Up-Regulation of Regulator of G Protein Signaling 4 Expression in a Model of Neuropathic Pain and Insensitivity to Morphine

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ABSTRACT

We hypothesized that the up-regulated expression of one or more members of the regulator of G protein signaling (RGS) family can cause an attenuation of signaling via Gi/Go-coupled opioid receptors, and thereby play a role in the development of hyperalgesia and accompanying insensitivity to morphine observed in animal models of neuropathic pain. Accordingly, we examined the mRNA expression of several RGS genes in a rat model of chronic neuropathic pain induced by partial ligation of the sciatic nerve. During the development of hyperalgesia, RGS4 was the only isoform examined whose mRNA levels increased significantly (up to 230%) in the lumbar spinal cord. In situ hybridization studies confirmed that RGS4 is present in the dorsal horn of the spinal cord where μ-opioid receptors (MORs) are also expressed. Overexpression of RGS4 in human embryonic kidney 293 cells stably expressing μ-opioid receptors predictably attenuated opioid agonist-induced inhibition of adenylyl cyclase. This inhibitory effect was overcome partially at high agonist concentrations, supporting the view that morphine insensitivity is promoted by RGS4 overexpression. These studies provide evidence that the up-regulation of RGS4 expression may contribute to changes in pain signal processing that lead to the development of hyperalgesia, and further affect its modulation by morphine.

Activation of the μ-opioid receptor (MOR) system leads to antinociception under most pain conditions, and a major site of opioid action is the dorsal horn of the spinal cord, which receives inputs from primary afferent fibers and integrates the multiple ascending and descending pathways that contribute to pain modulation (Ossipov et al., 1995). However, several studies have suggested that morphine is less effective at treating chronic neuropathic pain, as occurs after nerve injury in humans (Sindrup and Jensen, 1999) and in animal models (Wegert et al., 1997). Furthermore, the neuronal plasticity that underlies the development of neuropathic pain syndrome in some instances leads to a reduced morphine antinociception in drug-naïve conditions (Mao et al., 1995; Mayer et al., 1999).

The development of hyperalgesia (increased sensitivity to a repeated painful stimulus) in neuropathic pain states, and the insensitivity to morphine that has been observed to accompany it, are thought to share common mechanisms and neuronal substrates (Mayer et al., 1999). Although the details of these mechanisms are poorly understood, several adaptive changes in neuronal pathways have been proposed (Dickenson, 1997; Mayer et al., 1999), and changes in G protein-coupled receptor (GPCR) signaling in pain pathways have been established previously (Bohn et al., 1999; Przewlocka et al., 2002).

The coincident regulation of nociception and opioid responsiveness likely involves members of the regulator of G protein signaling (RGS) family of proteins, many of which are expressed in the nervous system (Gold et al., 1997) and serve to promote the attenuation of G protein signaling by stimulating the ability of Gα subunits to hydrolyze GTP and adopt an inactive GDP-bound state (Watson et al., 1996). The physiological importance of the known RGS isoforms in regulating nociception in rats has been suggested by the effects of specific antisense oligonucleotides injected into the lateral ventricle.
tricle of the brain, leading to a reduction in the expression of various RGS genes (Garzòn et al., 2001). The inhibition of RGS4, 7, 9, 12, 14, or 16 expression was shown in this study to increase to varying extents the acute antinociceptive effects of morphine and/or delay the loss of sensitivity to opioids.

RGS isoforms can act upon members of the Gi/o classes of heterotrimeric G proteins (Ross and Wilkie, 2000), which mediate the physiological effects of opioid receptors. RGS2 and RGS9 have been shown to attenuate signaling by MOR expressed heterologously in frog melanocytes (Rahman et al., 1999). Recombinant RGS4 has been shown to blunt the inhibitory activity of [Leu]-enkephalin, acting via the δ-opioid receptor on cAMP accumulation in NG108-15 membranes (Hepler et al., 1997).

Although RGS proteins have the potential to modulate nociceptive signaling pathways, it is unknown whether they are directly involved in physiological adaptations that lead to hyperalgesia and a reduction in morphine antinociception in neuropathic pain conditions. Defining a role for RGS proteins in the modulation of nociception requires, in part, demonstrating that their expression, localization, or function changes in relevant regions of the nervous system under conditions in which chronic neuropathic pain occurs.

In this study, we have determined whether the expression of several RGS genes is regulated in the spinal cord in concert with the development of neuropathic pain induced by partial ligation of the sciatic nerve. We report that RGS4 mRNA levels are specifically up-regulated in spinal cord when hyperalgesia is fully established and insensitivity to morphine is apparent. Moreover, we provide evidence that RGS4 is expressed in the dorsal horn of the spinal cord and show that RGS4 overexpression in vitro can attenuate MOR activity. These results suggest that dynamic regulation of RGS4 expression may contribute to the changes in pain signaling that occur in neuropathic pain conditions.

Materials and Methods

Materials. Drugs and chemicals used in this study were obtained from the following sources: methylene chloride (Salars, Como, Italy); [d-Pen²,d-Pen⁴]enkephalin, [d-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO), forskolin, 3-isobutyl-1-methylxantine, and poly-D-lysine hydrodromide (Sigma-Aldrich, Milan, Italy). S-(−)-2-(1-Pyrrolidinylmethyl)-1-(4-trifluoromethylphenyl)acetyl piperidine hydrochloride (BRL 52656) was synthesized as described by Brooks et al. (1993). Tissue culture plastics were purchased from NUNC (Milan, Italy); cell culture medium reagents, fetal bovine serum, hygromycin B, nonessential amino acids, FluGENE6 transfection reagent, Tn5Tnol DNA extraction reagent, oligo(dT)₁₂₋₁₈, and SuperscriptII reverse transcriptase were from Invitrogen (San Giuliano Milanese, Italy); and geneticin (G418 sulfate) was from Calbiochem (Milan, Italy). All PCR reagents were from Invitrogen. Tissue culture plastics were purchased from NUNC (Milan, Italy); cell culture medium reagents, fetal bovine serum, hygromycin B, nonessential amino acids, FluGENE6 transfection reagent, Tn5Tnol DNA extraction reagent, oligo(dT)₁₂₋₁₈, and SuperscriptII reverse transcriptase were from Invitrogen (San Giuliano Milanese, Italy); and geneticin (G418 sulfate) was from Calbiochem (Milan, Italy). All PCR reagents were from Invitrogen. Tissue culture plastics were purchased from NUNC (Milan, Italy); cell culture medium reagents, fetal bovine serum, hygromycin B, nonessential amino acids, FluGENE6 transfection reagent, Tn5Tnol DNA extraction reagent, oligo(dT)₁₂₋₁₈, and SuperscriptII reverse transcriptase were from Invitrogen (San Giuliano Milanese, Italy); and geneticin (G418 sulfate) was from Calbiochem (Milan, Italy). All PCR reagents were from Invitrogen. Tissue culture plastics were purchased from NUNC (Milan, Italy); cell culture medium reagents, fetal bovine serum, hygromycin B, nonessential amino acids, FluGENE6 transfection reagent, Tn5Tnol DNA extraction reagent, oligo(dT)₁₂₋₁₈, and SuperscriptII reverse transcriptase were from Invitrogen (San Giuliano Milanese, Italy); and geneticin (G418 sulfate) was from Calbiochem (Milan, Italy). All PCR reagents were from Invitrogen. Tissue culture plastics were purchased from NUNC (Milan, Italy); cell culture medium reagents, fetal bovine serum, hygromycin B, nonessential amino acids, FluGENE6 transfection reagent, Tn5Tnol DNA extraction reagent, oligo(dT)₁₂₋₁₈, and SuperscriptII reverse transcriptase were from Invitrogen (San Giuliano Milanese, Italy); and geneticin (G418 sulfate) was from Calbiochem (Milan, Italy). All PCR reagents were from Invitrogen. Tissue culture plastics were purchased from NUNC (Milan, Italy); cell culture medium reagents, fetal bovine serum, hygromycin B, nonessential amino acids, FluGENE6 transfection reagent, Tn5Tnol DNA extraction reagent, oligo(dT)₁₂₋₁₈, and SuperscriptII reverse transcriptase were from Invitrogen (San Giuliano Milanese, Italy); and geneticin (G418 sulfate) was from Calbiochem (Milan, Italy). All PCR reagents were from Invitrogen.
5'-gtgcctcggcaagcagg-3', 5'-gcctgccggtggtggac-3', 5'-gtcctgcggctgt-3', 5'-tcaccgaatccatcaactc-3'; RGS4 (U92279), 5'-tctcaccaagcctagcgg-3', 5'-tcacccgcaaatcagcagtg-3', RGS17 (AW105991), 5'-ttgggctgcgctgctgagc-3', 5'-ctgcggtgaatcagcagtg-3', 5'-gcctgccggtggtggac-3', 5'-gccctccggtggtgcag-3'; and GAIP (AF068136), 5'-atacctgctgagaaaggt-3', 5'-tagttcgctcgcagatg-3', 5'-gctgaacatcagcagtg-3'.

**Cloning of Rat RGS4 Plasmids and Riboprobes.** Rat RGS4 single-stranded cDNA was produced by reverse transcription using a gene-specific primer (5'-gtgcctcggcaagcagg-3', nucleotide 572, gene accession number NM_017214). This reaction product and the specific primers 5'-aaaatagctggcttagcatggagtag-3' and 5'-aaaaatagctggcttagcatggagtag-3' (BamHI and XbaI sites used for subsequent clone are underlined) were used to prepare a double-stranded RGS4 cDNA (282 base pairs) by PCR. After the PCR product was purified and cleaved with BamHI and XbaI, it was cloned into pBluescript SKII+ (Strategene, La Jolla, CA). The sequence of the RGS4 cDNA was checked by sequencing and this plasmid was used for subsequent clone are underlined) were used to prepare a double-stranded RGS4 cDNA (282 base pairs) by PCR. After the PCR product was purified and cleaved with BamHI and XbaI, it was cloned into pBluescript SKII+ (Strategene, La Jolla, CA). The sequence of the RGS4 cDNA was checked by sequencing and this plasmid was used for subsequent clone are underlined) were used to prepare a double-stranded RGS4 cDNA (282 base pairs) by PCR. After the PCR product was purified and cleaved with BamHI and XbaI, it was cloned into pBluescript SKII+ (Strategene, La Jolla, CA). The sequence of the RGS4 cDNA was checked by sequencing and this plasmid was used for subsequent clone are underlined) were used to prepare a double-stranded RGS4 cDNA (282 base pairs) by PCR. After the PCR product was purified and cleaved with BamHI and XbaI, it was cloned into pBluescript SKII+

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Results

Development of Hyperalgesia and Reduced Morphine Antinociception after Partial Sciatic Nerve Ligation. With the aim to establish that hyperalgesia was associated with reduced responsiveness to subsequent opioid treatment, we initially determined the effect of morphine in a rat model of neuropathic pain established in our laboratory. After partial ligation of the rat sciatic nerve (Seltzer et al., 1990), thermal hyperalgesia was fully established 3 days after ligation and was maximal 7 to 14 days after surgery (Fig. 1A). We compared the nociceptive effect of morphine (0.03, 0.1, and 0.3 mg/kg s.c.) on thermal hyperalgesia using the plantar test 1 day after ligation, at which time the animals exhibit a postoperative pain not related to neuropathic hyperalgesia, with the effect of morphine 7 days after surgical ligation of the sciatic nerve, at which time neuropathic pain is established (Fig. 1A). The antinociceptive effects of morphine (0.03 and 0.1 mg/kg s.c.) were significantly reduced by 27 and 57%, respectively, 7 days after surgery when hyperalgesia is maximal relative to 1 day after surgery (Fig. 1B). No changes in the antinociceptive effects of morphine were observed in sham-operated rats (Fig. 1C). This apparent reduction of morphine antinociception was completely overcome when a higher morphine dose (0.3 mg/kg s.c.) was used, demonstrating changes in the effective morphine dosage. These data, showing that hyperalgesia induced a reduction of morphine antinociception in the rat partial sciatic nerve ligation model of neuropathic pain, has been previously reported as well in the chronic nerve constriction injury model (Mao et al., 1995).

Changes in RGS Expression in Spinal Cord after Partial Nerve Ligation. To investigate whether RGS proteins are up-regulated specifically in the spinal cord in conditions of injury, we assessed the spinal expression levels of nine members of the RGS family, including RGS9, which had been implicated previously in the regulation of acute morphine activity (Garzón et al., 2001). As shown in Fig. 2, the mRNA levels of GAIP, RGS6, RGS11, and RGS17 were invariant in rats 7 days after surgery, relative to sham-operated control animals. In contrast, the expression level of three RGS genes were significantly decreased by 50% (RGS9 and RGS12) and 75% (RGS7) after nerve ligation. RGS8 and RGS14 were not expressed at detectable levels in the spinal cords of control or ligated rats. Therefore, among the 10 RGS members studied, only RGS4 showed a significant 2-fold increase in neuropathic rats, whereas spinal RGS4 mRNA levels in control (sham-operated) animals were invariant 3 and 7 days after surgery. In contrast, spinal RGS4 mRNA levels 3 and 7 days after ligation were about 2-fold higher than in sham-operated animals at the same time points. Fourteen days after liga-
tion, spinal RGS4 mRNA levels in nerve-ligated animals were similar to sham-operated control levels.

Expression of RGS4 in Dorsal Horn of Spinal Cord.
RGS4 and MOR expression in the spinal cord would be expected to overlap if the up-regulation of RGS4 mRNA expression in the spinal cord as a result of nerve injury directly plays a role in the loss of sensitivity to morphine in neuropathic pain conditions. We attempted to determine the location of RGS4 expression via in situ hybridization experiments using RGS4-specific probes (Fig. 4A). Although the RGS4 in situ hybridization signal in spinal cord was weaker than in rat brain (data not shown), RGS4 mRNA could be detected throughout much of the dorsal horn of the spinal cord of control rats. Experiments using a RGS4 sense probe confirmed that the signal observed in the dorsal horn was specific (Fig. 4B). Therefore, it is likely that the up-regulation of RGS4 mRNA expression after sciatic nerve ligation that we observed occurred in the dorsal horn of the spinal cord. However, it should be noted that at the level of resolution provided by this technique, we were unable to detect changes in the level or sites of RGS4 mRNA expression in spinal cords of neuropathic rats (data not shown). Because rat MOR mRNA is known to be expressed most abundantly in laminae I and II of the dorsal horn (Peckys and Landwehrmeyer, 1999), these results point to an overlap in RGS4 and MOR expression. Therefore, an up-regulation of RGS4 mRNA after sciatic nerve ligation could have the potential to regulate MOR signaling, contributing to the attenuation of morphine antinociception.

Attenuation of μ-Opioid Receptor Signaling by RGS4.
To establish a mechanistic basis for our results associating RGS4 up-regulation with reduced sensitivity to morphine, we further investigated whether RGS4 can affect MOR signaling at the cellular level. Studies were performed to evaluate the effects of RGS4 overexpression on the ability of MOR agonists to inhibit adenyl cyclase activity. We used a previously characterized transcriptional reporter cell line that derives from HEK293 cells and stably coexpresses hMOR (100 fmol/mg protein) and a cAMP-responsive CRE-luciferase gene-reporter construct (Fitzgerald et al., 1999).

As shown in Fig. 5, RGS4 overexpression reduced the inhibitory activity of DAMGO on luciferase expression. As a result, the inhibition of luciferase expression at the highest concentration of DAMGO tested (300 nM) was reduced from 69.4 ± 3.6% (n = 4) at 300 nM to 50 ± 4.5%. Changes in DAMGO potency (EC50 = 17 ± 1 nM) were not measured in these experiments. Similar profiles of RGS4 activity were observed when morphine was used in the assay (data not shown). Therefore, under the conditions used, RGS4 overexpression attenuated but did not completely block MOR signaling. This result is consistent with the hypothesis that RGS4 up-regulation could contribute to the reduced activity of morphine in the sciatic nerve ligation model of chronic neuropathic pain.

We further examined whether RGS4 expression exerts an effect similar to morphine insensitivity by comparing the extent of RGS4 inhibitory activity on DAMGO and morphine action at submaximal versus saturating concentrations of these agonists (Fig. 6). The extent of RGS4 inhibitory activity was calculated by the eq. 100 - [100 × IRGS4/Icontrol], where IRGS4 and Icontrol represent the extent of DAMGO-induced inhibition of FSK-stimulated luciferase expression in transfected and in control cells, respectively. At the highest level of RGS4-myc expression, the inhibitory activities of DAMGO and morphine at submaximal concentrations (30 nM DAMGO, 100 nM morphine) were reduced by 36 ± 1 and 48 ± 3%, respectively, relative to the response of control cells. When higher DAMGO (300 nM) and morphine (1000 nM) concentrations were used in these experiments the inhibitory activity of agonists on luciferase expression was only reduced by 26 ± 2% (DAMGO) and 17 ± 2% (morphine) in the presence of RGS4-myc. Therefore, by heterologously expressing RGS4 together with MOR in HEK293 cells, we have been able to demonstrate that RGS4 can reduce Gai-mediated MOR signaling and that this inhibitory effect of RGS4 can be partially overcome by increasing concentrations of opioid agonists.
The ability of RGS4 overexpression to modulate receptor signaling is not specific to MOR and can lead to an attenuation of Gi-signaling by MOR, DOR, and KOR opioid receptor subtypes. Activity of the DOR [d-Pen²,d-Pen⁵]-enkephalin toward FSK-stimulated luciferase expression was reduced from 69.5 ± 5.2% (control cells) to 47.3 ± 7.2% (n = 3) after transfection of RGS4-myc plasmid (3 µg) together with hDOR. Similarly, the overexpression of RGS4 in cells expressing hKOR resulted in a near complete loss in the ability of the selective KOR agonist BRL 52656 to inhibit activity, compared with the 58 ± 7.6% (n = 5) inhibition observed in control cells.

Discussion

This study has provided evidence suggesting that RGS4 overexpression is associated with the development of hyperalgesia and may further play a role in the altered response to morphine that can accompany the development of neuropathic pain by regulating G protein-coupled receptor signaling pathways involved in the control of nociception. After partial ligation of the rat sciatic nerve, we showed that both thermal hyperalgesia and reduced sensitivity to morphine develop and are associated with changes in RGS4 gene expression in the lumbar spinal cord. Furthermore, we demonstrated that RGS4 is expressed in the dorsal horn of the rat spinal cord and that RGS4 overexpression in cell culture strongly attenuates signaling by all subtypes of opioid receptor, including the µ-opioid receptor subtype that mediates the actions of morphine. Therefore, the up-regulation of RGS4 in lumbar spinal cord that occurs in response to nerve injury may potentially be part of a feedback mechanism to control nociceptive signaling events, including those modulated by µ-opioid receptors.

We were unable to demonstrate that RGS4 mRNA up-regulation corresponded to an increase in RGS4 protein, because of the low levels detected and lack of specificity of RGS4 antibodies. Previous studies using cell membrane fractions and recombinant RGS4 protein have suggested that RGS4 can affect opioid receptor signaling (Hepler et al., 1997), although these results were not reproduced in a coexpression system using frog melanocytes (Potenza et al., 1999). Our findings indicate that RGS4 overexpression in HEK293 cells can blunt Ga_i-mediated MOR signaling, a result that is consistent with the previously reported inhibition of dopamine D2 and serotonin 5-hydroxytryptamine1B receptor activity by overexpressed RGS4 (Yan et al., 1997; Leone et al., 2000). RGS4 could additionally affect G protein-coupled inwardly rectifying potassium channel currents through a Ga_i-mediated mechanism, as well as cAMP signaling responses, as shown in several Ga_i-coupled receptor systems (Inanobe et al., 2001; Keren-Raifman et al., 2001). Because the RGS domain of RGS4 and the Ga proteins are required for RGS modulation of G protein-coupled inwardly rectifying potassium channel and RGS modulation of GTPase activity, it is likely that spinal RGS4 up-regulation could completely block MOR signaling by affecting both Ga_i- and Gaβγ-mediated MOR signaling pathways.

Increases in RGS4 expression relative to that of the MOR, causing a change in the available pool of active (G protein-complexed) receptor, may lead in vivo to the observed losses in morphine antinociception. In support of this view, a study by Sora et al. (2001) has shown that changes in µ-opioid receptor reserve in vivo can effect morphine dose-response relationships. The extent of RGS4-mediated inhibition of
Gai-mediated MOR signaling in vitro can be partially overcome by saturating concentrations of agonist. This suggests that higher opioid concentrations would be needed in vivo to counteract RGS4 negative regulation and produce an antihyperalgesic effect. Indeed, reductions in rat neuropathic pain at 7 days postligation required the use of elevated morphine doses. The timing of RGS4 mRNA up-regulation after sciatic nerve ligation suggests its involvement in the initiation rather than the maintenance of hyperalgesia and/or development of insensitivity to morphine. The increased expression of RGS4 mRNA we observed occurs within 7 days after partial nerve ligation, by which time neuropathic pain is fully established and is associated with a reduced activity of morphine.

Studies by Nakagawa et al. (2001), showing that agonist activation of MOR and KOR results in a transient up-regulation of RGS4 mRNA in PC12 cells with a time-course profile that parallels that of opioid receptor desensitization, suggest that RGS4 can also be dynamically regulated by opioid receptors and act as a negative feedback regulator of opioid activity. Although RGS4 mRNA expression could be regulated by a variety of other pharmacological systems in our model, it is possible that RGS4 up-regulation results from the activation of antinociceptive opioid receptor systems in response to nerve injury and contributes to the development of a reduced functional activity.

Two weeks after ligation the relative levels of RGS4 mRNA were similar to that in control sham animals, even though hyperalgesia was still observed, suggesting that other regulatory mechanisms will have likely been invoked to maintain the observed hyperalgesia. RGS4 up-regulation could have broader effects on the regulation of nociception in the dorsal horn of the spinal cord beyond the modulation of opioid responsiveness. In addition to its ability to attenuate signaling by receptors coupled to Gi, including antinociceptive MOR, RGS4 may inhibit signaling by receptors coupled to Gq, such as group I metabotropic glutamate receptors (Saugstad et al., 1998) and 5-hydroxytryptamine receptors (Leone et al., 2000) that havepronociceptive effects.

The regulation of both pro- and antinociceptive pathways involved in the establishment of neuropathic pain and consequent reduced sensitivity to morphine may require orchestrated changes in the activity of several different RGS family members. Studies from Garzoñ et al. (2001) using oligonucleotide antisense probes suggest that blocking the expression of RGS4, RGS7, RGS9, or RGS12 increases the duration of acute morphine antinociception. Conversely, it would be expected that a reduction in morphine antinociception would be accompanied with an increase in RGS expression. However, it was our finding that RGS4 was the only family member shown to be up-regulated in vivo among the 10 RGS members studied and that RGS7, RGS9, and RGS12 seemed to be down-regulated in these studies. These RGSs may constitutively regulate antinociceptive receptors whose reduced expression and activity would contribute to increased hyperalgesia and neuropathic pain.

We suggest two models in which RGS4 up-regulation could contribute to the induction of hyperalgesia and reduced morphine antinociception. First, RGS4 up-regulation in nociceptive-specific neurons could sensitize postsynaptic responses to excitatory signals triggered by enhanced activity of C-fibers or excitatory interneurons. Postsynaptic sensitization could be due to direct inhibition of MOR signaling, thereby...
relieving μ-opioid-mediated inhibition of N-methyl-D-aspartate receptor activation, for example. Second, RGS4 up-regulation could exert its effects presynaptically in C-fibers or excitatory interneurons. In this model, RGS4 would attenuate signaling by GPCRs, including MOR that otherwise inhibit glutamate release; thus, up-regulation of RGS4 would facilitate glutamate release. Either mechanism could contribute to long-lasting adaptive changes, possibly coupling the induction and/or maintenance of hyperalgesia with morphine insensitivity. This is consistent with hypotheses proposing that plastic changes in the sensory and central nervous systems are closely associated with and are of critical importance to the development and maintenance of pathological pain states (Mayer et al., 1999).

It will be important to additionally determine which receptors involved in nociception besides MOR can be targeted by RGS4 inhibition. In this regard, recombinant RGS4 has been shown to preferentially attenuate signaling by muscarinic receptors relative to cholecystokinin receptors (Xu et al., 1999) in a perfused cell system, and we have found that in HEK293 cells RGS4 is able to attenuate signaling by κ- and δ-opioid receptors. The receptor targeting of RGS4 overexpression may both directly (as suggested in this study) or indirectly regulate MOR function. For instance, serotonin infusion in spinal cord can accelerate the development of morphine insensitivity by reducing the number of MOR binding sites (Li et al., 2001).

In conclusion, RGS proteins are beginning to emerge as important factors in a host of pathological conditions affecting the nervous system. These include anxiety and male aggression in mice lacking RGS2 (Olivera-Dos-Santos et al., 2000) and overexpression of RGS4 in certain populations of human schizophrenia (Mirmics et al., 2001). Our studies suggest that a family of RGS genes expressed in spinal cord may participate in the regulation of nociceptive GPCR signaling and adaptive changes of the nervous system that occur in association with hyperalgesia and accompanying morphine tolerance. Elucidation of these mechanisms may open new therapeutic directions by leading to the identification of small molecules that modulate the activities of these RGS proteins and enhance the efficacy of morphine in neuropain.

References
Dickenson AH (1997) Plasticity: implications for opioid and other pharmacological regulation of nociceptive GPCR signaling and adaptive changes phrenics (Mirnics et al., 2001). Our studies suggest that a family of RGS genes expressed in spinal cord may participate in the regulation of nociceptive GPCR signaling and adaptive changes of the nervous system that occur in association with hyperalgesia and accompanying morphine tolerance. Elucidation of these mechanisms may open new therapeutic directions by leading to the identification of small molecules that modulate the activities of these RGS proteins and enhance the efficacy of morphine in neuropain.


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