Molecular Biology of Thermoregulation
Some historical perspectives on thermoregulation

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Cooper, K. E. Some historical perspectives on thermoregulation. J Appl Physiol 92: 1717–1724, 2002; 10.1152/japplphysiol.01051.2001.—In this paper, selected historical aspects of thermoregulation and fever are presented as background to the application of molecular biology to thermoregulation. Temperature-sensing mechanisms, coordination of thermal information, thermoregulatory circuitry, efferent responses to thermal stimuli, set point mechanisms, and some of the mechanisms and consequences of fever and hyperthermia are highlighted. Neurotransmitters used in thermoregulatory circuits are also discussed. An attempt is made to include information from comparative physiological sources. Possible future avenues of research in the light of recent new technologies are also presented.

Body temperature ranges of mammals, birds, reptiles, insects, and fish and the diurnal temperature variations and the effects of exercise on body temperature. The exploitation of thermoelectricity by Becquerel and Breschet (4) in 1835 enabled Lefèvre (48) to use thermocouples to measure the thermal topography of the body. Lefèvre (48) also described whole animal and human calorimetry for determining metabolic rates, a technique used later by Dubois (23) to study fever and by Benzinger (6) as a partition calorimeter to investigate the central control of core temperature. Analytical equipment and neurophysiological and physicochemical techniques to explore the mechanisms of thermoregulation in the whole animal and at the molecular level have since multiplied almost exponentially. With the use of these new methods, researchers are now at the threshold of a much deeper understanding of the basic mechanisms of thermoregulation.

Knowledge of the mechanisms of thermoregulation has progressed from the crude localization of areas within the central nervous system involved in thermoregulation to fuller, but still incomplete, mapping of areas in the neural circuitry necessary to receive and process thermal inputs. Regulatory effector mechanisms have also been explored. Currie (18) and Liebermeister (49) knew that body temperature is a regulated entity. Ott (59) made brain stem sections and concluded that there are centers in the region of the corpora striata that affect thermoregulation. Ranson

THE INTENTION OF THIS PAPER is to outline some aspects of thermoregulation, setting them in historical context as well as making reference to some recent findings made at the cellular level. It is not intended to be an exhaustive review of all aspects of thermoregulation but to set the stage for papers on molecular biological studies. For greater breadth and depth, the reader should see the following reviews of the mechanisms of body temperature regulation and their disorders: Kuno (47), Kluger (44), Satinoff (65), Hellon and Townsend (34), Stitt (70), Moltz (55), Cooper (15), Zei-berger (76), Blatteis et al. (8), and Gisolfi and Mora (28).

Development of thermometers led to the scientific, quantitative study of thermoregulation. Mercury-in-glass thermometers were available to James Currie (18) in 1798, who used them as diagnostic and prognostic tools in his clinical studies, in his studies of fever, and in his experiments on cold water immersion. The wide use of thermometry in clinical practice came after the publication of Wunderlich's (74) Manual of Medical Thermometry in 1868. Wunderlich attempted to define the character of temperature changes associated with specific disease processes. He described the

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Characteristics of Iggo (39), and Hensel (35) investigated the coding of thermal information that projects to the extremely important regions within the hypothalamus (which respond to incoming thermally generated signals) has been studied. Dodt and Zotterman (21), Hardy (32), Iggo (39), and Hensel (35) investigated the coding of peripheral thermal sensation. They analyzed the characteristics of “warm” and “cold” receptor responses to thermal stimulation. However, the process of translation of neural firing patterns from peripheral temperature receptors into conscious sensations of heat and cold is still a mystery. One is reminded of a line in an old hymn: “the mystic harmony linking sense to sound and sight.”

Our knowledge of the complex circuits within the hypothalamus subserving thermoregulation has been greatly advanced by observations made on hypothalamic tissue slices (10). The molecular mechanisms of transduction of temperature sensing into neural firing patterns and the molecular events in the postsynaptic regions of the synapses, which cause sequential neural stimulation leading to the initiation of vascular smooth muscle responses or tissue heat production, are now being unraveled. It is generally accepted that the body temperature is regulated about a physiologically determined set point. The mechanism by which the set point is determined is still a mystery.

Studies in comparative physiology have added breadth to our concepts of thermoregulation. For example, thermoregulation occurs in ectotherms as well as in endotherms by behavioral means. Ectothermic animals can shuttle between direct sunlight and shade to maintain a remarkably steady core temperature during daylight hours. Some mammals have evolved the ability to hibernate, allowing them to survive in a state of torpor over cold winters. Grant’s Golden mole (Erimetaipa grantii), which lives in the Namib desert, lets its core temperatures fall over a large range while maintaining a surprising amount of hunting activity. It “swims” deeply into cool layers of very fine desert sand and reduces its body temperature and so lowers oxygen (and energy) expenditure and the need for vigorous breathing (25). Perhaps the lowered body temperature is, in part, a consequence of relative hypoxia. Some plants, e.g., the Eastern skunk cabbage, and some philodendras are able to maintain the temperature of the spadix or inflorescence well above ambient temperature during flowering. This elevated temperature is regulated to some degree and maintained by the appropriate alteration of the metabolic rate. The response appears to be hormonally mediated (45, 57). However, the sensory trigger for this thermal response awaits full elucidation at the molecular level. Some of the key elements of thermoregulation and fever are discussed below.

SENSING TEMPERATURE AND TEMPERATURE CHANGE

The ability of an organism to sense the temperature of its environment, and the temperatures of its body components, is fundamental to the control of its body temperature. There are specialized nerve endings in vertebrate endotherms and ectotherms that can detect and respond to both steady temperature and rapid changes in temperature. Some protozoa were shown to sense temperature and move to preferred temperature zones, e.g., Paramecium and Oxytricha (41, 52). The ability to sense temperature developed very early in phylogeny; by studying simple unicellular organisms, researchers can find clues to mechanisms that may parallel the events of thermal perception in multicellular organisms. Recent studies (40) in Paramecium have shown that various ion channels are involved in perception of temperature, such as different calcium channels responding to heating and cooling. It is possible that a temperature-detecting mechanism could involve both calcium channels and/or potassium channels shared with a mechanoreceptor (71), but this is yet to be determined. Discovery of temperature-sensitive ion channels has led to the study of the events that their activation brings about within the cell and the subsequent activation of the cell cilia. A recent study (56) suggests that prostaglandin I₂ may be a thermosensory mediator in Paramecium. In multicellular organisms having nervous systems, there are many neurons that exhibit temperature sensitivity. Although many of these form part of thermoregulatory control systems, the possession of thermosensitivity does not necessarily imply such a connection. For example, there are thermosensitive neurons in the sensorimotor cortex in the cat that appear not to have a role in thermoregulation (3). Thermosensitive neurons involved in thermoregulation respond to thermal stimulation by altering their pattern of spike discharges (36, 39). One can thus imagine that thermosensitive neurons behave as unicellular organisms; that is, when unable to move, they send out signals to be integrated with those of many other such cells, causing the whole organism to adjust to the changing thermal environment and preserve its internal thermal topography.

To be classified as a specific (peripheral) temperature receptor, four criteria must be met (35): 1) the neuron must exhibit a static sensitivity to constant temperature, 2) the neuron must show a dynamic response to temperature change with a positive coefficient in warm receptors and a negative coefficient in cold receptors, 3) the neuron must not be excited by moderate mechanical stimuli, and 4) neuron activity must occur within the innocuous or nonpainful range of temperature (35, 36). The pattern of neuron discharge in response to a change in temperature is both dynamic and static (see Refs. 35 and 39). The plot of static neuron discharge frequency against receptor temperature is bell shaped. The warm receptors increase their firing rate with increasing temperature, and the cold
receptors increase their discharge rate as the temperature falls (39). Both types of receptors show peaks of activity with a fall in discharge frequency beyond the peaks. The dynamic response of the warm receptors involves a short-lived burst of firing when the temperature is abruptly raised, whereas that of the cold receptor involves a transient rapid fall in firing frequency. The transduction of the temperature stimulus to neuron firing has been the subject of much study. Which ion channels are involved is still debated. One recent paper (51) provides evidence that a two-pore domain mechanogated potassium channel called TREK-1 may be one of the physiological temperature receptors. The roles of membrane proteins and second messenger substances in translating the opening of ion channels into nerve impulses are still under intensive investigation.

Downey et al. (22), using cooling cuffs around arteries, found evidence for thermosensitivity in the internal carotid artery of the rabbit. The use of direct neuronal recordings has also provided evidence for thermosensitive structures within the brain. Both “cold” and “warm” thermosensitive neurons were found in the hypothalamus, brain stem, and spinal cord (33, 35, 58, 67). It is interesting that the range of firing of cold receptors in the hypothalamus seems to be wider in hibernating animals (75). Hori (38) studied the transduction mechanisms of thermosensitive neurons in the preoptic area. He found that warm-sensitive neurons developed a tetrodotoxin-sensitive sodium current with a high $Q_{10}$, whereas cold-sensitive neurons experienced membrane depolarization due to reduction in potassium conductance. Boulant (11) studied the characteristics of hypothalamic thermosensitive neurons and the action of such endogenous substances as pyrogens, which reduce the activity of warm-sensitive neurons and increase the activity of cold-sensitive neurons. He also used a fluorescent dye (lucifer yellow) to show that dendrites of warm-sensitive neurons are arranged perpendicular to the third ventricle, whereas the temperature-insensitive neurons lie parallel to the third ventricle. This observation will be very useful in developing models of hypothalamic thermosensitivity. Boulant’s and other laboratories have also used axonally transported markers; perhaps some of the mystery of the afferent and efferent pathways used in thermoregulation will soon be unraveled. Burgon and Boulant (13) investigated the thermosensitive properties of some neurons in the rat suprachiasmatic nucleus. Such neurons could be important in providing clues to the mechanisms of circadian clock synchronization. This interesting physiological process requires further study as a means to build bridges between observations at the cellular level and the function of the whole animal. Thermosensitive neurons may exist in other deep body structures, and the weight given by the central controlling mechanism(s) to inputs from all areas of the body is still open to argument.

COORDINATION OF THERMAL INPUTS

The hypothalamus plays a vital role in controlling body temperature by coordinating thermal information from all body areas and directing the efferent signals to the appropriate heat production and heat conservation systems in mammals. The evidence for this comes from electrical stimulation and recordings (7, 33, 43) after parts of the hypothalamus were thermally stimulated and after hypothalamic lesioning (9, 31, 43). Hypothalamic disease has been found to be accompanied by disordered temperature regulation (26). The neurons in the anterior hypothalamus and the preoptic region may respond to thermal stimulation directly or to thermal stimulation of other connecting neurons. Boulant (10) proposed a hierarchy of neural structures controlling body temperature involving the brain stem and spinal cord, with the preoptic area acting as the highest coordinating center.

Evidence about the neurotransmitter substances used in hypothalamic and brain stem thermoregulatory synapses, particularly catecholamines and serotonin, was found in the 1960s (16, 24). Dopamine, GABA, glutamate, and acetylcholine were also found to be used as thermoregulatory neurotransmitters (9). The search for these neurotransmitters was spurred by Carlson and colleague’s discovery of norepinephrine in the hypothalamus by visualization with the formaldehyde condensation technique (quoted in Ref. 15, p. 22) and by pharmacological demonstration of high concentrations of catechol and other amines in the hypothalamus (1, 73). Bligh (9) proposed a neuronal model for sheep in which input from warm receptors acting through serotonergic synapses evoke heat loss mechanisms and from cold receptors acting through cholinergic synapses evoke heat production and conservation. In this model are cross-over neurons, possibly adrenergic, arranged so that on warm stimulation the cold receptor pathway is inhibited and vice versa. This system may be expanded to include synapses that use other neurotransmitters, with the transmitters varying according to the species. More recent evidence points to the role of nitric oxide in both normal thermoregulation and fever. These roles are presented and discussed by Gerstberger (27). Nitric oxide appears to be involved both in the central nervous system thermoregulatory regions and in the adjustment of peripheral vascular tone involved in temperature regulation. Cleavage of the heme molecule releases carbon monoxide. There is evidence that within the central nervous system carbon monoxide has a pyrogenic action (69). Although the prostaglandin E pathway in the appropriate central nervous system regions is central to the induction of fever, it is open to speculation whether the nitric oxide and carbon monoxide mechanisms provide an alternative fever pathway. Such a suggestion is yet to be validated.
There is evidence of the involvement of a histaminergic system involved in the brain stem subserving mechanisms of hibernation (60).

THE "SET POINT": ITS NATURE AND EVOLUTIONARY SELECTION

The notion of a regulated body temperature was clearly held by early researchers such as Currie (18), Liebemaster (49), Ott (59), and Lefèvre (48). Simple experiments in which human and animal subjects were first immersed in cold or hot water until their core temperatures changed and then put into a neutral thermal environment showed that the core temperature always returned to the initial level. The idea that, during fever, vide infra, the body regulates its temperature at a new high controlled level or "set point" was also supported (15, 49). For humans, the set point in health is $37^\circ C$. The regulated level of core temperature varies by $1^\circ C$ during the diurnal daily temperature swing. The advantage to humans of having a set point of $37^\circ C$ is unclear, especially when considering the different "set" levels in other mammals (range of 36–38°C), birds (38–43°C), marsupials (~35°C), and lizards during daylight hours (~32–38°C) (15, 44). Simple organisms such as paramecium are capable of maintaining a "regulated" temperature in a thermocline by selecting a thermal environmental preferendum or possibly selecting a region that avoids temperature extremes that would be harmful (see above). The molecular "switch(s)" for selection of thermal preferenda is still unclear. In a recent study (14) of the nematode worm Caenorhabditis elegans, which has three pairs of neurons involved in temperature sensing, a gene (ceh-14) was shown to confer thermosensory function. A groundbreaking model to explain the regulation of internal body temperature was proposed (Fig. 1) by Hammel (30), and he also used this model to develop a mechanism for a variable set point. Other engineering and neuronal models of set-point mechanisms have also been developed (see Chapters 11 and 12 in Ref. 9). One of these suggests that the intersection of the rising phases of the cold and warm receptors would be at the set point. These models will probably continue to undergo modification as new facts are discovered. Core temperature set point follows a diurnal cycle, changes during the menstrual cycle, and may even be altered during exercise.

Fig. 1. A schema for the controlling and controlled systems for the regulation of internal body temperature. ARAS, ascending reticular activating system; Aud vis, audiovisual; CNS, central nervous system; T, temperature; hypo, hypothalamus; $a_k$, proportionality factor for nonevaporative heat transfer; $a_m$, proportionality factor for shivering; EHL, evaporative heat loss; HP, heat production; HL, heat loss; $K$, coefficient for nonevaporative heat transfer; $T_c$, core temperature; $T_{op}$, operative temperature. [With permission from the Annual Review of Physiology, vol. 30, copyright 1968 by Annual Reviews, www.AnnualReviews.org (Ref. 30).]
SOME COORDINATED RESPONSES OF MULTICELLULAR ORGANISMS TO THERMAL STIMULI

An important response to changes in the environmental and body temperatures is the heat loss or conservation behavior (65). Moving to warmer or cooler places, donning or removing clothing, and adopting heat-exchange-altering postures are examples of behavioral thermoregulation. Physiological responses also occur (15, 36). These include alteration of the distribution of blood flow between the core and the skin so that surface heat loss is increased or reduced, the initiation of additional heat production by shivering, and the activation of nonshivering thermogenesis and the reduction of heat production during heat exposure. Physiological and behavioral responses to hypothalamic cooling were found to occur simultaneously in female rats (see p. 158–163 in Ref. 65). During long periods of cold exposure, some changes in basal metabolism are principally under the control of the thyroid hormones. Nonshivering thermogenesis occurs particularly in specialized fat depots known as brown adipose tissue (BAT). These fat pads are found in the newborn of several species and in adults of some animals. They are mainly located between the scapulae and around the kidneys. These fat cells are smaller than white fat cells, they contain high concentrations of mitochondria, and have a dense sympathetic innervation. They have been studied since the 1960s (37, 68); however, Dawkins and Hull (19) reported that Konrad von Gesner described brown fat in 1551. Recently, a group of proteins, the uncoupling proteins (UCPs), has been identified, a family of proteins, the uncoupling proteins (UCPs), has been shown to be paramount in the distribution of blood flow between the core and the skin so that surface heat loss is increased or reduced. This endogenous pyrogen was later found to be a fairly low-molecular-weight (15–18 kDa) peptide. We now recognize a family of cytokines; some of these, such as interleukin-1β and tumor necrosis factor-α, are pyrogenic. Cytokines enable cells of the immune system to talk to each other. The release of pyrogenic cytokines probably requires the action of the plasma complement cascade (8). The locus of action was first found to be in the vicinity of the anterior hypothalamus/preoptic region (15) and then more accurately localized in the organum vasculosum laminae terminalis (OVLT) (see Ref. 70). The discovery of a role of prostaglandin E as a final mediator of fever within the hypothalamus added an important piece to the puzzle of fever genesis (54). This notion was supported by the fact that aspirin and other antipyretics block the synthesis of prostaglandin E2 (72). It is thought that either the cytokines act within the OVLT to release prostaglandin E2, which then diffuses into the preoptic area, or they activate neurons in the OVLT, which release PGE2 in the preoptic area.

It has become apparent that fever is but one part of a coordinated first-phase response to infection and that the whole response includes mobilization of the immune system (20, 44). Kluger (44) showed that some ectotherms and some mammals have a reduced survival rate if the animal’s core temperature is not allowed to rise. It may be that the benefits of fever to the human are gained during the time between the onset of infection and the patient seeking medical help. Nevertheless, the wisdom of early use of antipyretics in fever is still debated.

A recent hypothesis was based on the absence of fever in animals given intraperitoneal endotoxin after subdiaphragmatic vagotomy. It was proposed that pyrogenic cytokines could act on vagal nerve endings in the liver. A pathway through the brain stem to the anterior hypothalamus/preoptic area then leads to the release of PGE2 to cause fever (see Ref. 8). There is still controversy over this proposition. Whether other peripheral neural inputs contribute to the initiation or maintenance of fever has not yet been explored. Fever caused by intravenous endotoxin in the human is often accompanied by skin hyperalgesia and aching in muscles and joints. Whether these unpleasant symptoms are caused by action of cytokines on peripheral nerve endings or nerves or on the gating mechanisms in their spinal or brain pathways remains virtually unknown but worthy of study. The possible protective role of heat shock proteins synthesized during fever is as yet little understood. Brown (12) reported the synthesis of a protein in the brain after induction of fever by bacterial pyrogen that was similar to a heat shock protein.

Evidence for the release of arginine vasopressin into the ventral septal brain area, where it acts as an endogenous antipyretic, came from studies by Kasting (42) after the discovery of the inability of newborn lambs to respond to intravenous endotoxin with fever (61). Other endogenous antipyretics include α-melano-
cyto stimulating hormone (α-MSH) (acting in the lateral septum) and corticosteroids (see Ref. 15).

HYPERTHERMIA

Hyperthermia can occur naturally or it may be artificially induced, for example, as an adjunct to cancer therapy. Naturally, it may occur during exercise in the heat, particularly if sweating is inhibited. This can occur if fluid intake or salt intake is restricted (46) or if minute amounts of bacterial pyrogen are present in the circulation (2).

Thermotolerance (heat resistance) can be related to the gene activation of proteins termed heat shock proteins. These proteins have been widely studied by molecular biologists (66). An original observation was the finding of stress-related genes activated on the Droso-phisla melanogaster salivary gland chromosome when the fly was given a heat shock (64). The gene switches for the synthesis of heat shock proteins and other stress proteins have yet to be fully elucidated; this discovery will add important information regarding the mechanisms of thermotolerance and fever. Raising oral or rectal temperatures in humans to 38.2–38.6°C in a hot tub for as little as 15 min triggered heat shock protein 72 gene expression (53). The possible therapeutic gains from this means of generating the protein requires further study.

QUO VADIS?

Attempting to predict the future of any scientific field is always dangerous. However, several lines of investigation would appear useful. Use of modern imaging techniques can show brain areas that become active during thermoregulatory responses or fever and antipyresis. After the localization of such regions by imaging, neurophysiological methods can be applied to determine connectivity. The use of axonally transported markers such as microbeads, immunochemical markers, and virus particles, which cross synapses, will add to the studies of the circuitry. The gene switches in the appropriate neuron pools and connecting neurons can then be related to thermoregulatory function. In addition, use of these materials could lead to identification of the range of ion channels in these neurons. Ion channels in peripheral thermoreceptors could then be revealed, as well as intracellular messenger systems that result from the ion fluxes. The use of knock-out animals is already extensive and should help unravel thermal mechanisms, such as the role of cyclooxygenase-2 in fever (8) at the cellular level. However, it should be noted that, in multifactorial control mechanisms, failure to prevent a function by one gene knockout may only mean that subsidiary mechanisms take over. Recent research has shown some gender differences in thermoregulatory responses and fever. More studies of these differences could lead to interesting knowledge of the actions of reproductive hormones in thermoregulatory circuits. Studies of the mechanisms of hibernation at the cellular and gene level could produce important knowledge about thermoregulatory control in both hibernators and nonhibernators. Perhaps other combinations of neurotransmitters will be found in thermoregulatory circuits, and discovery of these could lead to development of new drugs to manage thermoregulatory disorders. The combined role of fever and the immune system stimulation needs much further investigation. Finally, nature presents lesions that, when studied, could lead to identification of new knowledge about basic functions. Close collaboration with clinical colleagues will be of value. The past 50 or so years have brought enormous increases in our knowledge of thermoregulatory function and disorder, and truly the future of thermoregulatory research looks very exciting as researchers integrate the molecular aspects with the function of the whole organism.

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