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Activation of peripheral galanin receptors: Differential effects on nociception

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Abstract

Numerous reports suggest a significant role of peripheral galanin (GAL) in pain transmission; however, due to the lack of selective galanin receptor agonists and antagonists, the role of GAL receptors (GalR1-3) in pain transmission remains unclear. In this study, a new agonist, M617, that preferentially binds to GalR1, a GalR2 agonist (AR-M1896), and a GalR2 antagonist (M871) were tested in the periphery to elucidate the role of peripheral GalR1 and GalR2 in nociception. Ipsilateral, but not contralateral, hindpaw injection of M617 reduced capsaicin (CAP)-induced flinching by ~50%, suggesting that GalR1 activation produces anti-nociception. This anti-nociceptive effect was blocked by intraplantar injection of the non-selective GalR antagonist M35. In contrast ipsilateral, but not contralateral, intraplantar injection of GalR2 agonist AR-M1896 enhanced the CAP-induced nociception (1.7-fold). The GalR2 antagonist M871 blocked the pro-nociceptive effect of AR-M1896 in a dose-dependent manner. This antagonist had no effect on nociceptive behaviors induced by CAP alone. The data demonstrate that activation of peripheral GalR1 results in anti-nociception but activation of peripheral GalR2 produces pro-nociception. Thus, the use of these pharmacological tools may help to elucidate the contribution of GalR subtypes in nociceptive processing, identifying potential drug targets for the treatment of peripheral pain.

Keywords: Inflammatory pain; Pro-nociception; Anti-nociception; Capsaicin; Primary afferent

1. Introduction

The neuropeptide galanin (GAL) has a wide distribution in the central and peripheral nervous system of several species including mice, dogs, and rats (Ju et al., 1987; Meister et al., 1990; Perez et al., 2001; Skofitsch and Jacobowitz, 1986). Currently, several physiological and pathological functions have been attributed to GAL (see Berger et al., 2005; Brewer et al., 2005 for reviews). In regards to sensory input, there is a large body of evidence suggesting a role for GAL and its three receptor subtypes (GalR1, GalR2, and GalR3) in the transmission and modulation of pain (Bartfai et al., 2004; Blakeman et al., 2003; Hua et al., 2004; Jimenez-Andrade et al., 2004; Kerekes et al., 2003; Liu et al., 2001; Liu and Hökfelt, 2002; Lundström et al., 2005; Sun et al., 2003; see Wiesenfeld-Hallin et al., 2005, for review). In the spinal cord, GAL produces contrasting effects as both pro-nociception and anti-nociception following intrathecal administration have been observed (Cridland and Henry, 1988; Flatters et al., 2002; Hao et al., 1999; Hua et al., 2004; Kuraishi et al., 1991; Lundström et al., 2005; Post et al., 1988; Reeve et al., 2000; Wiesenfeld-Hallin et al., 1988; Yu et al., 2001). A possible explanation for these disparate findings could be the differential activation of GalRs: GalR1 and GalR3 are negatively coupled to adenylyl cyclase, and their activation results in hyperpolarization of neurons that could lead to anti-nociception. In contrast, GalR2s are coupled to the phospholipase C-protein kinase C (PKC) pathway and their activation predominantly causes excitatory effects (see Branchek et al., 2000; Liu and Hökfelt, 2002, for review). Low

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concentrations of GAL acting at GalR2 could result in excitatory effects (pro-nociceptive effects), while high concentrations of GAL might activate all three GalRs, ultimately producing mixed excitatory and inhibitory effects or inhibitory effects (Liu and Hökfelt, 2002).

While the role of GAL has been extensively studied in diverse pain models, the contribution of each GalR subtype to pain modulation is not fully understood partially due to the lack of selective agonists and antagonists for these receptors. We previously reported that intraplantar injection of GAL peptide or AR-M1896, a GalR2/3 agonist (Liu et al., 2001; Lu et al., 2005), potentiated capsaicin (CAP)-induced inflammatory pain behaviors and electrophysiological studies confirmed this pro-nociceptive action of GAL (Jimenez-Andrade et al., 2004). The GAL-induced potentiation was blocked by intraplantar injection of a PKC inhibitor and mimicked by a PKC activator (Jimenez-Andrade et al., 2005). Furthermore, Kerekes et al. (2003) showed an enhanced excitability of dorsal root ganglion (DRG) neurons following application of low doses of either AR-M1896 (1 nM) or GAL (1 fM). These functional studies are strongly supported by the high expression of GalR2 in DRG (Jimenez-Andrade et al., 2004; Kerekes et al., 2003) and unmyelinated axons from digital nerves (Jimenez-Andrade et al., 2004). These data suggest that peripheral GAL exerts an excitatory effect in inflammatory pain through activation of GalR2 and PKC intracellular pathways.

Recently synthesized compounds such as a GalR1 agonist M617 (Lundström et al., 2005) and a GalR2 antagonist M871 (Sollenberg et al., 2006) will very likely be useful in elucidating the role of GalRs in pain modulation. Intrathecal administration of M617 results in inhibition of C-fiber conditioning stimulation, suggesting that spinal GalR1 activation could result in inhibitory actions (Lundström et al., 2005). In order to better define the function of peripheral GalRs, we assessed the ability of intraplantar M617 to block CAP-induced pain. In addition, we evaluated the ability of M871 to block the GalR2-enhancement of CAP pain.

2. Materials and methods

All experiments were carried out in accordance to the ethical guidelines recommended by the International Association for the Study of Pain for experimental pain in conscious animals (Zimmermann, 1983). Moreover, all experimental protocols were approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados, México, DF, México).

2.1. Peptide synthesis

The peptides were synthesized in a stepwise manner in a 0.1 mmol scale on an automated peptide synthesizer (Applied Biosystems, Model 431A) using the *t*-Boc solid-phase peptide synthesis strategy. *tert*-Butyloxycarbonyl amino acids (Neosystem, Strasbourg, France) were coupled as hydroxybenzotriazole (HOBt) esters to a *p*-methylbenzylhydrylamine (MBHA) resin (Neosystem, Strasbourg, France) to obtain C-terminally amidated peptides. Deprotection of the *formyl* protecting group on tryptophane was carried out in 20% piperidine in DMF for 60 min, and deprotection of the dinitrophenol group on histidine was carried out by treatment of 20% thiophenole in DMF for 1 h. The peptide was finally cleaved from the resin using liquid HF at 0 °C for 1 h in the presence of *p*-cresol and *p*-thiocresol (1:1). The molecular weight was determined by MALDI-TOF mass spectrometry (Voyager-DE STR, Applied Biosystems, Framingham, USA).

2.2. Animal habituation

Male Wistar rats (250–300 g) from the campus breeding facilities (CINVESTAV-IPN) were used in this study. Animals had access to food and drinking water *ad libitum* before the experiments. The rats were habituated to the behavioral testing procedures by placement in open Plexiglas observation chambers for 1 h. Each rat was habituated twice before being placed in an experimental group.

2.3. Drugs

Galanin (porcine–galanin) was purchased from Peninsula Laboratories, Inc., (Belmont, CA, USA). The M617 [galanin(1-13)-Gln^{14}-bradykinin-(2-9)-amide], an agonist that preferentially binds to GalR1 (Lundström et al., 2005), M871 [galanin(2-13)–Glu–His–(Pro)3–(Ala–Leu)2–Ala amide], a selective GalR2 antagonist, (Sollenberg et al., 2006), and M35 [galanin-(1-13)-bradykinin-(2-9)-amide], a high-affinity non-selective GalR antagonist (Kask et al., 1995; Wiesenfeld-Hallin et al., 1992) were synthesized as above described. The GalR2 agonist AR-M1896 [Gal(2–11)Trp–Thr–Leu–Asn–Ser–Ala–Gly–Tyr–Leu–Leu–NH_2] (Liu et al., 2001) was kindly provided by Dr. Ralf Schmidt (Astra-Zeneca, Montreal, Quebec, Canada). The M871 was dissolved in distillated water, while all other drugs were dissolved in saline. A stock solution of 10% CAP (8-methyl-*N*-vanillyl-6-nonamide; Fluka Chemical Corp, Milwaukee, WI, USA) was made by dissolving 1 g of CAP in a mixture of 2 ml of ethanol, 0.7 ml of Tween-80 and 9.3 ml of saline. This solution was heated and stirred for 1–3 h until the final volume was 10 ml, indicating the ethanol had evaporated. This stock solution was diluted with CAP vehicle (7% Tween 80 in saline) to make working dilutions of CAP. For subcutaneous hindpaw injections of all drugs, a 29-gauge needle was attached to a Hamilton syringe with PE20 tubing. The needle punctured the plantar skin and was guided forward in the subcutaneous space to a site just proximal to the pads. Each animal was used only once ($n \geq 6$ per group), and the investigator was blinded as to which drugs were injected with CAP. At the end of the experiment, rats were euthanized with CO_2.

2.4. Study design

2.4.1. GAL effect on CAP-induced nociception

To determine if high doses of peripheral GAL modulated CAP-induced nociception, male Wistar rats received intraplantar injections of 10 μl 0.1% CAP+20 μl saline or 10 μl 0.1%