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# Prostaglandin $E_2$ release evoked by intrathecal dynorphin is dependent on spinal p38 mitogen activated protein kinase

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**Abstract**

Spinal dynorphin has been hypothesized to play a pivotal role in spinal sensitization. Although the mechanism of this action is not clear, several lines of evidence suggest that spinal dynorphin-induced hyperalgesia is mediated through an increase in spinal cyclooxygenase products via an enhanced *N*-methyl-D-aspartate (NMDA) receptor function. Spinal NMDA-evoked prostaglandin release and nociception has been linked to the activation of p38 mitogen activated protein kinase (p38). In the present work, we show that intrathecal delivery of an N-truncated fragment of dynorphin A, dynorphin A 2-17 ($dyn_{2\text{-}17}$), which has no activity at opioid receptors, induced a 8–10-fold increase in phosphorylation of p38 in the spinal cord. The increase in phosphorylated p38 was detected in laminae I–IV of the dorsal horn. Moreover, confocal microscopy showed that the activation of p38 occurred in microglia, but not in neurons or astrocytes. In awake rats, prepared with chronically placed intrathecal loop dialysis catheters, the concentration of prostaglandin $E_2$ in lumbar cerebrospinal fluid was increased 5-fold by intrathecal administration of $dyn_{2\text{-}17}$. Injection of SD-282, a selective p38 inhibitor, but not PD98059, an ERK1/2 inhibitor, attenuated the prostaglanin $E_2$ release. These data, taken together, support the hypothesis that dynorphin, independent of effects mediated by opioid receptors, has properties that can induce spinal sensitization and indicates that $dyn_{2\text{-}17}$ effects may be mediated through activation of the p38 pathway. These studies provide an important downstream linkage where by dynorphin may act through a non-neuronal link to induce a facilitation of spinal nociceptive processing.


*Keywords:* Dialysis; Dynorphin; $Dyn_{2\text{-}17}$; ERK; Intrathecal; Microglia; Pain; p38; Prostaglandin $E_2$; Spinal cord

## 1. Introduction

An extensive line of investigation suggests that dynorphin located in the spinal cord plays an important role in sensitization of nociceptive neurons (Dubner and Ruda, 1992; Laughlin et al., 2001). Increased levels of spinal prodynorphin and dynorphin mRNA and protein have been observed in pathological pain states (Iadarola et al., 1988; Kajander et al., 1990; Draisci et al., 1991; Dubner and Ruda, 1992; Malan et al., 2000; Abraham et al., 2001; Ji et al., 2002a; Shimoyama et al., 2005). Unlike other opioid peptides, dynorphin can be pronociceptive. Intrathecal injection of dynorphin produces nociceptive behavior in form of biting and licking (Tan-No et al., 2002) as well as hypersensitivity to thermal and tactile stimuli in mice (Laughlin et al., 1997) and rats (Vanderah et al., 1996; Kawaraguchi et al., 2004). Moreover, antiserum to dynorphin, as well as disruption of

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the dynorphin gene, prevents hyperalgesia associated with peripheral nerve injury (Nichols et al., 1997; Malan et al., 2000; Wang et al., 2001) and intrathecal injection of *N*-ethylmaleimide or dynorphin (Tan-No et al., 2005). The pronociceptive actions of dynorphin appears to be mediated through a non-opioid dependent mechanism since intrathecal administration of dynorphin A 2-17 ($dyn_{2-17}$), the N-truncated fragment of dynorphin that has no activity at opioid receptors (Walker et al., 1982; Bakshi and Faden, 1990), induces hyperalgesia that is not naloxone sensitive (Vanderah et al., 1996).

Intrathecal administration of *N*-methyl-D-aspartate (NMDA) induces hyperalgesia (Malmberg and Yaksh, 1992; Zochodne et al., 1994) and prostaglandin $E_2$ ($PGE_2$) release (Sorkin, 1993; Svensson et al., 2003a) both of which events are blocked by administration of cyclooxygenase (COX) inhibitors (Koetzner et al., 2004). Although the mechanism by which dynorphin promotes pain is not clear, increasing evidence supports the hypothesis that non-opioid properties of dynorphin are mediated in part through an enhancement of NMDA-ionophore function (Vanderah et al., 1996; Laughlin et al., 1997, 2001; Tan-No et al., 2002). Both direct and indirect effects of dynorphin on the NMDA receptor have been suggested. Binding studies indicate that dynorphin augments NMDA receptor activity by a direct interaction with multiple sites on the receptor complex (Laughlin et al., 2001). Alternately, the effect of dynorphin may be mediated by evoking release of glutamate (Bakshi and Faden, 1990; Skilling et al., 1992; Koetzner et al., 2002), leading to enhanced pain signaling.

Recent work showed that intrathecal $dyn_{2-17}$ also evokes $PGE_2$ release, and that the increase in extracellular $PGE_2$ concentration is blocked by pretreatment with NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5), as well as COX-1/2 inhibitors (Koetzner et al., 2004). These data support a mechanism for dynorphin-induced hypersensitivity mediated, at least in part, by NMDA receptor-dependent release of $PGE_2$.

An increasing number of reports suggest that p38 and ERK1/2, members of the mitogen activated protein (MAPK) family, are both involved in induction and maintenance of nerve injury- and inflammation-induced pain (Ji et al., 1999, 2002b; Kim et al., 2002; Milligan et al., 2003; Schafers et al., 2003; Svensson et al., 2003b, 2005; Tsuda et al., 2004). We have previously shown that NMDA activates spinal p38 and that NMDA-evoked $PGE_2$ release is attenuated by p38 inhibition (Svensson et al., 2003a). This raises the possibility that the p38 pathway also regulates the nociceptive action of dynorphin. In the present study, we show that intrathecal $dyn_{2-17}$ triggers phosphorylation (activation) of p38 and that $PGE_2$ release evoked by intrathecal $dyn_{2-17}$ can be blocked by inhibition of spinal p38 and finally that spinal $dyn_{2-17}$ activates p38 in microglia.

## 2. Materials and methods

### 2.1. Animals

Male Holzman Sprague-Dawley rats (300–350 g) were housed individually in micro isolator filter cages and maintained on a 12-h light/dark cycle with free access to food and water. To permit repeated bolus intrathecal drug delivery, chronic lumbar intrathecal injection catheters (single lumen PE-5, 8.5 cm in length) were implanted through a cisternal exposure under isoflurane anesthesia and externalized as described elsewhere (Yaksh and Rudy, 1976; Hayes et al., 2003). To permit intrathecal injection and dialysis of the lumbar intrathecal space, rats were prepared with chronic triple lumen loop dialysis catheters advanced 8.5 cm through a cisternal incision to the lumbar enlargement under isoflurane anesthesia and externalized (Marsala et al., 1995; Koetzner et al., 2004). The intrathecal portion of the dialysis probe consists of a tubular 3 cm cellulose dialysis fibers (Filtral AN69HF, Cobe Laboratories) bent double and connected at its ends to 7 cm of two lumen of the triple lumen catheter, and the third lumen permits the delivery of intrathecal drug without interrupting dialysis. Studies involving rats with chronic intrathecal dialysis catheters or single lumen injection catheter were undertaken 4–5 days after surgery. For bolus intrathecal injection, all agents were prepared to be delivered in 10 μl followed by 10 μl saline to flush the catheter. Rats were monitored daily and removed from the study if any neurological dysfunction was noted, if there was greater than 10% weight loss over 5 days or if the catheter was occluded. Fewer than 5% of the animals prepared were so excluded.

### 2.2. Intrathecal dialysis & $PGE_2$ assay

Dialysis experiments were conducted in unanesthetized rats 3–5 days after the implant. A syringe pump (Harvard, Natick, MA) was connected and dialysis tubing was perfused with artificial cerebrospinal fluid (ACSF) at a rate of 10 μl/min. The ACSF contained (mM) 151.1 $Na^+$, 2.6 $K^+$, 0.9 $Mg^{2+}$, 1.3 $Ca^{2+}$, 122.7 $Cl^-$, 21.0 $HCO_3$, and 2.5 $HPO_4$, and it was bubbled with 95% $O_2$/5% $CO_2$ before each experiment to adjust the final pH to 7.2. The efflux (20 min per fraction) was collected in an automatic fraction collector (Eicom, Kyoto, Japan) at 4 °C. Two baseline samples were collected following a 30-min washout, and additional three fractions after intrathecal injection of $dyn_{2-17}$ (3 and 30 nmol (5.9 and 59 μg) in 10 μl saline followed by 10 μl of saline to flush injection line). The concentration of $PGE_2$ in spinal dialysate was measured by ELISA using a commercially available kit (Assay Designs 90001; Assay Designs, Ann Arbor, MI). The antibody is selective for $PGE_2$ with less than 2.0% cross-reactivity to $PGF_{1\alpha}$, $PGF_{2\alpha}$, 6-keto-