PROSTAGLANDIN E$_2$ IN THE MEDIAL PREOPTIC AREA PRODUCES HYPERALGESIA AND ACTIVATES PAIN-MODULATING CIRCUITRY IN THE ROSTRAL VENTROMEDIAL MEDULLA

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Abstract—Prostaglandin E$_2$ (PGE$_2$) produced in the medial preoptic region (MPO) in response to immune signals is generally accepted to play a major role in triggering the illness response, a complex of physiological and behavioral changes induced by infection or injury. Hyperalgesia is now thought to be an important component of the illness response, yet the specific mechanisms through which the MPO acts to facilitate nociception have not been established. However, the MPO does project to the rostral ventromedial medulla (RVM), a region with a well-documented role in pain modulation, both directly and indirectly via the periaqueductal gray. To test whether PGE$_2$ in the MPO produces thermal hyperalgesia by recruiting nociceptive modulating neurons in the RVM, we recorded the effects of focal application of PGE$_2$ in the MPO on paw withdrawal latency and activity of identified nociceptive modulating neurons in the RVM of lightly anesthetized rats. Microinjection of a sub-pyrogenic dose of PGE$_2$ (50 fg in 200 nl) into the MPO produced thermal hyperalgesia, as measured by a significant decrease in paw withdrawal latency. In animals displaying behavioral hyperalgesia, the PGE$_2$ microinjection activated on-cells, RVM neurons thought to facilitate nociception, and suppressed the firing of off-cells, RVM neurons believed to have an inhibitory effect on nociception. A large body of evidence has implicated prostaglandins in the MPO in generation of the illness response, especially fever. The present study indicates that the MPO also contributes to the hyperalgesic component of the illness response, most likely by recruiting the nociceptive modulating circuitry of the RVM. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

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The illness response is a complex of physiological, neuroendocrine and behavioral changes including fever, anorexia, increased sleep, and activation of the hypothalamic–pituitary–adrenal axis. The different components of the illness response are thought to be adaptive, enhancing survival and recovery in the face of infection and immune challenge (Hart, 1988; Kent et al., 1992b; Dantzer, 2001;

Kelley et al., 2003). It has recently been suggested that hyperalgesia, broadly referring to increased responding to otherwise non-noxious stimuli and potentiated responding to normally noxious stimuli, is also an important element of the illness response (Watkins and Maier, 1999b; Maier and Watkins, 2003). This proposal is based on the observation that systemic administration of lipopolysaccharide or interleukin-1β, common experimental models of infection and immune activation, produces hyperalgesia as well as the other well-accepted components of the illness response (Maier et al., 1993; Watkins et al., 1994b; Yirmiya et al., 1994; Romanovsky et al., 1996; Watkins and Maier, 1999a).

Although the neural basis for the illness response is only partially understood, a substantial body of evidence points to the medial preoptic area (MPO) as a primary site at which the various elements of the response are organized (Elmquist et al., 1997; Konsman et al., 1999). A likely trigger is prostaglandin E$_2$ (PGE$_2$), produced in this region in response to immune signals (Kluger, 1991; Hopkins and Rothwell, 1995). A role for PGE$_2$ is based on several lines of evidence, although the primary focus has been fever. Prostaglandin E-type receptors are found within the MPO (Matsumura et al., 1992; Ek et al., 2000; Nakamura et al., 2000; Oka et al., 2000) and MPO neurons recorded *in vitro* respond to PGE$_2$ (Matsuda et al., 1992; Ranels and Griffin, 2003). Systemic administration of lipopolysaccharide or interleukin-1β results in an increase in PGE$_2$ levels (Sirko et al., 1989; Komaki et al., 1992; Cao et al., 1995; Sehic et al., 1996) and upregulation of cyclooxygenases (COX; Ivanov et al., 2002) in the MPO region. In addition, PGE$_2$ microinjected directly into the MPO produces fever (Amir and Schiavetto, 1990; Scammell et al., 1996; Oka et al., 1997, 2003b; Morrison, 2003), while direct application of COX inhibitors or EP receptor antagonists in the MPO blocks fever induced by lipopolysaccharide or interleukin-1β (Vaughn et al., 1979; Oka et al., 1997; Scammell et al., 1998). Direct application of PGE$_2$ or COX inhibitor in the MPO also implicates PGE$_2$ within this region in illness-induced hyperalgesia (Hosoi et al., 1997; Abe et al., 2001; Choi et al., 2003).

The pathways by which the MPO facilitates nociception are unknown. One plausible candidate is the rostral ventromedial medulla, which has been implicated in pain facilitation in inflammatory and neuropathic pain models (Urban and Gebhart, 1999; Porreca et al., 2002; Heinricher et al., 2003), and is required for hyperalgesia following systemic administration of lipopolysaccharide (Watkins et al., 1994a; Wiertelak et al., 1997). The rostral ventromedial

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medulla (RVM) also plays an important role in pain suppression, including opioid and stress-induced analgesia. The RVM receives input from the MPO, both directly, and indirectly via the midbrain periaqueductal gray (PAG; Chiba and Murata, 1985; Rizvi et al., 1996; Hermann et al., 1997; Murphy et al., 1999; Semenenko and Lumb, 1999). At least a subset of MPO neurons projecting to the RVM expresses the EP3 subtype of the prostaglandin receptor (Nakamura et al., 2002).

We recently provided direct evidence that "on-cells," a population of RVM neurons characterized by a burst of activity associated with nociceptive reflexes, exert a net facilitating effect on nociception (Neubert et al., 2004). This raises the possibility that activation of on-cells mediates illness-induced thermal hyperalgesia. If so, direct microinjection of PGE_2 into the MPO should activate on-cells in the RVM. The aim of the present experiments was to test whether focal application of PGE_2 in the MPO produces thermal hyperalgesia in lightly anesthetized rats, and to determine whether that hyperalgesia could be explained, at least in part, by activation of on-cells.

EXPERIMENTAL PROCEDURES

Animals and surgical preparation

All experimental procedures followed the guidelines of the Committee for Research and Ethical Issues of the IASP, and were approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University. All steps were taken to minimize the number of animals used and their suffering. Male Sprague–Dawley rats (Sasco, Portage, MI, USA; 250–300 g) were anesthetized with pentobarbital (60 mg/kg, i.p.), and a catheter inserted into an external jugular vein for administration of anesthetic. The rat was placed in a stereotaxic apparatus, a hole drilled in the skull over the cerebellum, and the dura removed to allow placement of an electrode in the RVM. A second small craniotomy was made to allow placement of a microinjection pipette into the MPO. Body temperature was maintained at approximately 37 °C by a circulating water pad.

Following surgery, the anesthetic level was allowed to lighten until a paw withdrawal reflex could be elicited by application of noxious heat using a feedback-controlled projector lamp focused on the blackened plantar surface of the paw. Following surgical preparation, the animals were then maintained in a lightly anesthetized state using a continuous infusion of methohexital at a rate (15–30 mg/kg per h, i.v.) that allowed a stable paw withdrawal (PW) latency and that prevented any signs of discomfort. The animals did not move spontaneously, nor did they vocalize or produce vigorous or prolonged withdrawal reflexes following noxious pinch. The rate was adjusted for each animal to allow a baseline PW of approximately 3 s. The protocol was begun after a stabilization period of at least 30 min, and infusion rate was not altered during the protocol.

Nociceptive testing and rectal temperature

Latency to paw withdrawal to heat was used as a measure of nociceptive responsiveness. Each trial consisted of a linear increase in temperature at approximately 1.8 °C/s from a holding temperature of 34 °C until the paw withdrawal occurred or to a maximum of 52 °C at 10.6 s. Trials were carried out at 5 min intervals throughout the experiment. The holding temperature obviates any concern that apparent effects on paw withdrawal latency were due to changes in plantar skin temperature potentially produced by PGE_2 in MPO. Rectal temperature was measured in

a small number of animals (TH5 thermometer Physitemp, Princeton, NJ, USA) in a separate set of experiments without RVM recording.

Recording and drug administration

A gold- and platinum-plated stainless steel recording microelectrode (Frederick Haer Co., Brunswick, ME, USA) was inserted into the RVM for extracellular single unit recording. A fresh glass infusion micropipette (75–100 μm, OD) was attached to a 1 μl Hamilton syringe with a length of PE-50 tubing for drug infusion and lowered into the MPO.

RVM neurons were classified as previously described (Fields et al., 1983). Spike waveforms were monitored and stored for off-line analysis (Datawave Systems, Thornton, CO, USA) to ensure that the unit under study was unambiguously discriminated throughout the experiment. Spike times were stored with a temporal resolution of 0.1 ms. Off-cells were characterized by an abrupt pause in ongoing activity beginning just prior to the occurrence of the PW. On-cells were identified by a sudden burst of activity beginning just prior to the occurrence of the PW. Cells of a third class, "neutral cells," were identified by no change in activity associated with paw withdrawal, and they did not respond to noxious or innocuous cutaneous stimulation.

Protocol and data analysis

PGE_2 was dissolved in DMSO at a concentration of 0.01 mg/ml and stocks were kept at -20 °C. An aliquot was thawed on the day of the experiment, and serially diluted in phosphate-buffered saline to achieve a final concentration of 50 fg/200 nl. Final concentration of DMSO in the microinjected solution was 0.0025%.

We determined the effects of PGE_2 microinjection into the MPO on PW latency and on the ongoing and reflex-related discharges of RVM neurons. Following three baseline PW trials, PGE_2 (50 fg; Cayman Chemical, Ann Arbor, MI, USA; in 200 nl vehicle) or vehicle (200 nl) was infused into the MPO over a period of approximately 2 min. PW latency and cell activity were then monitored for a period of 45 min. The dose of PGE_2 was chosen based on reports in awake animals (Hosoi et al., 1997) and on pilot data obtained in our lightly anesthetized preparation.

Only one protocol was performed in each animal. The average of the PW latencies and cell parameters obtained in the baseline period was compared with the average of the trials over the interval 30–45 min following the microinjection. This period was chosen for analysis because we were interested in examining cell activity at a time of significant thermal hyperalgesia, and pilot studies had indicated a gradual decrease in withdrawal latency, with a robust plateau at 30–45 min postinjection (see Fig. 5).

Three cell parameters were analyzed. *1) Ongoing activity.* Because off-cells and on-cells often show irregular alternations between periods of silence and activity, cell activity integrated over the 30 s prior to each PW trial was used as an overall index of ongoing firing. *2) On-cell PW-related burst.* Average firing rate in the 3 s period beginning 1 s before the PW was recorded for all PW trials. This approach, rather than counting the number of spikes or duration of the reflex-related burst, is necessary because a burst cannot be identified unless the neuron is inactive at the time of heat onset. *3) Duration of the off-cell pause.* Duration of the reflex-related pause was determined for those trials that fell at a time when the off-cell was not already silent at the time of heat onset.

Data are presented as mean±S.E.M. Wilcoxon's signed ranks and Mann-Whitney *U* tests were used for statistical analysis of cell parameters; Student's *t*-test for correlated means was used for comparing baseline and post-injection PW latencies. $P<0.05$ was considered significant.

Histology

At the conclusion of the experiments, recording sites were marked with an electrolytic lesion, and infusion sites by injection of Pontamine Sky Blue dye. Animals were killed with an overdose of methohexital, and perfused intracardially with physiological saline followed by 10% formalin. Tissue was stained with Neutral Red, and recording and infusion sites histologically verified and plotted on standardized sections (Paxinos and Watson, 1997). The MPO was considered to include the medial preoptic area and medial preoptic nucleus. Injection sites in the MPO and surrounding areas are shown in Fig. 1. The RVM was defined as the nucleus raphe magnus and adjacent reticular formation at the level of the facial nucleus. Recording sites were distributed in this region as in previous publications from this laboratory (Heinricher and Tortorici, 1994; Heinricher and Roychowdhury, 1997).

RESULTS

PGE$_2$ (50 fg) microinjected into the MPO produces thermal hyperalgesia in lightly anesthetized rats

The main goal of this experiment was to determine whether RVM neurons could mediate the hyperalgesic action of PGE$_2$ in the MPO, so we sought to focus on a single dose of PGE$_2$ that would produce a robust behavioral effect. Previous work in awake animals had shown that the dose-response curve for the hyperalgesic effect of PGE$_2$ in the MPO exhibited an inverted U-shape, with the maximum effect at 5–50 fg, and lesser or no effects at higher doses (Hosoi et al., 1997). We verified that 50 fg was an appropriate dose to produce hyperalgesia in lightly anesthetized animals, and that vehicle injections had no effect (Fig. 2). Microinjections into surrounding tissue (dorsal to anterior commissure or caudally in the anterior hypothalamus) or exiting the ventral surface of the brain were also ineffective (Fig. 2), although as reported by others (Hosoi et al., 1997), injections into the diagonal band of Broca also produced hyperalgesia (data not shown).

Microinjection of PGE$_2$ into the MPO recruits nociceptive modulatory circuitry of the RVM

Again, because we were interested in linking changes in RVM cell activity with hyperalgesia produced by focal application of PGE$_2$ in the MPO, animals in the PGE$_2$ microinjection group were divided into those showing a decrease in PW latency of at least 20% (28 of 35 animals tested in experiments in which we were able to successfully maintain cell isolation throughout the entire protocol) and those showing a lesser or no change (seven of 35 animals). Sites at which the injection failed to alter PW latency were interspersed among effective sites (Fig. 1).

Ten on-cells, ten off-cells and eight neutral cells were recorded in experiments in which microinjection of PGE$_2$ into the MPO produced at least a 20% decrease in PW latency. As shown in the example in Fig. 3A, on-cells displayed a significant increase in ongoing activity following infusion of PGE$_2$ ($P=0.03$, Wilcoxon's signed ranks test, Fig. 4). The overall increase in ongoing activity of these neurons reflected an increase in the proportion of time active (50.4±9.9% in baseline, 72.9±5.2% after PGE$_2$, $P=0.01$, Wilcoxon's signed ranks test), as well as a
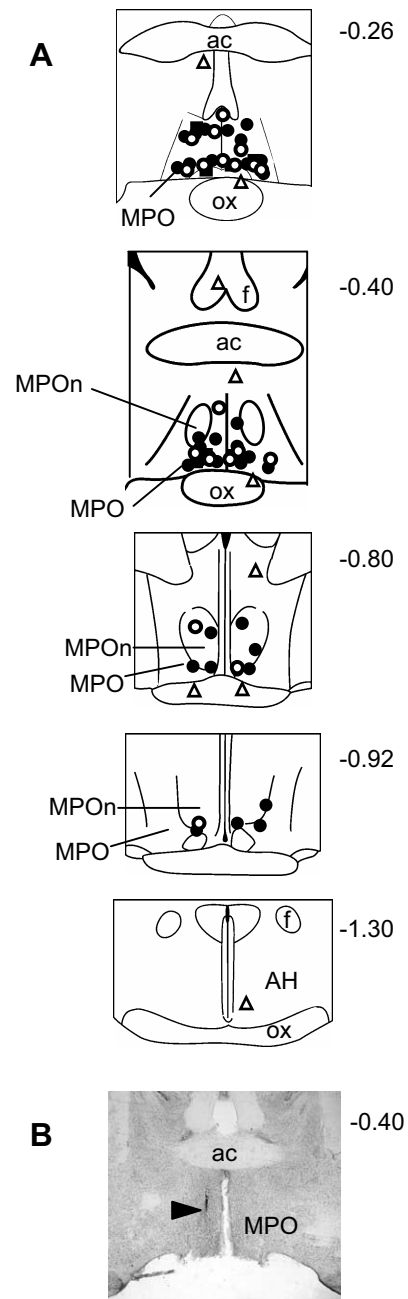

Fig. 1. (A) Histologically verified locations of infusion sites in experiments in which vehicle (open circles) or PGE$_2$ (filled symbols) was infused into the MPO. (Filled circles refer to sites at which PGE$_2$ infusion produced a decrease in PW latency of at least 20%, filled squares to sites at which there was a lesser or no change in latency.) ac, anterior commissure; AH, anterior hypothalamus; f, fornix; MPOn, medial preoptic nucleus; ox, optic chiasm. Distance from the interaural line is indicated. Missed placements (open triangles) were located caudal to the MPO (at the level of the anterior hypothalamus), dorsal (approaching or above the anterior commissure), or ventral (in the optic chiasm). (B) Injection site in the MPO (arrowhead).

relatively small, but significant, increase in firing rate during active periods (6.7±1.2 sp/s in baseline, 8.6±1.3 sp/s after PGE$_2$, $P=0.02$, Wilcoxon's signed ranks test). Firing rate of these neurons during the reflex-related burst showed a
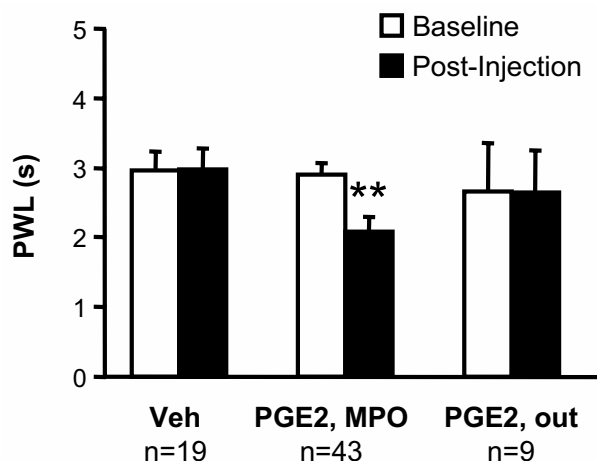
Fig. 2. Microinjection of PGE_2 into the MPO produces thermal hyperalgesia in lightly anesthetized rats. PGE_2 (50 fg in 200 nl) produced a significant decrease in PW latency when microinjected into the MPO. Vehicle (200 nl) had no effect, nor did injections dorsal or caudal to the MPO (PGE_2, out). There was no difference among the groups in baseline latencies (ANOVA). (** $P<0.01$ values averaged over 30–45 min post-injection time period compared with baseline, t-test for correlated means.) Note that neurons were successfully recorded in 35 of the 43 in the PGE_2 group, and 17 of 19 in the vehicle group.

comparable small but significant increase (12.3 ± 3.5 sp/s at the time of the reflex in baseline, 15.2 ± 3.5 sp/s after PGE_2, $P=0.03$, Wilcoxon's signed ranks test). The time course of the increase in on-cell firing closely paralleled the decrease in PW latency (Fig. 5). Vehicle microinjection in MPO had no effect on RVM on-cell discharge (Figs. 3A and 4).

In contrast to the activation of on-cells, off-cell firing was significantly depressed following microinjection of PGE_2 into the MPO ($P=0.03$, Figs. 3B and 4). This decrease was due primarily to a decrease in the proportion of time active ($71.3\pm7.2\%$ in baseline, $37.7\pm9.5\%$ after PGE_2, $P=0.01$, Wilcoxon's signed ranks test). Consistent with the increase in silent periods, the duration of the reflex-related pause was significantly increased, from an average of 29.6 ± 10.8 s in baseline to 54.8 ± 18.5 s following infusion of PGE_2 in the MPO ($P=0.01$, Wilcoxon's signed ranks test). Firing rate during active periods was not significantly changed (14.4 ± 3.2 sp/s in baseline, 11.5 ± 2.8 sp/s following PGE_2, $P=0.06$, Wilcoxon's signed ranks test). The time-course of the decrease in off-cell firing closely paralleled the decrease in PW latency (Fig. 5). Vehicle had no effect on off-cell discharge (Figs. 3B and 4).

Neutral cell firing was unaffected by PGE_2 in MPO (Figs. 3C and 4; $P=0.21$, Wilcoxon's signed ranks test).

The capacity of MPO microinjections of PGE_2 to alter the activity of identified RVM neurons was related to the behavioral effect. Thus, although the number of cells recorded in experiments in which PGE_2 had no or only a minor effect on reflex latency (i.e. less than 20% decrease in PW latency, Fig. 1 filled squares) was too small for statistical analysis (two on-cells, two off-cells and three neutral cells), the average ongoing firing of the two on-cells recorded following behaviorally ineffective injections was

clearly not increased (88% and 53% of baseline), and that of the two off-cells was not suppressed (169% and 161% of baseline).

Hyperalgesic dose of PGE_2 in the MPO does not produce hyperthermia

The dose of PGE_2 applied here is generally thought to be sub-pyrogenic (Scammell et al., 1996; Oka et al., 1997). However, previous investigators have not recorded nociceptive reflexes and body temperature simultaneously. In a separate set of experiments, we therefore recorded rectal temperature in a small number of animals throughout the hyperalgesia testing protocol to determine whether hyperalgesia was in fact dissociated from increased body temperature. These data demonstrated a clear dissociation of the hyperalgesic and hyperthermic effects of PGE_2 in the MPO (Table 1) in that 50 fg PGE_2 produced hyperalgesia without increasing rectal temperature. A higher dose of PGE_2 (50 ng in 200 nl, 2.5% DMSO) was microinjected as a positive control, and elicited the expected increase in body temperature without producing any decrease in paw withdrawal latency in the 45 min period over which these variables were monitored.

DISCUSSION

In awake animals, microinjection of COX inhibitors into the MPO prevents illness-induced hyperalgesia, whereas direct administration of PGE_2 into the MPO produces hyperalgesia (Hosoi et al., 1999; Abe et al., 2001). These observations indicate that prostaglandins acting in the MPO are critical mediators of the hyperalgesic component of the illness response. The principal finding of the present study was that thermal hyperalgesia produced by microinjection of PGE_2 into the MPO is associated with recruitment of identified nociceptive modulatory neurons in the RVM. On-cells become active a greater proportion of the time following PGE_2 microinjection, thus showing an overall increase in ongoing activity. By contrast, off-cells are active a smaller proportion of the time, and thus demonstrate an overall decrease in firing.

Role of RVM neurons in hyperalgesia produced by PGE_2 in the MPO

RVM on-cells are thought to have a net pro-nociceptive role in descending control. Direct, selective activation of on-cells produces hyperalgesia, and reduction of the threshold at which the on-cell burst is triggered is associated with a decrease in reflex latency. By contrast, activation of off-cells results in analgesia, and these neurons are generally thought to exert a net antinociceptive effect (Heinricher et al., 1994; Heinricher and Tortorici, 1994; McGaraughty et al., 2003; Neubert et al., 2004; Heinricher and Neubert, 2004). Ongoing activity of RVM neurons is also correlated with nociceptive responsiveness, such that periods of on-cell discharge and off-cell quiescence are associated with enhanced nociceptive behaviors (Heinricher et al., 1989; Ramirez and Vanegas, 1989; Bederson et al., 1990). In the present study, PGE_2 microinjection caused a shift in the balance between on- and
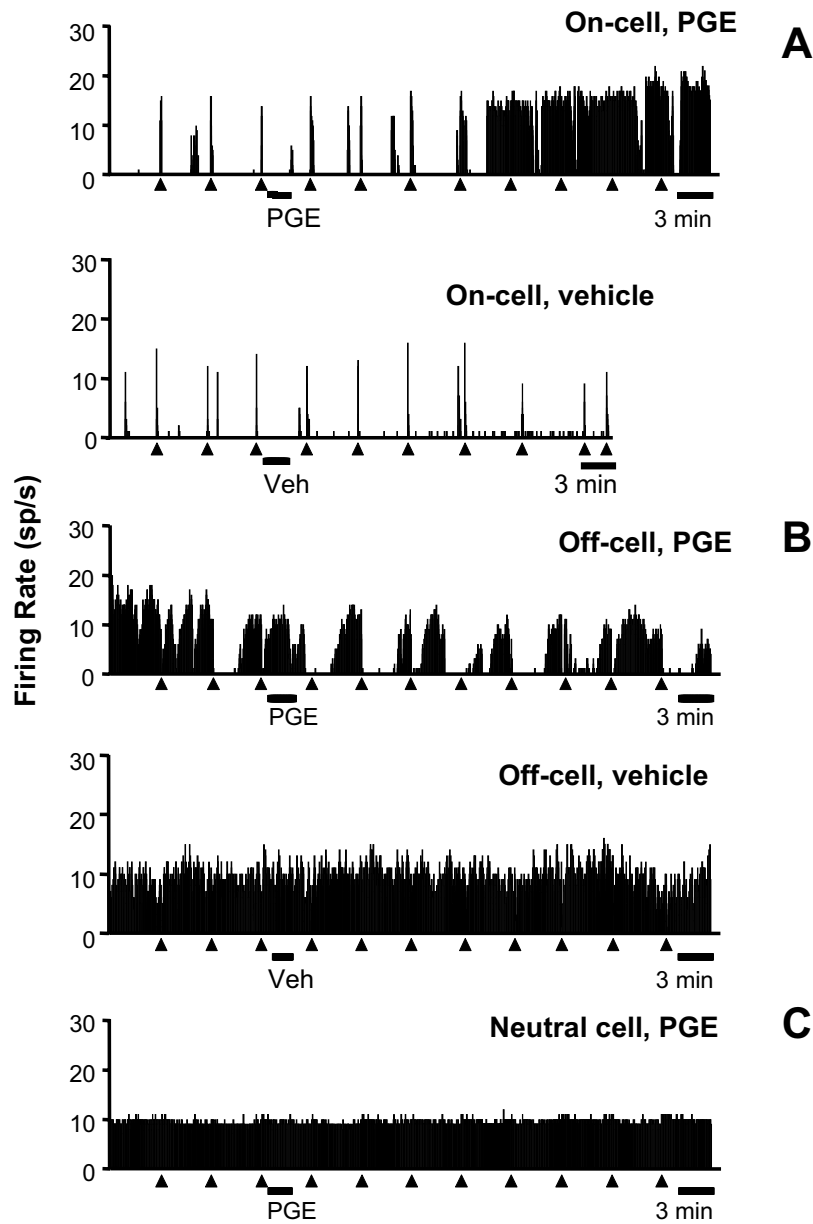
Fig. 3. Ratemeter records illustrate effects of PGE_2 in the MPO on ongoing discharge of identified RVM neurons. (A) Activation of an on-cell following PGE_2, but not vehicle, microinjection in the MPO. The cell in the upper trace had almost no spontaneous activity in baseline, but showed prolonged intervals of ongoing firing beginning approximately 23 min after the injection. Firing rate during active periods was not greatly increased. PW latency decreased to 29% of baseline following PGE_2 in this animal. Lower trace is an example of an on-cell recorded during infusion of vehicle. Baseline firing pattern was comparable to that of the on-cell shown in the upper trace, but infusion of vehicle into the MPO had no effect on firing pattern or rate. Triangles indicate PW trials, 1 s bins. (B) Suppression of off-cell firing following PGE_2, but not vehicle, microinjection in the MPO. Overall firing of the off-cell in the upper trace was decreased, to 51% of baseline in the period from 30 to 45 min post-injection. Note that the main change was in firing pattern, with a relatively minor effect on firing rate during active periods. PW latency decreased to 62% of this animal's baseline following PGE_2. Lower trace illustrates the lack of effect of vehicle on ongoing activity of a second off-cell. (C) Firing pattern and rate of this neutral cell were completely unaffected by MPO PGE_2. Overall firing in the period from 30 to 45 min post-injection was 105% of baseline. PW latency decreased to 72% of this animal's baseline following PGE_2. Triangles indicate PW trials, 1 s bins.

off-cell firing, so that on-cells were more likely to be in an active phase and off-cells in a quiescent phase at any given time following PGE_2. The noxious heat stimulus, delivered to the paw at 5-min intervals, was consequently more likely to fall at a time when the on-cell population was active and off-cells inactive. The reflex response to the heat therefore occurred at a shorter latency.

The correlation of decreased paw withdrawal latency with an increase in on-cell discharge and decrease in off-cell discharge is thus entirely consistent with earlier work and the respective roles of each cell class in nociceptive modulation. It should be emphasized that the present findings are correlative, and further work will be required to determine whether changes in either cell class
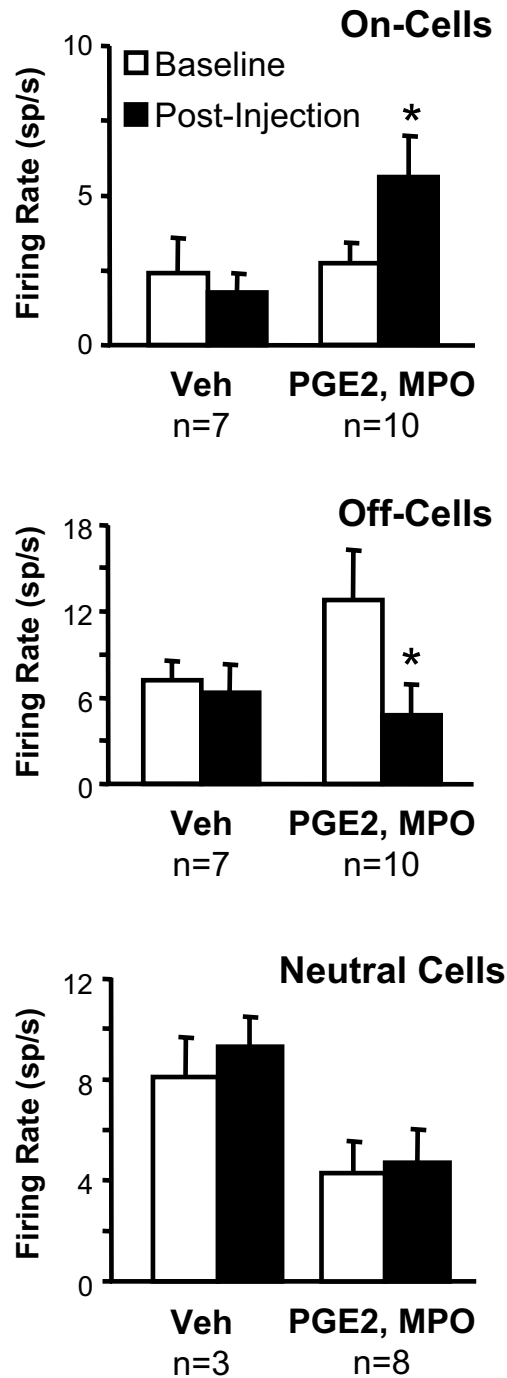
On-Cells

□ Baseline
■ Post-Injection

Firing Rate (sp/s)

Veh
n=7

PGE2, MPO
n=10

Off-Cells

Firing Rate (sp/s)

Veh
n=7

PGE2, MPO
n=10

Neutral Cells

Firing Rate (sp/s)

Veh
n=3

PGE2, MPO
n=8

Fig. 4. Ongoing firing of on- and off-cells and neutral cells in baseline compared with the post-injection period in animals microinjected with vehicle (left) or PGE_2 in the MPO. Mean ongoing discharge of RVM neurons in experiments in which PGE_2 produced at least a 20% decrease in PW latency. On-cells show a significant increase in on-going activity following a behaviorally significant injection of PGE_2, while off-cells display a significant decrease. Neutral cell discharge is unchanged by PGE_2 in the MPO. Baseline activity was comparable between vehicle and PGE_2 groups for each cell class ($P > 0.05$, Mann-Whitney U test, * $P < 0.05$, discharge following PGE_2 compared with that in baseline, Wilcoxon's signed ranks test).

Table 1. Dose-related dissociation of hyperalgesic and pyrogenic effects of PGE_2 in the MPO[a]

Dose PGE_2	Reflex latency (% of baseline)	Change in rectal temp	n
50 fg PGE_2	60±5%	−0.03±0.29°C	5
50 ng PGE_2	101±7%	1.68±0.24°C	5

[a] Body temperature was monitored throughout the hyperalgesia testing protocol in a small set of separate experiments. The 50 fg dose produced hyperalgesia without increasing body temperature. As a positive control, 50 ng was shown to produce hyperthermia. Hyperalgesia was not seen at this dose over the 45 min monitoring period.

play a causal role in hyperalgesia triggered by PGE_2 in the MPO. However, activation of on-cells is likely critical, since lesions of the RVM are known to interfere with illness-induced hyperalgesia (Watkins et al., 1994a; Wiertelak et al., 1997). Nevertheless, the overall reduction in the net antinociceptive influence of the off-cell population may also contribute to enhanced responsiveness.

The connection from the MPO to the RVM is both direct, and indirect, via the PAG (Chiba and Murata, 1985; Rizvi et al., 1996; Hermann et al., 1997; Murphy et al., 1999; Semenenko and Lumb, 1999). Our data provide no information on whether the changes in on- and off-cell discharge are due to a direct input from the MPO, or relayed through the PAG or some other site, such as the dorsomedial hypothalamus (Zaretskaia et al., 2003). However, Jiang and Behbehani (2001) noted that inactivation of the PAG attenuated, but did not block, the effects of non-selective MPO stimulation on RVM neurons, suggesting that at least some of the influence is independent of the PAG.

Early studies of the MPO and RVM emphasized their roles in a network ultimately mediating analgesia via the inhibition of dorsal horn neurons (Carstens et al., 1982; Mokha et al., 1987; Lumb and Cervero, 1989; Lumb, 1990; Workman and Lumb, 1997). Using electrical stimulation, Lumb and Morrison (1986) reported a strong excitatory connection from the MPO to spinally projecting neurons in the RVM, suggesting that activation of nociceptive inhibitory output neurons of the RVM could explain the antinociceptive effects of MPO stimulation. Jiang and Behbehani (2001) reported activation, inhibition and no effect of MPO stimulation on RVM neurons recorded in deeply anesthetized animals. They noted that neurons excited by noxious cutaneous stimulation, which likely overlap to at least some extent with the on-cells recorded here, were more likely to be excited by low-intensity electrical or neuroexcitant stimulation within the MPO. By contrast, neurons inhibited by noxious stimulation, which likely overlap with the off-cells recorded here, were more likely to be inhibited by stimulation within the MPO. However, the relationship between the neurons' responses to noxious stimulation and MPO stimulation was relatively weak.

Although our results are broadly consistent with these early reports, major differences in experimental conditions must be considered. First, animals in the earlier studies were deeply anesthetized, and it was thus not possible to
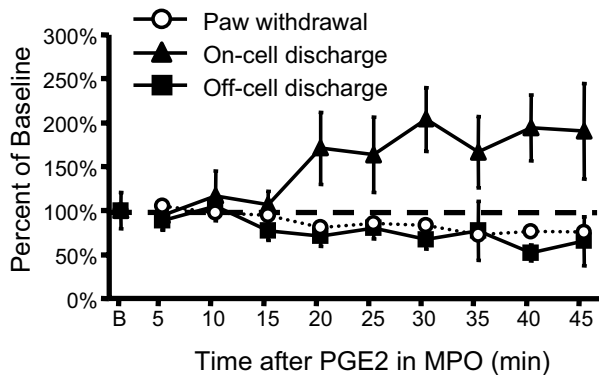
Fig. 5. Time course of changes in PW latency and on- and off-cell ongoing discharge. PW latency and on- and off-cell ongoing discharge are plotted as a percent of baseline. Alterations in on- and off-cell firing parallel the decrease in PW latency following microinjection of PGE$_2$ in the MPO.

determine whether the MPO manipulation produced analgesia, hyperalgesia, or had no behavioral effect. Our previous work has demonstrated a tight link between behavioral and RVM neuron responses to experimental manipulations, such as in opioid analgesia (Heinricher et al., 1994). In the present study, changes in on- and off-cell discharge were seen only in experiments in which there was behaviorally measurable hyperalgesia. The two on-cells and two off-cells recorded in four experiments in which the microinjection did not produce hyperalgesia showed no change in activity. Second, earlier studies used relatively non-specific methods to manipulate the MPO, such as electrical stimulation or neuroexcitant application. Such manipulations may influence circuitry involved in the host of other behavioral and physiological processes in which the MPO also plays a role, including sexual and maternal behavior, thermoregulation and autonomic control. Microinjection of a low dose of PGE$_2$ may be more specific, and more likely to recruit those MPO neurons specifically relevant to the illness response.

Technical considerations

Issues of drug diffusion and pharmacological specificity must be considered in any study in which the microinjection technique is used. Diffusion of drugs to sites distant from the intended target is a primary concern. That seems unlikely to be an issue in the present experiments because injections of identical volumes and doses of PGE$_2$ into regions immediately adjacent to the MPO had no effect on behavior or RVM neuronal activity. A second consideration is pharmacological specificity. No potent broad-spectrum EP receptor antagonist is presently available. However, the behavioral results in the present study were consistent with a significant body of work using various agonists, knock-out mice and COX inhibitors supporting a specific role of preoptic PGE$_2$ in fever and hyperalgesia (Hosoi et al., 1997; Abe et al., 2001; Choi et al., 2003; Oka et al., 2003a). The differential effect of PGE$_2$ on the three classes of RVM neurons, the coherent behavioral and neuronal

changes, and the lack of effect of the vehicle further argue in favor of a specific pharmacological action.

The possibility that anesthesia influenced the outcome of these experiments must also be considered. However, the change in PW latency without increased body temperature that we observed is entirely consistent with data obtained in awake behaving animals following microinjection of a range of doses of PGE$_2$ in the MPO (Scammell et al., 1996; Hosoi et al., 1997). This indicates that anesthesia per se is not blocking expression of fever with low doses of PGE$_2$. In addition, the fact that PW latency was unchanged in the saline-treated control group indicates that the anesthetic level was stable throughout the protocol.

Hyperalgesia, fever and the illness response

PGE$_2$ is not the only mediator, and the MPO is not the only brain structure implicated in the illness response. The various elements of the response are recruited by systemic or intracerebral administration of endotoxin or cytokines. These different components have distinct thresholds, dose-response relationships and time courses, which suggests differences in the underlying immune and neural mechanisms (Rothwell, 1989; Dunn et al., 1991; Kent et al., 1992a,b; Romanovsky et al., 1996; Avitsur et al., 1997; Luheshi et al., 1997; Montkowski et al., 1997; Sonti et al., 1997; Lenczowski et al., 1999). The relationship between altered nociception and fever is particularly complex. Fever following systemic administration of a pyrogen can be associated with hyperalgesia or analgesia (Mason, 1993; Yirmiya et al., 1994; Romanovsky et al., 1996; Morgan et al., 2004). Moreover, mechanistic studies point to important differences between fever and hyperalgesia induced using systemic pyrogen administration. Watkins, Maier and colleagues have shown that the hyperalgesia induced by systemic administration of a high dose of lipopolysaccharide (200 μg) is blocked by vagotomy. In contrast, fever (which was produced by lower doses of lipopolysaccharide, 1–50 μg) does not require vagal afferent transmission (Watkins et al., 1994a; Hansen et al., 2000). Induction of fever and hyperalgesia by interleukin-1β are similarly dissociated by dose and vagotomy (Watkins et al., 1994b; Hansen et al., 2001). When interleukin-1β is given via the cerebral ventricles, low doses produce hyperalgesia but not fever, whereas high doses result in fever with no hyperalgesia (Oka et al., 1993; Yabuuchi et al., 1996). Like interleukin-1β, PGE$_2$ has bidirectional effects on nociception when given intracerebroventricularly, producing hyperalgesia at low doses, and analgesia at high doses, likely mediated by EP$_3$ and EP$_1$ receptors, respectively (Oka et al., 1994).

Romanovsky et al. (1996) therefore suggest that a direct link between hyperalgesia and fever is too simplistic, and emphasize a more dynamic model that takes into account both the time course of the response to immune challenge and the magnitude of the challenge. Early or mild challenge appears to be associated with hyperalgesia. In contrast, later phases of the response, which are evoked by more intense challenges, are associated with

analgesia (see also Yirmiya et al., 1994). We saw no increase in body temperature associated with hyperalgesia following infusion of a low dose of PGE$_2$ in the MPO. This confirms previous work in awake animals showing similar dissociation of fever and hyperalgesia following PGE$_2$ in the MPO, with hyperalgesia obtained only at subpyrogenic doses (Hosoi et al., 1997). These authors suggested that the hyperalgesia obtained with extremely low doses of prostaglandin in the MPO serves as a warning for infection. It is not unreasonable to suggest that the modest early-phase fever associated with hyperalgesia following a systemic immune challenge is mediated by substances other than PGE$_2$ and/or structures other than the MPO. The loss or suppression of hyperalgesia observed here when a substantial fever (over 1.5 °C) was induced with the high dose of PGE$_2$ may be more closely related to the later phase of the illness response postulated by Romanovsky and colleagues (1996). Although the RVM has been implicated in illness-related hyperalgesia, the structures mediating the hypoalgesia reported by Romanovsky et al. (1996) and Yirmiya et al. (1994) in the later phase are unknown. Further work will be required to determine whether RVM neurons respond to a pyrogenic dose of PGE$_2$ in the RVM.

Relationship between pain modulation and fever in the RVM

Given the dissociation between the hyperalgesia and body temperature responses observed here, it is interesting that analysis of the circuitry mediating the pyrogenic effects of PGE$_2$ in the MPO has also focused on the rostral medial medulla, specifically the nucleus raphe pallidus (Madden and Morrison, 2003; Morrison, 2003). Raphe pallidus is located within the boundaries of the RVM, but is generally treated as a functionally discrete entity based on the high density of serotonergic neurons, compact location, distinct physiology, and some differential projections (Moore, 1981; Skagerberg and Björklund, 1985; Jacobs and Azmitia, 1992; Jacobs et al., 2002). The MPO projects densely to raphe pallidus, as well as more diffusely to other regions of the RVM (Hermann et al., 1997; Murphy et al., 1999), and sympathetic premotor neurons controlling brown adipose tissue are found in the RVM, although they are more densely concentrated in raphe pallidus (Bamshad et al., 1999; Cano et al., 2003). PGE$_2$ microinjected into the MPO in a dose sufficient to produce fever (50 ng) induces Fos expression in RVM, which is concentrated in, but not limited to, raphe pallidus (Nakamura et al., 2002). Furthermore, microinjections of GABA agonists centered on the raphe pallidus reverse hyperthermia produced by PGE$_2$ given via the cerebral ventricles or microinjected into the MPO (Nakamura et al., 2002; Morrison, 2003). Additional studies will be required to determine whether ventral medullary circuits regulating body temperature and those modulating nociception share common neural elements at the level of individual neurons. However, the differential dose-dependence of the hyperalgesic and pyrogenic actions of PGE$_2$ suggest that these two components of illness are mediated by different cell populations in the medulla.

Summary

The present study demonstrates that focal application of a low dose of PGE$_2$ in the MPO activates nociceptive facilitating neurons and suppresses the firing of nociceptive inhibiting neurons in the RVM, a region with a well-documented role in pain modulation (Fields, 2000; Porreca et al., 2002; Heinricher et al., 2003). A large body of evidence has implicated prostaglandins in the MPO in generation of the illness response, especially fever. The present study indicates that this region also contributes to the hyperalgesic component of the illness response, most likely by recruiting the nociceptive modulating circuitry of the RVM.

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