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ORIGINAL INVESTIGATION

Lisa A. Teather · Jane E. Magnusson
Richard J. Wurtman

Platelet-activating factor antagonists decrease the inflammatory nociceptive response in rats

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Abstract *Rationale:* Platelet-activating factor (PAF) is a membrane-derived phospholipid mediator that has biological effects on a variety of cells and tissues. A variety of stimuli, including those producing inflammation, promote the synthesis and release of PAF from various cell types. Evidence suggests that PAF exerts cellular actions through a plasma membrane receptor as well as via intracellular (microsomal) PAF binding sites. *Objective:* The present study was designed to: 1) investigate the role of PAF in a model of inflammatory nociception in rats (i.e. the formalin test), and 2) localize PAF's site(s) of action in nociception. To do this, we assessed the effect of administering two PAF antagonists (BN 52021 and BN 50730, which are selective for cell surface and intracellular PAF binding sites, respectively) on formalin-induced nociceptive responses. *Methods:* Forty minutes prior to formalin injection into the rat hindpaw, male Sprague-Dawley rats received systemic injections of BN 52021 (10, 1, or 0.1 mg/kg), BN 50730 (10, 1, or 0.1 mg/kg), or vehicle (45% 2-hydroxypropyl-β-cyclodextrin in distilled water, HBC) and the effects of the drugs on nociceptive behavioral responses were measured. *Results:* Rats receiving systemic BN 52021 or BN 50730 displayed a significant reduction of nociceptive responses in the late, but not early, phase of formalin-induced nociception. *Conclusions:* These findings suggest a role for endogenous PAF in nociceptive transmission, especially for persistent pain such as that which occurs in the late phase of the formalin test. The findings also indicate that both intracellular and cell surface PAF binding sites are involved in nociceptive modulation in rats, and that PAF antagonists might be useful for treating some patients with acute or chronic pain.

Keywords BN 52021 · BN 50730 · Formalin · Inflammation · Platelet-activating factor · Pain

L.A. Teather (✉) · R.J. Wurtman
Massachusetts Institute of Technology,
Department of Brain and Cognitive Sciences,
45 Carleton Street, E25-604, Cambridge, MA 02139, USA
e-mail: lteather@mit.edu
Tel.: +1-617-2536733, Fax: +1-617-2536882

J.E. Magnusson
Department of Clinical Neurosciences, University of Calgary,
Foothills Medical Center, Calgary, Alberta, Canada

Introduction

Platelet-activating factor (PAF; 1-0-alkyl-2-acetyl-sn-glycero-3-phosphocholine), a bioactive phospholipid mediator, participates in normal cell function, as well as in cellular pathology (for a review see Bazan 1994; Bazan et al. 1993, 1997). There is considerable evidence that PAF release from inflammatory cells can be a potent mediator of inflammatory responses (for a review, see Vane et al. 1998). Moreover, subplantar injection of PAF into the rat hindpaw increases pain sensitivity (i.e. decreases the nociceptive threshold) (Dallob et al. 1987), suggesting that PAF affects the processing of inflammation-related pain. The mechanism(s) of PAF action in inflammatory pain has not yet been established.

Ample evidence suggests that PAF exerts cellular actions through two high affinity intracellular membrane-binding sites, as well as through a low-affinity cell surface receptor (Marcheselli et al. 1990). The binding of PAF to cell surface receptors results in the activation of diverse intracellular signal transduction pathways that ultimately activate transcription factors and induce gene expression. For example, calcium, cyclic AMP (cAMP), inositol 1,4,5-triphosphate (IP_3), and diacylglycerol (DAG) can function as second messengers for signaling by the plasma membrane PAF receptor (for review, see Ishii and Shimizu 2000). Moreover, PAF also acts as an intracellular mediator (Bazan and Doucet 1993; Marcheselli and Bazan 1994), binding to intracellular sites which then elicit gene expression in neuronal and glial cell lines (Squinto et al. 1989; Bazan and Doucet 1993; Bazan et al. 1994). Furthermore, intracellular PAF binding sites are required in order for PAF to elicit the release of prostaglandin E_2 (PGE_2) from astrocytes

(Teather et al., unpublished data), cells thought to be critical for pathological pain processing (Watkins et al. 2001). Interestingly, PGE_2, and other arachidonic acid metabolites generated by the lipoxygenase and cylcooxygenase pathways, have also been implicated in pain processing (Dallob et al. 1987).

To assess the involvement of PAF in nociception, we used the formalin test, a commonly used model of inflammatory nociception in rats, which elicits a biphasic behavioral response (Dubison and Dennis 1977). The early phase starts immediately after injection of formalin, lasts about 5 min, and is thought to result from direct chemical stimulation of nocieptive fibers (Jongsma et al. 2001). The late phase is exhibited 15–70 min after formalin injection and appears to depend on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord (Tjolsen et al. 1992). To investigate the role of PAF in nociception, and the potential site(s) of its action, we administered two structurally distinct PAF antagonists systemically to rats 40 min prior to formalin injection, and measured their effects on the biphasic formalin response. BN 52021 is a competitive PAF antagonist that selectively inhibits the cell surface PAF receptor, while BN 50730 is believed to be a specific inhibitor for intracellular PAF binding sites (Marcheselli et al. 1990; Marcheselli and Bazan 1994).

Materials and methods

Animals

Sixty male Sprague-Dawley rats weighing 300–350 g (Charles River, Canada) were housed in groups of two or three per cage, in polycarbonate cages. Animals were maintained under standard environmental conditions (room temperature: 20–20°C; relative humidity: 55–60%; light/dark schedule: 12/12-h) with free access to standard laboratory chow and tap water.

Drug preparation and administration

BN 50730 (a generous gift from Biomeasure; Milford, Mass., USA) and BN 52021 (Biomol) were dissolved in 45% hydroxypropyl-β-cyclodextrin in distilled water (HBC). Drugs (at doses of 10, 1, or 0.1 mg/kg) or vehicle were administered intraperitoneally (IP) 40 min prior to formalin injection. The doses chosen were based on those found, in previous studies, to produce central effects after their peripheral administration (Bito et al. 1993; Marcheselli and Bazan 1994).

Formalin test

The following experiments were carried out in accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals. Behavioral testing was carried out in a blind manner. Nociceptive responses were examined in the formalin test described previously (Dubisson and Dennis 1977). In brief, animals were placed in a clear Plexiglas formalin test box (30×30×30 cm), with a mirror positioned at a 45° angle below the floor allowing for unobstructed observation of the animal's paw. Following a 10-min habituation period, animals were removed from the formalin box, at which time 50 µl of 1% formalin was injected subcutaneously (SC) into the plantar surface of the right hind paw with a 27-gauge needle. The amount of time that each rat elevated the injected paw was recorded in 5-min intervals during the 70-min period following formalin injection. Each animal was used once.

The 60-min formalin test produces a biphasic response consisting of an initial, rapidly decaying acute phase (early phase, 1–10 min after injection) followed by a slow rising and long-lived tonic phase (late phase, 15–60 min after injection). Typically, animals elevate their paws following injection (i.e. the early phase) followed by a reduction in this behavior. Approximately 15–20 min after injection, the inflammatory late phase begins and animals again elevate their paws to varying degrees for the remainder of the testing period. The amount of time animals elevate their injected paw is used as a behavioral measure of pain.

Data analysis

Data are expressed as means±SEM and P values <0.05 were considered statistically significant. Treatment groups were compared with vehicle-controls using one-way analysis of variance (ANOVA) followed by Fischer's PLSD post-hoc test to compare between groups if overall significance was found by ANOVA.

Results

BN 52021 effects on formalin-induced nociception

The nociceptive response (measured as time spent with the injected paw elevated) during the early phase (1–10 min post-formalin) was not significantly affected

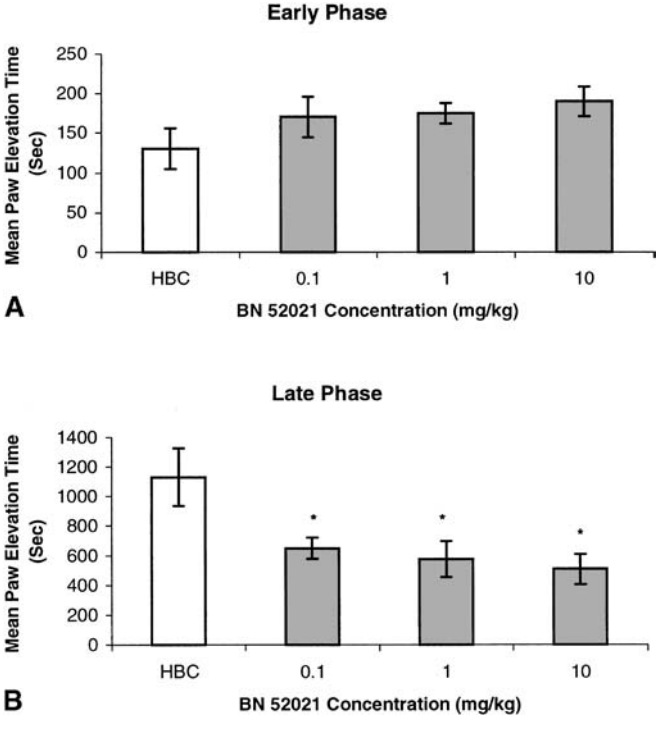

Fig. 1A, B Formalin-evoked nociceptive responses in rats that received systemic BN 52021 (10, 1 or 0.1 mg/kg) or control injections. Total paw elevation times in **A** the early phase (0–10 min after injection) and **B** the late phase (10–60 min after injection) of formalin-induced nociception. Data are expressed as means±SEM. *P<0.05; Fisher's PLSD test versus control. *HBC* 45% hydroxypropyl-B-cyclodextrin (in distilled water)

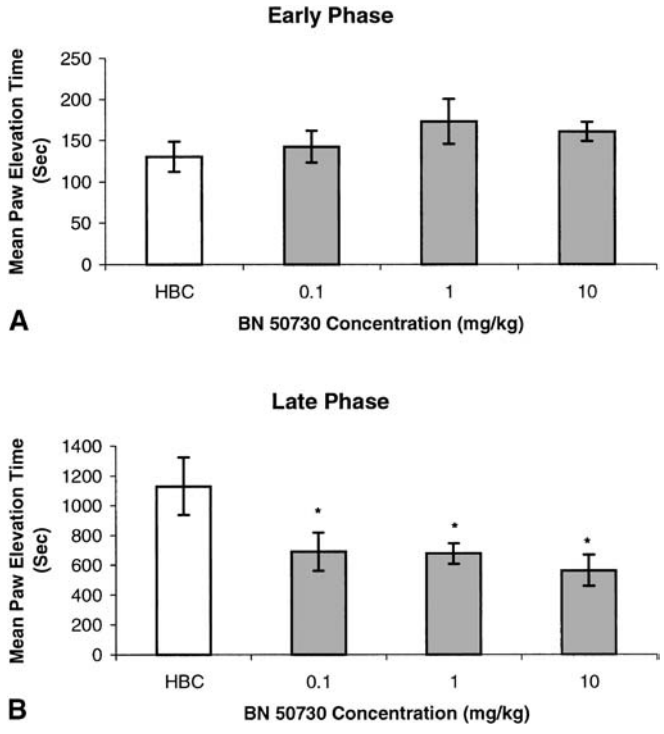

Fig. 2A, B Formalin-evoked nociceptive responses in rats that received systemic BN 50730 (10, 1 or 0.1 mg/kg) or control injections. Total paw elevation times in **A** the early phase (0–10 min after injection) and **B** the late phase (10–60 min after injection) of formalin-induced nociception. Data are expressed as means±SEM. **P*<0.05; Fisher's PLSD test versus control. *HBC* 45% hydroxypropyl-B-cyclodextrin (in distilled water)

by BN 52021 administration (Fig. 1A), although rats that received BN 52021 tended to elevate their paws for longer periods of time than did vehicle-treated controls. During the late phase (10–60 min), BN 52021-treated rats elevated their paws for significantly shorter times than did control-treated rats [$F(3,30)=3.831$, $P<0.05$; Fig. 1B]. Fisher's PLSD post-hoc analysis revealed that the responses of rats receiving 10 ($P=0.008$), 1 ($P=0.013$) or 0.1 ($P=0.0366$) mg/kg BN 52021 differed significantly from those of control-treated rats.

BN 50730 effects on formalin-induced nociption

The nociceptive response (measured as time spent with the injected paw elevated) during the early phase (1–10 min post-formalin) was not significantly affected by BN 50730 administration (Fig. 2A). During the late phase (10–60 min), BN 50730-treated rats exhibited significantly shorter paw elevation times than did control-treated rats [$F(3,30)=2.933$, $P<0.05$; Fig. 1B]. Fisher's PLSD post-hoc analysis revealed that the behavior of rats receiving 10 ($P=0.016$), 1 ($P=0.046$) or 0.1 ($P=0.049$) mg/kg BN 50730 differed significantly from those of control-treated rats.

Discussion

These data show that systemic administration of PAF antagonists, which act selectively on cell surface or intracellular PAF binding sites (BN 52021 and BN 50730, respectively), decreases nociceptive behavior during the late, but not the early, phase of the formalin test in rats (Figs 1B and 2B). Although three doses were used for each antagonist, we did not reveal a dose-response relationship for either drug (i.e. all three doses of BN 52021 and BN 50730 decreased nociceptive behavior in a similar fashion). We believe that these results attest to the significance of endogenous PAF in nociception. PAF is extremely potent and tightly regulated; perhaps the lowest dose of each antagonist was sufficient to block enough of the binding sites to prevent endogenous PAF from carrying out its nociceptive function(s) at both intracellular and plasma membrane sites.

A feature of the formalin test in rodents is that animals show two distinct phases of nociceptive behavior, which seem to depend on different mechanisms (Dubisson and Dennis 1977). Substance P and bradykinin participate in the early phase, while histamine, serotonin and prostanoids appear to be involved in the late phase (Shibata et al. 1989). The early phase of formalin-induced nociception (also known as the acute phase) starts immediately after its injection, and is thought to result from direct chemical stimulation of chemosensitive nociceptors (Dubisson and Dennis 1977; Hatakeyama et al. 2001; Jongsma et al. 2001). The second phase (also known as the tonic phase) is thought to result from peripheral inflammatory processes, and from sensitization in the spinal cord produced by the first phase (Tjolsen et al. 1992), as well as from functional changes in central processing (Coderre et al. 1990). As both antagonists tended to increase noiceptive responses (albeit not significantly, Figs 1A and 2A) during the early phase, the decrease in nociceptive responses during the late phase cannot be attributed to a reduction in the early phase of formalin-induced nociception.

Our use of peripheral injections as the means of administering the PAF antagonists does not allow conclusions to be drawn concerning their sites of action in attenuating late phase nociceptive responses. Cell surface PAF receptor mRNA expression (Mori et al. 1996) and the density of PAF binding sites (Bito et al. 1993) are predominant in the cerebral cortex and hippocampus, and there is considerable evidence suggesting the involvement of the hippocampus in pain processing in humans (Ploghaus et al. 2000; Wei et al. 2000), and nociceptive behaviors in rodents (Blanchard and Fial 1968; Yeung et al. 1977; Prado and Roberts 1985). Moreover, the hippocampus is known to have a mediatory role in the late, but not the early, phase of formalin-induced nociception (McKenna and Melzack 1992). Thus the antinociceptive effect of PAF antagonists during the late phase may have resulted from blockade of hippocampal PAF binding sites. PAF also exerts a variety of biological effects through actions at intracellular and cell surface

binding sites in hippocampus (for a review, see Bazan et al. 1993, 1997; Bazan 1994).

The antinociceptive effects of BN 52021 and BN 50730 may also result from blockade of the actions of endogenous PAF within the spinal cord, or at peripheral nervous system sites. Peripheral inflammation activates dorsal horn astrocytes (i.e. upregulated expression of activation markers) (Fu et al. 2000) and activated astrocytes maintain late, but not early, phase pain (for review, see Watkins et al. 2001) possibly by releasing prostaglandins (and other proinflammatory mediators) (Watkins et al. 1997), We have recently shown that PAF increases prostaglandin E_2 (PGE_2) release from astrocytes (Teather et al., unpublished data), suggesting that PAF antagonists may decrease nociceptive responses in the late phase by decreasing astrocytic PGE_2 release in the dorsal horn of the spinal cord.

In conclusion, the nociceptive responses to subcutaneous formalin injection were significantly reduced in rats receiving PAF antagonists that acted on intracellular or cell surface PAF binding sites. Selective PAF antagonists might thus be effective in the treatment of certain forms of acute and chronic pain.

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