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Effects of Temperature and Neuroactive Substances on Hypothalamic Neurones in vitro: Possible Implications for the Induction of Fever

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Summary

This paper reviews some of our findings which have shown the usefulness of *in vitro* methods in the study of hypothalamic neurones. (1) Membrane current analyses of dispersed neurones of the rat preoptic and anterior hypothalamus (POA) during thermal stimulation have revealed that warm-sensitive neurones are endowed with a non-inactivating Na^+ channel having a high Q_{10} in the hyperthermic range (35–41 °C). (2) A brain slice study has shown that neurones in the organum vasculosum lamina terminalis (OVLT) region have much higher sensitivity to PGE_2 than POA neurones. This provides further evidence of a critical role of the OVLT in translation of blood-borne cytokine signals into brain signals for fever induction. (3) Local application of IL-1β and IFNα altered the activity of thermosensitive (TS) neurones and glucose responsive (GR) neurones *in vitro* in an appropriate way to produce fever and anorexia. While the responses to IL-1β required the local release of prostaglandins, the responses to IFNα were found to be mediated by opioid receptor mechanisms. (4) The responses of POA TS neurones and VMH GR neurones to IL-1β but not those to IFNα, were reversibly blocked by αMSH, an endogenous antipyretic peptide. Thus, immune cytokines and their related neuroactive substances may affect hypothalamic TS and GR neurones thereby producing elaborately regulated changes in homeostatic functions such as thermoregulation (fever) and feeding (anorexia), which are considered as host defence responses.

Key words

Thermosensitivity – Interleukin-1 – Interferon – αMSH – Prostaglandin E_2

Thermosensitive (TS) neurones in the preoptic and anterior hypothalamus (POA), which respond to small changes in local temperature, form synaptic networks almost exclusively with TS neurones in the other brain areas, and integrate thermal signals arising from different parts of the body, thereby playing a central role in thermoregulation. Since the discovery of TS neurones in the POA (Nakayama *et al.* 1961), an issue whether TS neurones are specific central thermosensors or whether some neuroneal circuits somehow possess thermosensitivity had been unresolved. With the advent of brain slice techniques, we first demonstrated the existence of warm-sensitive and cold-sensitive neurones having an inherent thermosensitivity which is preserved during synaptic suspension (Hori *et al.* 1980). Since then, *in vitro* studies of hypothalamic TS neurones utilizing tissue slices and dispersed cell preparations have proved to be highly useful in analyzing the thermal transduction mechanisms (Kiyohara *et al.* 1990, Hori 1991), the multimodal sensitivity to temperature, osmolality and glucose (Nakashima *et al.* 1985, Boulant *et al.* 1989, Hori *et al.* 1988), the responsiveness to neuroactive substances (Hori 1991) and the local circuitry of TS neurones within the hypothalamus (Boulant *et al.* 1989).

The present paper gives a brief review of some of our findings which have shown the usefulness of *in vitro* methods in the study of rat hypothalamic neurones. These include (1) the membrane mechanisms of thermal transduction of POA TS neurones, (2) the responsiveness of neurones in the organum vasculosum lamina terminalis (OVLT) region and POA to PGE_2 in relation to the concept of critical involvement of OVLT in mediating the febrile responses to blood-borne cytokines, (3) the responsiveness of hypothalamic TS and glucose-responsive (GR) neurones to immune cytokines for the production of fever and the underlying neuroneal mechanisms, and (4) the modulatory actions of αMSH on interleukin-1 (IL-1) induced activity of hypothalamic TS and GR neurones.

Analyses of thermal transduction mechanisms of POA neurones *in vitro*

The thermal transduction mechanisms in invertebrate neurones and mammalian peripheral thermoreceptors have been attributed to the temperature dependence of the Na^+/K^+ permeability ratio, the electrogenic Na^+ pump and the effects of Ca^{2+} (Carpenter 1981, Schaefer 1987). Quite recently we investigated the ionic current responses of 108 dispersed cells from POA of neonatal rats, using the whole cell configuration (Kiyohara *et al.* 1990). The Na^+ current during changes in cellular temperature was observed after suppressing Ca^{2+} and K^+ currents by internal perfusion of F^- and by replacing K^+ with Cs^+, respectively. About one fourth of neurones showed a non-linear increase in an inward current with high Q_{10} characteristics (4.3-7.0) in the hyperthermic range (35-40 °C) and low Q_{10} characteristics (1.9-2.2) in the hypothermic range (32-35 °C). On the basis of the thermal sensitivity, the shape of thermal response curves and the population, this type of neurones are taken to be warm-sensitive neurones. The remaining three quarters of neurones were non-TS neurones showing a linear increase in an inward current with Q_{10} of 2 (1.3-2.0) during warming over the entire range of temperatures (32-40 °C).

Furthermore, tetrodotoxin (TTX, 500 nM) could reversibly block the high Q_{10} characteristics of the current responses in the hyperthermic range of warm-sensitive neurones, but not the low Q_{10} characteristics in the hypothermic range. The inward current responses to temperature of non-TS neurones were not affected by TTX. The results suggest that the warm-sensitivity of POA neurones is brought about by non-inactivating, TTX-sensitive Na^+ channels having a high Q_{10} in the hyperthermic range (35-40 °C). This conclusion agrees with our findings that warm-sensitive neurones in the septum and the dorsal motor nucleus of the vagus (DMV) decreased the input membrane resistance to a rise in temperature in a TTX-sensitive way.

Although we have not yet elucidated the ionic mechanisms of the cold-sensitivity of hypothalamic neurones, our results on cold sensitive neurones in the DMV suggested that cooling-induced depolarization may be attributed to a reduction in K^+ conductance.

Critical involvement of the region of the organum vasculosum lamina terminalis (OVLT) in the production of fever

It has long been a matter of debate how blood-borne endogenous pyrogens (EP) such as IL-1, tumor necrosis factor (TNF) and interferon a (IFNα) eventually reach or otherwise signal their receptors in the brain to produce fever. Recent studies have proposed the critical role of the OVLT region as a possible site of entry and action of blood-borne EP for the production of fever and ACTH release. The placement of lesions in the OVLT region in rats and rabbits affects the febrile response and ACTH release to intravenous injection of EPs/IL-1 (Stitt 1985, Katuura *et al.* 1990). A microinjection of indomethacin into the OVLT region suppresses the ACTH release which is induced by systemic injection of IL-1b in the rat (Katuura *et al.* 1990). The hyperthermic action of PGE_2 is more potent when microinjected into the OVLT region than when injected into the POA (Stitt 1986). This is compatible with the highest PGE_2 binding found in the regions surrounding the OVLT (Matsumura *et al.* 1990). Based on these findings, it has been suggested that blood-borne EPs/IL-1 affects some as yet unidentified cells in the OVLT region which release PGE_2, then PGE_2 either diffuses into the adjacent POA neurones or acts on the neurones in the OVLT region which send the signals synaptically to POA neurones thereby producing fever (Stitt 1986).

To investigate whether the OVLT neurones actually possess a high sensitivity to

PGE_2 as the OVLT hypothesis assumes, we observed the responsiveness of neurones in the OVLT region and the POA to local application of PGE_2 in rat OVLT and POA tissue slices which were isolated from each other. Perfusion with PGE_2 in doses between 1 nM and 250 nM altered the firing rate in 35 of 42 OVLT neurones and 24 of 43 POA neurones dose dependently. The responses to PGE_2 were observed even during synaptic suspension. OVLT neurones in general were found to be more sensitive to PGE_2 than POA neurones. The approximate threshold concentration of PGE_2 to alter the firing rate of the OVLT neurones (4.9 ± 1.8 nM S.E.M.) was significantly lower than that of the POA neurones (40.6 ± 15.8 nM). There was a significantly higher incidence of inhibitory responses to PGE_2 among warm-sensitive neurones in the OVLT region, but no clear correlation between the type of thermosensitivity and the type of responses to PGE_2 in POA neurones. The lower threshold responses to PGE_2 and higher incidence of PGE_2 responsiveness among OVLT neurones are consistent with the OVLT hypothesis which suggests a PGE_2-mediated process in the OVLT region which translates the blood-borne signals conveyed by EPs/IL-1 into brain signals.

Involvement of brain immune cytokines in the induction of fever and anorexia

Immune cytokines such as IL-1β and IFNα are now known to be synthesized in the brain mainly from glial cells during both peripheral and central infections and brain injury (Fontana *et al.* 1984, Giulian and Lachman 1985, Larsson *et al.* 1978). Although the brain-derived cytokines act on astrocytes and promote their growth (Giulian and Lachman 1985), there is some evidence that the brain-derived cytokines function as neuromodulators in the brain and may be involved, at least partly, in the production of fever, anorexia, sleep and analgesia (Dinarello 1988, Hori *et al.* 1991). By the use of brain tissue slices, we found that these cytokines decreased and increased the activity of warm-sensitive neurones and cold-sensitive neurons in the POA, respectively (Nakashima *et al.* 1988, 1989), and decreased the activity of glucoreceptor neurones in the ventromedial hypothalamic nucleus (VMH) (Kuriyama *et al.* 1990). In view of the functions ascribed to the POA TS neurones and the VMH glucoreceptor neurones, these responses may explain, at least in part, the fever and anorexia induced by brain-derived cytokines.

The neuroneal responses to IL-1b, but not to IFNα, were abolished by concurrent application of sodium salicylate, suggesting that the local synthesis of cyclooxygenase metabolites is essential in mediating the local actions of IL-1β on hypothalamic neurones. On the other hand, the responses of POA and VMH neurones to IFNα, but not those to IL-1β, were reversibly blocked by naloxone, suggesting the involvement of opioid receptor mechanisms in the action of IFNα on hypothalamic neurones (Nakashima *et al.* 1988, Kuriyama *et al.* 1990). Indeed, it has been demonstrated that IFNα, but not IFNγ, binds to the μ opioid receptor in the mouse brain (Blalock and Smith 1981), and produces analgesia, catalepsy and the suppression of abstinence of morphine addiction (Blalock and Smith 1981, Dafny and Reyes-Vazquez 1985). Furthermore, we found that systemic injections of naltrexone could suppress the fever which was induced by central injection of IFNα at least for the first one hour. The opioid receptor mediation of IFNα's action has been demonstrated in the peripheral immune system as well. The enhancing action of cytotoxic activity of natural killer cells in the spleen is blocked by naloxone (Kay *et al.* 1984). These findings represent an example showing the common signal molecules and the common receptor mechanisms between brain cells and immune cells.

αMSH as an endogenous antipyretic and an endogenous antagonist against IL-1

αMSH, a hypothalamic peptide, has been suggested as one of the endogenous antipyretics which may work to limit excessive fever. αMSH is released in the brain specifically during EP-induced fever, but not during passive hyperthermia, and it suppresses EP-induced fever, but not passive hyperthermia. Quite recently, we found that the responses of POA TS neurones and VMH glucose responsive neurones in tissue slices to local application of IL-1β, but not those to IFNα, were reversibly blocked by simultaneous application of αMSH (Kuriyama *et al.* 1990, Mizuno *et al.* 1989). The suppression of IL-1-induced responses by αMSH has been observed in the peripheral

tissues as well as in the CNS. It was recently demonstrated that aMSH suppressed the hyperalgesia induced by subcutaneous injection of IL-1 (Follenfant *et al.* 1989), IL-1-induced inflammatory responses (Lipton 1989) and the IL-1-induced enhancement of lymphocyte proliferation, but not the IL-2-induced responses of immunological cells (Cannon *et al.* 1986). These findings suggest that αMSH is an endogenous antagonist against IL-1 both in the nervous system and the immune system. It thus appears that IL-1 that causes fever, inflammation, acute phase responses and immunological activation evokes central and peripheral release of αMSH, and this peptide in turn acts as an endogenous antagonist against IL-1 both in the CNS and the periphery to modulate IL-1-induced host defense responses.

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