cAMP and cGMP Contribute to Sensory Neuron Hyperexcitability and Hyperalgesia in Rats With Dorsal Root Ganglia Compression

Xue-Jun Song, Zheng-Bei Wang, Qiang Gan, and Edgar T. Walters

Department of Neurobiology, Parker College Research Institute, Dallas; and Department of Integrative Biology and Pharmacology, University of Texas at Houston, Medical School, Houston, Texas

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Song, Xue-Jun, Zheng-Bei Wang, Qiang Gan, and Edgar T. Walters. cAMP and cGMP contribute to sensory neuron hyperexcitability and hyperalgesia in rats with dorsal root ganglia compression. J Neurophysiol 95: 479–492, 2006. Numerous studies have implicated the cAMP-protein kinase A (PKA) pathway in producing hyperexcitability of dorsal root ganglia (DRG) sensory neurons under conditions associated with pain. Evidence is presented for roles of both the cAMP-PKA and cGMP-protein kinase G (PKG) pathways in maintaining neuronal hyperexcitability and behavioral hyperalgesia in a neuropathic pain model: chronic compression of the DRG (CCD treatment). Lumbar DRGs were compressed by a steel rod inserted into the intervertebral foramen. Thermal hyperalgesia was revealed by shortened latencies of foot withdrawal to radiant heat. Intracellular recordings were obtained in vitro from lumbar ganglia after in vivo DRG compression. Activators of the cAMP-PKA pathway, 8-Br-cAMP and 8-pCPT-cAMP, and of the cGMP-PKG pathway, 8-Br-cGMP and Sp-cGMP, increased the hyperexcitability of DRG neurons already produced by CCD treatment, as shown by further decreases in action potential threshold and increased repetitive discharge during depolarization. The adenylyl cyclase inhibitor, SQ22536, the PKA antagonist, Rp-cAMPS, and the guanylate cyclase inhibitor, ODQ, and the PKG inhibitor, Rp-8-pCPT-cGMPS, reduced the hyperexcitability of CCD DRG neurons. In vivo application of PKA and PKG antagonists transiently depressed behavioral hyperalgesia induced by CCD treatment. Unexpectedly, application of these agonists and antagonists to ganglia of naïve, uninjured animals had little effect on electrophysiological properties of DRG neurons and no effect on foot withdrawal, suggesting that sensitizing actions of these pathways in the DRG are enabled by prior injury or stress. The only effect observed in uncompressed ganglia was modest depolarization of DRG neurons by PKA and PKG agonists. CCD treatment also depolarized DRG neurons, but CCD-induced depolarization was not affected by agonists or antagonists of these pathways.

INTRODUCTION

Injury and inflammation involving axons or somata of dorsal root ganglion (DRG) sensory neurons often cause neuropathic pain (reviewed by Devor 1994, 1999; Zimmermann 2001). The pain is produced in part by ectopic spontaneous activity and hyperexcitability in affected DRG neurons (Abdulla and Smith 2001a; Bennett and Xie 1988; Devor 1994; Hu and Xing 1998; Kim and Chung 1992; Ma and LaMotte 2005; Seltzer et al. 1990; Song et al. 1999, 2003a,b; Stebbing et al. 1999; Wall and Gutnick 1974; Zhang et al. 1999). Hyperexcitability may involve alterations of TTX-sensitive and TTX-resistant Na+ currents (TTX-R INa) (Rizzo et al. 1995; Waxman 1999), K+ currents (Everill and Kocsis 1999; Yao et al. 2003), and an N-type Ca2+ current (Abdulla and Smith 2001b; Baccei and Kocsis 2000), and might depend on transcriptional changes (e.g., Costigan et al. 2002; Valder et al. 2003; Xiao et al. 2002). Important questions remain about the intracellular signals that induce and maintain these persistent alterations.

The cAMP-protein kinase A (PKA) pathway mediates peripheral hyperalgesic actions of inflammatory mediators (Aley and Levine 1999; Ferreira and Nakamura 1979; Taiwo et al. 1989). In isolated DRG neurons, prostaglandin E2 (PGE2) enhances soma excitability by stimulating cAMP synthesis (Hingtgen et al. 1995) and producing PKA-mediated decreases in both INa (Evans et al. 1999) and an afterhyperpolarization current (Fowler et al. 1985), as well as increases in TTX-R INa (England et al. 1996; Gold et al. 1996), vanilloid receptor (VR-1) current (Caterina et al. 1997; Lopshire and Nicol 1998), and hyperpolarization-activated cation current (Ih) (Ingram and Williams 1994). An unanswered question is whether the cAMP-PKA pathway is important for neuropathic pain. Transient translocation of PKA or other PKA-dependent signals into the nucleus after nerve injury might induce long-term alterations by altering gene expression, as occurs during some forms of learning and memory (e.g., Kandel 2001) and perhaps in the spinal cord after noxious stimulation (Ma et al. 2003; Malmberg et al. 1997; Miletic et al. 2002). Also, continuing activation of PKA might persistently maintain hyperalgesia and hyperexcitability (e.g., Aley and Levine 1999, 2002; Bolyard et al. 2000; Liao et al. 1999).

Much less attention has been paid to cGMP in studies of neuropathic pain. Although the cAMP-PKA and cGMP-protein kinase G (PKG) pathways often produce antagonistic effects, similarities between these cyclic nucleotides and between their corresponding protein kinases suggest that manipulations directed at one pathway could affect the other (e.g., Jiang et al. 1992). Thus some effects attributed to the cAMP-PKA pathway might involve the cGMP-PKG pathway. Interestingly, cGMP can contribute to peripheral sensitization in DRG neurons (Aley et al. 1998) and to long-term hyperexcitability of nociceptor somata in Aplysia ( Lewin et al. 1997). However, evidence for hyperalgesic contributions of the cGMP-PKG pathway is greater at the spinal level than in the DRG or periphery (e.g., Kress et al. 1996; Tegeder et al. 2004), and this pathway also produces hypoalgesic effects (see DISCUSSION).

DRG compression, which represents a significant clinical problem in humans, provides a useful animal model for study-
ing neuropathic pain. DRG neurons are normally electrically silent in the absence of stimulation, but after DRG compression or nerve injury, 10% of DRG neurons become spontaneously active (Devor 1999; Song et al. 1999; Zhang et al. 1999). Interestingly, after DRG compression, the cAMP-PKA pathway enhances spontaneous activity of large A-β neurons (Hu et al. 2001), which may sensitize central neurons and cause hyperalgesia (Ma and Woolf 1996; Mannion et al. 1999; Neumann et al. 1996). Hyperexcitability of medium-sized (A-delta fiber) and small (C fiber) neurons also occurs (Song et al. 2003a,b; Zhang et al. 1999). We now report that, in chronically compressed ganglia, both the cAMP-PKA and cGMP-PKG pathways help to maintain hyperalgesia and hyperexcitability in DRG neurons of all sizes. Surprisingly, in uncompressed ganglia, these pathways have little immediate effect on DRG neuron excitability or withdrawal behavior.

**METHODS**

**Surgical procedures**

All investigations were conducted in conformity with APS Guiding Principles in the Care and Use of Animals and were approved by the Parker College Research Institute’s Committee on the Care and Use of Experimental Animals.

Experiments were performed on adult, male Sprague-Dawley rats (n = 305, 200–220 g at the start of each experiment). The rats were housed in groups of four in plastic cages (40 × 60 × 30 cm) with soft bedding and free access to food and water under a 12-h day/12-h night cycle. They were kept 5–7 days under these conditions, before and ≤4 wk after surgery. All surgeries were done under pentobarbital sodium (40–50 mg/kg, ip) anesthesia. After surgery, the muscle and skin layers were sutured.

Hollow stainless steel rods were surgically implanted unilaterally into the intervertebral foramen (IVF) at L₄ and L₅ to chronically compress the DRG (CCD) (Song et al. 1999). In brief, rats (n = 48) were anesthetized, paraspinous muscles were separated from the mamilary and transverse processes, and the IVF of L₄ and L₅ was exposed. A hollow, stainless steel, L-shaped rod (4 mm in length and 0.66 mm in diameter) was implanted chronically into the IVF. In separate animals, the rod was implanted into the IVF at L₄ (n = 96) or at L₅ and L₆ (n = 6) and connected to silicon tubing (0.51 mm ID, 0.94 OD, 30–40 mm length; Dow Corning, Medical Materials Division) filled with saline (~10 μl). The other end of the tubing was sealed, except when injecting drugs. Unlike the solid rods used previously (Song et al. 1999, 2003a), the hollow stainless steel rods were made from 25-gauge needles with one hole drilled on each side and one outlet on each end to permit delivery of drugs to the DRG during compression.

To deliver drugs or saline to the DRG in previously unoperated, naïve rats, a sharp, stainless steel needle, 0.4 mm in diameter (with a right angle to limit penetration), was inserted ~4 mm into the IVF at L₅ (n = 80) after the IVF was exposed. Injections took 1.5–2 min, and the needle was withdrawn. Each of another group of unoperated, naïve (n = 6) rats received injection into the IVF at the L₄ and L₅, respectively. For sham injections, the needle was left in the IVF for the same period of time. Sham surgery (n = 8) involved identical surgical procedures to those described but without insertion of the rod or the needle.

**Behavioral testing**

Thermal hyperalgesia was determined by measuring foot withdrawal latency during heat stimulation (Hargreaves et al. 1988; Song et al. 2003a). Each rat was placed in a box (22 × 12 × 12 cm) containing a smooth glass floor. The temperature of the glass was measured and maintained at 26 ± 0.5°C. A heat source (ITTC Model 336 Analgesia Meter, Life Science) was focused on a portion of the hindpaw that was flush against the glass, and a radiant thermal stimulus was delivered to that site. The stimulus shut off automatically when the hindpaw moved (or after 20 s to prevent tissue damage). The intensity of the heat stimulus was maintained constant throughout all experiments. In control rats, elicited paw movements occurred at a latency of 9–12 s. Thermal stimuli were delivered four times to each hind paw at 5- to 6-min intervals. To reduce contributions from preexisting differences among individuals in thermal responsiveness, the withdrawal latencies were normalized by subtracting each value on the treated side from the corresponding value on the contralateral side, and the results were expressed as difference scores. The rats were tested on each of 2 successive days before surgery. Postoperative tests were conducted 1, 3, 5, 7, and 10 days after surgery. Additional tests were conducted 2, 6, 12, 24, and 36 h after injection of drugs or saline into the IVF on the third day after surgery. For the rats receiving transient needle insertion, the additional tests were conducted 6, 12, 24, and 36 h after surgery. For the rats used for electrophysiological studies, postoperative tests were conducted 1, 3, and 5 days after surgery and on the day of electrophysiological recording (days 8–28).

**Electrophysiological studies**

In vitro preparations of DRGs were made from L₄ and/or L₅ ganglia for electrophysiological studies from 48 CCD, 49 naïve, and another 4 sham surgery rats. The experimenters who performed electrophysiological recordings and analyzed data were blinded to the source of the cells. The procedure was similar to that described previously (Song et al. 2003a). In brief, the rat was anesthetized with pentobarbital sodium after the final behavioral test. The sciatic nerve was isolated from surrounding tissue and transected at the mid-thigh level, and its proximal portion was traced to the ganglia. A laminectomy was performed, and L₄ and L₅ DRGs were identified. The location of the rod was checked immediately after exposing the ganglia. Oxygenated artificial cerebrospinal fluid (ACSF), consisting of (in mM) 130 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 24 NaHCO₃, 10 dextrose, 1.2 MgCl₂, and 1.2 CaCl₂ (pH = 7.3) was dripped periodically onto the surface of the ganglion during the surgical procedure to prevent drying and hypoxia. The ganglion was removed from the rat and placed in a 35-mm petri dish filled with oxygenated ACSF. Under a dissecting microscope, the perineurium and epineurium were peeled away from the ganglion with fine forceps and the attached sciatic nerve and dorsal roots transected adjacent to the ganglion. The ganglion was placed in the recording chamber and mounted on the stage of an upright microscope (BX50-WI, Olympus). A U-shaped stainless steel rod with four pieces of silver wire crossing from one side to the other was used to hold the ganglion gently in place. The DRG was incubated in oxygenated ACSF. The temperature was maintained at 35–36°C by a temperature controller (TC-344B, Warner Instruments, Hamden, CT).

Intracellular recordings were made from the DRG somata using conventional bridge-balance techniques (Axoclamp-2B, Axon Instruments, Foster City, CA) and analyzed with PCLAMP-8 under Windows 98 (Axon Instruments). DRG neurons were visualized under a microscope using differential interference contrast. Somata of the DRG neurons were classified visually by their diameters as small (≤30 μm), medium (31–49 μm), or large (≥50 μm). Glass microelectrodes were fabricated with a Flaming/Brown micropipette puller (Model P-97/PC, Sutter Instruments.) and filled with 2 M potassium acetate (pH = 7.2). Satisfactory recordings were obtained with electrodes having DC resistances of 20–60 MΩ.

Resting membrane potential (RMP) was taken 2–3 min after RMP had stabilized. All neurons accepted for testing exhibited a stable RMP of ~40 mV or more negative. Action potential (AP) properties were examined by delivering depolarizing currents of 0.05–4 nA (50-ms duration) in increments of 0.05–0.2 (for small neurons), 0.1–0.2 (for medium neurons), or 0.1–0.4 nA (for large neurons) until
an AP was evoked. AP current threshold was defined as the minimum current required for evoking an AP. AP threshold voltage was defined as the first point on the upstroke of an action potential where the rising rate exceeded 50 mV/ms (Anderson et al. 1987). AP amplitude was measured from the AP threshold to the peak. AP duration was measured at threshold voltage. Afterhyperpolarization (AHP) amplitude was measured from the RMP to the peak hyperpolarization, and AHP duration was measured as the interval from onset until the AHP had recovered by 50%. Input resistance (R_{in}) for each neuron was obtained from the slope of the steady-state I-V relationship during a

FIG. 1. Chronic compression depolarizes resting membrane potential (RMP) in dorsal root ganglia (DRG) neurons of all sizes. A: distribution of RMP values in uncompressed DRG neurons from naïve animals. B: RMP distribution from sham-treated animals. C: RMP distribution from chronic compression of the DRG (CCD treatment) ganglia. D: mean values of RMP in each group (*P < 0.05, 1-way ANOVA and Dunnett’s tests). *Comparison of CCD and sham to naïve groups.
series of 100-ms hyperpolarizing currents delivered in steps of 0.1–0.2 nA from –2 to 0.5 nA. Repetitive discharge of each neuron was measured by counting the spikes evoked by intracellular injection of standardized depolarizing currents at 2.5 times threshold strength (×1000 ms). Discharge patterns of DRG neurons were classified into two groups: 1) neurons firing either one or two APs and 2) neurons firing more than two APs (Song et al. 2003a).

**Drug application**

All chemicals were obtained from Sigma. Drugs were applied in the bath to excised DRG in vitro at the final concentrations indicated: the cAMP analog, 8-Br-cAMP (10–500 μM); the PKA activator, Sp-cAMPS (10–500 μM); the adenylate cyclase activator, forskolin (1 and 10 μM); the adenylate cyclase inhibitor, SQ22536 (10–500 μM); the PKA inhibitor, Rp-8-pCPT-cGMPS (10–500 μM); the cGMP analog, 8-Br-cGMP (10–500 μM); the PKG activator, Sp-cGMPS (10–500 μM); the guanylate cyclase inhibitor, ODQ (5 and 10 μM), and the PKG inhibitor, Rp-8-pCPT-cGMP (10–500 μM). Forskolin and ODQ were dissolved in DMSO to make a 10 and 20 mM stock solution, respectively, and subsequently diluted with oxygenated ACSF before application. The final concentration of DMSO was 0.01–0.1%. We tested DMSO at 0.01, 0.1, and 0.2% and found that none of these concentrations produced significant effects on the RMP or excitability of DRG neurons (data not shown). Water-soluble drugs were diluted in ACSF just before application or diluted initially with pure water for a stock solution that was subsequently diluted with oxygenated ACSF before application. Application began 10–45 min before and continued during the 3–4 h of electrophysiological recordings. In vivo delivery (50 μl) of antagonists (500–1,000 μM) and agonists (1–500 μM) was by injection into the L4 intervertebral foramen through silicon tubing connected to the hollow rod or through the fine needle as described above. Saline was delivered as a control. Injection was finished in ~1.5–2 min. Rats were only included in the analysis if the rod, with the tubing attached, remained within the IVF throughout the experiment.

**Statistical tests**

Changes in withdrawal latencies over time were tested with two-way ANOVA with repeated measures followed by Bonferroni post hoc tests. One-way ANOVA followed by Dunnett’s tests were used to test the hypothesis that RMP and excitability of DRG neurons in CCD groups were significantly different from both the naïve and sham control groups. Individual t-test were used to test specific hypotheses about differences between each operated or drug-treated group and its corresponding control group for each electrophysiological parameter tested. Fisher’s exact test (for smaller groups) and χ² tests (for larger groups) were used to identify differences in the incidence of effects. All data are presented as means ± SE. Unless otherwise stated, statistical results are considered significant if P < 0.05.

**RESULTS**

**Chronic compression produces thermal hyperalgesia**

We began by confirming and extending earlier demonstrations (Hu and Xing 1998; Song et al. 1999, 2003a,b; Zhang et al. 1999) that CCD treatment produces pain and hyperalgesia. In our first study, all CCD rats used for electrophysiological analysis showed clear behavioral indications of pain and thermal hyperalgesia. Withdrawal latencies of the feet ipsilateral to CCD treatment decreased from preoperative values of 9.6 ± 0.21 (mean of the 2 preoperative tests) to 6.2 ± 0.31 s (P < 0.01, n = 37) on the day of electrophysiological recordings, 1–4 wk after treatment. Withdrawal latencies of the contralateral feet showed no significant change during the same period, going from 10.2 ± 0.22 to 9.7 ± 0.25 s (n = 37). In a second study (see Fig. 5), 96 additional CCD rats also showed thermal hyperalgesia. In both studies, all the CCD rats developed varying degrees of abnormality in gait and posture, which presumably serve to minimize aversive sensory stimulation (Song et al. 1999). None of the naïve or sham-operated rats used in these studies showed any significant changes in foot withdrawal responses to the radiant heat stimulation.

**Chronic compression produces depolarization and hyperexcitability of DRG neuron somata**

Intracellular recordings from DRG neurons were made from 236, 194, and 96 neurons of different sizes recorded from 10 CCD, 10 naïve, and 4 sham surgery rats, respectively. The CCD DRG neurons, compared with those from naïve and sham ganglia, exhibited significant alterations in RMP and excitability. Although previous studies have failed to find effects of CCD treatment on RMP (Song et al. 2003a; Zhang et al. 1999), these studies had only analyzed cells with RMP ≤ –50 mV. In this study, we noticed a shift in the distribution of RMP after CCD treatment toward the –40 to –50-mV range (Figs. 1, A–C), and therefore set the cut-off for analysis at ≤ –40 mV. Mean RMP was significantly less negative in CCD neurons than in neurons from naïve and sham surgery animals in each size range (≤ –40 mV; P < 0.01, t-test; Fig. 1D). When using a more hyperpolarized cut-off of ≤ –50 mV, significantly depolarized RMP was found in the medium-sized neurons but not in the large or small neurons (data not shown).

As previously observed (Song et al. 1999), we found that the incidence of spontaneous activity was greater in CCD ganglia (16 of 236 neurons, 6.4%) than in ganglia from sham-treated animals (2 of 96 neurons, 2%) or naïve animals (2 of 194 neurons, 1%). A neuron was defined as spontaneously active if AP discharge lasted ≥2 min after a stable recording was first obtained. All the spontaneously active neurons had RMP less negative than –55 mV (mean RMP in CCD cells, –43.7 ± 1.1 mV, n = 15).

As found in previous studies of DRG neurons after CCD injury (Song et al. 1999, 2003a,b; Zhang et al. 1999) or sciatic nerve injury (Devor 1994; Wall and Devor 1983), significant alterations were found in the CCD neurons in AP current and voltage thresholds, patterns of repetitive discharge, and AP...
duration. Figure 2 summarizes these effects and shows examples of electrophysiological responses to intracellular test stimuli applied to different sizes of DRG neuron. The mean AP current threshold decreased significantly in CCD DRG neurons of all sizes regardless of their RMP (Fig. 2, A and B; \( P < 0.01 \), t-test). Hyperexcitability of DRG neurons was also revealed as an enhancement of repetitive discharge evoked by a 50-ms depolarizing current pulse normalized to AP current threshold. Approximately 60% of the CCD DRG neurons responded with three or more APs (data not shown).

Because neurons from sham-operated \( (n = 96) \) and naïve \( (n = 194) \) ganglia showed no significant differences from each other in any of the electrophysiological properties measured and because previous studies revealed no behavioral differences between naïve and sham-treated animals at the timepoints tested in this study (Hu and Xing 1998; Song et al. 1999; 2003a), we used DRG neurons from naïve animals as controls in the following electrophysiological experiments. Changes in AP current threshold and repetitive discharge similar to the ones we find after CCD treatment have often been observed after various forms of injury to DRG neurons (Abdulla and Smith 2001a; Gallego et al. 1987; Gurtu and Smith 1988; Song et al. 2003a,b; Stebbing et al. 1999; Study and Kral 1996; Zhang et al. 1999). In the following studies, RMP, AP current threshold, and repetitive discharge of CCD DRG neurons with RMP \( \leq -40 \) mV were used to evaluate the effects of activators and inhibitors of the cAMP-PKA and cGMP-PKG pathways on excitability of sensory neurons after DRG compression.

**cAMP-PKA pathway contributes to compression-induced hyperexcitability**

Membrane-permeant activators of PKA, 8-Br-cAMP (50 \( \mu \)M, \( n = 52; 200 \mu \)M, \( n = 48; 500 \mu \)M, \( n = 36 \)) and Sp-cAMPS (50 \( \mu \)M, \( n = 59; 200 \mu \)M, \( n = 51; 500 \mu \)M, \( n = 32 \)) significantly increased the hyperexcitability of CCD DRG neurons, as shown by a further decrease in AP current threshold and an increased incidence of repetitive discharge to depolarizing current pulses (Fig. 3). Conversely, the adenylate cyclase inhibitor, SQ22536 (50 \( \mu \)M, \( n = 56; 200 \mu \)M, \( n = 54; 500 \mu \)M, \( n = 37 \)) and the PKA antagonist, Rp-cAMPS (50 \( \mu \)M, \( n = 63; 200 \mu \)M, \( n = 45; 500 \mu \)M, \( n = 47 \)), reduced the
hypereexcitability of CCD DRG neurons when applied 1–4 h after removal from the animal (and release of the compression). Both the facilitatory and inhibitory effects of these agents were exhibited in all sizes of CCD DRG neurons (Fig. 3). However, neither the activators nor the inhibitors produced any change in RMP, which had already been depolarized by CCD treatment (data not shown, see also Fig. 1). These results indicate that activation of the cAMP-PKA pathway enhances DRG neuron excitability and that the hypereexcitability, but not depolarization, after CCD treatment involves continuing activation of this pathway.

cGMP-PKG pathway contributes to compression-induced hypereexcitability

Membrane-permeant activators of PKG, 8-Br-cGMP (50 μM, n = 60; 200 μM, n = 44; 500 μM, n = 43) and Sp-cGMPS (50 μM, n = 53; 200 μM, n = 56; 500 μM, n = 39) significantly increased the hypereexcitability of all sizes of CCD DRG neurons, as evidenced by a further decrease in AP current threshold and an increased incidence of repetitive discharge to depolarizing current pulses (Fig. 4). Furthermore, an inhibitor of guanylate cyclase, ODQ (5 μM, n = 73; 10 μM, n = 54), and an inhibitor of PKG, Rp-8-pCPT-cGMPS (50 μM, n = 57; 200 μM, n = 46; 500 μM, n = 55) reduced the hypereexcitability of all sizes of CCD DRG neurons. Again, neither the activators nor the inhibitors of the cGMP-PKG pathway produced further changes in RMP, which had been depolarized by CCD treatment (data not shown). These results indicate that activation of the cGMP-PKG pathway can enhance DRG neuron excitability and that the hypereexcitability, but not depolarization, after CCD treatment involves continuing activation of this pathway.

cAMP and cGMP pathways depolarize DRG neurons in control ganglia but have little immediate effect on excitability

Given the lack of effect of activators of the cAMP-PKA and cGMP-PKG pathways on RMP in CCD neurons, it was a surprise to find that activation of these pathways significantly depolarized uncompressed DRG neurons from naïve rats. The mean RMP depolarized ~5 mV in each case (Fig. 5A; P < 0.01, t-test). No significant differences were found among the different doses of each drug on RMP. Another surprise was that the significant effects of these pathways on RMP were associated with very little change in neuronal excitability (Fig. 5, B and C). Although the excitability of control DRG neurons tended to increase slightly, as indicated by small decreases in AP current thresholds and increases in repetitive discharge, no statistically significant differences in excitability were found between neurons treated with ACSF (n = 194) and those treated for 1–4 h with a 50-fold range of doses of the activators. 8-Br-cAMP (10 μM, n = 57; 50 μM, n = 63; 200 μM, n = 59; 500 μM, n = 48), Sp-cAMPS (10 μM, n = 58; 50 μM, n = 61; 200 μM, n = 65; 500 μM, n = 69), 8-Br-cGMP (10 μM, n = 48; 50 μM, n = 56; 200 μM, n = 70; 500 μM, n = 40), Sp-cGMPS (10 μM, n = 48; 50 μM, n = 53; 200 μM, n = 57; 500 μM, n = 42), as well as a 10-fold range of doses of the direct activator of adenylyl cyclase, forskolin (1

![Figure 4](http://jn.physiology.org/10.1152/jn.00177.2005)


\[ \mu M, \ n = 75; \ 10 \ \mu M, \ n = 71 \]. The only exception was a significant decrease in AP threshold current in medium-sized neurons during 8-Br-cAMP treatment (Fig. 5Bb). The lack of consistent effects of a wide range of doses of PKA and PKG activators on AP threshold current and repetitive discharge to depolarizing current pulses can be seen in the dose-response relations shown in Fig. 5, D and E.

Inhibitors of cAMP-PKA and cGMP-PKG pathways failed to produce significant effects on RMP and also failed to affect excitability in each size of DRG neuron in uncompressed ganglia from naïve rats. The RMP, AP threshold current, and repetitive discharge to depolarizing test pulses exhibited no significant differences in neurons treated with ACSF compared with those treated for 1–4 h with the inhibitors SQ22536 (10 \( \mu M, n = 65; 50 \ \mu M, n = 49; 200 \ \mu M, n = 51; 500 \ \mu M, n = 57 \)), Rp-cAMPS (10 \( \mu M, n = 66; 50 \ \mu M, n = 54; 200 \ \mu M, n = 62; 500 \ \mu M, n = 58 \)), ODO (5 \( \mu M, n = 67; 10 \ \mu M, n = 74 \)), or Rp-8-pCPT-cGMPs (10 \( \mu M, n = 55; 50 \ \mu M, n = 64; 200 \ \mu M, n = 51; 500 \ \mu M, n = 33 \)). These results indicate that both the cAMP-PKA and cGMP-PKG pathways can depolarize RMP of uncompressed DRG neurons without significantly affecting excitability and that neither pathway contributes directly to the RMP or excitability of uncompressed DRG neurons taken from naïve animals and tested in situ.

In vivo delivery of inhibitors of PKA and PKG depresses thermal hyperalgesia

Because our electrophysiological findings indicate that the cAMP-PKA and cGMP-PKG pathways are important for maintaining hyperexcitability of DRG neurons during DRG compression and this hyperexcitability is likely to contribute to hyperalgesia, we asked whether application of blockers or agonists of these pathways alters the hyperalgesia produced by DRG compression in vivo.

The PKA inhibitor, Rp-cAMPS (0.5 \( \mu M, n = 6; 1 \ \mu M, n = 8 \)), and the PKG inhibitor, Rp-8-pCPT-cGMPs (0.5 \( \mu M, n = 6; 1 \ \mu M, n = 8 \)), were administered into the IVF of L4 on the third day after injury when thermal hyperalgesia was well developed. Each inhibitor significantly interrupted the ongoing hyperalgesia, as evidenced by transient recovery of the shortened latencies of foot withdrawal (Fig. 6A). Before surgery, there were no significant differences in the latencies of withdrawal between the left and right hind feet, and therefore the difference scores from both preoperative test sessions in each group clustered around zero. Beginning on the first postoperative day, CCD-treated rats in all groups showed profound thermal hyperalgesia, expressed as significantly reduced latency of foot withdrawal to heat stimulation on the treated side. This hyperalgesia was transiently but significantly depressed by Rp-cAMPS and Rp-8-pCPT-cGMPs, respectively. The inhibition started within 2 h, peaked at 6 h, and lasted about 24 h.

Despite their enhancement of sensory neuron excitability in compressed ganglia, activators of PKA and PKG failed to enhance hyperalgesic behavior in CCD rats. The activators 8-Br-cAMP (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 8 \)), Sp-cAMPS (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 8 \)), 8-Br-cGMP (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 8 \)), and Sp-cGMPs (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 8 \)), as well as the adenylate cyclase activator forskolin (1 \( \mu M, n = 6; 10 \ \mu M, n = 6 \)), did not further increase CCD-induced thermal hyperalgesia (Fig. 6B). The lack of additional enhancement by these agonists might be caused by a ceiling effect because the latencies of foot withdrawal responses after CCD treatment were very low compared with those found after other hyperalgesia-inducing treatments, including carrageenan application to the DRG, sciatic nerve constriction, and partial dorsal rhizotomy (Hu and Xing 1998; Song et al. 2003a). The lack of effect of the agonists was not a consequence of applying the drugs to L4 only, because additional experiments showed that injection of 8-Br-cAMP (1 \( \mu M, n = 6 \)) into L4 as well as L5 in CCD rats again failed to augment the CCD-induced hyperalgesia (data not shown).

An interesting question is whether, in the absence of chronic compression, delivery of PKA or PKG activators to the DRG in vivo can produce thermal hyperalgesia. We attempted to answer this question by briefly inserting a fine needle into the IVF in the L4 DRG of naïve animals to transiently introduce the activators. No hyperalgesia was observed after sham surgery. Needle insertion by itself produced significant hyperalgesia, which started within 6–12 h and lasted for 2–3 days in seven rats and 5 days in one rat (see also Song et al. 1999).

Needle insertion plus injection of saline (n = 8), forskolin (1 \( \mu M, n = 4; 10 \ \mu M, n = 4 \)), 8-Br-cAMP (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 4 \)), 8-Br-cGMP (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 4 \)), Sp-cAMPS (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 4 \)), Sp-cGMPs (0.1 \( \mu M, n = 4; 1 \ \mu M, n = 4 \)), and the PKG inhibitor, Rp-8-pCPT-cGMPS (0.5 \( \mu M, n = 4; 1 \ \mu M, n = 4 \)), did not significantly enhance the hyperalgesia produced by needle insertion alone (Fig. 6C). When injections were made into L4 and L5 DRG of naïve rats, 8-Br-cAMP (1 \( \mu M, n = 6 \)) injection was associated with a modest enhancement of the transient hyperalgesia compared with that induced by injection into L5 alone (peak latency difference score -2.13 ± 0.35 vs. -1.56 ± 0.27 s after injection into L4 alone). However, this slight augmentation does not permit conclusions to be drawn about the sufficiency of agonist application to induce hyperalgesia because it might have been caused by the additional injury resulting from needle insertion into the second IVF rather than from additional agonist effects on the second DRG. The fact that the degree and duration of hyperalgesia were clearly less after needle insertion than after CCD treatment (where the peak difference scores were around -4; Fig. 6C) suggests that activation of either the PKA or PKG pathway in a DRG from naïve animals is relatively ineffective in inducing hyperalgesia.

In contrast to the effects of PKA and PKG inhibitors in CCD rats, in naïve rats neither Rp-cAMPS (0.5 \( \mu M, n = 6; 1 \ \mu M, n = 6 \)) nor Rp-8-pCPT-cGMPs (0.5 \( \mu M, n = 6; 1 \ \mu M, n = 6 \)) significantly altered the latencies of foot withdrawal ipsilateral.
eral or contralateral to the side of the needle insertion. Moreover, there was no significant difference between animals treated and untreated with these inhibitors (Fig. 6C). These results indicate that application of PKA and PKG inhibitors to the DRG is not normally analgesic, but instead acts to reduce the sensitization induced by CCD treatment.

**DISCUSSION**

These studies indicate that both the cAMP-PKA and cGMP-PKG pathways help to maintain hyperexcitability of DRG neurons and behaviorally expressed hyperalgesia caused by DRG compression. In contrast to the effects seen in compressed ganglia, activation of either of these pathways in uncompressed ganglia from naïve animals depolarized the DRG neurons but failed to produce significant immediate neuronal hyperexcitability or behavioral hyperalgesia.

**cAMP-PKA pathway contributes to hyperexcitability in compressed ganglia**

We showed previously that spontaneous activity of A-beta neurons after CCD treatment is enhanced and inhibited, respectively, by agonists and antagonists of the cAMP-PKA pathway (Hu et al. 2001). Spontaneous activity of A-beta neurons may produce central sensitization after peripheral inflammation or nerve injury, leading to hyperalgesia or allodynia (Liu et al. 2000; Ma and Woolf 1996; Mannion et al. 1999; Neumann et al. 1996). Although 90% of injured DRG neurons are silent in the absence of stimulation, many of these are hyperexcitable (Abdulla and Smith 2001a; Ma et al. 2003; Song et al. 2003a,b; Zhang et al. 1999). Peripheral activation of sensory neurons having hyperexcitable somata might trigger extra spikes in the somata that could contribute to sensitization and hyperalgesia (see Amir et al. 2002, 2005; Clatworthy and Walters 1993; Gasull et al. 2005). Hyperexcitability of medium-sized (A-delta) and small (C) nociceptive neurons is also likely to be important for pain and hyperalgesia (e.g., Devor 1994, 1999; Zimmermann 2001). These results show that CCD-induced hyperexcitability of DRG neurons is reduced by antagonists of the cAMP-PKA pathway and further increased by agonists of this pathway and that these effects occur in all sizes of DRG cells. Thus the cAMP-PKA pathway regulates sensory neuron excitability after injury.

Compression-induced hyperexcitability may involve changes in synthesis of ion channels (Ishikawa et al. 1999; Kim et al. 2002; Waxman 1999; Waxman et al. 1994, 1999, 2000; Zhang et al. 1997). However, rapid attenuation of the hyperexcitability of CCD neuron somata by application of Rp-cAMPS into the IVF indicates that it depends at least partly on continuing sensitization of the sensory neurons’ peripheral processes by local inflammation (Aley and Levine 1999). Indeed, it is possible that inflamm-
formation of the DRG is induced by the implanted rod, causing
the release of inflammatory mediators such as PGE_{2} that
activate PKA, thus increasing the excitability of the DRG
neurons by altering I_{k}, TTX-R I_{Na}, and I_{h}.

cGMP-PKG pathway contributes to hyperexcitability in
compressed ganglia

Our studies indicate that the cGMP-PKG pathway also
contributes to hyperexcitability in compressed ganglia. A
cGMP analog, 8-Br-cGMP, and a PKG agonist, Sp-cGMPs,
enhanced hyperexcitability in all sizes of CCD neurons,
whereas inhibitors of guanylate cyclase (ODQ) and of PKG
(Rp-8-pCPT-cGMPS) reduced the hyperexcitability produced
by CCD treatment. In principle, the enhanced excitability
produced by PKG agonists might be explained by cross-
activation of PKA (just as effects of PKA agonists might
involve cross-activation of PKG). However, the reduction of
hyperexcitability by inhibitors that are highly selective for each
pathway strongly indicates that, in the compressed DRG, both
the cAMP-PKA and cGMP-PKG pathways help to maintain
hyperexcitability.

cGMP and PKG in the spinal cord are important for hyper-
algesia (e.g., Meller and Gebhart 1993; Niedbala et al. 1995;
Salter et al. 1996; Schmidtke et al. 2003; Tegeder et al. 2004).
However, there has been almost no evidence that this pathway
operates in the DRG to produce hyperalgesia. In fact, activation
of the cGMP-PKG pathway in somatic sensory neurons
has often been reported to have depressive rather than sensi-
tizing effects (e.g., Duarte et al. 1992; Kress et al. 1996; Liu et
al. 2004; Sachs et al. 2004). Different results might be ac-
counted for by different effects of the cGMP-PKG pathway in
different subsets of DRG neurons (Vivancos et al. 2003).
Evidence consistent with sensitizing effects of the cGMP-PKG
pathway are observations that cGMP can increase the excit-
ability of dissociated DRG neurons (Liu and Simon 2003;
Pollock et al. 2003) and that type 1 PKG is expressed in both
developing and mature DRG neurons (Qian et al. 1996).
In addition, cGMP-PKG signaling is important for guidance and
connectivity of sensory axons during development (e.g.,
Schmidt et al. 2002; Song et al. 1998). Peripheral processes of
adult DRG neurons are involved in some forms of hyperalgesia
that depend on peripheral NO release and local cGMP synthe-
sis (Aley et al. 1998). NO causes cGMP synthesis in cultured
DRG neurons, but in ganglia, NO-induced cGMP synthesis is
suggested to occur primarily in glial cells rather than DRG
neurons (e.g., Morris et al. 1992; Shi et al. 1998; Thippeswamy
and Morris 2001). However, significant levels of cGMP can be
difficult to detect because of high levels of phosphodiesterase
activity (e.g., Honda et al. 2001). An interesting possibility is
that compression leads to cGMP synthesis and activation of
PKG in stressed sensory axons and subsequent retrograde
transport of the active PKG to neuronal somata in the DRG.
This occurs in Aplysia sensory neurons after nerve injury and
results in hyperexcitability of the sensory neuron soma (Sung
et al. 2004). Potential sources of NO for stimulating cGMP
synthesis in the compressed DRG and adjacent nerves include
cytokines and inflammatory mediators such as bradykinin
(Bauer et al. 1995; Hess et al. 1993).

cAMP and cGMP pathways have little immediate influence
on DRG neuron excitability in the absence of prior injury

Agonists and antagonists of the cAMP-PKA and cGMP-
PKG pathways had little or no effect on the excitability of
neurons sampled in uncompressed ganglia from naïve animals.
This was true for all of the agonists and antagonists tested,
across a wide range of doses. The lack of a clear effect on
excitability was particularly interesting for the cAMP-PKA
pathway because it appears to conflict with earlier reports. This
discrepancy might reflect, in part, our relatively long delay
(3–4 h) between beginning the application of agonists or
agonists and delivery of tests to DRG neurons. Previous
studies used brief, acute applications of activators and inhibi-
tors, and compared responses in the presence of each drug to
the predrug baseline responses. However, the first cells we
tested (within 10–45 min) showed no more evidence of hy-
perexcitability than did cells tested later and, of course, these
agents had strong effects during the same prolonged treatment
periods in compressed ganglia. Another possibility is that
previous studies have examined the effects of perturbing
cAMP-PKA pathways in neurons that had previously been
injured by either dissociation or prior compression. The largest
numbers of such studies have been performed on isolated
sensory neurons in culture, which have shown that activation
of the cAMP-PKA pathway enhances TTX-R I_{Na} (Aley et al.
1998; Cardenas et al. 2001; England et al. 1996; Gold et al.
1996) and I_{k} (Ingram and Williams 1996), while depressing
I_{K} (Evans et al. 1999). In addition, in dissociated DRG neurons,
this pathway enhances discharge induced by bradykinin (Cui
and Nicol 1995; Smith et al. 2000) or capsaicin (Lopshire and
Nicol 1998) and enhances heat-induced responses by modula-
tion of the VR-1 receptor (Caterina et al. 1997; Rathee et al.
2002).

Interestingly, the only reports of cAMP-induced hyperexcit-
ability in DRG neurons recorded in the ganglion have been in
ganglia that had been chronically compressed (Hu et al. 2001).
This difference indicates that, in the absence of prior injury or
stress, activation of the cAMP-PKA or cGMP-PKG pathways
is not sufficient to induce hyperexcitability of the DRG neuron
soma, although the cAMP-PKA pathway is sufficient to induce
hyperexcitability of peripheral terminals of DRG neurons
(Aley and Levine 1999; Ferreira and Nakamura 1979; Taiwo et
al. 1989). It may be that these pathways are normally only
linked to excitability mechanisms in the periphery (near the
sensory terminals in the case of the cAMP-PKA pathway and
perhaps in peripheral axons in the case of the cGMP-PKG
pathway; see Sung et al. 2004), but nerve or ganglion injury
produced by compression, inflammation, axotomy, or disso-
ciation causes the pathways to become linked to excitability
mechanisms in the soma, where they can become persistently
activated to maintain soma hyperexcitability (see Aley and
Levine 1999, 2002; Bolyard et al. 2000; Liao et al. 1999;
Muller 2000). Interestingly, an “inflammatory soup” of several
mediators applied to a previously compressed DRG increases
the firing rate and depolarization of DRG neurons and elicits
ectopic discharge in some neurons, but these effects fail to
occur in uncompressed ganglia from naïve animals (Song et al.
2003c). Because several of these inflammatory mediators ac-
tivate the cAMP-PKA pathway to produce hyperexcitability in
dissociated neuron or peripheral preparations (Cui and Nicol

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Although they did not affect DRG neuron excitability in naïve animals, agonists of both the cAMP-PKA and cGMP-PKG pathways significantly depolarized the RMP of sensory neurons in uncompressed ganglia. CCD treatment also depolarized DRG neurons but, surprisingly, this depolarization was not altered by either agonists or antagonists of the cAMP-PKA and cGMP-PKG pathways. This indicates that both pathways can depolarize RMP under control conditions, but neither contributes significantly to RMP after CCD treatment. Furthermore, changes in RMP produced by these pathways and by CCD treatment are relatively independent of changes in neuronal excitability.

**cAMP and cGMP pathways contribute to hyperalgesia produced by ganglion compression**

Much evidence indicates that hyperexcitability of DRG neurons contributes to hyperalgesia in chronic pain states (Burchiel 1984; Hu and Xing 1998; Ji and Woolf 2001; Kajander and Bennett 1992; Song et al. 1999, 2003a,b; Wall and Devor 1983). We have shown that in vivo delivery of antagonists of PKA or PKG selectively to the DRG transiently and Devor 1983). We have shown that in vivo delivery of antagonists of PKA or PKG selectively to the DRG transiently produces significant hyperalgesia after in vivo delivery of PKA and PKG antagonists. This indicates that both pathways contribute significantly to RMP after CCD treatment. Furthermore, changes in RMP produced by these pathways and by CCD treatment are relatively independent of changes in neuronal excitability.

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