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G protein-coupled receptor kinase 6 controls post-inflammatory visceral hyperalgesia ☆

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ABSTRACT

Post-inflammatory pain is a poorly understood phenomenon. G protein-coupled receptors are involved in regulating pain signaling in the context of inflammation. G protein-coupled receptor kinases (GRK) modulate signaling through these receptors. We investigated whether GRK6 contributes to post-inflammatory visceral hyperalgesia.

Colitis was induced in female mice by 1% dextran sodium sulphate in drinking water for 7 days. Disease score, colon length, and colonic cytokines were determined. On day 49, when animals had recovered from colitis, we induced visceral pain by intracolonic capsaicin instillation. Behavioral responses to capsaicin were monitored for 20 min. Referred hyperalgesia was measured using von Frey hairs. Spinal cord c-Fos was visualized by immunohistochemistry.

In contrast to our earlier observations in male GRK6$^{-/-}$ and wild type (WT) mice, we did not detect differences in the course of colitis or in expression of colonic cytokines between female GRK6$^{-/-}$ and WT mice. After recovery from colitis, capsaicin-induced behavioral pain responses and spinal cord c-Fos expression were more pronounced in female GRK6$^{-/-}$ than WT mice. Naive GRK6$^{-/-}$ and WT animals did not differ in pain and c-Fos responses to capsaicin. Capsaicin-induced referred hyperalgesia post-colitis was increased in GRK6$^{-/-}$ compared to WT mice. However, referred hyperalgesia post-colitis was not affected by ablation of GRK6. Furthermore, in vitro IL-1β sensitized the capsaicin receptor TRPV1 and this process was inhibited by over-expression of GRK6.

We describe the novel concept that GRK6 inhibits post-inflammatory visceral hyperalgesia but does not contribute to visceral pain in naive animals. We propose that GRK6 regulates inflammation-induced sensitization of TRPV1.

1. Introduction

Pain and discomfort in association with altered bowel habits are characteristics of irritable bowel syndrome. Although precise mechanisms responsible for visceral pain are unknown, it is generally accepted that altered processing of information from visceral afferents plays an important role (Lembo et al., 1994; Bernstein et al., 1996; Chang et al., 2000). Animal studies have shown that inflammation can modulate processing of visceral afferent signals and thereby cause visceral hyperalgesia (Burton and Gebhart, 1995; Coutinho et al., 1996; Gschossmann et al., 2002; Verma-Gandhu et al., 2007). In addition, visceral hyperalgesia can persist after resolution of acute intestinal inflammation. For example, transient trinitrobenzene sulphonic acid (TNBS)-induced colitis in rats caused increased sensitivity to colorectal distension up to 17 weeks after induction of colitis (Gschossmann et al., 2004). Moreover, *Trichinella spiralis* infection increased visceral sensitivity to pain until several weeks after inflammation had subsided (Bercik et al., 2004). We recently described that visceral sensitivity of mice, determined as behavioral pain responses and spinal cord neuronal activation after intracolonic capsaicin, was enhanced after recovery from dextran sodium sulphate (DSS) colitis (Eijkelkamp et al., 2007b).

Sensitization of neuronal afferents by inflammatory mediators is one of the mechanisms which may underlie post-inflammatory hyperalgesia (Bueno and Fioramonti, 2002; Cervero and Laird, 2004). Neuronal sensitization is characterized by increased signaling of receptors involved in pain transmission (Thompson et

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al., 1994; Bird et al., 2006). Many of these receptors, such as the receptors for substance P (SP), glutamate, calcitonin gene-related peptide (CGRP), prostaglandin E2 (PGE2), and chemokines, belong to the G protein-coupled receptor (GPCR) family. The activity of GPCRs can be regulated by the family of GPCR kinases (GRK1-7). GRKs phosphorylate agonist-activated GPCRs, thereby inducing rapid uncoupling of the receptor from the G protein, a process called homologous receptor desensitization. GRK-mediated GPCR phosphorylation facilitates binding of arrestin proteins, resulting in GPCR desensitization and internalization (Pitcher et al., 1998; Ferguson, 2001). Several studies have shown that the intracellular level of GRK determines the extent of receptor desensitization and internalization, thereby determining GPCR sensitivity (Menard et al., 1997; Premont and Gainetdinov, 2007).

The specific substrate receptors for one ubiquitously expressed kinase, GRK6, are still largely unknown, but it has been shown that GRK6 is involved in desensitization of e.g. the chemokine receptor CXCR4, the BLT1 receptor for the leukotriene LTB4, and the CGRP receptor. Moreover, reduction of intracellular levels of GRK6 increases signaling via the above-mentioned receptors (Aiyar et al., 2000; Kavelaars et al., 2003; Vroon et al., 2004, 2006). Finally, it is known that GRKs interact with a variety of key downstream signaling molecules thereby regulating cellular signaling as well (Reiter and Lefkowitz, 2006). Regulation of downstream signaling molecules by GRKs may therefore also affect non-GPCR-induced hyperalgesia by e.g. the cytokines IL-1β and TNF-α.

We speculated that GRK6 may play a key role in post-inflammatory hyperalgesia. We thus set out to study post-inflammatory pain in a chemically-induced colitis model (DSS-colitis) in mice with a targeted deletion of the GRK6 gene. Recently, we have demonstrated that in male mice with a genetic deletion of GRK6, the course of DSS-induced colitis was more severe than in male WT mice (Eijkelkamp et al., 2007a). However, we show here that in female mice the course and intensity of DSS-colitis did not differ between GRK6$^{-/-}$ mice and WT mice. Therefore, female mice were used in the present study to investigate the role of GRK6 in post-inflammatory visceral pain. To quantify post-inflammatory visceral sensitivity to pain, we determined behavioral responses to intracolonic capsaicin instillation after resolution of colitis. This method has been described to be a valid way to investigate colonic sensitivity to painful stimulation (Laird et al., 2001; Eijkelkamp et al., 2007b) and measures an affective response to pain. Moreover, as an objective measure to assess neuronal activation in response to painful stimulation, we analyzed capsaicin-induced spinal cord neuronal c-Fos expression. The behavioral pain responses correlated well with this more objective measure of neuronal activation (Eijkelkamp et al., 2007b). Unlike the frequently used pseudo-affective visceromotor response to colonic distension, the method we used here does not require surgery. In addition, using capsaicin as the noxious stimulus, we were able to obtain information on referred somatic hyperalgesia as an indicator of central sensitization before and after resolution of DSS-colitis.

2. Materials and methods

2.1. Animals

GRK6-deficient C57BL/6 and WT control animals (Eijkelkamp et al., 2007a) were housed in the Utrecht University, Central Animal Facility. Experiments were performed in accordance with international guidelines and approved by the Experimental Animal Committee of the University Medical Center Utrecht.

2.2. Experimental design

Female WT and GRK6$^{-/-}$ littermates of 12–14 weeks were randomly assigned to naive or colitis groups. As described before, animals received drinking water with 1% Dextran Sodium Sulphate for 7 days to induce transient colitis ((Eijkelkamp et al., 2007b); mol. wt. 40,000; ICN Biomedicals, Eschwege, Germany). From day 8 onwards all animals received normal drinking water. Body weight, stool consistency and fecal blood loss (Hemocult tests, Beckman Coulter, Eschwege, Germany) were recorded daily. The disease activity index (DAI) was calculated (Table 1).

Table 1
Disease activity index

Score	Weight loss	Stool consistency[a]	Fecal Blood
0	None	Normal	Normal
1	1–5 (%)		
2	5–10 (%)	Loose stool	Hemoccult+
3	10–15 (%)		
4	15–20 (%)	Diarrhea	Gross Bleeding

The disease activity index reflects the combined scores of weight loss, stool consistency and bleeding, leading to a maximum DAI of 12.
[a] Normal stool = firm and well-formed pellets, loose stool = pasty, and semi-formed stools which do not stick to the anus, diarrhea = liquid stools that stick to the anus.

At day 49, naive animals and animals post-colitis were randomly assigned to two groups and subjected to the following procedures:

Group 1: capsaicin treatment and measurement of capsaicin-induced behavioral responses from 5 till 25 min after capsaicin treatment. At 30 min after capsaicin treatment capsaicin-induced referred hyperalgesia was determined using von Frey hairs. At 2 h after capsaicin treatment the animals were sacrificed, and spinal cords were collected for c-Fos staining.

Group 2: After determination of referred hyperalgesia using von Frey hairs, animals were sacrificed and the colon was collected. After measurement of colon length, colons were longitudinally divided into two samples. One sample was fixed in 4% paraformaldehyde, paraffin embedded and stained with hematoxylin and eosin for histological examination. The other half of the colon was used for RNA and protein extraction.

2.3. Behavioral pain responses

Behavioral responses to intracolonic capsaicin were determined as described (Laird et al., 2001; Eijkelkamp et al., 2007b). Briefly, animals were placed on a raised wire mesh under a clear plastic box (30 × 20 × 15 cm) for 30 min prior to capsaicin instillation. Capsaicin (30 μL; 0.1% wt/vol in 10% ethanol/10% Tween 80/80% saline; Sigma. St. Louis, MA, USA) or vehicle was administered intracolonically under isoflurane anesthesia (3% in N_2O:O_2 1:1). After 5 min, behavior was recorded during 20 min for analysis by two separate observers (inter-observer reliability: 90%) who were blinded to the experimental conditions. Postures defined as pain-related behaviors (abdominal (i) licking, (ii) stretching, (iii) retractions, and (iv) squashing of lower abdomen against the floor) were each counted as one. Two hours after capsaicin instillation, animals were sacrificed and perfused with 4% paraformaldehyde and spinal cords were collected.

2.4. Referred hyperalgesia

Withdrawal responses to application of von Frey hairs (8, 20, 40, 70, 160, 400, and 600 mg; Stoelting, Wood Dale, IL, USA) to the abdomen were examined as a measure of referred hyperalgesia (Eijkelkamp et al., 2007b). Prior to capsaicin instillation, animals were placed on a raised wire mesh (5 × 5 mm apertures) under a clear plastic box (10 × 10 × 5 cm) for 30 min. Von Frey hairs were applied up through the wire mesh to the lower to mid abdomen of the freely moving animals for 5 s in ascending order of force