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BRAIN RESEARCH

Short Communication

Inhibition of platelet-activating factor receptors in hippocampal plasma membranes attenuates the inflammatory nociceptive response in rats

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ARTICLE INFO

Article history:
Accepted 13 March 2006
Available online 13 June 2006

Keywords:
Formalin test
Hippocampus
Inflammation
Nociception
Pain
Platelet-activating factor

ABSTRACT

Evidence suggests that platelet-activating factor (PAF) is a mediator in inflammatory-based pain. Using the biphasic formalin model in rats, we recently demonstrated that PAF antagonists which were selective for either intracellular or plasma membrane PAF receptors decreased the late-phase of the nociceptive response. Inasmuch as both of the PAF antagonists previously used were administered systemically, and reportedly are able to cross the blood–brain barrier, the anatomic locations at which PAF affects pain processing remained to be elucidated. Since PAF is required for hippocampal-dependent memory consolidation, and since the hippocampus has been shown to mediate the late-phase of formalin-induced nociception, the present study investigated the effects on nociception of administration of PAF antagonists within the hippocampus, and of using agents specific for either plasma membrane (BN 52021) or intracellular (BN 50730) PAF binding sites. Intrahippocampal injections of BN 52021 decreased the late-phase of the nociceptive response in a concentration-dependent manner. In contrast, intrahippocampal administration of BN 50730 had no effect on inflammatory nociception. These findings suggest that hippocampal plasma membrane PAF receptors, but not intracellular PAF binding sites, mediate tonic inflammatory pain processing in rats.

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Platelet-activating factor (PAF; 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a potent phospholipid mediator that participates in inflammatory responses (for review, see Vane et al., 1998), including formalin-induced nociception in rats (Teather et al., 2002a,b). Evidence suggests that PAF exerts cellular actions through two high affinity intracellular (i.e. microsomal) binding sites and a low-affinity plasma membrane receptor (Marcheselli et al., 1990). Activation of the plasma membrane PAF receptors, which are coupled to G-proteins, modulates several intracellular signal transduction cascades, including calcium, cyclic AMP (cAMP), inositol 1,4,5-triphosphate (IP_3), and diacylglycerol (DAG) (for review, see Ishii and Shimizu, 2000). PAF also acts as an intracellular mediator (Marcheselli and Bazan, 1994; Bazan and Doucet, 1993), by binding to microsomal sites to elicit gene expression in neuronal and glial cell lines (Squinto et al., 1989; Bazan and Doucet, 1993; Bazan et al., 1994). These intracellular PAF binding sites are also required for PAF-induced prostaglandin E_2 (PGE_2) release from astrocytes (Teather and Wurtman, 2003; Teather et al., 2002a,b).

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doi:10.1016/j.brainres.2006.03.036

In previous work using the formalin test, we found that PAF—acting at both intracellular and cell surface binding sites—can mediate the processing of tonic inflammatory-based pain in rats (Teather et al., 2002a,b). The formalin test is a widely accepted model for the study of inflammatory nociception that permits the evaluation of acute and tonic pain. Subcutaneous (s.c.) injections of formalin into the rat hindpaw elicit a biphasic behavioral response (Dubuisson and Dennis, 1977). The early phase (i.e. acute pain) starts immediately after injection, lasts about 5 min, and is thought to result from direct chemical stimulation of nociceptive fibers (Jongsma et al., 2001). The late phase (i.e. tonic or persistent pain) is exhibited 15–60 min after formalin injection and appears to depend on the inflammatory reaction in the peripheral tissue plus functional changes in the dorsal horn of the spinal cord (Tjolsen et al., 1992). Moreover, the late phase of nociception is also influenced by higher central processing (Coderre et al., 1990). The hippocampus, for instance has been shown to play a significant role in tonic pain processing (for review, see Teather, in press).

To further investigate the potential site(s) of PAF action in inflammatory-based pain, we administered two distinct PAF antagonists into the contralateral hippocampus (with respect to the injected paw) of rats 20 min prior to formalin injection, and measured their effects on the biphasic formalin response. BN 52021 is a competitive PAF antagonist that selectively inhibits cell surface PAF receptors, while BN 50730 is believed to be a specific inhibitor for intracellular PAF binding sites (Marcheselli and Bazan, 1994; Marcheselli et al., 1990).

The following experiments were carried out in accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Sixty male Sprague–Dawley rats (300–350 g) were housed in groups of 2–3 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. After 1 week of handling (5 min per day), animals were anesthetized (50 mg/kg sodium pentobarbitol), and unilateral guide cannulae (23 gauge) were implanted into the left hippocampus. Coordinates for the guide cannulae (AP = –3.1 mm, ML = 1.5 mm from bregma, and DV = –2.0 mm from the skull surface) were chosen based on previous evidence that these PAF antagonists impair memory when administered into this CA1 region (Teather et al., 2001). The guide cannulae were attached to the skull using jeweler's screws and dental acrylic. After surgery, stylets were inserted and left in place to ensure cannula patency. The formalin test was conducted 7–10 days post-surgery.

BN 50730 (a gift from Biomeasure; Milford, MA) and BN 52021 (Biomol; Plymouth Meeting, PA) were dissolved in 45% hydroxy-β-cyclodextrin in distilled water (HBC). The concentrations used in this study (10, 1, or 0.1 μg/0.5 μl injection volume) were based on preliminary work, as well as studies in which these concentrations were proven to be effective at impairing hippocampal-dependent memory processing (Teather et al., 2001).

The formalin test was carried out in a similar fashion as previously described (Teather et al., 2002a,b). Briefly, animals were placed in a clear Plexiglas®open field box (30 cm × 30 cm × 35 cm), with a mirror positioned at a 45° angle below the floor allowing for unobstructed observation of the animal's paw. The day prior to formalin testing, rats were placed in the boxes for a 15 min habituation period. The day of testing, vehicle or PAF antagonists were administered into the left hippocampus (total volume of 0.5 μl) and the rats were placed in the boxes for 20 min. At this time, animals were removed from the box, and 50 μl of 1% formalin (0.4% formaldehyde) was injected s.c. into the plantar surface of the right hindpaw with a 27-gauge needle. Immediately after injection, each animal was exposed to the open field box for 60 min and the amount of time they elevated their injected paw was recorded (i.e. a behavioral measure of pain).

Upon completion of testing, animals were overdosed with sodium pentobarbitol and perfused with saline followed by 10% formalin solution. Brains were removed, fixed, and cut into 20-μm coronal sections throughout the cannula tract. Sections were then mounted, stained with cresyl violet, and coverslipped. Slides were examined using light microscopy for verification of injection needle tip location using the atlas of Paxinos and Watson (1986). The behavioral data for 4 rats were discarded from the study due to incorrect cannulae placement. Fig. 1 illustrates the placement of cannulae into the dorsal hippocampus from the remaining rats.

Data are expressed as means ± SEM and P values <0.05 were considered statistically significant. Experimental groups were compared using a one-way analysis of variance (ANOVA) with repeated measures (5 min blocks of time) followed by Scheffe's post hoc test. Independent t tests were used to assess the effects of the PAF antagonists on the individual 5 min bins of time post-formalin as well.

The late-phase of the nociceptive response in rats was significantly affected by intrahippocampal administration of BN 52021 (Fig. 2). ANOVA analysis indicated a significant main effect of time [$F(11,25) = 4.852$, $P < 0.001$], as would be expected considering the dynamic nature of the biphasic response. Moreover, a main effect of group was also revealed [$F(3,25) = 9.124$, $P < 0.001$]. Scheffe's post hoc analysis indicated that the nociceptive responses of rats receiving 10 ($P = 0.001$) or 1 ($P = 0.005$) μg BN 52021 were significantly diminished compared with those of control-treated rats. Independent t tests indicated that rats receiving the 10 and 1 μg concentrations of BN 52021 had significantly attenuated levels of paw elevation between 30 and 45 min post-formalin; the 1 μg concentration also exhibited decreased paw elevation at 50 min post-formalin.

The nociceptive response in rats was not significantly affected by intrahippocampal BN 50730 administration (Fig. 3), as ANOVA analysis indicated no significant group effect [$F(3,27) = 1.29$, P = n.s.]. There was a significant main effect of time [$F(11,27) = 11.86$, $P < 0.0001$], due to the dynamic nature of the biphasic nociceptive response.

These data show that intrahippocampal injection of BN 52021, but not BN 50730, decreases nociceptive behavior during the tonic or late phase of the formalin test (Figs. 2 and 3), suggesting that hippocampal cell surface, but not intracellular, PAF binding sites mediate inflammatory-based nociception in rats.

Considerable evidence suggests the involvement of the hippocampus (among other structures) in pain processing in

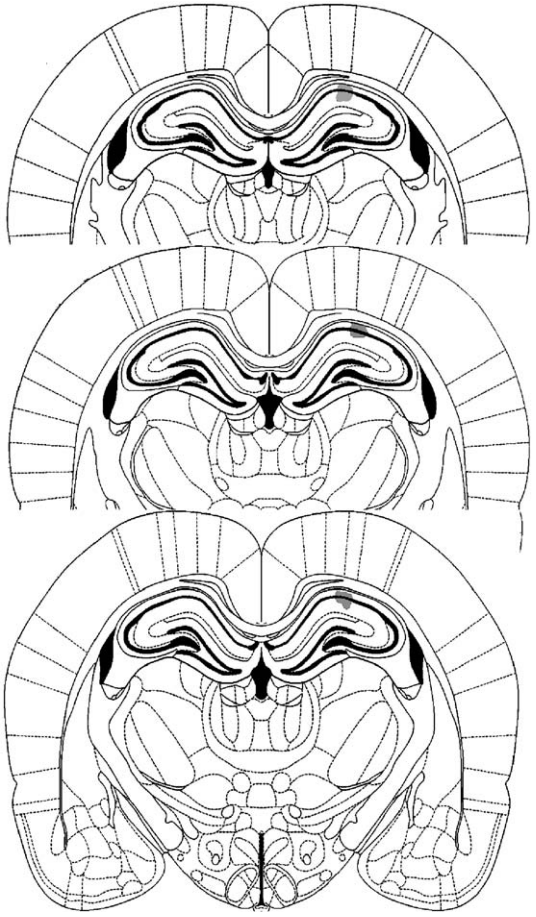

Fig. 1 – Dorsal hippocampal cannulae placements (with overlap among injection sites). Placements ranged from –2.56 to –3.30 mm AP from bregma. Top: –2.80 mm; Middle: –3.14 mm; Bottom: –3.30 mm. Verification from the atlas of Paxinos and Watson (1986).

humans (Ploghaus et al., 2000; Wei et al., 2000), and nociceptive behaviors in rodents (Blanchard and Fial, 1968; Prado and Roberts, 1985; Yeung et al., 1977). Moreover, the hippocampus is known to have a mediatory role in the late, but not in the early, phase of formalin-induced nociception (McKenna and Melzack, 1992). In fact, hindpaw injection of formalin selectively excited a few CA1 pyramidal cells (the area where PAF antagonists were administered in the present study) against a background of widespread pyramidal cell suppression, enhancing the signal-to-noise response in the CA1 region for upwards of 60 min (Khanna and Sinclair, 1992). These findings suggest that the CA1 region processes nociceptive information throughout the entire 60 min after formalin injection (Zheng and Khanna, 2001).

We have recently shown that intracellular and cell surface PAF binding site antagonists decrease nociception when administered peripherally (Teather et al., 2002a,b). As the levels of PAF binding sites are relatively high in the hippocampus (Bito et al., 1993; Mori et al., 1996), we hypothesized that the PAF antagonists may be alleviating nociception in rats via actions on hippocampal PAF receptors. Indeed, intrahippocampal administration of BN 52021, which inhibits the

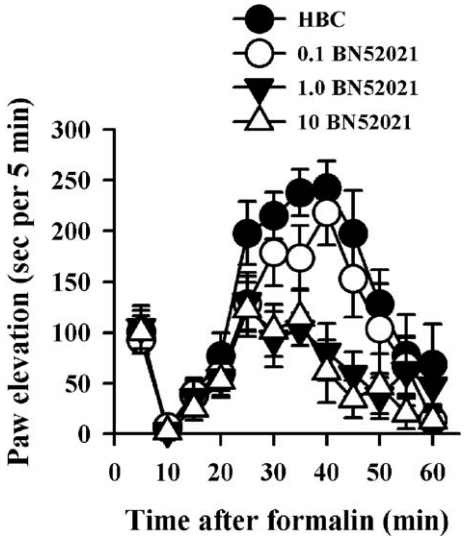

Fig. 2 – Formalin-evoked nociceptive responses in rats that received intrahippocampal injections of BN 52021 (10, 1, or 0.1 μg/0.5 μl injection volume) or control 20 min prior to formalin. Data are expressed as means ± SEMs. Indications of significance for single 5 min bins were not included in order to maintain the clarity of the figure.

G-protein-coupled PAF receptors on plasma membranes, attenuates the late-phase of the nociceptive response, similar to the effect of systemic BN 52021. These findings suggest that endogenous PAF binds to plasma membrane PAF receptors within the hippocampus to mediate the processing of painful information of an inflammatory nature. In contrast, intrahippocampal injection of the intracellular PAF binding site inhibitor BN 50730 had no effect on nociception. While systemic BN 50730 attenuates

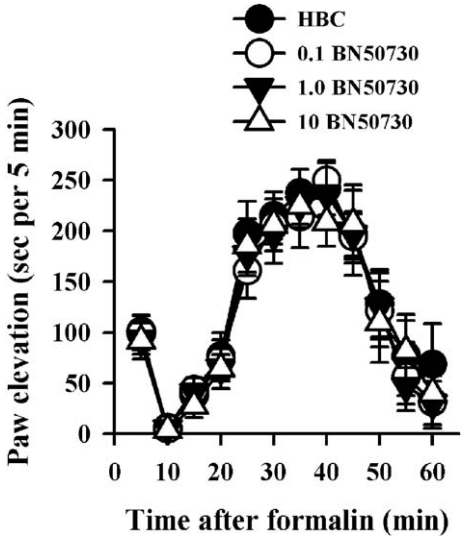

Fig. 3 – Formalin-evoked nociceptive responses in rats that received intrahippocampal injections of BN 50730 (10, 1, or 0.1 μg/0.5 μl injection volume) or control 20 min prior to formalin. Data are expressed as means ± SEMs.

the late phase of nociception (Teather et al., 2002a,b), this antagonist is without effect when administered directly into the CA1 region of the hippocampus. Recent in vitro findings demonstrate that PAF elicits PGE_2 release from astrocytes via actions at intracellular binding sites (Teather and Wurtman, 2003; Teather et al., 2002a,b). Considering the important role for spinal cord astrocytes in the nociceptive response to formalin (Watkins et al., 1997, 2001), it has been suggested that systemically administered BN 50730 attenuates the nociceptive response by blocking intracellular PAF binding sites within spinal astrocytes, ultimately preventing the inflammatory-mediated release of PGE_2 (Teather, in press).

Acknowledgments

This study was supported in part by grants from The Natural Science and Engineering Research Council (Grant No.283351-04 and 300789-04; L.A.T), The National Institutes of Mental Health (Grant No. 5-RO1 MH28783-24), and The Center for Brain Sciences and Metabolism Charitable Trust. The authors wish to thank Dr. Jane Magnusson for assistance with pilot studies.

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