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THE EFFECTS OF PROSTAGLANDIN E_2 ON THE NEURONS OF THE VENTROMEDIAL PREOPTIC AREA OF THE HYPOTHALAMUS: A MECHANISM OF FEVER

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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by

Heather J. Ranels

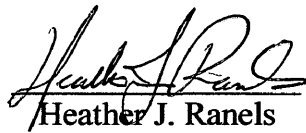
2002

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

The requirements for the degree of

Master of Arts



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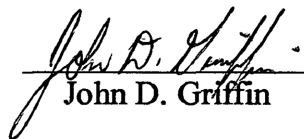

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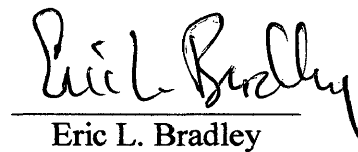

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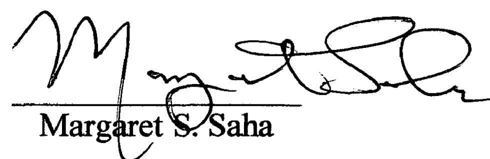

Margaret S. Saha

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LIST OF ABBREVIATIONS

APFx:	anterior perifornical area
COX2:	cyclooxygenase 2
EP:	prostaglandin E receptors
Il-1:	interleukin-1
LPS:	lippopolysaccharide
OVLT:	organum vasculosum terminalis
PGs:	prostaglandins
PGE_2:	prostaglandin E_2
PKA:	protein kinase A
POA:	preoptic area
PVH, PVN:	paraventricular nucleus
VMH:	ventral medial hypothalamus
VMPO:	ventromedial preoptic area

NOTE TO THE READER

The work presented in this thesis consists of two related projects using different techniques to explore the effects of PGE_2 on neurons in the ventromedial preoptic area of the hypothalamus. The first technique is extracellular single-unit recording. The findings of this investigation are reported in the form of a manuscript titled *The Effects of Prostaglandin E_2 on the Firing Rate Activity of Thermosensitive and Temperature Insensitive Neurons in the Ventromedial Preoptic Area of the Rat Hypothalamus* (by H.J. Ranels & J.D. Griffin). Presented here in Chapter II., this manuscript has been submitted to *Brain Research.*

A second manuscript titled *The Effects of Prostaglandin E_2 on the Cellular Properties of Thermally Classified Neurons in the Ventromedial Preoptic Area of the Rat Hypothalamus* (by H.J. Ranels & J.D. Griffin) is in progress. This manuscript is presented here in Chapter III and utilized the technique of whole-cell patch clamp recording. Due to the format chosen for this thesis, some of the information provided in the manuscripts (Chapter II and III) may also be found in the Introduction and the Conclusions (Chapter I and IV, respectively). However, all appropriate considerations concerning experimental findings of this thesis are addressed in Chapter IV, and the reader is encouraged to refer to this chapter for a comprehensive examination of the results related to the current literature.

ABSTRACT

Fever, an elevation in body temperature, is thought to play an adaptive role in the immune system's attempts to fight invading infectious organisms through the actions of endogenous pyrogens, such as prostaglandin E_2 (PGE_2) directly on the central nervous system. A suggested mechanism for the induction of fever is the up regulation of the thermostatic set-point for body temperature achieved through the integration of thermal information by neurons in the hypothalamus that can be classified as either warm sensitive of temperature insensitive.

The ventromedial preoptic area of the hypothalamus (VMPO) has been confirmed morphologically and physiologically as a site of fever induction, including through the local production of PGE_2. Within the VMPO, there is overlapping expression of prostaglandin receptor subtypes, EP_3 and EP_4. Both of these receptor subtypes are activated during a fever response and result in either a decrease or an increase in intracellular cAMP. This may result in changes in ionic conductance may alter the activity of hypothalamic neurons.

To examine firing rate activity and thermosensitivity extracellular single- unit recordings from the VMPO were made. Neurons were classified as either warm sensitive ($m \geq 0.8$ imp$\cdot s^{-1}\cdot{}^{\circ}C^{-1}$) or temperature insensitive. Hypothalamic tissue slices were maintained at a constant temperature of ~36°C and perfused with PGE_2 (200nM). This study provided evidence that the majority of temperature insensitive neurons responded to PGE_2 with an increase in firing rate activity, while warm sensitive neurons showed a reduction in firing rate. This suggests that both warm sensitive and temperature insensitive neurons in the VMPO may play critical and contrasting roles in the production of a fever during an acute phase response to infection.

To characterize the cellular properties of VMPO neurons, whole-cell patch clamp recordings were made in hypothalamic tissue slices perfused with PGE_2 (200nM or 1μM). In response to both 200 nM and 1 μM, all neurons showed a decrease in input resistance. However in response to a depolarizing current, temperature insensitive neurons responded to PGE_2 with an increased firing rate frequency and a decreased delay in the onset of action potential generation, while warm sensitive neurons decreased firing rate frequency. Temperature insensitive neurons treated with PGE_2 also showed depolarized membrane potential. Changes in firing rate activity were only seen in response to 1 μM PGE_2. Insensitive neurons showed an increase in firing rate activity, while warm sensitive neurons showed a decrease in firing rate activity. In addition there was no change in local synaptic input in response to temperature or PGE_2.

THE EFFECTS OF PROSTAGLANDIN E_2 ON THE NEURONS OF THE VENTROMEDIAL PREOPTIC AREA OF THE HYPOTHALAMUS: A MECHANISM OF FEVER

CHAPTER I

INTRODUCTION

The regulation of body temperature is highly efficient and employs regulatory changes in cardiovascular and respiratory activity, as well as osmotic, metabolic and behavioral responses. The neural pathway of this control system involves the hypothalamus, limbic system, lower brainstem, reticular formation, spinal cord, and sympathetic ganglion (Boulant, 1991). Within the hypothalamus, a major autonomic center, neurons integrate afferent sensory information from thermoreceptors in the skin and body core with inherent responses to local brain temperature. Through the integration of this information, these neurons initiate thermoregulatory responses to maintain a constant body temperature within a narrow range, except in rare instances such as fever, exercise induced hyperthermia, and other pathological states. With a rise in temperature, hypothalamic thermoregulatory neurons initiate heat loss responses, ranging from panting, sweating, and an increase in skin blood flow, to behavioral responses such as fanning and seeking shade. If body temperature falls, these neurons initiate heat production or retention responses, which include shivering, non-shivering thermogenesis, cutaneous vasoconstriction, and changes in behavior (Boulant, 1998).

1. Thermosensitivity in the Hypothalamus

Evidence for central thermosensitivity comes from studies in which discrete neural areas of the hypothalamus were thermally stimulated, and from electrophysiological studies which characterized the temperature sensitivity of hypothalamic neurons. Within the hypothalamus, approximately 60% of the neurons are classified temperature insensitive, 30% are warm sensitive, and less then 10% are cold sensitive (Boulant, 1998). To thermally classify neurons based on changes in spontaneous firing rate, two differing criteria have been used: neuronal Q_{10} and the slope of the thermoresponsive curve. Neuronal $Q_{10,}$ in its simplest form, is the ratio of a response before and after a theoretical 10°C change in temperature. If the response doubles during a 10°C increase then the Q_{10} is considered to be 2.0. The slope of the thermoresponsive curve is preferred for most neural integration studies, as it can be applied to the activity of a neuron in response to local changes in temperature, as well as responses to afferent input concerning peripheral and visceral temperature (Boulant, 1989).

Although the hypothalamus is a major center of thermoregulation, the majority of neurons in the hypothalamus are characterized as temperature insensitive. These neurons show little or no change in firing rate activity in response to local changes in temperature. The activity of these neurons may not be directly correlated with thermoregulatory responses, but it has been proposed for many years that synaptic input from temperature insensitive neurons serves as a steady-state reference signal to thermoregulatory effector neurons (Hammel, 1965; Boulant & Dean, 1986; Boulant, 1998). In addition, a recent study showed that temperature insensitive neurons have distinct dendritic morphology. These neurons tend to have dendritic projections, which parallel the third ventricle

(Griffin et al., 2001). This is in contrast to warm sensitive neurons, which orient their dendrites towards major sites of afferent pathways, both medially and laterally. The contrasting morphology of temperature insensitive and warm sensitive neurons suggests a different functional role depending on thermosensitivity classification.

Neurons are considered to be warm sensitive if their firing rate substantially increases during local warming or decreases during local cooling. To classify a neuron as warm sensitive, the minimum criterion is a regression coefficient of 0.8 impulses$\cdot s^{-1} \cdot {}^{\circ}C^{-1}$ (Boulant, 1980; Boulant & Dean, 1986). This criterion is based on the result of several *in vivo* studies (Boulant & Bignall, 1973; Boulant & Hardy, 1974; Boulant & Dean, 1986). In particular, Boulant & Hardy (1974) studied the integration of peripheral thermal information and thermoregulatory responses that corresponded with changes in neuronal sensitivity to local, preoptic temperature. Although this criteria for warm sensitivity is widely accepted, several investigators still use no criteria or criteria far below accepted minimums, suggesting that many studies overestimate thermosensitive hypothalamic cell populations (Janský et al., 1992; Matsuda et al., 1992; Morimoto, 1988).

2. Intracellular Mechanism of Neuronal Thermosensitivity

Initial intracellular recording studies have suggested that the basis of neuronal temperature sensitivity in the hypothalamus depends on thermal changes in membrane potential (Gorman & Marmor, 1970; Carpenter, 1981; Kobayashi & Takahashi, 1993). However, Griffin & Boulant (1995) provided evidence that indicated membrane potential remains stable in all neuronal classes during warming and cooling. Instead Griffin et al. (1996) characterized a temperature dependant, depolarizing prepotential, which may be

the primary determinant of thermosensitivity for warm sensitive neurons. Warming increases the rate the depolarization of this prepotential, which in turn decreases the interspike interval and increases the firing rate of warm sensitive neurons. Further evidence suggests that the depolarization rate of the prepotential in warm sensitive neurons may be due to the inactivation of a potassium A current (Griffin et al., 1996).

3. Hypothalamic control of Temperature Regulation

A proposed model for neuronal control of thermoregulation shows the development of a hypothalamic set-point temperature, which can account for changes in thermoregulatory responses and apparent shifts in body temperature (Figure I.1.; Hammel, 1965). Hammel suggests that each type of thermoregulatory response is controlled by groups of effector neurons. These effector neurons are located in the hypothalamus or brainstem nuclei and control specific thermoregulatory responses through a change in firing rate activity. Insensitive and warm sensitive neurons in the hypothalamus integrate peripheral and central temperature information with their own inherent spontaneous activity to produce mutually antagonistic synaptic input to each effector neuron. Through the integration of this synaptic information, the activity of effector neurons determines a set-point, and the level of activation of specific thermoregulatory responses (Boulant, 1980).

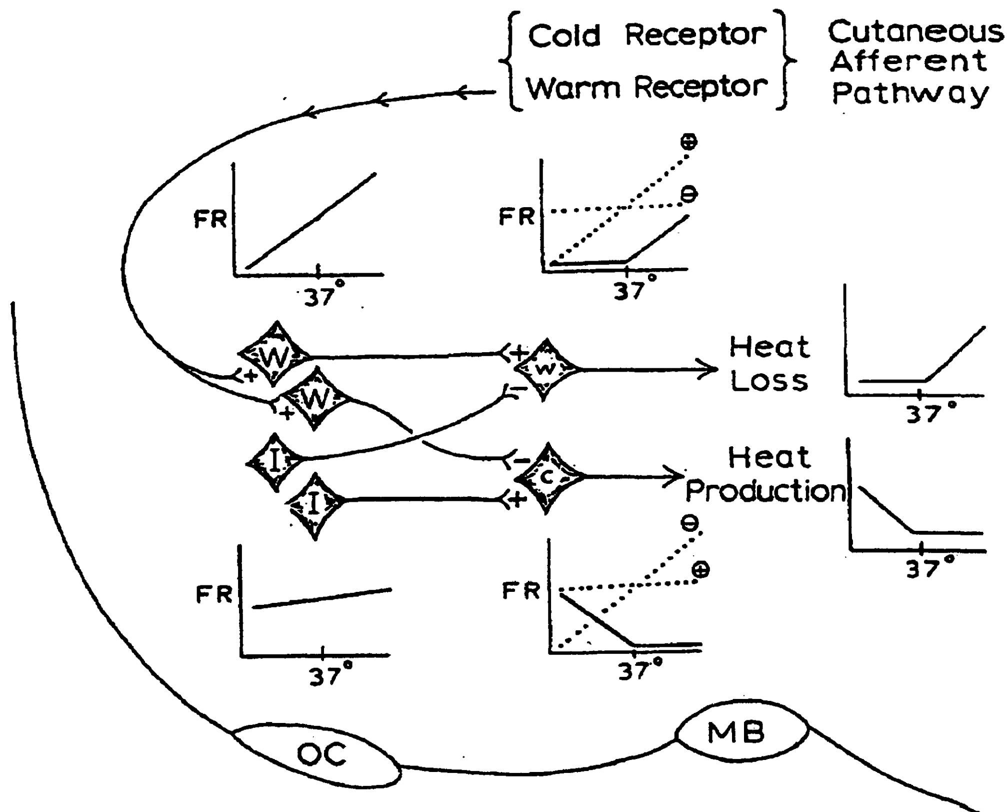

Figure I.1. Hammel's proposed model for explaining temperature regulation

Synaptic inputs from warm sensitive neurons (W) and temperature insensitive neurons (I) in the preoptic area of the hypothalamus are integrated by warm (w) and cold (c) effector neurons, which trigger thermoregulatory responses, which correlates with changes in temperature from the integrated set- point temperature. An increase in firing rate of warm sensitive neurons will trigger a heat loss response by warm effectors neurons, and a decrease in firing rate of warm sensitive neurons will trigger a heat production response from cold effector neurons. OC, optic chiasm, MB, mammillary body (Adapted from Boulant, 1991).

4. Fever

With respect to Hammel's model (Figure I.1.), an upward shift in the given hypothalamic set-point will result in a fever. Fever, which is part of the acute phase response to infection or inflammation, is characterized by an elevation of body temperature of 1-4°C (Saper & Breder, 1994). Several studies have shown that endotoxins, such as lippopolysaccharide (LPS), a complex glycolipid found in the outer membrane of most gram-negative bacteria, stimulate leukocytes and other cells to produce certain mediators of inflammation that have a pyrogenic effect (Elmquist et al., 1996; Scammell et al., 1996; Scammell et al., 1998). This has made the use of LPS a good experimental model for the acute phase response of infection. It triggers the release and secretion of endogenous pyrogens such as Interleukin-1 (IL-1), tumor necrosis factor α, interferon β, and interferon γ (Saper & Breder, 1994). Experimentally, these endogenous pyrogens tend to inhibit the firing rate activity of warm sensitive neurons while having little effect on insensitive neurons. However, studies indicate that they do not act directly on hypothalamic neurons, but induce the production of prostaglandin E_2 (PGE_2) in the preoptic area (POA) of the hypothalamus. PGE_2 may act on the neural pathways to raise body temperature by directly inhibiting warm sensitive neurons controlling heat loss responses or through the activation of temperature insensitive neurons. This suggests that the mechanism for the induction of a fever appears to be the up regulation of the thermostatic set-point for body temperature in the POA (Saper & Breder, 1994; Boulant, 1998) (Figure I.2.).

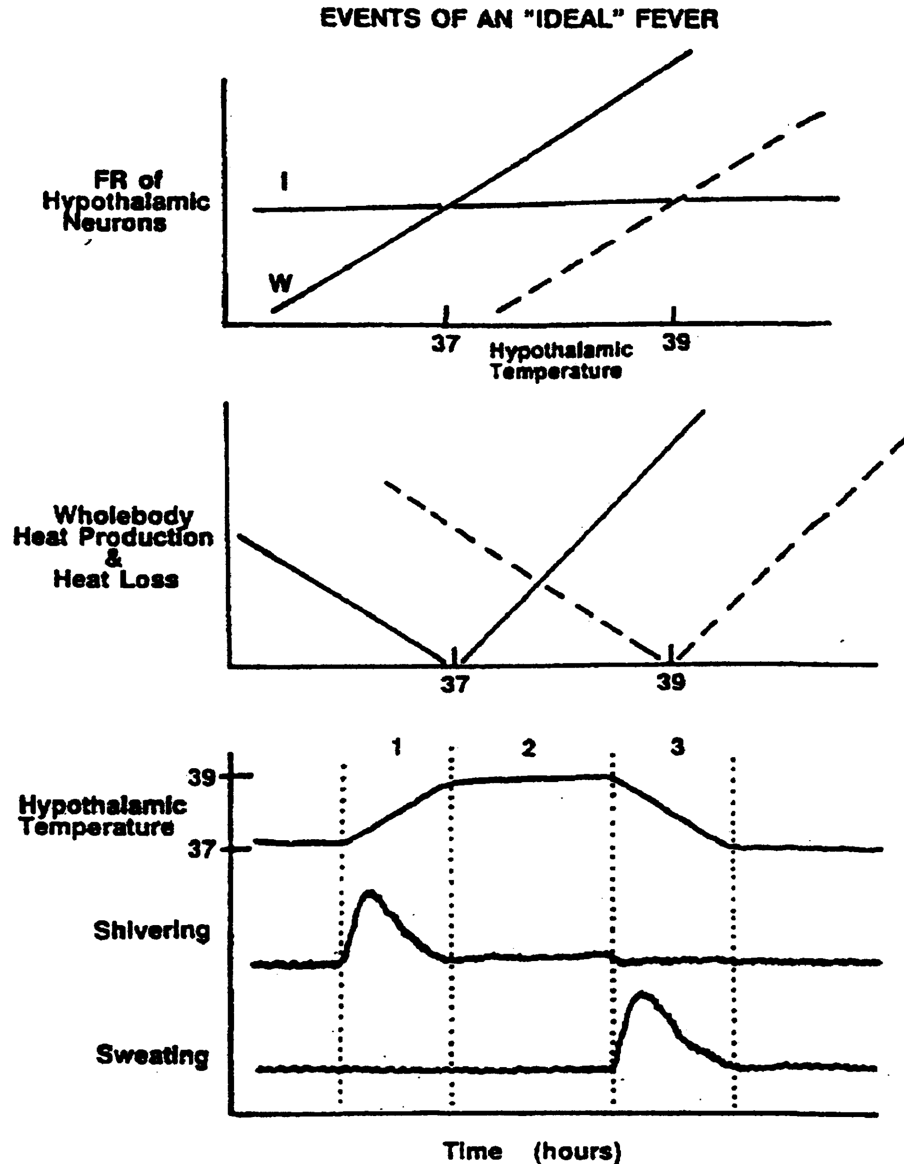

Figure I.2. Responses of neurons and whole body before, during, and after fever

Top: the integration of the normal firing rates (FR) of temperature insensitive neurons (I) and warm sensitive neurons (W) yielding a set-point of 37°C. An upregulation of W sensitive neurons raises the set-point inducing a fever (dashed line). Middle: the degree of thermoregulatory responses as a function of hypothalamic temperature under normal conditions (solid line) and fever (dashed line). Bottom: Temperature changes during fever corresponding with bodily responses during (1) initiation of fever steady, (2) stated of fever and, (3) decline in fever (Adapted from Boulant, 1991).

5. Prostaglandin E_2

Prostaglandins (PGs) comprise a diverse family of autocoids, whose synthesis is initiated by cyclooxygenase-mediated metabolism of the unsaturated 20-carbon fatty acid arachidonic acid, generating five primary bioactive prostanoids including PGE_2 (Breyer et al., 2001). PGs are produced by a variety of cells in response to both physiological and pathological stimuli, and are released into the interstitial space immediately after synthesis. They then act locally to stimulate cellular activity via specific G-protein-coupled receptors (Breyer et al., 2001; Ushikubi et al., 1995).

PGE_2 is a major product of cyclooxygenase-initiated arachidonic acid metabolism and may have multiple, and at times apparently opposing, functional effects on given target tissues. For example, it exerts vasodilator effects on both arterial and venous beds, and causes smooth muscle relaxation or constriction dependant on the location (Coleman et al., 1990). Molecular cloning has now confirmed the existence of multiple PGE_2 receptor subtypes, encoded by distinct genes. These receptors are EP_1, EP_2, EP_3, and EP_4 and likely account for the diverse effects of PGE_2 (Ek et al., 2000).

To characterize the role of PGE_2 in the production of a fever, Morimoto et al. (1988) performed *in vivo* studies in which PGE_2 was injected directly into the POA and ventral medial hypothalamus (VMH) of the rat while monitoring rectal temperature. They also investigated the effect of PGE_2 on neuronal activity in the POA and VMH in tissue slice preparations. This study reported that regardless of thermosensitivity, neurons in the VMH decreased their firing rates in response to PGE_2; while in the POA, neurons increased their firing rates. From this evidence, the investigators concluded that responses to PGE_2 could not be characterized on the basis thermosensitivity. However,

this study used a thermocoefficient of 0.5 impulses·s^{-1}·°C^{-1} as a minimum criterion for classification of warm sensitive neurons. This may have resulted in the classification of insensitive neurons as warm sensitive. In addition, the slice preparations were often treated with multiple consecutive exposures of PGE_2 at varying concentrations. Finally, the specific locations of the neurons were poorly characterized.

Another prominent study in 1992 (Matsuda et al.) used tissue slices containing the organum vasculosum of the lamina terminalis (OVLT) region of the hypothalamus to characterize the effect of PGE_2 on neuronal activity. The tissue slices were perfused with varying concentrations of PGE_2 during the recording of extracellular neuronal responses. This study reported that in response to PGE_2, the majority of warm sensitive neurons decreased their firing rates. However, some warm sensitive cells did show increases in firing rate in response to PGE_2. Once again, this study used an alternative thermocoefficient of only 0.7 impulses·s^{-1}·°C^{-1} to classify a neuron as warm sensitive. In addition, PGE_2 was only perfused for periods of two minutes, and responses had extremely variable times of onset and duration. As in the previous studies, the characterization of the specific location of recorded neurons was vague and without any morphological confirmation. Varying minimum criterion for classification of warm sensitive neurons and broad generalized characterization of a neuron's location in the hypothalamus are recurrent problems in several other similar studies and may be reasons for reported variability in neuronal response to PGE_2 (Hori et al., 1988; Nakashima et al., 1989; Janský et al., 1992).

6. Cellular actions of PGE_2

Throughout the nervous system, studies have focused on characterizing the mechanism through which PGE_2 can alter neuronal activity. Experimental findings support the notion that PGE_2-induced sensitization of sensory neurons results from activation of a cAMP transduced cascade (Lopshire & Nicol, 1998). Evidence for this is three-fold: PGE_2 increases intracellular levels of cAMP in sensory neurons, exogenous application of membrane-permeant analogs of cAMP mimics the sensitizing effect of PGE_2, and inhibition of the cAMP-dependant protein kinase (PKA) blocks sensitization to PGE_2. This study also showed that PGE_2, through the activation of the cAMP-PKA signaling pathway, increases whole-cell currents that are elicited by capsaicin. In contrast Evans et al. (1999) demonstrated that PGE_2 attenuated whole cell potassium currents in sensory neurons and that this suppression was also dependent on the activation of the cAMP-PKA transduction cascade. Within the hypothalamus, Griffin et al., (1990) showed that neurons increased firing rate activity with an increase of intracellular cAMP. This evidence suggest that changes in ionic conductance, due to cAMP, may be a possible mechanism for altering the activity of hypothalamic neurons. Therefore, within the hypothalamus, PGE_2 induced changes in neuronal activity may be due to cAMP dependant mechanisms.

7. Sites of Fever Induction

As stated previously, the POA has been identified as playing a primary role in thermoregulation (Boulant, 1991). Numerous studies over 50 years indicate that approximately 40% of the neurons in the POA are thermosensitive and have control over

thermoregulatory responses. Early lesion studies demonstrated that neurons in the POA are affected by pyrogenic substances (for reviews see, Boulant, 1991; Saper & Breder, 1994). More specifically, evidence suggests that the OVLT is a site at which circulating cytokine signals are transduced into the central nervous system and trigger change in neuronal activity, resulting in a typical fever response to infection (Elmquist et al., 1996). However, these early lesion studies generally removed more than just the OVLT, including several prominent nuclei in the POA (Blatteis et al., 1983; Katsura et al., 1990; Stitt, 1986).

To more precisely characterize the site of fever activation, Scammell et al. (1998) reported that an intravenous injection of LPS rapidly induced production of a critical enzyme involved in PG production, cyclooxygenase-2 (COX2). COX2 is seen in the perivascular microglia and leptomenigal macrophages and catalyzes the formation of PGs that can diffuse into adjacent brain regions to influence neural activity. With microinjection of keterolac, a COX2 inhibitor, this study was able to identify potential production sites of PGs and found that PG synthesis in the POA is necessary for fever after the intravenous injection of LPS. This research identified a specific cell group adjacent to the OVLT that is activated by LPS and important in the production of fever, the ventromedial preoptic area (VMPO).

8. The Ventromedial Preoptic Area

The role of VMPO as a site of fever induction has also been confirmed anatomically (Elmquist et al., 1996; Scammell et al., 1996). With injections of LPS, specific areas activated during a fever show labeling with Fos, an immediate early gene

product. Fos is expressed with increased levels of cellular activation and is used as a marker to identify extended neural systems activated by specific stimuli. Using this anatomical approach, the VMPO was identified as being physiologically activated during the generation of a fever (Elmquist et al., 1996). In addition, Scammell et al. (1996) utilized Fos to identify the pattern of neural activation induced by preoptic injection of PGE_2. Fos induction was seen in the VMPO as well as the paraventricular nucleus (PVH). This evidence suggests that PGE_2 activates the VMPO, which in turn stimulates the PVH to produce a fever response (Scammell et al., 1996; Figure I.3.).

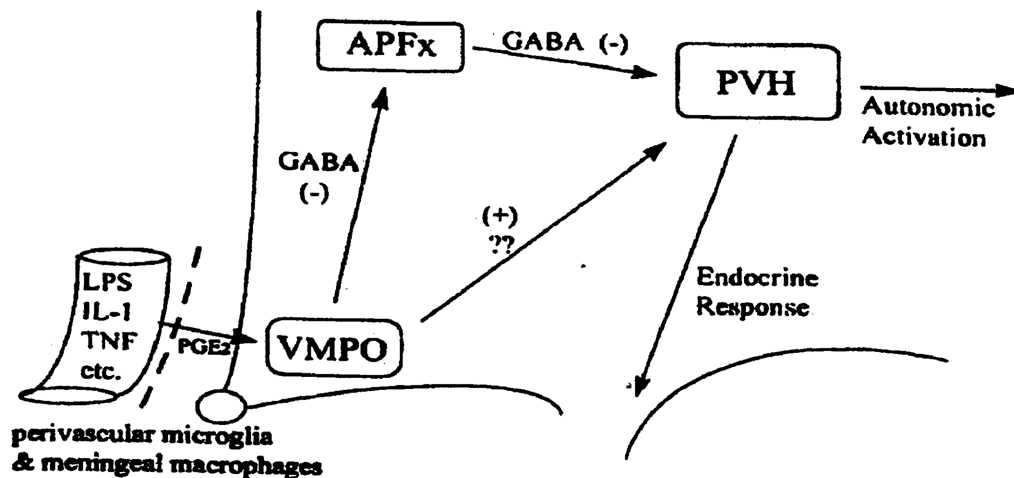

Figure I.3. A proposed model for the generation of fever

Abbreviations: APFx, anterior perifornical area; PVH, paraventricular nucleus; GABA, gamma amino butyric acid; VMPO, ventral medial preoptic area; LPS lippopolysaccharide; IL-1, interleukin-1β, PGE_2, prostaglandin E_2; TNF, tumor necrosis factor.

Further support for the importance of the VMPO in fever is found in recent studies that identified the location and activation of the specific PGE_2 receptor subtypes: EP_1, EP_2, EP_3, and EP_4. All four subtypes have been identified in the hypothalamus (Oka et al., 2000; Ushikubi et al., 2000). Some areas important to thermoregulation, like the POA, show overlapping expression of EP_1, EP_3, and EP_4 (Ek et al., 2000). The receptor subtypes that may be involved in febrile response remain hotly debated, partly because of this diffuse pattern of receptor expression, and partly because individual neurons may alter expression and affinity of receptors subtypes during the course of an inflammatory response (Ek et al., 2000; Oka et al., 2000).

9. Importance of EP_3 and EP_4 in the Generation of a Fever

Ushikubi et al. (2000) studied the responses of mice lacking the specific EP receptor subtypes to different pyrogenic substances. Mice lacking the EP_3 receptor did not develop a fever in response to centrally administered PGE_2. There was also no generation of a fever in response to peripheral or central administration of Il-1 or LPS. This evidence suggests that EP_3 is essential for a febrile response to infection. Another principal study used a combination of in situ hybridization and immunohistochemical techniques to determine the central distribution of EP_3 mRNA within the rat central nervous system, with an emphasis on Il-1 responsive cell groups (Ek et al., 2000). This study showed that the mRNA probe for the EP_3 receptor is expressed in a very restrictive manner in the brain, which included expression in the VMPO. Oka et al. (2000) further investigated the roles of all the receptor subtypes in association with LPS activated Fos. However, this research showed that the EP_3 receptor subtype did not appear within the

VMPO or OVLT in correlation with LPS activated Fos. This may be due to the possibility that Fos depends on the activation of cellular mechanisms. The activation of the EP_3 subtype has been shown to decrease the intracellular cAMP levels and may lead to the inhibition of cellular mechanisms and a lack of Fos activation (Oka et al., 1994; Oka et al., 2000).

This same study did, however, show a strong relationship between the expression of EP_4 receptors and Fos expression in the VMPO during intravenous LPS (Oka et al., 2000). Evidence also suggests that EP_4 activation will result in an increase in intracellular cAMP (Ek et al., 2000). Based on this data, the presence of EP_4 in the VMPO and apparent activation of Fos with LPS injection, the EP_4 receptor subtype has been implicated as having a role in altering the activity of neurons in the VMPO, resulting in a febrile response.

8. Summary and Hypotheses

Since previous studies only characterized the indirect responses of hypothalamic neurons to endogenous cytokines, this thesis will focus directly on characterizing the thermosensitivities of VMPO neurons and their responses to PGE_2. Research has shown PGE_2 is produced within the central nervous system in response to systemic LPS, followed by the generation of a fever. The presence and activation of PGE_2 receptors, and the activation of Fos during an immune system challenge in the VMPO, further emphasizes the importance of this area in the generation of a fever. However, there are no current studies looking at the neuronal activity specific to this region. Furthermore, little is known about the cellular conductances, which are involved in the changes of firing rate

activity in the hypothalamus that occurs in response to PGE_2, and how this may alter thermoregulatory responses to produce a fever.

From *in vitro* tissue slice preparations using single-unit extracellular recording techniques I proposed to characterize the firing rate activity of neurons in the VMPO with respect to temperature and PGE_2. Once this initial study was completed, I used a whole-cell patch recording technique examine the cellular conductances of VMPO neurons in response to PGE_2.

Hypothesis 1: In response to the local production of PGE_2, *temperature insensitive neurons in the VMPO will increase their spontaneous firing rate activity while warm sensitive neurons will decrease their spontaneous firing rate activity.*

Specific Aim 1: To determine the neurophysiologic responses of the VMPO neurons to local changes in temperature and exposure to PGE_2, which has been shown to be produced locally in response to a LPS immune system challenge.

Hypothesis 2: The VMPO neuronal changes in firing rate in response to PGE_2 *result from changes in cellular conductances that alter input resistance, membrane potential, and the generation of action potentials.*

Specific Aim2: To determine the cellular conductances involved in the changing spontaneous firing rate of the VMPO neurons in response to PGE_2 by examining input resistance, membrane potential, the generation of action potential, and synaptic input.

CHAPTER II

The Effects of Prostaglandin E_2 on the Firing Rate Activity of Thermosensitive and Temperature Insensitive Neurons in the Ventromedial Preoptic Area of the Rat Hypothalamus.

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Abstract

In response to an immune system challenge with lipopolysacharide (LPS), recent work has shown that Fos immunoreactivity is displayed by neurons in the ventromedial preoptic area of the hypothalamus (VMPO). In addition, neurons in this region show distinct axonal projections to the anterior perifornical area (APFx) and the paraventricular nucleus (PVN). It has been hypothesized that neurons within the VMPO may integrate their local responses to temperature, with changes in firing activity that result from LPS induced production of prostaglandin E_2 (PGE_2). This may play a critical role in altering the set-point regulation of thermoeffector neurons in the APFx and PVN, resulting in hyperthermia. To characterize the firing rate activity of VMPO neurons, single-unit recordings were made of neuronal extracellular activity in rat hypothalamic tissue slices. Based on the slope of firing rate as a function of tissue temperature, neurons were classified as either warm sensitive or temperature insensitive. Neurons were then treated with PGE_2 (200 nM) while tissue temperature was held at a constant level (~36°C). The

location of each neuron was later confirmed through histochemical staining. The majority of temperature insensitive neurons responded to PGE_2 with an increase in firing rate activity, while warm sensitive neurons showed a reduction in firing rate. This suggests that both warm sensitive and temperature insensitive neurons in the VMPO may play critical and contrasting roles in the production of a fever during an acute phase response to infection.

1. Introduction

The acute phase response is a multi-system coordinated reaction to immune stimulation that includes a wide variety of metabolic, endocrine, autonomic, and behavioral responses, which are controlled by the central nervous system (Saper & Breder, 1994). Current theories suggest that these responses are triggered by the actions of circulating endogenous pyrogens on vagal sensory pathways, as well as by altering the activity of hypothalamic neurons that are in close proximity to the organum vasculosum of the lamina terminalis (OVLT; Blatties, 2000). These varying pathways of activation may allow for specific immune conditions to elicit unique patterns of stimulation.

Fever, which is a component of the acute phase response, is an elevation in body temperature of 1-4°C. A suggested mechanism for this response is the up regulation of a thermostatic set-point (Boulant, 1998). This set-point is achieved through the integration of both central and afferent thermal information by neurons in the anterior regions of the hypothalamus, that can be classified as either inherently thermosensitive or temperature insensitive. These neurons regulate the activity of efferent pathways, to control thermoregulatory responses. During a fever, changes in the activity of neurons in the

anterior hypothalamus may be the mechanism by which the thermostatic set-point is adjusted into the hyperthermic range.

Several studies have shown that endotoxins such as lippopolysaccharide (LPS), a complex glycolipid found in the outer membrane of most gram-negative bacteria, stimulate leukocytes and other cells to produce certain endogenous substance that have pyrogenic effects (for review, Blatteis, 2000). These endogenous pyrogens may not act directly on hypothalamic neurons, but induce the production of prostaglandin E_2 (PGE_2) within specific regions of the hypothalamus. This hypothesis is supported by data indicating that in addition to a fever, intravenous injection of LPS resulted in an increased production within the hypothalamus of cyclooxygenase-2 (COX2), a primary enzyme in the synthesis of PGE_2 (Scammell et al., 1998). Further experiments using microinjections of keterolac (a COX2 inhibitor) identified discrete sites of activation that are necessary for fever. More specifically, this research identified a critical cell group adjacent to the OVLT that is now known as the ventromedial preoptic area (VMPO).

The role of the VMPO as a site of fever induction has also been confirmed anatomically. In response to intravenous injection of LPS or microinjection of PGE_2 directly into the VMPO, neurons in this region showed expression of Fos, an immediate early gene product that is present during increased levels of cellular activation (Elmquist et al., 1996; Scammell et al., 1996). The ability of neurons in the VMPO to respond to the local production of PGE_2 is also supported by evidence that all four PGE_2 receptor subtypes are present within the hypothalamus (Oka et al., 2000). Within the anterior regions of the hypothalamus, including the VMPO, there is overlapping expression of EP_1, EP_3, and EP_4 receptor subtypes (Ek et al., 2000). With respect to the generation of a

fever, recent work indicates that both EP_3 and EP_4 receptor subtypes are selectively important (Ushikubi et al., 2000; Ek et al., 2000; Oka et al., 1994).

If an increased concentration of PGE_2 in the anterior hypothalamus is responsible for the production of a fever, then it would be expected that a correlation exists between the thermosensitivity of neurons in this region and responses to PGE_2. Although several extracellular single-unit recording studies have characterized the effects of PGE_2 on the firing rate activity of hypothalamic neurons, no correlation with thermosensitivity has been reported. Matsuda et al. (1992) showed that PGE_2 decreased the firing rates of some warm sensitive neurons, while having no effect or increasing the firing rates of other warm sensitive neurons. This is in contrast to similar studies, which indicated that some cytokines selectively decreased the firing rates of warm sensitive neurons (Hori et al., 1988; Nakashima et al., 1989). However, these previous studies used varying criteria to define warm sensitivity and did not limit their recording locations to any specific functional nuclei within the anterior hypothalamus. Using a well established functional criteria to define thermosensitivity, our study has characterized the firing rate activity of VMPO neurons in response to temperature and PGE_2.

2. Materials and Methods

Hypothalamic tissue slices containing the VMPO were prepared from male Sprague-Dawley rats (100-150 grams in weight), which were housed under standard conditions and given food and water *ad lib.* Prior to each recording session, an animal was anesthetized using isoflourene and sacrificed by decapitation, according to procedures approved by the Animal Care and Use Committee of the College of William and Mary.

The brain was then quickly removed and a tissue block containing the hypothalamus was cut using a vibratome. Two or three coronal slices of the anterior hypothalamus, containing the VMPO, were sectioned and placed in a tissue chamber (Kelso et al., 1982). Slices were allowed to equilibrate for 2 hours before recordings were attempted.

Throughout the recording session, the tissue slices were perfused with a pyrogen free nutrient medium consisting of (mM): 124 NaCl, 26 $NaHCO_3$, 10 glucose, 5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, and 1.24 KH_2PO_4. This medium was oxygenated (95% O_2 - 5% CO_2), warmed to a constant temperature of approximately 36°C, and allowed to flow into the chamber at 1-1.5 $ml \cdot min^{-1}$ (Kelso et al., 1983). A small thermocouple was positioned just below the tissue slices to continuously monitor temperature.

Extracellular single-unit recordings were made from neurons in the VMPO using glass microelectrodes with tip diameters of less than 1 μm and filled with 3M NaCl. All recordings were made using an Xcell-3 Microelectrode Amplifier (FHC Inc.) and stored along with temperature on digital tape for later analysis. Once the activity of a neuron was isolated (signal : noise $\geq$ 3 : 1) and stable for several minutes, temperature in the recording chamber was varied 2-3°C above and below 36°C to determine responses to temperature. Neuronal thermosensitivity ($impulses \cdot s^{-1} \cdot {}^{\circ}C^{-1}$) was characterized by plotting firing rate as a function of temperature to determine the regression coefficient (m) of this plot. As in previous studies (Kelso et al., 1982; Griffin et al., 2001), warm sensitivity was defined as a regression coefficient of at least 0.8 $impulses \cdot s^{-1} \cdot {}^{\circ}C^{-1}$. All other neurons in this study were defined as temperature insensitive.

After the thermosensitivity of a neuron had been characterized, each neuron was tested for its response to PGE_2. Once a stable temperature was achieved (~36°C), the

perfusion medium was switched to one containing PGE_2 (200 nM, Sigma Chemical Co.). The duration of exposure to PGE_2 ranged from 5 - 15 minutes. Treatment was stopped prior to 10 minutes only when a change in firing rate (impulses·s^{-1}) was $\geq$ 15%. Exposure to PGE_2 was followed by a control period of at least 10 minutes. After the recording session, one minute samples of stable firing rate activity were digitized (60 Hz) for comparison (pClamp Software, Axon Instruments). These samples were collected during baseline conditions (just prior to perfusion with PGE_2), at the end of perfusion with PGE_2 or at the peak of a change in firing rate, and at the end of a 10 minute control period or when firing rate returned to baseline levels. For each sample of firing rate activity, a mean and standard error were calculated (Sigmaplot Software, SPSS Inc.). To characterize a significant response to perfusion with PGE_2, firing rate had to change at least 15% from baseline levels. In addition, the response had to be significant ($p \leq 0.05$), when compared to baseline levels using a standard T test.

Once a recording had been completed, a dissection microscope was used to visually confirm the location of the electrode. Tissue slices were then removed from the chamber and fixed in a 10% formalin solution for at least 2 hours. This was followed by at least 2 hours in a 30% sucrose solution and then tissue slices were sectioned again to a thickness of 50 μm. A giemsa staining procedure was used to identify specific hypothalamic areas so that the location of each electrode within the VMPO could be reconfirmed (Griffin et al., 2001).

3. Results

Thermosensitivity:

The thermosensitivities of 30 VMPO neurons were characterized and their firing rate activities studied in response to PGE_2. The majority of these neurons were classified as temperature insensitive (n=25; Table II.1.). Based on their responses to PGE_2, this class was divided into two groups: low-slope temperature insensitive neurons (n=19), which had thermosensitivities of 0.49 impulses$\cdot s^{-1}\cdot {}^{o}C^{-1}$ or less; and high-slope temperature insensitive neurons (n=6), which had thermosensitivities in a range of 0.5 - 0.79 impulses$\cdot s^{-1}\cdot {}^{o}C^{-1}$. All other neurons were classified as warm sensitive (n=5). There was no specific pattern to the distribution of these neurons throughout the VMPO (Figure II.1.).

Table II.1. Thermosensitivity of neurons in VMPO.

		Thermosensitivity: (impulses$\cdot s^{-1}\cdot {}^{o}C^{-1}$)		
Classification	N	Criteria	Mean $\pm$ S.E.	Range
Low-Slope Temperature Insensitive	19	$\leq$ 0.49	0.08 $\pm$ 0.05	-0.42 to 0.40
High-Slope Temperature Insensitive	6	0.5 - 0.79	0.58 $\pm$ 0.04	0.50 to 0.78
Warm Sensitive	5	$\geq$ 0.8	1.18 $\pm$ 0.10	0.95 to 1.50

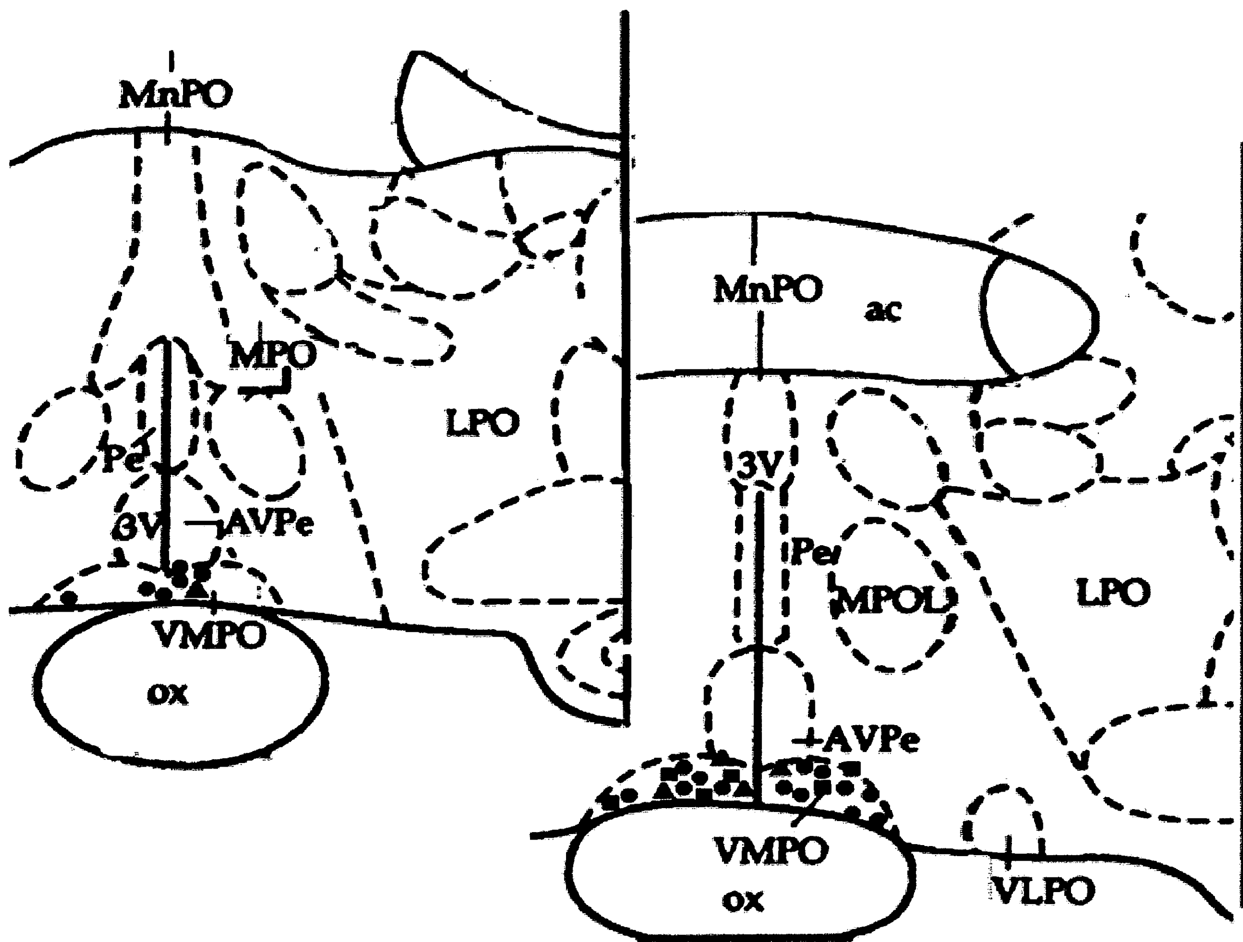

Figure II.1. Neuronal location of VMPO neurons shown in coronal sections

Key: ● are low-slope temperature insensitive neurons; ■ are high-slope temperature insensitive neurons; ▲ are warm sensitive neurons. 3V, third ventrical; ac, anterior commisure; AVPe anterior periventricular nucleus; LPO, lateral preoptic area; MnPO, medial preoptic area; ox, optic chiasm; Pe, periventriclar hypothalamic nucleus; VMPO, ventromedial preoptic nucleus. (Adapted from Paxinos & Watson, 1998).

Firing Rate Activity and Responses to PGE_2:

The mean baseline firing rate for all recorded VMPO neurons in this study was 4.41 ± 0.62 impulses·s^{-1}. Although the mean baseline-firing rate of low-slope temperature insensitive neurons was lower than high-slope temperature insensitive neurons, this difference was not significant (Table II.2.). Warm sensitive neurons also had a similar mean baseline firing rate, however, there was a large range within this class (1.87 -16.0 impulses·s^{-1}).

Table II.2. Effect of PGE_2 on the Spontaneous Firing Rate Activity of VMPO Neurons.

	Firing Rate: (impulses·s^{-1}± S.E.)		
Classification	Baseline	PGE_2 (200 nM)	Control
Low-Slope Temperature Insensitive	3.34 ± 0.60	6.39 ± 0.84*	3.59 ± 0.81
High-Slope Temperature Insensitive	6.49 ± 0.73	6.47 ± 0.76	6.51 ± 0.71
Warm Sensitive	5.29 ± 2.35	0.69 ± 0.68*	1.59 ± 1.41

*Significantly different from Baseline Firing Rate ($P< 0.01$)

In response to PGE_2, 17 of 19 low-slope temperature insensitive neurons showed a significant increase in firing rate, with an average increase of 90% above baseline. (Table II.2.). Figure II.2. shows the firing rate activity of a low-slope temperature insensitive neuron that relative to other neurons in this group, had a high baseline firing rate. In response to PGE_2, firing rate increased from 4.90 ± 0.16 impulses·s^{-1} to 11.63 ± 0.31 impulses·s^{-1}, and lasted approximately 20 minutes beyond when perfusion with PGE_2 was stopped.

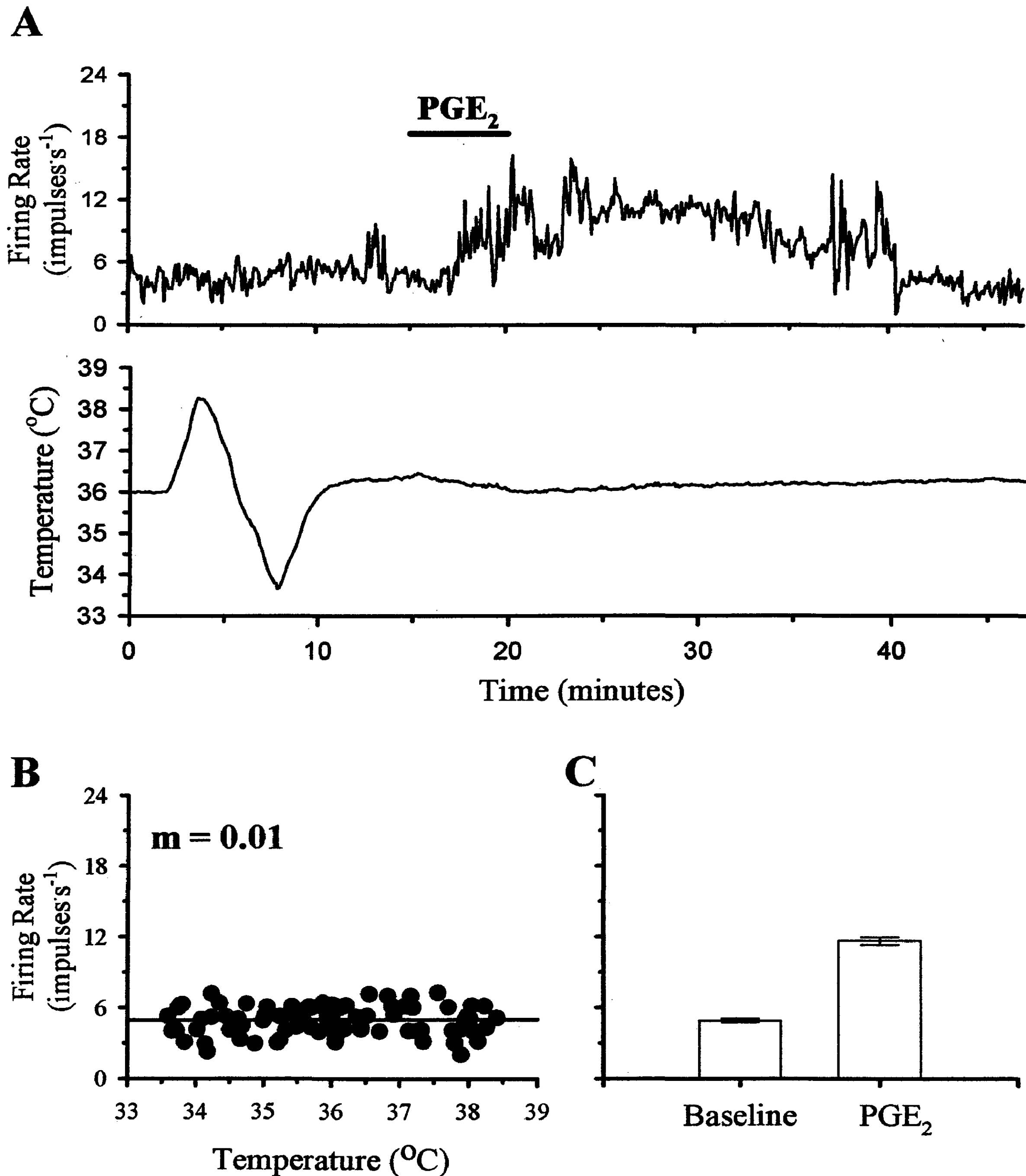

Figure II.2. The effects of temperature and PGE_2 on the firing rate activity of a VMPO low-slope temperature insensitive neuron

A shows the firing rate during changes in temperature and during perfusion with PGE_2. In **B**, firing rate is plotted as a function of temperature. In **C**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline; 4.90 ± 0.16) and during the peak of the response (PGE_2; 11.63 ± 0.31). For each plot in **C**, the error bars may be obscured.

Figure II.3. shows the firing rate activity of a low-slope temperature insensitive neuron, which had a low baseline firing rate. In response to PGE_2, firing rate increased from 0.27 $\pm$ 0.05 impulses$\cdot s^{-1}$ to 4.57 $\pm$ 0.15 impulses$\cdot s^{-1}$. This increase in firing rate lasted more than 45 minutes before returning to baseline levels.

Although high-slope temperature insensitive neurons showed some degree of thermosensitivity none of these neurons responded to PGE_2 with a significant change in firing rate (n=6; Table II.2.). Figure II.4. shows the firing rate activity of a high-slope temperature insensitive neuron. After determining this neuron's thermosensitivity, PGE_2 was added to the perfusion medium for 10 minutes, during which there was no change in firing rate.

All five VMPO neurons that were classified as warm sensitive responded to PGE_2 with a significant decrease in firing rate (Table II.2.). This inhibition had a long duration and only two neurons showed a return to baseline firing rate levels before recordings were terminated. Figure II.5. shows the firing rate activity of a warm sensitive neuron, which during perfusion with PGE_2, firing rate decreased by 95.6%. The activity of second warm sensitive neuron is detailed in Figure II.6. In response to PGE_2, firing rate decreased from 16.0 $\pm$ 0.07 to 0.11 $\pm$ 0.02 impulses$\cdot s^{-1}$ (99.3%). This response lasted for 25 minutes, without showing any indication of a return to the baseline level of activity.

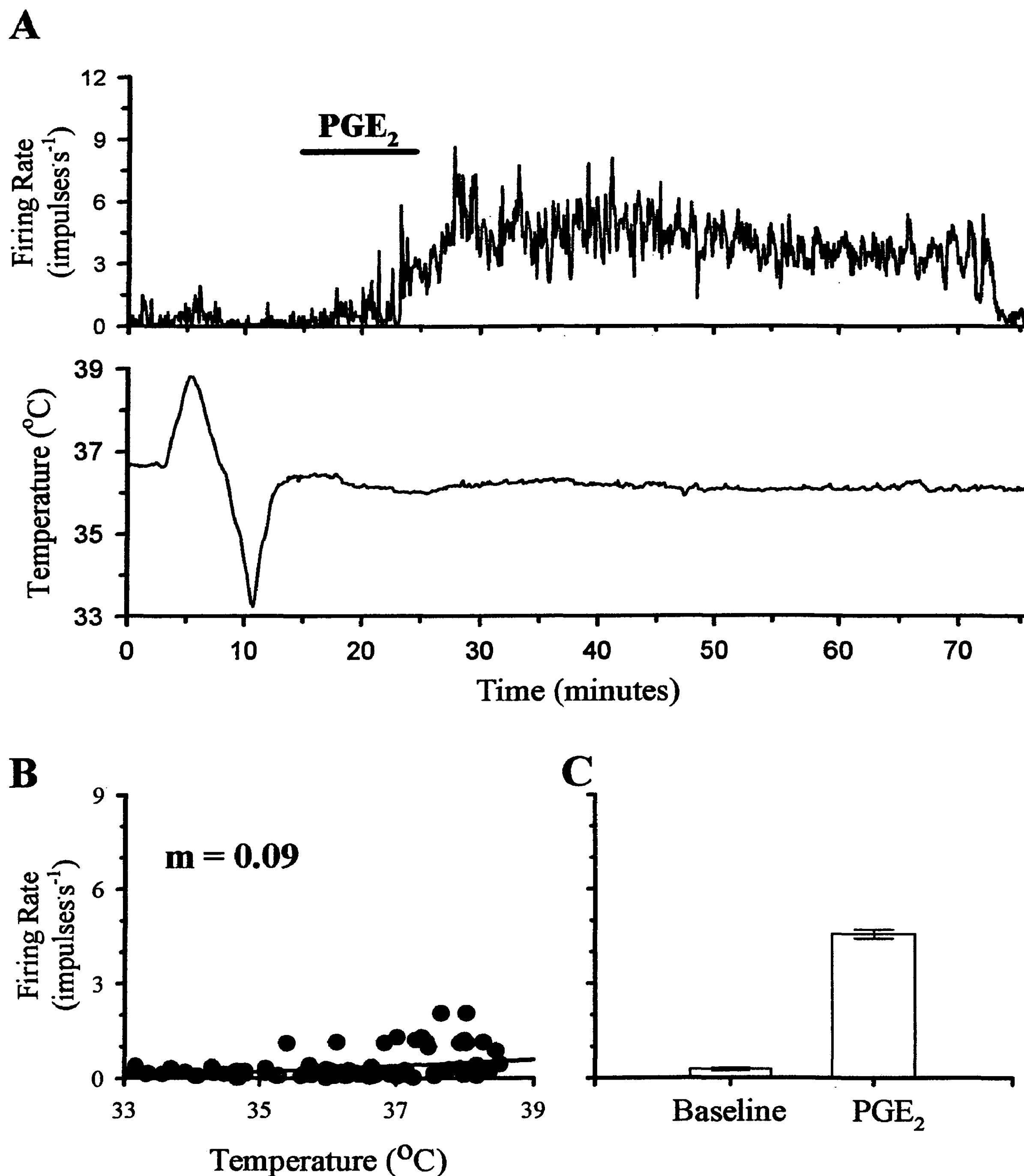

Figure II.3. The effect of temperature and PGE_2 on the firing rate activity of a VMPO low-slope temperature insensitive neuron

A shows the firing rate during changes in temperature and during perfusion with PGE_2. In **B**, firing rate is plotted as a function of temperature. In **C**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline; 0.27 ± 0.05) and during the peak of the response (PGE_2; 4.57 ± 0.15). For each plot in **C**, the error bars may be obscured.

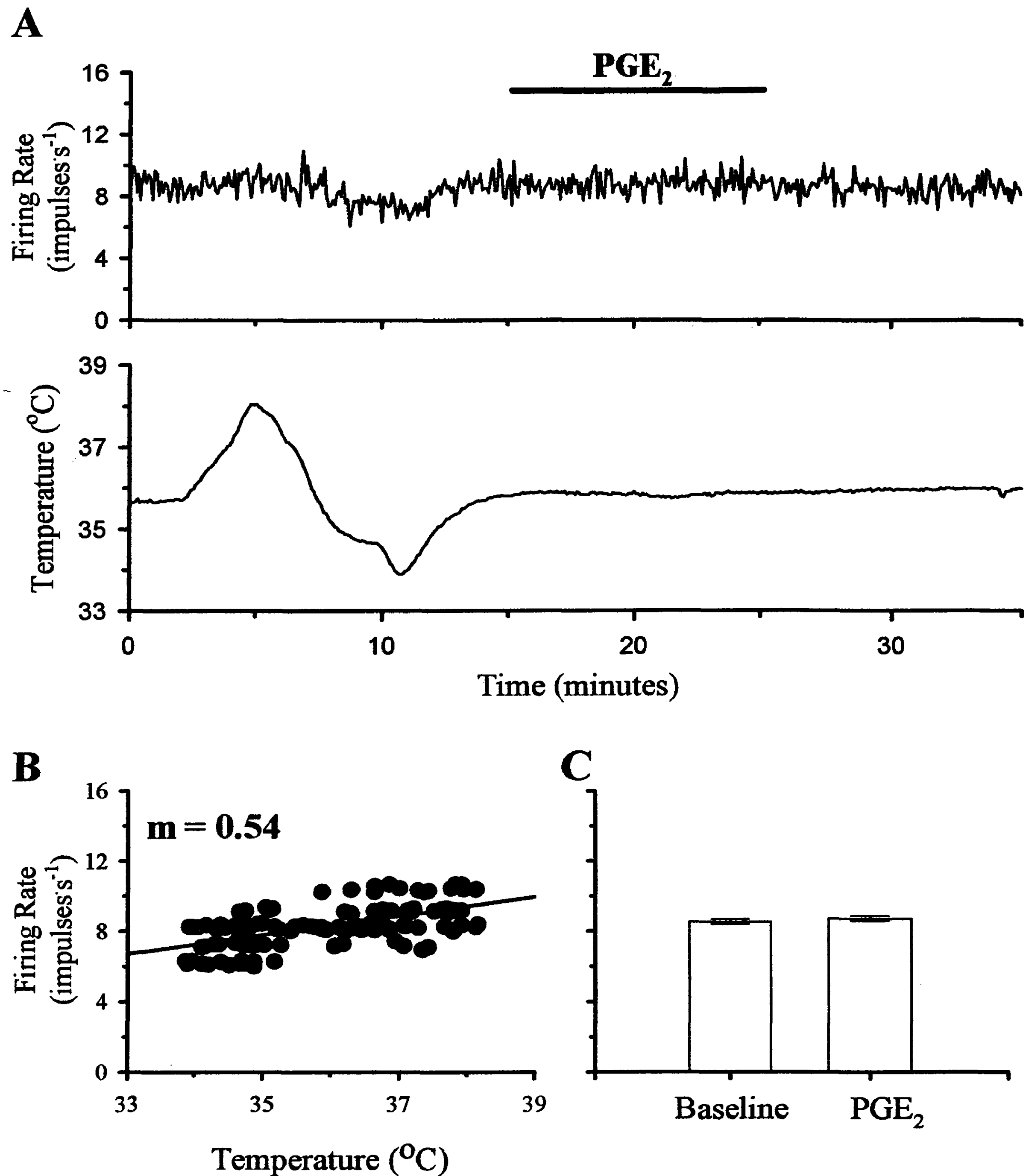

Figure II.4. The effect of temperature and PGE_2 on the firing rate activity of a VMPO high-slope temperature insensitive neuron

A shows the firing rate during changes in temperature and during perfusion with PGE_2. In B, firing rate is plotted as a function of temperature. In C, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline; 8.51 ± 0.14) and during the peak of the response (PGE_2; 8.71 ± 0.13). For each plot in C, the error bars may be obscured.

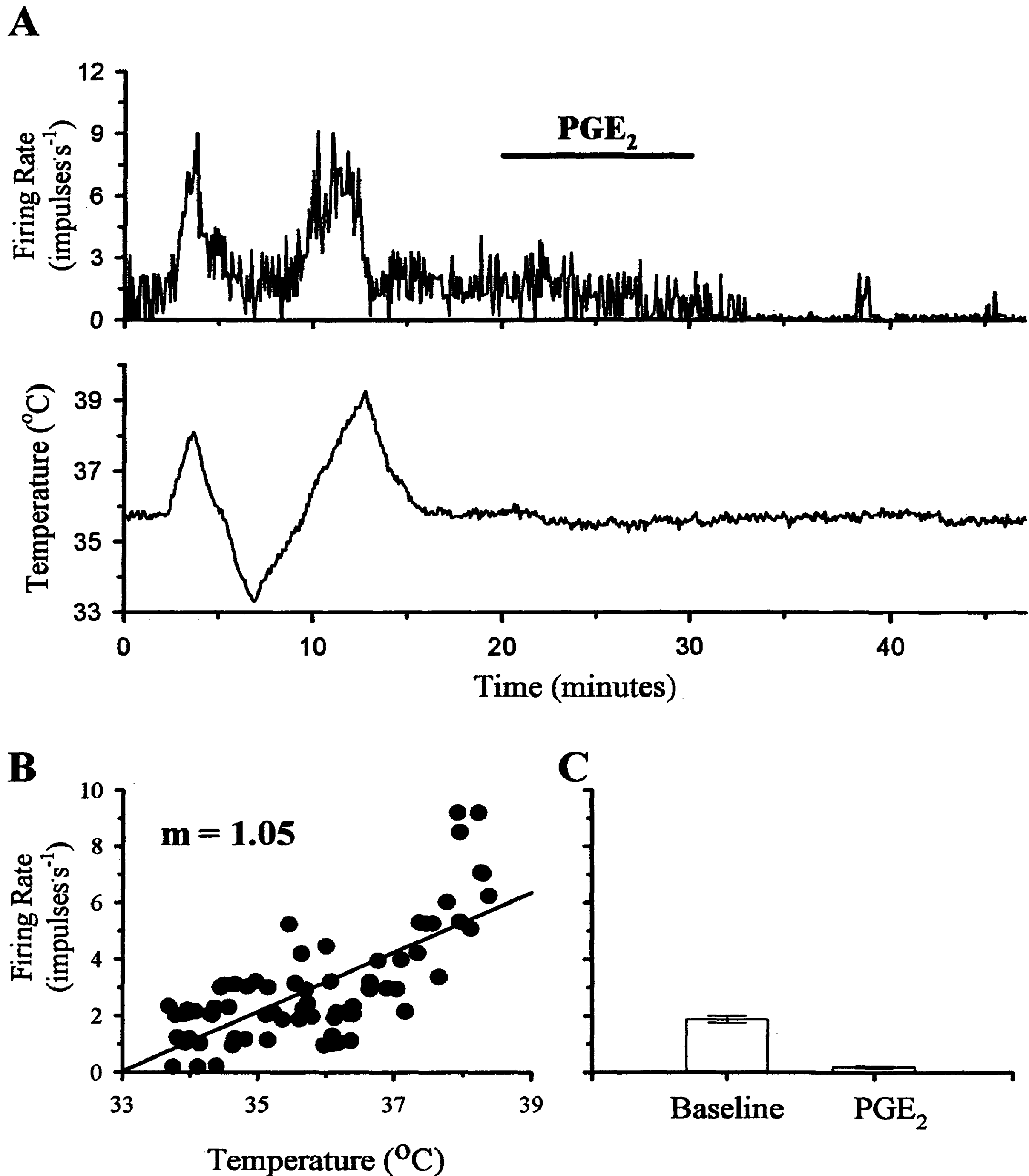

Figure II.5. The effect of temperature and PGE_2 on the firing rate activity of a VMPO warm sensitive neuron

A shows the firing rate during changes in temperature and during perfusion with PGE_2. In **B**, firing rate is plotted as a function of temperature. In **C**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline;1.59 $\pm$ 0.12) and during the peak of the response (PGE_2; 0.07 $\pm$ 0.03). For each plot in **C**, the error bars may be obscured.

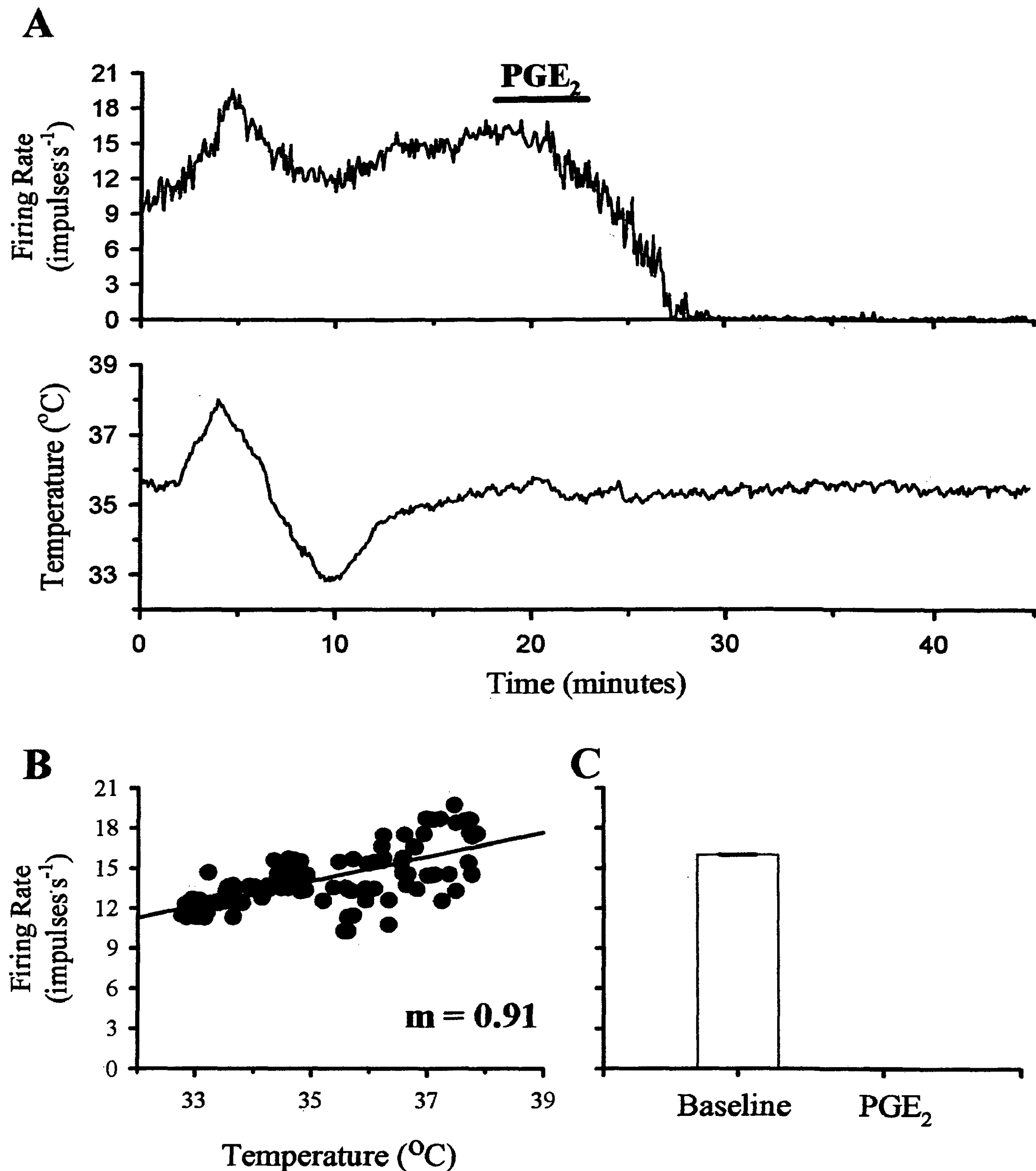

Figure II.6. The effect of temperature and PGE_2 on the firing rate activity of a VMPO warm sensitive neuron

A shows the firing rate during changes in temperature and during perfusion with PGE_2. In **B**, firing rate is plotted as a function of temperature. In **C**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline; 15.98 $\pm$ 0.07) and during the peak of the response (PGE_2; 0.11 $\pm$ 0.02). For each plot in **C**, the error bars may be obscured.

4. Discussion

An enduring model of temperature regulation suggests that a set-point for body temperature is achieved through the integration of both central and afferent thermal information by neurons within the hypothalamus (Hammel, 1965). This model has been supported by numerous studies, which indicate that the anterior hypothalamus has control over virtually all thermoregulatory responses, and is sensitive to changes in local temperature (Boulant, 1991). Within this region, approximately 35% of the neurons can be classified as warm sensitive. These neurons are not only inherently thermosensitive, but many are influenced by peripheral temperature and show a direct correlation of their firing rate activity with the stimulation of specific thermoregulatory responses. Recent data also indicates that warm sensitive neurons receive local synaptic input primarily from temperature insensitive neurons (Griffin et al., 2001). This input may provide a reference signal that contributes to the establishment of the thermostatic set-point.

It can then be hypothesized that either an increase in the firing rate activity of temperature insensitive neurons or a decrease in the firing rate activity of warm sensitive neurons could result in an adjustment of the set-point into the hyperthermic range. Although this model would suggest a strong correlation between inherent thermosensitivity and responses to PGE_2, previous electrophysiology studies have not presented support for this theory. As stated earlier, this may have been due to the lack of standard criteria for defining warm sensitivity and a general sampling of neurons from the anterior regions of the hypothalamus.

For the present study we used a criteria of $m \geq 0.8$ impulses$\cdot s^{-1} \cdot {}^{o}C^{-1}$ to define warm sensitivity. This is based on *in vivo* recordings indicating that neurons which meet

this criteria not only responded to local changes in temperature, but many were directly affected by afferent thermal input or showed a correlation in firing rate and the activation of specific thermoregulatory responses (Boulant & Bignall, 1973; Boulant & Hardy, 1974). Recent work also indicates that these warm sensitive neurons show a distinct pattern of dendritic morphology, which may be functionally significant (Griffin et al., 2001). Previous studies that investigated the effects of PGE_2 on the activity of hypothalamic neurons used criteria that ranged from 0.5 – 0.7 impulses$\cdot s^{-1} \cdot {}^{o}C^{-1}$ to define war sensitivity (Morimoto et al., 1988; Matsuda et al., 1992). Although we found that the VMPO had a smaller population of warm sensitive neurons compared to other regions of the anterior hypothalamus, all of these neurons responded to perfusion with PGE_2 with a significant decrease in firing rate (Table II.2.).

Based on their responses to PGE_2, temperature insensitive neurons were divided into two groups (Table II.2). High-slope temperature insensitive neurons did not respond significantly to PGE_2, suggesting that they may not be directly involved in the stimulation of a fever. However, the majority of low-slope temperature insensitive neurons responded to PGE_2 with a significant increase in firing rate activity. In a similar manner to the responses of warm sensitive neurons, PGE_2 dependent changes in firing rate had a slow onset and lasted for at least 10 minutes after perfusion with PGE_2 had ended. This would suggest an indirect mechanism of cellular activation, such as is typical with the activation of a second messenger pathway. Both the activation of EP_3 and EP_4 receptor subtypes, which have been linked to the development of a fever, have been shown to decrease intracellular cAMP or increase intracellular cAMP respectively (Ek et al., 2000; Oka et al., 1994; Oka et al., 2000).

Although neurons throughout the anterior hypothalamus may be responsive to endogenous substances such as PGE_2, there is evidence that discrete neuronal populations within this region may specifically regulate homeostatic mechanisms such as those involved in temperature regulation. With respect to the acute phase response to infection, neural activation of the VMPO has been established as a critical step in the production of a fever (Elmquist et al., 1997). In addition, two distinct groups of LPS induced Fos activated neurons in the VMPO have been characterized, which form local efferent projections that may underlie the adjustment of the thermostatic set-point that results in fever.

The larger of these two efferent pathways from the VMPO is an inhibitory projection to the anterior perifornical region (APFx), an area of the hypothalamus, which contains a high proportion of warm sensitive neurons (Elmquist & Saper, 1996; Dean & Boulant, 1989). From the APFx, Roland & Sawchencko (1993) have shown that there is a similar inhibitory projection to the autonomic parvicellular division of the PVN. Therefore, activation of this efferent pathway may result in the inhibition of warm-sensitive neurons in the APFx, decreasing the level of inhibition to the PVN. Consistent with this hypothesis and the current model of set-point thermoregulation, our data would suggest that this efferent pathway is made up of temperature insensitive neurons from the VMPO, that respond to PGE_2 with an increase in firing rate activity (Table 1).

A more restricted efferent projection also exists directly from the VMPO to the PVN (Elmquist & Saper, 1996). In contrast to previous electrophysiology studies of the anterior hypothalamus, we found that only a small percentage of neurons in the VMPO are warm sensitive. We also report that all of these warm sensitive neurons responded to

PGE_2 with a significant decrease in firing rate (Table II.2.). Thus, it may be suggested that warm sensitive neurons provide excitatory input to the PVN through this direct efferent projection. In response to increased concentrations of PGE_2 in the VMPO, the level of excitation would be reduced which may limit the activation of heat loss thermoregulatory responses (i.e., vasodilatation) and result in a fever.

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CHAPTER III

The Effects of Prostaglandin E_2 on the Cellular Properties of Thermally Classified Neurons in the Ventromedial Preoptic Area of the Rat Hypothalamus

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Abstract

Physiological and morphological evidence suggest that in response to an immune system challenge with lipopolysacharide, activation of the ventromedial preoptic area of the hypothalamus (VMPO) is important in the generation of fever. Extracellular single-unit recordings from the VMPO have provided evidence that prostaglandin E_2 (PGE_2) dependent changes in firing rate can be correlated with thermosensitivity. We have hypothesized that neuronal response to PGE_2 result from changes in cellular conductances that lead to a change in firing rate. To characterize the cellular properties of VMPO neurons, whole-cell patch clamp recordings were made in hypothalamic tissue slices. Neurons were classified as either warm sensitive ($m \geq 0.8$ imp·s^{-1}·°C^{-1}) or temperature insensitive. At a constant temperature of ~36°C, tissue slices were perfused with PGE_2 (200nM or 1µM). In response to either 200 nM or 1 µM concentration of PGE_2, all recorded neurons showed a decrease in input resistance. However in response to a depolarizing current, temperature insensitive neurons responded to PGE_2 with an increased firing rate frequency and a decreased delay in the onset of action potential

generation, while warm sensitive neurons decreased firing rate frequency. The membrane potential of temperature insensitive neurons also depolarized in response to PGE_2. Changes in firing rate activity were only seen in response to 1 μM PGE_2. Insensitive neurons showed an increase in firing rate activity, while warm sensitive neurons showed a decrease in firing rate activity. In addition there was no change in local synaptic input in response to temperature or PGE_2.

1. Introduction

Fever, an elevation in body temperature, is thought to play an adaptive role in the immune system's attempts to fight invading infectious organisms throug the actions of endogenous pyrogens directly on the central nervous system (Saper & Breder, 1994). A suggested mechanism for the induction of fever is the up regulation of the thermostatic set-point for body temperature (Saper & Breder, 1994; Boulant, 1998). This set-point is achieved through the integration of both central and afferent thermal information by neurons in the anterior regions of the hypothalamus, that can be classified as either inherently thermosensitive or temperature insensitive (Boulant, 1980). These neurons regulate the activity of efferent pathways that originate in the paraventricular nucleus (PVN), to control the responses of thermoregulatory effector mechanisms.

During a fever, changes in the inherent activity of neurons in the anterior hypothalamus may be the mechanism by which the thermostatic set-point is adjusted into the hyperthermic range. Evidence suggests that this response is due to endogenously produced cytokines, such as prostaglandin E_2 (PGE_2; Saper & Breder, 1994; Boulant, 1991). It has also been established that fever can be generated by the stimulation of

neural activity across the organum vasculosum lamina terminalis (OVLT), which is in close proximity to the thermoregulatory control centers of the hypothalamus. One of these control centers, the ventromedial preoptic area of the hypothalamus (VMPO) has been shown to be activated by the local production of PGE_2 (Scammell et al., 1996).

The role of the VMPO as a site of fever induction has also been confirmed anatomically. In response to intravenous injection of LPS or microinjection of PGE_2 directly into the VMPO, neurons in this region showed expression of Fos, an immediate early gene product that is present during increased levels of cellular activation (Elmquist et al., 1996; Scammell et al., 1996). The ability of neurons in the VMPO to respond to the local production of PGE_2 is also supported by evidence that all four PGE_2 receptor subtypes are present within the hypothalamus (Oka et al., 2000). Within the VMPO, there is overlapping expression of EP_3 and EP_4. Both of these receptor subtypes are activated during a fever response and result in a decrease in intracellular cAMP or an increase in intracellular cAMP, respectively (Ek et al., 2000; Oka et al., 1994; Oka et al., 2000; Ushikubi et al., 2000). Changes in ionic conductance, due to cAMP, may be a possible mechanism for altering the activity of hypothalamic neurons. (Lopshire & Nicole, 1998; Evans et al., 1999; Griffin et al., 1990).

Physiological and morphological evidence suggests that in response to an immune system challenge, activation of neurons in the VMPO is an important component in the generation of fever. Single-unit recordings from the VMPO have provided evidence that PGE_2 dependent changes in firing rate can be correlated with thermosensitivity, in which warm sensitive neurons are inhibited and temperature insensitive neurons are excited. These contrasting responses of VMPO neurons may be explained by the presence of

different PGE_2 receptors, which may alter the activity of specific cellular conductances. The present study utilizes intracellular whole-cell patch recording techniques to examine changes in cellular conductances that may lead to PGE_2 induced changes in the firing rate activity of the VMPO neurons.

2. Materials and Methods

Hypothalamic tissue slices containing the VMPO were prepared from male Sprague-Dawley rats weighing 100-150 grams. All animals were housed under standard conditions and given food and water *ad lib*. Prior to each recording session, an animal was anesthetized using isoflourene and sacrificed by quick decapitation, according to procedures approved by the Animal Care and Use Committee of the College of William and Mary. Following removal of the brain, a tissue block containing the hypothalamus was cut using a vibratome into 400 μm thick slices (Kelso et al., 1982). Two or three coronal slices of the anterior hypothalamus containing the VMPO were then placed in a recording chamber and allowed to equilibrate 1-2 hours before recordings were attempted.

Slices were continually perfused with pyrogen free artificial cerebral spinal fluid (aCSF), which consisted of (in mM), 124 NaCl, 26 $NaHCO_3$, 10 glucose, 5 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, and 1.24 KH_2PO_4. This nutrient medium was oxygenated (95% O_2-5% CO_2) and warmed to a constant temperature of ~36°C by a thermoelectric assembly and allowed to flow into the chamber at 1-1.5 $ml \cdot min^{-1}$ (Kelso et al., 1983). A small thermocouple was positioned just below the tissue slices to continuously monitor temperature.

Tight-seal whole-cell recordings (WCR) were made using glass microelectrodes with tip diameters of 2 μm, filled with a solution that consisted of (in mM) 130 K-gluconate, 10 EGTA, 2 ATP, 1 $MgCl_2$, 1CaCl, having a pH of 7.2 and an osmolarity of 295 mOsmols. Acceptable recordings consisted of action potential amplitudes through zero and stable recordings for at least 20 minutes. As described previously (Griffin & Boulant, 1995), the liquid junction potential was experimentally determined to be 12.0 mV, and was subtracted from all recorded potentials.

Recordings were made using an integrated patch-clamp amplifier (Axopatch 200B amplifier, Axon Instruments). Specific protocols to measure input resistance and action potential activity were generated by a Pentium computer that was interfaced with the amplifier (using pClamp software and digital interface, Axon Instruments). Continuous recordings of membrane potential activity and temperature were also stored on digital tape for later analysis.

A stereomicroscope was used to place each recording electrode into the VMPO. Once the electrode was positioned against the surface of a neuron and a gigaohm seal achieved, the cell membrane was ruptured by suction, establishing an intracellular recording. When the activity of the neuron was stable for several minutes, temperature in the recording chamber was varied 2-3°C above and below 36°C. Neuronal thermosensitivity (impulses$\cdot s^{-1} \cdot °C^{-1}$) was characterized by plotting firing rate as a function of temperature to determine the regression coefficient (m) of this plot. As in previous studies (Kelso et al., 1982; Griffin et al., 2001), warm sensitivity was defined as a regression coefficient of at least 0.8 impulses$\cdot s^{-1} \cdot °C^{-1}$. All other neurons in this study were defined as temperature insensitive.

After the thermosensitivity of a neuron had been characterized, each neuron was tested for its response to PGE_2 (200nM or 1μM). At a stable temperature (~36°C), the perfusion medium was switched to one containing PGE_2. The duration of exposure to PGE_2 ranged from 5-15 minutes with treatment being stopped prior to 10 minutes only if a notable response had occurred. Exposure to PGE_2 was followed by a perfusion with the pyrogen free aCSF for a control period for at least 10 minutes. A standard input resistance test (Griffin & Boulant, 1995) and a small depolarizing current (50pA, 500 mSec) were administered every 5 minutes throughout the recording session.

To determine if PGE_2 had a significant effect on firing rate activity, one-minute samples of stable activity were digitized (60 Hz) for comparison (pClamp Software, Axon Instruments). These samples were collected during baseline conditions (just prior to perfusion with PGE_2) at the end of perfusion with PGE_2, and at the end of a 10-minute control period (or when firing rate returned to baseline levels). For each sample, a mean and standard error were calculated (Sigmaplot Inc. Software). To characterize a significant response to perfusion with PGE_2, firing rate had to change at least 15 % from baseline levels. In addition, the response had to be significant ($p \leq 0.05$), when compared to baseline levels using a standard T test.

Input resistance was determined by the slope of a current-voltage plot obtained from a standard test in which ten hyperpolarizing current injections (ranging from –10 to –100 pA) were administered Current pulses of 210 mSec durations were used to insure that the membrane capacitance was fully charged, and only the linear portion of the potentials were plotted. In response to a depolarizing current (50 pA, 500 mSec), a change in action potential frequency was determined by the difference in the number of

potential before and during the depolarizing current. In addition the delay to onset of action potential generation was measured from the start of the depolarizing current to the peak of the first action potential.

A baseline membrane potential was also determined by measuring the midpoint between start of the prepotential and threshold. When comparing changes in these characteristics, significant differences were determined using a paired t-test ($p \geq 0.05$), and are reported as a mean $\pm$ standard error.

Synaptic input was also measured and classified as excitatory post-synaptic potentials (EPSPs) and inhibitory post-synaptic potentials (IPSPs). Individual potentials were identified as rapid changes in membrane potential of at least 1mV greater then background noise (Burgoon & Boulant, 1998). Frequencies of EPSPs and IPSPS were determined at baseline, during perfusion with PGE_2 exposure, and during control. For each sample, the number of postsynaptic potentials were counted over for 20 seconds. From these data, frequency averages and standard errors were obtained and reported. Thermosensitivities of synaptic input was determined by frequencies collected at three different temperatures (33°C, 36°C, and 38°C). From these data, frequency averages and standard errors were obtained and plotted as a function of temperature.

Once a recording had been complete, a dissection microscope was used to visually confirm the location of the electrode. Tissue slices were then removed from the recording chamber and fixed in a 10% formalin solution for at least 2 hours (Viana et al., 1990). This was followed by at least 2 hours in a 30% sucrose solution and sectioned again to a thickness of 50 μm. Sections were then stained with giemsa to identify specific

hypothalamic areas (Griffin et al., 2001). The location of each recording electrode within the VMPO was then reconfirmed.

3. Results

Thermosensitivity:

The thermosensitivities of 26 VMPO neurons were classified and cellular properties characterized in response to PGE_2. Intracellularly recorded neurons in the VMPO had similar characteristics to neurons recorded from this region using extracellular single-unit techniques. The majority of these neurons were classified as temperature insensitive (n=19; Table III.1.). Based on their responses to PGE_2 and previous extracellular recordings, these neurons were divided into two classes: low-slope temperature insensitive neurons (n=14), which had thermosensitivities of 0.49 impulses$\cdot s^{-1} \cdot {}^{\circ}C^{-1}$ or less; and high-slope temperature insensitive neurons (n=5), which had thermosensitivity in a range of 0.5-0.79. All other neurons were classified as warm sensitive (n=7). There was no significant difference in the membrane potentials or baseline firing rates between these classes of neurons. There was also no specific pattern to the distribution of these neurons throughout the VMPO (Figure III.1).

Table III.1 Thermosensitivity, baseline firing rate activity, and membrane potential of VMPO neurons.

Class	**N**	Thermosensitivity (impulses·s^{-1}·°C^{-1})	Firing Rate (impulses·s^{-1})	Membrane Potential (mV)
Low-Slope Temperature Insensitive	14 (54%)	0.21 ± 0.05	6.65 ± 1.60	-42.39 ± 2.64
High-Slope Temperature Insensitive	5 (19%)	0.62 ± 0.02	8.94 ± 1.52	-40.44 ± 1.13
Warm	7 (27%)	1.10 ± 0.12	12.54 ± 2.48	-40.40 ± 3.69

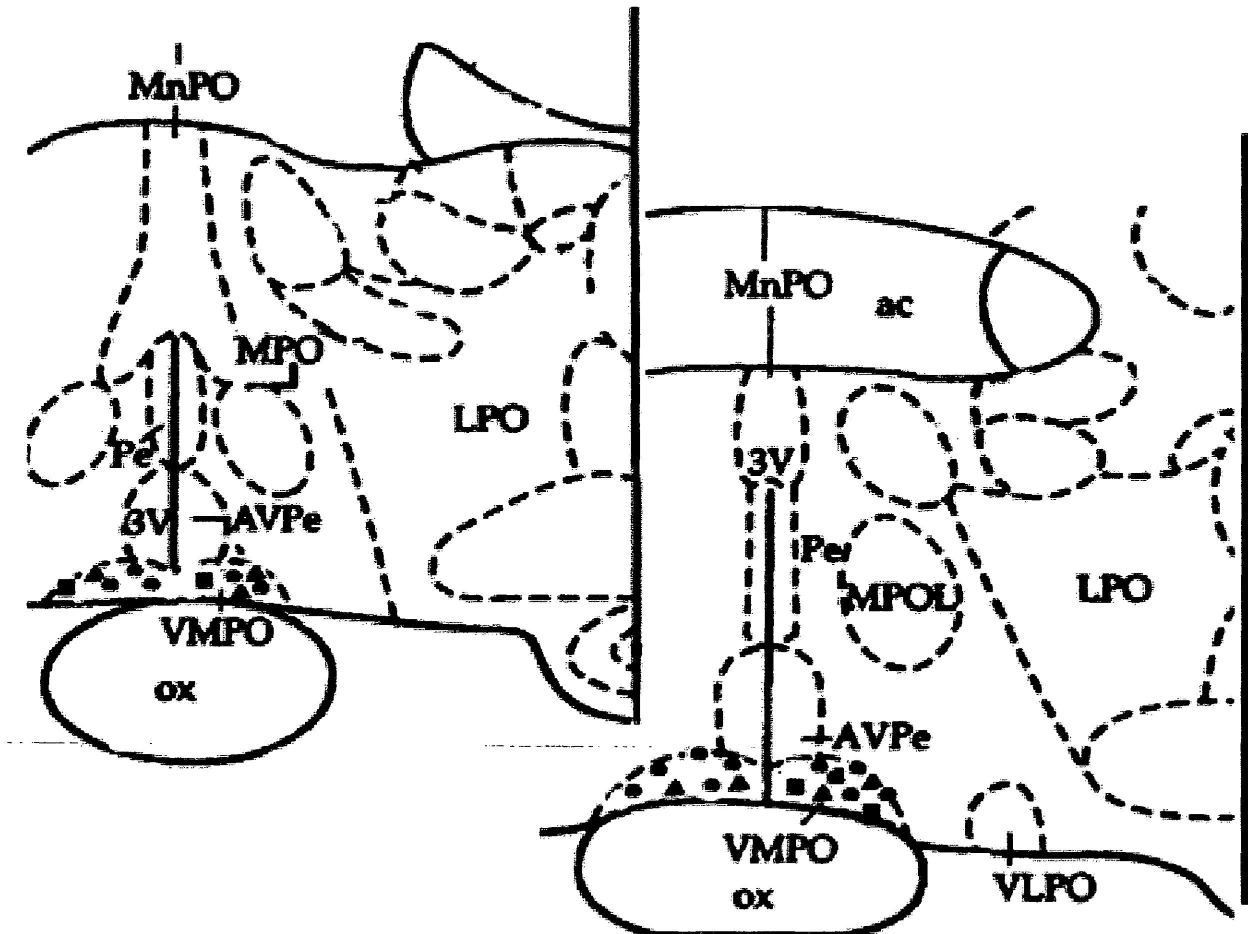


Figure III.1. Neuronal location of VMPO neurons shown in a coronal section.

Key: ● are low-slope temperature insensitive neurons; ■ are high-slope temperature insensitive neurons; ▲ are warm sensitive neurons. Abbreviations: 3V, third ventrical; ac, anterior commisure; AVPe anterior periventricular nucleus; LPO, lateral preoptic area; MnPO, medial preoptic area; ox, optic chiasm; Pe, periventriclar hypothalamic nucleus; VMPO, ventromedial preoptic nucleus.

Intracellular properties:

Regardless of thermosensitivity, all neurons tested showed a significant decrease in input resistance from 286.8 $\pm$ 12.3 mOhms during baseline conditions to 258.0 $\pm$ 11.6 mOhms during perfusion to PGE_2 (200 nM or 1 μM). In response to a depolarizing current (50pA, 500 mSec), low-slope temperature insensitive neurons showed a significant increase in firing rate frequency during perfusion with PGE_2 (200 nM or 1 μM) from 22.1 $\pm$ 2.0 imp·s^{-1} during baseline to 26.0 $\pm$ 1.6 imp·s^{-1} in response to PGE_2. In contrast, warm sensitive neurons showed a decrease in firing rate frequency (25.6 $\pm$ 4.3 imp·s^{-1} to 13.6 $\pm$ 5.2 imp·s^{-1}). High-slope temperature insensitive neurons showed little or no change firing rate frequency due to depolarizing current during perfusion with PGE_2.

In addition to changes in firing rate frequency, low-slope temperature insensitive neurons showed significant decrease in delay onset of action potential generation during a depolarizing current (50 pA, 500 mSec) in response to PGE_2 (200nM or 1 μM; Table III.2.). Figure III.2. shows the effect of depolarizing current and PGE_2 on the activity of a low-slope temperature insensitive neuron. The baseline to delay to the onset of action potential generation is 8.0 mSec (Figure III.2.A). In response to PGE_2, the delay to the onset of action potential generation decreased to 3.0 mSec (Figure III.2.B.) In addition the firing rate frequency increased from 32.0 impulses·s^{-1} to 34.0 impulses·s^{-1} during perfusion with PGE_2.

In contrast, warm sensitive neurons did not show a change in delay to the onset of action potential generation in response to a depolarizing current, during perfusion with PGE_2 (Table III.2). Figure III.3 shows the effect of depolarizing current and PGE_2 on the activity of a warm sensitive neuron. The delay to the onset of action potential generation

was 5.0 mSec during both baseline conditions and perfusion with PGE_2. However firing rate frequency decreased from 36 impulses·s^{-1} to 2.0 impulses·s^{-1}. High-slope temperature insensitive neurons did not show a change in onset of action potential generation or firing rate frequency, in response to depolarizing current and perfusion with PGE_2.

Table III.2. Delay to the onset of action potential generation due to depolarizing current (50pA, 500 mSec) during baseline, PGE_2 (200 nM or 1 uM), and control periods.

Class	N	Baseline	PGE_2 (200 nM or 1 μM)
Low-Slope Insensitive	13	9.31 ± 1.41 mSec	6.69 ± 0.67 mSec*
High-Slope Insensitive	5	10.82 ± 1.56 mSec	10.42 ± 1.62 mSec
Warm Sensitive	6	6.83 ± 1.47 mSec	8.50 ± 1.71 mSec

*Significantly different from Baseline.

Figure III.2. The effect of depolarizing current and PGE_2 on the activity of a VMPO low-slope temperature insensitive neuron.

A shows the action potential activity during baseline conditions, in response to a depolarizing current injection (50 pA, 500 msec). The onset of the current is indicated on the line at the bottom of the figure and only the initial 62.0 mSec are shown. The delay to the onset of action potential generation was 8.0 mSec and the change in firing rate frequency was 32.0 impulses$\cdot s^{-1}$. In **B**, the action potential activity in response to a depolarizing current injection is shown during perfusion with PGE_2 (1 μM). The delay to onset of action potential generation was 3.0 mSec and the change in firing rate frequency was 34.0 impulses$\cdot s^{-1}$.

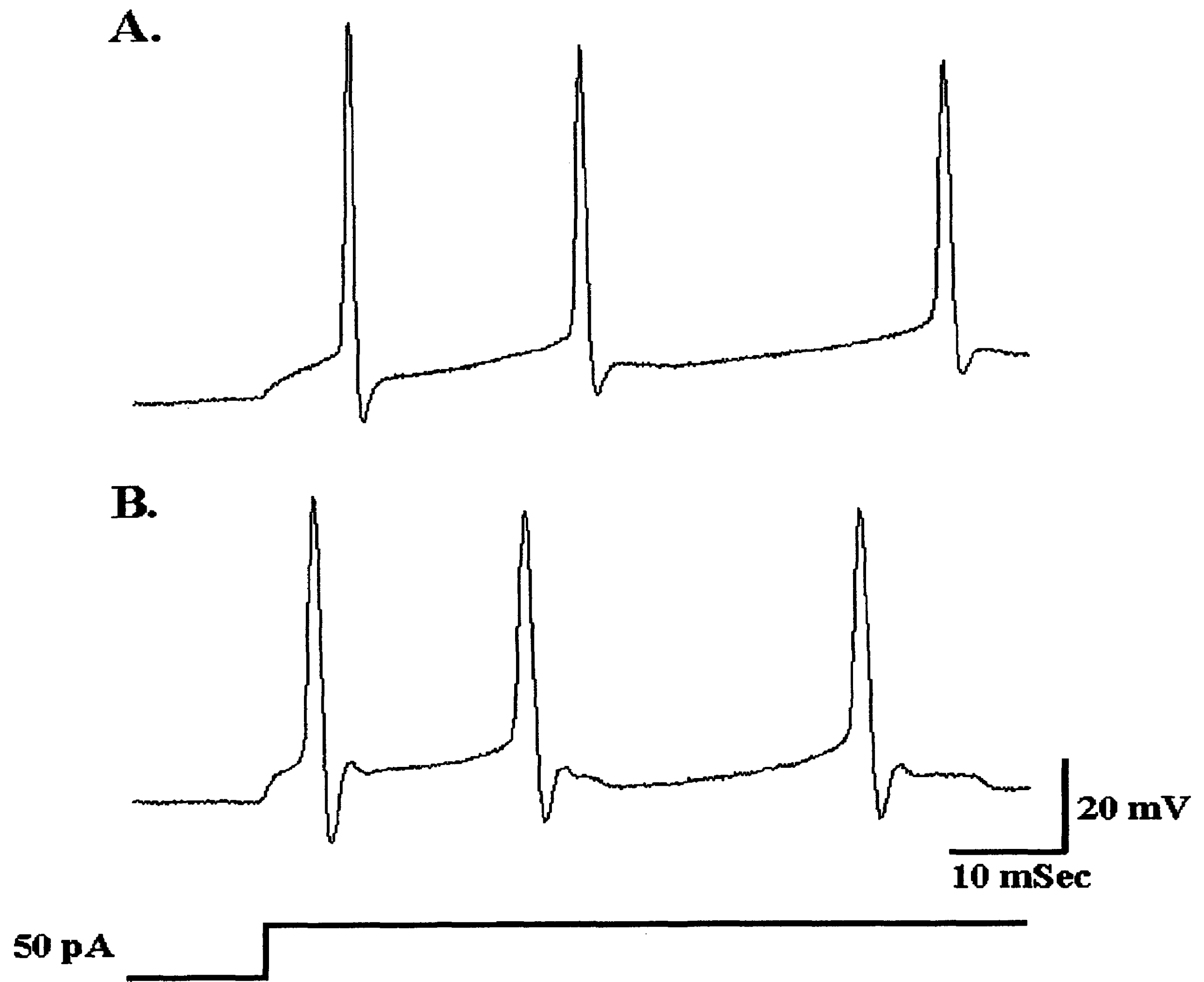
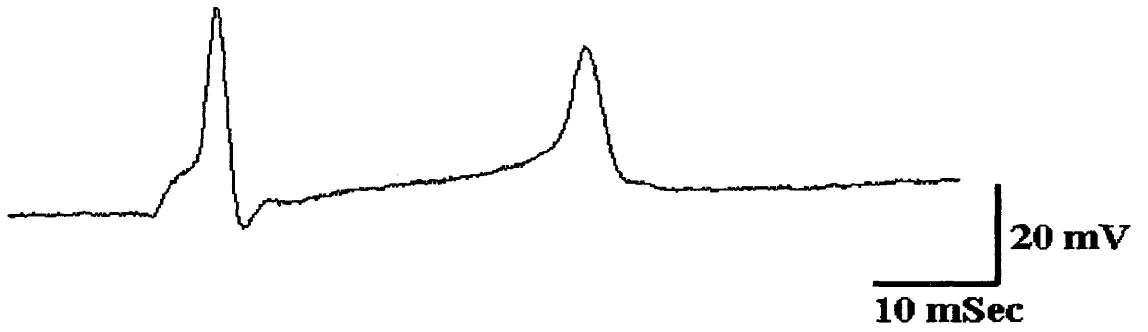

A.
B.
20 mV
10 mSec
50 pA

Figure III.3. The effect of depolarizing current and PGE_2 on the activity of a VMPO warm sensitive neuron.

A shows the action potential activity during baseline conditions, in response to a depolarizing current injection (50 pA, 500 msec). The onset of the current is indicated on the line at the bottom of the figure and only the initial 62.0 mSec are shown. The delay to the onset of action potential generation was 5.0 mSec and the change in firing rate frequency was 36.0 impulses$\cdot s^{-1}$. In **B**, the action potential activity in response to a depolarizing current injection is shown during perfusion with PGE_2 (1 μM). The delay to onset of action potential generation was 5.0 mSec and the change in firing rate frequency was 2.0 impulses$\cdot s^{-1}$.

A.

B.



50 pA

Firing Rate Activity Responses to PGE_2:

Regardless of thermosensitivity, no significant changes in firing rate were recorded during perfusion with 200 nM PGE_2. However in response to 1 μM PGE_2, all neurons classified as low-slope temperature insensitive showed a significant increase in firing rate, while warm sensitive neurons showed a decrease in firing rate (Table III.3.). There were no high-slope temperature insensitive neurons recorded from during the perfusion with 1 μM PGE_2.

Table III.3. Firing Rate responses to 1 μM PGE_2

Class	N	Baseline	PGE2 (1 uM)
Low-Slope Insensitive	6	5.63 ± 2.93 imp $\cdot s^{-1}$	7.01 ± 3.26 imp $\cdot s^{-1}$*
Warm Sensitive	2	14.01 ± 8.18 imp $\cdot s^{-1}$	10.01 ± 8.83 imp $\cdot s^{-1}$

*Significantly different from Baseline.

Figure III.4. shows the thermosensitivity and the effect of 1 μM PGE_2 on the firing rate activity of a VMPO low-slope temperature insensitive neuron, that had a low baseline firing rate of 0.15 ± 0.03 impulses$\cdot s^{-1}$, which increased in response to PGE_2 to 2.76 ± 0.10 impulses$\cdot s^{-1}$. In Figure III.5 the firing rate activity of a low-slope temperature insensitive neuron that had a high baseline firing rate of 3.80 ± 0.12 impulses$\cdot s^{-1}$ is shown. In response to PGE_2, firing rate significantly increased to 8.13 ± 0.03 impulses$\cdot s^{-1}$.

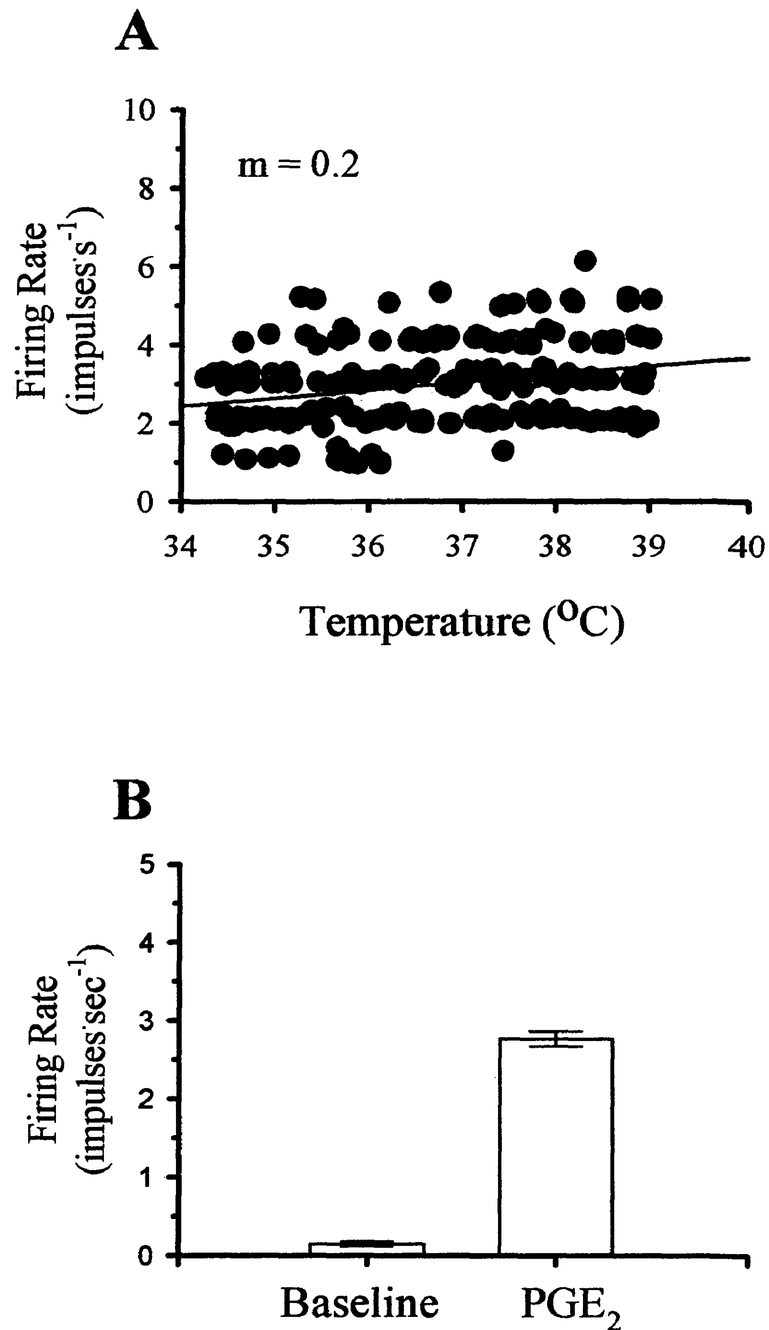
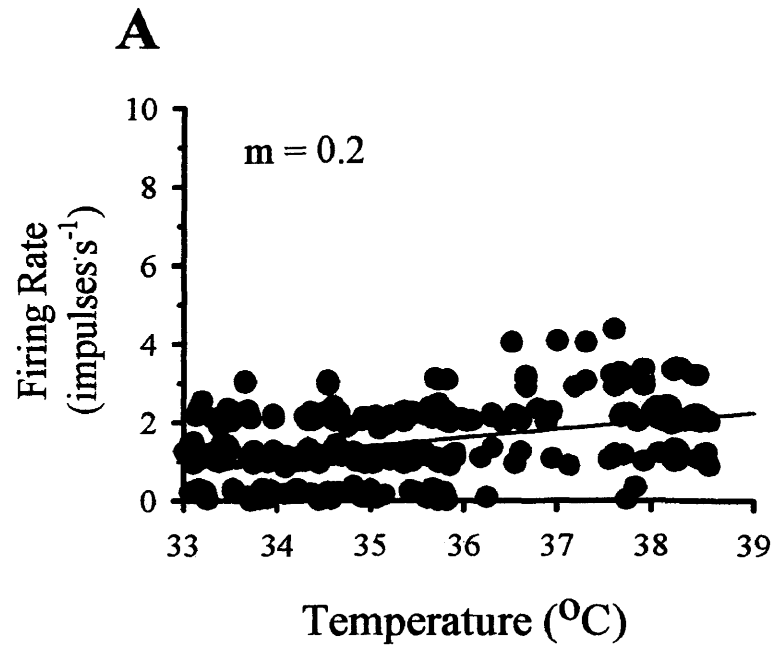
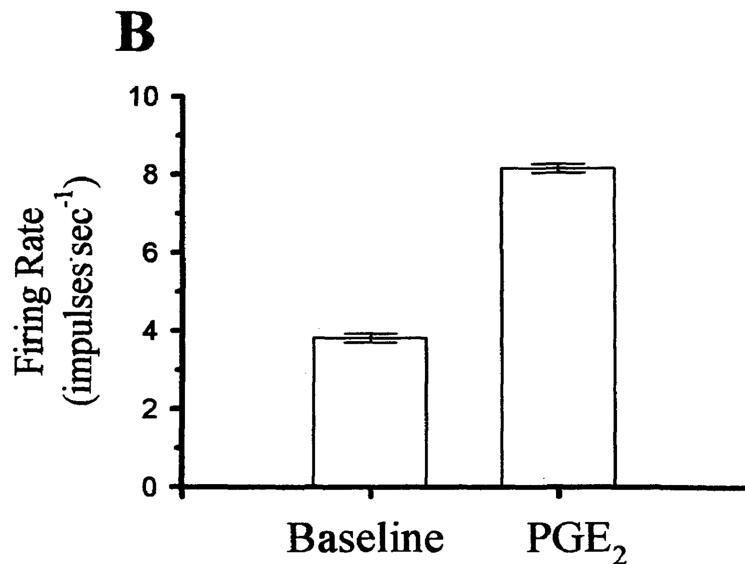


Figure III.4. The effect of temperature and PGE_2 on the firing rate activity of a VMPO low-slope temperature insensitive neuron

A shows the firing rate plotted as a function of temperature. In **B**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline; 0.15 $\pm$ 0.03 impulses·s^{-1}) and during the peak of the response (PGE_2; 2.76 $\pm$ 0.10 impulses·s^{-1}). For each plot in **B**, the error bars may be obscured.





III.5. The effect of temperature and PGE_2 on the firing rate activity of a VMPO low-slope temperature insensitive neuron

A shows the firing rate plotted as a function of temperature. In **B**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline; 3.80 $\pm$ 0.12 impulses·s^{-1}) and during the peak of the response (PGE_2; 8.13 $\pm$ 0.03 impulses). For each plot in **B**, the error bars may be obscured.

Figure III.6. shows the thermosensitivity and the effect of 1 μM PGE_2 on the firing rate activity of a VMPO warm sensitive neuron with a low baseline firing rate of 5.83 $\pm$ 0.12 impulses·s^{-1}, which significantly decreased in response to PGE_2 to 0.98 $\pm$ 0.11 impulses·s^{-1}. In Figure III.7., a warm sensitive neuron that had a high baseline firing rate of 23.85 $\pm$ 0.10 impulses·s^{-1} is shown. In response to PGE_2 firing rate significantly decreased to 17.64 $\pm$ 0.12 impulses·s^{-1}.

The increase in firing rate activity that was recorded from low-slope temperature insensitive neurons, in response to PGE_2 (1 μM), may have been due to a depolarization of the membrane potential. Membrane potential of warm sensitive neurons remained steady throughout treatment. Figure III.8. shows the effect of PGE_2 on the interspike interval activity of low-slope temperature insensitive neuron in which the interspike interval decreased during PGE_2. During the interspike interval the membrane potential was depolarized, which led to a shortened interval and an increase in firing rate.

In response to PGE_2 (1 μM), a decrease in firing rate recorded from warm sensitive neurons, this may not have been due to a change in membrane potential, but a change in the properties of an active conductance such as the potassium A current. Figure III.9. shows the effect of PGE_2 on the interspike interval activity of a warm sensitive neuron. Although changes in membrane potential were consistent during baseline conditions and PGE_2, the slope of the prepotential decreased in response to PGE_2.

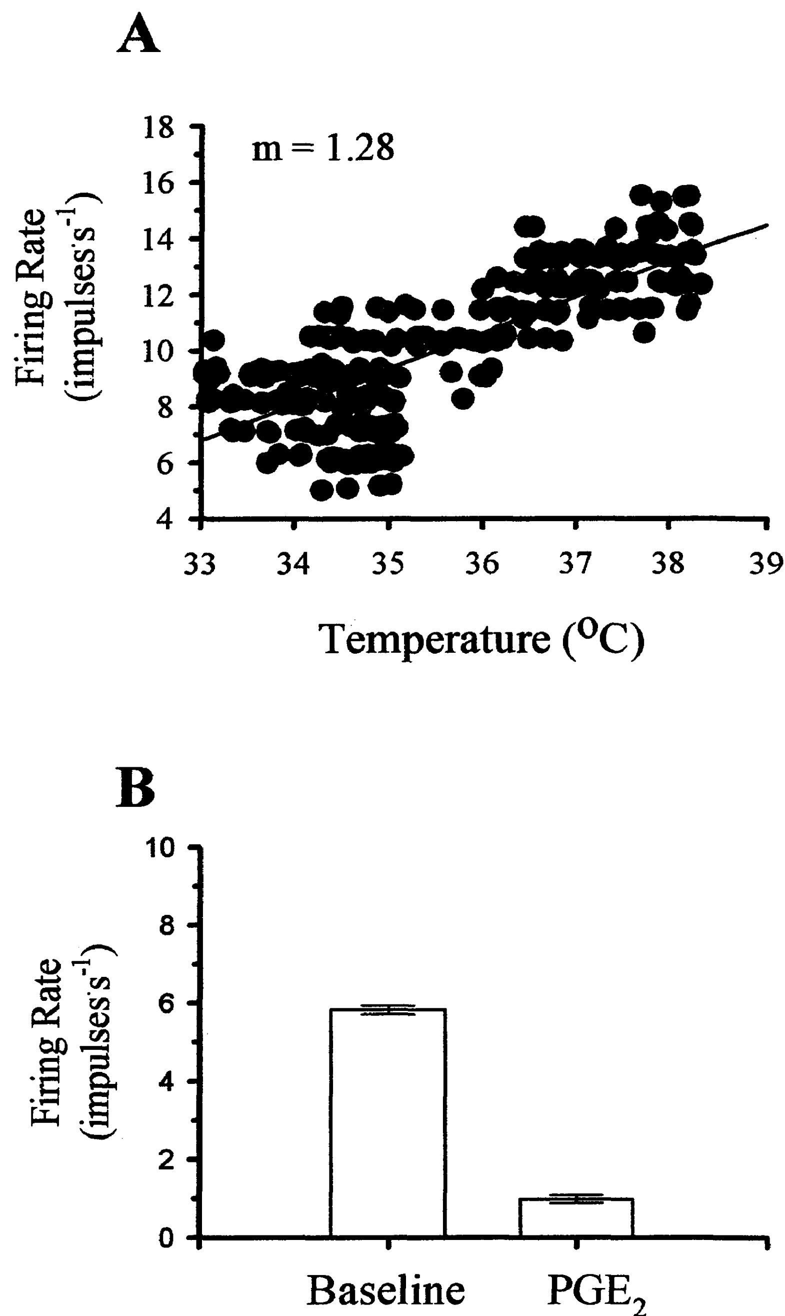


Figure III.6. The effect of temperature and PGE_2 on the firing rate activity of a VMPO warm sensitive neuron

A shows the firing rate plotted as a function of temperature. In **B**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline; 5.83 ± 0.12 impulses·s^{-1}) and during the peak of the response (PGE_2; 0.98 ± 0.11 impulses·s^{-1}). For each plot in **B**, the error bars may be obscured

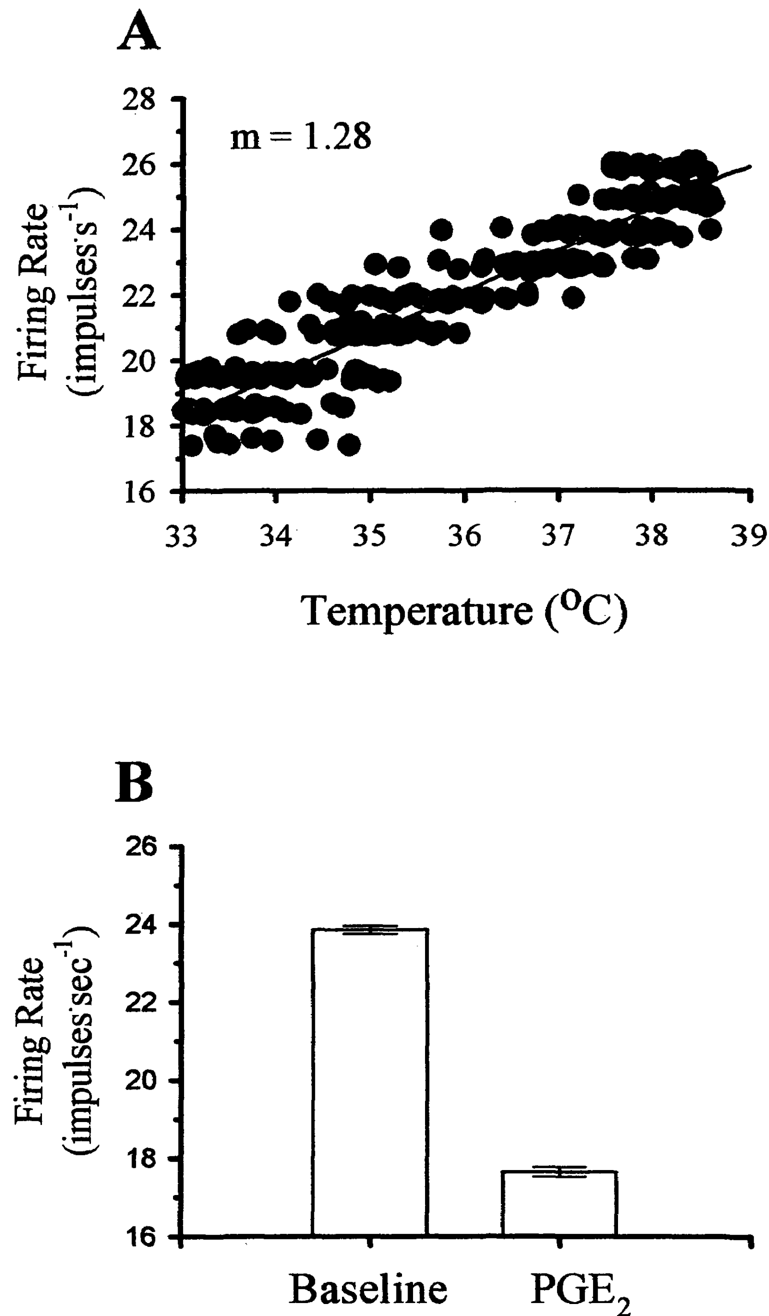


Figure III.7. The effect of temperature and PGE_2 on the firing rate activity of a VMPO warm sensitive neuron

A shows the firing rate plotted as a function of temperature. In **B**, one minute samples of firing rate activity are plotted as individual bar graphs, just before perfusion with PGE_2 (Baseline;; 23.85 $\pm$ 0.10 impulses·s^{-1}) and during the peak of the response (PGE_2; 17.64 $\pm$ 0.12 impulses·s^{-1}). For each plot in **B**, the error bars may be obscured.

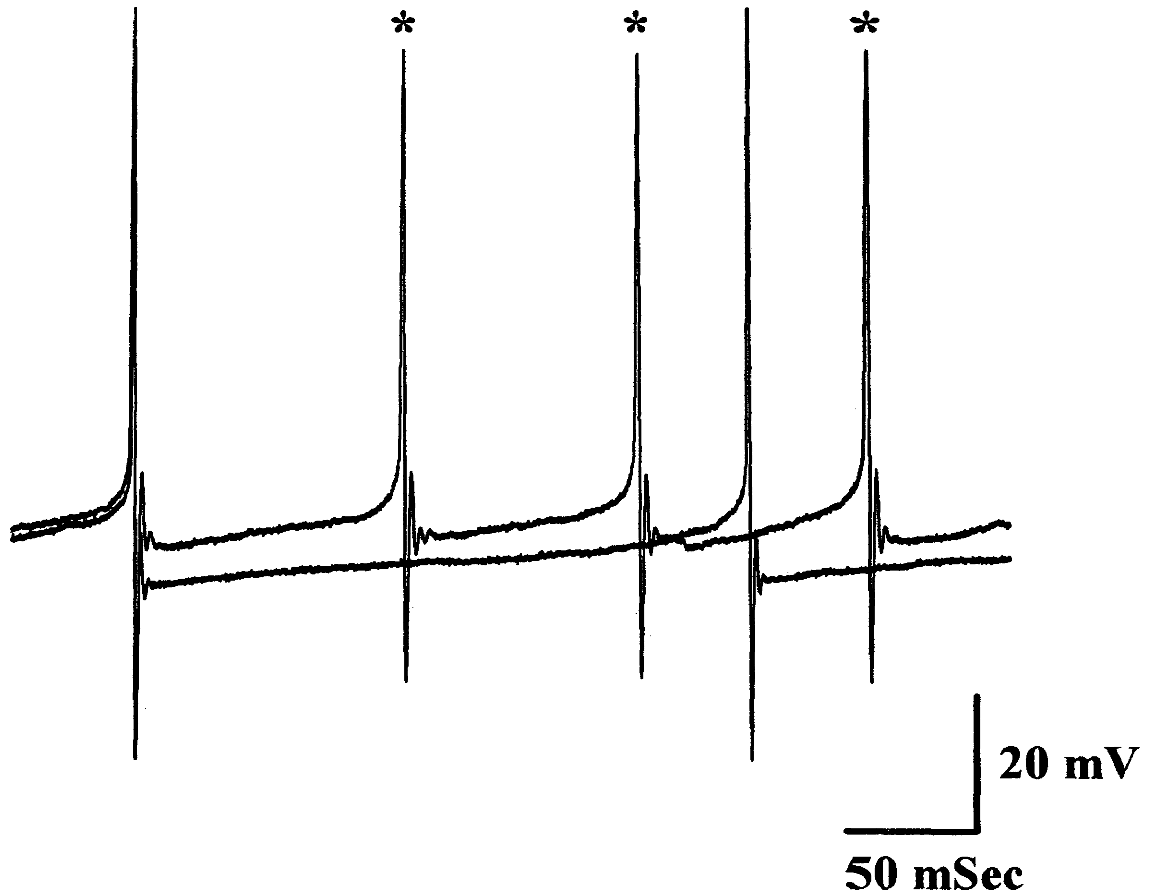


Figure III.8. The effect of PGE_2 on the interspike interval activity of a VMPO low-slope temperature insensitive neuron

For baseline conditions and perfusion with PGE_2 (action potentials are marked with asterisks), superimposed records show individual action potentials followed by subsequent changes in membrane potential and action potentials.

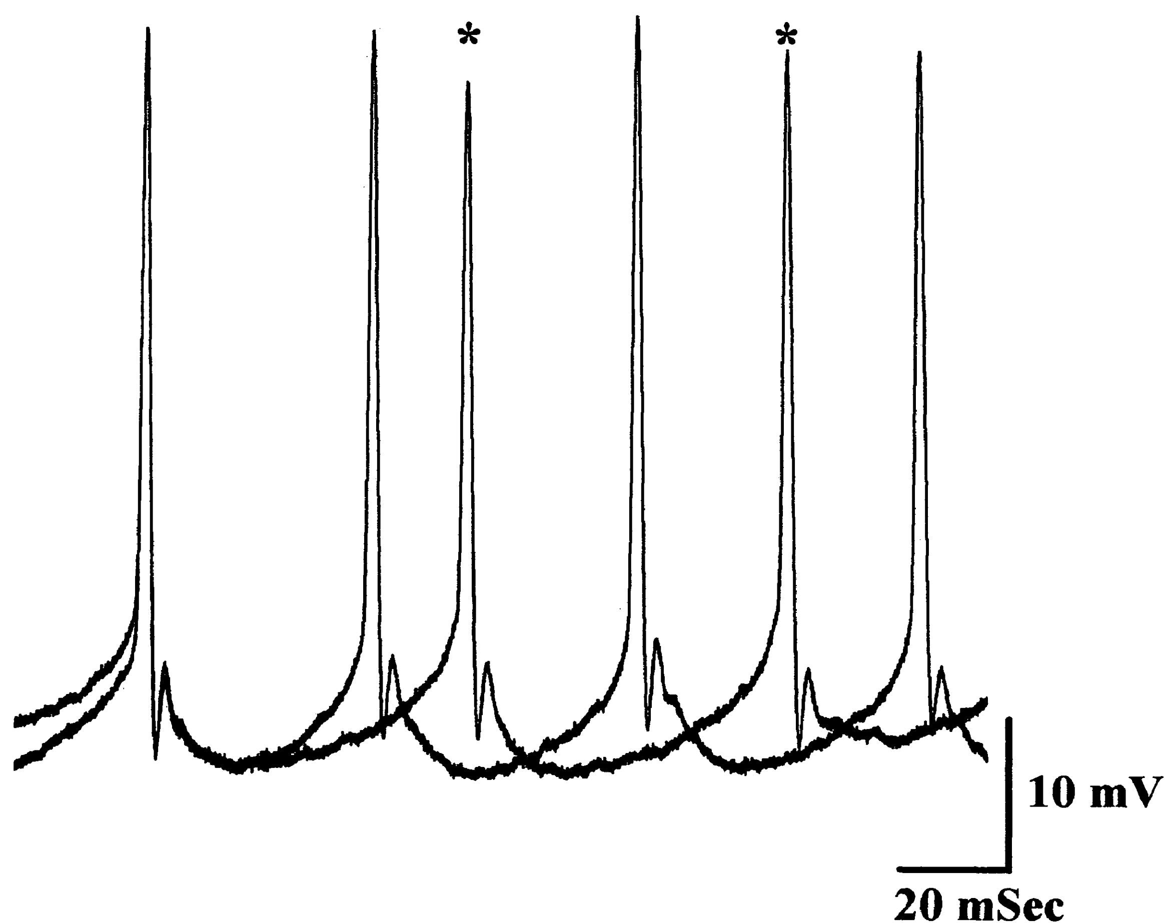


Figure III.9. The effect of PGE_2 on the interspike interval activity of a VMPO warm sensitive neuron

For baseline conditions and perfusion with PGE_2 (action potentials are marked with asterisks), superimposed records show individual action potentials followed by subsequent changes in membrane potential and action potentials.

As seen previously for all regions of the anterior hypothalamus (Griffin, et al., 2001) local synaptic input did not show a change in frequency in response to temperature. Regardless of thermosensitivity, synaptic input did not change significantly in response to PGE_2 (200 nM or 1 uM; Table III.4.).

Table III.4. Thermosensitivity and frequency of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) recorded from VMPO neurons in response to PGE2 (200 nM or 1 uM).

Class	N	Thermosensitivity ($imp \cdot s^{-1} \cdot {}^{\circ}C^{-1}$)	Frequency (postsynaptic potentials$\cdot s^{-1}$) Baseline	PGE_2	Control
EPSPs:					
Low-Slope Temperature Insensitive	12	0.19 ± 0.06	1.81 ± 0.34	1.93 ± 0.32	1.92 ± 0.24
High-Slope Temperature Insensitive	4	0.07 ± 0.07	2.24 ± 0.53	2.36 ± 0.65	2.64 ± 0.51
Warm Sensitive	5	0.10 ± 0.04	1.85 ± 0.68	2.23 ± 1.50	1.99 ± 0.75
IPSPs:					
Low-Slope Temperature Insensitive	12	0.26 ± 0.09	5.12 ± 0.92	6.07 ± 0.89	5.65 ± 0.81
High-Slope Temperature Insensitive	4	0.41 ± 0.16	7.67 ± 2.32	8.21 ± 1.42	7.89 ± 1.67
Warm Sensitive	5	0.15 ± 0.06	4.82 ± 2.17	4.50 ± 2.22	5.45 ± 1.77

4. Discussion

Morphological and physiological evidence has shown the VMPO to be an important area in the generation of a fever (Elmquist et al., 1996; Scammell et al., 1996). As a model for temperature regulation suggests, the integration of synaptic input from warm sensitive neurons and temperature insensitive neurons in the anterior hypothalamus creates a set-point for temperature control (Hammel, 1996). The generation of fever is a result of an upregulation of this set-point. Previously, extracellular single-unit recordings from the VMPO showed a strong correlation between neuronal thermosensitivity and effect of PGE_2, an endogenous pyrogen, which inhibited warm sensitive neurons and excited temperature insensitive neurons (Chapter II.). This new evidence supports the hypothesis of an up-regulation of thermoregulatory set-point into hyperthermic range.

Intracellularly recorded neurons in the VMPO had similar proportions of temperature insensitive neurons and warm sensitive neurons as extracellular single-unit recordings in the VMPO and no unique pattern of location within the VMPO. The present study also found similar correlation of thermosensitivity and changes in firing rate activity in response to PGE_2 (1 μM; Chapter II.). Since it is likely that PGE_2 stimulates cellular activity through a second messenger signaling pathway, it is conceivable that with the intracellular whole-cell patch recording technique, the required concentrations needed to induce firing rate changes were not obtained when treated with a lower dose of PGE_2 (200 nM).

In response to PGE_2 (1 μM) low-slope temperature insensitive VMPO neurons responded to PGE_2 with an increase in firing rate activity, while warm sensitive neurons responded with a decrease in firing rate activity (Table III.3.). This change in activity was

not related to changes in local synaptic input, as the frequencies IPSPs or EPSPs recorded from both warm sensitive and temperature insensitive did not change significantly in response to PGE_2 (Table III.4.).

Stimulation protocols were used to determine input resistance and response to depolarizing current. All neurons that were tested, regardless of thermosensitivity, responded to PGE_2 with a significant decrease in input resistance. This decrease in resistance would indicate an increase in membrane capacitance leading to the conclusion that ion channels were opening. In response to a small depolarizing current, low-slope temperature insensitive neurons increased their firing rate frequency, while warm sensitive neurons decreased their firing rate frequency. This would suggest that different channels were being affected by PGE_2. Low-slope temperature neurons were also observed to show a decrease in the delay to the onset of the generation of action potentials in response to PGE_2 (Figure III.3.). In contrast, warm sensitive neurons showed little if any change in this delay. Several studies have shown that this delay to is due to the presence of a Ca^{++} dependant K^+ conductance (Fan et al., 2000; Tanaka, et al., 1998; Zhang et al., 1999). Thus, we might suggest that PGE_2 is altering the activity of this conductance in VMPO temperature low-slope insensitive neurons.

In response to PGE_2 (1 μM) distinct changes in the intracellular activity to low-slope temperature insensitive neurons were recorded. Low-slope temperature insensitive neurons showed an increase in firing rate activity that is likely due to a depolarization of membrane potential (Figure III.8). In contrast, warm sensitive neurons do not show a change in membrane potential in response to PGE_2 (Figure III.9). The decrease in firing rate activity is most likely due to a decrease in the rate of depolarization of the

prepotential that precedes each action potential. Recent work from Griffin et al. (1996) has provided evidence that this prepotential is mediated by a slow inactivation potassium A current. A recent study demonstrated that PGE_2 attenuated a whole cell potassium current in sensory neurons (Evans et al., 1999). Suppression of this potassium current is dependent on activation of the cAMP-PKA transduction cascade. This suggests an inhibitory mechanism that can be activated by PGE_2, which may result in a decrease in neuronal firing rate activity.

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CHAPTER IV

CONCLUSIONS

The studies in this thesis, examined the effects of PGE_2 on the activity of neurons in the VMPO by using both *in vitro* extracellular single-unit and whole-cell patch clamp recording techniques in rat hypothalamic tissue slices. More specifically, these studies uncovered the strong correlation between neuronal thermosensitivity in the VMPO and responses to PGE_2, and examined changes in cellular conductances that might lead to the different responses in firing rate activity.

Using extracellular single-unit recordings, this study demonstrated that low-slope temperature insensitive neurons in the VMPO increased their firing rate activity in response to PGE_2 (200 nM) and that warm sensitive neurons decreased their firing rate activity. Likewise, intracellular whole-cell patch recordings in the VMPO showed the same correlation of thermosensitivity and response to PGE_2 (1 μM). These responses fit well with the current model for the control of thermoregulation with an inhibition of warm sensitive neurons or excitation of temperature insensitive neurons resulting in an upregulation of the normal set-point into hyperthermic range.

My data indicates that the observed changes in firing rate activity in the presence of PGE_2 were not due to changes in local synaptic input. Synaptic input was primarily from temperature insensitive neurons and showed little change in response to PGE_2. In

addition, all neurons in the VMPO, regardless of thermosensitivity, significantly decreased input resistance with exposure to PGE_2 (200 nM or 1 μM). These findings support the hypothesis that changes in firing rate activity in responses to PGE_2 are due to changes in cellular conductances.

In response to a depolarizing current, low-slope temperature insensitive neurons showed an increase in firing rate frequency and a decrease in the delay to the onset action potential generation (Figure III.4). In addition, these neurons showed depolarization of the membrane in response to PGE_2. These responses may be due to affects on a Ca^{++} dependant K^+ channel resulting in an increase firing rate activity.

Alternatively, warm sensitive neurons decreased their firing rate frequencies in response to PGE_2 with little change in the delay to the onset of action potential generation of action potentials (Figure III.3., and III.8.). These neurons also showed no change in membrane potential during PGE_2 exposure. However, the rate of rising slope of the prepotential appears to decrease. This prepotential has been linked to the potassium A current and is considered to play an important role in determining warm sensitivity (Griffin et al., 1996). This might indicate that the observed decrease in firing rate in response to PGE_2 of warm sensitive neurons may not have been due to a change in membrane potential, but a change in the properties of an active conductance, such as the potassium A current.

Warm sensitive neurons accounted for approximately ~ 20 % of the sampled population in the VMPO. This is lower than the 35% seen throughout the anterior hypothalamus. However, this distinct population difference might be correlated to the two different local pathways known to be involved in the production of fever. These

pathways are based on two distinct groups of LPS induced Fos activated neurons in the VMPO that have been characterized, which form local efferent projections to the APFx and PVN which may underlie the adjustment of the thermostatic set-point that results in fever.

A small, restricted efferent projection exists directly from the VMPO to the PVN, which might consist of the warm sensitive neurons in the VMPO. Since all of the warm sensitive neurons in the VMPO responded to PGE_2 with a significant decrease in firing rate, it might be suggested that these neurons provide excitatory input to the PVN through this direct efferent projection. In response to increased concentrations of PGE_2 in the VMPO, the level of excitation would limit the activation of heat loss thermoregulatory responses (i.e., vasodilatation) and resulting in a fever.

In contrast, the major pathway from the VMPO is an inhibitory projection to the APFx, which contains a high proportion of warm sensitive neurons (Elmquist & Saper, 1996; Dean & Boulant, 1989). From the APFx, another inhibitory projection to the autonomic parvicellular division of the PVN has been characterized (Roland & Sawchencko, 1993). Therefore, activation of this efferent pathway may result in the inhibition of warm sensitive neurons in the APFx, decreasing the level of inhibition to the PVN. Consistent with this hypothesis and the current model of set-point thermoregulation, this would suggest that this efferent pathway is made up of temperature insensitive neurons from the VMPO, that respond to PGE_2 with an increase in firing rate activity (Figure IV.1.).

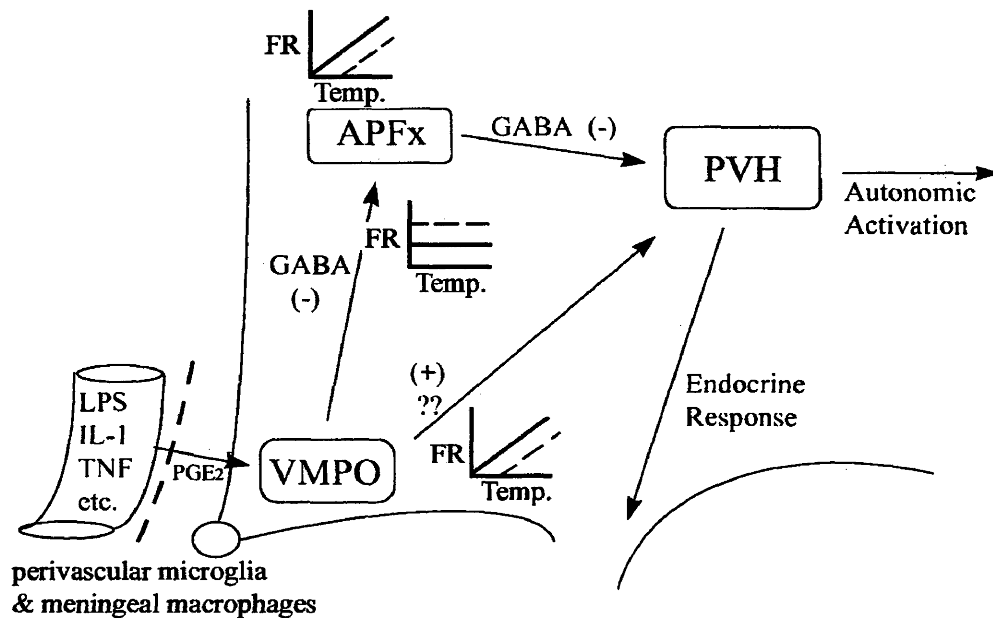

Figure IV.1. Proposal for how responses of the VMPO neurons to PGE_2 and the current model for the generation of fever

Warm sensitive neurons in the VMPO decrease firing rate activity in response to PGE_2 (dashed lines), which limits excitatory input to the PVH resulting in a decreased activation of heat loss responses. Meanwhile low-slope temperature insensitive neurons in the VMPO increase their firing rate activity in response to PGE_2 (dashed lines), possibly inhibiting warm sensitive neurons in the APFx. This inhibition of warm sensitive neurons might results in a decreased level of inhibition of the PVH that might then cause heat retention. Abbreviations: APFx, anterior perifornical area; PVH, paraventricular nucleus; GABA, gamma amino butyric acid; VMPO, ventral medial preoptic area; LPS lippopolysaccharide; IL-1, interleukin-1β, PGE_2, prostaglandin E_2; TNF, tumor necrosis factor.

Future directions for the study of the mechanism of fever might include additional whole-cell recordings to determine the specific conductances effected by PGE_2 in warm sensitive neurons and temperature insensitive neurons. Specific conductances to examine would be the potassium A current in warm sensitive neurons and Ca^{++} dependant K^+ channels in temperature insensitive neurons. In addition, the examination of the dendritic projections of neurons from the VMPO would be functionally important. Previous research has shown the pathway to the APFx to be inhibitory and the smaller pathway directly to the PVN that is possibly excitatory. If our hypothesis is correct then temperature insensitive neurons in the VMPO would be GABAergic and warm sensitive neurons might be glutaminergic. This last hypothesis is presently being examined in the Griffin Lab.

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