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RESEARCH PAPER

Increased susceptibility of annexin-A1 null mice to nociceptive pain is indicative of a spinal antinociceptive action of annexin-A1

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Background and purpose: Annexin-A1 (ANXA1), a glucocorticoid-regulated protein, mediates several of the anti-inflammatory actions of the glucocorticoids. Previous studies demonstrated that ANXA1 is involved in pain modulation. The current study, using ANXA1 knockout mice (ANXA1$^{-/-}$), is aimed at addressing the site and mechanism of the modulatory action of ANXA1 as well as possible involvement of ANXA1 in mediating the analgesic action of glucocorticoids.
Experimental approach: The acetic acid-induced writhing response was performed in ANXA1$^{-/-}$ and wild-type (ANXA1$^{+/+}$) mice with spinal and brain levels of prostaglandin E_2 (PGE_2) examined in both genotypes. The effect of the ANXA1 peptomimetic Ac2-26 as well as methylprednisolone on the writhing response and on spinal cord PGE_2 of ANXA1$^{+/+}$ and ANXA1$^{-/-}$ was compared. The expression of proteins involved in PGE_2 synthesis, cytosolic phospholipase A_2 ($cPLA_2$) and cyclooxygenases (COXs), in the spinal cord of ANXA1$^{+/+}$ and ANXA1$^{-/-}$ was also compared.
Key results: ANXA1$^{-/-}$ mice exhibited a significantly greater writhing response and increased spinal cord levels of PGE_2 compared with ANXA1$^{+/+}$ mice. Ac2-26 produced analgesia and reduced spinal PGE_2 levels in ANXA1$^{+/+}$ and ANXA1$^{-/-}$ mice, whereas methylprednisolone reduced the writhing response and spinal PGE_2 levels in ANXA1$^{+/+}$, but not in ANXA1$^{-/-}$ mice. The expression of $cPLA_2$, COX-1, COX-2 and COX-3 in spinal cord tissues was upregulated in ANXA1$^{-/-}$compared with ANXA1$^{+/+}$.
Conclusions and implications: We conclude that ANXA1 protein modulates nociceptive processing at the spinal level, by reducing synthesis of PGE_2 by modulating $cPLA_2$ and/or COX activity. The analgesic activity of methylprednisolone is mediated by spinal ANXA1.
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Keywords: annexin-A1; antinociceptive; cyclooxygenase; methylprednisolone; prostaglandin E_2; spinal cord

Abbreviations: ANXA1, annexin-A1; COX, cyclooxygenase; $cPLA_2$, cytosolic PLA_2; PGE_2, prostaglandin E_2

Introduction

Annexin-A1 (ANXA1; formerly known as lipocortin-1), a 37 kDa glucocorticoid-regulated protein (Buckingham and Flower 1997), is a member of the annexin family of calcium and phospholipids-binding proteins and is found in the central nervous system (CNS) (Bolton *et al.*, 1990). It has been demonstrated that ANXA1 mediates the anti-inflammatory actions of glucocorticoids in many experimental models (Podgorski *et al.*, 1992; Yang *et al.*, 1997, 2004) and also to possess antipyretic actions (Davidson *et al.*, 1991).

Prostanoids are lipid mediators involved in transmission of nociceptive pain. Synthesis of the prostanoids is initiated by the generation of arachidonic acid by phospholipase A_2 (PLA_2), which is metabolized by cyclooxygenase (COX) enzymes to generate short-lived mediators that act as precursors for the synthesis of the biologically active prostanoids: prostaglandin E_2 (PGE_2), prostaglandin D_2, prostaglandin I_2, prostaglandin $F_{2\alpha}$ or thromboxane A_2. Two isoforms of COX are known to exist, COX-1 and COX-2. COX-1 is constitutively expressed and is generally responsible for the production of prostanoids involved in homoeostatic regulation of body functions (Crofford, 1997), whereas COX-2 is an inducible isoform (Xie *et al.*, 1991) in most tissues (although substantial pools of the enzyme exist constitutively in some organs for example the CNS) and is responsible for production of prostanoids involved in pathological processes such as inflammation (Tomlinson *et al.*, 1994). A third isoform, COX-3, was recently identified as a splice variant of COX-1 and is predominantly expressed in the CNS (Chandrasekharan *et al.*, 2002).

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In peripheral tissues, PGE_2 and prostaglandin I_2 were shown to be hyperalgesic, that is, they sensitize nerve endings to other pain producing agents such as bradykinin by lowering the activation threshold (Ferreira *et al*., 1974; Taiwo and Levine, 1990). At the spinal level, it was demonstrated that facilitation of nociception is mediated by PGE_2 synthesized by constitutive COX-1 and COX-2 (Willingale *et al*., 1997; Yamamoto and Nozaki-Taguchi, 1997; Dirig *et al*., 1998). Within the brain, injection of PGE_2 into the pre-optic area resulted in the generation of thermal hyperalgesia (Hori *et al*., 2000).

Few studies have addressed the question of whether ANXA1 is involved in the activation/modulation of pain pathways. Ferreira *et al*. (1997) showed that ANXA1 peptidomimetics produced analgesia in inflamed rat paws and that neutralizing antisera to ANXA1 prevented the anti-hyperalgesic activity of glucocorticoids. Using a rat model of C-fibre modulated bradykinin-induced plasma extravasation, Green *et al*. (1998) suggested that the inhibitory action of corticosterone on C-fibre activation was mediated by the release of ANXA1. More recently, Pieretti *et al*. (2004) demonstrated that inhibition of the formalin-induced nociceptive behaviour by ANXA1 peptidomimetics, administered locally or centrally, is dependent on activation of the receptors of the formylated peptide family.

We have previously observed that ANXA1 null ($ANXA1^{-/-}$) mice were more susceptible to nociceptive pain induced by the intraperitoneal (i.p.) injection of acetic acid compared with wild-type ($ANXA1^{+/+}$), suggesting that ANXA1 modulates nociceptive pain. In addition, increased levels of PGE_2 in the spinal cord of $ANXA1^{-/-}$ compared with $ANXA1^{+/+}$ mice suggest that ANXA1 modulates nociceptive processing at the spinal level by downregulating PGE_2 spinal nociceptive facilitation. Evidence is also presented to demonstrate that ANXA1 mediates the analgesic action of the glucocorticoid, methylpredinsolone.

Methods

Animals

All animal procedures were in accordance with UK Home Office regulations. Male and female $ANXA1^{+/+}$ and $ANXA1^{-/-}$ mice on a C57BL/6 background (20–25 g body weight; Hannon *et al*., 2003), maintained on a standard chow pellet diet with tap water *ad libitum*, were used for all experiments. The animals were housed five per cage in a room with controlled lighting (lights on from 0800 to 2000 hours), in which the temperature was maintained at 21–23 °C.

Writhing test and tissue collection

Nociceptive responses were induced in $ANXA1^{+/+}$ or $ANXA1^{-/-}$ mice by the i.p. injection of 0.6% (v/v) glacial acetic acid in 0.9% (w/v) saline. In response to acetic acid, the mice develop the 'writhing' reaction, which is characterized by patterns of waves of constriction and elongation passing caudally along the abdominal wall followed by extension of the hind limbs (Collier *et al*., 1968). The number of writhes was counted for a 20-min period following the administration of the stimulus. In some experiments, the animals were pretreated with either acetyl 2–26 (Ac2-26; 200 μg, i.p., 10 min before) or 60 mg kg^{-1} 6α-methylprednisolone 21-hemisuccinate (subcutaneously, 1 h before) to test for analgesic activity. At the end of the 20-min observation period, the animals were killed by cervical dislocation, the peritoneal cavity lavaged with 1 mL of 0.9% (w/v) saline and the samples frozen. The whole spinal cord was removed and immediately frozen. Whole brains were removed and dissected into the cerebral cortex, mid-brain, brain stem and cerebellum, and frozen immediately. PGE_2 was measured in the peritoneal fluid, dissected brains and whole spinal cords using conventional enzyme immunoassay techniques after extraction with C18 Sep-Pak columns.

Extraction of PGE_2 from peritoneal fluid, spinal cord and dissected brain

The procedure was carried out as a modification of the protocol described by Powell (1980). Frozen brain and spinal cord tissues were pulverized with a nitrogen bomb. Peritoneal fluid was centrifuged at 800 *g* for 10 min at 4 °C to remove cells. One millilitre of 15% (v/v) ethanol in distilled water (pH 3) was added to pulverized tissues or peritoneal supernatants and samples were stored at 4 °C for 10 min and then centrifuged at 375 *g* for 10 min at 4 °C. C-18 Sep-Pak columns were conditioned with 4 mL ethanol followed by 4 mL distilled water at a flow rate of 5–10 mL min^{-1}. The supernatant from homogenates was then applied to the columns at a flow rate of 5 mL min^{-1}. The columns were then washed with 4 mL distilled water followed by 4 mL of 15% (v/v) ethanol in distilled water. The samples were then eluted with 2 mL of ethyl acetate at a flow rate of 5 mL min^{-1}. The samples were dried and then stored at −80 °C ready for prostaglandin measurement.

PGE_2 enzyme immunoassay

Measurement of peritoneal, brain and spinal cord PGE_2 was carried out by using a commercial enzyme immunoassay kit from Amersham Biosciences (Buckinghamshire, UK) according to the manufacturer's protocol. Briefly, extracted PGE_2 was incubated on a goat anti-mouse IgG-coated plate along with anti-PGE_2 antibody and horseradish peroxidase-labelled PGE_2. The blue colour developed with 3,3′,5,5′-tetramethylbenzidine substrate was read colometrically at 630 nm. The concentration of PGE_2 in the samples was determined by comparing the calculated percentage binding of PGE_2 in the samples with a standard PGE_2 curve (0.05–6.4 ng mL^{-1}).

Western blotting

The protein concentration in pulverized spinal cord tissues was determined using the method developed by Bradford (1976). Briefly, spinal cord tissue homogenates from $ANXA1^{+/+}$ and $ANXA1^{-/-}$ were pooled together ($n = 5$), then sonicated for 40 s after reconstitution in 200 μL of protease inhibitory cocktail containing 104 mM 4-(2-aminoethyl) benzenesulphonylflouride, 0.08 mM aprotinin, 2.1 mM leupeptin, 3.6 mM bestatin, 1.5 mM pepstatin A and 1.4 mM

E-64, in 50 mM Tris buffer (pH 7.4). The protein concentration was quantified using the Bradford reagent (Bio Rad, Hemel Hempstead, Hertfordshire, UK).

Protein samples (15 μg) were analysed with 10 or 7% sodium dodecyl sulphate-polyacryamide gels using the Bio-Rad mini protean II gel electrophoresis system. The proteins were separated electrophoretically at 180 V and then transferred to nitrocellulose membranes electrophoretically at 100 V for 1 h. The blots were blocked with 5% (w/v) milk and 0.2% (w/v) BSA in Tris-buffered saline containing 0.1% (v/v) Triton X-100 (TTBS) overnight at 4 °C. They were then washed for 20 min in TTBS and incubated with one of the following primary antibodies: monoclonal anti-cytosolic PLA_2 ($cPLA_2$, 0.2 μg mL^{-1}), monoclonal anti-COX-1 (0.2 μg mL^{-1}), polyclonal anti-COX-2 (2 μg mL^{-1}), polyclonal anti-COX-3 (2 μg mL^{-1}) or monoclonal anti-β-actin (1:10 000 dilution) at room temperature for 1 h, washed again and incubated with 0.2 μg mL^{-1} of anti-rabbit (used with polyclonal primary antibodies) or anti-mouse (used with monoclonal primary antibodies) horse-radish peroxidase-conjugated secondary antibodies. The blots were finally washed and developed on X-ray films using the western blotting ECL plus reagent.

The bands were scanned and quantified using Image J software (NIH Image) and expressed as the ratio of β-actin protein.

Statistical analysis

The results were analysed using Graph Pad Prism 3.0 (San Diego, CA, USA) and results expressed and presented graphically as means ± s.e.mean. Statistical analysis was performed by comparing the vehicle-treated group with the drug-treated group using a one-tailed unpaired *t*-test. A *P*-value $\leqslant 0.05$ was considered to be statistically significant.

Materials

The following drugs, reagents and kits were used: 6α-methylprednisolone 21-hemisuccinate and anti-β-actin antibody (Sigma, Dorset, Poole, UK), Ac2-26 peptide, which corresponds to amino acids 2–26 of the N-terminus region of ANXA1 protein, was synthesized at Imperial College London, glacial acetic acid (VWR, Leicestershire, UK), C18 Sep-Pak columns (Waters, Milford, MA, USA), PGE_2 EIA kit (GE Healthcare, Buckinghamshire, UK), anti-ANXA1 antibody (Zymed Laboratories, San Francisco, CA, USA), anti-$cPLA_2$ antibody, goat anti-rabbit and goat anti-mouse secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-COX-1 and anti-COX-2 antibodies (Cayman Chemicals, Ann Arbor, MI, USA), anti-COX-3 antibody (Autogen Bioclear, Mile Elm, Wiltshire, UK).

Results

ANXA1 modulates nociceptive pain in mice

The writhing response induced by acetic acid in ANXA1$^{-/-}$ mice, lacking a functional ANXA1 protein, was significantly greater compared with ANXA1$^{+/+}$ mice (Figures 1a and b). This effect was observed in both male ANXA1$^{+/+}$ compared with male ANXA1$^{-/-}$ mice ($P<0.01$) and in female ANXA1$^{+/+}$ compared with female ANXA1$^{-/-}$ mice ($P<0.05$).

In an attempt to investigate the observed difference in the writhing response between ANXA1$^{+/+}$ and ANXA1$^{-/-}$ mice, the levels of PGE_2 were measured in the cerebral cortex, mid-brain, cerebellum, brain stem, spinal cord and peritoneal washouts. In spinal cord tissues of ANXA1$^{-/-}$ mice, the levels of PGE_2 were significantly greater ($P<0.05$) compared with ANXA1$^{+/+}$ mice (Figure 2e). There was no significant difference in the levels of PGE_2 between ANXA1$^{+/+}$ and ANXA1$^{-/-}$ in any brain tissue studied (Figures 2a–d). In peritoneal washouts, PGE_2 was significantly higher in ANXA1$^{+/+}$ compared with ANXA1$^{-/-}$ mice (Figure 2f).

The levels of PGE_2 were significantly higher in the spinal cord tissues of ANXA1$^{-/-}$ compared with ANXA1$^{+/+}$ mice; hence, we proposed that ANXA1 protein modulates nociception by downregulation of PGE_2 synthesis in the cord. Therefore, for the rest of the current investigation, we only examined differences in PGE_2 in at the spinal level.

The ANXA1 peptidomimetic, Ac2-26, which corresponds to amino acids 2–26 of the N-terminal region of ANXA1 protein, has been shown to mimic the anti-inflammatory actions of the full-length protein. Ac2-26 (200 μg)

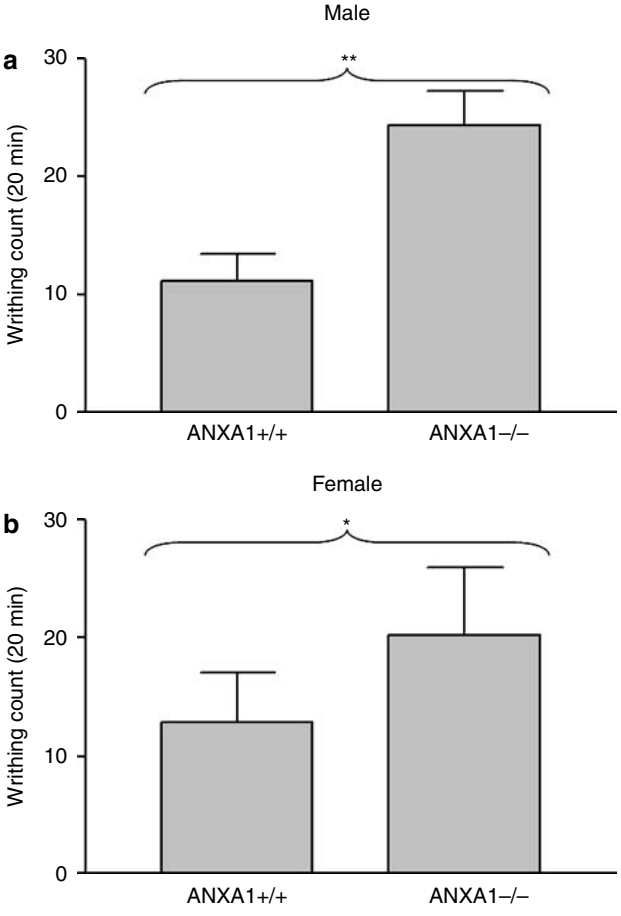

Figure 1 The acetic acid-induced writhing response in male (**a**) and female (**b**) ANXA1$^{-/-}$ mice compared with ANXA1$^{+/+}$ mice. The writhing response was induced by the i.p. administration of 0.6% (v/v) of acetic acid and the writhing behaviour count for 20 min. *$P<0.05$, **$P<0.01$, $n=6$. ANXA1, annexin-A1.

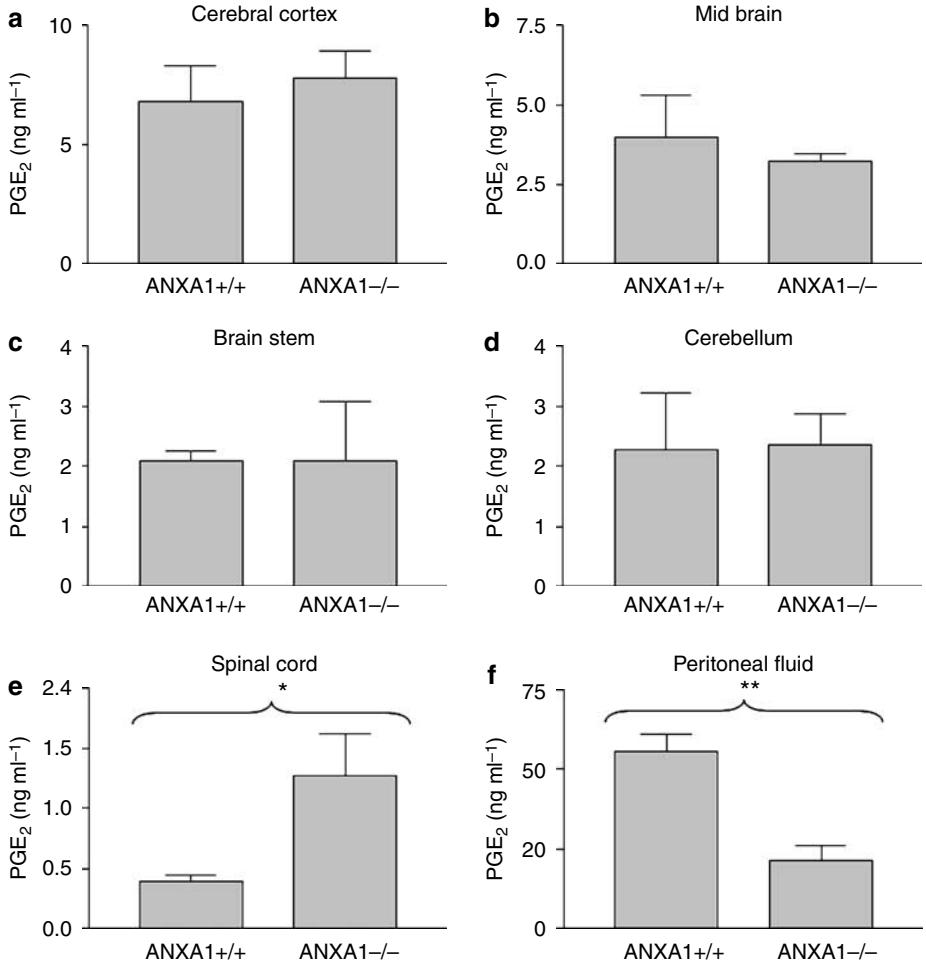

Figure 2 Comparison of the levels of PGE_2 between ANXA1$^{+/+}$ and ANXA1$^{-/-}$ mice writhing to 0.6% acetic acid in cerebral cortex (**a**), mid-brain (**b**), brain stem (**c**), cerebellum (**d**), spinal cord (**e**) and peritoneal washouts (**f**). Following the i.p. administration of acetic acid, the writhing response was observed for 20 min, after which the animals were killed by cervical dislocation, brain and spinal cord tissues dissected out and snap-frozen in liquid nitrogen. PGE_2 was extracted using C18 columns and measured with a PGE_2 EIA kit. *$P<0.05$, **$P<0.01$, $n=6$. ANXA1, annexin-A1; PGE_2, prostaglandin E_2.

administered i.p. to ANXA1$^{+/+}$ and ANXA1$^{-/-}$ mice, 10 min before the induction of writhing with acetic acid, produced a significant analgesic effect ($P<0.05$) in both genotypes (Figure 3a). The analgesic effect of Ac2-26 was coupled with significant reduction in the levels of PGE_2 in spinal cord tissues of ANXA1$^{-/-}$ mice and a nonsignificant reduction in ANXA1$^{+/+}$ mice (Figure 3b).

ANXA1 mediates the analgesic action of methylprednisolone

Methylprednisolone is a synthetic glucocorticoid used in the treatment of inflammatory conditions. Some of its anti-inflammatory actions were shown to be mediated through the induction of ANXA1 (Podgorski *et al*., 1992; Solito *et al*., 1993). The aim of the present experiments was to test whether ANXA1 also mediates the analgesic actions of this glucocorticoid. Thus, at the therapeutic dose of 60 mg kg^{-1} in rodents (Kim and Jahng, 2004), methylpredinsolone administered 1 h before the induction of the writhing response significantly reduced the writhing counts ($P<0.05$) in ANXA1$^{+/+}$ mice but not in ANXA1$^{-/-}$ mice (Figure 4a). Methylprednisolone produced an inhibitory trend on spinal cord PGE_2 levels ($P>0.05$) in ANXA1$^{+/+}$, whereas no such trend was seen in tissues from ANXA1$^{-/-}$ (Figure 4b).

$cPLA_2$, COX-1, COX-2 and COX-3 proteins are upregulated in spinal cord tissues of ANXA1$^{-/-}$ mice

The expression of some of the enzymes involved in synthesis of PGE_2 namely $cPLA_2$, COX-1, COX-2 and COX-3 was examined in spinal cord tissues of ANXA1$^{+/+}$ and ANXA1$^{-/-}$ mice. The expression of all four proteins increased in the spinal cords of ANXA1$^{-/-}$ mice compared with ANXA1$^{+/+}$ animals. $cPLA_2$ increased by almost 100% (Figure 5a; 95%), whereas COX-1 increased by 21% (Figure 5b), COX-2 increased by 48% (Figure 5c) and COX-3 by 38% (Figure 5d).

Discussion

The present study demonstrated that ANXA1 is an endogenous modulator of acute nociceptive pain, as loss of a fully functional ANXA1 protein in ANXA1 null mice resulted in

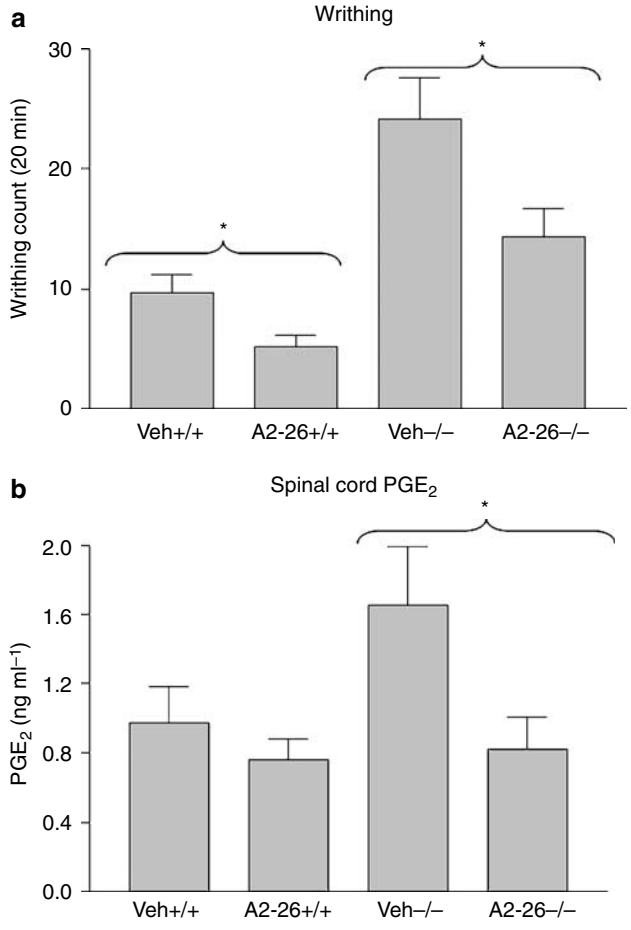

Figure 3 The effect of the ANXA1 peptidomimetic Ac2-26 on the writhing response induced with acetic acid (**a**) and on spinal cord PGE_2 levels (**b**) of ANXA1$^{+/+}$ and ANXA1$^{-/-}$. Acetic acid (0.6%) was administered intraperitoneally 10 min after the administration of 200 μg of Ac2-26 and the writhing response observed for 20 min. The animals were killed by cervical dislocation, whole spinal cord tissues dissected and snap-frozen in liquid nitrogen. Spinal cord PGE_2 levels were measured using a PGE_2 EIA kit after extraction with C18 columns. *$P<0.05$, $n=6$. ANXA1, annexin-A1; PGE_2, prostaglandin E_2.

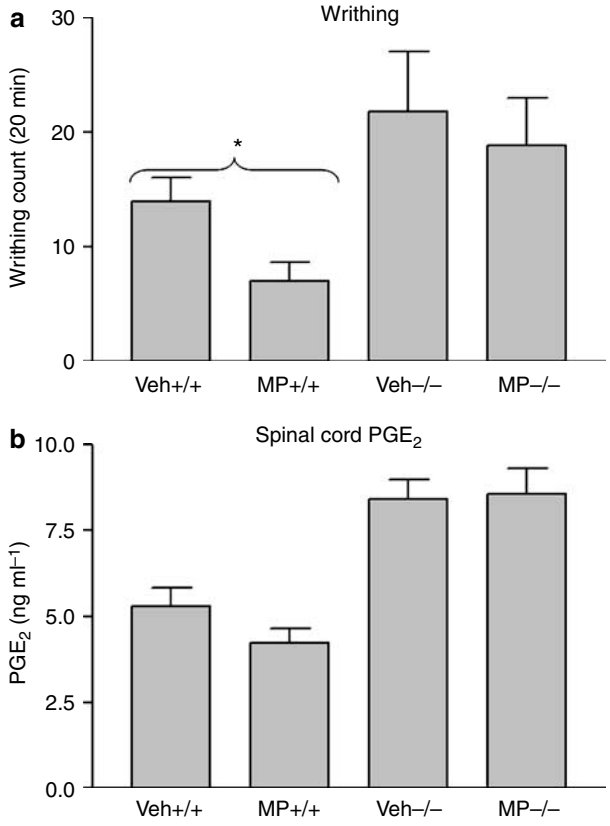

Figure 4 The effect of 6α-methylprednisolone 21-hemi-succinate (MP) on the writhing response induced with acetic acid (**a**) and on spinal cord PGE_2 levels (**b**) in ANXA1$^{+/+}$ and ANXA1$^{-/-}$ mice. Acetic acid (0.6%) was administered intraperitoneally 1 h after the administration of 60 mg kg^{-1} of MP and the writhing response observed for 20 min. The animals were killed by cervical dislocation, whole spinal cord tissues dissected and snap-frozen in liquid nitrogen. Spinal cord PGE_2 levels were measured using a PGE_2 EIA kit after extraction with C18 columns. *$P<0.05$, $n=6$. ANXA1, annexin-A1; PGE_2, prostaglandin E_2.

an exacerbation in the acetic acid-induced writhing pain compared with wild-type mice. The increase in the writhing response in ANXA1 null mice was coupled with an increase in the spinal levels of PGE_2, suggesting that the observed antinociceptive action of ANXA1 is dependent on down-regulation of the pathway leading to synthesis of PGE_2. This eicosanoid is an important nociceptive mediator within the dorsal horn of the spinal cord (Willingale *et al*., 1997) and is involved in mediating the writhing response. Classical and antipyretic COX inhibitors have been shown to inhibit significantly the writhing response coupled with reduction in PGE_2 synthesis in the CNS (Ayoub *et al*., 2006). The reduction of the writhing response and spinal cord PGE_2 in ANXA1$^{+/+}$ and ANXA1$^{-/-}$ animals by the ANXA1 N-terminal peptide Ac2-26 provides further support for the idea that the antinociceptive action of ANXA1 is dependent on reduction of PGE_2 synthesis at the spinal level.

The observed reduction in the levels of PGE_2 in the peritoneal washouts of ANXA1$^{-/-}$ compared with ANXA1$^{+/+}$ mice could be due to the leukopaenia observed in the peritoneum of ANXA1$^{-/-}$ mice challenged with zymosan (Hannon *et al*., 2003).

Methylprednisolone is a synthetic anti-inflammatory glucocorticoid classified as a steroidal anti-inflammatory drug. It is commonly used topically for the treatment of inflammatory conditions of the eye, ear and nose and also for rheumatic diseases (British National Formulary 49, 2005). Methylprednisolone exhibited an analgesic action in ANXA1$^{+/+}$ mice writhing to acetic acid and showed a tendency to reduce the spinal cord levels of PGE_2, both effects believed to be mediated through ANXA1, as loss of the protein in ANXA1$^{-/-}$ mice resulted in loss of the analgesic action and in the PGE_2 inhibitory action.

At the molecular level, the glucocorticoids possess genomic and non-genomic actions (Saklatvala, 2002). At the genomic level, the glucocorticoids repress transcription of a number of pro-inflammatory gene products that include the pro-inflammatory cytokines, interleukins 1–6 (Cavaillon, 1995) and pro-inflammatory enzymes such as inducible NOS (Matsumura *et al*., 2001), $cPLA_2$ (Croxtall *et al*., 1996) and COX-2 (Hay and de Belleroche, 1998). Glucocorticoids are

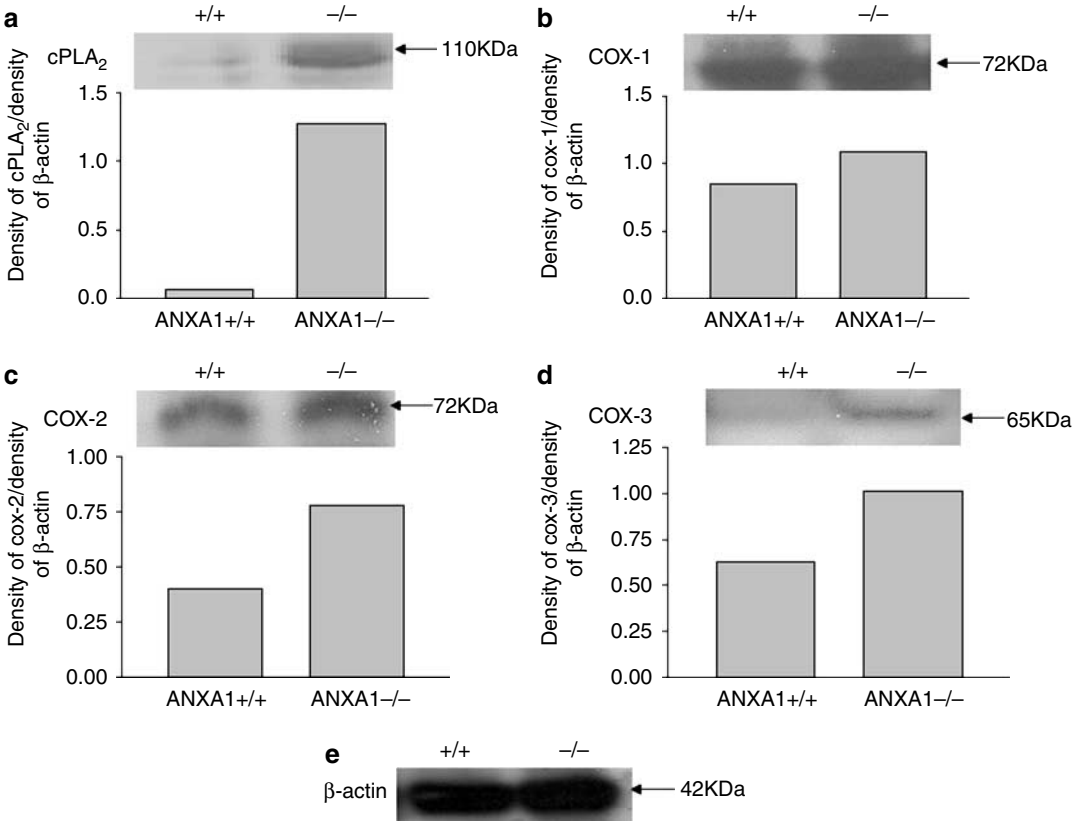

Figure 5 The expression profile of $cPLA_2$ (**a**), COX-1 (**b**), COX-2 (**c**) and COX-3 (**d**) in the spinal cord tissues of $ANXA1^{+/+}$ and $ANXA1^{-/-}$ mice expressed as density units and normalized to the density of β-actin (**e**) acting as a housekeeping protein. Whole spinal cord tissues were dissected from untreated mice and snap-frozen in liquid nitrogen. Tissues with the same genotype were pooled together ($n=5$) and crushed using a nitrogen gun. The protein concentration was determined and samples were constituted in Lammelli buffer. The proteins were detected using standard electrophoresis and immunoblotting techniques. ANXA1, annexin-A1.

also involved in the induction of anti-inflammatory products such as interleukin-10 (Mozo *et al*., 2004) and ANXA1 (Podgorski *et al*., 1992; Croxtall and Flower, 1994). These genomic actions of glucocorticoids are dependent on binding, activation and nuclear localization of the glucocorticoid receptor expressed in most cells, which is a time-dependent process. The non-genomic actions of the glucocorticoids are very rapid occurring within minutes and are dependent on cytoplasmic glucocorticoid receptor (Buckingham, 2006).

Methylprednisolone was pre-administered 1 h before the induction of writhing pain, suggesting that the analgesic effect of this drug is unlikely to be explained by *de novo* synthesis of ANXA1 protein as it characteristically takes glucocorticoids more than 2 h to induce synthesis of ANXA1 (Solito *et al*., 1993; Parente and Solito, 1994). In addition, ANXA1 was shown to be constitutively expressed in CNS tissues (including spinal cord) of $ANXA1^{+/+}$ mice, as also previously demonstrated by Strijbos *et al*. (1990), and that expression was reduced dramatically in $ANXA1^{-/-}$ mice. Croxtall *et al*. (2002) showed that in the human lung carcinoma cell line, A549, glucocorticoids reduced PGE_2 biosynthesis by either inhibition of $cPLA_2$ activity or inhibition of COX-2 protein induction, the former effect considered to be a non-genomic, and the latter, a genomic effect. Some of the drugs tested were able to do both (Croxtall *et al*., 2002). Methylprednisolone was shown to reduce $cPLA_2$ activity, but did not inhibit COX-2 protein induction. Therefore, it is possible that direct inhibition of $cPLA_2$ activity by methylprednisolone could explain its analgesic action in this model.

However, as we have shown that the analgesic action of methylpredinsolone is dependent on ANXA1, we postulate that methylprednisolone is more likely to be working through the increasing externalization of ANXA1. This phenomenon of exportation of stored ANXA1 through the plasma membrane from the intracellular pool of cytoplasmic ANXA1 into the pericellular pool (attached to the outer surface of the cell membrane) by glucocorticoids occurs within minutes and contributes to the anti-inflammatory effects of the glucocorticoids (Buckingham, 1996; Philip *et al*., 1997).

In an attempt to dissect out the mechanism by which ANXA1 reduces spinal cord PGE_2 biosynthesis, we examined the expression of enzymes involved in the biosynthesis of PGE_2. Within the spinal cord, the expression of $cPLA_2$, COX-1, COX-2 and COX-3 proteins was higher in $ANXA1^{-/-}$ mice compared with $ANXA1^{+/+}$ mice, which correlates with the observed pattern of PGE_2 release. Thus, the higher levels of PGE_2 in the spinal cords of $ANXA1^{-/-}$ mice could be the result of increased activity of these enzymes as a result of their increased expression. These results suggest that ANXA1 modulates spinal nociceptive processing by reducing activity

of one or some of these enzymes. The current investigation does not provide evidence into which enzyme is inhibited by ANXA1; however, it has already been shown that ANXA1 reduces $cPLA_2$ activity (Solito *et al*., 1998; Tommasini and Cantoni, 2004). The increased expression of cPLA2 is likely to be a consequence of ANXA1 deletion, as shown previously (Hannon *et al*., 2003).

In this model, we have already shown that both COX-1 and COX-3, but not COX-2, are involved in nociceptive transmission at the spinal level; hence, it is also possible that ANXA1 inhibits activity or synthesis of these enzymes (Ayoub *et al*., 2006).

The current findings demonstrate that ANXA1 plays an antinociceptive role within the spinal cord, which provides a novel drug target for the development of analgesics. In addition, the current study provides a possible mechanism for the analgesic action of the glucocorticoids, which are routinely given to patients with postoperative pain associated with procedures such as third molar extraction, tonsillectomy and breast augmentation (Schultze-Mosgau *et al*., 1995; Stewart *et al*., 2002; Bamgbose *et al*., 2005; Romundstad *et al*., 2006).

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Conflict of interest

The authors state no conflict of interest.

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