Calcitonin Gene-Related Peptide Receptor Activation Produces PKA- and PKC-Dependent Mechanical Hyperalgesia and Central Sensitization

Rui-Qing Sun, Yi-Jun Tu, Nada B. Lawand, Jing-Yin Yan, Qing Lin, and William D. Willis

Department of Neuroscience and Cell Biology, The University of Texas Medical Branch, Galveston, Texas 77555-1069

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Sun, Rui-Qing, Yi-Jun Tu, Nada B. Lawand, Jing-Yin Yan, Qing Lin, and William D. Willis. Calcitonin gene-related peptide receptor activation produces PKA- and PKC-dependent mechanical hyperalgesia and central sensitization. J Neurophysiol 92: 2859–2866, 2004; 10.1152/jn.00339.2004. Calcitonin gene-related peptide (CGRP), acting through CGRP receptors, produces behavioral signs of mechanical hyperalgesia in rats and sensitization of wide dynamic range (WDR) neurons in the spinal cord dorsal horn. Although involvement of CGRP receptors in central sensitization has been confirmed, the second-messenger systems activated by CGRP receptor stimulation and involved in pain transmission are not clear. This study tested whether the hyperalgesia and sensitizing effects of CGRP receptor activation on WDR neurons are mediated by protein kinase A or C (PKA or PKC) signaling. Intrathecal injection of CGRP in rats produced mechanical hyperalgesia, as shown by paw withdrawal threshold tests. CGRP-induced hyperalgesia was attenuated significantly by the CGRP1 receptor antagonist, CGRP(8–37). The effect was also attenuated significantly by a PKA inhibitor (H89) or a PKC inhibitor (chelerythrine chloride). Electrophysiological experiments demonstrated that superfusion of the spinal cord with CGRP-induced sensitization of spinal dorsal horn neurons. The CGRP effect could be blocked by CGRP(8–37). Either a PKA or PKC inhibitor (H89 or chelerythrine) also attenuated this effect of CGRP. These results are consistent with the hypothesis that CGRP produces hyperalgesia by a direct action on CGRP1 receptors in the spinal cord dorsal horn and suggest that the effects of CGRP are mediated by both PKA and PKC second-messenger pathways.

INTRODUCTION

Calcitonin gene-related peptide (CGRP) has been implicated in the processing of nociceptive information in the spinal cord (Bennett et al. 2000; Neugebauer et al. 1996; Satoh et al. 1992; Sun et al. 2003a,b), where it is found in the terminals of unmyelinated (C) and thinly myelinated (Aδ) afferent fibers (see review by Willis and Coggeshall 2004). CGRP can enhance the release of glutamate and aspartate in the spinal cord (Kangrga and Randic 1990; Smullin et al. 1990), increase the responses of WDR and nociceptive specific neurons to N-methyl-D-aspartate (NMDA) or (R,S)-α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) (Ebersberger et al. 2000), and facilitate the actions of substance P in behavioral and electrophysiological experiments by potentiating the release and inhibiting the degradation of substance P (Biella et al. 1991; LeGreves et al. 1985; Mao et al. 1992a; Oka et al. 1987; Schaible et al. 1992; Wiesenfeld-Hallin et al. 1984; Woolf and Wiesen-Hallin 1986). Together, these studies suggest that CGRP may produce its effect on pain transmission and modulation through interactions with substance P and excitatory amino acids. Alternatively, CGRP may be involved in pain transmission independently of substance P and excitatory amino acids. It has been reported that CGRP itself produces a depolarization (Miletic and Tan 1988; Ryu et al. 1988). Our previous studies demonstrated that the effects of CGRP in hyperalgesia and central sensitization are mediated by CGRP1 receptors (Sun et al. 2003a,b). The CGRP receptor is considered to be a protein formed by a complex of seven transmembrane domains and includes a calcitonin receptor-like receptor (CRLR), a receptor activity-modifying protein (RAMP) that defines the relative potency of ligands for the receptor, and a receptor component protein (RCP) that defines the G protein to which the receptor couples (Chakravarty et al. 2000; Evans et al. 2000; McLatchie et al. 1998). The CGRP1 receptor, which binds CGRP and CGRP(8–37) with highest affinity, is made up of CRLR, RAMP1, and RCP, which couples the CGRP receptor to cellular signal transduction machinery (McLatchie et al. 1998). Among the second messengers produced by CGRP receptor activation is cAMP and its down-stream protein kinase A (PKA) (Baidan et al. 1992; Parsons and Seybold 1997; Parameswaran et al. 2000; Poyner 1992; Seybold et al. 2003). However, little is known regarding the signaling molecules and the second-messenger pathways involved in CGRP-mediated secondary hyperalgesia and central sensitization. In this study, we first found that the PKA inhibitor H89 could prevent the hyperalgesia and the sensitization of WDR neurons induced by CGRP. Considering that CGRP also stimulated phosphoinositide turnover and a Ca2+-signaling pathway (Aiyar et al. 1999; Lauer and Changeux 1989) and that protein kinase C (PKC) is involved in the development of central sensitization and nociceptive transmission, we tested whether the PKC pathway is also the downstream of CGRP receptor activation. Our behavioral and electrophysiological results showed that inhibition of PKA and PKC blocked the mechanical hyperalgesia and central sensitization induced by CGRP.

METHODS

Experimental animals

Male Sprague-Dawley rats weighing 270–320 g were used in this study. The experiments were approved by the Institutional Animal Care and Use Committee and were consistent with the ethical guidelines of the National Institutes of Health and of the International Association for the Study of Pain.

Address for reprint requests and other correspondence: W. D. Willis, Dept. of Neuroscience and Cell Biology, The University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-1069 (E-mail: wdwillis@utmb.edu).

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All experimental animals were housed and maintained in accordance with the guidelines of the University of Texas Medical Branch Animal Care and Use Committee.

Placement of intrathecal catheters for behavioral experiments

Externally accessible PE-10 intrathecal catheters were implanted in rats to be used for behavioral testing according to a modification of the technique described by Storkson et al. (1996). After anesthesia with pentobarbital sodium (50 mg/kg ip), a catheter (PE 10, 0.61 mm OD) was inserted into the subarachnoid space through a guide cannula connected to a 20-gauge needle, which punctured the dura at the level of the cauda equina. The catheter was then carefully implanted rostrally, aiming its tip just dorsal to the lumbar enlargement. The position of the catheter was checked postmortem.

General preparation and anesthesia in electrophysiological experiments

Rats were anesthetized initially with pentobarbital sodium (50 mg/kg ip) for performing surgery. The trachea was cannulated to provide unobstructed ventilation, and a catheter was inserted into the external jugular vein for maintenance of anesthesia during experiment by continuous infusion of sodium pentobarbital (10 mg·kg⁻¹·h⁻¹). The level of anesthesia was frequently checked during the experiment by examining corneal and withdrawal reflexes and observation of end-tidal CO₂ level. Once a stable level of surgical anesthesia was reached, the animals were paralyzed with an initial dose of pancuronium (1 mg/kg), and they were ventilated artificially to maintain the end-tidal CO₂ between 3.5 and 4.5%. Paralysis of the musculature was reached, the animals were paralyzed with an initial dose of pancuronium (1 mg/kg), and they were ventilated artificially to maintain the end-tidal CO₂ between 3.5 and 4.5%. Paralysis of the musculature was maintained by continuous infusion of pancuronium (0.4–0.6 mg·kg⁻¹·h⁻¹). A laminectomy was performed to expose the spinal cord at the T₁₋₃–L₂ vertebral level. The spinal cord was continually bathed in a pool of warm (37°C) mineral oil except for those spinal segments to be superfused. Core body temperature was maintained at 37°C by a servo-controlled heating blanket.

Administration of drugs

CGRP₁₋₃₇ (Sigma), CRGP (TOCRIS), and H89 (an inhibitor of PKA, Calbiochem) were dissolved in artificial cerebrospinal fluid (ACSF), and chelerythrine chloride (CC, an inhibitor of PKC, Calbiochem) was dissolved in 0.2% DMSO. In behavioral experiments, drugs were administered by intrathecal injection. In electrophysiological experiments, drugs were applied by controlled superfusion of the spinal cord at the segments from which recordings were made. A specially synthesized silicone rubber was used to form a small well on the cord dorsum at the recording level for controlled superfusion of the spinal cord (Beck et al. 1995). H89 or CC was applied in the silicone well after control responses were recorded. Thirty minutes later, H89 or CC was removed, and CGRP was applied in the well. Thirty minutes after that, the responses were measured to test whether the effect produced by CGRP would be blocked by H89 or CC. Then ACSF was used several times to wash out CGRP left on the cord. Thirty minutes later, the responses were measured again.

Behavioral testing

Animals were tested for responses to von Frey filament (Stoelting) application. Animals were placed in plastic cages on an elevated screen and allowed to acclimate to the testing environment for 30 min. The 50% paw withdrawal threshold in response to von Frey hair probing was detected using the up-down method (Chaplan et al. 1994). Beginning with the 2.00 g filament, which is in the middle of the series of eight von Frey filaments (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10 g) with logarithmically incremental stiffness ranging from 0.41 to 15.1 g (numbers 3.61–5.18), each filament was applied with enough force to cause slight bending and this force was held for ~6–8 s. A positive response was recorded if the paw was sharply withdrawn. The pattern of positive and negative responses was converted to a 50% threshold value by using the formula given by Dixon (1980). The observer was blinded to the treatment of rats.

Recording from wide dynamic range neurons

Recordings were made from wide dynamic range (WDR) neurons in the dorsal horn. These neurons responded to both innocuous and noxious mechanical stimulation, but best to noxious stimuli. Cells were searched for under the small well for controlled superfusion of the spinal cord at the L₃ segment using low-impedance (3–5 MO) carbon filament electrodes (Kation Scientific, Minneapolis, MN) and an electronic micromanipulator that advanced in 5-μm steps. Search stimuli consisted of mechanical stimulation (innocuous stimuli consisted of brushing in a stereotyped manner with a camel’s hair brush, noxious stimuli were mild pinching with the experimenter’s fingers). WDR neurons with receptive fields (RFs) located on the plantar surface of the hindpaw were recorded extracellularly in the L₃ segment of the spinal cord. Data were analyzed only from recordings of the activity of single neurons the spike amplitude of which could be easily discriminated from spikes of neighboring units. Electrophysiological activity was amplified and displayed on a storage oscilloscope before being sent to a data analysis system (CED 1401, PC) for data collection using Spike-2 software, which enables computation and storage of peristimulus time histograms. Throughout the experiment, spike size and configuration were continuously monitored on the digital oscilloscope and with the use of Spike-2 software to confirm that the same WDR neuron was recorded and that the relationship of the recording electrode to the neuron remained constant.

Evoked response measures and experimental design

After the RF of the identified WDR neuron was mapped using brush and mild pinch stimuli, graded mechanical stimuli were applied. These consisted of brushing the skin with a camel hair brush in a stereotyped manner (20 strokes/10 s), and then sustained application of an arterial clip to a fold of skin. The clip produced a sensation of firm pressure (press, 144 g/mm²) near the threshold for pain when applied to human skin. The background activity was recorded for 5 min before the application of the mechanical stimuli. Each stimulus (brush, press) was applied for 10 s followed by a 2-min pause. Responses to mechanical stimuli were calculated by subtracting the mean background discharge from the total activity that occurred during the 10-s stimulus. Three baseline responses were determined for brush and pressure, and an average response was calculated as baseline. To minimize a potential “human factor” bias, the experimenter who applied the mechanical stimuli did not observe the oscilloscope or the computer monitor and was unaware of the response magnitude.

Statistical analysis

In electrophysiological experiments, the baseline activity (background activity and responses to brush and press) was regarded as control (100%) in each group. The activity of each WDR neuron after drug or ACSF administration was expressed as a percentage of baseline. Values are presented as means ± SE. Comparison between the means was analyzed with repeated-measures ANOVA followed by Newman-Keuls test. In all tests, significance was accepted at the level P < 0.05.
RESULTS

Inhibition of PKA or PKC blocked the effect of CGRP receptor activation in nociceptive behavior experiment

H89 inhibits the effect of CGRP. To test whether the effect of CGRP (0.5 μM, 10 μl) on nociception was dependent on the PKA second-messenger system, H89 (10 μM, 10 μl) was given 20 min before CGRP application. As a control for the effect of H89 or CGRP, the same volume of ACSF was used. Thirty rats were divided into five groups randomly. The groups were ACSF + ACSF, H89 + ACSF, ACSF + CGRP, ACSF + CGRP + CGRP8–37, and H89 + CGRP. H89 (10 μM, 10 μl) was given 20 min before CGRP (0.5 μM, 10 μl) application. As shown in Fig. 1, there was no significant difference among 50% mechanical thresholds at any time point after injection of ACSF, i.e., 5, 10, 30, 50, 70, and 90 min. H89 given alone did not alter the baseline mechanical threshold, which was consistent with a previous report (Sluka and Willis, 1997). After injection of CGRP (0.5 μM, 10 μl), 50% paw withdrawal threshold decreased significantly. The decrease in the 50% paw withdrawal threshold was maintained for ≥50 min after injection of CGRP and peaked at or before 30 min (Fig. 1A). CGRP8–37 (10 μM, 5 μl) blocked the effect of CGRP (1 μM, 5 μl); the mean 50% paw withdrawal threshold in the CGRP + CGRP8–37 group was 14.03 ± 0.52 g), which was consistent with previous results (Sun et al., 2003b). Application of H89 before CGRP completely blocked CGRP-induced mechanical hyperalgesia (Fig. 1). These results suggest that the effect of CGRP receptor activation in nociception is dependent on the PKA pathway.

Chelerythrine chloride inhibits the effect of CGRP. To test whether the effect of CGRP on nociception was dependent on the PKC second-messenger system, CC (0.1 mM, 10 μl) was given 20 min before CGRP (0.5 μM, 10 μl) application. As a control for the effect of CC or CGRP, the same volume of 0.2% DMSO (dissolved in ACSF) or ACSF was used. Twenty-six rats were divided into four groups randomly. The groups were 0.2% DMSO + ACSF, CC + ACSF, 0.2% DMSO + CGRP, and CC + CGRP. As shown in Fig. 2, after injection of 0.2% DMSO, there were no significant changes in paw withdrawal threshold at any time point, i.e., 5, 10, 30, 50, 70, and 90 min. CC given alone did not alter baseline nociceptive threshold; this is consistent with a previous report (Granados-Soto et al., 2000). After injection of CGRP (0.5 μM, 10 μl), 50% paw withdrawal threshold decreased significantly. The decrease of 50% paw withdrawal threshold was maintained for ≥50 min after injection of CGRP (Fig. 2). Application of CC before CGRP completely blocked CGRP-induced mechanical hyperalgesia (Fig. 2). This result suggests that the effect of CGRP in nociception is also dependent on the PKC pathway.

Blockade of CGRP-induced enhancement of responses of dorsal horn neurons to mechanical stimuli when the spinal cord was pretreated with a PKA or PKC inhibitor

In 36 rats, neurons with afferent input from the plantar surface of the hindpaw were recorded in segment L5 of the spinal cord at depths of 260–800 μm from the cord dorsum. Three neurons were located at depths of 260–320 μm, 8 at 320–500 μm, and 25 at 500–800 μm. Most neurons in the sample were estimated to be distributed widely in laminae V and VI (500–800 μm). All the recorded neurons were of the WDR type and exhibited spontaneous activity. The activity after drug or ACSF administration is expressed as a percentage of baseline.

The following experiments tested whether blockade of PKA or PKC could prevent the enhancement of the responses of the WDR neurons by CGRP. In two experimental groups, the spinal cord was pretreated with H89 (10 μM, 15 μl) or CC (0.1 mM, 15 μl) before CGRP (0.5 μM, 15 μl) was infused. In a control group, ACSF was applied instead of H89 or CC. To test whether the CGRP1 receptor antagonist blocks the effect of CGRP, CGRP (1 μM, 7.5 μl) and CGRP8–37 (20 μM, 7.5 μl) were applied together. In another control group, only ACSF was infused.

The activity of a representative WDR neuron in the dorsal horn after application of CGRP is shown by rate histograms in Fig. 3. The top row shows the baseline background activity and responses to brush and press. After the baseline activity was recorded, ACSF was applied, and 30 min later CGRP was administered. There was no difference between the baseline...
activity (Fig. 3, top) and the activity 30 min after ACSF superfusion (2nd row). However, 30 min after superfusion of CGRP, the representative WDR neuron showed an increase in background activity and in responses to press but not in responses to brush (Fig. 3, 3rd row) compared with the baseline activity (Fig. 3, top). Furthermore, the increases in background activity and in responses to press outlasted the drug infusion period for more than half an hour (Fig. 3, bottom).

After CGRP superfusion, neurons were considered responsive to CGRP when changes in background activity and responses to mechanical stimuli were increased by 25% from baseline value. According to this criterion, in three of six WDR neurons tested, there was an increase in background activity after CGRP superfusion. A summary of the grouped data is shown in Fig. 4. At 30 min after superfusion with ACSF (15 μl), there were no significant changes in the background activity and responses to mechanical stimuli of naïve WDR neurons. At 30 min after superfusion of CGRP (0.5 μM, 15 μl), responses to press significantly increased to 160.1 ± 7.5% (repeated-measures ANOVA followed by Newman-Keuls test, P < 0.05), but the responses to brush stimuli were not obviously changed.

Moreover, after washout, the increases in responses to press induced by CGRP lasted at least half an hour. Because CGRP produced inconsistent effects on the background activity of neurons, background activity was not significantly increased. At 30 min after superfusion of CGRP (1 μM, 7.5 μl) + CGRP8-37 (20 μM, 7.5 μl), the background activity and responses to brush and press of WDR neurons was 113.1 ± 9.6, 94.5 ± 8.5, and 106.9 ± 12.8% of baseline, respectively. CGRP8-37 attenuated the effect of CGRP completely, consistent with our previous study (unpublished details). There were no significant changes in the responses of the WDR neurons at 30, 60, and 90 min after ACSF compared with baseline. Figure 5A contains rate histograms of a representative WDR neuron that showed a blockade of CGRP-induced enhancement by H89. H89 pretreatment itself produced no significant changes in the background activity or in responses to brush or press stimuli (middle). However, the enhanced response to noxious mechanical stimuli produced by CGRP superfusion was nearly completely blocked (bottom). A PKC inhibitor, CC, also blocked the enhanced responses induced by CGRP. Figure 5B shows a typical experiment on one WDR neuron. The grouped data are summarized in Fig. 6.

**DISCUSSION**

Effects of CGRP, PKA, and PKC in central sensitization

Our previous behavioral and electrophysiological studies demonstrated that activation of CGRP1 receptors in the dorsal horn contributes to the development of central sensitization associated with cutaneous inflammation (Sun et al. 2003a,b). PKA and PKC have both been shown to be involved in the...
CGRP after CGRP stimulation at concentrations of previous study has demonstrated an increased production of CGRP produces its effect on neurons in the dorsal horn. A possible mechanism by which CGRP exerts its effect on dorsal horn neurons is through activation of cAMP and its downstream PKC, blocked the mechanical hyperalgesia and central sensitization induced by CGRP.

Intrathecal inhibitors of spinal PKA, PKC

Intrathecal injection of H89, a PKA inhibitor, or CC, a PKC inhibitor, did not produce a change in mechanical paw withdrawal threshold. However, when either H89 or CC was given intrathecally 20 min before CGRP was delivered, the inhibitor blocked the mechanical hyperalgesia induced by CGRP. These results suggest that the decreased nociceptive threshold induced by CGRP may be mediated by the activation of PKA or PKC.

An important issue is whether the effects of H89 or chelerythrine in fact reflect their actions as PKA or PKC inhibitors. First, the inhibitors of PKA (H89) and PKC (CC) were used at a specific dose chosen based on the results of other comparable studies (Begon et al. 2001; Granados-Soto et al. 2000; Guo et al. 2002; Li and Chen 2003; Seybold et al. 2003; Sluka and Willis 1997; Sluka et al. 1997). Moreover, CGRP increases the expression of NK1 receptors by a cAMP-dependent pathway (Seybold et al. 1997). Furthermore, CGRP also leads to extracellular-signal-regulated kinase (ERK) activation by a PKA-dependent pathway (Parameswaran et al. 2000), and ERK contributes to the establishment and maintenance of persistent inflammatory heat and mechanical hypersensitivity (Ji et al. 2003). All the results concerning the signaling pathway downstream of CGRP receptor activation were obtained from in vitro studies. A possible mechanism by which CGRP exerts its effect on dorsal horn neurons is through activation of cAMP and its downstream PKC (Baidan et al. 1992; Parsons and Seybold 1997; Parameswaran et al. 2000; Poyner 1992; Seybold et al. 2003), a signal transduction mechanism well known to contribute to the sensitization of STT neurons (Lin et al. 2002; Sluka et al. 1997). In this study, our behavioral and electrophysiological results showed that not only inhibition of PKA, but also of PKC, blocked the mechanical hyperalgesia and central sensitization induced by CGRP.

Intrathecal injection of H89, a PKA inhibitor, or CC, a PKC inhibitor, did not produce a change in mechanical paw withdrawal threshold. However, when either H89 or CC was given intrathecally 20 min before CGRP was delivered, the inhibitor blocked the mechanical hyperalgesia induced by CGRP. These results suggest that the decreased nociceptive threshold induced by CGRP may be mediated by the activation of PKA or PKC.
responses of WDR neurons in the dorsal horn to noxious stimuli produced by CGRP were blocked by H89 and significantly attenuated by chelerythrine. The results of the electrophysiological experiments on dorsal horn neurons are consistent with the behavioral data and suggest a direct action of CGRP, H89, and CC on spinal sensory neurons.

Are PKA and PKC activated directly or indirectly?

In this study, we found that the effects of CGRP are significantly blocked by a PKA or PKC inhibitor. It appears that both PKA and PKC are necessary for the effects of CGRP. Is there a cross-talk between PKA and PKC? Indeed, in skeletal muscle, CGRP was shown to stimulate phosphoinositide turnover, most likely through the CGRP-induced increase in levels of cAMP as this effect was mimicked by other cAMP-mobilizing agents such as forskolin (Lauffer and Changgeux 1989). In a similar fashion, CGRP was reported to increase intracellular cAMP levels in human ocular ciliary epithelial cells (Crook and Yabu 1992). This increase was apparently modulated by PKC because the interaction of the latter with CGRP receptors prevented a further increase in cAMP levels. This suggests that both the PKA and PKC pathways may be downstream of CGRP receptor activation. The activation of multiple intracellular pathways might be due to coupling of CGRP receptors to different transduction mechanisms or to a close interaction between different second-messenger pathways. In fact, several studies have indicated that the PKC and PKA pathways do not function in isolation but rather interact in an intricate intracellular network to achieve the obtained response (Choi and Toscano 1988; Kubota et al. 2003; Sugita et al. 1997). Furthermore, co-activation of PKA and PKC pathways is also triggered by activation of other receptors. For example, co-activation of PKA and PKC is necessary for the effects of prostaglandin E2 (Ren et al. 1996), estrogen (Kelly et al. 1999), β-adrenergic (Khasar et al. 1999), and bradykinin receptor activation (Kohno et al. 2003). Unfortunately, we cannot deduce whether these pathways are being activated in parallel or in a certain sequence as a result of CGRP receptor activation because of the experimental design in our study. Further examination of these mechanisms would be necessary in future studies.

There is also the possibility that the effect of the PKC inhibitor could reflect an indirect effect of CGRP in this study. Previous studies demonstrated that CGRP receptors are coupled predominantly to adenylyl cyclase via a G protein to induce the formation of cAMP (Poyner 1992; review; Parsons and Seybold 1997). However, NK1 receptors and group I mGluRs are linked with phospholipase C. Activation of NK1 receptors and group I mGluRs leads to generation of diacylglycerol (DAG) and inositol 1,4,5-trisphosphate, and DAG is an important factor regulating the activity of PKC (Yokota et al. 1989; Hershey and Krause 1990; Conn and Pin 1997). CGRP can potentiate the release of substance P and glutamate from rat spinal dorsal horn neurons during mechanical nociception, suggesting the presence of presynaptic CGRP receptors on substance P and glutamate neurons (Kangra and Randic 1990; Oku et al. 1987). In this situation, the PKA pathway may be triggered by activation of presynaptic CGRP receptors on substance P and glutamate neurons, and the PKC pathway may be triggered by SP activation of neurokinin receptors or glutamate activation of metabotropic receptors (Wajima et al. 2000).

However, we cannot exclude the possibility that the effect of CGRP is postsynaptic. Several studies agree that CGRP can produce depolarization of neurons in spinal dorsal horn slices (Miletic and Tan 1988; Ryu et al. 1988). Pre- and postsynaptic CGRP receptors have been localized in the spinal cord (Ye et al. 1999). Thus the effect of CGRP could be pre- or postsynaptic or both. We hypothesize that central sensitization induced by CGRP depends on a cascade or a combination of crucial events in the neurons. Each of these steps may depend on the action of specific receptor and signaling pathway. This suggests that different second-messenger pathways must be co-activated in a coordinated fashion. In this study, both PKA and PKC inhibitors have been shown to block the effect of CGRP in mechanical hyperalgesia and central sensitization.

In conclusion, our data suggest that activation of CGRP receptors in the spinal cord produces mechanical hyperalgesia and sensitizes WDR neurons in the dorsal horn in the rat. These effects of CGRP are mediated by at least two second-messenger systems involving PKA and PKC.

GRANTS

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