e agents, i.t. administration of a maximal effective dose was made 2 n, 3 hr, 6 hr and 24 hr before (pretreatment) and 9 min after (post-<br>atment; before the second phase of the formalin response) formalin<br>iection.<br>Dose-res

min, 3 hr, 6 hr and 24 hr before (pretreatment) and 9 min after (post-<br>treatment; before the second phase of the formalin response) formalin<br>injection.<br>Dose-response curves were carried out with the agents administered<br>i.t injection.<br>
Dose-response curves were carried out with the agents administe<br>
i.t. immediately before (2 min) the formalin injection, an interval<br>
maximum effect based on the initial time course studies. The co<br>
pounds used 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 i.t. immediately before  $(2 \text{ min})$  the formalin injection, an interval of salmaximum effect based on the initial time course studies. The compounds used for i.t. administration are the following: ASA, indometh-<br>acin, flurb maximum effect based on the initial time course studies. The compounds used for i.t. administration are the following: ASA, indometh-<br>acin, flurbiprofen, ketorolac, zomepirac, acetaminophen, ibuprofen (racemic),  $S(+)$ ibup maximum effect based on the initial time course steads. The com-<br>pounds used for i.t. administration are the following: ASA, indometh-<br>acin, flurbiprofen, ketorolac, zomepirac, acetaminophen, ibuprofen (ra-<br>cemic),  $S(+)$ ib acin, 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% cyclode differentially control groups, rate 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 sel 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 i 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 i effect studies was selected on the basis of two factors: 1) when the dos<br>did not differ in effect by more than 10% of the maximum effec<br>achieved by the immediately lower dose (*i.e.*, the plateau effect of th<br>drug was pres 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.<br> **HP test.** With several of the more pote

drug was presumably reached), and 2) when it was impossible to dissolve the drug in a higher concentration.<br> **HP test.** With several of the more potent agents (*i.e.*, ketorolac, zomepirac, indomethacin, flurbiprofen and A dissolve the drug in a higher concentration.<br> **HP test.** With several of the more potent agents<br>
zomepirac, indomethacin, flurbiprofen and ASA), the<br>
drug which was used in the formalin test was given i.t<br>
upon the HP (52. Systemic action of NSAID. Intraperiones assessed.<br>
Systemic action of NSAID. Intraperiones are carried and ASA), the highest dose of the HP (52.5°C) response latency was assessed.<br>
Systemic action of NSAID. Intraperitonea

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.<br>Systemic action of NSAID. Intra tion. In this study, the NSAID used were: ASA, indomethacin, keto-<br>response are formula injection of NSAID. Intraperitoneal injections were carriec<br>out 2 min before the formulin injection in order to determine the dose-<br>re **rolacy of the flurbind of NSAID.** Intraperitoneal injections were carried out 2 min before the formalin injection in order to determine the dose-<br>response and time vs. response of the agents after systemic administra-<br>tio out 2 min before the formal in injection in order to determine the dose-<br>response and time *vs.* response of the agents after systemic administra-<br>tion. In this study, the NSAID used were: ASA, indomethacin, keto-<br>rolac, f rolac, flurbiprofen, zomepirac and acetaminophen. These were selected<br>because of their potency or reference compounds. For the appropriate<br>control group, rats were given i.p. 5% cyclodextrin and 5% ethanol. In<br>addition, th

### Statistical Analysis

ated rats in the formalin test. The highest doses used in this series<br>is determined as described for the i.t. route mentioned above.<br>**atistical Analysis**<br>The time-response data are presented as the mean flinches per min<br>d was determined as described for the i.t. route mentioned above.<br> **Statistical Analysis**<br>
The time-response data are presented as the mean flinches per min<br>
and the S.E.M. for the period of 1 to 2 min, 5 to 6 min and at 5-m **Statistical Analysis**<br>The time-response data are presented as the mean flinches per min<br>and the S.E.M. for the period of 1 to 2 min, 5 to 6 min and at 5-min<br>intervals after that up to 60 min. Dose-response curves are pres The time-response data are presented as the mean flinches per m<br>and the S.E.M. for the period of 1 to 2 min, 5 to 6 min and at 5-m<br>intervals after that up to 60 min. Dose-response curves are present<br>as the sum of flinches 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 flin 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 do lines for phase in (or or min) and phase zar (to obter him), respectively]. The cumulative flinching response was calculated for each rat and the ISS. The data were examined by one-way ANOVA with a Dunnett's test ( $P < .05$ 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<sub>50</sub> (inhibitory dose result (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 Mu 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

inding (1907). The poetic of the formalized according<br>to Tallarida and Murray (1987) for the relative potency of the agent<br>administered i.t. vs. i.p.<br>Inspection of the results clearly revealed a plateau of activity for all

138 Malmberg and Yaksh<br>endpoints, separate animals were also examined on the HP. Rats were ANOVA was used. To define the maximum inhibition, dose-response<br>placed on the 52.5°C hot plate. The amount of time licking the hind ANOVA was used. To defme the maximum inhibition, dose-response Vol. 263<br>ANOVA was used. To define the maximum inhibition, dose-response<br>curves were inspected. When two sequential NSAID doses differing by<br>0.5 log units produced effects differing by 10% or less, the effect Vol. 263<br>
ANOVA was used. To define the maximum inhibition, dose-response<br>
curves were inspected. When two sequential NSAID doses differing by<br>
0.5 log units produced effects differing by 10% or less, the effect<br>
produced 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 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 gi Dunnett's test was used. All statistical significance was considered at<br>a critical value of  $P < .05$ .<br><br>**Results**<br>**General Behavior**<br>The formalin injected s.c. into the dorsal surface of the right

## **Results**

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



*1992*<br>any effect on general behavior or motor function when admin-<br>istered i.t. or i.p. Thus, there were no changes in placing/ 1992<br>any effect on general behavior or motor function when admin-<br>istered i.t. or i.p. Thus, there were no changes in placing/<br>stepping, righting reflexes or ambulation. In 50% of the animals 1992<br>any effect on general behavior or motor function when admin-<br>istered i.t. or i.p. Thus, there were no changes in placing/<br>stepping, righting reflexes or ambulation. In 50% of the animals<br>receiving the highest doses o istered i.t. or i.p. Thus, there were no changes in placing/ potent in inhibiting the first phase as the second phase of the stepping, righting reflexes or ambulation. In 50% of the animals formalin response  $[ID<sub>50</sub>: 3$ any effect on general behavior or motor function when administered i.t. or i.p. Thus, there were no changes in placing/<br>stepping, righting reflexes or ambulation. In 50% of the animals<br>receiving the highest doses of indome allodynia with squeaking and agitation. In 50% of the animals<br>allodynia with squeaking and agitation evoked by touching of<br>the flank was observed.<br>Although there was no effect upon motor function, the i.t. ceiving the highest doses of indomethacin i.t. (28 nmol) and<br>torolac i.t. (27 nmol) after 1 and 3 hr, respectively, a transient<br>lodynia with squeaking and agitation evoked by touching of<br>e flank was observed.<br>Although ther

ketorolac i.t. (27 nmol) after 1 and 3 hr, respectively, a transient<br>allodynia with squeaking and agitation evoked by touching of<br>the flank was observed.<br>Although there was no effect upon motor function, the i.t.<br>injection allodynia with squeaking and agitation evoked by touching of<br>the flank was observed.<br>hthough there was no effect upon motor function, the i.t.<br>injection of the several NSAID resulted in a potent suppression<br>of the flinchin the flank was observed.<br>
Although there was no effect upon motor function, the i.t.<br>
injection of the several NSAID resulted in a potent suppression<br>
of the flinching behavior observed during phase 2, and to a<br>
much lesser Although there was no effect upon motor function, the i.t.<br>injection of the several NSAID resulted in a potent suppression<br>of the flinching behavior observed during phase 2, and to a<br>much lesser extent in the phase 1 port injection of the several NSAID resulted in a potent suppression<br>of the flinching behavior observed during phase 2, and to a<br>much lesser extent in the phase 1 portion of the algogenic<br>activity observed after the s.c. injec of the flinching behavior observed during phase 2, and to a<br>much lesser extent in the phase 1 portion of the algogenic<br>activity observed after the s.c. injection of formalin. A typical<br>time course of effect, with differen activity observed after the s.c. injection of formalin. A typical<br>time course of effect, with different doses of  $S(+)$ ibuprofen<br>given i.t. 2 min before the injection of formalin, is presented in<br>figure 1. In contrast, the time course of effect, with different doses of  $S(+)$ ibuprofen<br>given i.t. 2 min before the injection of formalin, is presented in<br>figure 1. In contrast, the stereoisomer  $R(-)$ ibuprofen was with-<br>out effect on the flinching

figure 1. In contrast, the stereoisomer  $R(-)$ ibuprofen was with-<br>out effect on the flinching behavior in the formalin test (fig. 1).<br>Typically, the first portion of the second phase (phase 2a;<br>10–39 min after formalin) wa out effect on the flinching behavior in the formalin test (fig. 1).<br>Typically, the first portion of the second phase (phase 2a;<br>10–39 min after formalin) was observed to be most sensitive to<br>the inhibitory effects of thes Typically, the first portion of the second phase (phase 2a;<br>10–39 min after formalin) was observed to be most sensitive to<br>the inhibitory effects of these agents. In contrast, even at the<br>highest doses, there was no stati responses. 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.<br>**Potency and Efficacy** 

**Formalin test.** As indicated for  $S(+)$ ibuprofen in figure 1, **Potency and Efficacy of i.t. NSAID**<br>**Potency and Efficacy of i.t. NSAID**<br>**Formalin test.** As indicated for  $S(+)$ ibuprofen in figure<br>the suppressant effects of these i.t. agents on the appearal<br>and magnitude of the second **Potency and Emcacy or i.t. NSAID** bo<br> **Formalin test.** As indicated for  $S(+)$ ibuprofen in figure 1, not<br>
the suppressant effects of these i.t. agents on the appearance<br>
and magnitude of the second phase activity were dos **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. dosethe suppressant effects of these i.t. agents on the appearance<br>and magnitude of the second phase activity were dose depend-<br>ent. Figure 2 presents the i.t. dose-response curves for the<br>neveral NSAID determined on the phas and magnitude of the second phase activity were dose dependent. Figure 2 presents the i.t. dose-response curves for the noiseveral NSAID determined on the phase 2a (10-39 min) re-<br>sponse evoked by s.c. formalin. The rank several NSAID determined on the phase 2a (10–39 min) re-<br>sponse evoked by s.c. formalin. The rank order of potency<br>(defined by the ID<sub>50</sub> in nmol) of i.t. NSAID on the phase 2a<br>flinching response was found to be: indometh several NSAID determined on the phase 2a (10–35 min) re-<br>sponse evoked by s.c. formalin. The rank order of potency<br>(defined by the ID<sub>50</sub> in nmol) of i.t. NSAID on the phase 2a<br>flinching response was found to be: indometh (defined by the ID<sub>so</sub> in nmol) of i.t. NSAID on the phase 2a<br>flinching response was found to be: indomethacin  $(1.9) \ge$ <br>flurbiprofen  $(2.1) >$  ketorolac  $(5.2) \ge$  zomepirac  $(5.9) >$ <br> $S(+)$ ibuprofen  $(15.7) \ge$  ibuprofen(rac flurbiprofen  $(2.1)$  > ketorolac  $(5.2)$  ≥ zomepirac  $(5.9)$  >  $S(+)$ ibuprofen  $(15.7)$  ≥ ibuprofen  $(\text{vacemic})$   $(18.9)$  > acetylsalicylic acid  $(27)$  > acetaminophen  $(257)$  >  $R(-)$ ibuprofen  $(>270)$  or  $= 0$  (table 1). For  $S(+)$ ibuprofen (15.7)  $\geq$  ibuprofen (racemic) (18.9)  $>$  acetylsali-<br>cylic acid (27)  $>$  acetaminophen (257)  $> R(-)$ ibuprofen ( $>270$ ) or 24 hr<br>= 0 (table 1). For comparison, the potency of morphine to for keto<br>inhibit t cylic acid  $(27)$  > acetaminophen  $(257)$  >  $= 0$  (table 1). For comparison, the pot<br>inhibit the second phase of the formalin r<br>magnitude as the more potent NSAID [m<br>CI: 3.8  $(2.6-5.4)$  nmol, data not shown].<br>A very modes 0 (table 1). For comparison, the potency of morphine to hibit the second phase of the formalin response is of the same agnitude as the more potent NSAID [morphine  $ID_{50}$  and  $95\%$  : 3.8 (2.6–5.4) nmol, data not shown]. inhibit the second phase of the formal in response is of the same<br>magnitude as the more potent NSAID [morphine  $ID_{50}$  and 95%<br>CI: 3.8 (2.6–5.4) nmol, data not shown].<br>A very modest reduction of the flinching response was

magnitude as the more potent NSAID [morphine ID<sub>60</sub> and 95% re<br>CI: 3.8 (2.6–5.4) nmol, data not shown].<br>A very modest reduction of the flinching response was frequently observed in phase 1 (0–9 min), although a statistica



**profen and acetaminophen, presented as the cumulative number of formalin evoked flinches during the phase 2a interval (10–39 min). Each Example 19 Concept formally the phase (nmol, IT)**<br>**Fig. 2.** Dose-response curves for i.t. NSAID: ASA, ketorolac, zomepirac, istra<br>indomethacin, flurbiprofen, ibuprofen(racemic), S(+)-ibuprofen,  $R(-)$ -ibu-<br>profen and aceta **Fig. 2.** Dose-response curves for i.t. NSAID: ASA, ketorolac, zomepirac, istration indomethacin, flurbiprofen, ibuprofen(racemic), S(+)-ibuprofen,  $R(-)$ -ibu-<br>profen and acetaminophen, presented as the cumulative number of **Fig. 2.** Dose-response curves for i.t. NSAID: ASA, ketorolac, zorindomethacin, flurbiprofen, ibuprofen(racemic), S(+)-ibuprofen, *F* profen and acetaminophen, presented as the cumulative nuroformalin evoked flinches durin

Spinal Action of NSAIDs 139<br>and 95% CI: 24 (5–130) nmol]. For comparison, morphine is as<br>potent in inhibiting the first phase as the second phase of the **Spinal Action of NSAIDs 139**<br>and 95% CI: 24 (5-130) nmol]. For comparison, morphine is as<br>potent in inhibiting the first phase as the second phase of the<br>formalin response  $[ID_{so}: 3.7 (1.6–8.1) \text{ nmol, data not shown}].$ **Spinal Action of NSAIDs** 139<br>and 95% CI: 24 (5-130) nmol]. For comparison, morphine is a<br>potent in inhibiting the first phase as the second phase of the<br>formalin response  $[ID_{so}: 3.7 (1.6-8.1)$  nmol, data not shown].<br>As in d 95% CI: 24 (5–130) nmol]. For comparison, morphine is as<br>tent in inhibiting the first phase as the second phase of the<br>rmalin response  $[ID_{60}: 3.7 (1.6–8.1)$  nmol, data not shown].<br>As indicated in the dose-response curve

uch isser extent in the phase 1 portion of the algogenic the i.t. administration of indomethacin, flurbiprofen, ketorolac, tivity observed after the s.c. injection of formalin. A typical  $\frac{1}{30\%}$  of control. These val  $S(+)$ ibuprofen at the highest dose on the phase 1 or phase 2<br>
Formalin test. As indicated for  $S(+)$ ibuprofen in figure 1, and  $S(+)$  is able to totally diminish<br> **Potency and Efficacy of i.t. NSAID**<br> **Potency and Efficacy** and 95% CI: 24 (5-130) nmol]. For comparison, morphine is<br>potent in inhibiting the first phase as the second phase of t<br>formalin response  $[ID_{50}: 3.7 (1.6-8.1)$  nmol, data not shown<br>As indicated in the dose-response curves 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].<br>As indicated in the dose-response curves for i.t. agents, all agents on the phase 1 and phase formalin response  $[ID_{50}: 3.7 (1.6-8.1)$  nmol, data not shown].<br>As indicated in the dose-response curves for i.t. agents, all<br>agents on the phase 1 and phase 2 endpoints showed a limita-<br>tion in the maximum achievable supp 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 an 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 w tion in the maximum achievable suppression of the flinch<br>behavior (e.g., efficacy). Intrathecal and i.p. dose-response<br>curves showed similar distinct plateaus with numerically small<br>but statistically significant differenc behavior (e.g., efficacy). Intrathecal and i.p. dose-response<br>curves showed similar distinct plateaus with numerically small<br>but statistically significant differences between the agents. As<br>indicated in figure 3 (top), the curves showed similar distinct plateaus with numerically small<br>but statistically significant differences between the agents. As<br>indicated in figure 3 (top), the inhibitory effect obtained with<br>the i.t. administration of in 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 approach  $30\%$  of control. These values were only 40 to  $50\%$  of control 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 ac zomepirac and ASA approached a level which was about 2<br>30% of control. These values were only 40 to 50% of cor<br>for ibuprofen,  $S(+)$ ibuprofen and acetaminophen, and 95%<br> $R(-)$ ibuprofen, which were without effect. Less diffe 30% of control. These values were only 40 to 50% of control for ibuprofen,  $S(+)$ ibuprofen and acetaminophen, and 95%  $R(-)$ ibuprofen, which were without effect. Less difference volumed between i.p. administered drugs, alth 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 acetamino-<br>phen resulted in a significantly le  $R(-)$ ibuprofen, which were without effect. Less difference was<br>found between i.p. administered drugs, although acetamino-<br>phen resulted in a significantly less maximal possible suppres-<br>sion than ketorolac and flurbiprofe phen resulted in a significantly less maximal possible suppres-<br>sion than ketorolac and flurbiprofen (fig. 3; bottom). However, phen resulted in a significantly less maximal possible suppres-<br>sion than ketorolac and flurbiprofen (fig. 3; bottom). However,<br>comparing the i.t. with the i.p. administration route for a single<br>agent did not reveal any di sion than ketorolac and flurbiprofen (fig. 3; bottom). However,<br>comparing the i.t. with the i.p. administration route for a single<br>agent did not reveal any difference except for indomethacin,<br>which showed slightly more sup both the first and second phases of the formalin response (data not 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 not **shown).** hich showed slightly more suppression after i.t. injection. In ntrast, spinal morphine (80 nmol) is able to totally diminish th the first and second phases of the formalin response (data tt shown).<br>**HP** test. Even with the

contrast, spinal morphine (80 nmol) is able to totally diminish<br>both the first and second phases of the formalin response (data<br>not shown).<br>**HP test.** Even with the highest doses of i.t. ketorolac, zo-<br>mepirac, indomethaci both the first and second phases of the formalin response (data<br>not shown).<br>**HP test.** Even with the highest doses of i.t. ketorolac, zo-<br>mepirac, indomethacin, flurbiprofen and ASA, these agents did<br>not produce any signif not shown).<br> **HP test.** Even with the highest doses of i.t. ketorolac, zomepirac, indomethacin, flurbiprofen and ASA, these agents did<br>
not produce any significant effect on the HP latency (table 2).<br>
In comparison, morphi the vestilized (80 nmol), elevates the response latency (table in comparison, morphine, in a dose similar to the NSAID d<br>tested (80 nmol), elevates the response latency to 60 sec. In comparison, morphine, in a dose similar to the NSAID doses<br>tested (80 nmol), elevates the response latency to 60 sec.<br>**Time Course of Action of i.t. NSAID** 

To define the time course of the effects of i.t. NSAID, drugs<br>were delivered at intervals of 9 min after and 2 min, 3 hr, 6 hr tested (80 nmol), elevates the response latency to 60 sec.<br> **Time Course of Action of i.t. NSAID**<br>
To define the time course of the effects of i.t. NSAID, drugs<br>
were delivered at intervals of 9 min after and 2 min, 3 hr, Time Course of Action of i.t. NSAID<br>To define the time course of the effects of i.t. NSAID, drugs<br>were delivered at intervals of 9 min after and 2 min, 3 hr, 6 hr<br>or 24 hr before the injection of formalin. As shown in figu To define the time course of the effects of i.t. NSAID, drugs<br>were delivered at intervals of 9 min after and 2 min, 3 hr, 6 hr<br>or 24 hr before the injection of formalin. As shown in figure 4<br>for ketorolac, administration o were delivered at intervals of 9 min after and 2 min, 3 hr, 6 hr<br>or 24 hr before the injection of formalin. As shown in figure 4<br>for ketorolac, administration of the NSAID at different inter-<br>vals before and at 9 min after 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 o for ketorolac, administration of the l<br>vals before and at 9 min after the<br>revealed that the maximum inhibition<br>did not differ, whether the agent was<br>9 min after the injection of formalin.<br>Increasing the pretreatment interv Is before and at 9 min after the application of forms vealed that the maximum inhibition of the phase 2a respond not differ, whether the agent was delivered 2 min before min after the injection of formalin.<br>Increasing the

did not differ, whether the agent was delivered 2 min before or<br>9 min after the injection of formalin.<br>Increasing the pretreatment intervals resulted in a progres-<br>sive reduction in the suppressive effects upon the phase 1 did not differ, whether the agent was delivered 2 min before  $9$  min after the injection of formalin.<br>
Increasing the pretreatment intervals resulted in a progre<br>
sive reduction in the suppressive effects upon the phase 1 9 min after the injection of formalin.<br>Increasing the pretreatment intervals resulted in a progres-<br>sive reduction in the suppressive effects upon the phase 1 and<br>phase 2b effect, as compared to the 2-min pretreatment inte 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 fo examined, the maximum pretreatment intervals during which<br>the effect obtained was not statistically less than the maximally<br>achieved suppression (measured with the 2-min pretreatment phase 2b effect, as compared to the 2-min pretreatment intervals. Thus, as indicated in figure 5 for the several agent examined, the maximum pretreatment intervals during which the effect obtained was not statistically les vals. 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 th relation of measured with the 2-min pretreatment<br>the effect obtained was not statistically less than the maximally<br>achieved suppression (measured with the 2-min pretreatment<br>interval) were: ASA (100 nmol), 3 hr; flurbiprof hieved suppression (measured with the 2-min pretreatmenterval) were: ASA (100 nmol), 3 hr; flurbiprofen (27 nmol); zomepirac (34 nmol), 3 hr; and ketorolac (27 nmol), 6 hr<br>importantly, postformalin treatment, just before t

achieved suppression (measured with the 2-min pretreatment<br>interval) were: ASA (100 nmol), 3 hr; flurbiprofen (27 nmol), 3<br>hr; zomepirac (34 nmol), 3 hr; and ketorolac (27 nmol), 6 hr.<br>Importantly, postformalin treatment, interval) were: ASA (100 nmol), 3 hr; flurbiprofen (27 nmol)<br>hr; zomepirac (34 nmol), 3 hr; and ketorolac (27 nmol), 6 hr<br>Importantly, postformalin treatment, just before the init<br>tion of the second phase (9 min after form hr; zomepirac (34 nmol), 3 hr; and ketorolac (27 nmol), 6 hr.<br>Importantly, postformalin treatment, just before the initia-<br>tion of the second phase (9 min after formalin injection),<br>decreased the flinch behavior in phase 2 Importantly, postformal in treatment, just before the initiation of the second phase  $(9 \text{ min after formal in injection})$ , decreased the flinch behavior in phase  $2a$  by the same magnitude as the pretreatment of  $2 \text{ min}$  before the formal in (f tion of the second phase  $(9 \text{ min after formula injection})$ ,<br>decreased the flinch behavior in phase 2a by the same magni-<br>tude as the pretreatment of 2 min before the formalin (fig. 2).<br>The lack of a difference between the 2-min pretreatment decreased the flinch behavior in phase 2a by the same mag<br>tude as the pretreatment of 2 min before the formalin (fig.<br>The lack of a difference between the 2-min pretreatment t<br>the 9-min post-treatment on the phase 2a effec tude as the pretreatment of 2 min before the formalin (fig. 2).<br>The lack of a difference between the 2-min pretreatment and<br>the 9-min post-treatment on the phase 2a effects of any of the<br>agents examined emphasizes that the The lack of a difference between the 2-min pretreatment and<br>the 9-min post-treatment on the phase 2a effects of any of the<br>agents examined emphasizes that these agents after i.t. admin-<br>istration have a very short latency 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 phas 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 postistration have a very short latency to<br>were no differences between the phas<br>function of time of drug pre- or post-tr<br>the differential effects of i.t. NSAID<br>independent of administration time.

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**140** Malmb<br>TABLE 1<br>Inhibitory effects Inhibitory effects of i.t. and i.p. administered NSAID on the 2a phase (10-39 mm) of the formalin test, presented as percent of control **response** TABLE 1<br>Inhibitory effects of i.t. and i.p. administered NSAID on the 2a phase (1)<br>response<br>ID<sub>so</sub> values and 95% CI calculated from regression lines shown in figures 2 and 6.

ID<sub>50</sub> values and 95% CI calculated from regression lines shown in figures 2 and 6.





**Fig. 3.** Maximal inhibitory effect obtained by i.t. (top)<br>and i.p. (bottom) injection, presented as percent of<br>the phase 2a control response. The results of the Fig. 3. Maximal inhibitory effect obtained by i.t. (top)<br>and i.p. (bottom) injection, presented as percent of<br>the phase 2a control response. The results of the<br>statistical analysis are indicated by the black bars Fig. 3. Maximal inhibitory effect obtained by i.t. (top)<br>and i.p. (bottom) injection, presented as percent of<br>the phase 2a control response. The results of the<br>statistical analysis are indicated by the black bars<br>at the bo **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 The phase 2a control response. The results of the back bars<br>at the bottom. Drug effects not joined by a common<br>or overlapping line are different (P < .05). Abbrevi-<br>ations: . INDO, indomethacin; KETO, ketorolac;<br>ZOM, zomep ations: INDO, indomethacin; KETO, ketorolac;<br>ZOM, zomepirac; FLUR, flurbiprofen; IBU, ibupro-<br>fen; ACET, acetaminophen. The bars present the<br>mean and S.E.M. for 4 to 10 rats for the two highest<br>doses that did not differ mo fect).

The i.p. administration of ASA (2.7-83 μmol/kg), ketorolac<br>The i.p. administration of ASA (2.7-83 μmol/kg), ketorolac<br>7-83 μmol/kg), zomepirac (2.7-83 μmol/kg), indomethacin **Example 13.433 Effects of i.p. NSAID**<br>The i.p. administration of ASA (2.7-83  $\mu$ mol/kg), ketorolac<br>(2.7-83  $\mu$ mol/kg), zomepirac (2.7-83  $\mu$ mol/kg), indomethacin<br>(2.7-27  $\mu$ mol/kg), flurbiprofen (2.7-46  $\mu$ mol/kg) a **Behavioral Effects of i.p. NSAID**<br>
The i.p. administration of ASA (2.7-83  $\mu$ mol/kg), ketor<br>
(2.7-83  $\mu$ mol/kg), zomepirac (2.7-83  $\mu$ mol/kg), indometh<br>
(2.7-27  $\mu$ mol/kg), flurbiprofen (2.7-46  $\mu$ mol/kg) and acetar The i.p. administration of ASA  $(2.7-83 \mu \text{mol/kg})$ , ketorolac  $(2.7-83 \mu \text{mol/kg})$ , zomepirac  $(2.7-83 \mu \text{mol/kg})$ , indomethacin  $(2.7-27 \mu \text{mol/kg})$ , flurbiprofen  $(2.7-46 \mu \text{mol/kg})$  and acetaminophen  $(2.7-27 \mu \text{mol/kg})$  had no detecta The I.p. administration of<br>(2.7–83  $\mu$ mol/kg), zomepirad<br>(2.7–27  $\mu$ mol/kg), flurbiprofe<br>ophen (2.7–27  $\mu$ mol/kg) had<br>function or general behavior<br>Intraperitoneal injection of  $(2.7-27 \mu \text{mol/kg})$ , flurbiprofen  $(2.7-46 \mu \text{mol/kg})$  and acetaminophen  $(2.7-27 \mu \text{mol/kg})$  had no detectable effect upon motor function or general behavior.<br>Intraperitoneal injection of these agents did, however, pro-<br>duce a s

(2.1-21  $\mu$ mol/kg), introlproten (2.1-46  $\mu$ mol/kg) and acetamin-<br>ophen (2.7-27  $\mu$ mol/kg) had no detectable effect upon motor<br>function or general behavior.<br>Intraperitoneal injection of these agents did, however, pro-<br> function or general behavior.<br>Intraperitoneal injection of these agents did, he<br>duce a significant suppression of the phase 2a forma<br>and, to a lesser extent, the phase 1 response, ii<br>similar to that described above for i.t

ACET<br> **Potency and Efficacy of i.p. NSAID**<br>
The i.p. administration of the NSAID resulted in a dose-THE INCORDING THE INCORRETTER<br>The i.p. administration of the NSAID resulted in a dose-<br>pendent inhibition of the phase 2a formalin response, wit Potency and Efficacy of i.p. NSAID<br>The i.p. administration of the NSAID resulted in a dose-<br>dependent inhibition of the phase 2a formalin response, with<br>the rank of order of potency  $(ID_{so}$  in  $\mu$ mol, total dose) being: **Potency and Efficacy of i.p. NSAID**<br>The i.p. administration of the NSAID resulted in a dose-<br>dependent inhibition of the phase 2a formalin response, with<br>the rank of order of potency (ID<sub>50</sub> in  $\mu$ mol, total dose) being The i.p. administration of the NSAID resulted in a dose-<br>dependent inhibition of the phase 2a formalin response, with<br>the rank of order of potency (ID<sub>50</sub> in  $\mu$ mol, total dose) being:<br>ketorolac (3.0)  $\geq$  flurbiprofen dependent inhibition of the phase 2a formalin response, with<br>the rank of order of potency  $(ID_{50}$  in  $\mu$ mol, total dose) being:<br>ketorolac  $(3.0) \geq$  flurbiprofen  $(3.1) \geq$  zomepirac  $(5.5) \geq$  acet-<br>aminophen  $\geq$  acet the rank of order of potency  $(D_{50} \text{ in } \mu \text{mol})$ , total dose) being:<br>ketorolac (3.0)  $\geq$  flurbiprofen (3.1)  $\geq$  zomepirac (5.5)  $\geq$  acet-<br>aminophen  $\geq$  acetylsalicylic acid (8.0). Dose-response lines are<br>presented presented in micromoles per kilogram (fig. 6), and the mean value (and S.E.M.) for the weight of the rats used was  $309 \pm 5$  g. Note that the i.p. dose is in micromoles as compared to the i.t. dose in nanomoles.  $a$ minophen  $\geq$  acetylsalicylic acid  $(8.0)$ . Dose-response lines are

*1992* TABLE 2 **Maximal Possible Effect (%MPE) of i.t. NSAID on the 52.5°C HP**<br>**Maximal Possible Effect (%MPE) of i.t. NSAID on the 52.5°C HP**<br>Dose what + SEM \*

Drug	Dose	%MPE $\pm$ S.E.M."
	nmol	
ASA	100	$14.6 \pm 3.0$
Ketorolac	80	$13.3 \pm 2.5$
Flurbiprofen	80	$10.3 \pm 2.1$
Indomethacin	30	$9.0 \pm 5.6$
Zomepirac	80	$6.5 \pm 4.2$
Morphine	30	$80.0 \pm 10$



Fig. 4. Time effect curve with i.t. ketorolac (27 nmol) administered 2 min,<br>3, 6 and 24 hr before and 9 min after the formalin. Number of flinches<br>per min is plotted vs. the time after the formalin injection into the dorsa **ime** (min)<br>Fig. 4. Time effect curve with i.t. ketorolac (27 nmol) administered 2 min,<br>3, 6 and 24 hr before and 9 min after the formalin. Number of flinches<br>per min is plotted vs. the time after the formalin injection in Fig. 4. Time effect cu<br>3, 6 and 24 hr befor<br>per min is plotted vs.<br>surface of the right<br>includes 20 animals;<br>of 4 to 6 animals.



**Example 19** 124 hrs **can be a community of the set of the effect on the phase 2a formalin esponse** induction through the effect on the phase 2a formalin response induction of the effective i.t. dose of ketorolac (27 nmol **Intrathecal injection times relative to formalin** the principal of the effect on the phase 2a formalin response is produced by a just maximally effective i.t. dose of ketorolac (27 nmol), is flurbiprofen (27 nmol), zomep **Fig. 5.** Time course of the effect on the phase 2a formalin response in produced by a just maximally effective i.t. dose of ketorolac (27 nmol), in flurbiprofen (27 nmol), zomepirac (34 nmol) or ASA (100 nmol). Data are **Fig.** 5. Time course of the effect on the phase 2a formalin response ineproduced by a just maximally effective i.t. dose of ketorolac (27 nmol), irrflurbiprofen (27 nmol), zomepirac (34 nmol) or ASA (100 nmol). Data are 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 i 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 formali diative matter and the intervals of the inhibition of the flinching response and with administration immediately before (2 min) formalin. Each point the graph represents the mean and S.E.M. of four to six animals.<br>Similar

found with administration immediately before (2 min) formalin. Each point not the graph represents the mean and S.E.M. of four to six animals.<br>Similar to the i.t. administration paradigm, the major effect of the i.p. NSAID on the graph represents the mean and S.E.M. of four to six animals.<br>
Similar to the i.t. administration paradigm, the major effect<br>
of the i.p. NSAID on the formalin response was found in phase<br>
2a. Although the phase 1 wa Similar to the i.t. administration paradigm, the major effect<br>of the i.p. NSAID on the formalin response was found in phase<br>2a. Although the phase 1 was mildly attenuated by the highest<br>doses used (see below), the effect o



10 100<br> **Dose (umol/kg, IP)**<br> **Fig. 6.** Dose-response curves for i.p. NSAID: ASA, ketorolac, zomepirac,<br>
indomethacin, flurbiprofen and acetaminophen, presented as the cumulative number of formalin evoked flinches during p **Dose (umol/kg, IP)**<br>Fig. 6. Dose-response curves for i.p. NSAID: ASA, ketorolac, zomepirac,<br>indomethacin, flurbiprofen and acetaminophen, presented as the cumu-<br>lative number of formalin evoked flinches during phase 2a fo Eig. 6. Dose-response curves for i.p. NSAID: ASA, ketorolac, zomepiral<br>indomethacin, flurbiprofen and acetaminophen, presented as the cumulative number of formalin evoked flinches during phase 2a formal<br>response. Each dose flative number of formalin evoked flinches during phase 2a formalin<br>response. Each dose on the graph represents the mean and S.E.M. of<br>4 to 6 rats except for the control group, which includes 20 animals.<br>found to be dose d

drugs.

4 to 6 rats except for the control group, which includes 20 animals.<br>
found to be dose dependent for any of the i.p. administered<br>
drugs.<br>
Typically, there were no differences in the maximal inhibi-<br>
tory effect found betw in a biology of the F.p. administered<br>drugs.<br>Typically, there were no differences in the maximal inhibi-<br>tory effect found between the highest doses of the tested NSAID<br>in phase 2a, with the exception of acetaminophen, for Typically, there were no differences in the maximal inhibitory effect found between the highest doses of the tested NSAID<br>in phase 2a, with the exception of acetaminophen, for which<br>the maximum suppression was statisticall

## **Discussion**

and  $\frac{1}{1}$  aloses, these agents had little effect on the escape response<br>  $\frac{1}{1}$  aloses, these agents had little effect on the escape response<br>  $\frac{1}{1}$  aloses, these agents had little effect on the escape response In the present study, we sought to define the antinociceptive<br>In the present study, we sought to define the antinociceptive<br>tency and efficacy of spinally and systemically administered **potency and ST ANDU ST ANDU ST ANDU ST ANDU ST ANDU ST AND S Discussion**<br>In the present study, we sought to define the antinociceptive<br>potency and efficacy of spinally and systemically administere<br>NSAID on the formalin test. As indicated, a variety of struc-<br>turally diverse agents 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 In the present study, we sought to define the antihociceptive<br>potency and efficacy of spinally and systemically administered<br>NSAID on the formalin test. As indicated, a variety of struc-<br>turally diverse agents were examine potency and emcacy of spinally and systemically administered<br>NSAID on the formalin test. As indicated, a variety of struc-<br>turally diverse agents were examined and found to result in a<br>reliable, dose-dependent, stereospeci turally diverse agents were examined and found to result in a<br>reliable, dose-dependent, stereospecific suppression of specific<br>phases of the formalin response after both spinal and systemic<br>administration. The principle ob renable, dose-dependent, stereospectic suppression of specific<br>phases of the formalin response after both spinal and systemic<br>administration. The principle observations were: 1) agents were<br>approximately 100 to 1000 times 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 administration. The principle observations were: 1) agents were<br>approximately 100 to 1000 times more potent after spinal than<br>after systemic administration; 2) in spite of the significant<br>difference in potency, all agents approximately 100 to 1000 times more potent after spinal than<br>after systemic administration; 2) in spite of the significant<br>difference in potency, all agents displayed surprisingly similar<br>efficacy (*i.e.*, the same maxima 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.; a efficacy (*i.e.*, the same maximal degree of suppression), and the maximal suppression was similar whether the agent was a ministered systemically or i.t.; and 3) given i.t. at the highe doses, these agents had little eff 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 ministered systemically of i.t., and 3) given i.t. at the ingnest<br>doses, these agents had little effect on the escape response<br>generated by the acute thermal stimulus. Jointly, these obser-<br>vations provide strong support t generated by the actue thermal stimulus. Jointly, these observations provide strong support that the structurally diverse<br>class of molecules classified as NSAID can produce a powerful<br>and direct effect upon spinal nocicept class of molecules classified as NSAID can produce a powerful<br>and direct effect upon spinal nociceptive processing and that<br>this effect reflects upon a characteristic of the spinal processing<br>induced by the ongoing afferen and direct effect upon spinal nociceptive processing and that<br>this effect reflects upon a characteristic of the spinal processing<br>induced by the ongoing afferent barrage generated by the<br>irritant stimulus. Such observation this effect reflects upon a characteristic of the spinal processing<br>induced by the ongoing afferent barrage generated by the<br>irritant stimulus. Such observations are consistent with pre-<br>vious reports in which it was shown 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 aci nor altered the response on the HP (Yaksh, 1982).<br>Spinal NSAID Action: Cyclooxygenase Inhibition or zomepirac would diminish the acetic acid evoked writhing<br>response in rats at doses which were neither systemically active<br>nor altered the response on the HP (Yaksh, 1982).<br>**Spinal NSAID Action: Cyclooxygenase Inhibition** sponse in rats at doses which were neither systemically active<br>
In altered the response on the HP (Yaksh, 1982).<br> **Sinal NSAID Action: Cyclooxygenase Inhibition**<br>
In the present study, we found a considerable variation in response. Each to see on the granh represents the mean and S.E.M. of<br>4 to 6 rats except for the control group, which mchoises 20 animals<br>for found to be dose dependent for any of the i.p. administered<br>flure,<br>found to be do

nor altered the response on the HP (Yaksh, 1982).<br> **Spinal NSAID Action: Cyclooxygenase Inhibition**<br>
In the present study, we found a considerable variation in<br>
the apparent potency of the NSAID. The relative i.t. potency **Spinal NSAID Action: Cyclooxygenase Inhibition**<br>In the present study, we found a considerable variation in<br>the apparent potency of the NSAID. The relative i.t. potency<br>in attenuating the phase 2 response was: indomethaci

**142 Malmberg and Yaksh**<br> $S(+)$ ibuprofen (1.7) > ibuprofen (1.4) > ASA (1.0) > acetam<br>ophen (0.1) >  $R(-)$ ibuprofen (>0.1). As all drugs were ads **142 Malmberg and Yaksh**<br> $S(+)$ ibuprofen  $(1.7)$  > ibuprofen  $(1.4)$  > ASA  $(1.0)$  > acetam<br>ophen  $(0.1)$  >  $R(-)$ ibuprofen  $(>0.1)$ . As all drugs were adm<br>istered i.t. and showed a rapid onset of action, it appear **142 Malmberg and Yaksh**<br> $S(+)$ ibuprofen  $(1.7) >$ ibuprofen  $(1.4) >$  ASA  $(1.0) >$  acetamin-<br>ophen  $(0.1) > R(-)$ ibuprofen  $(>0.1)$ . As all drugs were admin-<br>istered i.t. and showed a rapid onset of action, it appears<br>unlikel  $S(+)$ ibuprofen (1.7) > ibuprofen (1.4) > ASA (1.0) > acetamin-<br>ophen (0.1) >  $R(-)$ ibuprofen (>0.1). As all drugs were admin-<br>istered i.t. and showed a rapid onset of action, it appears<br>unlikely that the blood-brain barrie ophen  $(0.1) > R(-)$ ibuprofen  $(>0.1)$ . As all drugs were admin-<br>istered i.t. and showed a rapid onset of action, it appears systemlikely that the blood-brain barrier, significant acute metab-<br>olism or differential protein b istered 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) a olism or differential protein binding (given the low levels oprotein in the cerebrospinal fluid) are variables contributing this marked differential potency, as would occur after system administration.<br>In spite of their st

protein in the cerebrospinal fluid) are variables contributing<br>this marked differential potency, as would occur after systen<br>administration.<br>In spite of their structural diversity, these molecules des<br>nated as NSAID share this marked differential potency, as would occur after systemic<br>administration.<br>In spite of their structural diversity, these molecules desig-<br>thated as NSAID share the property of cyclooxygenase inhibi-<br>tion. A positive c administration.<br>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 the 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. antinoci nated 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 tion. A positive correlation of their relative potency in blocking<br>this enzyme with their i.t. antinociceptive potency would pro-<br>vide corollary support for the role of cyclooxygenase inhibition<br>in mediating their observed 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 crelative potency as cyclooxyge vide corollary support for the role of cyclooxygenase inhibition were  $\alpha$  in mediating their observed spinal actions. Assessment of their clearar relative potency as cyclooxygenase inhibitors in the commonly which used i Tradive potency as cyclooxygenase infinitions in the commonly used in vitro assay of bovine seminal vesicles reveals the relative increments in the commention (100)  $> S(+)$ ibuprofen (10)  $>$  ASA [(1.0)  $>$  acetaminophen (0 potency to be: flurbiprofen  $(200) >$  zomepirac  $(110) \geq$  indo-<br>methacin  $(100) > S(+)$ ibuprofen  $(16) >$ ibuprofen  $(10) >$ ASA p<br> $(1.0) >$  acetaminophen  $(0.3) > R(-)$ ibuprofen  $(0.02)$  (Adams *et* fal., 1975, 1976; Garcia-Rafanel methacin  $(100) > S(+)$ ibuprofen  $(16) >$ ibuprofen  $(10) >$ .<br>  $(1.0) >$  acetaminophen  $(0.3) > R(-)$ ibuprofen  $(0.02)$  (Adan<br> *al.*, 1975, 1976; Garcia-Rafanell and Forn, 1979; Pruss et<br>
1980; Shen, 1979). Comparable potencies hav  $d(1.0) >$  acetaminophen  $(0.3) > R(-)$ ibuprofen  $(0.02)$  (Adams *et in*, *i*, 1975, 1976; Garcia-Rafanell and Forn, 1979; Pruss *et al.*, the 1980; Shen, 1979). Comparable potencies have been reported 1 for the brain. Thus al., 1975, 1976; Garcia-Rafanell and Forn, 1979; Pruss *et al.*, to 1980; Shen, 1979). Comparable potencies have been reported 1 for the brain. Thus, the endogenous biosynthesis of prostaglandins by brain tissue slices in 1980; Shen, 1979). Comparable potencies have been reported L<br>for the brain. Thus, the endogenous biosynthesis of prostaglan-<br>dins by brain tissue slices *in vitro* showed the relative inhibitory m<br>potency to be: indometha for the brain. Thus, the endogenous biosynthesis of prostaglandins by brain tissue slices in vitro showed the relative inhibitory measured potency to be: indomethacin (400) > ASA (1) > acetaminophen clo (0.1) (Wolfe *et a* dins by brain tissue slices in vitro showed the relative inhibitory metaphore potency to be: indomethacin (400) > ASA (1) > acetaminophen close (0.1) (Wolfe *et al.*, 1976). Additionally, in vivo suppression of no prostag 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  $F_{2\alpha}$ , afte (0.1) (Wolfe *et al.*, 1976). Additionally, *in vivo* suppression of no<br>prostaglandin biosynthesis, measured as prostaglandin  $E_2$  and Co<br>prostaglandin  $F_{2\alpha}$ , after oral administration showed the rank of<br>order of pote prostaglandin  $F_{2a}$ , after oral administration showed the rank<br>order of potency of inhibition in rat brain to be indomethacin<br> $\geq$  flurbiprofen  $>$  ibuprofen  $>$  acetaminophen (Fitzpatrick and<br>Wynalda, 1976); whereas t prostaglandin  $F_{2\alpha}$ , after oral administration showed the rank<br>order of potency of inhibition in rat brain to be indomethacin<br> $\geq$  flurbiprofen  $>$  ibuprofen  $>$  acetaminophen (Fitzpatrick and<br>Wynalda, 1976); whereas order of potency of inhibition in rat brain to be indometh.<br>  $\geq$  flurbiprofen > ibuprofen > acetaminophen (Fitzpatrick<br>
Wynalda, 1976); whereas the post mortem increase in pro<br>
glandin  $E_2$  synthesis in mouse brain *ex*  $\geq$  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 Wynalda, 1976); whereas the post mortem increase in prosta-<br>glandin E<sub>2</sub> synthesis in mouse brain *ex vivo* after inhibition of<br>orally administered NSAID was demonstrated to be indometh-<br>acin (280)  $\ge$  zomepirac (115)  $>$ glandin  $E_2$  synthesis in mouse brain *ex vivo* after inhibition of a orally administered NSAID was demonstrated to be indometh-<br>acin (280)  $\ge$  zomepirac (115)  $>$  ibuprofen (16)  $>$  ASA (1.0)  $\ge$  i. acetaminophen (0.3 acin (280) ≥ zomepirac (115) > ibuprofen (16) > ASA (1.0) ≥ i.t. vs. i.p. potencies, the relative differences in potency observed<br>acetaminophen (0.3) (Ferrari *et al.*, 1990). These orders of by each route were consisten acetaminophen (0.3) (Ferrari *et al.*, 1990). These orders of by<br>potency are comparable to the rank order of potency observed di<br>in the present studies. In addition, considering those cycloox-<br>in<br>ygenase inhibition studies potency are comparable to the rank order of potency observed<br>in the present studies. In addition, considering those cycloox-<br>ygenase inhibition studies where complete dose-response curves<br>have been established, all agents in the present studies. In addition, considering those cycloox-<br>ygenase inhibition studies where complete dose-response curves<br>have been established, all agents examined show the same<br>maximum suppression, though their rela ygenase inhibition studies where complete dose-response curves<br>have been established, all agents examined show the same<br>maximum suppression, though their relative potencies differed.<br>Similarly, as noted above, examination have been established, all agents examined show the same<br>maximum suppression, though their relative potencies differed.<br>Similarly, as noted above, examination of the ability of these<br>agents in vivo to suppress the phase 2 maximum suppression, though their relative potencies differed.<br>Similarly, as noted above, examination of the ability of these<br>agents in vivo to suppress the phase 2 behavior revealed that<br>the active drugs resulted in a com 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 beha agents *in vivo* to suppress the phase 2 behavior revealed that an the active drugs resulted in a comparable, subtotal reduction be in the formalin-evoked flinch behavior. These parallels, al-<br>though not proving, are consi the active drugs resulted in a comparable, subtotal reduction be<br>in the formalin-evoked flinch behavior. These parallels, al-<br>though not proving, are consistent with the hypothesis that<br>cyclooxygenase inhibition is an inte in the formalin-evoked flinch behavior. These parallels, al-<br>though not proving, are consistent with the hypothesis that<br>cyclooxygenase inhibition is an intervening variable in the<br>spinal actions of these agents in modify though not proving, are consistent with the hypothesis that mexiclooxygenase inhibition is an intervening variable in the list spinal actions of these agents in modifying phase 2 of the aformalin response, and that failure spinal actions of these agen<br>formalin response, and that<br>produce a complete suppress<br>effect) does not reflect upon a<br>suppression of cyclooxygenas<br>The stereospecificity of th rmalin response, and that failure of all of the agents to oduce a complete suppression (*i.e.*, demonstrates a plateau fect) does not reflect upon an inability to produce a complete ppression of cyclooxygenase.<br>The stereo produce a complete suppression (*i.e.*, demonstrates a platea effect) does not reflect upon an inability to produce a complet suppression of cyclooxygenase.<br>The stereospecificity of the effects observed in the presen expe

effect) does not reflect upon an inability to produce a complete and<br>suppression of cyclooxygenase.<br>The stereospecificity of the effects observed in the present<br>experiments provides further supportive evidence for the spec suppression of cyclooxygenase.<br>
The stereospecificity of the effects observed in the present<br>
experiments provides further supportive evidence for the spec-<br>
In<br>
ificity of the observed spinal NSAID activity. Ibuprofen is 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 experiments provides further supportive evidence for the specificity of the observed spinal NSAID activity. Ibuprofen is a formpound exhibiting optical isomerism. *In vitro* studies show that only the  $S(+)$ enantiomers is that only the  $S(+)$ enantiomers is effective as a prostaglandin psynthesis inhibitor (Adams *et al.*, 1976). In vivo after oral we administration in rats and mice, stereoselectivity was not found rebecause conversion of th 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* administration in rats and mice, stereoselectivity was not found<br>because conversion of the inactive  $R(-)$ isomer to the active<br> $S(+)$  form can occur (Adams *et al.*, 1976). Importantly, in the<br>present studies, the *R*-isome because conversion of the inactive  $R(-)$ isomer to the active to  $S(+)$  form can occur (Adams *et al.*, 1976). Importantly, in the spresent studies, the  $R$ -isomer was without effect, emphasizing a that unlike the systemic

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not contain significant quantities of the converting enzyme and<br>
that the spinally administered agent does not reach significant<br>
systemic concentrations where such conversion could most Vol. 263<br>not contain significant quantities of the converting enzyme and<br>that the spinally administered agent does not reach significant<br>systemic concentrations where such conversion could most<br>certainly occur. Acetaminoph not contain significant quantities of the converting enzyme and<br>that the spinally administered agent does not reach significant<br>systemic concentrations where such conversion could most<br>certainly occur. Acetaminophen appear not contain significant quantities of the converting enzyme at that the spinally administered agent does not reach significa<br>systemic concentrations where such conversion could movertainly occur. Acetaminophen appears to b 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 per systemic concentrations where such conversion could most certainly occur. Acetaminophen appears to block the enzyme<br>only in an environment that is low in peroxides (*e.g.*, hypo-<br>thalamus), which might explain the poor ant certainly occur. Acetaminophen appears to block the enzyme<br>only in an environment that is low in peroxides (*e.g.*, hypo-<br>thalamus), which might explain the poor anti-inflammatory<br>capacity in inflamed tissue peripherally 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 aceta administration. capacity in inflamed tissue peripherally (Marshall *et al.*, 1987).<br>Importantly, however, we found acetaminophen to be active in the formalin test, with significant intrinsic activity after spinal administration.<br>Though c

Importantly, however, we found acetaminophen to be active in<br>the formalin test, with significant intrinsic activity after spinal<br>administration.<br>Though clearance studies have not been accomplished, the<br>duration of action o the formalin test, with significant intrinsic activity after spinal<br>administration.<br>Though clearance studies have not been accomplished, the<br>duration of action of the several spinally administered NSAID<br>were on the order o administration.<br>Though clearance studies have not been accomplished, the<br>duration of action of the several spinally administered NSAID<br>were on the order one would anticipate based on the kinetic<br>clearance of the agents fro 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 sp 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 irreversib 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 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 t which are known to produce an irreversible inhibition, such indomethacin flurbiprofen and ASA (Roth *et al.*, 1975; Kant and Hampton, 1978; Rome and Lands, 1975), did not show particularly longer analgesic effect than age 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-competit Lands, 1975). Tricularly longer analgesic effect than agents such as ibup<br>n, which produce a substrate-competitive, reversible inhi<br>nn of cyclooxygenase (Smith and Lands, 1971; Rome ands, 1975).<br>It is interesting to note that detailed r

fen, which produce a substrate-competitive, reversible inhibition of cyclooxygenase (Smith and Lands, 1971; Rome and Lands, 1975).<br>It is interesting to note that detailed reviews of the experimental literature have emphasi tion of cyclooxygenase (Smith and Lands, 1971; Rome and Lands, 1975).<br>
It is interesting to note that detailed reviews of the experimental literature have emphasized the dissociation of the cyclooxygenase-inhibiting activ Lands, 1975).<br>It is interesting to note that detailed reviews of the experi-<br>mental literature have emphasized the dissociation of the cy-<br>clooxygenase-inhibiting activity of these agents and their anti-<br>nociceptive action It is interesting to note that detailed reviews of the experi-<br>mental literature have emphasized the dissociation of the cy-<br>clooxygenase-inhibiting activity of these agents and their anti-<br>nociceptive actions (Brune *et a* mental literature have emphasized the dissociation of the cyclooxygenase-inhibiting activity of these agents and their anti-<br>nociceptive actions (Brune *et al.*, 1991; Clisshold, 1986; Mc-<br>Cormack and Brune, 1991). Compar clooxygenase-inhibiting activity of these agents and their anti-<br>nociceptive actions (Brune *et al.*, 1991; Clisshold, 1986; Mc-<br>Cormack and Brune, 1991). Comparison in the present studies<br>of the magnitude of the relative nociceptive actions (Brune *et al.*, 1991; Clisshold, 1986; Mc-Cormack and Brune, 1991). Comparison in the present studies of the magnitude of the relative potencies reveals a close rank ordering. It should be stressed, ho of the magnitude of the relative potencies reveals a close rank<br>ordering. It should be stressed, however, that the greatest<br>observed dissociation between analgesic and cyclooxygenase<br>activity is often noted in studies with of the magnitude of the relative potencies reveals a close rank<br>ordering. It should be stressed, however, that the greatest<br>observed dissociation between analgesic and cyclooxygenase<br>activity is often noted in studies with 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 ab 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 posit 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 r 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 consi 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 t 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.<br>drug delivery. Given the probable spinal actions of these agents<br>in this model and the absence of systematic data on brain levels<br>of the several agents, re drug delivery. Given the probable spinal actions of these agents<br>in this model and the absence of systematic data on brain levels<br>of the several agents, relative potency estimates after systemic<br>administration might be con in this model and the absence of systematic data on brain levels<br>of the several agents, relative potency estimates after systemic<br>administration might be considered potentially misleading.<br>Such differences in potency are f of the several agents, relative potency estimates after systemic<br>administration might be considered potentially misleading.<br>Such differences in potency are frequently observed between<br>systemic and i.t. or intracerebral dos administration might be considered potentially misleading.<br>Such differences in potency are frequently observed between<br>systemic and i.t. or intracerebral dose drug potency (see Herz<br>and Teschemacher, 1971, for example). Ne Such differences in potency are frequently observed betwee systemic and i.t. or intracerebral dose drug potency (see Her and Teschemacher, 1971, for example). Nevertheless, it should be emphasized that all of these agents systemic and i.t. or intracerebral dose drug potency (see H and Teschemacher, 1971, for example). Nevertheless, it sho<br>be emphasized that all of these agents may have a variety<br>effects which could influence neuronal funct and Teschemacher, 1971, for example). Nevertheless, it should<br>be emphasized that all of these agents may have a variety of<br>effects which could influence neuronal function. Thus, indo-<br>methacin, in low concentrations, will effects which could influence neuronal function. Thus, indo-<br>methacin, in low concentrations, will directly inhibit phospho-<br>lipase  $A_2$  in rabbit polymorphonuclear leukocytes (Kaplan *et*<br>*al.*, 1978). Indomethacin and methacin, 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 sys lipase A<sub>2</sub> in rabbit polymorphonuclear leukoc<br>*al.*, 1978). Indomethacin and ASA may also dir<br>cyclic AMP second messenger system by a<br>related to phospholipase or cyclooxygenase in<br>and Hampton, 1978; Dinnendahl *et al.*, 1 *Functional Mechanisms Underlying Actions of Spinal NSAID*<br>Functional Mechanisms Underlying Actions of Spinal NSAID<br>Functional Mechanisms Underlying Actions of Spinal NSAID<br>In spite of the limitation of the effect to the p

compound exhibiting optical isomerism. In vitro studies show the HP test, we do not believe that these results reflect upon a<br>that only the  $S(+)$ enantiomers is effective as a prostaglandin peripheral anti-inflammatory act lated to phospholipase or cyclooxygenase inhibition (Kantor<br>In Hampton, 1978; Dinnendahl *et al.*, 1973).<br>Inctional Mechanisms Underlying Actions of Spinal NSAID<br>In spite of the limitation of the effect to the phase 2 of t and Hampton, 1978; Dinnendahl *et al.*, 1973).<br>**Functional Mechanisms Underlying Actions of Spinal NSAID**<br>In spite of the limitation of the effect to the phase 2 of the<br>formalin response with little or no effect upon the p **Functional Mechanisms Underlying Actions of Spinal NSAID**<br>In spite of the limitation of the effect to the phase 2 of the<br>formalin response with little or no effect upon the phase 1 or<br>the HP test, we do not believe that t **Functional Mechanisms Underlying Actions of Spinal NSAID**<br>In spite of the limitation of the effect to the phase 2 of the<br>formalin response with little or no effect upon the phase 1 or<br>the HP test, we do not believe that In spite of the limitation of the effect to the phase 2 of the formal<br>in response with little or no effect upon the phase 1 or<br>the HP test, we do not believe that these results reflect upon a<br>peripheral anti-inflammatory a formalin response with little or no effect upon the phase 1 or<br>the HP test, we do not believe that these results reflect upon a<br>peripheral anti-inflammatory action of the agents. First, doses<br>which were in excess of 100 ti the HP test, we do not believe that these results reflect upon a peripheral anti-inflammatory action of the agents. First, doses<br>which were in excess of 100 times those active spinally were<br>required to produce the same effects after spinal injection. If<br>the spinal effects were mediated which were in excess of 100 times those active spinally were<br>required to produce the same effects after spinal injection. If<br>the spinal effects were mediated by a peripheral redistribution,<br>such differences in potency woul the spinal effects were mediated by a peripheral redistribution,<br>such differences in potency would not be anticipated. Moreover,<br>as noted above, conversion of the inactive isomer of ibuprofen<br>was not observed to occur, emp

1992<br>examination of the pathophysiology observed after intradermal<br>formalin injection reveals increased paw volume and the apformalin injection of the pathophysiology observed after intradermal<br>formalin injection reveals increased paw volume and the ap-<br>pearance of inflammatory cells at intervals considerably later 1992<br>examination of the pathophysiology observed after intradermal<br>formalin injection reveals increased paw volume and the ap-<br>pearance of inflammatory cells at intervals considerably later<br>than 2 to 3 hr (Wheeler-Aceto examination of the pathophysiology observed<br>formalin injection reveals increased paw voi<br>pearance of inflammatory cells at intervals of<br>than 2 to 3 hr (Wheeler-Aceto *et al.*, 1990).<br>If the action of these NSAID correlates amination of the pathophysiology observed after intradermal tractural transmultion reveals increased paw volume and the ap-<br>arance of inflammatory cells at intervals considerably later prom<br>an 2 to 3 hr (Wheeler-Aceto *et* 

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 ab pearance of inflammatory cells at intervals considerably later<br>than 2 to 3 hr (Wheeler-Aceto *et al.*, 1990). a<br>If the action of these NSAID correlates with their ability to<br>attenuate cyclooxygenase activity, and this effe than 2 to 3 hr (Wheeler-Aceto *et al.*, 1990).<br>If the action of these NSAID correlates with their ability to<br>attenuate cyclooxygenase activity, and this effect is not me-<br>diated by a peripheral action, then the explanatio If the action of these NSAID correlates with their ability t<br>attenuate cyclooxygenase activity, and this effect is not me<br>diated by a peripheral action, then the explanation for the<br>observation must include several points: 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 in diated by a peripheral action, then the explanation for this affections of the spinal cord must be increased by some property of the particular stimulus state induced by intradermal of formalin; and 2) spinal prostaglandin 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 m thesis within the spinal co<br>erty of the particular stin<br>formalin; and 2) spinal p<br>hyperesthetic state. Severa<br>will be considered below.<br>**Formation of prostag** ty of the particular stimulus state induced by intradermal<br>rmalin; and 2) spinal prostaglandins must act to induce a<br>peresthetic state. Several issues pertinent to this hypothesis<br>Il be considered below.<br>**Formation of pros** 

formalin; and 2) spinal prostaglandins must act to induce<br>hyperesthetic state. Several issues pertinent to this hypothe<br>will be considered below.<br>**Formation of prostaglandins.** It is commonly appreciat<br>that increasing the hyperesthetic state. Several issues pertinent to this hypothesis hyp<br>will be considered below. Lev<br>We Formation of prostaglandins. It is commonly appreciated oral<br>that increasing the intracellular Ca<sup>++</sup> will result in the will be considered below.<br> **Formation of prostaglandins.** It is commonly appreciated<br>
that increasing the intracellular Ca<sup>++</sup> will result in the activa-<br>
tion of phospholipase, leading to a subsequent increase in<br>
cytosol **Formation of prostaglandins.** It is commonly appreciated or that increasing the intracellular  $Ca^{++}$  will result in the activa-<br>tion of phospholipase, leading to a subsequent increase in an eytosolic arachidonic acid. In that increasing the intracellular  $Ca^{++}$  will result in the activion of phospholipase, leading to a subsequent increase cytosolic arachidonic acid. In the presence of molecular oxyg membrane-bound cyclooxygenase rapidly c 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, 1 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, membrane-bound cyclooxygenase rapidly converts such free<br>pools of arachidonate to subsequent elements in the prosta-<br>glandin cascade (see Leslie and Watkins, 1985). Membrane the<br>depolarization leading to an opening of volt pools of arachidonate to subsequent elements in the proglandin cascade (see Leslie and Watkins, 1985). Memb<br>depolarization leading to an opening of voltage-sensitive (channels (see Janis *et al.*, 1987), as well as the occ glandin cascade (see Leslie and Watkins, 1985). Membrane the<br>depolarization leading to an opening of voltage-sensitive Ca<sup>++</sup> app<br>channels (see Janis *et al.*, 1987), as well as the occupancy of a neu<br>variety of receptor ( depolarization leading to an opening of voltage-sensitive Ca<sup>+</sup><br>channels (see Janis *et al.*, 1987), as well as the occupancy of<br>variety of receptor ionophores, such as for the N-methyl-I-<br>aspartate receptor (MacDermott channels (see Janis *et al.*, 1987), as well as the occupancy of a new variety of receptor ionophores, such as for the N-methyl-D-<br>iconspartate receptor (MacDermott *et al.*, 1986), will lead to marked increases in cytoso variety of receptor ionophores, such as for the N-methyl-D-<br>aspartate receptor (MacDermott *et al.*, 1986), will lead to<br>marked increases in cytosolic Ca<sup>++</sup>. Although it appears prob-<br>able that at least some of the releas 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 marked increases in cytosolic Ca<sup>++</sup>. Although it appears pable that at least some of the release of prostanoids in l<br>may derive from neurons (Gonzales *et al.*, 1989), releasency prostaglandins has been demonstrated in ra able that at least some of the release of prostanoids in brain<br>may derive from neurons (Gonzales *et al.*, 1989), release of<br>prostaglandins has been demonstrated in rat spinal cord astro-<br>cyte cultures in response to treat 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  $P$  (M prostaglandins has been demonstrated in rat spinal cord astrocyte cultures in response to treatment with low (submicromo-<br>lar) concentrations of substance P (Marriott *et al.*, 1990).<br>Consistent with the effects of excitat cyte cultures in response to treatment with low (submicromolar) concentrations of substance P (Marriott *et al.*, 1990).<br>Consistent with the effects of excitation on prostaglandin syn-<br>thesis, increasing neuronal activity lar) concentrations of substance P (Marriott *et al.*, 1990).<br>Consistent with the effects of excitation on prostaglandin syn-<br>thesis, increasing neuronal activity has been shown to increase<br>the extracellular levels of pros Consistent with the effects of excitation on prostaglandin synthesis, increasing neuronal activity has been shown to increase<br>the extracellular levels of prostanoids in brain ventricular su-<br>perfusion *in vivo* (Romero *e* thesis, increasing neuronal activity has been shown to increase<br>the extracellular levels of prostanoids in brain ventricular su-<br>perfusion *in vivo* (Romero *et al.*, 1984; Navarro *et al.*, 1988,<br>1989), and in spinal perf 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.* perfusion *in vivo* (Romero *et al.*, 1984; Navarro *et al.*, 1988, p<br>1989), and in spinal perfusates after high threshold afferent d<br>stimulation (Ramwell *et al.*, 1966), increased potassium (Yaksh, s<br>1982) and noxious t 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 stimulation (Ramwell *et al.*, 1966), increased potassium (Yaksh, sensition 1982) and noxious thermal stimulation (Coderre *et al.*, 1990a). Sin recent work, it was found in lumbar spinal dialysates in rats Pro and primat 1982) and noxious thermal stimulation (Coderre *et al.*, 1990a).<br>In recent work, it was found in lumbar spinal dialysates in rats<br>and primates that there was a significant increase in prostanoid<br>release after protracted e In recent work, it was found in full bar significant increase in prostanoid<br>
release after protracted electrical activation of C fibers or in<br>
the presence of peripheral inflammation of the knee joint (L.<br>
S. Sorkin, unpub the presence of peripheral inflammation of the knee joint (L.<br>S. Sorkin, unpublished observation). These characteristics<br>would suggest that prostanoids might be optimally elaborated<br>under conditions associated with ongoing 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 would suggest that prostanoids might be optimally elaborated (video the respective paw reveals), measurement of neuronal outing flow from dorsal horn neurons excited by formalin injections into the respective paw reveals t flow from dorsal horr<br>into the respective pay<br>effect: an initial burst<br>the injection, followed<br>and Sullivan, 1987).<br>As post-treatment to the respective paw reveals two components to the formalin<br>fect: an initial burst lasting several minutes associated with<br>a e injection, followed by a second protracted phase (Dickenson et<br>d Sullivan, 1987).<br>As post-trea

effect: an initial burst lasting several minutes associated with<br>the injection, followed by a second protracted phase (Dickenson<br>and Sullivan, 1987).<br>As post-treatment with NSAIDs are able to diminish the<br>second phase resp the injection, followed by a second protracted phase (Dickenson<br>and Sullivan, 1987).<br>As post-treatment with NSAIDs are able to diminish the<br>second phase response, it appears likely that the processes of<br>spinal prostaglandi and Sullivan, 1987). The recommends are able to diminish the years second phase response, it appears likely that the processes of leustinal prostaglandin generation, release and clearance occur at cyter of the active prost As post-treatment with NSAIDs are able to diminish the ylat second phase response, it appears likely that the processes of leul spinal prostaglandin generation, release and clearance occur at cyta a high rate. The rapid t 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 convers spinal prostaglandin generation, release and clearance occur at<br>a high rate. The rapid turnover of the active prostanoids may<br>be metabolic (*i.e.*, by conversion of the active form to an<br>inactive product, such as occurs w be metabolic (*i.e.*, by conversion of the active form to an o inactive product, such as occurs with prostaglandin  $I_2$  being b converted rapidly to 6-keto-prostaglandin  $F_{1\alpha}$ , or by bulk difficion). Because of their inactive product, such as occurs with prostaglandin  $I_2$  being bee<br>converted rapidly to 6-keto-prostaglandin  $F_{1\alpha}$ , or by bulk dif-<br>fusion). Because of their lipid solubility, such extraparenchymal neu<br>clearance will converted rapidly to 6-keto-prostaglan<br>fusion). Because of their lipid solubility,<br>clearance will be very rapid. Thus, the<br>prostaglandin  $F_{2\alpha}$ , for example, after i.c<br>the order of 8 min (Hagen *et al.*, 1977).<br>**Effects** sion). Because of their fiplo solubility, such extraparenchym-<br>barance will be very rapid. Thus, the estimated half-life obtaglandin  $F_{2\alpha}$ , for example, after i.c.v. administration is o<br>e order of 8 min (Hagen *et al.* 

Spinal Action of NSAIDs 14<br>tracellular prostanoids can significantly influence the excite-<br>bility of neurons. Thus, by a receptor-mediated mechanis Spinal Action of NSAIDs 143<br>tracellular prostanoids can significantly influence the excita<br>bility of neurons. Thus, by a receptor-mediated mechanism<br>prostaglandins of the E series will stimulate adenylate cyclase **prostage Spinal Action of NSAIDs** 143<br>tracellular prostanoids can significantly influence the excita-<br>bility of neurons. Thus, by a receptor-mediated mechanism,<br>prostaglandins of the E series will stimulate adenylate cycl (see Axelrod et al., 1988). As will be discussed below, the local tracellular prostanoids can significantly influence the excita-<br>bility of neurons. Thus, by a receptor-mediated mechanism,<br>prostaglandins of the E series will stimulate adenylate cyclase<br>and activate protein kinase A *via* bility 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 prostaglandins of the E series will stimulate adenylate cyclase<br>and activate protein kinase A *via* an increase in cyclic AMP<br>(see Axelrod *et al.*, 1988). As will be discussed below, the local<br>application of lipidic acids 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 (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 behaviora application of lipidic acids can induce an augmented release<br>afferent neurotransmitters (Geppetti *et al.*, 1991; Nicol *et*<br>1992). The behavioral relevance of these membrane events<br>emphasized by the observation that the afferent neurotransmitters (Geppetti *et al.*, 1991; Nicol *et a* 1992). The behavioral relevance of these membrane events emphasized by the observation that the i.t. injection of a variet of cyclooxygenase products, such 1992). The behavioral relevance of these membrane events is<br>emphasized by the observation that the i.t. injection of a variety<br>of cyclooxygenase products, such as prostaglandin  $E_2$ , prosta-<br>glandin  $F_{2\alpha}$  or prostagla emphasized by the observation that the i.t. injection of a variety<br>of cyclooxygenase products, such as prostaglandin  $E_2$ , prosta-<br>glandin  $F_{2\alpha}$  or prostaglandin  $D_2$  results in a dose-dependent<br>hyperalgesia (Ferreira or cyclooxygenase products, such as prostaglandin  $E_2$ , prostaglandin  $F_{2a}$  or prostaglandin  $D_2$  results in a dose-dependent hyperalgesia (Ferreira *et al.*, 1978; Ferreira, 1983; Taiwo and Levine, 1986; Uda *et al.* 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 a 1987). E have been reported to be antinociceptive in the formalin test<br>and in the acetic acid-induced writhing test (Drower *et al.*,<br>1987).<br>**Mechanisms of facilitatory actions of prostaglandins.**<br>As emphasized above, afferent i

and in the acetic acid-induced writhing test (Drower *et al.*, 1987).<br> **Mechanisms of facilitatory actions of prostaglandins.**<br>
As emphasized above, afferent input can evoke an increase in<br>
the extracellular levels of the As emphasized above, afferent input can evoke an increase in 1987). Mechanisms of facilitatory actions of prostaglandins.<br>As emphasized above, afferent input can evoke an increase in<br>the extracellular levels of the several prostanoids, and it is<br>apparent that these lipidic acids can Mechanisms of facilitatory actions of prostagland<br>As emphasized above, afferent input can evoke an increase<br>the extracellular levels of the several prostanoids, and<br>apparent that these lipidic acids can exert a direct effe As emphasized above, afferent input can evoke an increase in<br>the extracellular levels of the several prostanoids, and it is<br>apparent that these lipidic acids can exert a direct effect upon<br>neuronal function. The mechanism e extracellular levels of the several prostanoids, and parent that these lipidic acids can exert a direct effect in uronal function. The mechanism underlying this augment are not understood, but three alternatives appear l

apparent that these lipidic acids can exert a direct effect upon<br>neuronal function. The mechanism underlying this augmenta-<br>tion are not understood, but three alternatives appear likely.<br>First, prostanoids may directly au neuronal function. The mechanism underlying this augmentation are not understood, but three alternatives appear likely.<br>First, prostanoids may directly augment, by a receptor-me-<br>diated effect, the release of excitatory n tion are not understood, but three alternatives appear likely.<br>First, prostanoids may directly augment, by a receptor-me-<br>diated effect, the release of excitatory neurotransmitter. Vasko<br>and colleagues have shown that pro First, prostanoids may directly augment, by a receptor-ine-<br>diated effect, the release of excitatory neurotransmitter. Vasko<br>and colleagues have shown that prostaglandin  $E_2$  will augment<br>a voltage-sensitive Ca<sup>++</sup> curre and colleagues have shown that prostaglandin  $E_2$  will augmen<br>a voltage-sensitive  $Ca^{++}$  current in dorsal root ganglion ce<br>cultures and facilitate the depolarization-evoked release of th<br>C afferent neurotransmitter subs a voltage-sensitive  $Ca^{++}$  current in dorsal root ganglion cell<br>cultures and facilitate the depolarization-evoked release of the<br>C afferent neurotransmitter substance P (Nicol *et al.*, 1992).<br>Arachidonic acid has been sh cultures and facilitate the depolarization-evoked release of the C afferent neurotransmitter substance P (Nicol *et al.*, 1992).<br>Arachidonic acid has been shown to evoke calcitonin generalated peptide (CGRP) release from c C afferent neurotransmitter substance P (Nicol *et al.*, 1992).<br>Arachidonic acid has been shown to evoke calcitonin gene-<br>related peptide (CGRP) release from capsaicin-sensitive pri-<br>mary afferents, and the release was inh Arachidonic acid has been shown to evoke calcitonin generelated peptide (CGRP) release from capsaicin-sensitive primary afferents, and the release was inhibited in the presencof indomethacin (Geppetti *et al.*, 1991). Sim related peptide (CGRP) release from capsaicin-sensitive pri-<br>mary afferents, and the release was inhibited in the presence<br>of indomethacin (Geppetti *et al.*, 1991). Similarly, infusion of<br>prostaglandin I<sub>2</sub> and prostaglan mary anerents, and the release of indomethacin (Geppetti et prostaglandin  $I_2$  and prostagla dependent manner, suggesting sensory neuropeptide release.<br>Second, prostanoids may a prostaglandin  $I_2$  and prostaglandin  $E_2$  released CGRP in a dose-<br>dependent manner, suggesting that a prostanoid can mediate<br>sensory neuropeptide release.<br>Second, prostanoids may serve to diminish an inhibition.<br>Prosta

prostaglandin  $I_2$  and prostaglandin  $E_2$  released CGRP in a dose-<br>dependent manner, suggesting that a prostanoid can mediate<br>sensory neuropeptide release.<br>Second, prostanoids may serve to diminish an inhibition.<br>Prosta dependent manner, suggesting that a prostanoid can mediate<br>sensory neuropeptide release.<br>Second, prostanoids may serve to diminish an inhibition.<br>Prostaglandins of the E series act presynaptically to inhibit<br>noradrenalin r sensory neuropeptide release.<br>Second, prostanoids may serve to diminish an inhibition.<br>Prostaglandins of the E series act presynaptically to inhibit<br>noradrenalin release (Bergström *et al.*, 1973; Hedqvist, 1973).<br>The hyp Second, prostanoids may serve to diminish an inhibition.<br>Prostaglandins of the *E* series act presynaptically to inhibit<br>noradrenalin release (Bergström *et al.*, 1973; Hedqvist, 1973).<br>The hyperalgesic effect of i.t. pros 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), suggest mine or the catecholaminergic neurotoxin 6-hydroxydopamine inhibition. ine or the catecholaminergic neurotoxin 6-hydroxydopamine<br>'aiwo and Levine, 1988), suggesting that afferent-evoked re-<br>ase of prostanoids may inhibit a reflex-evoked bulbospinal<br>hibition.<br>Third, in addition to the evidence

(Taiwo and Levine, 1988), suggesting that afferent-evoked release of prostanoids may inhibit a reflex-evoked bulbospinal<br>inhibition.<br>Third, in addition to the evidence for prostaglandin release<br>into the extracellular space lease of prostanoids may inhibit a reflex-evoked bulbospinal<br>inhibition.<br>Third, in addition to the evidence for prostaglandin release<br>into the extracellular space to act as neurohormones, these<br>agents play an intracellula inhibition.<br>
Third, in addition to the evidence for prostaglandin release<br>
into the extracellular space to act as neurohormones, these<br>
agents play an intracellular role in signal transduction (Axelrod<br> *et al.*, 1988). Pr Third, in addition to the evidence for prostaglandin releas<br>into the extracellular space to act as neurohormones, thes<br>agents play an intracellular role in signal transduction (Axelro<br>et al., 1988). Prostaglandins may dir 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 ac 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 aden-<br>ylate cyclase activation ( *et al.*, 1988). Prostaglandins may directly (*i.e.*, not through a receptor) mediate GTPase activation not associated with aden-<br>ylate cyclase activation (Bitonti *et al.*, 1980). Prostaglandin E<sub>2</sub>,<br>leukotrienes and lip receptor) mediate GTPase activation not associated with ade ylate cyclase activation (Bitonti *et al.*, 1980). Prostaglandin leukotrienes and lipoxine A have been demonstrated to increacy tosolic Ca<sup>++</sup> independent of inos yiate cyclase activation (Bitonii et at., 1560). Frostagianum E2,<br>leukotrienes and lipoxine A have been demonstrated to increase<br>cytosolic Ca<sup>++</sup> independent of inositol phosphate<sub>3</sub> formation<br>(Wolf *et al.*, 1986; Halush beta shownless and positive A nave been demonstrated to increase<br>cytosolic Ca<sup>++</sup> independent of inositol phosphate<sub>3</sub> formation<br>(Wolf *et al.*, 1986; Halushka and Burch, 1984). For the metab-<br>olites produced by the lipoxy (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 olites 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 Gr been shown that they can affect the activity of membrane ion<br>channels and protein kinases and are possibly involved in<br>neurotransmitter release (Piomelli and Greengard, 1990). The<br>arachidonic acid metabolites from the 5-li

**144 Malmberg and Yaksh**<br>phopholipase  $A_2$  inhibitors and with lipoxygenase inhibitors<br>but not with the cyclooxygenase inhibitor indomethacin (Piom **144 Malmberg and Yaksh**<br>phopholipase  $A_2$  inhibitors and with lipoxygenase inhibito<br>but not with the cyclooxygenase inhibitor indomethacin (Pior<br>elli *et al.*, 1987). Given the cyclooxygenase selectivity of t **144 Malmberg and Yaksh**<br>phopholipase  $A_2$  inhibitors and with lipoxygenase inhibitors, po<br>but not with the cyclooxygenase inhibitor indomethacin (Piom-<br>elli *et al.*, 1987). Given the cyclooxygenase selectivity of the phopholipase  $A_2$  inhibitors and with lipoxygenase inhibitors,<br>but not with the cyclooxygenase inhibitor indomethacin (Piom-<br>elli *et al.*, 1987). Given the cyclooxygenase selectivity of the<br>agents used in the present st phopholipase  $A_2$  inhibitors and with lipoxygenase inhibitors,<br>but not with the cyclooxygenase inhibitor indomethacin (Piom-<br>elli *et al.*, 1987). Given the cyclooxygenase selectivity of the<br>agents used in the present st but not with the cycloox<br>elli *et al.*, 1987). Given<br>agents used in the prese:<br>on nociception mediate<br>remains to be assessed.<br>As outlined above, tl ii *et al.*, 1987). Given the cyclooxygenase selectivity of the ents used in the present study, the role of the central changes a nociception mediated by neuronal lipoxygenase products mains to be assessed.<br>As outlined ab

agents used in the present study, the role of the central changes<br>on nociception mediated by neuronal lipoxygenase products<br>remains to be assessed.<br>As outlined above, the current body of data suggests that<br>under conditions on nociception mediated by neuronal lipoxygenase products in<br>remains to be assessed. <br>As outlined above, the current body of data suggests that p<br>under conditions of a modestly protracted period of stimulation lo<br>of small Femants to be assessed.<br>
As outlined above, the current body of data suggests that pro-<br>
under conditions of a modestly protracted period of stimulation<br>
of small afferents, there will be an increase in the formation of<br>
p under conditions of a modestly protracted period of stimulation<br>of small afferents, there will be an increase in the formation of<br>prostaglandins. Extracellular movement of these lipidic acids<br>would then, by specific recept of small afferents, there will be an increase in the formation of<br>prostaglandins. Extracellular movement of these lipidic acids<br>would then, by specific receptor-mediated mechanisms, act to<br>augment the excitability of the c prostaglandins. Extracellular movement of these lipidic acids a would then, by specific receptor-mediated mechanisms, act to a augment the excitability of the cells to the ongoing afferent riput by facilitating the release 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 dire augment the excitability of the cells to the ongoing afferent<br>input by facilitating the release of neurotransmitter, perhaps<br>from the primary afferent, by a direct postsynaptic action or<br>by intracellular mechanisms wherein from the primary afferent, by a direct postsynaptic action or vations provide grounds for considering that at least some of<br>by intracellular mechanisms wherein changes in ion channel the events brought into play by the loc from the primary afferent, by a direct postsynaptic action or<br>by intracellular mechanisms wherein changes in ion channel<br>permeability lead to reduced transmembrane voltage stability.<br>Based on these conditions, cyclooxygena Based on these conditions, cyclooxygenase inhibitors would of a cyclooxygenase-mediated central facilitation. In recent<br>serve to alter the behavioral consequences of a noxious stimulus studies, we have shown that the i.t. permeability lead to reduced transmembrane voltage stability.<br>Based on these conditions, cyclooxygenase inhibitors would<br>serve to alter the behavioral consequences of a noxious stimulus<br>only under those stimulus conditions Based on these conditions, cyclooxygenase inhibitors would<br>serve to alter the behavioral consequences of a noxious stimulus<br>only under those stimulus conditions which bring the above<br>characteristics into play. The plateau serve to alter the behavioral consequences of a noxious stimulus<br>only under those stimulus conditions which bring the above<br>characteristics into play. The plateau in the antinociceptive<br>effects observed after spinal NSAID only under those stimulus conditions which bring the above veharacteristics into play. The plateau in the antinociceptive effects observed after spinal NSAID administration likely reflects upon the fact that the prostanoi characteristics into play. The plateau in the effects observed after spinal NSAID administers flects upon the fact that the prostanoids since regulate the excitability of the membrane and control its sensitivity to the af flects upon the fact that the prostanoids simply serve to up-<br>regulate the excitability of the membrane and not absolutely<br>control its sensitivity to the afferent barrage.<br>**Pharmacology of the Formalin Test** gulate the excitability of the membrane and not absolutely<br>ntrol its sensitivity to the afferent barrage.<br>**armacology of the Formalin Test**<br>Formalin injected s.c. results in an immediate and intense rease in the spontaneou

control its sensitivity to the afferent barrage.<br> **Pharmacology of the Formalin Test**<br>
Formalin injected s.c. results in an immediate and intens<br>
increase in the spontaneous activity of C fiber afferents, evok<br>
ing a fast **Formalin injected s.c. results in an immediate and intense**<br>
increase in the spontaneous activity of C fiber afferents, evoking a fast transmitter monosynaptic input to projection neurons<br>
in the spinal cord, followed by Formalin injected s.c. results in an immediate and intense<br>increase in the spontaneous activity of C fiber afferents, evok-<br>ing a fast transmitter monosynaptic input to projection neurons<br>in the spinal cord, followed by a 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 b ing a fast transmitter monosynaptic input to projection neurons<br>in the spinal cord, followed by a second-phased afferent barrage<br>(Heapy *et al.*, 1987). This biphasic excitatory input is mirrored<br>in the activation of wide in the spinal cord, followed by a second-phased afferent barrage<br>
(Heapy *et al.*, 1987). This biphasic excitatory input is mirrored<br>
in the activation of wide dynamic range dorsal horn neurons<br>
(Dickenson and Sullivan, 1 (Heapy *et al.*, 1987). This biphasic excitatory input is mirrored horing the activation of wide dynamic range dorsal horn neurons the (Dickenson and Sullivan, 1987). Protracted (sec to min) C fiber clout not A fiber) sti in the activation of wide dynamic range dorsal horn neurons the (Dickenson and Sullivan, 1987). Protracted (sec to min) C fiber clout not A fiber) stimulation, aside from its direct excitatory effect, evokes a central faci (Dickenson and Sullivan, 1987). Protracted (sec to min) C fiber (but not A fiber) stimulation, aside from its direct excitato effect, evokes a central facilitated state in which the widynamic range cell shows an exaggerate (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, effect, evokes a central facilitated state in which the wide<br>dynamic range cell shows an exaggerated discharge to a given<br>C fiber input ("wind-up"; Mendell, 1966). Of particular signif-<br>icance, i.t. injections of opiates a dynamic range cell shows an exaggerated discharge to a given cheap.<br>C fiber input ("wind-up"; Mendell, 1966). Of particular significance, i.t. injections of opiates are able to suppress the aug-<br>mented response only at dos icance, i.t. injections of opiates are able to suppress the mented response only at doses which block the initial C<br>burst. This observation corresponds to the ability of opiat<br>block the release of primary afferent peptides mented response only at doses which block the initial  $C$  iber<br>burst. This observation corresponds to the ability of opiates to<br>block the release of primary afferent peptides (see Sabbe and<br>Yaksh, 1990). In contrast, antag block the release of primary afferent peptides (see Sabbe and Yaksh, 1990). In contrast, antagonists for the N-methyl-D-<br>aspartate receptor fail to block the initial evoked activity, but<br>prevent the development of the seco Yaksh, 1990). In contrast, antagonists for the N-methyl-D-<br>aspartate receptor fail to block the initial evoked activity, but<br>prevent the development of the second facilitated state (*i.e.*,<br>wind-up) (Dickenson and Sulliva aspartate receptor fail to block the initial evoked activity, but<br>prevent the development of the second facilitated state (*i.e.*,<br>wind-up) (Dickenson and Sullivan, 1990). These spinal re-<br>sponses appear to parallel the bi prevent the development of the second facilitated state (*i.e.*, 1 wind-up) (Dickenson and Sullivan, 1990). These spinal responses appear to parallel the biphasic behavior observed after position is imilar injections of f wind-up) (Dickenson and Sullivan, 1990). These spinal re-<br>sponses appear to parallel the biphasic behavior observed after<br>similar injections of formalin in the unanesthetized rat. In-<br>trathecal opioids administered before sponses appear to parallel the biphasic behavior observed after phase esimilar injections of formalin in the unanesthetized rat. In-<br>trathecal opioids administered before phase 1 can completely and Y<sub>i</sub><br>suppress in a dosesimilar injections of formalin in the unanesthetized rat. In-<br>trathecal opioids administered before phase 1 can completely and<br>suppress in a dose-dependent fashion the first and second phase<br>response. Spinal N-methyl-D-asp trathecal opiolos administered before phase 1 can completely and<br>suppress in a dose-dependent fashion the first and second phase<br>response. Spinal N-methyl-D-aspartate antagonists at low<br>doses have minimal effect upon phase response. Spinal N-methyl-D-aspartate antagonists at low doses have minimal effect upon phase 1 and produce only a 7 to 80% reduction in the phase 2 response. Interestingly, the administration of opioids after the phase 1 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 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-<br>treatment with N-methyl-D-asparta administration of opioids after the phase 1 but before the phase 2 response readily blocks the phase 2 response, but such post-<br>treatment with N-methyl-D-aspartate antagonists is without<br>effect (Yamamoto and Yaksh, 1992; T 2 response readily blocks the phase 2 response, but such potent treatment with N-methyl-D-aspartate antagonists is with effect (Yamamoto and Yaksh, 1992; T. J. Coderre, person communication). Identical results have been ob treatment with N-methyl-D-aspartate antagonists is without for<br>effect (Yamamoto and Yaksh, 1992; T. J. Coderre, personal ger<br>communication). Identical results have been observed with i.t. has<br>antagonists for the neurokinin 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 communication). Identical results have been observed with i.t. hands and a relation of the neurokinin-1 tachykinin receptor (Yama-<br>moto and Yaksh, 1991). Importantly, in the present study, we hidemonstrated that NSAID were antagonists for the neurokinin-1 tachykinin receptor (Yama<br>moto and Yaksh, 1991). Importantly, in the present study, we<br>demonstrated that NSAID were equally effective in suppressing<br>the second phase after both pre- and pos

Vol. 263<br>porting the hypothesis that afferent C fiber barrages generated<br>by the formalin stimulus, leading to a spinal release of glutamate (Skilling et al., 1988) and substance P (Kuraishi et al., Vol. 263<br>porting the hypothesis that afferent C fiber barrages generated<br>by the formalin stimulus, leading to a spinal release of gluta-<br>mate (Skilling *et al.*, 1988) and substance P (Kuraishi *et al.*,<br>1989), among other porting the hypothesis that afferent C fiber barrages generated<br>by the formalin stimulus, leading to a spinal release of gluta-<br>mate (Skilling *et al.*, 1988) and substance P (Kuraishi *et al.*,<br>1989), among other mediator porting the hypothesis that afferent C fiber barrages generated<br>by the formalin stimulus, leading to a spinal release of gluta-<br>mate (Skilling *et al.*, 1988) and substance P (Kuraishi *et al.*,<br>1989), among other mediator by the formalin stimulus, leading to a spinal release of gluta-<br>mate (Skilling *et al.*, 1988) and substance P (Kuraishi *et al.*,<br>1989), among other mediators, may give rise to an increase in<br>intracellular Ca<sup>++</sup>, an inc mate (Skining et al., 1966) and substance I (Kutaisin et al., 1989), among other mediators, may give rise to an increase in intracellular Ca<sup>++</sup>, an increase in cytosolic arachidonate and the subsequent generation of diffu intracellular  $Ca^{++}$ , an increase in cytosolic arachidonate and<br>the subsequent generation of diffusable prostanoids. These<br>prostanoids may then evoke a facilitated excitability of the<br>local neuronal population by mechanis Intracentiar Ca  $\alpha$ , an increase in cycosonc arachitomate and<br>the subsequent generation of diffusable prostanoids. These<br>prostanoids may then evoke a facilitated excitability of the<br>local neuronal population by mechanism prostanoids may then evoke a facilitated excitability of the local neuronal population by mechanisms discussed above.<br>Interestingly, i.t. substance P has been shown to produce mild agitation behavior and a subsequent hyper local neuronal population by mechanisms discussed above.<br>Interestingly, i.t. substance P has been shown to produce mild<br>agitation behavior and a subsequent hyperalgesia (Moochhala<br>and Sawynok, 1984; Yasphal et al., 1982). Interestingly, i.t. substance P has been shown to produce minitation behavior and a subsequent hyperalgesia (Moochhand Sawynok, 1984; Yasphal et al., 1982). Hunskaar et al. (198 reported that the agitation behavior of i.t. agitation behavior and a subsequent hyperalgesia (Moochhala<br>and Sawynok, 1984; Yasphal *et al.*, 1982). Hunskaar *et al.* (1985)<br>reported that the agitation behavior of i.t. substance P can be<br>attenuated by systemic ASA an 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 consi reported that the agitation behavior of i.t. substance P can be<br>attenuated by systemic ASA and acetaminophen. Such obser-<br>vations provide grounds for considering that at least some of<br>the events brought into play by the lo 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 m vations provide grounds for considering that at least some of<br>the events brought into play by the local activation of receptors<br>postsynaptic to the primary afferent may involve the generation<br>of a cyclooxygenase-mediated c postsynaptic to the primary afferent may involve the generation of a cyclooxygenase-mediated central facilitation. In restudies, we have shown that the i.t. injection of NSAID in d which block the phase 2 of the formalin t 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 studies, we have shown that the i.t. injection of NSAID in doses<br>which block the phase 2 of the formalin test will also block the<br>thermal hyperesthesia occurring secondary to i.t. N-methyl-D-<br>aspartate and substance P. Thi 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 identic ermal hyperesthesia occurring secondary to i.t. N-methyl-Ipartate and substance P. This effect is stereospecific an hieved by doses which are identical to those which diminise phase 2 of the formalin test (Malmberg and Yak

postsynaptic to the primary afferent may involve the generation<br>studies, we have shown that the i.t. injection of NSAID in doses<br>which block the phase 2 of the formalin test will also block the<br>thermal hyperesthesia occur aspartate and substance P. This effect is stereospecific and<br>achieved by doses which are identical to those which diminish<br>the phase 2 of the formalin test (Malmberg and Yaksh, 1992).<br>Although it appears certain that the o achieved by doses which are identical to those which diminish<br>the phase 2 of the formalin test (Malmberg and Yaksh, 1992).<br>Although it appears certain that the ongoing state of facili-<br>tation may be initiated by the stimul the phase 2 of the formal in test (Malmberg and Yaksh, 1992).<br>Although it appears certain that the ongoing state of facili-<br>tation may be initiated by the stimulatory effects of substance<br>P and glutamate, the continued act Although it appears certain that the ongoing state of facili-<br>tation may be initiated by the stimulatory effects of substance<br>P and glutamate, the continued activity of the respective N-<br>methyl-D-aspartate and neurokinin-1 tation may be initiated by the stimulatory effects of substance P and glutamate, the continued activity of the respective N-<br>methyl-D-aspartate and neurokinin-1 receptors is not necessary<br>for the maintenance of the spinal P and glutamate, the continued activity of the respective N<br>methyl-D-aspartate and neurokinin-1 receptors is not necessary<br>for the maintenance of the spinal facilitated state, as indicated<br>by the failure of post-treatment methyl-D-aspartate and neurokinin-1 receptors is not necessary<br>for the maintenance of the spinal facilitated state, as indicated<br>by the failure of post-treatment to effectively blunt the second<br>phase response. We believe i for the maintenance of the spinal facilitated state, as indicated<br>by the failure of post-treatment to effectively blunt the second<br>phase response. We believe it likely that the initial stimulation,<br>however, yields an incre by the failure of post-treatment to effectively blunt the second<br>phase response. We believe it likely that the initial stimulation,<br>however, yields an increase in cytosolic arachidonic acid and<br>the initiation of an ongoing phase response. We believe it likely that the initial stimulation however, yields an increase in cytosolic arachidonic acid an the initiation of an ongoing and prolonged generation of cyclooxygenase intermediaries which se 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 t the initiation of an ongoing and prolonged generation of cy-<br>clooxygenase intermediaries which serve to exert a facilitatory<br>effect upon the excitability within the dorsal horn. It is inter-<br>esting to note the similarities 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 B esting to note the similarities between synaptic plast<br>changes of long-term potentiation in the hippocampus after<br>afferent barrage (Collingridge and Bliss, 1987), where are<br>donic acid and nitric oxide are proposed to serve 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 activat afferent barrage (Collingridge and Bliss, 1987), where a donic acid and nitric oxide are proposed to serve as inteary mechanisms induced by the activation of the N-methyl-paspartate receptor (Williams *et al.*, 1989; Gart donic 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 tran ary mechanisms induced by the activation of the N-methyl-D-<br>aspartate receptor (Williams *et al.*, 1989; Garthwaite *et al.*,<br>1988), and spinal nociceptive transmission. N-methyl-D-aspar-<br>tate activation results in a Ca<sup>++</sup> aspartate receptor (Williams *et al.*, 1989; Garthwaite *et a* 1988), and spinal nociceptive transmission. N-methyl-D-aspatate activation results in a Ca<sup>++</sup>-dependent increase in cyclGMP through the production of nitric 1988), and spinal nociceptive transmission. N-methyl-D-aspartate activation results in a Ca<sup>++</sup>-dependent increase in cyclic GMP through the production of nitric oxide (Garthwaite *et al.*, 1988). In recent studies, we hav tate 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 abl 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 th 1988). In recent studies, we<br>itors of nitric oxide are a<br>phase of the formalin test is<br>N-methyl-D-aspartate in a<br>and Yaksh, in preparation **COLOGE STATE CALCE AT SPACE CONSTRANCE IN A SPACE CONSTRANCE IN A SPACE CONSTRANCE CONSTRANCE CONSTRANCE TO THE C** -methyl-D-aspartate in a dose-dependent manner (Malmberg<br>id Yaksh, in preparation).<br>**inical Significance**<br>The developing scenario outlined above offers insights into<br>e probable organization of the pain state which develops

and Yaksh, in preparation).<br> **Clinical Significance**<br>
The developing scenario outlined above offers insights into<br>
the probable organization of the pain state which develops in<br>
the immediate period after the generation of Clinical Significance<br>The developing scenario outlined above offers insights into<br>the probable organization of the pain state which develops in<br>the immediate period after the generation of a C fiber barrage.<br>It has now bee **Ith Character Character Character 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. I** The developing scenario outlined above offers insights into<br>the probable organization of the pain state which develops in<br>the immediate period after the generation of a C fiber barrage.<br>It has now been demonstrated in beh the probable organization of the pain state which develops in<br>the immediate period after the generation of a C fiber barrage.<br>It has now been demonstrated in behavioral studies of the<br>formalin response that the second late the immediate period after the generation of a C fiber barrage.<br>It has now been demonstrated in behavioral studies of the<br>formalin response that the second late phase depends upon the<br>generation of an initial afferent bar It has now been demonstrated in behavioral studies of t<br>formalin response that the second late phase depends upon t<br>generation of an initial afferent barrage. Coderre *et al.* (1990<br>have shown that local anesthetics given formalin response that the second late phase depends upon<br>generation of an initial afferent barrage. Coderre *et al.* (199<br>have shown that local anesthetics given before and shortly a<br>the formalin will prevent the facilita generation of an initial afferent barrage. Coderre et *ut.* (15500)<br>have shown that local anesthetics given before and shortly after<br>the formalin will prevent the facilitation, reflecting a mecha-<br>nism perhaps mediated by 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 an

1992<br>operative analgesic requirements (McQuay *et al.*, 1988). Simi-<br>larly, the observation that cyclooxygenase inhibitors may serve<br>to markedly diminish the second phase component of the pain Speciality dialigy of determining the cyclos of the cyclos of the cyclos of the passe. Biochem. Pharmacol. 22: 223-2228, 1973.<br>
Larly, the observation that cyclooxygenase inhibitors may serve the pain DROWER, E. J., STAPEL 1992<br>
operative analgesic requirements (McQuay *et al.*, 1988). Simi-<br>
larly, the observation that cyclooxygenase inhibitors may serve  $\sum_{n=1}^{\infty}$ <br>
to markedly diminish the second phase component of the pain<br>
response operative analgesic requirements (McQuay *et al.*, 1988). Simi-<br>larly, the observation that cyclooxygenase inhibitors may serve<br>to markedly diminish the second phase component of the pain<br>response appears to reflect upon t 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 presponse appears to reflect upon t larly, the observation that cyclooxygenase inhibitors may serve<br>to markedly diminish the second phase component of the pain<br>response appears to reflect upon the growing appreciation of<br>the relative potency of NSAID in mana to markedly diminish the second phase component of the pain<br>response appears to reflect upon the growing appreciation of<br>the relative potency of NSAID in managing at least certain<br>types of postoperative pain. Thus, concur 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 postsur the relative potency of NSAID in managing at least certatypes of postoperative pain. Thus, concurrent administratiof certain NSAID clearly reduces the amount of opiate requir for the complete relief of the postsurgical pai of certain NSAID clearly reduces the amount of opiate required<br>for the complete relief of the postsurgical pain state (for ex-<br>ample, Reasbeck *et al.*, 1982; Gillies *et al.*, 1987). Importantly,<br>in recent studies, we hav for the complete relief of the postsurgical pain state (for  $\alpha$  ample, Reasbeck *et al.*, 1982; Gillies *et al.*, 1987). Important in recent studies, we have observed, using isobolographic p tocols, that concurrent spinal ample, 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 yie in recent studies, we have observed, using isobolographic pro-<br>tocols, that concurrent spinal administration of morphine and<br>NSAID in rats will indeed yield a remarkable synergy (Malm-<br>that a powerful analgesia can be obt tocols, 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 NSAID in rats will indeed yield a remarkable synergy (Malm<br>berg and Yaksh, unpublished observations). The demonstration<br>that a powerful analgesia can be obtained in humans in the<br>absence of changes in inflammatory signs an berg and Yaksh, unpublished observations). The demonstration<br>that a powerful analgesia can be obtained in humans in the<br>absence of changes in inflammatory signs and that the clinical<br>analgesic potency of NSAID do not covar that a powerful analgesia can be obtained in humans in absence of changes in inflammatory signs and that the clini<br>analgesic potency of NSAID do not covary with their are implammatory effects (McCormack and Brune, 1991) is analgesic potency of NSAID do not covary with their anti-<br>inflammatory effects (McCormack and Brune, 1991) is in ac-<br>cord with the hypothesis that there are important NSAID-<br>sensitive processes relevant to pain processing analgesic potency of NSAID do not covary with their anti-<br>inflammatory effects (McCormack and Brune, 1991) is in ac-<br>cord with the hypothesis that there are important NSAID-<br>sensitive processes relevant to pain processing inflammatory effects (McCormack and Brune, 1991) is in accord with the hypothesis that there are important NSAID-<br>sensitive processes relevant to pain processing that are not<br>related to changes in peripheral inflammatory cord with the hypothesis that there are important NSAID sensitive processes relevant to pain processing that are no related to changes in peripheral inflammatory processes. In deed, limited studies in humans have suggested 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 AS related to changes in peripheral infla<br>deed, limited studies in humans have a<br>present work, the spinal delivery of A;<br>gesic effects (Devoghel, 1983). Furth<br>toxicity and kinetics appear warranted<br>References

- XICIty and kinetics appear warranted.<br>
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