Hypothalamic Neuronal Responses to Cytokines

MASAAKI SHIBATA, Ph.D.

Department of Physiology and Biophysics, University of Tennessee, Memphis, Memphis, Tennessee

Received June 29, 1989

Fever has been extensively studied in the past few decades. The hypothesis that hypothalamic thermosensitive neurons play a major role in both normal thermoregulation and in fever production and lysis has particularly helped to advance our understanding of the neuronal mechanisms underlying the response to pyrogens. Furthermore, new data in the study of host defense responses induced by pyrogenic cytokines such as interleukin 1, interferon α, tumor necrosis factor α, and interferon 6 have demonstrated that those factors have multiple, yet coordinated, regulatory activities in the central nervous system, so that our understanding of the role of the brain in the activity of these agents requires a new perspective and dimension. Thus, recent evidence from our laboratory indicates that blood-borne cytokines may be detected in the organum vasculosum laminae terminalis and transduced there into neuronal signals. Such signals may then affect distinct, but partially overlapping, sets of neuronal systems in the preoptic area of the anterior hypothalamus, mediating directly and/or indirectly the array of various host defense responses characteristic of infection that are thought to be induced by blood-borne cytokines.

INTRODUCTION

The cytokine interleukin 1 (IL-1) induces various centrally mediated host defense responses to infectious pathogens. These include, among others, fever, acute-phase glycoproteinemia, increased counts of white blood cells and levels of adrenocorticotropic hormone (ACTH), and enhanced slow-wave sleep. Fever has been the most studied of these responses, perhaps because it is the most manifest and often the earliest sign of infection. Indeed, central nervous system (CNS) mechanisms of fever have been extensively studied for the past 30 years. More recent studies have revealed that fever can be induced, in addition to IL-1, by a variety of other cytokines also secreted by certain activated immune cells, e.g., interferon α (IFN), tumor necrosis factor α (TNF), and IL-6. Moreover, these factors also modulate in the CNS several of the other host defense responses. The aim of this paper is to review very briefly the central neuronal mechanisms of thermoregulation and fever and, using this knowledge as a stepping stone, to discuss our latest findings in terms of centrally mediated host defense responses generally induced by blood-borne cytokines.

Abbreviations: ACTH: adrenocorticotropic hormone  CNS: central nervous system  CSF: cerebrospinal fluid  EP: endogenous pyrogen  5HT: serotonin  FR: firing rate  icv: intracerebroventricular  IFN: interferon  IL-1: interleukin 1  iPOA: intraPOA  iv: intravenous  OVLT: organum vasculosum laminae terminalis  p: purified  PGE: prostaglandin E  POA: preoptic area  Tbw: body temperature  TNF: tumor necrosis factor

Address reprint requests to M. Shibata, Ph.D., Dept of Physiology and Biophysics, University of Tennessee, Memphis, 894 Union Avenue, Memphis, TN 38163

Copyright © 1990 by The Yale Journal of Biology and Medicine, Inc.
All rights of reproduction in any form reserved.
ROLES OF HYPOTHALAMIC THERMOSENSITIVE NEURONS

In Vivo: Neuronal Substrates of Thermoregulation

The involvement of the hypothalamus in body temperature regulation was first established in the late 1930s, mainly on the basis of data from animals in which the hypothalamus was lesioned or surgically separated from the rest of the brain. Such animals were unable to maintain their body temperatures (T_{bo}) in various ambient temperatures [1]. More direct evidence implicating the hypothalamus in T_{bo} regulation was derived from experiments in which the anterior hypothalamus was heated [2-4] or cooled [3-5] through implanted devices. In these studies, hypothalamic heating induced panting, cutaneous vasodilation, sweating, and a fall in T_{b}. Conversely, hypothalamic cooling elicited shivering, cutaneous vasoconstriction, and an rise in T_{b}. The latter condition mimics fever production, and the former fever lysis. These observations thus predicted the existence of specific hypothalamic elements, the excitability of which could be affected by a local temperature change.

In the early 1960s, hypothalamic neurons sensitive to small, local temperature changes were found in anesthetized cats and dogs [6-9]. Two types of neurons were described: warm-sensitive neurons, which increased their firing rates (FR) with higher than normal (ca. 37°C) hypothalamic temperature, and cold-sensitive neurons, which increased their FR with lower than normal hypothalamic temperatures. Two criteria for assessment of neuronal thermosensitivity have been proposed: the Q_{10} and the thermal coefficient (imp/s/°C). Neurons are classified as warm-sensitive if their Q_{10} is larger than 2.0 [10,11] or their positive thermal coefficient is at least 0.8 imp/s/°C, or cold-sensitive if they exhibit a negative thermal coefficient of at least -0.6 imp/s/°C [12-14].

According to the "Glossary of Terms for Thermal Physiology" [Pflügers Arch 410:567-587, 1987], the Q_{10} denotes "the ratio of the rate of a physiological process at a particular temperature to the rate at a temperature 10°C lower, when the logarithm of the rate is an approximately linear function of temperature." The thermal coefficient, which represents thermosensitivity of a neuron, is, according to Boulant and Hardy [14], determined by its change in firing rate (imp/s) for a given change in temperature (°C). In practice, the thermal coefficient is customarily determined over a 4-5°C range of temperature in which the individual neuron appears most thermosensitive. The Q_{10} expression for the thermosensitivity has its own shortcomings. For example, the Q_{10} cannot be applied to cold-sensitive neurons because it has no negative values. It produces a bias in favor of neurons with low FR. By definition, it would be inappropriate if the Q_{10} were applied to a temperature range of less than 10°C over which the neurons appear most warm-sensitive. On the other hand, the thermal coefficient gives more weight to thermosensitive neurons with high FR. The criteria for assessment of neuronal thermosensitivity differ from laboratory to laboratory. From the theoretical point of view, it would therefore seem more appropriate to use both the Q_{10} and the thermal coefficient to eliminate all the biases associated with these criteria.

The medial preoptic area (POA) of the hypothalamus was found to contain the largest number of such thermosensitive neurons, apparently scattered randomly within this region. Proportions of warm-sensitive, cold-sensitive, and thermally insensitive neurons were 30 percent, 10 percent, and 60 percent, respectively [15]. Nearly 70 to 80 percent of the thermosensitive neurons in the POA also responded to peripheral thermal stimulation. They were usually affected in the same direction by thermally
stimulating the POA and by ambient temperatures [16,17], suggesting convergence of thermal signals on these neurons in the POA. Based on this and other evidence, the hypothesis was proposed that homeostatic thermal balance is controlled by hypothalamic thermosensitive neurons that integrate central and peripheral thermal signals [18]. According to this hypothesis, warm-sensitive neurons receive excitatory synaptic inputs from peripheral warm receptors and from local warm signals, while cold-sensitive neurons receive excitatory synaptic inputs from peripheral cold receptors and local inhibitory inputs from warm-sensitive neurons. There is, as yet, however, no direct evidence to establish a functional role for these neurons in thermoregulation.

Fever

Other substances that cause \( T_{bo} \) rises include exogenous and endogenous pyrogens and prostaglandin E (PGE), a putative fever mediator. Responses of thermosensitive neurons in the POA to these substances are generally consistent with their observed thermoregulatory effects. Of these, exogenous (e.g., endotoxin) and endogenous pyrogens (EPs) have been the most studied since the first report in the early 1960s that the activity of hypothalamic warm-sensitive and cold-sensitive neurons decreased and increased, respectively, in conjunction with fever after intravenous (iv) administration of bacterial or EPs [19,20]. These activity changes started between 15 and 30 minutes after pyrogen injection, and returned to their preinjection levels 75 to 115 minutes afterward. Administration of acetylsalicylate, an antipyretic, facilitated their recovery coincident with the fall in \( T_{bo} \). When microinjected directly into the POA, leukocytic pyrogen (a mixture of pyrogenic cytokines) decreased the FR of warm-sensitive neurons and increased that of cold-sensitive neurons within a short time [21]. Sodium acetylsalicylate, similarly microinjected, blocked the pyrogen-induced changes [21]. These results indicate that hypothalamic thermosensitive neurons are themselves sensitive to EP and suggest the possibility that the pyrogen might directly affect these neurons. Lately, a similar result was reported in anesthetized rats in which minute amounts of purified human (p) IL-1 were iontophoretically applied in the immediate vicinity of thermosensitive POA neurons [22]; viz., pIL-1 affected thermosensitive neurons consistently for over 40 minutes with an onset latency of six minutes. Sodium acetyl salicylate co-applied iontophoretically blocked the IL-1 effect. Thus, it was concluded that thermosensitive neurons in the POA may play a major role not only in the central control of thermoregulation but also in fever production. It is, however, important to note again that no direct evidence exists to support this hypothesis. Moreover, it does not take into account that circulating pyrogens cannot enter the brain and directly affect the activity of hypothalamic thermosensitive neurons (see the final section of this paper). Although the amount of iontophoretically applied IL-1 was extremely small, the injected IL-1 could diffuse and affect a number of neurons in addition to thermosensitive neurons. The question, therefore, still remains as to whether thermosensitive neurons are per se sensitive to IL-1 or are driven transsynaptically by other neurons or glial cells sensitive to IL-1. The latter hypothesis is of particular interest since these cells are able to synthesize IL-1 in the brain [23].

Since the discoveries that PGE induces hyperthermia after injection into the third ventricle of cats, rabbits, and rats [24–26] and that antipyretics reduce both fever and levels of PGE in ventricular or cisternal cerebrospinal fluid (CSF) [27–29], the view has been widely held that PGE is synthesized in the brain and mediates fever production. Levels of PGE\(_2\) increase in the CSF of various species during fever rise
PGE was also detected as early as six to nine minutes after the beginning of incubation of rat hypothalamic slices with leukocytic pyrogen [33]. Studies in rats, rabbits, guinea pigs, cats, and monkeys have demonstrated that the sites most sensitive to PGE are located in and around the POA [26,34–37,62]. Despite this fact, the neuroelectrophysiological effects of PGE on thermosensitive neurons in the POA have not unanimously supported the PG hypothesis of fever. A gross form of PGE application such as intraPOA (iPOA) microinjection [38] or intracerebroventricular (icv) injection [39] decreased the FR of warm-sensitive neurons and increased that of cold-sensitive neurons. Iontophoretically applied PGE, however, increased the FR of a small number of POA neurons regardless of thermosensitivity [40] or that of the majority of warm-sensitive, but not cold-sensitive, neurons [41]. This result may suggest that PGE does not act directly on thermosensitive neurons. It is, however, unlikely that such conflicting electrophysiological results are accounted for by the different anesthetics used since a study using POA slice preparations (that contain no anesthetic) also produced inconsistent results [42].

**In Vitro: Neuronal Studies**

The development of the brain slice method for electrophysiological study has helped to address some of these issues since the thermosensitivity of POA neurons in slice preparations [12,43], and also tissue cultures [44,45], is unchanged compared to that in in vivo preparations. Thus, it was found that some warm-sensitive neurons retained their thermosensitivity in a synaptic-blocking medium that contained high Mg++ and low Ca++, suggesting that they were inherently thermosensitive. It has been suggested, however, that only warm-sensitive neurons are inherently thermosensitive, with cold sensitivity merely being the result of the inhibitory drive exerted by warm-sensitive neurons upon cold-sensitive neurons [13,14]. On the other hand, other results have suggested that inherently thermosensitive neurons include both warm-sensitive and cold-sensitive neurons [46]; in the latter case, Ca++ was completely removed from the medium to enhance the synaptic blocking effect. Several studies have, however, demonstrated that lowering the calcium concentration of the medium induces synaptic blockade without affecting nerve conduction [47], while removing the calcium altogether causes hyperexcitability of the tissues [48]. Thus, the conclusion that cold sensitivity is also intrinsic may have been due to the different compositions of the media. Intracellular recordings from thermosensitive POA neurons of rats [49,50] and green sunfish [51] showed that no cold-sensitive neurons in either species exhibited characteristics prototypical of true thermoreceptors, further supporting the hypothesis that only warm-sensitive neurons are inherently thermosensitive. As to whether thermosensitive POA neurons are themselves sensitive to cytokines, the latest evidence suggests that IFN affects thermosensitive POA neurons in a calcium-free/high-magnesium medium [52]. The interpretation of this result, however, requires caution since IL-1 and TNF do not stimulate the release of phospholipase A₂ in a calcium-free medium [53,54].

**Differential Cytokine Effects**

It was found that although all three cytokines, IL-1, TNF, and IFN, cause fever when microinjected iPOA, the febrile responses each evokes differ. Thus, IL-1β elicits fevers with rapid onsets and relatively short durations, whereas the fevers after IFN are more delayed in onset and longer in duration, and those after TNF are bimodal [55].
was also found that IL-1β elevates plasma Cu levels as much as 67 percent over control, but that IFN and TNF are inactive in this respect [56]. We asked, therefore, whether such differential effects could be similarly expressed by thermosensitive POA neurons in slice preparations. In conformity with previous observations, the addition to the medium of IL-1β, IFN, or TNF decreased the FR of the majority of warm-sensitive neurons and increased that of most cold-sensitive neurons in the preparations. When the responses of individual thermosensitive neurons to two or more of these cytokines were examined, however, nearly two-thirds of all neurons responded differentially; e.g., a warm-sensitive neuron was inhibited by IL-1β but excited by TNF. This result, therefore, does not contradict the possibility that each of these cytokines may stimulate a different population of thermosensitive neurons that possess partially overlapping characteristics. Observations that not every POA thermosensitive neuron exhibits sensitivity equally to osmotic, glucose, and reproductive steroid stimulation [57–59] strengthen our finding. It is conceivable that different neuronal sets composed of units with various combinations of sensitivities are responsive to one or more of these cytokines and mediate various responses.

Our results in POA slices showed that a significant number of some thermally insensitive neurons also increase or decrease their FR in response to the cytokines. A similar effect of crude leukocytic pyrogen on thermally insensitive POA neurons in slices of guinea pig brain has been previously reported [60]. The importance of these results may lie in the possibility that thermally insensitive POA neurons may mediate nonfebrile cytokine-induced responses. For example, fever induced by ivc IL-1 is blocked by antipyretics, but the enhanced slow-wave sleep is unaffected by this treatment [61]. Similarly, the hyperproteinemia induced by iPOA IL-1 is not blocked by antipyretics [62]; indeed, POA thermal stimulation does not evoke acute-phase responses [63]. These results suggest that thermally insensitive hypothalamic neurons may be involved in the modulation of other, nonfebrile host defense responses mediated by the cytokines.

HOW ARE BLOOD-BORNE CYTOKINE SIGNALS TRANSDUCED INTO NEURONAL SIGNALS IN THE CNS?

Numerous attempts have been made to demonstrate entry of circulating pyrogens [64,65] or IL-1 [66–68] into the brain, but so far unsuccessfully, suggesting that circulating pyrogens may not, in fact, pass into the brain. Yet most host defense responses [69–71] induced by systemic cytokines apparently involve the hypothalamus, in particular the POA. Recent studies have demonstrated that lesions of the frontal wall of the third ventricle including the circumventricular organ, organum vasculosum laminae terminalis (OVLT, located outside the blood-brain barrier), suppressed not only the febrile but also the acute-phase glycoproteinemic responses to systemic endotoxin and EP [72–74]. In contrast, a marked enhancement of the febrile response to systemic crude IL-1 was reported in animals with smaller OVLT lesions [75], which did not include the vascular plexus of the OVLT. The reason for this apparent discrepancy is not clear; however, both results unquestionably suggest the importance of the OVLT for fever production by circulating pyrogens. The enhanced febrile response to systemic EP was also observed after injection of immunoadjuvants, zymosan, lipopolysaccharide, and muramyl dipeptide, iv, or into the OVLT, but not into the POA [76,77]. This result again suggests a role of the OVLT in fever. In this context, PGE was suggested as a mediator for the febrile response acting within the
OVLT [76,78]. It has, therefore, been proposed that the OVLT may be a site where blood-borne cytokines might interact with the CNS. As the OVLT contains abundant serotonin (5HT) terminals [79,80], we examined the possibility that OVLT neurons might respond to both cytokines and 5HT by recording extracellular single-unit activities in slice preparations from guinea pig brain. We found [81] that some OVLT neurons increased their FR for more than 47 minutes after TNF, with an onset latency of 6.5 minutes. These neurons also augmented their FR after 5HT for over 44 minutes. The majority of OVLT neurons, however, decreased their FR for more than 37 minutes after 5HT, but these neurons did not respond to TNF. A long-term FR decrease after 5HT was often preceded by an increased FR recovery or decreased FR recovery period. The response characteristics of the OVLT neurons to TNF were identical to those of POA thermosensitive neurons to this cytokine. By contrast, the long-term FR change of OVLT neurons observed after 5HT was unusual. Hypothalamic neurons tested with the same dose of 5HT in our system changed their activity over no more than three to ten minutes. It is not known as yet whether the responses of OVLT neurons to 5HT and TNF are synaptically mediated; however, the neurons inhibited by 5HT did not similarly decrease their FR after TNF. 5HT-induced inhibition is, therefore, probably not of post-synaptic origin. It is interesting to speculate that the observed excitatory responses of OVLT neurons to both 5HT and TNF may indicate that 5HT is a transmitter of OVLT neurons sensitive to TNF, thereby transducing the message of this circulating cytokine into neuronal signals in the OVLT for transfer into the POA and other brain areas.

In conclusion, several important issues remain to be addressed. These are important because they concern the fundamental yet unanswered question of what hypothalamic thermosensitivity really is. First, neurons sensitive to temperature changes are also found in regions outside the hypothalamus. These regions include the sensorimotor cortex [85] and at least 18 nuclei in the diencephalon, including the POA and the anterior hypothalamus [86,87], yet thermal stimulation of the sensorimotor cortex and of some of these nuclei other than the POA and the anterior hypothalamus induces no apparent thermoregulatory response. Second, hypothalamic thermosensitive neurons exhibit sensitivity to at least 13 different substances [82–84]. All hypothermizing and hyperthermizing substances excite, respectively, POA warm- and cold-sensitive neurons. In addition, thermosensitive neurons are also sensitive to glucose, reproductive steroids, osmotic changes, baro/volume receptor inputs, and aversive/emotional stimuli [83–84]. The results are taken to indicate that the hypothalamic thermosensitive neurons play more than one role and are involved in the interactions between homeostatic systems. There is, however, no direct evidence to support the idea that the hypothalamic thermosensitive neurons are, in fact, involved in other homeostatic functions. Therefore, taken together, these two issues raise the following questions: (a) Is thermosensitivity site-specific to the hypothalamus, and is it so because only the hypothalamic thermosensitive neurons may possess efferent connections to thermoeffectors? (b) Are hypothalamic thermosensitive neurons uniquely sensitive to multiple modalities, or is thermosensitivity merely one of many properties shared by neurons generally, irrespective of their location? And if so, to what purpose?

Finally, it is important to note that the evidence that blood-borne cytokines induce fever by their action on, for example, the OVLT, and not on the hypothalamic thermosensitive neurons. It is highly unlikely that circulating cytokines actually enter the brain and act directly on these neurons. Furthermore, it is interesting to note that
circulating cytokines induce an array of host defense responses specific to infections, one of which is fever. Many of these responses are centrally mediated, particularly through the hypothalamus, and they seem to be functionally interconnected [69–71]. It is, however, not known to what extent the OVLT is involved, in addition to fever induction and possibly acute-phase glycoproteinemia, in the the host defense responses induced by circulating cytokines, nor what system operates within the OVLT.

ACKNOWLEDGEMENTS

This study was supported, in part, by an award from the University of Tennessee Neuroscience Center of Excellence to me and by NIH grant NS-22716 to Dr. C.M. Blatteis. I wish to thank Dr. Blatteis for his help in the preparation of this paper.

REFERENCES

1. Bligh J: Temperature Regulation in Mammals and Other Vertebrates. Amsterdam/London/New York, North-Holland Publishing Company, 1973
2. Magoun HW, Harrison F, Brobeck JR, Ranson SW: Activation of heat loss mechanisms by local heating of the brain. J Physiol 1:101–114, 1938
3. Strom G: Effect of hypothalamic cooling on cutaneous blood flow in the unanesthetized dog. Acta Physiol Scand 21:271–277, 1950
4. Freeman WJ, Davis DD: Effects on cats of conductive hypothalamic cooling. Am J Physiol 197:145–148, 1959
5. Hammel HT, Hardy JD, Fusco MM: Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. Am J Physiol 198:481–486, 1960
6. Nakayama T, Eisenman JS, Hardy JD: Single unit activity of anterior hypothalamus during local heating. Science 134:560–561, 1961
7. Nakayama T, Hammel HT, Hardy JD, Eisenman JS: Thermal stimulation of electrical activity of single units in the preoptic region. Am J Physiol 204:1122–1126, 1963
8. Hardy JD, Hellon RF, Sutherland JA: Temperature-sensitive neurons in the dog’s hypothalamus. J Physiol 175:242–253, 1964
9. Cunningham DJ, Stolwijk JAJ, Murakami N, Hardy JD: Responses of neurons in the preoptic area to temperature, serotonin, and epinephrine. Am J Physiol 213:1570–1581, 1967
10. Eisenman JS, Jackson DC: Thermal response patterns of septal and preoptic neurons in cats. Exp Neurol 19:33–45, 1967
11. Eisenman JS: Unit activity studies of thermosensitive neurons. In Essays on Temperature Regulation. Edited by J Bligh, RE Moore. Amsterdam/London, North-Holland Publishing Company, 1972, pp 55–69
12. Kelso SR, Perlmutter MN, Boulant JA: Thermosensitive single-unit activity by in vitro hypothalamic slices. Am J Physiol 242:R77–R84, 1982
13. Kelso SR, Boulant JA: Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. Am J Physiol 243:R480–R490, 1982
14. Boulant JA, Hardy JD: The effect of spinal and skin temperatures on the firing rate and thermosensitivity of preoptic neurons. J Physiol 240:639–660, 1974
15. Hellon RF: Central thermoreceptors and thermoregulation. In Handbook of Sensory Physiology, Vol 3. Edited by E Neil. Heidelberg/New York, Springer, 1972, pp 161–186
16. Hellon RF: The stimulation of hypothalamic neurons by changes in ambient temperature. Pfügers Arch 321:56–66, 1970
17. Nakayama T, Ishikawa Y, Tsurutani T: Projection of scrotal thermal afferents to the preoptic and hypothalamic neurons in rats. Pfügers Arch 380:59–64, 1979
18. Boulant JA, Dean JB: Temperature receptors in the central nervous system. Ann Rev Physiol 48:639–654, 1986
19. Wit A, Wang SC: Temperature-sensitive neurons in preoptic/anterior hypothalamic region: Actions of pyrogens and acetylsalicylate. Am J Physiol 215:1160–1169, 1968
20. Eisenman JS: Pyrogen-induced changes in the thermosensitivity of septal and preoptic neurons. Am J Physiol 216:330–334, 1969
21. Schoener EP, Wang SC: Leukocyte pyrogen and sodium acetylsalicylate on hypothalamic neurons in the cat. Am J Physiol 229:185–190, 1975
22. Hori T, Shibata M, Nakashima T, Yamasaki M, Asami A, Asami T, Koga H: Effects of interleukin-1 and arachidonate on the preoptic and anterior hypothalamic neurons. Brain Res Bull 20:75–92, 1988
23. Giulian D, Baker TJ, Shih L-CN, Lachman LB: Interleukin 1 of the central nervous system is produced by ameboid microglia. J Exp Med 164:594–604, 1986
24. Milton AS, Wendlandt S: Effect on body temperature of prostaglandins of the A, E and F series on injection into the third ventricle of unanesthetized cats and rabbits. J Physiol 218:325–336, 1971
25. Feldberg W, Saxena PN: Fever produced by prostaglandin E1, J Physiol 217:547–556, 1971
26. Feldberg W, Saxena PN: Further studies on prostaglandin E1 fever in cats. J Physiol 219:739–745, 1971
27. Flower RJ, Vane JR: Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). Nature 240:410–411, 1972
28. Feldberg W, Gupta KP: Pyrogen fever and prostaglandin-like activity in cerebrospinal fluid. J Physiol 228:41–53, 1973
29. Feldberg W, Gupta KP, Milton AS, Wendlandt S: Effect of pyrogen and antipyretics on prostaglandin activity in cisternal C.S.F. of unanaesthetized cats. J Physiol 234:279–303, 1973
30. Cocconi F, Lees J, Bishai I: Further evidence implicating prostaglandin E1 in the genesis of pyrogen fever. Am J Physiol 254:R463–R469, 1988
31. Sirko S, Bishai I, Cocconi F: Prostaglandin formation in the hypothalamus in vivo: Effect of pyrogens. Am J Physiol 256:R616–R624, 1989
32. Bernheim HA, Gilbert TM, Stitt JT: Prostaglandin E levels in third ventricular cerebrospinal fluid of rabbits during fever and changes in body temperature. J Physiol 301:69–78, 1980
33. Scott IM, Fertel RH, Boulant JA: Leukocytic pyrogen effects on prostaglandins in hypothalamic tissue slices. Am J Physiol 253:R71–R76, 1987
34. Lipton JM, Welch JP, Clark WG: Changes in body temperature produced by injecting prostaglandin E1, EGTA and bacterial endotoxin into the PO/AH region and the medulla oblongata of the rat. Expinencia 29:806–808, 1973
35. Crawshaw LI, Stitt JT: Behavioral and autonomic induction of prostaglandin E1 fever in squirrel monkeys. J Physiol 244:197–206, 1975
36. Williams JW, Rudy TA, Yaksh TL, Viswanathan CT: An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. Brain Res 120:251–262, 1977
37. Stitt JT: Prostaglandin E, fever induced in rabbits. J Physiol 232:163–179, 1973
38. Schoener EP, Wang SC: Effects of locally administered prostaglandin E1 on anterior hypothalamic neurons. Brain Res 117:157–162, 1976
39. Gordon CJ, Heath JE: The effect of prostaglandin E1 on the firing rate of thermally sensitive and insensitive neurons in the preoptic/anterior hypothalamus of unanesthetized rabbits. Fed Proc 38:1295, 1979
40. Stitt JT, Hardy JD: Microelectrophoresis of PGE1 onto single units in rabbit hypothalamus. Am J Physiol 229:240–245, 1975
41. Jell RM, Sweatman P: Prostaglandin-sensitive neurons in cat hypothalamus: Relation to thermoregulation and to biogenic amines. Can J Physiol Pharmacol 55:560–567, 1977
42. Boulant JA, Scott IM: Comparison of prostaglandin E1 and leukocytic pyrogen on hypothalamic neurons in tissue slices. In Homeostasis and Thermal Stress. Edited by KE Cooper, P Lomax, E Schönbaum, WL Veale. Basel, Karger, 1986, pp 78–80
43. Hori T, Nakashima T, Hori N, Kiyohara T: Thermosensitive neurons in hypothalamic tissue slices in vitro. Brain Res 186:207–207, 1980
44. Nakayama T, Hori Y, Suzuki M, Yonezawa T, Yamamoto K: Thermo-sensitive neurons in preoptic and anterior hypothalamic tissue cultures in vitro. Neurosci Lett 9:23–26, 1978
45. Baldino F Jr, Geller HM: Electrophysiological analysis of neuronal thermosensitivity in rat preoptic and hypothalamic tissue cultures. J Physiol 327:173–184, 1982
46. Hori T, Nakashima T, Kiyohara T, Shibata M: Effect of calcium removal on thermosensitivity of preoptic neurons in hypothalamic slices. Neurosci Lett 20:171–175, 1980
47. Bagust J, Kerkut GA: Some effects of magnesium ions upon conductance and synaptic activity in the isolated spinal cord of the mouse. Brain Res 177:410–413, 1979
48. Richards CD, Sercombe R: Calcium, magnesium and the electricity activity of guinea pig olfactory cortex in vitro. J Physiol 211:571–584, 1970
49. Curras MC, Kelso SR, Boulant JA: Intracellular recordings of preoptic temperature sensitive and insensitive neurons (Abstract). FASEB J 2:746, 1988
50. Perlmuter MN, Boulant JA: Intracellular recordings from temperature-sensitive septal and hypothalamic neurons. Soc Neurosci Abst 9:517, 1983
51. Nelson DO, Prosser CL: Intracellular recordings from thermosensitive preoptic neurons. Science 213:787–789, 1981
52. Nakashima T, Hori T, Kuriyama K, Matsuda T: Effects of interferon-α on the activity of preoptic thermosensitive neurons in tissue slices. Brain Res 454:361–367, 1988
53. Gilman SC, Chang J, Zeigler PR, Uhl J, Mochan E: Interleukin-1 activates phospholipase A\textsubscript{2} in human synovial cells. Arthritis Rheumat 31:127–130, 1988
54. Peilschiffer J, Pignat W, Vosbeck K, Marki F: Interleukin 1 and tumor necrosis factor synergistically stimulate prostaglandin synthesis and phospholipase A\textsubscript{2} release from rat renal mesangial cells. Biochem Biophys Res Commun 159:383–394, 1989
55. Shibata M, Blatteis CM, Krueger JM, Obal F Jr, Opp M: Pyrogenic, inflammatory, and somnogenic responses to cytokines: Differential modes of action. In Thermoregulation: Research and Clinical Applications. Edited by P Lomax, E Schönbaum. Basel, Karger, 1988, pp 69–73
56. Blatteis CM, Ahokas RA, Dinarello CA, Ungar A: Thermal and plasma Cu responses of guinea pigs to intraperitoneally injected rIL1, rIL2, rIFNα, and rTNFα. Fed Proc 46:683, 1987
57. Silva NL, Boulant JA: Effects of osmotic pressure, glucose, and temperature on neurons in preoptic tissue slices. Am J Physiol 247:R335–R345, 1984
58. Silva NL, Boulant JA: Effects of testosterone, estradiol, and temperature on neurons in preoptic tissue slices. Am J Physiol 250:R625–R632, 1986
59. Boulant JA, Silva NL: Interactions of reproductive steroids, osmotic pressure, and glucose on thermosensitive neurons in preoptic tissue slices. Can J Physiol Pharmacol 65:1267–1273, 1987
60. Boulant JA, Scott IM: Effects of leukocytic pyrogen on hypothalamic neurons in tissue slices. In Environment, Drugs and Thermoregulation. Edited by P Lomax, E Schönbaum. Basel, Karger, 1983, pp 125–127
61. Krueger JM, Walter J, Dinarello CA, Wolff SM, Chedid L: Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am J Physiol 246:R994–R999, 1984
62. Blatteis CM, Hunter WS, Llanos-Q J, Ahokas RA, Mashburn TA Jr: Activation of acute-phase responses by intraperitoneal injections of endogenous pyrogen in guinea pigs. Brain Res Bull 12:689–695, 1984
63. Hunter WS, Blatteis CM, Llanos-Q J, Mashburn TA Jr, Ahokas RA: Thermal stimulation of the hypothalamus does not evoke the acute-phase reaction. Brain Res 19:69–74, 1987
64. Rowley D, Howard JG, Jenkin CR: The fate of 32p labelled bacterial lipopolysaccharide in laboratory animals. Lancet i:366–367, 1956
65. Dascombe MJ, Milton AS: Study on the possible entry of bacterial endotoxin and prostaglandin E\textsubscript{2} into the central nervous system from the blood. Brit J Pharmacol 66:565–572, 1979
66. Dinarello CA, Weiner P, Wolff SM: Radiolabeling and disposition in rabbits of purified human leukocytic pyrogen. Clin Res 26:522A, 1978
67. Cocca F, Lees J, Dinarello CA: Occurrence of interleukin-1 in cerebrospinal fluid of the conscious cat. Brain Res 446:245–250, 1988
68. Blatteis CM, Dinarello CA, Shibata M, Llanos-Q J, Quan N, Busija D: Does circulating interleukin-1 enter the brain? In Thermal Physiology. Edited by JB Mercer. Amsterdam, Elsevier, 1989, pp 385–390
69. Dinarello CA: Biology of interleukin 1. FASEB J 2:108–115, 1988
70. Dinarello CA: Interleukin 1. Rev Infect Dis 6:51–95, 1984
71. Blatteis CM: Neuronal mechanisms in the pyrogenic and acute phase responses to interleukin-1. Int J Neurosci 38:223–232, 1988
72. Blatteis CM, Bealer SL, Hunter WS, Llanos-Q J, Ahokas RA, Mashburn TA Jr: Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. Brain Res Bull 11:519–526, 1983
73. Blatteis CM, Hales JRS, McKinley MJ, Fawcett AA: Role of the anteroventral third ventricle region in fever in sheep. Can J Physiol Pharmacol 65:1255–1260, 1987
74. Blatteis CM, Llanos-Q J, Howell RD, Quan N: Effects of anteroventral 3rd ventricle region (AV3V) lesions on the febrile response of guinea pigs to endogenous pyrogens (EP). Proc IUPS 17:328, 1989
75. Stitt JT: Evidence for the involvement of the organum vasculosum laminae terminalis in the febrile responses of rabbits and rats. J Physiol 368:501–511, 1985
76. Stitt JT, Shimada SG: Immunoadjuvants enhance the febrile response of rats to endogenous pyrogen. J Appl Physiol 67:1734–1739, 1989
77. Stitt JT, Shimada SG: Enhancement of the febrile responses of rats to endogenous pyrogen occurs within the OVLT region. J Appl Physiol 67:1740–1746, 1989
78. Stitt JT: Prostaglandin E as the neural mediator of the febrile response. Yale J Biol Med 59:137–149, 1986
79. Bosler O: Radioautographic identification of serotonin axon terminals in the rat organum vasculosum laminae terminalis. Brain Res 150:177–181, 1978

80. Calas A, Bosler O, Arlusion M, Bouchard C: Serotonin as a neurohormone in circumventricular organs and supraependimal fibers. In Brain-Endocrine Intreaction. III. Neural Hormones and Reproduction. Edited by DE Scott, GP Kozlowski, A Weindl. Basel, Karger, 1978, pp 238–240

81. Shibata M, Blatteis CM: Neurons in the organum vasculosum laminae terminalis respond to tumor necrosis factor and serotonin in slice preparations. In Thermal Physiology. Edited by JB Mercer. Amsterdam, Elsevier, 1989, pp 413–414

82. Nakayama T: Neuronal activities related to thermoregulation. Yale J Biol Med 59:189–195, 1986

83. Hori T, Kiyohara T, Nakashima T: Thermosensitive neurons in the brain—the role in homeostatic functions. In Thermal Physiology. Edited by JB Mercer. Amsterdam, Elsevier, 1989, pp 3–12

84. Boulant JA, Silva NL: Neuronal sensitivities in preoptic tissue slices: Interactions among homeostatic system. Brain Res Bull 20:871–878, 1988

85. Baker JL, Carpenter DO: Thermosensitivity of neurons in the sensorimotor cortex of the cat. Science 169:597–598, 1970

86. Dean JB, Boulant JA: In vitro localization of thermosensitive neurons in the rat diencephalon. Am J Physiol 257:R57–R64, 1989

87. Inenega K, Osaka T, Yamashita H: Thermosensitivity of neurons in the paraventricular nucleus of the rat slice preparation. Brain Res 424:126–132, 1987