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ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 273–280
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Activation of peripheral galanin receptors: Differential effects on nociception

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Received 17 June 2006; received in revised form 9 August 2006; accepted 9 August 2006
Available online 20 September 2006

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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doi:10.1016/j.pbb.2006.08.008

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%

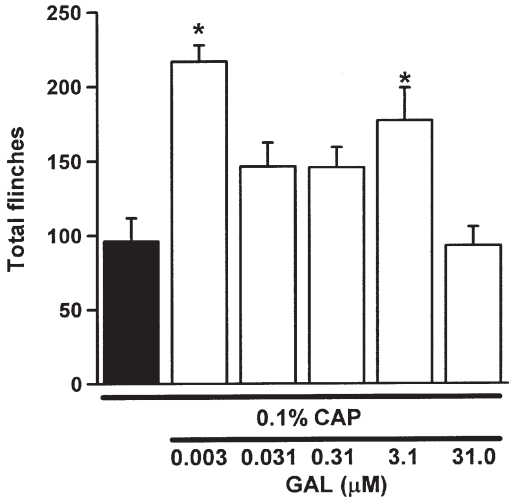

Fig. 1. Concentration–response relationship for peripheral galanin (GAL, 0.003–31.0 μM). Intraplantar GAL at 0.003 μM increases the capsaicin (CAP)-induced flinching behavior; however, GAL at higher concentrations does not modify the flinching behavior. Bars represent the mean ± S.E.M. of the total sum of flinches during 60 min ($n \geq 6$ in each group). *$P<0.05$, significant difference from CAP alone group, one-way ANOVA followed by Tukey's test.

CAP+20 μl GAL (0.003, 0.031, 0.31, 3.1 and 31.0 μM). Flinching (a nociceptive behavior) of the injected hindpaw was assessed for 60 min (Jimenez-Andrade et al., 2004, 2005). Neither the needle puncture nor injection of saline caused nociceptive responses (data not shown).

2.4.2. GalR1 effect on CAP-induced nociception

To determine whether activation of peripheral GalR1 modulated CAP-induced nociception, 20 μl M617 (0.01, 0.1 or 1.0 μM) was injected subcutaneously into the hindpaw followed by 10 μl 0.1% CAP. Flinching behavior was assessed for 60 min post-injection. From this dose–response study, we selected the dose of M617 (1.0 μM) that produced the maximum reduction in CAP-induced responses and used it for the rest of the studies. Possible systemic effects produced by intraplantar injection of M617 were evaluated by injecting 20 μl 1.0 μM M617+10 μl CAP vehicle into one hindpaw, followed immediately by injection of 10 μl 0.1 % CAP+20 μl saline into the contralateral hindpaw. There is no selective GalR1 antagonist; however, it has been reported that low concentrations of the antagonist M35 (<10 nM) block spinal GAL effects *in vivo* (Wiesenfeld-Hallin et al., 1992). Due to the high sequence homology between M35 and M617 (Lundström et al., 2005), it is plausible to speculate that M35 might antagonize the peripheral anti-nociceptive effect of M617. So, 30 μl M35 (0.1 and 1.0 nM) was injected into the hindpaw 20 min prior to injection of 10 μl 0.1% CAP+20 μl 1.0 μM M617. The administration schedule for M35 was selected based on pilot experiments in our laboratory. Control experiments demonstrated that 30 μl of saline did not modify the M617 anti-nociception (data not shown), indicating that the additional 30 μl volume had no effect on nociceptive behaviors. A possible tonic effect of endogenous GAL was evaluated by administration of 30 μl 0.1 nM M35 20 min before injection of 10 μl 0.1% CAP+20 μl saline.

2.4.3. GalR2 effect on CAP-induced nociception

To determine whether the putative GalR2 antagonist M871 (Sollenberg et al., 2006) modulated GalR2-induced behaviors, rats received intraplantar injections of 10 μl 0.1% CAP followed by 20 μl saline or 20 μl AR-M1896 (0.0017, 0.017 or 0.17 μM). Flinching behavior was evaluated for 60 min after CAP injection. From this dose–response study, the working dose of AR-M1896 (0.017 μM) was determined. Then, 30 μl of different concentrations of M871 (0.01, 0.1, or 1.0 μM) were injected 20 min prior to 10 μl 0.1% CAP+20 μl 0.017 μM AR-M1896. Control experiments showed that 30 μl of distillated water (M871 vehicle) had no effect on CAP-induced nociception (data not shown).

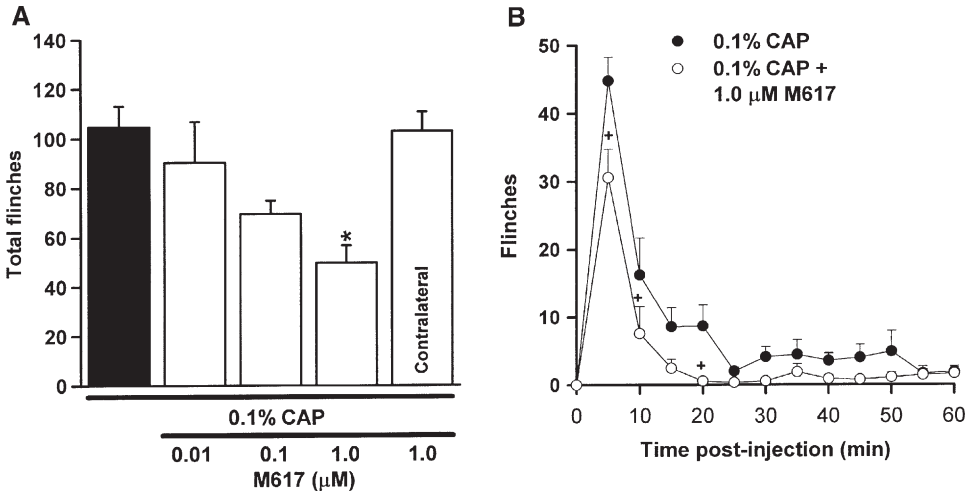

Fig. 2. (A) Dose–response relationship for peripheral M617, a GalR1-preferring agonist. (B) Time course of flinching behavior produced by 0.1% capsaicin (CAP) and 0.1% CAP+1.0 μM M617. Bars and time points represent the mean ± S.E.M. of the total sum of flinches during 60 min and of the number of flinches in 5 min intervals ($n \geq 6$ in each group). *$P<0.05$, compared to CAP alone group, one-way ANOVA followed by Tukey's test. $^{+}P<0.05$, compared to respective time point in CAP alone group, two-factor repeated-measures ANOVA followed by Tukey's test.

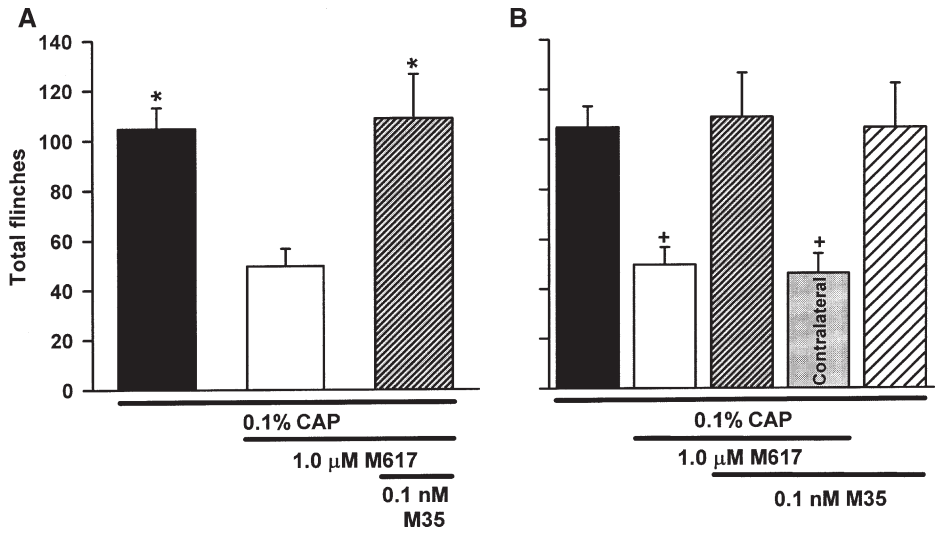

Fig. 3. (A) Intraplantar 0.1 nM M35 reversed the anti-nociceptive effect of M617 on capsaicin (CAP)-induced nociception. (B) The administration of M35 into the contralateral hindpaw had no effect. Furthermore, CAP-induced pain was not altered by M35 alone. Bars represent the mean ± S.E.M. of the total sum of flinches during 60 min ($n \geq 6$ in each group). *$P<0.05$, compared to CAP alone, $^{+}P<0.05$, significant difference from CAP + 1.0 μM M617, one-way ANOVA followed by Tukey's test.

2.5. Statistical analysis

All data were expressed as mean ± standard error of the mean (S.E.M.). Sigma Stat 2.03 was used to perform all statistical analysis. Time-courses of flinching behavior induced by CAP alone or CAP plus various drugs were constructed by plotting the mean number of flinches in 5 min intervals as a function of time. The total number of flinches occurring during 60 min was obtained and plotted as bar graphs versus treatment. Comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by a Tukey's test. Pairwise comparisons in the time course data for flinching were analyzed using a two-factor repeated measures ANOVA followed by a Tukey's test. A $p<0.05$ was considered significant.

3. Results

3.1. GAL effect on CAP-induced nociception

The co-injection of 0.003 μM GAL + CAP resulted in a significant increase of CAP-induced flinching ($F_{(5,\ 53)}=8.8$, $p<0.001$); however, higher concentrations (0.031, 0.31, and 3.1 μM) of GAL resulted in varying levels of enhancement of CAP-induced flinching but the highest concentration (31.0 μM) had no effect (Fig. 1).

3.2. GalR1 effect on CAP-induced nociception

Intraplantar injection of M617 (GalR1-preferring agonist) reduced CAP-induced flinching in a dose-dependent manner;

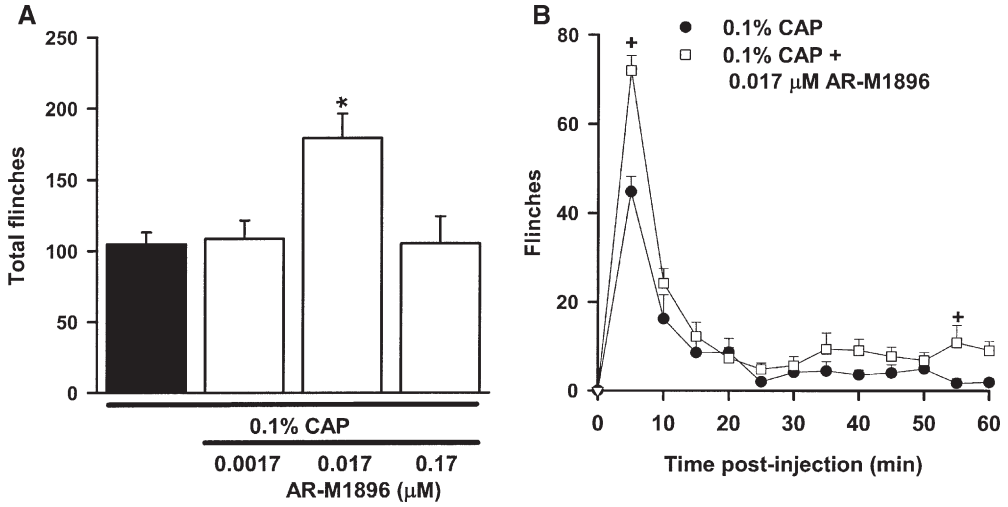

Fig. 4. (A) Dose–response relationship for peripheral AR-M1896, a GalR2 agonist. AR-M1896 at 0.017 μM potentiated the capsaicin (CAP)-induced flinching. (B) Time course of flinching behavior produced by 0.1% CAP and 0.1% CAP + 0.017 μM AR-M1896. Bars and time points represent the mean ± S.E.M. of the total sum of flinches during 60 min and of the number of flinches in 5 min intervals ($n \geq 6$ in each group). *$P<0.05$, compared to CAP alone, one-way ANOVA followed by Tukey's test. $^{+}P<0.05$, compared to respective time point in CAP alone group, two-factor repeated-measures ANOVA followed by Tukey's test.

however, this effect was statistically significant only when the highest concentration of M617 was administered (1.0 μM, Fig. 2A, $F_{(4,\ 32)}=6.3$, $p<0.001$). The time course for flinching behavior indicated a significant group ($F_{(1,\ 176)}=25.5$, $p<0.001$), time ($F_{(11,\ 176)}=41.3$, $p<0.001$), and group×time interaction ($F_{(1,\ 176)}=1.63$, $p=0.094$). Pairwise comparisons between CAP alone and CAP+GalR1 groups indicated that both magnitude and duration of the CAP-induced flinching behavior were significantly reduced by 1.0 μM M617 (Fig. 2B). Injection of the working dose of M617 into one hindpaw did not affect the CAP-induced flinching behavior in the contralateral paw indicating that M617 was producing its anti-nociceptive effect through activation of local GalR1 receptors (Fig. 2A, "Contralateral").

Pre-treatment with 0.1 nM M35 reversed the M617 anti-nociceptive effect ($F_{(2,\ 23)}=8.4$, $p=0.002$) since these animals displayed flinching behavior that was no different from animals receiving CAP alone (Fig. 3A). The effect of M35 on M617 was not due to its systemic circulation since rats treated with 0.1% CAP+M617 in one hindpaw and M35 in the contralateral hindpaw showed flinching behavior similar to that seen in animals injected with CAP+M617 alone (Fig. 3B). The CAP-induced flinching behavior was not altered following intraplantar injection of M35 alone (Fig. 3B).

3.3. GalR2 effect on CAP-induced nociception

The CAP-induced flinching behavior was significantly increased after intraplantar injection of 0.017 μM AR-M1896 (GalR2 agonist, $F_{(3,\ 27)}=6.6$, $p=0.002$) but not 0.0017 μM or 0.17 μM AR-M1896 (Fig. 4A). Two-factor repeated-measures ANOVA for the time course data for flinching behavior revealed significant group ($F_{(1,\ 228)}=12.6$, $p=0.002$), time ($F_{(12,\ 228)}=70.9$, $p<0.001$) and group×time interaction ($F_{(12,\ 228)}=4.02$, $p<0.001$). Pairwise comparisons between CAP alone and CAP+GalR2 groups demonstrated that intraplantar injection of the GalR2 agonist enhanced the magnitude of flinching behaviors induced by CAP (Fig. 4B). Co-injection of M871 (GalR2 antagonist) reduced the pro-nociceptive effect of AR-M1896 in a dose-dependent manner (Fig. 5A, $F_{(4,\ 34)}=11$, $p<0.001$). For further studies, 0.1 μM M871 was selected as the working dose. Intraplantar injection of 30 μl 0.1 μM M871 in the contralateral paw followed by injection of 0.1% CAP+0.017 μM AR-M8196 in the other paw did not block the AR-M1896 effect indicating that M871 was producing its effect through a local, not a systemic action (Fig. 5B). Animals treated with M871+CAP showed behavior similar to that seen in animals treated with CAP alone (Fig. 5B).

4. Discussion

The present study confirms previous reports that intraplantar GAL at low concentrations (nM) induces pro-nociceptive effects (Jimenez-Andrade et al., 2004, 2005). However, GAL at high doses (μM) in the periphery does not produce anti-nociceptive effects, in contrast to what is observed in the spinal cord (Wiesenfeld-Hallin et al., 1989; Xu et al., 1998). Furthermore, we demonstrate that peripheral activation of GalR1 with M617, a GalR1-preferring agonist, reduces CAP-induced inflammatory pain, while peripheral activation of GalR2 enhances this inflammatory pain.

Although significant progress in our understanding of the role of GAL in nociception comes from studies using mice lacking GAL peptide (for references, see Wiesenfeld-Hallin et al., 2005), information gained from GalR1 and GalR2 knock-out mice is controversial. In GalR1 knock-out mice, no or modest hyperalgesia develops under inflammatory and neuropathic conditions (Blakeman et al., 2003; Grass et al., 2003; Malkmus et al., 2005). Likewise, no changes in thermal or mechanical responses in GalR2 knock-out mice were reported (Gottsch et al., 2005; Shi

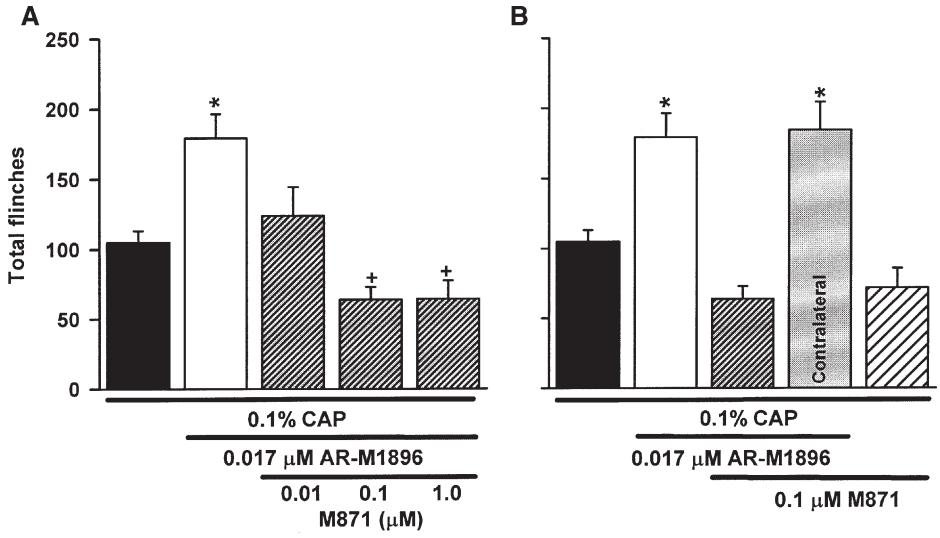

Fig. 5. (A) Dose–response relationship for peripheral M871, a GalR2 antagonist. Intraplantar M871 blocked the AR-M1896 (GalR2 agonist) enhancement of capsaicin (CAP)-induced flinching in a dose-dependent manner. (B) Ipsilateral but not contralateral M871 reversed the pro-nociceptive effect of AR-M8196. M871 alone did not modify the CAP-induced nociception. Bars represent the mean±S.E.M. of the total sum of flinches during 60 min ($n\geq 6$ in each group). *$P<0.05$, significant difference from CAP alone; $^{+}P<0.05$, significant difference from CAP+AR-M1896, one-way ANOVA followed by Tukey's test.

et al., 2006). Compensatory changes in these genetically altered animals might mask the role of GalRs in nociception. Thus, the availability of receptor subtype-selective agents helps to clarify GalR contributions.

There is a large body of evidence supporting a role for GAL in pain transmission in the spinal cord. However, both pro-nociceptive (after low doses, ng) and anti-nociceptive effects (following high doses, μg) have been reported following spinal GAL (Wiesenfeld-Hallin et al., 1989; Xu et al., 1998). A differential activation of GalR subtypes has been suggested as the mechanism underlying these dose-dependent, mixed effects. For example, low concentrations of GAL produces pro-nociceptive effects due to activation of mainly GalR2 while high concentrations of GAL results in mixed or inhibitory effects due to activation of GalR1, 2 and 3 (Liu and Hökfelt, 2002). This hypothesis is strongly supported by the different intracellular signaling pathways of GalRs: GalR2 activation is coupled to Gq/11 proteins that may result in neuronal excitation (Branchek et al., 2000). In contrast, GalR1 and GalR3 activation is coupled to inhibitory Gi/Go proteins that most likely result in hyperpolarization of neurons and neuronal inhibition. Efforts to evaluate this hypothesis and elucidate contributions of each receptor subtype in pain transmission have been hampered by a lack of selective agonist and/or antagonist to GalRs.

In the present study, ipsilateral, but not, contralateral intraplantar injection of M617 resulted in a significant decrease in CAP-induced inflammatory pain suggesting that local activation of GalR1 results in analgesia. This agonist has a 25-fold higher affinity for GalR1 compared to GalR2 (Lundström et al., 2005). This finding confirmed a previous observation that spinal administration of M617 resulted in an inhibitory effect on spinal cord hyperexcitability induced by a C-fiber conditioning stimulus (Lundström et al., 2005). Similarly, this result agrees with previous studies performed in the spinal cord, where intrathecal administration of peptide antisense directed against GalR1 mRNA reduced the spinal inhibitory effect of intrathecal GAL (Pooga et al., 1998; Rezaei et al., 2001). Other pharmacological studies have also suggested an inhibitory role for GAL acting at spinal GalR1; however, this assumption is derived from indirect evidence (Hua et al., 2004; Liu et al., 2001). Thus, using a GalR1-preferring agonist, our studies (Lundström et al., 2005; the present report) provide direct evidence for an anti-nociceptive role of GalR1 in the periphery.

To date, there is no selective GalR1 antagonist to further confirm the analgesic effect mediated by M617. The peptide M35 has been widely used as a non-selective GalR antagonist (Hao et al., 1999; Wiesenfeld-Hallin et al., 1992; Xu et al., 1998). There is high sequence homology between M35 and M617; thus, it is possible that M35 could block M617 effects. Low concentrations of M35 (0.1 and 1.0 nM) were used in this study because agonist properties have been reported with high concentrations (10 and 100 nM, Kask et al., 1995; Mahoney et al., 2003b). In the present study, M35 blocked the anti-nociceptive effect induced by M617 in a dose-dependent manner suggesting a GalR-mediated effect. On the other hand, M35 alone did not modify CAP-induced nociceptive behavior, suggesting that endogenous GAL plays a minor role in CAP-induced pain.

We recently reported that peripheral activation of GalR2 resulted in a significant enhancement of CAP-induced pain in Sprague–Dawley rats, as AR-M1896 potentiated the flinching behavior induced by intraplantar CAP (Jimenez-Andrade et al., 2004). This selective agonist (Liu et al., 2001) has allowed elucidation of the diverse physiological functions mediated by GalR2 (Jimenez-Andrade et al., 2004; Kerekes et al., 2003; Ma et al., 2001; Mahoney et al., 2003a). However, recent evidence suggests that this agonist has affinity for GalR3 and thus might be a pharmacological tool to study the actions mediated by non-GalR1 receptors (Lu et al., 2005). Thus, the pro-nociceptive effect observed in this study following intraplantar AR-M1896 could result from activation of GalR2 and GalR3. However, it is highly unlikely that AR-M1896 would produce these effects through GalR3 since there is low or no expression of GalR3 receptor found in primary afferent neurons (Mennicken et al., 2002; Smith et al., 1998; Waters and Krause, 2000), and GalR3 activation results predominantly in decreased neuronal activity and/or neurotransmitter release (Branchek et al., 2000; Smith et al., 1998).

In this study, GAL at low doses reduced inflammatory pain induced by CAP, confirming our previous observations (Jimenez-Andrade et al., 2004, 2005). The pro-nociceptive effect induced by GAL was mimicked by intraplantar administration of low doses of AR-M1896 both in Sprague–Dawley (Jimenez-Andrade et al., 2004) and Wistar rats (present study). Furthermore, this pro-nociceptive effect was blocked by PKC inhibition and mimicked by PKC activation (Jimenez-Andrade et al., 2005). Taken together, these findings suggest that peripheral GAL administered at low doses activates GalR2 receptors resulting in pro-nociception through activation of PKC intracellular pathways.

When higher concentrations of GAL [31.0 μM ~2 μg (Fig. 1) and 310.0 μM ~20 μg (data not shown)] were injected intraplantar, they failed to produce an anti-nociceptive effect. These results are in contrast with previous studies performed in the spinal cord, where low doses facilitated the nociceptive flexor reflex but high doses (1 and 10 μg) inhibited the flexor reflex activity (Wiesenfeld-Hallin et al., 1989; Xu et al., 1998). The reason for this discrepancy is not clear but is likely due to a differential expression of GalRs in the periphery compared to the spinal cord. The GalR2 mRNA and the GalR2 protein are highly expressed in DRGs (75%) and in peripheral terminals of nociceptors (68%) (Jimenez-Andrade et al., 2004; Kerekes et al., 2003). In contrast, only 43% of DRG neurons express GalR1 mRNA (Kerekes et al., 2003) and very low levels or no expression of GalR3 mRNA is found (Mennicken et al., 2002; Smith et al., 1998; Waters and Krause, 2000). Therefore, the lack of analgesia after high doses (μg) of GAL might be due to an abundance of GalR2s (excitatory) compared to GalR1 and GalR3s (inhibitory) in peripheral terminals of nociceptors. The fact that M617 does exert an analgesic effect in inflammatory pain is a line of evidence that this peptide binds to GalR1. In fact, in vitro culture experiments indicate that GalR1 has a higher affinity for M617, than for GAL itself (Lundström et al., 2005). However, at the concentrations used in the current study, GalR1 and GalR2 may be equally activated by M617 (Lundström et al., 2005). Also, it is unknown at this time whether M617 binds to or activates GalR3. As mentioned above, however, there is a paucity of GalR3

expression in the DRG so there would be little or no GalR3 involvement following local peripheral injection of M617.

Our knowledge of the role of GalR subtypes in pain signaling has been significantly limited by the lack of pharmacological agents. The use of ligands that preferentially bind to various GalR subtypes has revealed that activation of peripheral GalR1 reduces, but activation of GalR2 potentiates, inflammatory pain. Although GalR3 antagonists have recently become available (Swanson et al., 2005), the paucity of GalR3 expression in DRGs will limit the use of these drugs in the periphery. However, further characterization of GalR function in different pain models may provide new therapeutics potentials for the treatment of pain.

Acknowledgements

The authors thank Vicki Wilson for her excellent secretarial assistance. This work was supported by NIH NS40700 and NS27910 to SMC and the Swedish Research Council (VR-Med). JMJA is a CONACyT fellow from Mexico.

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