PLATELET-ACTIVATING FACTOR AFFECTS NOCICEPTION IN RATS AT CEREBRAL SITES OF ACTION

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Abstract

Systemic administration of selective platelet-activating factor (PAF) antagonists that block either plasma membrane or intracellular PAF binding sites attenuate the late-phase nociceptive response to formalin in rats. Inasmuch as these antagonists both cross the blood-brain barrier, and selectively attenuate the late-phase of nociception, we have suggested that PAF may be an important central mediator of inflammatory-based pain. In the present study we investigated the effect of central administration of PAF antagonists on formalin-induced nociception. Intra-cerebral ventricular (i.c.v.) injections of either BN 52021 (a plasma membrane PAF receptor antagonist) or BN 50730 (an intracellular PAF binding site antagonist) diminished the late-phase nociceptive response to formalin. These findings suggest that PAF acts within the central nervous system (CNS) - at both plasma membrane and intracellular sites - to mediate inflammatory-based pain processing.

Background

Platelet-activating factor (PAF; 1-*0*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a potent phospholipid mediator with widespread pathophysiological effects [1]. PAF exerts cellular actions through two high affinity intracellular membrane-binding sites and via a low-affinity plasma membrane receptor [2]. The binding of extracellular PAF to plasma membrane receptors activates diverse intracellular signal transduction pathways, including those involving calcium, cyclic AMP (cAMP), inositol 1,4,5-triphosphate (IP_3), and diacylglycerol (DAG) [3]. Intracellular PAF can bind to microsomal sites to elicit gene expression in neuronal and glial cell lines [4,5]. The intracellular PAF binding sites

are also the locus at which PAF elicits the release of prostaglandin E_2 (PGE_2) from astrocytes [6,7].

Subplantar injection of PAF into the rat's hindpaw has been shown to increase sensitivity to pain [8], suggesting that PAF modulates pain by acting at the site of injury. We previously demonstrated that systemic administration of distinct PAF antagonists attenuated the late-phase, but not early-phase, of the nociceptive response to formalin in rats [9]. While PAF has the potential to act peripherally to mediate inflammatory processes, we hypothesized that this phospholipid could also be acting centrally, considering that the PAF antagonists used in this study readily cross the blood-brain barrier, and that PAF binding sites are known to be present in the central nervous system (CNS) [10].

We recently demonstrated that intra-hippocampal administration of BN52021 (an antagonist selective for plasma membrane PAF receptors) attenuated the late-phase nociceptive response. In contrast, BN 50730 (which selectively inhibits intracellular PAF binding sites) administered into the hippocampus had no influence on the behavioral expression of nociception [11]. These findings suggest that PAF mediates nociception, in part, by activating plasma membrane PAF receptors within the hippocampus. Intracellular PAF binding sites within the hippocampus do not appear to be involved in the processing of nociceptive information. In the present study, we investigated the effects on nociception of intra-cerebral ventricular (i.c.v.) injections of the structurally-distinct PAF antagonists, BN 52021 and BN 50730. We found that the central administration of either PAF antagonists alleviated the late-phase nociceptive response,

suggesting the involvement of both plasma membrane and intracellular PAF binding sites in the central processing of inflammatory-based nociception.

Methods

Animals

Fifty-six male Sprague Dawley rats weighing 300-350 g (Charles River; Wilmington, MA) were housed in groups of 2-3 in polycarbonate cages. Animals were maintained under standard environmental conditions (room temperature: 20-20 °C; relative humidity: 55-60%; light/dark schedule: 12/12 hr) with free access to standard laboratory chow and tap water. All experiments were carried out in accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Ethical Guidelines for investigation of experimental pain in conscious animals issued by the ad-hoc Committee of the International Association for the Study of Pain.

Surgical Procedures

Rats were anesthetized with sodium pentobarbital (55 mg/kg i.p.), and placed into a stereotaxic instrument, with the skull on an even horizontal plane. The coordinates used for placement of the 27-guage guide cannula were from bregma: 1.0 mm posterior, 1.5 mm lateral to the left, and 4.5 mm ventral from the skull, according to the atlas of Paxinos and Watson [12]. Cannulae were secured to the skull with jewelers' screws and dental cement. A stainless steel stylet maintained cannula patency during the 7-10 day recovery period.

Drug Injections

BN 50730 (a generous gift from Biomeasure; Milford, MA) and BN 52021 (Biomol; Plymouth Meeting, PA) were dissolved in 45% hydroxy-β-cyclodextrin in

distilled water (HBC) (Sigma-Aldrich; St. Louis, MO). Drugs (at concentrations of 1, 10, or 20 μg/0.5 μl) or HBC vehicle were administered into the left lateral ventricle 20 min prior to formalin injection. The concentrations of the antagonists used in this study were based on previous studies that found them to be centrally effective at impairing various forms of memory processing [13,14].

Formalin Test

Nociceptive responses were examined using the formalin test described previously [15]. The Plexiglas® formalin test box (32cm x 30cm x 35cm) had mirrors in the lower chamber and behind the box to provide unobstructed observation of paws. Animals were habituated to the test chamber for 45 min on the day prior to formalin testing. On the day of testing, each rat was placed in a towel and the vehicle or PAF antagonist (either BN 52021 or BN 50730) administered. The rat was then placed in the test box for 20 min, at which time the rat was removed from the box and injected subcutaneously (s.c.) (i.e. into the plantar surface of the right hind paw) with 50 μl of 1% formalin using a 27-gauge needle. Immediately after injection the animal was exposed to the test box for 60 min, and the amount of time it elevated the injected paw was scored as a behavioral measure of pain. Behavioral scoring was carried out by experimenters blinded to treatment condition.

Histology

At the completion of behavioral testing, rats were deeply anesthetized with a 1.0-ml injection of sodium pentobarbital, and perfused with saline followed by 10% formalin. Brains were removed and subsequently sectioned through the cannula-tract region. The 20-μm sections were then stained with Cresyl violet, and the gelatinized slides

dehydrated and coverslipped. Cannula placements were examined for verification of needle tip location using the atlas of Paxinos and Watson [12].

Statistical Analysis

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 measure (5 min blocks of time) followed by Scheffe's post hoc test. Independent t-tests were used to examine the effects of the PAF antagonists on the individual 5 min bins of time post-formalin as well.

Results

The late-phase of the nociceptive response in rats was significantly affected by intra-cerebral administration of BN 52021 (Fig. 1). ANOVA analysis indicated a significant main effect of Time [$F(11,308) = 5.62$, $p < 0.001$], as would be expected considering the dynamic nature of the formalin response. A significant Treatment effect was also revealed [$F(3,28) = 3.77$, $p < 0.05$]. Scheffe's post hoc analysis indicated that the responses of rats receiving 20, 10, or 1 μg BN 52021 differed significantly from those of control-treated rats (p's < 0.05). Independent t-tests revealed that rats receiving BN 52021 (20, 10, or 1 μg) exhibited significantly attenuated levels of paw elevation between 30 and 45 min post-formalin; the highest two concentrations also decreased paw elevation at 50 min post-formalin (p's < 0.05).

The late-phase of the nociceptive response was also significantly affected by intra-cerebral BN 50730 administration (Fig. 2). ANOVA analysis revealed significant main effects of Time [$F(11,297) = 5.39$, $p < 0.001$] and Treatment [$F(3,27) = 3.4$, $p < 0.05$]. Scheffe's post-hoc analysis revealed that the nociceptive responses of rats

receiving either 20 or 10 μg of BN 50730 differed significantly from the HBC-treated control rats (p's < 0.5). Independent t-tests indicated that rats receiving the two highest concentrations of BN 50730 exhibited significantly attenuated levels of paw elevation between 35 and 45 min post-formalin (p's < 0.05).

Discussion

These data show that the central administration of two structurally-distinct PAF antagonists - which act to block either plasma membrane or intracellular PAF binding sites - attenuated the late-phase nociceptive response to formalin in rats (Figs 1 and 2).

A feature of the formalin test in rodents is that animals display two behaviorally distinct phases. The early-phase starts immediately after injection, and is the result of direct chemical stimulation of chemosensitive nociceptors by the irritant formalin [15,16] The late-phase is thought to result from peripheral inflammatory processes at the site of injury, sensitization within the spinal cord, as well as from functional changes in that occur in supraspinal regions [17].

PAF appears act at the site of injury to modulate pain processing, as evidenced by findings that show PAF injections into the rat hindpaw increase sensitivity to other painful stimuli and to those that are not normally of painful [8]. However, we found that systemic administration of BN 50730 or BN 52021 increased the early nociceptive response (albeit not significantly) to formalin [9]. Thus, the decrease in late-phase nociception caused by PAF antagonists delivered systemically cannot be attributed to a reduction in the early-phase response. Since BN 50730 and BN 52021 readily cross the blood-brain barrier, and PAF receptors are expressed in the CNS [10], we hypothesized that PAF could be acting as an endogenous central nociceptive mediator.

Considerable evidence suggests the involvement of the hippocampus in pain processing in humans [18] and in nociceptive behaviors in rodents [19]. The hippocampus appears to have a selective role in the late-phase of formalin-induced nociception [20], and contains relatively high levels of PAF receptor mRNA and protein and PAF binding sites [10]. In previous work, we investigated the significance of plasma membrane and intracellular PAF binding sites in hippocampal nociceptive processing. While plasma membrane PAF receptors are required for hippocampal processing of nociceptive information, the intracellular PAF binding sites were deemed to have little influence on hippocampal processing [11].

The current findings suggest that both plasma membrane and intracellular PAF binding sites are involved in the processing of nociceptive information at central sites of action. We have previously illustrated the importance of hippocampal plasma membrane PAF receptors in nociception [11]; the central sites of intracellular PAF action in the processing of painful information remain to be determined. We have hypothesized that PAF might be required to activate intracellular PAF sites within the spinal cord to mediate spinal sensitization, and thus behavioral nociception [9,11].

Peripheral inflammation activates dorsal horn astrocytes, causing these reactive cells to produce inflammatory mediators such as prostaglandins [21]. Reactive astrocytes are involved in the maintenance of the late-phase of the nociceptive response [22]. We have shown that PAF - acting at intracellular binding sites - rapidly increases PGE_2 release from primary rat astrocytes [6,7]. Thus, centrally-administered BN 50730 may decrease the late-phase nociceptive response to formalin by decreasing astrocytic PGE_2 release in the dorsal horn of the spinal cord. Indeed, PGE_2 is produced in the spinal cord

after inflammation or tissue injury and may facilitate nociceptive transmission in the spinal cord [23].

Conclusion

In conclusion, the late-phase of the nociceptive response to formalin was significantly reduced in rats receiving intra-cerebral injections of structurally-distinct PAF antagonists. These findings suggest that both plasma membrane and intracellular PAF binding sites are involved in the central processing of information related to the processing of inflammatory-related nociception.

Abbreviations:

ANOVA = analysis of variance
cAMP =cyclic adenosine monophosphate
CNS = central nervous system
DAG = diacylglycerol
HBC = 45% hydroxyl-β-cyclodextrin
i.c.v. = intra-cerebral ventricular
PAF = platelet-activating factor
PGE_2 = prostaglandin E_2

Competing interest: The authors have applied for a patent related to the use of PAF antagonists as potential anti-inflammatory agents. The data collected and presented in this manuscript provide basic knowledge as to PAF's site of action in nociceptive processing.

Authors's contributions: LAT and RJW participated in the conception and interpretation of the study. All authors read and approved the final manuscript.

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Figure Headings

Fig. 1. Formalin-evoked nociceptive responses in rats that received BN 52021 (20, 10 or 1 μg/0.5 μl) or vehicle control (HBC = hydroxy-β-cyclodextrin) by injection into the lateral ventricle 20 min prior to paw injections. 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 (see results in text).

Fig. 2. Formalin-evoked nociceptive responses in rats that received BN 50730 (20, 10 or 1 μg/0.5 μl) or vehicle control (HBC = hydroxy-β-cyclodextrin) by injection into the lateral ventricle 20 min prior to paw injections. 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 (see results in text).

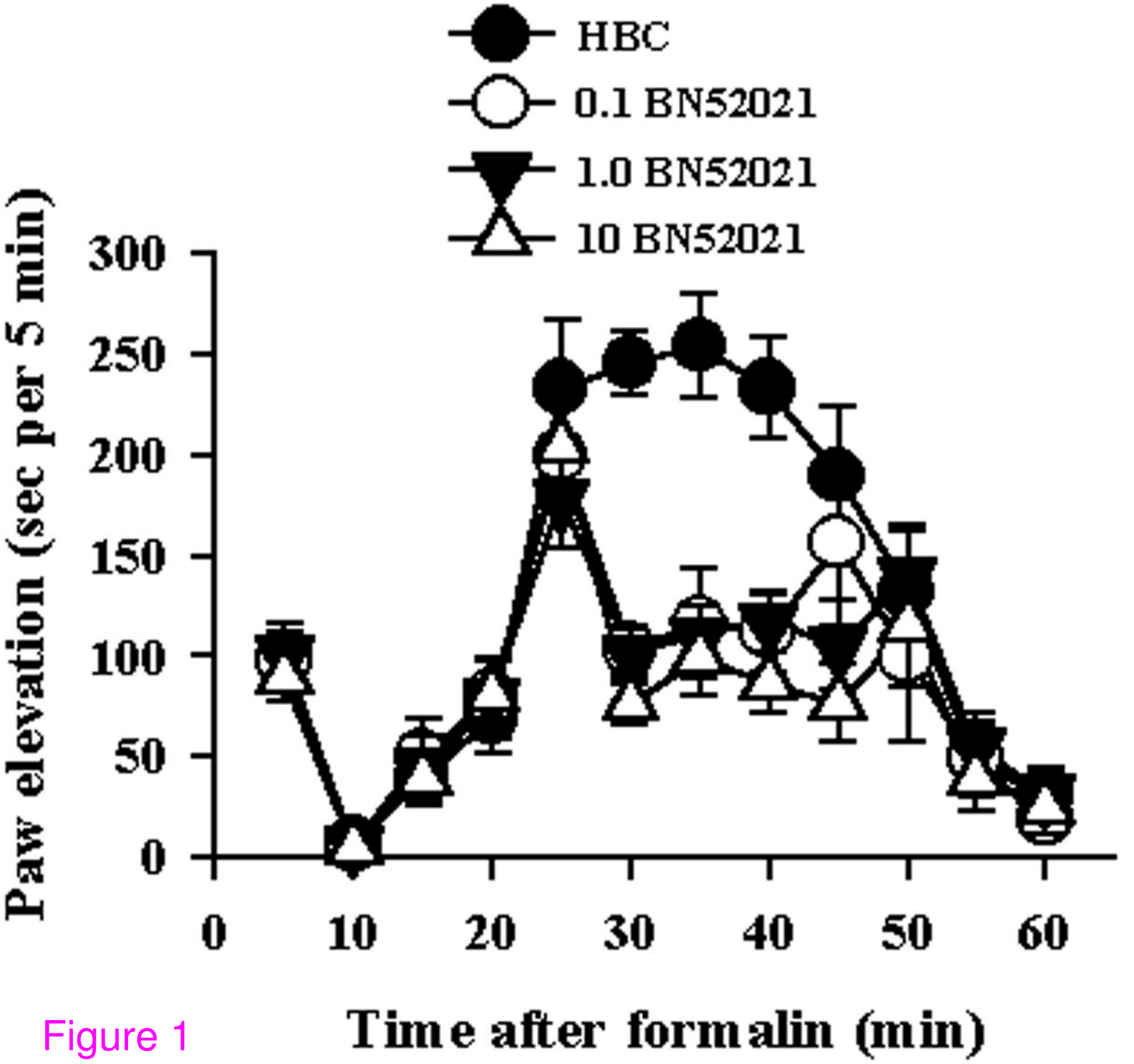
HBC
0.1 BN52021
1.0 BN52021
10 BN52021
Paw elevation (sec per 5 min)
300
250
200
150
100
50
0
0
10
20
30
40
50
60
Time after formalin (min)

Figure 1

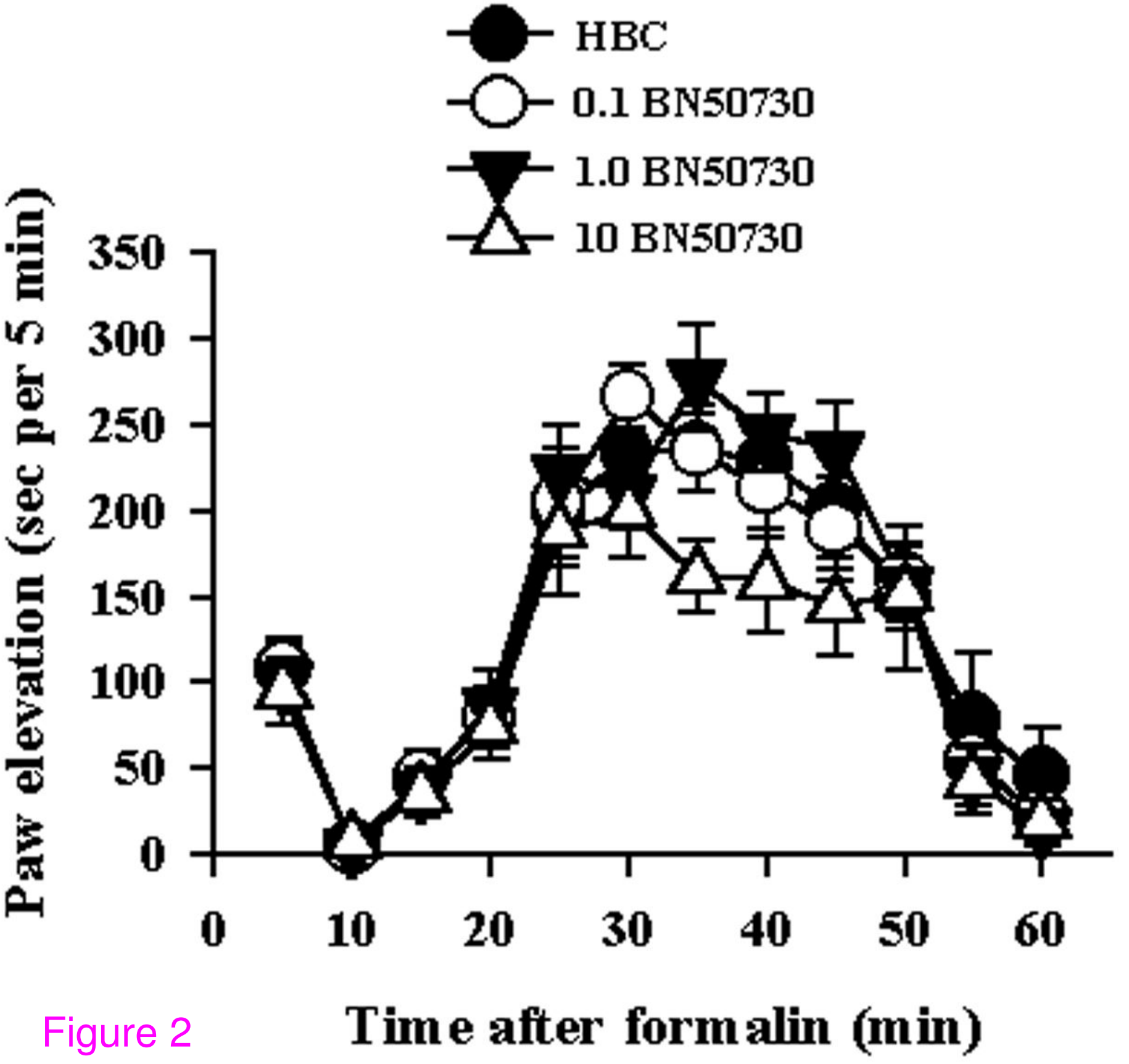
HBC
0.1 BN50730
1.0 BN50730
10 BN50730
Paw elevation (sec per 5 min)
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
Time after formalin (min)

Figure 2