THE YALE JOURNAL OF BIOLOGY AND MEDICINE 63 (1990), 147–156

Hypothalamic Neuronal Responses to Cytokines

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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_2, tumor necrosis factor α, and interleukin 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 laboraotry 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_2 (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 T_{bo}: body temperature TNF: tumor necrosis factor

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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_{bo}. Conversely, hypothalamic cooling elicited shivering, cutaneous vasoconstriction, and a rise in T_{bo}. 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 coefficent 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

[28–32]. 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 thermodetectors, 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 A_2 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]. It

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 icv 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 mintues. 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.

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