Capsaicin Infused Into the PAG Affects Rat Tail Flick Responses to Noxious Heat and Alters Neuronal Firing in the RVM

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Submitted 6 May 2003; accepted in final form 7 June 2003

McGaraughty, Steve, Katharine L. Chu, Robert S. Bitner, Brenda Martino, Rachid El Kouhen, Ping Han, Arthur L. Nikkel, Edward C. Burgard, Connie R. Faltynek, and Michael F. Jarvis. Capsaicin infused into the PAG affects rat tail flick responses to noxious heat and alters neuronal firing in the RVM. J Neurophysiol 90: 2702–2710, 2003. First published June 18, 2003; 10.1152/jn.00433.2003. It is well established that the vanilloid receptor, VR1, is an important peripheral mediator of nociception. VR1 receptors are also located in several brain regions, yet it is uncertain whether these supraspinal VR1 receptors have any influence on the nociceptive system. To investigate a possible nociceptive role for supraspinal VR1 receptors, capsaicin (10 nmol in 0.4 μl) was microinjected into either the dorsal (dPAG) or ventral (vPAG) regions of the periaqueductal gray. Capsaicin-related effects on tail flick latency (immersion in 52°C water) and on neuronal activity (ON-, OFF-, and neutral cells) in the rostral ventromedial medulla (RVM) were measured in lightly anesthetized rats. Administration of capsaicin into the dPAG but not the vPAG caused an initial hyperalgesic response followed later by analgesia (125 ± 20.96 min postinjection). The tail flick–related burst in ON-cell activity was triggered earlier in the hyperalgesic phase and was delayed or absent during the analgesic phase. Spontaneous activity of ON-cells increased at the onset of the hyperalgesic phase and decreased before and during the analgesic phase. The tail flick–related pause in OFF-cell activity as well as spontaneous firing for these cells was unchanged in the hyperalgesic phase. During the analgesic phase, OFF-cells no longer paused during noxious stimulation and had increased levels of spontaneous activity. Neutral cell firing was unaffected in either phase. Pretreatment with the VR1 receptor antagonist, capsazepine (10 nmol in 0.4 μl), into the dPAG blocked the capsaicin-induced hyperalgesia as well as the corresponding changes in ON- and OFF-cell activity. VR1 receptor immunostaining was observed in the dPAG of untreated rats. Microinjection of capsaicin likely sensitized and then desensitized dPAG neurons affecting nocifensive reflexes and RVM neuronal activity. These results suggest that supraspinal VR1 receptors in the dPAG contribute to descending modulation of nociception.

INTRODUCTION

Intradermal administration of capsaicin, a piquant component of red peppers, initially causes the sensation of burning pain and hyperalgesia followed by localized insensitivity to noxious stimuli (LaMotte et al. 1991, 1992; Simone et al. 1987). The distinct behavioral phases are correlated with activation and subsequent desensitization of subsets of sensory Aδ- and C-fibers (Baumann et al. 1991). The so-called capsaicin receptor, VR1, is a member of the TRP family of ion channels (TRPV1) and is widely found on small- and medium-sized primary afferent neurons (Caterina et al. 1997; Guo et al. 1999; Ma 2002; Tominaga et al. 1998). The primary afferent VR1 receptor has a significant role in the transmission of nociceptive signals and is postulated to be a molecular integrator because of its heterologous activation and/or modulation by heat, protons, and other endogenous matter released during tissue injury (Caterina et al. 1997; Chuang et al. 2001; Tominaga et al. 1998, 2001; Vellani et al. 2001; Vyklicky et al. 1998). Furthermore, VR1 receptors are upregulated in states of chronic inflammation (Carlton and Coggeshall 2001) and neuropathy (Fukuoka et al. 2002; Hudson et al. 2001) and thus may contribute to the neuropathology of these sensitized states. Endogenous ligands such as anandamide, N-arachidonoyl-dopamine (NADA), and lipoxigenase derivatives may activate VR1 receptors located both inside and outside of the CNS (Di Marzo et al. 2002; Huang et al. 2002; Hwang et al. 2000; Zygmunt et al. 1999).

In addition to a peripheral nociceptor site of action, VR1 receptors are also located in several regions of the CNS. In the spinal cord, VR1 receptors are found both presynaptically in lamina I and postsynaptically in lamina II (Gou et al. 1999; Valtzschanoff et al. 2001). Spinal administration of the VR1 receptor antagonist capsazepine inhibits evoked activity of wide dynamic range neurons in both noninflamed and carrageenan-inflamed rats (Kelly and Chapman 2002). In the brain, VR1 expression and radiolabeled vanilloid binding has been observed in several sites including the hypothalamus, hippocampus, and cortical regions (Acs et al. 1996; Mezey et al. 2000; Szabo et al. 2002). Administration of capsaicin into the hypothalamus and nucleus of the solitary tract modulates thermal regulation and respiratory frequency, respectively (Jancso-Gabor et al. 1970; Mazzone and Geraghty 1999). VR1-immunopositive cells have also been found in supraspinal regions, such as the locus coeruleus, central amygdala, and the habenula, that are implicated in nociceptive modulation (Mezey et al. 2000). However, the contribution of VR1 receptors to nociceptive transmission or modulation in these and other supraspinal regions is relatively unknown. Early work by Bodnar et al. (1982, 1983) demonstrated antianalgesic actions of intraventricularly administered capsaicin, and more recently, intraventricular injections of VR1 receptor antagonists were...
shown to attenuate spontaneous nocifensive behaviors evoked by the intradermal injection of capsaicin into a hindpaw of a mouse (Santos and Calixto 1997).

In another recent study, Palazzo et al. (2002) induced hypalgesia by microinjecting capsaicin into the dorsolateral periaqueductal gray (PAG). The observed effects were absent in capsazepine-pretreated animals, indicating that the VR1 receptor may contribute to this antinociception. In this report, intra-PAG administration of capsazepine was shown to evoke a localized glutamatergic response, an effect similar to that observed in vitro after capsazepine application to hypothalamic, substantia nigra, and locus coeruleus tissue (Marinelli et al. 2002, 2003; Sasaki et al. 1998).

The PAG has a key role in descending modulation of nociception (Behbehani 1995; Fields 2000; Yakh 1997). The PAG is thought to mediate nonopiod and opioid antinociception through its dorsal and ventral regions, respectively (Belloewan and Helsemstetter 1998; Cannon et al. 1982; Lovick 1991). However, the PAG has limited direct projections to the spinal cord (Sandkuhl et al. and Gebhart 1984). The rostral ventromedial medulla (RVM), a site that projects directly to the spinal cord dorsal horn, is an important intermediate site in PAG-related modulation (Basbaum and Fields 1984; Fields et al. 1995). Three populations of RVM neurons with distinct roles in nociceptive modulation have been identified (Fields et al. 1983; Heinricher and McCarlhugothy 1999). Cells of one class, OFF-cells, are defined by an abrupt pause in firing that begins just before the occurrence of nociceptive reflexes, and they become continuously active after systemic or local morphine administration into the RVM, PAG, or amygdala (Cheng et al. 1986; Fields et al. 1983; Heinricher et al. 1994; McCarlhugothy and Heinricher 2002; McCarlhugothy et al. 1993). OFF-cells are likely to exert a net inhibitory influence on spinal nociceptive processing. Cells of a second class, ON-cells, are identified by a burst of activity that occurs just before a nociceptive reflex. The activity of ON-cells is depressed after systemic, iontophoretic, or site-specific (RVM, PAG, and amygdala) application of opioids (Barbaro et al. 1986; Cheng et al. 1986; Heinricher et al. 1992, 1994; McCarlhugothy and Heinricher 2002; McCarlhugothy et al. 1993). ON-cells are likely to have a facilitating effect on nociception. Cells of a third class, neutral cells, are unaffected by morphine administration (Barbaro et al. 1986), and whether this cell class contributes to nociceptive modulation is unknown. However, phenotypic changes in this cell class have been recently observed in chronically inflamed animals (Miki et al. 2002).

The goal of the present study was to further characterize the modulatory effects of capsazepine-sensitive neurons in the PAG. To this end, we tested the effects of microinjections of capsazepine into either the dorsal-dorsolateral (dPAG) or ventrolateral (vPAG) regions of the PAG on the activity of identified ON-, OFF-, and neutral cells in the RVM. Additionally, we determined whether changes in cell activity were associated with changes in nociceptive responsiveness.

METHODS

Surgical preparation

All animal handling and experimental protocols were approved by Abbott’s Institutional Animal Care and Use Committee (IACUC), and were conducted in accordance with the ethical principles for pain-related animal research of the American Pain Society. Male Sprague–Dawley rats (300–350 g) were initially anesthetized with pentobarbital (60 mg/kg, ip), and a catheter was placed in the left external jugular vein. The animals were then secured in a stereotaxic apparatus. Two small craniectomies were made to allow stereotaxic placement of a recording electrode in the RVM and an infusion cannula (28 gauge) into either the dPAG or vPAG. Body temperature was maintained at about 37°C by placing the animals on a circulating water blanket. After surgery, animals were allowed to recover from the initial anesthesia to the point at which a tail flick reflex could be elicited. The rats were then maintained in a lightly anesthetized state by a continuous infusion of propofol at a rate of 6–12 mg/kg/h (iv). The anesthetic state was considered stable if the animals maintained a control tail flick latency during infusing period before recording. Nociceptive testing and single-unit recording were begun immediately after anesthetic stability was determined.

Neuronal recording and nociceptive testing

Platinum-plated stainless steel microelectrodes (Frederick Haer, Brunswick, ME) were used to record neuronal activity in the RVM. Spike activity was sampled at 20 kHz. Spike waveforms were monitored on an oscilloscope throughout the experiment, digitized (32 points), and then stored for off-line analysis (Datawave Technologies, Longmont, CO) to ensure that the unit under study was unambiguously discriminated throughout the experiment. Units were characterized according to the classification systems of Fields et al. (1983, 2000). OFF-cells displayed a sudden pause and ON-cells showed an abrupt burst of firing just before the tail flick reflex. Neutral cells showed no reliable tail flick–related change in activity. Neurons were considered neutral cells and included in the analysis only if identified in a penetration in which an ON- or OFF-cell was also encountered.

Tail flick latencies were measured after immersion of the animal’s tail (4 cm from the tip) into a glass reservoir filled with noncirculating water. The reservoir was the inner section of a double-walled glass–tempering beaker. The temperature of the stimulus (52.0 ± 0.2°C) was maintained by water circulating through an enclosed external chamber. The temperature of the circulating water was feedback regulated by a flow-through heater (Polyscience, Niles, IL). If the animal failed to flick away from the noxious stimulus within 15 s, the tail was removed from the water. Between tail flick trials, the animal’s tail was dried and warmed to approximately 35°C while placed within a thermal blanket.

Testing procedures

After an ON-, OFF-, or neutral cell was isolated within the RVM, spontaneous and tail flick–related firing were monitored during baseline (15 min). Tail flick trials (3) were separated by 5 min in baseline. Capsaicin (10 nmol) or vehicle (20% EtOH, 10% polyoxyethylene sorbitan monoooleate, and saline) was then microinjected into either the dPAG or vPAG in a volume of 0.4 μl over a period of 1 min. Each injection cannula was attached to a 1-μl Hamilton syringe by a length of PE 50 tubing. After capsaicin infusion, tail flick trials were conducted 1 min, 5 min, and every 15 min thereafter for the duration of the experiment (65 min postcapsaicin). In a separate group of animals, the VR1 receptor antagonist capsazepine (10 nmol) was administered into the dPAG 10 min before capsaicin (10 nmol). To achieve this sequence of drug administration, the drugs were separated within the same PE 50 tubing by 1 μl of air volume and injected into the dPAG at the different times through the same cannula. A 5-μl Hamilton syringe was used for these injections. In a final group of animals, the effects of capsaicin were investigated over a longer period of time. To this end, the effects of capsaicin (10 nmol) were measured for 215 min after microinjection into either the dPAG or vPAG. In these experiments, capsaicin was infused into the PAG with two different vol-
umes, 0.4 and 4.0 μl. The larger volume was administered to increase the population of PAG neurons affected by capsaicin.

Except for 6 experiments in which 2 easily distinguished neurons were simultaneously recorded on one electrode, only one cell was studied in each experiment. In experiments in which cell isolation was not maintained, only tail flick data were kept for subsequent analysis.

At the end of the each experiment, the RVM recording sites were marked with an electrolytic lesion and Chicago Sky Blue dye was injected through the cannulae. Rats were then killed with an overdose of propofol and perfused intracardially with physiological saline followed by 10% formalin. All RVM recording sites were verified histologically. Infusion sites are shown in Fig. 1.

Data analysis

Baseline spontaneous activity for all cell classes was measured for 5 min before the first tail flick trial. After drug injection, spontaneous cell activity was represented by cell firing in the 60 s before each tail flick trial and was expressed as a percentage of baseline spontaneous activity. Onset to the tail flick–related on-cell burst and the off-cell pause was also measured. The onset to on-cell burst was defined as the time elapsed between initiating tail immersion and the first 1-s bin during which on-cell activity was at least double that of baseline spontaneous activity. Time from tail immersion to the first 1-s bin without cell activity represented the onset to the off-cell pause. Pause data were obtained only for those trials on which the neuron was spontaneously active before heat onset. Data are presented as mean ± SE; statistical significance for cell activity was established by using either a Friedman’s rank test for correlated samples followed by a Wilcoxon’s matched-pairs test for comparisons with individual baseline scores or a Kruskal–Wallis ANOVA followed by a Mann–Whitney U test for comparison across groups (P < 0.05). An ANOVA (repeated-measures or independent groups) followed by a Fisher’s PLSD was used to evaluate differences in tail flick latencies (P < 0.05).

Immunohistochemical detection of VR1

Untreated rats were deeply anesthetized with pentobarbital and perfused through the aorta with buffered saline (3 min, 25 ml/min) followed by 10% formalin (12 min, 30 ml/min). The brains were removed and postfixed in 10% formalin for 12–24 h before immunohistochemical analysis. After postfixation, brains were processed and embedded in paraffin, sectioned (6 μm), and mounted on aminosilane-coated glass slides (Newcomer Supply). Specifically, coronal sections from the brain stem that contained the PAG were mounted.

The immunohistochemical procedure used consisted of a 3-step ABC (avidin–biotin complex)–peroxidase technique. Sections were first deparaffinized through a xylen and graded alcohol series, followed by a 20-min incubation in blocking serum. The sections were then incubated with ABC reagent (Vectastain Elite, Vector). The sections were visualized by incubation for 2–8 min in a peroxidase substrate solution (diaminobenzidine) and counterstained with hematoxylin. Sections were examined and photographed with a light microscope (Leica, DMRB).

RESULTS

Hyperalgesic phase

Microinjection of 10 nmol of capsaicin into the dPAG but not vPAG produced a significant decrease in tail flick latencies (Fig. 2). This hyperalgesia occurred between 5 and 20 min after the injection of capsaicin and lasted, in most cases, for at least another 45 min. The tail flick latency was decreased by about 1 s from baseline, and was not observed in animals pretreated with 10 nmol of capsaicpine.

The tail flick–related on-cell burst in neuronal activity was triggered significantly earlier (0.9–1.6 s), compared with vehicle-treated rats, after injection of capsain into the dPAG but not vPAG (Fig. 3A). The timing of this effect corresponded with the occurrence of hyperalgesia and was absent in animals pretreated with 10 nmol of capsaicpine. The onset to the tail flick–related pause in off-cell activity was unaffected by capsaicin administration into either region of the PAG (Fig. 3B).

Both on- (Fig. 4) and off-cells (Fig. 5) exhibited significant changes in spontaneous activity within the first minute after the injection of capsain into the dPAG but not vPAG. off-cell activity decreased, whereas on-cell activity increased during this time. These neuronal effects were evident despite no...
observable changes in tail flick latencies during the first minute after capsaicin infusion. After this first minute, off-cell spontaneous activity returned to baseline levels for the remainder of the 65-min recording period. On-cells exhibited another significant peak in spontaneous activity 20 min after capsaicin injection into the dPAG, which corresponded with the onset of hyperalgesia. However, during the remainder of the hyperalgesic phase, on-cell activity decreased to levels that were not significantly different from baseline or vehicle-treated rats. Animals pretreated with capsazepine into the dPAG did not exhibit capsaicin-related changes in the spontaneous activity of on- and off-cells.

The spontaneous firing of neutral cells (n = 4) was unaffected by infusion of capsaicin into the dPAG. The firing rate of neutral cells was 11.4 ± 1.8 spikes/s in baseline and ranged from 11.7 ± 1.9 to 12.9 ± 2.7 spikes/s during the 65-min recording period after the microinjection of capsaicin.

Analgesic phase

In a separate group of animals, capsaicin was administered into the dPAG to examine its effects over an extended period of time. Administration of capsaicin (10 nmol in 0.4 μl) into the dPAG initially caused the expected hyperalgesic response that lasted 35 to 155 min postinjection. In the majority of animals tested (11 out of 19), the hyperalgesic phase was then followed by an analgesic phase (Fig. 6). In the analgesic phase, animals failed to exhibit a tail flick response by the 15-s cutoff time. The mean time to the first analgesic response was 125.0 ± 20.96 min postinjection. In every analgesic animal, the transition to the analgesic phase was abrupt, occurring between 2 consecutive trials (Fig. 6). Of the 11 animals that were analgesic, tail flick latencies of 6 animals had returned to baseline levels before the induction of analgesia. The remaining 5 animals were hyperalgesic up to the first analgesic response. Similar biphasic behavioral effects were observed after the infusion of 4.0 μl of capsaicin (10 nmol) into the dPAG. However, at this larger volume, the first analgesic response occurred earlier (93.5 ± 24.9 min postinjection) and in a proportionately higher percentage of animals (10 out of 11).

Administration of capsaicin into the dPAG did not trigger analgesia in every animal. In these nonanalgesic animals (n =
9, both volumes), tail flick latencies returned to baseline levels by the end of the experiment (4.5 ± 0.39 s in baseline to 4.3 ± 0.89 s at 215 min postinjection) despite having significantly lower latencies (3.49 ± 0.39, P < 0.05) 20 min after the microinjection of capsaicin into the dPAG.

Tail flick latencies were not significantly affected during the extended testing period by administration of vehicle into the dPAG (5.46 ± 0.67 s in baseline to 5.36 ± 1.15 s at 215 min postinjection, n = 3) or capsaicin, at either volume, into the vPAG (4.1 ± 0.68 s to 4.88 ± 0.62 s, n = 8).

In analgesic animals, the effects of the 0.4 and 4.0 μl capsaicin infusions on RVM neuronal activity were the same; therefore data from the two groups were combined. Microinjection of capsaicin into the dPAG affected tail flick–related neuronal activity of both ON- and OFF-cells. Both the ON-cell burst (8 of 9) and the OFF-cell pause (7 of 8) in activity were absent in most animals 215 min after the infusion of capsaicin (Fig. 7). Spontaneous activity of ON- and OFF-cells was significantly decreased or increased, respectively, by the microinjection of capsaicin into the dPAG (Fig. 7).

In nonanalgesic animals (dPAG or vPAG microinjections), all measured parameters of ON- and OFF-cell activity, 215 min after the injection of capsaicin, were not significantly different from baseline levels. Neutral cell activity (n = 6) remained unchanged over the entire recording period after infusion of capsaicin into the dPAG (18.4 ± 6.7 spikes/s in baseline to 18.2 ± 6.0 spikes/s 215 min postinjection).

VR1 expression in the dPAG

To examine the expression of the VR1 receptor in the rat dPAG, immunohistochemical staining was conducted in brain stem sections using a polyclonal Ab against the amino terminus of the VR1 receptor. Positive staining throughout the cytosol was seen in several cells of the dPAG (Fig. 8A), as well as in other PAG subregions including the vPAG. In addition to the PAG, VR1 cellular immunostaining was observed in other brain stem nuclei that included the locus coeruleus and dorsal raphe nucleus (data not shown). Immunostaining was completely eliminated by preincubating the VR1 Ab with the peptide immunogen (50 μg/ml; Fig. 8B), as well as by omitting the use of the VR1 primary Ab (data not shown).

FIG. 6. Example, from one animal, of transition between hyperalgesic and analgesic periods. Hyperalgesic (H) and analgesic (A) phases are highlighted by shaded areas. Tail flick latencies of this animal decreased after infusion of capsaicin into dPAG. For this rat, hyperalgesia lasted about 65 min before latencies returned to baseline levels. Abrupt analgesic period then followed (170 min postinjection) in which the animal failed to respond to noxious stimulation by cutoff time (15 s). In this example, spontaneous activity of the ON-cell increased at onset of the hyperalgesic phase then decreased to baseline levels. Just before the analgesic phase, ON-cell activity began to decrease, and then was not spontaneously active during analgesic phase.

FIG. 5. A: infusion of 10 nmol of capsaicin into dPAG (n = 13) initially caused decrease in spontaneous OFF-cell activity. This effect was not observed after vPAG (n = 13) injection or after capsazepine (10 nmol, n = 8) pretreatment. Capsaicin effect was significant but short-lived as cell activity quickly returned to baseline levels. **P < 0.01 compared with baseline (Friedman’s followed by Wilcoxon’s test) as well as vehicle- and capsazepine-treated animals (Kruskal–Wallis followed by a Mann–Whitney U test). Baseline spontaneous firing for OFF-cells was 5.9 ± 1.2 spikes/s; vehicle-treated rats, n = 9. B: ratemeters show individual examples of capsaicin-induced, short-lasting decrease in OFF-cell activity (top) and capsazepine block of this effect (bottom). △ indicates tail flick trials in B.
DISCUSSION

Discrete microinjections of the vanilloid receptor agonist capsaicin into the cerebral ventricles or specific brain regions can result in a multitude of effects. Some of these include increased respiratory frequency, either hyper- or hypothermia, enhanced gastric acid secretion, and abolished fictive retching (Hajos et al. 1983; Mazzone et al. 1999; Minowa et al. 2001; Osaka et al. 2000; Shiroshita et al. 1997). Nociceptive responsiveness is also affected. Bodnar et al. (1982, 1983) reported that intraventricular capsaicin decreased the analgesic effectiveness of both morphine and a cold-water swim. Additionally, capsaicin injected into the dPAG increased paw withdrawal latencies after noxious thermal stimulation (Palazzo et al. 2002). In the present study, microinfusion of capsaicin into the dPAG caused a bidirectional response to noxious thermal stimulation of the tail. Initially, tail flick latencies decreased by about 1 s from baseline levels and remained at those lower latencies for at least 1 h postinjection. Some animals then returned to baseline tail flick latencies, whereas others remained hyperalgesic before an abrupt transition to an analgesic phase that occurred about 2 h after the injection of capsaicin. In the analgesic phase, animals no longer responded to the noxious stimulation.

These bidirectional effects of dPAG-capsaicin are similar to those observed after peripheral administration of capsaicin in that initial hyperalgesia can be followed by subsequent hypoalgesia (LaMotte et al. 1991, 1992; Simone et al. 1987). Indeed, biphasic capsaicin-induced actions are not limited to peripheral tissue. Selective injections of capsaicin into other brain sites have similar, time-dependent, bidirectional actions on nonnociceptive endpoints (Jancso-Gabor et al. 1970; Shiroshita et al. 1997). These “polar” behavioral outcomes have been attributed to sensitization and desensitization, respectively, of relevant neurons (Baumann et al. 1991; Shiroshita et al. 1997). Thus the hyperalgesic and subsequent analgesic phases observed after microinjection of capsaicin into the dPAG might represent respective sensitization and desensitization of affected capsaicin-sensitive neurons. Both of these behavioral effects appear to be the result of selective manipulations to the dorsal regions of the PAG, given that injections of capsaicin into the vPAG did not alter tail flick latencies.

These results are in agreement with the data from Palazzo et al. (2002) that show that capsaicin-sensitive mechanisms in the dPAG modulate nociceptive activity. However, our observation of an initial hyperalgesic phase contrasts with the early hypoalgesia reported by this group. Technical differences may

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FIG. 7. Firing of on- \((n = 9)\) and off-cells \((n = 8)\) was altered during analgesic phase. At last time point tested (215 min postinjection), in analgesic animals, spontaneous activity of on- and off-cells was significantly decreased and increased, respectively, from baseline levels after infusion of capsaicin into dPAG. Baseline spontaneous firing for these on- and off-cells was 5.8 ± 1.2 and 4.7 ± 1.2 spikes/s, respectively. Tail flick–related activity of both cell classes was affected in most animals; off-cell pause and on-cell burst were each observed from only one rat. *P < 0.05, **P < 0.01 compared with baseline (Friedman’s followed by Wilcoxon’s test).

FIG. 8. Expression of VR1 in dPAG as determined by immunohistochemical detection. Immunostaining of VR1 was observed in dPAG (A) using rabbit polyclonal Ab against amino terminus. Immunostaining was completely eliminated (B) by preincubating VR1 Ab with peptide immunogen (50 μg/ml). Scale bar = 25 μm.
contribute to these discrepant outcomes. Palazzo et al. administered 0.2 μl of capsaicin into the dPAG at a rate 6 times greater than our rate of infusion (0.1 μl/2.5 s compared with 0.1 μl/15 s). Because the desensitization rate of capsaicin-sensitive neurons is concentration-dependent (Liu and Simon 1996; Liu et al. 1996), this rapid delivery of a smaller volume of a highly concentrated capsaicin solution may be sufficient to quickly desensitize a small population of PAG neurons causing the relatively small increase in withdrawal latencies. Alternatively, it must also be considered whether the hyperalgesia induced by infusion of a larger volume of capsaicin into the dPAG was due to compound diffusion to other sites given the proximity of the cerebral aqueduct. This is unlikely because capsaicin was without effect after injection into the vPAG, which is a region equidistant to the aqueduct.

Neurons in the RVM likely have a substantial role in the behavioral outcome after administration of capsaicin into the dPAG. The PAG has limited direct projections to the spinal cord (Sandkuhler and Gebhart 1984); however, neurons in the dPAG project, both directly and indirectly, to the RVM (Fardin et al. 1984; Keay and Bandler 2001; Van Bockstaele et al. 1991). In the present study, the occurrence of hyperalgesia after infusion of capsaicin into the dPAG was correlated with an earlier activation of the tail flick–related on-cell burst. Thus capsaicin may have sensitized dPAG neurons and reduced the input needed to trigger the on-cell burst. It has been proposed that on-cells facilitate nociception (Fields 1992; Heinricher and Roychowdhury 1997; Heinricher et al. 1992), and thus, the earlier onset of the on-cell burst may have been critical to the occurrence of hyperalgesia. Shifts in RVM cell activity were likely important to the analgesic phase as well. Changes to RVM cell activity during this phase resembled “typical” firing patterns observed in RVM-related analgesia (Fields et al. 1983; Heinricher and McGaraughty 1999). Spontaneous activity of on- and off-cells decreased and increased, respectively, whereas the reflex-related on-cell burst and off-cell pause were absent in most animals. It has been suggested that the off-cell pause, or the elimination thereof, is a critical element of RVM-related analgesia (Heinricher et al. 1994; McGaraughty and Heinricher 2002). The present data are consistent with this proposal. Furthermore, the current data demonstrate that hyperalgesia, albeit a small 1-s decrease in response latency, may be induced without altering the onset of the off-cell pause. Therefore alterations of on- and off-cell activity just before a nociceptive response, the burst or pause, are likely key physiological parameters that specifically contribute to shifting nociceptive state toward hyperalgesia or hypoalgesia, respectively.

Changes in spontaneous on- and off-cell firing did not correlate with nociceptive responsiveness during capsaicin-induced hyperalgesia. After an initial decline, off-cell firing returned to baseline levels during animal hyperalgesia. On-cell activity had similar peaks in activity during periods with and without hyperalgesia and did not sustain a high level of activity during the hyperalgesic phase. Thus the degree of spontaneous activity for these cell classes was not predictive of behavioral responsiveness. It has been previously reported that drug-induced shifts in the spontaneous firing of RVM neurons may not always predict the drug-related changes to behavior (McGaraughty and Heinricher 2002). Cell activity in the hyperalgesic phase also demonstrated that pharmacological manipulation of the dPAG can affect the activity of one class of RVM neurons without affecting the other. Spontaneous on-cell activity increased at the onset of hyperalgesia, whereas off-cell activity was unaffected, and dPAG-capsaicin triggered an earlier on-cell burst but was without effect on the onset of the off-cell pause. Barbaro et al. (1989) reported that the firing patterns of RVM on- and off-cells are inversely correlated and it was hypothesized that this may be the result of local inhibitory intraneuronal synapses between the two cell classes. The lack of coordinated activity between on- and off-cells during the hyperalgesic phase suggests that these RVM neurons may be affected independently by projections from the dPAG and that there are circumstances when on- and off-cell activity is not inversely correlated.

Like the RVM, the dPAG is “wired” for bidirectional nociceptive modulation. Classes of cells with similar nociceptive characteristics to RVM on- and off-cells have been identified throughout the PAG and may have similar respective modulatory actions (Heinricher et al. 1987). Infusion of capsaicin into the dPAG may have influenced both of these classes of dPAG neurons either directly or indirectly through release of local neurotransmitters like glutamate. Microinjection of selective glutamatergic receptor agonists and antagonists into the dPAG can modulate nociceptive responsiveness (Berrino et al. 2001; Maione et al. 1998, 2000). Although this research is still in its early stages, the direction of the modulation (pronociceptive or antinociceptive) may be related to the activation of specific subtypes of glutamatergic receptors within the dPAG (Berrino et al. 2001; Maione et al. 1998, 2000). A capsaicin–glutamate interaction has been demonstrated in several supraspinal sites (Marinelli et al. 2002, 2003; Sasamura et al. 1998). The hyperalgesia observed by Palazzo et al. (2002) after infusion of capsaicin into the dPAG was blocked by selective NMDA and mGlu (mGlu1 and mGlu5) receptor antagonists. However, given that specific glutamatergic receptors in the dPAG may contribute to pronociceptive activity (Maione et al. 1998, 2000), a capsaicin–glutamate interaction in this region may also factor into the hyperalgesia observed in the present study.

Capsaicin is a well-documented agonist of the VR1 receptor (Caterina et al. 1997; for a review see Szallasi and Blumberg 1999). In the present study, both behavioral and neuronal actions induced by local administration of capsaicin were absent in animals pretreated with capsazepine, a competitive VR1 receptor antagonist. These findings suggest that capsaicin may modulate nociception by VR1 receptors located in the dPAG. VR1 receptors have been localized to several brain regions (Acosta et al. 1996; Mezey et al. 2000; Szabo et al. 2002); however, VR1 receptor localization to the PAG has not been previously reported. Our immunohistological data demonstrate that the VR1 receptor can be expressed in the PAG, although the staining was less intense than that observed in the locus coeruleus. Notably, the expression of the VR1 receptor was found in both the dPAG and vPAG. Because administration of capsaicin into the vPAG was without effect on RVM neurons or on the tail flick reflex, it may be reasoned that VR1 receptors in this region contribute to nonnociceptive endpoints or are not functionally active within the current experimental parameters.

Thus these data and those presented by Palazzo et al. (2002) suggest that VR1 receptors located in the dPAG contribute to descending modulation of nociception. Existence of the VR1 receptor in the dPAG should necessitate a ligand native to this
region. One endovanilloid candidate is anandamide (Di Marzo et al. 2002), which is a weak but full agonist of the VR1 receptor (Smart et al. 2000; Zygmunt et al. 1999). Indeed, anandamide is released into the dPAG after both electrical stimulation of this region and also after a peripheral chemical insult (Walker et al. 1999). Furthermore, these effects may be specific to the dorsolateral and not the ventrolateral region of the PAG (Martin et al. 1995). Both capsaicin and anandamide have been shown to evoke glutamate release in substantia nigra tissue, and both of these actions were selectively attenuated by a VR1 receptor antagonist (Marinelli et al. 2003). This study by Marinelli et al. also revealed that, in the presence of selective cannabinoid antagonists, membranes from substantia nigra neurons were able to bind \[^3H\]anandamide, suggesting a VR1 receptor site of action. Nonetheless, anandamide is a potent agonist of cannabinoid receptors (Devane et al. 1992), which are also located in the dPAG (Herkenham et al. 1991). Therefore, anandamide actions in the dPAG are most likely not limited to an interaction with VR1 receptors.

In summary, administration of the VR1 receptor agonist, capsaicin, into the dPAG, but not vPAG, caused initial hyperalgesia, followed by analgesia. During the hyperalgesic phase, the tail flick–related on-cell burst occurred earlier. The earlier burst was likely a consequence of capsaicin-induced sensitization of dPAG neurons. During the analgesic phase, off-cell spontaneous firing increased whereas activity of on-cells decreased. Both the off-cell pause and the on-cell burst were virtually eliminated during this phase, possibly due to capsaicin-induced desensitization of dPAG neurons. All actions of capsaicin were antagonized by the injection of capsazepine into the dPAG, suggesting a role for the VR1 receptor in this region. The dPAG could be an important supraspinal site involved in VR1-related descending modulation of spinal nociceptive signals.

We thank S. Stec for help on the figures.

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REFERENCES


Bellgowan PS and Helmstetter FJ. The role of mu and kappa opioid receptors within the periaqueductal gray of the rat: re- \(J\) Neurophysiol, 2709PAG CAPSAICIN


