[Frontiers in Bioscience 10, 2193-2216, September 1, 2005]

FEVER AND HYPOTHERMIA IN SYSTEMIC INFLAMMATION: RECENT DISCOVERIES AND REVISIONS

Andrej A. Romanovsky [1], Maria C. Almeida [1], David M. Aronoff [2], Andrei I. Ivanov [3], Jan P. Konsman [4], Alexandre A. Steiner [1], and Victoria F. Turek [5]

[1]Systemic Inflammation Laboratory, Trauma Research, St. Joseph's Hospital and Medical Center, 350 West Thomas Road, Phoenix, AZ 85013, USA, [2]Division of Infectious Diseases, Department of Internal Medicine, University of Michigan Health System, 1500 West Medical Center Drive, Ann Arbor, MI 48109, USA, [3]Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA 30322, USA, [4]Laboratoire de Neurobiologie Integrative, Centre National de la Recherche Scientifique FRE 2723/Institut National de la Recherche Agronomique UR 1244, Institut Francois Magendie, 33077 Bordeaux, France, and [5]Department of Behavioral Neuroscience, Oregon Health and Science University, 3181 South West Sam Jackson Park Road, Portland, OR 97239, USA

TABLE OF CONTENTS

1. ABSTRACT

Systemic inflammation is accompanied by changes in body temperature, either fever or hypothermia. Over the past decade, the rat and mouse have become the predominant animal models, and new species-specific tools (recombinant antibodies and other proteins) and genetic manipulations have been applied to study fever and hypothermia. Remarkable progress has been achieved. It has been established that the same inflammatory agent can induce either fever or hypothermia, depending on several factors. It has also been established that experimental fevers are generally polyphasic, and that different mechanisms underlie different febrile phases. Signaling mechanisms of the most common pyrogen used, bacterial lipopolysaccharide (LPS), have been found to involve the Toll-like receptor 4. The roles of cytokines (such as interleukins-1beta and 6 and tumor necrosis factor-alpha) have been further detailed, and new early mediators (*e.g.*, complement factor 5a and platelet-activating factor) have

been proposed. Our understanding of how peripheral inflammatory messengers cross the blood-brain barrier (BBB) has changed. The view that the *organum vasculosum* of the *lamina terminalis* is the major port of entry for pyrogenic cytokines has lost its dominant position. The vagal theory has emerged and then fallen. Consensus has been reached that the BBB is not a divider preventing signal transduction, but rather the transducer itself. In the endothelial and perivascular cells of the BBB, upstream signaling molecules (*e.g.*, pro-inflammatory cytokines) are switched to a downstream mediator, prostaglandin (PG) E_2. An indispensable role of PGE_2 in the febrile response to LPS has been demonstrated in studies with targeted disruption of genes encoding either PGE_2-synthesizing enzymes or PGE_2 receptors. The PGE_2-synthesizing enzymes include numerous phospholipases (PL) A_2, cyclooxygenases (COX)-1 and 2, and several newly discovered terminal PGE synthases (PGES). It has been realized that the "physiological," low-scale production of PGE_2 and the accelerated synthesis of PGE_2 in inflammation are catalyzed by different sets of these enzymes. The "inflammatory" set includes several isoforms of PLA_2 and inducible isoforms of COX (COX-2) and microsomal (m) PGES (mPGES-1). The PGE_2 receptors are multiple; one of them, EP3 is likely to be a primary "fever receptor." The effector pathways of fever start from EP3-bearing preoptic neurons. These neurons have been found to project to the raphe pallidus, where premotor sympathetic neurons driving thermogenesis in the brown fat and skin vasoconstriction are located. The rapid progress in our understanding of how thermoeffectors are controlled has revealed the inadequacy of set point-based definitions of thermoregulatory responses. New definitions (offered in this review) are based on the idea of balance of active and passive processes and use the term balance point. Inflammatory signaling and thermoeffector pathways involved in fever and hypothermia are modulated by neuropeptides and peptide hormones. Roles for several "new" peptides (*e.g.*, leptin and orexins) have been proposed. Roles for several "old" peptides (*e.g.*, arginine vasopressin, angiotensin II, and cholecystokinin) have been detailed or revised. New pharmacological tools to treat fevers (*i.e.*, selective inhibitors of COX-2) have been rapidly introduced into clinical practice, but have not become magic bullets and appeared to have severe side effects. Several new targets for antipyretic therapy, including mPGES-1, have been identified.

2. INTRODUCTION

Although the thermoregulatory manifestations of systemic inflammation, *viz.*, fever and hypothermia, have been studied for years, our understanding of their molecular and physiological mechanisms has substantially advanced over the past decade (1995-2004). This decade has also changed the standards for animal models to study fever and hypothermia and produced new definitions of these thermoregulatory responses. Perhaps most importantly, new pharmacological tools to treat clinical fevers have been introduced. To describe these and other advances in research on the febrile and hypothermic responses to inflammatory stimuli is the purpose of this review. This review also introduces a series of articles (1-20) on fever and hypothermia published as a special issue of the *Frontiers in Bioscience* and available online at http://www.bioscience.org/current/special/romanov.htm.

3. NEW TERMINOLOGY

This review focuses on two responses to infectious, inflammatory, and other stimuli: fever and hypothermia. Fever (also known as the febrile response) and hypothermia (also known as anapyrexia or regulated hypothermia) are commonly studied in the laboratory by injecting animals with bacterial lipopolysaccharide (LPS) or mediators of its action, including pro-inflammatory cytokines [*e.g.*, interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha] and prostaglandins (PGs) of the E series. Fever used to be defined as an increase in deep body temperature (T_b) occurring due to an increase in the thermoregulatory set point (21-23), whereas anapyrexia was defined as a decrease in T_b due to a decrease in the set point (21, 23-25). Those definitions were based on a model of T_b control requiring a single set point, either obvious (physiological) or hidden (mathematical). More than two decades ago, Satinoff (26) proposed that thermoeffectors are controlled largely independent of each other, and Werner (27) demonstrated that all setpoint concepts are built on unnecessary and unproven assumptions. Werner (27) also proposed a more general concept that is based on the balance of active (controlling) and passive (controlled) processes and requires neither an obvious nor a hidden set point. Over the last decade, further evidence of independent control of thermoeffectors has been accumulated (28), the inadequacy of models of the thermoregulatory system with a single set point has been demonstrated (29), and many cases of independent recruitment of thermoeffectors in thermoregulatory responses have been documented (11, 30-33). In view of these developments, Romanovsky (34) suggested to modify definitions of fever and regulated hypothermia. These new, modified definitions are listed in Table 1. They are based not on comparing T_b with a nonexistent set point, but on determining at which value T_b would balance in a given response.

4. EXPERIMENTAL MODELS AND PHENOMENOLOGY

4.1. Studying Fever: Species Used and the Response Latency

Traditionally, fever was studied almost exclusively in larger mammals, from guinea pigs and rabbits to sheep, pigs, goats, dogs, and monkeys. The rat was generally considered a species that does not respond to LPS with fever, whereas some authors believed that special "tricks" (such as priming with LPS) are required to induce the febrile response in this species (35). The fundamental study by Székely & Szelényi (36) clarified the issue: well-adapted rats studied in a thermocouple setup under thermoneutral conditions have been shown to respond to small doses of LPS with marked, reproducible fevers. The introduction of telemetric thermometry simplified studying thermoregulation in rats and propelled research in these

Table 1. Definitions of thermoregulatory states or responses

Term[1]	Definition	Notes and guides for usage
Classifying principle 1: Level of T_b		
Normothermia (euthermy, cenothermy)	A state characterized by a "normal" T_b	Terms **normo-**, **hyper-**, and **hypothermia** mean that T_b is at (within a few tenths of a degree C), above, or below its "normal" level for a given species under thermoneutral conditions. These terms should not be used to pinpoint the type of T_b regulation in a state or response or to clarify the relationship between T_b and T_{eq} (see below). A widespread opinion that **fever** should not be called **hyperthermia** is hardly justifiable. Similarly to how any state characterized by an increased blood pressure is hypertension (regardless of the mechanisms involved), any state characterized by a high T_b is **hyperthermia**. In fact, the terms **normo-**, **hyper-**, and **hypothermia** have the advantage of being applicable to states and responses that involve unknown and/or multiple thermoregulatory mechanisms.
Hyperthermia	A state or response characterized by a higher than normal T_b	
Hypothermia	A state or response characterized by a lower than normal T_b	
Classifying principle 2: Type of T_b regulation		
Homeothermy (homoiothermy)	A state in which all thermoeffector responses have similar (within a few tenths of a degree C) threshold T_bs.	In **homeothermy**, T_b is regulated (defended) with a narrow dead band (tightly). Theoretically, the **homeothermic** type of T_b regulation can be utilized in **normothermy**, as well as in **hyperthermic** or **hypothermic** responses. However, **hypothermic** responses occurring with narrow dead band regulation have not been demonstrated.
Poikilothermy	A state in which thermoeffector responses have different (usually by several degrees C) threshold T_bs.	In **poikilothermy**, T_b is regulated with a wide dead band (loosely). When T_b is between the threshold T_b's for triggering cold- and heat-defense responses, it is not defended, *i.e.*, is the result of passive heat transfer between the body and its environment. The **poikilothermic** type of T_b regulation can be utilized in **normo-**, **hyper-**, and **hypothermic** states.
Classifying principle 3: Relationship between T_b and T_{eq}		
Fever (**febrile** response or state)	A state in which T_{eq} is above the normal level of T_b, or a response in which T_b temporarily balances above its normal level.	Before **fever** occurs, $T_b = T_{eq}$. Then T_{eq} increases, which leads to $T_b < T_{eq}$ at the onset of **fever**, and $T_b = T_{eq}$ at a plateau (this is a **febrile** state in its pure form). Thereafter, T_{eq} decreases, which leads to $T_b > T_{eq}$ during deffervescence, and then $T_b = T_{eq}$ when **fever** is resolved. The old definition of **fever** assumed that T_b is tightly defended during this response. It is now known that **fevers** can be characterized by either the **homeothermic** or **poikilothermic** type of regulation (see *Section 4*). Caution should be exercised to use the term **fever** in such a way that it does not imply exclusively the **homeothermic** type of regulation.
Anapyrexia (regulated hypothermia, cryexia)	A state in which T_{eq} is below the normal level of T_b, or a response in which T_b temporarily balances below its normal level.	All earlier definitions of **anapyrexia** assumed that T_b is tightly defended during this response, *i.e.*, implied the **homeothermic** type of T_b regulation. However, many quantitative studies show that this response is characterized by the **poikilothermic** type of T_b regulation. Because **anapyrexia**, as originally defined, does not exist, this term should not be used without clarifying the type of T_b regulation. In the absence of such a clarification, the terms **poikilothermy** (when talking about the type of T_b regulation) and **hypothermia** (when talking about a decreased T_b) can be used to describe this response.

[1] Synonyms are listed in parentheses. Throughout the table, recommended terms are shown in bold. Abbreviations used: T_b, deep body temperature; T_{eq}, equilibrium (or balance) point.

animals in the 1980s and 1990s. However, researchers were puzzled over the discrepancy between a long (~1 h) latency of the febrile response to LPS in rats studied in telemetric setups and a short (15-30 min) latency of the febrile response to LPS in all larger mammals, as well as the rats in the study of Székely & Szelényi (36). This discrepancy was explained only recently (37). It was confirmed that rats readily respond to LPS with a short-latency fever when studied at a neutral ambient temperature and injected with LPS in a stress-free manner, through a preimplanted catheter (Figure 1A). However, it was found that the initial febrile rise (called the first febrile phase, or *Phase I*) can be readily overlooked when the ambient temperature is below neutral, which is exactly what happens in many telemetric studies. Telemetry studies are typically conducted in rats housed in their home cages, *i.e.*, at room temperature, which is normally subneutral for this species (38). Furthermore, most telemetry studies involve an acute, stressful injection of a pyrogen *vs.* stress-free administration through a preimplanted catheter used by Székely & Szelényi (36). It appeared that injection-associated stress hyperthermia masks *Phase I* (37; see Figure 1B). Solving the latency puzzle has led to gradual acceptance of the fact that the febrile response of rats resembles that of larger animals, and from this, the rat has become the primary model to study thermoregulatory manifestations of systemic inflammation. With the spread of genetically modified mice, the mouse is also becoming a common species to study thermoregulatory responses (42-49).

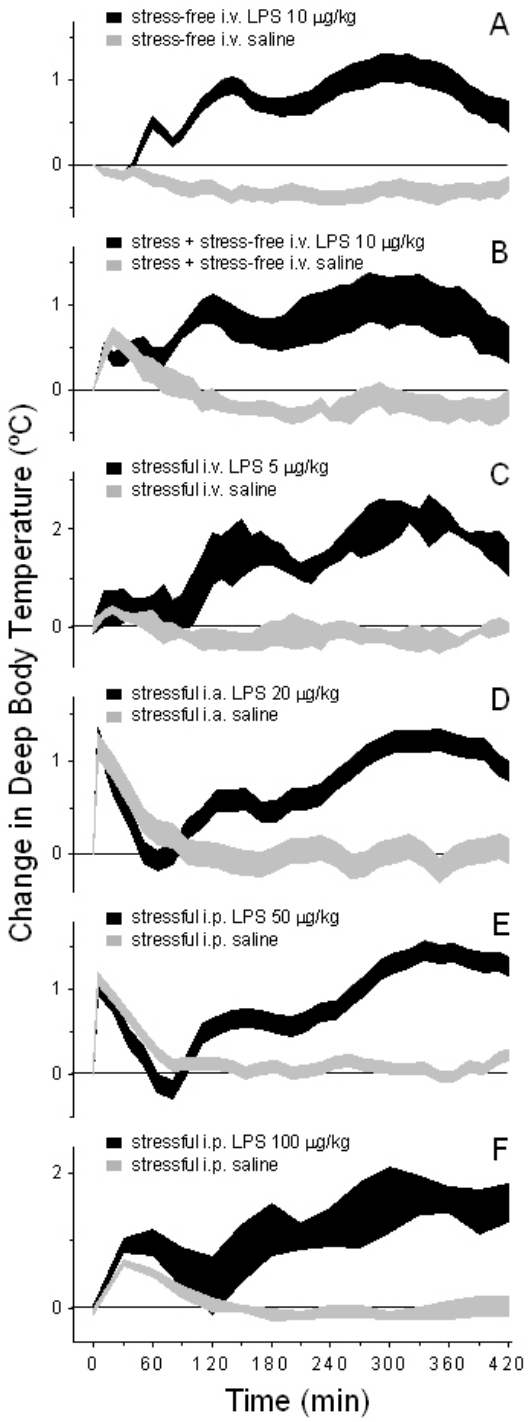

Figure 1. The phases of polyphasic lipopolysaccharide (LPS) fever in rats are characterized by remarkably consistent timing. This timing is insensitive to many factors, including the route of LPS administration, which can be intravenous (i.v.; panels A-C), intraarterial (i.a.; panel D), or intraperitoneal (i.p., panels E, F). However, *Phase I* of the response can be clearly seen only when caution is taken to inject LPS in a stress-free way, *i.e.*, through a preimplanted catheter, from outside the experimental chamber, and without touching the animal (A). When LPS is administered i.v. exactly as in panel A, but the rat is handled and pricked with a needle in the abdomen (to simulate a typical, stressful LPS administration), *Phase I* is masked by stress hyperthermia (B). When LPS administered in a stressful way (involving handling and/or pain of variable extent), stress hyperthermia of variable height and duration similarly masks *Phase I* (C-F). Note, that *Phases II* and *III* can be seen regardless of whether initial stress hyperthermia occurs. Data are re-plotted from Romanovsky *et al.* (37; panels A, B), Elmquist *et al.* (39; C), Caldwell *et al.* (40; D, E), and Wachulec *et al.* (41; F), with appropriate permissions.

4.2. Counting Febrile Phases

It is now accepted that the thermoregulatory response to LPS is much more complex than previously thought. In fact, an intravenous (i.v.) injection of LPS causes several different thermoregulatory responses in experimental animals, depending on the dose, ambient temperature, and other factors (50). This is not a technical nicety but a fact of fundamental importance: as we demonstrate throughout this review, different responses have different mechanisms and different adaptive values. It is, therefore, imperative to provide at least brief descriptions of these responses.

When a small, near-threshold dose (1 microgram/kg, in the case of the rat) is administered at a neutral or near-neutral (27-32°C) ambient temperature, a so-called monophasic fever typically occurs: it consists of a single burst of thermoeffector activity and a single rise of T_b peaking at 1-1.5 h postinjection. If the ambient temperature remains near-neutral, but the dose increases, the response changes in an intriguing way: a single, bolus injection of LPS now produces several sequential bursts in the activity of thermoregulatory effectors and, consequently, several rises in T_b (febrile phases). These phases have remarkably precise timing (36), which remains the same for different preparations of LPS and different rat strains (51). For a very narrow dose range (from slightly below to slightly above 3 microgram/kg), the febrile response of rats to LPS consists of two T_b rises: *Phase I* (peaks ~1 h postinjection) and *Phase II* (peaks at 2-2.5 h). If the dose increases further (from ~5 microgram/kg to lethal), the response becomes at least triphasic with *Phase III* peaking at 5-6 h postinjection (51). In many experiments conducted at room temperature (subneutral for rats) or involving an acute, stressful injection of a pyrogen, *Phase I* is missed (37; also see Figure 1B-F), and *Phases II* and *III* are misnamed as *Phases I* and *II*. However, the timing of the remained phases (*II* and *III*) is always the same, regardless of the experimental setup and even the route of pyrogen administration (Figure 1). The authors of a recent study in mice (49) have reported seeing polyphasic fevers in this species as well. In larger animals, *Phase III* has not been reported, possibly because only relatively low doses of LPS (up to 10 times higher than those causing monophasic fever) have been used, whereas in rats doses up to 10,000 higher have been studied (37, 50, 52).

The thermoregulatory mechanism of *Phase I* (and possibly of monophasic fever) is a parallel upward shift of the threshold T_bs for activation of different thermoregulatory effectors (for review, see Ref. 11). Such a shift leads to precise regulation of T_b but at a new, elevated level. Hence, according to the new definitions (Table 1), this hyperthermic response can be termed fever and is characterized by the homeothermic type of T_b regulation. The thermoregulatory mechanism of *Phase II* (and speculatively *Phase III*) involves a so-called threshold dissociation: the threshold T_b for activation of heat-defense effectors remains elevated (as it was during *Phase I*), but the threshold T_b for activation of cold-defense effectors decreases by several degrees (53). The development of threshold dissociation means that T_b regulation switches to the poikilothermic type (Table 1). When this happens, autonomic effectors are no longer used for thermoregulation in a wide range of T_bs, and the thermoregulatory behavior becomes the only thermoregulatory tool available, like in poikilothermic animals. This also means that the animal's T_b becomes highly sensitive to the ambient temperature, and that hypothermia readily occurs at subneutral ambient temperatures.

4.3. Studying Hypothermia: from "Thermoregulatory Failure" to Specific Mechanisms

Even though inflammation-associated hypothermia has been recognized for a long time and is of substantial clinical significance (54), this response has been considered as a thermoregulatory "failure" reflecting the inability of the brain to regulate T_b in shock. Specific thermoregulatory mechanisms of this response (selective, drastic decrease in the threshold T_b for activation of heat production coupled with cold-seeking behavior; see Ref. 30) have been revealed only during the past decade. According to Table 1, this is a hypothermic response with the poikilothermic type of T_b regulation. Because of the development of the poikilothermic type of T_b regulation, it is not surprising that i.v. LPS causes hypothermia in a cold environment. What is surprising is the timing of this response. In the rat, this hypothermia occurs very early after LPS administration and has a consistent nadir at ~90 min postinjection. This first, short-lasting hypothermic "phase" (corresponds to *Phases I* and *II* of the febrile response) is often followed by another, long-lasting decrease in T_b occurring at the same time as febrile *Phase III*. Perhaps, the hypothermic response to LPS is also polyphasic. Recently, LPS-induced hypothermia has become a focus of research in several laboratories (55-59). However, the hypothermic response is still studied much less than the febrile response. Reflecting this discrepancy, the present review pays substantially more attention to the mechanisms of fever than those of hypothermia.

5. HOW THE THERMOREGULATORY RESPONSES TO BACTERIAL PYROGENS ARE INITIATED

5.1. Signaling of Bacterial Pyrogens

Over the last decade, a large family of mammalian receptors termed Toll-like receptors (TLR) has been discovered and identified as receptors for LPS and other microbial pyrogens, thus starting a revolution in understanding the mechanisms of LPS recognition and signaling (for review, see Refs. 60, 61). Cellular TLR4 recognizes and responds to LPS, but only after LPS interacts with the CD14 protein; importantly, LPS-binding protein and myeloid differentiation protein-2 act as "adaptor" molecules and accelerate LPS binding to TLR4 and its intracellular signaling (62, 63). Until recently, TLR2 was also thought by some to recognize LPS (64) and mediate LPS-induced fever (65). However, Hirschfeld *et al.* (66) and Lee *et al.* (67) have demonstrated that it is not LPS *per se* but rather a highly bioactive lipopeptide contaminant of common LPS preparations ("endotoxin protein") that signals through TLR2. Endotoxin protein is

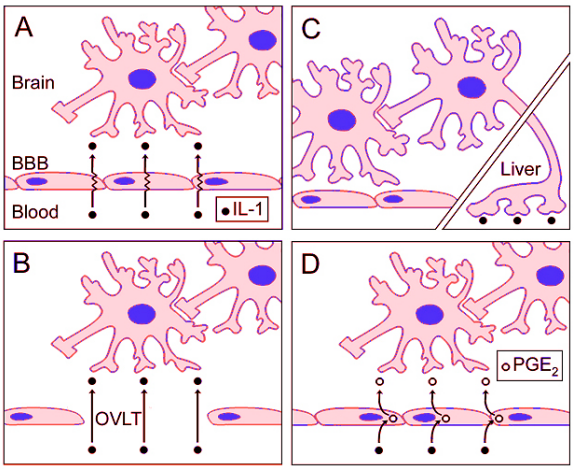

Figure 2. Schematic presentation of four major mechanisms by which peripheral proinflammatory molecules, most notably cytokines (such as interleukin-1, IL-1), have been proposed to reach the brain: saturable transport across the blood-brain barrier (BBB) (A); entry though the *organum vasculosum* of the *lamina terminalis* (OVLT) (B); signal transduction by sensory nerves, primarily the hepatic vagus (C); and synthesis of prostaglandin (PG) E_2 in cells forming the BBB (D). Note that the BBB can be viewed either as a major obstacle (restrictive barrier) for immune signaling to the brain (A-C) or as an active transducer that switches one signaling molecule to another (D).

not involved in non-thermoregulatory responses (such as anorexia) to common LPS preparations (68). Whether all thermoregulatory responses to such preparations (monophasic fever, *Phases I*, *II*, and *III* of polyphasic fever, and hypothermia) are caused by LPS *per se* remains to be established. In addition to the TLR4 mechanism, LPS recognition may involve other receptors, such as CD11/CD18 beta-2 integrin (69) and cell-surface proteins known as scavenger receptors (70). Gioannini *et al.* (71) list several more examples of proteins that may participate in cellular activation by LPS depending on specific structural features of particular LPS species, the host cell types examined, and the response studied. It is possible, therefore, that different receptors contribute to the development of different thermoregulatory responses to LPS. The ability of LPS to cause predominantly fever in a neutral or supraneutral environment but predominantly hypothermia in a subneutral environment has been speculated (72) to reflect different distribution of the blood in the body at different ambient temperatures and, consequently, different distribution of LPS and its recognition by different cells, possibly via different receptors. In addition to LPS, other microbial pyrogens are recognized by members of the TLR family. Wall constituents of gram-positive bacteria (*e.g.*, muramyl dipeptide) signal through TLR2 (in combination with either TLR1 or TLR6); double-stranded viral RNA is recognized by TLR3; and bacterial DNA interacts with TLR9 (61, 73-75).

5.2. Early Mediators

Substantial progress has also been achieved in identification of the critical early mediators of the thermoregulatory responses. New tools, including recombinant endogenous antagonists and genetically modified animals, have been instrumental in clarifying the roles of the pro-inflammatory cytokines, most importantly IL-1beta, IL-6, and TNF-alpha, in LPS fever and hypothermia (5, 7, 76). Other cytokines, such as ciliary neurotrophic factor and interferons alpha and gamma may be involved as well (76, 77). However, it is unclear whether and which of those cytokines are synthesized fast enough to trigger febrile *Phase I* or hypothermia (3, 11). Being the earliest cytokine to surge in the blood after LPS administration, TNF-alpha is a good candidate; however, neutralization of TNF-alpha with its type 1 soluble receptor does not affect *Phase I* of LPS fever in guinea pigs (78). Furthermore, clinical fevers often occur without any increase in the levels of circulating cytokines (79). Not surprisingly, several mediators other than cytokines have been proposed to trigger fever; these include circulating PGE_2 (see *Section 7*), anaphylatoxic component 5a of the complement cascade (3), and platelet-activating factor (PAF; 72, 80). The latter is known to appear in the circulation within minutes after i.v. LPS administration (81) and has been shown to possess an extremely high pyrogenic activity, higher than that of PGE_2 (72, 80). At least one PAF receptor antagonist (BN-52021) has been shown to attenuate i.v. LPS-induced fever (72). It can be speculated that PAF acts on brain endothelial cells expressing its receptors to stimulate PG synthesis (82). Interestingly, PAF can be involved in the genesis not only of the febrile, but also of the hypothermic, response to LPS (83, 84). For a while, peripheral nitric oxide was thought to be an early febrigenic mediator, but this hypothesis has been recently rejected (85).

6. FROM THE PERIPHERY TO THE BRAIN

6.1. Transport

Four major mechanisms by which peripheral pro-inflammatory molecules, most notably cytokines, signal the brain have been proposed: 1) saturable transendothelial transport; 2) entry though the *organum vasculosum* of the *lamina terminalis* (OVLT) and possibly other circumventricular organs; 3) signal transduction by sensory nerves, primarily the vagus; and 4) PG synthesis in cells that form the blood-brain barrier (BBB). These mechanisms are schematically outlined in Figure 2.

The transport theory (Figure 2A) states that circulating cytokines can cross the BBB by carrier transport (86). Although the list of cytokines utilizing carrier transport to cross the BBB has been extended substantially over the last decade (87), the physiological significance of this mechanism is difficult to prove. A recent addition to this theory, or perhaps a deviation from it, has been the proposition that cytokines can reach brain tissue by diffusion through basal laminae (88).

6.2. Entry through the *Organum Vasculosum* of the *Lamina Terminalis*

The OVLT theory (Figure 2B) suggests that cytokines enter the brain through the circumventricular

organs, primarily the OVLT, in which capillaries are fenestrated resulting in a "leaky" BBB. This theory was proposed by Blatteis *et al.* in 1983 (89) based on the findings that electrolytic lesions of the anterior wall of the third ventricle weaken the animals' ability to respond to pyrogens with fever. However, subsequent studies designed to test the OVLT signaling hypothesis produced contradictory results. Indeed, whereas several "lesion studies" and "nonlesion studies" suggested an important role for the OVLT in immune-to-brain signaling (for review, see Ref. 15), other lesion (*e.g.*, 90) and nonlesion (*e.g.*, 91, 92) studies failed to support such a role, at least for the specific experimental conditions tested. In fact, lesion studies were found to have all three possible outcomes with respect to the febrile response, *viz.*, exaggeration, blockade, and no effect (for review, see Ref. 93). However, even the outcome supposed to support the theory, blockade, can be explained by those side effects of OVLT lesioning (dehydration, malnutrition, hyperthermia, etc.) that attenuate the febrile response via mechanisms unrelated to the passage of the immune signal across the BBB (93). Furthermore, many findings that are typically cited in support of the "signaling through the OVLT" hypothesis (*e.g.*, that microinjection of pyrogens or antipyretic substances in the vicinity of the OVLT causes fever or antipyresis, respectively; see Refs. 94-97) do not actually support it. These findings show that the OVLT and/or other structures within the *lamina terminalis* (the anteroventral periventricular nucleus, ventromedial preoptic area, and medial preoptic nucleus) are crucial for fever genesis, but they do not differentiate by which of the four mechanisms these structures are activated during systemic inflammation. For nearly two decades, the results of lesion studies were viewed as proof of the physiological significance of the OVLT mechanism. When the proof did not withstand the scrutiny of careful examination, the theory lost its dominant position.

6.3. Vagal Signaling

The last decade has also witnessed a rise and apparent decline of another theory, the vagal (Figure 2C). First experimental hints implying that neural signaling via unidentified sensory nerves may be involved in the early, but not late, stages of the febrile/inflammatory response were obtained by Morimoto *et al.* (98) and Cooper and Rothwell (99). Several studies published in 1992-1993 suggested that at least some sensory nerve fibers conveying febrigenic signals to the brain travel within the vagus nerve (for review, see Refs. 14, 50). A "breakthrough" happened in 1995, when Watkins *et al.* (100) demonstrated that sub-diaphragmatic vagotomy leads to an attenuation of the febrile response of rats to intraperitoneal (i.p.) administration of IL-1. The same year, Székely *et al.* (101) reported that desensitization of intra-abdominal chemosensitive afferents (this procedure is sometimes referred to as "chemical vagotomy") with small i.p. doses of capsaicin (a vanilloid receptor VR1/TPRV-1 agonist) decreases the febrile response of rats to i.v. LPS, mostly its *Phase I*. Several groups reproduced these initial demonstrations (reviewed by Refs. 14, 50).

Although surgical vagotomy was found by many to attenuate or completely block some or even all febrile phases in rats and guinea pigs, this surgery can lead to severe "side effects," including malnutrition, thermoeffector deficiency, and other thermoregulatory impairments that can affect the febrile response (102). Many earlier studies ignored this issue. When caution was exercised to prevent malnutrition and associated disorders (102) and to produce vagotomized animals fully capable of increasing their T_b (103, 104), surgical vagotomy was found to still block monophasic fever, but it attenuated neither any phases of the polyphasic febrile response nor the hypothermic response to LPS (52). It is, therefore, tempting to conclude that malnutrition and other adverse effects of vagotomy might have been responsible for many cases of fever attenuation observed in early studies in vagotomized animals. Such a conclusion is supported by the fact that several later studies did not find any attenuation of the polyphasic febrile response in rats (40, 105, 106); some of these studies were conducted by the same groups that reported an attenuation of fever by vagotomy in their earlier papers. As for the capsaicin desensitization, the ability of this procedure to block *Phase I* of the febrile response to LPS has been confirmed (107, 108), but it has been found to be due to a non-neural, non-VR1-mediated mechanism (108, 109). This action, therefore, has nothing to do with the proposed vagal signaling.

In light of these recent findings, vagal signaling does not appear to play any significant role in the polyphasic febrile or hypothermic response to inflammatory stimuli. It may, however, be involved in triggering the monophasic febrile response, *i.e.*, the response to very low, near-threshold doses of a pyrogen. It has been found that both total subdiaphragmatic vagotomy and selective transection of the hepatic vagal branch (a small, predominantly afferent nerve servicing the liver and its portal vein) attenuate monophasic LPS fever (52, 102, 110). The liver with its Kupffer cells has long been known to be responsible for the clearance of peripherally injected pyrogens (111, 112) and has been suspected of contributing to the pathogenesis of fever (113). This suspicion has been recently reinforced by a large amount of data (for review, see Refs. 14, 114), including a demonstration of the early induction by LPS of hepatic synthesis of the ultimate downstream mediator of fever, PGE_2 (115). It has also been shown that intraportal infusion of IL-1 increases the discharge rate of hepatic vagal afferents (116), and IL-1 and PG receptors are present on vagal paraganglia associated with the hepatic branch and in the nodose ganglion that contains cell bodies of vagal sensory neurons (117, 118). It could be concluded that febrigenic chemical signals (possibly including IL-1 and PGE_2) originate in the liver and bind to the appropriate receptors on the hepatic vagus. The proposed mechanism seems important for triggering the febrile response not only to low doses of i.v. LPS (52), but also low doses of i.p. IL-1beta (119).

6.4. The Blood-Brain Barrier as a Signal Transducer

Our view on the role of the BBB in pyrogenic signal transduction has changed drastically over the last decade: the BBB is no longer considered an obstacle (restrictive barrier) for such signaling (Figure 2A-C); instead, it is viewed as an active signal transducer (a place

for switching from one signaling molecule to another; Figure 2D). Indeed, peripheral administration of LPS, IL-1beta, or TNF-alpha has been shown to rapidly induce expression of PGE_2-synthesizing enzymes in the vascular endothelium and perivascular cells throughout the brain (120, 121). Because brain parenchyma is essentially devoid of PG-catabolizing activity, PGE_2 produced by these cells can freely reach neurons of thermoeffector circuitries to trigger fever. The type of cells responsible for the pyrogenic signal transduction has been a subject of the most vivid scientific exchange (122). Research conducted by Matsumura and his co-authors (120, 123, 124) suggested that endothelial cells are the main source of PG synthesis after i.p. injection of IL-1 or LPS. However, Elmquist *et al.* (125) argued that perivascular cells, a type of brain macrophage, constitute the major PGE_2-producing population after i.v. administration of the same pyrogens. Recent studies show that both cell types are involved (121), but that their involvement depends on the nature of the pyrogenic stimulus, its dose, and time post-administration (126, 127). The evolution of views on the cellular substrate of blood-to-brain signal transduction is discussed in greater detail by Schiltz and Sawchenko (1), and Matsumura and Kobayashi (9).

7. PROSTAGLANDIN E_2

7.1. Prostaglandin E_2 as a Mediator of Fever

The involvement of PGs of the E series in the febrile response was established in the early 1970s, when Milton and Wendlandt (128) found a pyrogenic activity of PGE_1, and Vane (129) showed that aspirin-like drugs exert their antipyretic action by inhibiting synthesis of PGs from arachidonic acid. For a long time, however, it remained disputed whether PGs of the E series, most notably PGE_2, are obligatory mediators of fever. This dispute could not be resolved by using traditional pharmacological tools, which never have absolute specificity. Indeed, even the "classical" inhibitors of PGE_2 synthesis, aspirin and salicylates, can exert anti-inflammatory activities via unrelated mechanisms (for review, see Ref. 130), such as prevention of signaling via the pro-inflammatory transcription factor NF-kappaB (131), increase in the plasma concentration of a neutralizing receptor for IL-1, the type-2 soluble receptor (132), and stimulation of nitric oxide release (133). The long-lived dispute about the importance of PGE_2 in LPS fever was ended by experiments in gene knockouts. Targeted disruption of genes encoding either the PGE_2 receptor (45, 49) or PGE_2-synthesizing enzymes (46, 48) provided undisputable evidence that PGE_2 is indispensable for mounting the febrile response, at least to LPS (for comprehensive reviews of such gene knockout studies, see Refs. 3, 13). Together with traditional pharmacological studies (reviewed by Ref. 10), these studies suggest that PGE_2 mediates all phases of the polyphasic LPS fever (also see *Section 7.3* below).

7.2. Do Eicosanoids Mediate Hypothermia?

It has been known for some time that enzymes synthesizing pyrogenic PGE_2 may also be involved in production of mediators causing hypothermia in systemic inflammation; one such mediator may be PGD_2 (134, 135). Another candidate has been identified recently as 15-deoxy-delta$^{12\text{-}14}$ PGJ_2, a metabolite of PGD_2. It has been found that late stages of experimental inflammation are accompanied by accelerated synthesis of 15-deoxy-delta$^{12\text{-}14}$ PGJ_2 (136), that the intrabrain level of 15-deoxy-delta$^{12\text{-}14}$ PGJ_2 is elevated during LPS fever, and that central administration of this PG decreases T_b of febrile rats (137). Products of two alternative pathways of arachidonic acid metabolism, namely the lipoxygenase and epoxygenase pathways, possess marked hypothermic activity in afebrile and febrile animals; they have been implicated in either reducing T_b or preventing its rise in systemic inflammation (for review, see Ref. 12).

7.3. Synthesis of Prostaglandin E_2 in Systemic Inflammation

A critical role of PGE_2 in at least one thermoregulatory response, fever, brings into focus the mechanisms regulating production of this mediator, and the past few years have resulted in enormous progress in understanding these mechanisms (for review, see Ref. 10). The PGE_2-synthesizing cascade consists of three reactions catalyzed by: 1) phospholipases A_2 (PLA_2), 2) cyclooxygenases (COX), and 3) terminal PGE synthases (PGES) (10, 138). While PLA_2 and COX enzymes were purified and cloned from mammalian tissues in the late 1980s-early 1990s, the distal gap in the PGE_2-synthesizing cascade was filled only during the past decade, when three PGES, *viz.*, microsomal (m) PGES-1, mPGES-2, and cytosolic PGES, were cloned and/or isolated from mammalian tissues (for review, see Refs. 10, 138).

Cloning and characterization of the PGE_2-synthesizing enzymes led to a novel concept, which is critical for understanding mechanisms of the inflammatory response. This concept implies that the physiological, low-scale production of PGE_2 and the accelerated synthesis of PGE_2 in inflammation are catalyzed by different subsets of enzymes. The 'inflammatory' subset includes several isoforms of secretory PLA_2 ($sPLA_2$) (*viz.*, $sPLA_2$ IIA, IID, IIE, IIF and V), as well as inducible isoforms of COX (COX-2) and terminal synthase (mPGES-1) (10, 138). Synthesis of these isoforms are stimulated by LPS and pro-inflammatory cytokines, both *in vitro* and *in vivo*. Robust transcriptional co-expression of "inflammatory" isoenzymes (*viz.*, $sPLA_2$ IIA, COX-2, and mPGES-1) has been recently found during the triphasic febrile response to LPS in rats (115). As shown in Figure 3, such a coordinated expressional upregulation of PGE_2-synthesizing genes occurs both in the brain and in peripheral tissues and lasts almost through the entire febrile course. Recent *in vitro* studies (for review, see Refs. 10, 138) suggest that the inducible isoforms of $sPLA_2$, COX-2, and mPGES-1 are not simply co-stimulated by inflammatory agents, but are functionally coupled to effectively channel arachidonic acid through the PGE_2-synthesizing cascade. Such functional coupling explains predominant synthesis of PGE_2 over other prostanoids during the febrile response. The indispensable roles of COX-2 and mPGES-1 in LPS fever have been recently demonstrated using gene knockouts (46, 48). Which PLA_2

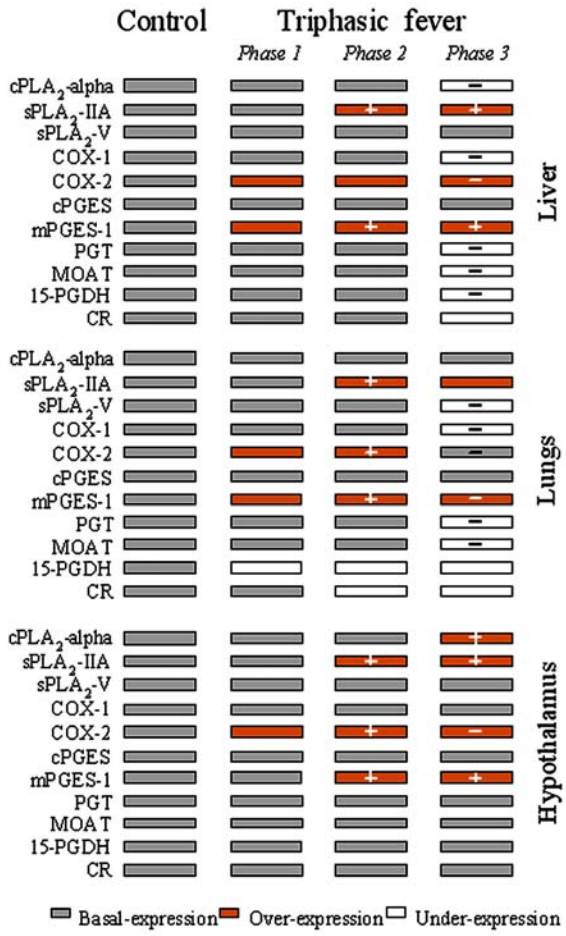

Figure 3. Schematic summary of our recent studies (115, 141). Up-regulation of a gene involved in prostaglandin (PG) E_2 metabolism in lipopolysaccharide (LPS)-treated rats at a given febrile phase (compared to the corresponding saline-treated rats) is shown in red; downregulation is shown in white; gray represents no statistically significant changes. +, statistically significant upregulation of a gene at the given febrile phase compared to the preceding phase; -, statistically significant downregulation of a gene at the given febrile phase compared to the preceding phase. Note that some genes are upregulated at *Phase III* as compared to their expression in saline-treated rats, but downregulated as compared to their expression at *Phase II*. For full name of the genes listed, see *Abbreviations* at the end of the article. Reproduced from Ivanov & Romanovsky (10).

isoform supplies arachidonic acid for synthesis of febrigenic PGE_2 has not been determined decisively.

It should also be noted that each febrile phase (as well as each underlying burst of PGE_2 synthesis) is characterized by a distinct pattern of the transcriptional regulation of PGE_2-synthesizing enzymes. For LPS fever in rats, these patterns have been revealed in a study by Ivanov *et al.* (115) and are presented in Figure 3. The most remarkable event of *Phase I* is a strong transcriptional upregulation of the functionally coupled COX-2 and mPGES-1 in the peripheral LPS-processing organs, *viz.*, the liver and lungs. It can be suggested that the synthesis of PGE_2 in the periphery is the major mechanism of this phase. *Phase II* is characterized by a robust transcriptional upregulation of the entire "inflammatory" subset (*viz.*, $sPLA_2$-IIA, COX-2, and mPGES-1) in the periphery and in the brain. Hence, PGE_2 synthesized both inside and outside the brain likely contributes to this phase. *Phase III* involves further transcriptional upregulation of $sPLA_2$-IIA and mPGES-1 in the liver and brain and upregulation of cytosolic PLA_2-alpha in the hypothalamus. Similar to *Phase II*, this phase is likely to be mediated by both peripheral and central bursts of PGE_2 synthesis. Figure 3 also shows that mechanisms of *Phases II* and *III* involve transcriptional downregulation of proteins involved in carrier-mediated uptake and catabolism of PGE_2 in peripheral organs (see *Section 7.4* below).

7.4. Catabolism

It was demonstrated more than three decades ago that the level of PGE_2 in body fluids is determined not only by the rate of its synthesis but also by the rate of its intracellular uptake and degradation (139). A study by Coggins *et al.* (140) confirmed this notion by showing that the genetic elimination of 15-hydroxy-PG dehydrogenase, a rate-limiting enzyme of PG catabolism, in mice results in elevated tissue levels of PGE_2 and embryonic mortality. The role of catabolic events in the regulation of PGE_2 level during the febrile response has gained attention only recently, when Ivanov *et al.* (141) found that expression of PGE_2 transmembrane transporters and catabolizing enzymes (15-hydroxy-PG dehydrogenase and carbonyl reductase) is drastically downregulated in the liver and lungs during *Phases II* and *III* of LPS fever (Figure 3). We conjecture that decreased carrier-mediated uptake and catabolism of PGE_2 in peripheral organs increases the blood-to-brain gradient of PGE_2, thus allowing blood-borne PGE_2 to more readily enter the brain during febrile *Phases II* and *III*. That i.v. LPS facilitates influx of circulating PGE_2 into the brain has been demonstrated (142). The roles of intracellular uptake and catabolism in the regulation of PGE_2 activity during the febrile response are further discussed by Ivanov & Romanovsky (10).

7.5. Mechanism of Action of Prostaglandin E_2

As for the mechanism of PGE_2 action, studies involving genetically modified mice lacking PG receptors have shown that fevers induced by LPS, IL-1, and PGE_2 are primarily mediated by the EP3 receptor (45, 49). In the case of polyphasic LPS fever, a study by Oka *et al.* (49) suggests that this receptor mediates all febrile phases studied, but it is unclear whether the authors were able to separate *Phase I* from the injection-associated hyperthermia; the latter hyperthermia was not affected by the lack of the EP3 receptor. The EP3 receptor is the most abundant PGE_2 receptor in the preoptic area of the hypothalamus (143, 144). Preoptic neurons expressing the EP3 receptor project (presumably polysynapticaly) to the raphe pallidus (145), where premotor sympathetic neurons driving thermogenesis (145-147) and skin vasoconstriction (148, 149) are located. Signaling cascades triggered by

activation of EP3 receptor are discussed elsewhere (2, 13, 150, 151).

Whereas *Phases II* and *III* of the polyphasic response to LPS are likely to involve the EP3 receptor in the brain and the abovementioned mechanism, mediation of other thermoregulatory responses to LPS is largely speculative. It is possible that the EP1 receptor is involved in some phases (49, 152). Based on circumstantial evidence (*i.e.*, the presence of the EP3 receptor on the hepatic vagus; see Ref. 118), the vagal EP3 receptor may mediate monophasic fever, but this speculation remains to be elaborated by further studies. In contrast to the febrile response, the hypothermic response to LPS involves neither the EP3, nor EP1 receptor (49).

8. NEURONAL CIRCUITRY OF FEVER AND HYPOTHERMIA

All of the hypothalamic temperature-sensitive neurons can be divided in two large classes: warm-sensitive (more abundant) and cold-sensitive (relatively rare). For a long time, it was assumed that all thermoregulatory responses, including those to inflammatory stimuli, could be triggered by either activation of one class of the temperature-sensitive neurons or inhibition of the other, and that the roles of cold- and warm-sensitive neurons are reciprocal and "symmetrical" (153). During the past decade, this misconception was put to rest. Studies involving combination of thermal and chemical stimulation of preoptic neurons showed that both cold-defense and heat-defense responses are initiated by the corresponding change in the activity of warm-sensitive neurons (154, 155). Anatomical characterization of these neurons has been another major achievement. It appeared that these cells are characterized by the horizontal orientation of their dendrites: towards the third ventricle medially and the medial forebrain bundle laterally (156). Because neurons conveying temperature signals from the body surface and viscera enter hypothalamic nuclei via the periventricular stratum and medial forebrain bundle (157), such an orientation seems ideal for receiving input from both populations of spinohypothalamic neurons.

Another major advance has been made in delineating the neuronal circuitries connecting the warm-sensitive hypothalamic neurons and thermoeffectors, most notably the vasculature of specialized heat-exchange organs (such as the rat's tail) and the brown adipose tissue (Figure 4). These two effectors are the major autonomic thermoeffectors that bring about the febrile response (36), at least in small rodents, the species of laboratory animals that are now widely used to study thermoregulatory responses to systemic inflammation. Inhibition of thermogenesis is also a principal mechanism of all hypothermic (anapyretic) responses (34), including LPS-induced hypothermia (30). The progress in identifying the pathways controlling non-shivering thermogenesis and tail skin vasoconstriction in the rat has been propelled by the development of transsynaptic retrograde tracing techniques employing pseudorabies virus, along with the refinement of techniques for discrete lesion/ stimulation of neural structures. The revealed pathways appeared complex, and their detailed description is beyond the scope of this paper (for review, see Refs. 28, 158). However, a few points deserve comment. It was discovered that premotor sympathetic neurons that project directly to the intermediolateral column of the spinal cord and control brown fat thermogenesis are located primarily in the raphe pallidus, whereas premotor neurons that control skin vasoconstriction are located in both the raphe pallidus and magnus. These medullary neurons are under the control of superior structures of the neural axis, including the dorsomedial and paraventricular nuclei in the hypothalamus, the periaqueductal gray matter, retrorubral field, and ventral tegmental area in the midbrain, and the locus coeruleus in the pons (159-161). Through these pathways, thermoeffectors are controlled relatively independently of each other (28, 162), and certain portions of the pathways may be recruited in a thermoregulatory response in a stimulus-specific fashion. The latter speculation is supported by findings that the paraventricular nucleus (163) and locus coeruleus (164) seem to mediate LPS- and PGE_2- induced, but not cold-induced, thermogenesis.

The thermoregulatory responses to inflammatory stimuli involve not only autonomic thermoeffector responses, but also behavioral ones, including warmth-seeking behavior in fever (for recent articles, see Refs. 165-167) and cold-seeking behavior in hypothermia (30). Although becoming a subject of keen attention (168), neurocircuitries of these responses remain largely unknown.

9. PEPTIDE MODULATORS OF FEVER AND HYPOTHERMIA

9.1. Leptin

Over the past decade, there has been a boom in the literature on peptide functions of hormones and neuropeptides, as many new ones have been discovered, and the known ones have been redefined. The adipocyte-derived, IL-6-like peptide, leptin, and its receptor have been the focus of many studies. It has been shown that LPS and pro-inflammatory cytokines increase the expression of the leptin gene and the concentration of leptin in the blood (169-171), whereas leptin exerts multiple actions on the network of pro- and anti-inflammatory cytokines (172, 173). It also has multiple thermoregulatory effects. Specifically, administration of leptin upregulates uncoupling protein transcription in the brown adipose tissue (174), activates thermogenesis (175, 176), and, at least according to two studies, produces an IL-1-dependent (177), alpha-melanocyte stimulating hormone (MSH)-sensitive (178) fever. However, exogenous leptin has been found apyrogenic in a study by Kelly et al. (77). Recent studies in genetically modified animals (Zucker *fatt*y rats with a largely nonfunctional leptin receptor and Koletsky *f/f* rats that do not have the leptin receptor) shed some light on the role of leptin and its receptor in the thermoregulatory responses to inflammatory stimuli (59, 179). It appears that the febrile response to LPS in both mutants is normal in a thermoneutral environment, but that

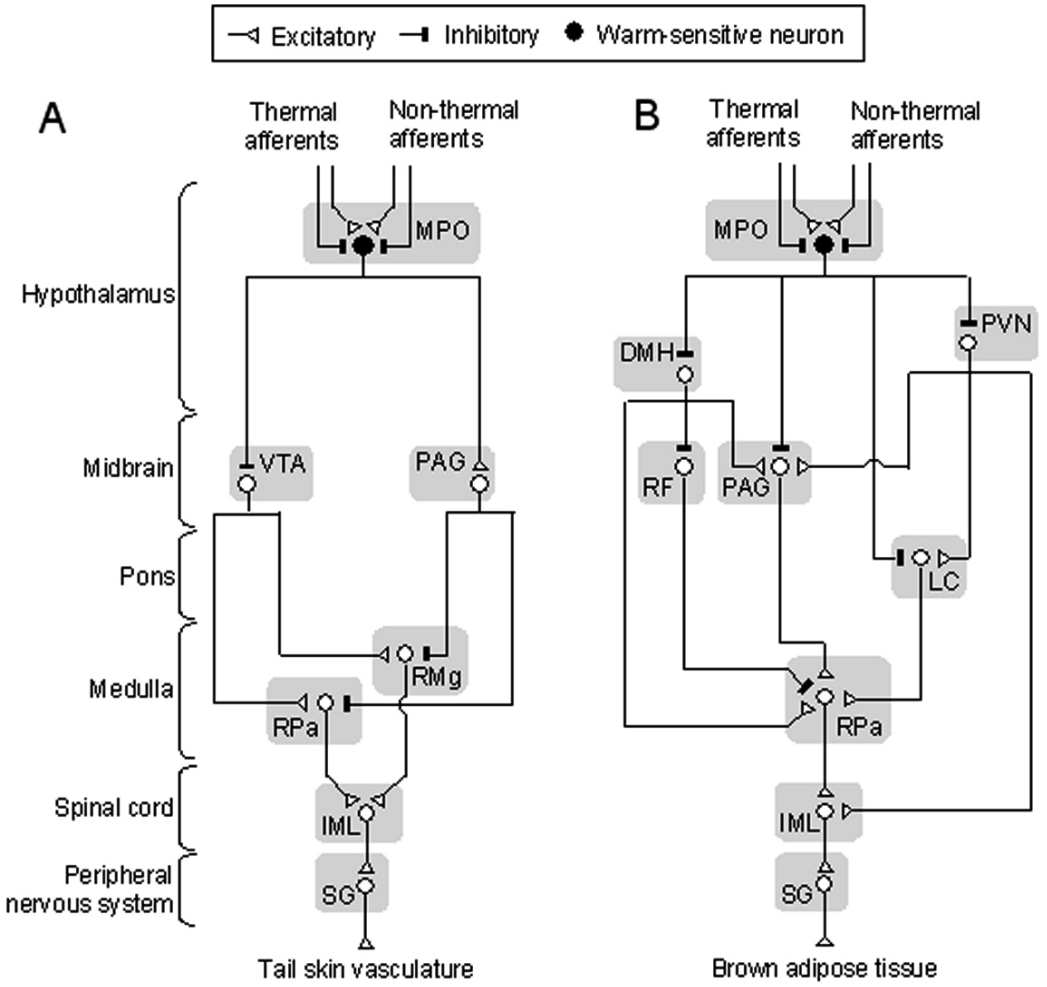

Figure 4. Possible neuronal circuitries controlling the vasomotor tone of tail skin (A) and brown fat thermogenesis (B) in the rat; based on reviews by Nagashima *et al.* (28) and Morrison (158). Abbreviations used: DMH, dorsomedial hypothalamus; IML, intermediolateral column; LC, locus coeruleus; MPO, medial preoptic region; PAG, periaqueductal gray matter; PVN, paraventricular nucleus; RF, retrorubral field; RMg, Raphe Magnus; RPa, Raphe Pallidus; SG, sympathetic ganglion; VTA, ventral tegmental area.

the hypothermic response to LPS in a cool environment is drastically prolonged in leptin receptor-deficient Koletsky rats. Hence, the leptin receptor and its endogenous ligand(s) are likely to mediate a T_b rise not during LPS fever but during the recovery from LPS hypothermia. It can be speculated that the attenuated fevers of mutant Zucker rats in a cool environment reported earlier by Dascombe *et al.* (180) and others (reviewed in Ref. 179) were due to a prolongation of an obvious or a hidden hypothermic component of the overall thermoregulatory response to LPS or the cytokines used. This story is another illustration of the crucial importance of a tight control of ambient temperature for the outcome of thermoregulatory studies in small animals. Leptin receptor-dependent mechanisms of the recovery from LPS hypothermia include activation of the anti-inflammatory hypothalamo-pituitary-adrenal axis, inhibition of both the production and hypothermic action of TNF-alpha, and suppression of inflammatory (via NF-kappaB) signaling in the brain (59).

9.2. Orexins and Neuropeptide Y

Two other newly discovered peptides, orexins (hyposecretins)-A and B, have received much attention (for a comprehensive review, see Ref. 17). Orexin A has been found to cause immediate hypothermia and hyperphagia followed by late hyperthermia and hypophagia; these responses are thought to be mediated, at least partially, by neuropeptide Y (NPY) acting on its Y1 receptor (181-183). However, the mechanisms of the hypothermic action of orexin-A (peripheral vasodilation) are different from those of the hypothermic action of NPY (metabolic depression), even though both responses occur only in a subneutral environment (184). In contrast to orexin-A, orexin-B produces hyperthermia only and does not lead to hyperphagia (182). Whether any of these peptides are involved in fever or systemic inflammation-associated hypothermia is largely unknown. The T_b-decreasing action of exogenous orexin-A can be revealed not only under afebrile (normal) conditions, but also during fever (182).

Downregulation of the NPY system in the hypothalamus has been speculated to be involved in the development of responses to LPS, including anorexia (185) and fever (17).

9.3. Corticotropin-releasing Factor and Urocortins

The role of corticotropin-releasing factor (CRF) in thermoregulation has continued to be of interest over the last decade (for review, see Ref. 17). Even though it is well established that intracerebroventricular (i.c.v.) CRF activates thermogenesis and increases T_b, studies of the role of CRF in fever led to controversial conclusions. It seems that brain CRF may be involved in both T_b-increasing (pyretic) and T_b-decreasing (antipyretic) mechanisms during fever (17, 186). A pyretic mechanism may be CRF-mediated activation of paraventricular hypothalamic neurons; these neurons are rich in CRF and its receptor (187) and mediate PGE_2- and LPS-induced fever (163). An antipyretic mechanism may be CRF-mediated activation of the anti-inflammatory hypothalamo-pituitary-adrenal axis (188); activation of this axis inhibits the febrile response (189). An important recent discovery has been the identification of the novel endogenous ligands of CRF receptors, urocortins 1, 2, and 3. Urocortins have both hyperthermic/thermogenic (190) and anti-thermogenic (191) effects, and there is evidence that urocortin 1 plays a role in ethanol-induced hypothermia (192). Whether urocortins are involved in the thermoregulatory responses to inflammatory stimuli remains unknown.

9.4. Angiotensin II and Cholecystokinin

The roles of many previously catalogued hormones and neuropeptides in the thermoregulatory responses to inflammatory stimuli have been clarified over the past decade. The list of such neuropeptides includes angiotensin (ANG) II and cholecystokinin (CCK). Exogenously administered ANG II was known for a long time to generate baroreflex-mediated hypothermia in different species (for review, see Ref. 20). Recent studies have revealed that endogenous ANG II has a different thermoregulatory function in systemic inflammation: this peptide appears to enhance fever via multiple mechanisms. Upstream of PGE_2 synthesis, ANG II stimulates LPS-induced production of IL-1beta by acting on the type-1 ANG II receptor; downstream of PGE_2 synthesis, ANG II promotes fever by acting on its type-2 receptor in the brain (20, 193).

Exogenous CCK has been found to induce both hypo- and hyperthermia, depending on the route of administration, dose, ambient temperature, and other factors (for review, see Ref. 19). There are more than two types of CCK receptors, but the CCK-1 (formerly, CCK-A receptor; predominantly peripheral) and CCK-2 (formerly, CCK-B receptor; predominantly central) remain the two major ones. It has been shown that central application of CCK causes hyperthermia via action on the CCK-2 receptor, while peripheral administration results in CCK-1 receptor-mediated hypothermia (194). There is also a study providing evidence that the CCK-2 receptor in the brain may mediate LPS fever (195). Because intra-abdominal vagal fibers (known to bear CCK-1 receptors) were thought to trigger *Phase I* of LPS fever (see *Section 6.3*), it was investigated whether the CCK-1 receptor may mediate this febrile phase. However, studies involving pharmacological analysis (196) and mutant rats deficient in the CCK-1 receptor (197) ruled out such mediation. Whether CCK and its receptors are involved in inflammation-associated hypothermia remains unknown.

9.5. The "Classical" Endogenous Antipyretics

Alpha-MSH and arginine vasopressin (AVP) have been considered endogenous antipyretics for a long time (for review, see 16, 17). Recent studies have confirmed the antipyretic property of alpha-MSH and established that its antipyretic (as well as anorexic) action during LPS fever is mediated predominantly by the melanocortin type-4 receptor (198-200).

In early studies, the antipyretic effect of AVP was thought to be mediated by the V_1 receptor in the area of the brain located just rostral to the anterior hypothalamus and ventral to the lateral septum; this area is often referred to as the ventral septal area (VSA) (201). The antipyretic action of AVP was used to explain several "antipyretic phenomena," such as the decreased ability of pregnant animals to mount the febrile response. Recent experiments in rats have found no effect of i.c.v. AVP on the febrile response to i.p. or i.c.v. LPS (202). Recent experiments in rabbits have also failed to reveal an antipyretic action of intra-VSA AVP on the febrile response to i.v. LPS or intra-VSA PGE_2, but instead, found a V_2-mediated "hyperpyretic" (fever-enhancing) effect (202). Interestingly, six earlier studies conducted in AVP-deficient Brattleboro rats (for references, see 51) produced contradictory results that, on the whole, do not support an involvement of AVP in endogenous antipyresis. There is also an earlier study conducted in rabbits showing that AVP produces a hyperpyretic, rather than antipyretic, effect (203). Furthermore, the pregnancy-associated antipyresis is no longer considered to be mediated by AVP (204), and several new hypotheses to explain this phenomenon have been recently proposed (205-207).

10. BIOLOGICAL VALUE AND ANTIPYRETIC THERAPY

10.1. Biological Value

Since the time of Hippocrates (*c.* 460-*c.* 375 B.C.), the adaptive *vs.* maladaptive value of fever has remained a subject of heated debate and polarizing the medical community. Indeed, the old literature is full of categorical statements both proclaiming a beneficial role of this response and urging to actively fight this foe (for review, see Ref. 208). In contrast, the role of hypothermia in systemic inflammation has been almost completely neglected. On those occasions when the question of biological value of hypothermia in inflammation did surface, the response was usually considered unconditionally "bad" (54). In basic research, the progress of the last decade has been the development of a view that both fever and hypothermia have intrinsic adaptive values, yet can be harmful under particular circumstances (208). In other words, the question should be not *whether* fever (or hypothermia) is good (or bad), but *when* the particular

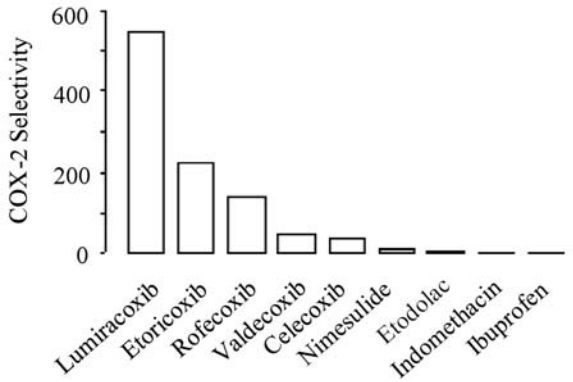

Figure 5. Selectivity of novel inhibitors of cyclooxygenase (COX)-2 is shown as a ratio of inhibitory potencies (COX-1/COX-2) determined in human whole blood assays. The data represent mean values from the articles by Riendeau *et al.* (220) and Tacconelli *et al.* (221).

response is beneficial, and *when* the same response is harmful. In clinical research, such a view translated into trying to refine the indications and contraindications for the use of a large arsenal of modern pharmacological and physical tools affecting T_b regulation (8, 209). A group of patients for whom aggressive fever suppression has quite recently been advocated are critically ill neurological and neurosurgical patients (210). In such patients, elevated T_b has been associated with a longer hospital stay and higher mortality rate. However, as with other groups of patients, there is no evidence from randomized trials to support the use of antipyretics in febrile patients with neurological insult (211). It is hoped that future prospective clinical studies will provide more definitive information regarding which patients and under what conditions may benefit most (or least) from T_b corrections by specific physical or pharmacological tools.

10.2. Antipyretic Therapy

Significant advances in antipyretic pharmacology over the recent past have followed the cloning and identification of the second isoform of COX by Xie *et al.* (212), Kujubu *et al.* (213), and O'Banion *et al.* (214). It was soon realized that COX-2 is involved in inflammation and fever ("bad" COX), whereas COX-1 has essential housekeeping functions under normal conditions ("good" COX) and does not mediate fever (also see *Section 7.3*). It was further realized that nonsteroidal anti-inflammatory drugs could exhibit isoform selectivity (215). This realization sparked the search for selective COX-2 inhibitors, which would suppress COX-2-mediated inflammation and fever, while having minimal adverse effects related to inhibition of COX-1 (such as gastrointestinal and renal toxicity).

Within a few years, reports of selective COX-2 inhibitors began to appear (216, 217), and animal experiments with these agents demonstrated potent antipyretic effects with reduced gastrointestinal toxicity (218, 219). Recently, a number of highly selective COX-2 inhibitors have been approved for clinical use or are in the process of approval in the United States and Europe (Figure 5). However, adverse renal effects have been observed with these agents, similar to those seen with nonselective inhibitors, which may be in part due to inhibition of constitutively expressed COX-2 in the kidney (222). When taken on a daily basis at high doses, these drugs also have adverse cardiovascular effects (see, *e.g.*, 223). While this review was in preparation, one drug shown in Figure 5, Vioxx® (rofecoxib), was withdrawn from the market, and two other, Celebrex® (celecoxib) and Bextra® (valdecoxib), appeared in the US Food and Drug Administration's Public Health Advisory suggesting that these COX-2 inhibitors can increase the risk of heart attack and stroke.

Although the development of selective COX-2 inhibitors represents the largest area of growth in antipyretic pharmacology, a series of recent reports has also enhanced our understanding of the mechanism of action of acetaminophen, a drug that has been in clinical use for more than 100 years (for review, see Ref. 4). In many experimental paradigms, acetaminophen is a relatively weak inhibitor of both COX-1 and COX-2 (224), but it is highly effective in blocking PGE_2 synthesis, fever, and pain *in vivo*. It has been shown that COX activity within the central nervous system is more sensitive to inhibition by acetaminophen than in peripheral tissues (225), and has long been hypothesized that a particularly acetaminophen-sensitive isoform of COX (different from COX-1 and COX-2) exists within the brain and is involved in fever genesis (for review, see Ref. 226). Indeed, such an isoform was described in canine (and possibly human) tissues and named COX-3 (227). It represents a splicing variant of COX-1 and is now commonly refereed to as COX-1 variant retaining intron 1 (COX-$1V_1$; 228, 229), or COX-1b (230). However, it appears unlikely that COX-$1V_1$ is a pharmacological target for acetaminophen's analgesic and antipyretic effects. Although the canine COX-$1V_1$ mRNA maintains an open reading frame (227), the mouse (231), rat (230), and human (232) COX-$1V_1$ transcripts are shifted out of frame by intron 1 retention and would not be expected to yield an active enzyme. That the rat COX-$1V_1$ protein does not have COX activity has been recently demonstrated (230). Furthermore, COX-$1V_1$ does not appear to be induced by acute inflammatory stimulation in rodent models (231, 233), and PG synthesis in a variety of rat tissues depends solely on the actions of COX-1 and COX-2, with no evidence for the involvement of an acetaminophen-sensitive isoform (234). In spite of these findings, two very recent studies give consideration to the role of COX-$1V_1$ in chronic inflammatory diseases, such as human Alzheimer (235) and collagen-induced arthritis in rats (229).

11. INSTEAD OF CONCLUSIONS

The recent troubles with selective COX-2 inhibitors will certainly accelerate the search for new drugs to suppress the thermoregulatory symptoms of systemic inflammation. For dealing with fever, several promising targets have been recently suggested (for review, see Ref. 10). The most downstream position of mPGES-1 in the PGE_2-synthesizing cascade, the highest magnitude of

upregulation of this enzyme among all PGE_2-synthesizing enzymes studied during LPS fever, and the long duration of this upregulation (115) make mPGES-1 an attractive target for antipyretic therapy. The fact that mPGES-1 is strongly upregulated when expression of COX-2 declines (115) adds to the attractiveness of this pharmacological target, and so does recently established fact that the role of mPGES-1 in fever is indispensable (48). The recently demonstrated massive transcriptional downregulation of hepatic and pulmonary (but not cerebral) PGE_2 transporters and degrading enzymes during LPS fever (141) is likely to result in an increased blood-to-brain gradient (decreased brain-to-blood gradient) of PGE_2 and, hence, its facilitated penetration into the central nervous system (its depressed elimination from the brain). This largely unrecognized mechanism may constitute a novel target for antipyretic and anti-inflammatory therapy. As is the case with expression of mPGES-1, tissue expression of PGE_2 transporters and dehydrogenases is suppressed most dramatically at the time when COX-2 expression is declining. In addition to enzymes involved in the metabolism of PGE_2, proteins responsible for the synthesis, transport, clearance, and action of other mediators of fever and hypothermia mentioned in this review should also be considered as potential therapeutic targets. As information regarding the pathogenesis of fever and hypothermia continues to emerge, the development of novel antipyretic agents and discoveries of anti-hypothermic drugs will follow.

12. ACKNOWLEDGEMENTS

Maria C. Almeida is a visiting Ph.D. student from the Medical School of Ribeirão Preto, University of São Paulo, and fellow of CAPES Foundation, Brazil. Graphics and secretarial assistance by Karla P. Scarff and Jeffrey J. Burmeister are gratefully appreciated.

13. REFERENCES

1. Schiltz J. C. & P. E. Sawchenko: Signaling the brain in systemic inflammation: the role of perivascular cells. *Front Biosci* 8, 1321-1329 (2003)

2. Steiner A. A. & L. G. S. Branco: Fever and anapyrexia in systemic inflammation: intracellular signaling by cyclic nucleotides. *Front Biosci* 8, s1398-s1408 (2003)

3. Blatteis C. M, S. Li, Z. Li, V. Perlik & C. Feleder: Signaling the brain in systemic inflammation: the role of complement. *Front Biosci* 9, 915-931 (2004)

4. Botting R: Antipyretic therapy. *Front Biosci* 9, 956-966 (2004)

5. Conti B, I. Tabarean, C. Andrei & T. Bartfai: Cytokines and fever. *Front Biosci* 9, 1433-1449 (2004)

6. Gourine A. V, N. Dale, V. N. Gourine & K. M. Spyer: Fever in systemic inflammation: roles of purines. *Front Biosci* 9, 1011-1022 (2004)

7. Leon L. R: Hypothermia in systemic inflammation: role of cytokines. *Front Biosci* 9, 1877-1888 (2004)

8. Mackowiak P. A: The febrile patient: diagnostic, prognostic, and therapeutic considerations. *Front Biosci* 9, 2297-2301 (2004)

9. Matsumura K. & S. Kobayashi: Signaling the brain in inflammation: the role of endothelial cells. *Front Biosci* 9, 2819-2826 (2004)

10. Ivanov A. I. & A. A. Romanovsky: Prostaglandin E_2 as a mediator of fever: synthesis and catabolism. *Front Biosci* 9, 1977-1993 (2004)

11. Janský L. & S. Vybíral: Thermal homeostasis in systemic inflammation: modulation of neuronal mechanisms. *Front Biosci* 9, 3068-3084 (2004)

12. Kozak W. & V. Fraifeld: Non-prostaglandin eicosanoids in fever and anapyrexia. *Front Biosci* 9, 3339-3355 (2004)

13. Oka T: Prostaglandin E_2 as a mediator of fever: the role of prostaglandin E (EP) receptors. *Front Biosci* 9, 3046-3057 (2004)

14. Romanovsky A. A: Signaling the brain in the early sickness syndrome: are sensory nerves involved? *Front Biosci* 9, 494-504 (2004a)

15. Roth J, E. M. Harre, C. Rummel, R. Gerstberger & T. Hubschle: Signaling the brain in systemic inflammation: role of sensory circumventricular organs. *Front Biosci* 9, 290-300 (2004a)

16. Roth J, E. Zeisberger, S. Vybíral & L. Janský: Endogenous antipyretics: neuropeptides and glucocorticoids. *Front Biosci* 9, 816-826 (2004b)

17. Székely M, E. Pétervári & Z. Szelényi: Orexigenic *vs.* anorexigenic peptides and feeding status in the modulation of fever and hypothermia. *Front Biosci* 9, 2746-2763 (2004)

18. Szelényi Z. & M. Székely: Sickness behavior in fever and hypothermia. *Front Biosci* 9, 2447-2456 (2004)

19. Szelényi Z, M. Székely, Z. Hummel, M. Balaskó, A. A. Romanovsky & E. Pétervári: Cholecystokinin: possible mediator of fever and hypothermia. *Front Biosci* 9, 301-308 (2004)

20. Watanabe T, M. Miyoshi & T. Imoto: Angiotensin II: its effects on fever and hypothermia in systemic inflammation. *Front Biosci* 9, 438-447 (2004)

21. Cabanac M. & B. Massonnet: [Pathology of thermoregulation.] *Rev Neurol (Paris)* 136, 285-302 (1980) (in French)

22. Kluger M. J: Fever: role of pyrogens and cryogens. *Physiol Rev* 71, 93-127 (1991)

23. The Commission for Thermal Physiology of the International Union of Physiological Sciences (IUPS Thermal Commission): Glossary of terms for thermal physiology: third edition. *Jpn J Physiol* 51, i-xxxvi, (2001)

24. Gordon C. J: The therapeutic potential of regulated hypothermia. *Emerg Med J* 18, 81-89 (2001)

25. Steiner A. A. & L. G. S. Branco: Hypoxia-induced anapyrexia: implications and putative mediators. *Annu Rev Physiol* 64, 263-288 (2002)

26. Satinoff E: Neural organization and evolution of thermal regulation in mammals. *Science* 201, 16-22 (1978)

27. Werner J: The concept of regulation for human body temperature. *J Therm Biol* 5, 75-82 (1979)

28. Nagashima K, S. Nakai, M. Tanaka, & K. Kanosue: Neuronal circuitries involved in thermoregulation. *Auton Neurosci* 85, 18-25 (2000)

29. Kanosue K, A. A. Romanovsky, T. Hosono, X.-M. Chen, and Y.-Z. Zhang: "Set point" revisited. In: *Thermal Physiology 1997* (Eds: Nielsen Johannsen B. & R. Nielsen), The August Krogh Institute, Copenhagen, Denmark, pp. 39-43 (1997)

30. Romanovsky A. A, O. Shido, S. Sakurada, N. Sugimoto, & T. Nagasaka: Endotoxin shock: thermoregulatory mechanisms. *Am J Physiol Regul Integr Comp Physiol* 270, R693-R703 (1996)

31. Sessler D. I: Mild perioperative hypothermia. *N Engl J Med* 336, 1730-1737 (1997)

32. Kobayashi A, T. Osaka, Y. Namba, S. Inoue, T. H. Lee, & S. Kimura: Capsaicin activates heat loss and heat production simultaneously and independently in rats. *Am J Physiol* 275, R92-R98 (1998)

33. Sakurada S, O. Shido, N. Sugimoto, Y. Hiratsuka, T. Yoda, & K. Kanosue: Autonomic and behavioral thermoregulation in starved rats. *J Physiol* 526, 417-424 (2000)

34. Romanovsky A. A: Do fever and anapyrexia exist? Analysis of set point-based definitions. *Am J Physiol Regul Integr Comp Physiol* 287, R992-R995 (2004b)

35. Splawiński J. A, E. Zacny, & Z. Górka: Fever in rats after intravenous E. coli endotoxin administration. *Pflügers Arch* 368, 125-128 (1977)

36. Székely M. & Z. Szelényi: Endotoxin fever in the rat. *Acta Physiol Acad Sci Hung* 53, 265-277 (1979)

37. Romanovsky A. A, V. A. Kulchitsky, C. T. Simons & N. Sugimoto: Methodology of fever research: why are polyphasic fevers often thought to be biphasic? *Am J Physiol Regul Integr Comp Physiol* 275, R332-R338 (1998a)

38. Romanovsky A. A, A. I. Ivanov & Y. P. Shimansky: Selected contribution: ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality. *J Appl Physiol* 92, 2667-2679 (2002)

39. Elmquist J. K, T. E. Scammell, C. D. Jacobson & C. B. Saper: Distribution of Fos-like immunoreactivity in the rat brain following intravenous lipopolysaccharide administration. *J Comp Neurol* 371, 85-103 (1996)

40. Caldwell F. T. Jr, D. B. Graves & B. H. Wallace: Humoral *versus* neural pathways for fever production in rats after administration of lipopolysaccharide. *J Trauma* 47, 120-129 (1999)

41. Wachulec M, E. Peloso, E. Satinoff: Individual differences in response to LPS and psychological stress in aged rats. *Am J Physiol Regul Integr Comp Physiol* 272, R1252-R1257 (1997)

42. Chai Z, S. Gatti, C. Toniatti, V. Poli & T. Bartfai: Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopoly-saccharide or IL-1beta: a study on IL-6-deficient mice. *J Exp Med* 183, 311-316 (1996)

43. Leon L. R, C. A. Conn, M. Glaccum & M. J. Kluger: IL-1 type I receptor mediates acute phase response to turpentine, but not lipopolysaccharide, in mice. *Am J Physiol* 271, R1668-R1675 (1996)

44. Horai R, M. Asano, K. Sudo, H. Kanuka, M. Suzuki, M. Nishihara, M. Takahashi & Y. Iwakura: Production of mice deficient in genes for interleukin (IL)-1alpha, IL-1beta, IL-1alpha/beta, and IL-1 receptor antagonist shows that IL-1beta is crucial in turpentine-induced fever development and glucocorticoid secretion. *J Exp Med* 187, 1463-1475 (1998)

45. Ushikubi F, E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ichikawa, T. Tanaka, N. Yoshida & S. Narumiya: Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395, 281-284 (1998)

46. Li S, Y. Wang, K. Matsumura, L. R. Ballou, S. G. Morham & C. M. Blatteis: The febrile response to lipopolysaccharide is blocked in cyclooxygenase-2(-/-), but not in cyclooxygenase-1(-/-) mice. *Brain Res* 825, 86-94 (1999)

47. Kozak W. & A. Kozak: Differential role of nitric oxide synthase isoforms in fever of different etiologies: studies using NOS gene-deficient mice. *J Appl Physiol* 94, 2534-2544 (2003)

48. Engblom D, S. Saha, L. Engstrom, M. Westman, L. P. Audoly, P. J. Jakobsson & A. Blomqvist: Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. *Nat Neurosci* 6, 1137-1138 (2003)

49. Oka T, K. Oka, T. Kobayashi, Y. Sugimoto, A. Ichikawa, F. Ushikubi, S. Narumiya & C. B. Saper: Characteristics of thermoregulatory and febrile responses in mice deficient in prostaglandin EP1 and EP3 receptors. *J Physiol* 551, 945-954 (2003a)

50. Romanovsky A. A, C. T. Simons & V. A. Kulchitsky: "Biphasic" fevers often consist of more than two phases. *Am J Physiol Regul Integr Comp Physiol* 275, R323-R331 (1998b)

51. Romanovsky A. A: Thermoregulatory manifestations of systemic inflammation: lessons from vagotomy. *Auton Neurosci* 85, 39-48 (2000)

52. Romanovsky A. A, C. T. Simons, M. Székely & V. A. Kulchitsky: The vagus nerve in the thermoregulatory response to systemic inflammation. *Am J Physiol Regul Integr Comp Physiol* 273, R407-R413 (1997a)

53. Vybíral S, M. Székely, L. Janský, & L. Černý: Thermoregulation of the rabbit during the late phase of endotoxin fever. *Pflügers Arch* 410, 220-202 (1987)

54. Clemmer T. P, C. J. Fisher Jr, R. C. Bone, G. J. Slotman, C. A. Metz, F. O. Thomas, & The Methylprednisolone Severe Sepsis Study Group: Hypothermia in the sepsis syndrome and clinical outcome. *Crit Care Med* 20, 1395-1401 (1992)

55. Dogan M. D, H. Ataoglu & E. S. Akarsu: Effects of selective cyclooxygenase enzyme inhibitors on lipopolysaccharide-induced dual thermoregulatory changes in rats. *Brain Res Bull* 57, 179-185 (2002)

56. Giusti-Paiva A, M. De Castro, J. Antunes-Rodrigues & E. C. Carnio: Inducible nitric oxide synthase pathway in the central nervous system and vasopressin release during experimental septic shock. *Crit Care Med* 30, 1306-1310 (2002)

57. Zhang Y. H, J. Lu, J. K. Elmquist & C. B. Saper: Specific roles of cyclooxygenase-1 and cyclo-oxygenase-2 in lipopolysaccharide-induced fever and Fos expression in rat brain. *J Comp Neurol* 463, 3-12 (2003)

58. Azab A. N. & J. Kaplanski: Involvement of eicosanoids in the hypothermic response to lipopolysaccharide during endotoxemia in rats. *Prostaglandins Leukot Essent Fatty Acids* 70, 67-75 (2004)

59. Steiner A. A, M. D. Dogan, A. I. Ivanov, S. Patel, A. Y. Rudaya, D. H. Jennings, M. Orchinik, T. W. W. Pace, K. A. O'Connor, L. R. Watkins & A. A. Romanovsky: A new function of the leptin receptor: mediation of the recovery from lipopolysaccharide-induced hypothermia. *FASEB J* 18, 1949-1951 (2004)

60. Beutler B. & E. T. Rietschel: Innate immune sensing and its roots: the story of endotoxin. *Nat Rev Immunol* 3, 169-176 (2003)

61 Qureshi S. T. & R. Medzhitov: Toll-like receptors and their role in experimental models of microbial infection. *Genes Immun* 4, 87-94 (2003)

62. Poltorak A, X. He, I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton & B. Beutler: Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085-2088 (1998)

63. Nagai Y, S. Akashi, M. Nagafuku, M. Ogata, Y. Iwakura, S. Akira, T. Kitamura, A. Kosugi, M. Kimoto & K. Miyake: Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol* 3, 667-672 (2002)

64. Yang R. B, M. R Mark, A. Gray, A. Huang, M. H. Xie, M. Zhang, A. Goddard, W. I. Wood, A. L. Gurney & P. J. Godowski: Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signaling. *Nature* 395, 284-288 (1998)

65. Dinarello C. A, S. Gatti & T. Bartfai: Fever: links with an ancient receptor. *Curr Biol* 9, R147-R150 (1999)

66. Hirschfeld M, Y. Ma, J. H. Weis, S. N. Vogel & J. J. Weis: Cutting edge: repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. *J Immunol* 165, 618-622 (2000)

67. Lee H. K, J. Lee & P. S. Tobias: Two lipoproteins extracted from Escherichia coli K-12 LCD25 lipopolysaccharide are the major components responsible for Toll-like receptor 2-mediated signaling. *J Immunol* 168, 4012-4017 (2002)

68. Von Meyenburg C, B. H. Hrupka, D. Arsenijevic, G. J. Schwartz, R. Landmann & W. Langhans: Role for CD14, TLR2, and TLR4 in bacterial product-induced anorexia. *Am J Physiol Regul Integr Comp Physiol* 287, R298-R305 (2004)

69. Perera P. Y, T. N. Mayadas, O. Takeuchi, S. Akira, M. Zaks-Zilberman, S. M. Goyert & S. N.Vogel: CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and taxol-inducible gene expression. *J Immunol* 166, 574-581 (2001)

70. Pearson A. M: Scavenger receptors in innate immunity. *Curr Opin Immunol* 8, 20-28 (1996)

71. Gioannini T. L, A. Teghanemt, K. A. Zarember & J. P. Weiss: Regulation of interactions of endotoxin with host cells. *J Endotoxin Res* 9, 401-408 (2003)

72. Ivanov A. I, S. Patel, V. A. Kulchitsky & A. A. Romanovsky: Platelet-activating factor: a previously unrecognized mediator of fever. *J Physiol* 553, 221-228 (2003a)

73. Takeuchi O, K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda & S. Akira: Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 11, 443-451 (1999)

74. Yoshimura A, E. Lien, R. R. Ingalls, E. Tuomanen, R. Dziarski & D. Golenbock: Cutting edge: recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J Immunol* 163, 1-5 (1999)

75. Janssens S. & R. Beyaert: Role of Toll-like receptors in pathogen recognition. *Clin Microbiol Rev* 16, 637-646 (2003)

76 Dinarello C. A: Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *J Endotoxin Res* 10, 201-222 (2004)

77. Kelly J. F, C. F. Elias, C. E. Lee, R. S. Ahima, R. J. Seeley, C. Bjorbaek, T. Oka, C. B. Saper, J. S. Flier & J. K. Elmquist: Ciliary neurotrophic factor and leptin induce distinct patterns of immediate early gene expression in the brain. *Diabetes* 53, 911-920 (2004)

78. Roth J, D. Martin, B. Storr & E. Zeisberger: Neutralization of pyrogen-induced tumor necrosis factor by its type 1 soluble receptor in guinea pigs: effects on fever and interleukin-6 release. *J Physiol* 509, 267-275 (1998)

79. Netea M. G, B. J. Kullberg & J. W. Van der Meer: Circulating cytokines as mediators of fever. *Clin Infect Dis* 31, Suppl 5, S178-S184 (2000)

80. Ivanov A. I, A. A. Steiner, S. Patel, A. Y. Rudaya & A. A. Romanovsky: Albumin in not an irreplaceable carrier for amphipathic mediators of thermoregulatory responses to lipopolysaccharide: compensatory role of alpha$_1$-acid glycoprotein. *Am J Physiol Regul Integr Comp Physiol* (December 2, 2004; doi:10.1152/ ajpregu.00514.2004).

81. Han S. J, H. M. Ko, J. H. Choi, K. H. Seo, H. S. Lee, E. K. Choi, I. W. Choi, H. K. Lee & S. Y. Im: Molecular mechanisms for lipopolysaccharide-induced biphasic activation of nuclear factor-kappa B (NF-kappa B). *J Biol Chem* 277, 44715-44721 (2002)

82. Marrache A. M, F. Gobeil Jr., S. G. Bernier, J. Stankova, M. Rola-Pleszczynski, S. Choufani, G. Bkaily, A. Bourdeau, M. G. Sirois, A. Vazquez-Tello, L. Fan, J. S. Joyal, J. G. Filep, D. R. Varma, A. Ribeiro-Da-Silva & S. Chemtob: Proinflammatory gene induction by platelet-activating factor mediated via its cognate nuclear receptor. *J Immunol* 169, 6474-6481 (2002)

83. Libert C, W. Van Molle, P. Brouckaert & W. Fiers: Alpha1-Antitrypsin inhibits the lethal response to TNF in mice. *J Immunol* 157, 5126-5129 (1996)

84. Ephgrave K, T. Kremer, K. Broadhurst & J. Cullen: The role of platelet-activating factor in conscious, normotensive endotoxemia. *J Surg Res* 68, 170-174 (1997)

85. Steiner A. A, A. Y. Rudaya, A. I. Ivanov & A. A. Romanovsky. Febrigenic signaling to the brain does not involve nitric oxide. *Br J Pharmacol* 141, 1204-1213 (2004)

86. Banks W. A, A. J. Kastin & D. A. Durham: Bidirectional transport of interleukin-1 alpha across the blood-brain barrier. *Brain Res Bull* 23, 433-437 (1989)

87. Banks W. A, S. A. Farr & J. E. Morley: Entry of blood-borne cytokines into the central nervous system: effects on cognitive processes. *Neuroimmunomodulation* 10, 319-327 (2002-2003)

88. Mercier F, J. T. Kitasako & G. I. Hatton: Fractones and other basal laminae in the hypothalamus. *J Comp Neurol* 455, 324-340 (2003)

89. Blatteis C. M, S. L. Bealer, W. S. Hunter, J. Llanos-Q, R. A. Ahokas & T. A. Mashburn Jr: Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. *Brain Res Bull* 11, 519-526 (1983)

90. Takahashi Y, P. Smith, A. Ferguson & Q. J. Pittman: Circumventricular organs and fever. *Am J Physiol Regul Integr Comp Physiol* 273, R1690-R1695 (1997)

91. Sehic E, R. Gerstberger & C. M. Blatteis: The effect of intravenous lipopolysaccharide on NADPH-diaphorase stainning (= nitric oxide synthase activity) in the organum vasculosum laminae terminalis of guinea pigs. *Ann N Y Acad Sci* 813, 383-391 (1997)

92. Cartmell T, G. N. Luheshi & N. J. Rothwell: Brain sites of action of endogenous interleukin-1 in the febrile response to localized inflammation in the rat. *J Physiol* 518, 585-594 (1999)

93. Romanovsky A. A, N. Sugimoto, C. T. Simons & W. S. Hunter: The *organum vasculosum laminae terminalis* (OVLT) in immune-to-brain febrigenic signaling: a reappraisal of lesion experiments. *Am J Physiol Regul Integr Comp Physiol* 285, R420-R428 (2003)

94. Stitt J. T. & S. G. Shimada: Site of action of calcium channel blockers in inhibiting endogenous pyrogen fever in rats. *J Appl Physiol* 71, 956-960 (1991)

95. Scammell T. E, J. K. Elmquist, J. D. Griffin & C. B. Saper: Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. *J Neurosci* 16, 6246-6254 (1996)

96. Scammell T. E, J. D. Griffin, J. K. Elmquist & C. B. Saper: Microinjection of a cyclooxygenase inhibitor into the anteroventral preoptic region attenuates LPS fever. *Am J Physiol Regul Integr Comp Physiol* 274, R783-R789 (1998)

97. Lin M. T. & J. H. Lin: Involvement of tyrosine kinase in the pyrogenic fever exerted by NOS pathways in *organum vasculosum laminae terminalis*. *Neuropharmacology* 39, 347-352 (2000)

98. Morimoto A, N. Murakami, T. Nakamori & T. Watanabe: Evidence for separate mechanisms of induction of biphasic fever inside and outside the blood-brain barrier in rabbits. *J Physiol* 383, 629-637 (1987)

99. Cooper A. L. & N. J. Rothwell: Mechanisms of early and late hypermetabolism and fever after localized tissue injury in rats. *Am J Physiol Endocrinol Metab* 261, E698-E705 (1991)

100. Watkins L. R, L. E. Goehler, J. K. Relton, N. Tartaglia, L. Silbert, D. Martin & S. F. Maier: Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 183, 27-31 (1995)

101. Székely M, M. Balaskó & A. A. Romanovsky: Capsaicin-sensitive neural afferents in fever pathogenesis (Abstract). *Pflügers Arch* 440, R64 (1995)

102. Romanovsky A. A, V. A. Kulchitsky, C. T. Simons, N. Sugimoto & M. Székely: Febrile responsiveness of vagotomized rats is suppressed even in the absence of malnutrition. *Am J Physiol Regul Integr Comp Physiol* 273, R777-R783 (1997b)

103. Romanovsky A. A, V. A. Kulchitsky, C. T. Simons, N. Sugimoto & M. Székely: Cold defense mechanisms in vagotomized rats. *Am J Physiol* 273, R784-R789 (1997c)

104. Sugimoto N, C. T. Simons, A. A. Romanovsky: Vagotomy does not affect thermal responsiveness to intrabrain prostaglandin E_2 and cholecystokinin octapeptide. *Brain Res* 844, 157-163 (1999)

105. Hansen M. K, S. Daniels, L. E. Goehler, R. P. Gaykema, S. F. Maier & L. R. Watkins: Subdiaphragmatic vagotomy does not block intraperitoneal lipopolysaccharide-induced fever. *Auton Neurosci* 85, 83-87 (2000)

106. Luheshi G. N, R. M. Bluthe, D. Rushforth, N. Mulcahy, J. P. Konsman, M. Goldbach & R. Dantzer: Vagotomy attenuates the behavioural but not the pyrogenic effects of interleukin-1 in rats. *Auton Neurosci* 85, 127-132 (2000)

107. Székely M, M. Balaskó, V. A. Kulchitsky, C. T. Simons, A. I. Ivanov & A. A. Romanovsky: Multiple neural mechanisms of fever. *Auton Neurosci* 85, 78-82 (2000)

108. Dogan M. D, S. Patel, A. Y. Rudaya, A. A. Steiner, M. Székely & A. A. Romanovsky: Lipopolysaccharide fever is initiated via a capsaicin-sensitive mechanism independent of the subtype-1 vanilloid receptor. *Br J Pharmacol* 143, 1023-1032 (2004)

109. Dogan M. D, V. A. Kulchitsky, S. Patel, E. Petervari, M. Szekely & A. A. Romanovsky: Bilateral splanchnicotomy does not affect lipopolysaccharide-induced fever in rats. *Brain Res* 993, 227-229 (2003)

110. Simons C. T, V. A. Kulchitsky, N. Sugimoto, L. D. Homer, M. Székely & A. A. Romanovsky: Signaling the brain in systemic inflammation: which vagal branch is involved in fever genesis? *Am J Physiol Regul Integr Comp Physiol* 275, R63-R68 (1998)

111. Mathison J. C. & R. J. Ulevitch: The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. *J Immunol* 123, 2133-2143 (1979)

112. Ruiter D. J, J. van der Meulen, A. Brouwer, M. J. Hummel, B. J. Mauw, J. C. van der Ploeg & E. Wisse: Uptake by liver cells of endotoxin following its intravenous injection. *Lab Invest* 45, 38-45 (1981)

113. Dinarello C. A, P. T. Bodel & E. Atkins: The role of the liver in the production of fever and in pyrogenic tolerance. *Trans Assoc Am Physicians* 81, 334-344 (1968)

114. Li Z. & C. M. Blatteis: Fever onset is linked to the appearance of lipopolysaccharide in the liver. *J Endotoxin Res* 10, 39-53 (2004)

115. Ivanov A. I, R. S. Pero, C. Scheck & A. A. Romanovsky: Prostaglandin E_2-synthesizing enzymes in fever: differential transcriptional regulation. *Am J Physiol Regul Integr Comp Physiol* 283, R1104-R1117 (2002)

116. Niijima A: The afferent discharges from sensors for interleukin-1beta in the hepatoportal system in the anesthetized rat. *J Auton Nerv Syst* 61, 287-291 (1996)

117. Goehler L. E, J. Relton, D. Dripps, R. Kiechle, N. Tartaglia, S. F. Maier & L. R. Watkins: Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull* 43, 357-364 (1997)

118. Ek M, M. Kurosawa, T. Lundeberg, M. Heilig & A. Ericsson: Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins. *J Neurosci* 18, 9471-9479 (1998)

119. Hansen M. K, K. A. O'Connor, L. E. Goehler, L. R. Watkins & S. F. Maier: The contribution of the vagus nerve in interleukin-1beta-induced fever is dependent on dose. *Am J Physiol Regul Integr Comp Physiol* 280, R929-R934 (2001)

120. Yamagata K, K. Matsumura, W. Inoue, T. Shiraki, K. Suzuki, S. Yasuda, H. Sugiura, C. Cao, Y. Watanabe & S. Kobayashi: Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J Neurosci* 21, 2669-2677 (2001)

121. Ek M, D. Engblom, S. Saha, A. Blomqvist, P. J. Jakobsson & A. Ericsson-Dahlstrand: Inflammatory response: pathway across the blood-brain barrier. *Nature* 410, 430-431 (2001)

122. Rivest S: What is the cellular source of prostaglandins in the brain in response to systemic inflammation? Facts and controversies. *Mol Psychiatry* 4, 500-507 (1999)

123. Cao C, K. Matsumura, K. Yamagata & Y. Watanabe: Endothelial cells of the rat brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1 beta: a possible site of postaglandin synthesis responsible for fever. *Brain Res* 733, 263-272 (1996)

124. Matsumura K, C. Cao, M. Ozaki, H. Morii, K. Nakadate & Y. Watanabe: Brain endothelial cells express cyclooxygenase-2 during lipopolysaccharide-induced fever: light and electron microscopic immunocytochemical studies. *J Neurosci* 18, 6279-6289 (1998)

125. Elmquist J. K, C. D. Breder, J. E. Sherin, T. E. Scammell, W. F. Hickey, D. Dewitt & C. B. Saper: Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. *J Comp Neurol* 381, 119-129 (1997)

126. Schiltz J. C. & P. E. Sawchenko: Distinct brain vascular cell types manifest inducible cyclooxygenase expression as a function of the strength and nature of immune insults. *J Neurosci* 22, 5606-5618 (2002)

127. Konsman J. P, S. Vigues, L. Mackerlova, A. Bristow & A. Blomqvist: Rat brain vascular distribution of interleukin-1 type-1 receptor immunoreactivity: relationship to patterns of inducible cyclooxygenase expression by peripheral inflammatory stimuli. *J Comp Neurol* 472, 113-129 (2004)

128. Milton A. S. & S. Wendlandt: A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *J Physiol* 207, 76P-77P (1970)

129. Vane J. R: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* 231, 232-235 (1971)

130. Tegeder I, J. Pfeilschifter & G. Geisslinger: Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J* 15, 2057-2072 (2001)

131. Yin M. J, Y. Yamamoto & R. B. Gaynor: The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. *Nature* 396, 77-80 (1998)

132. Daun J. M, R. W. Ball, H. R. Burger & J. G. Cannon: Aspirin-induced increases in soluble IL-1 receptor type II concentrations *in vitro* and *in vivo*. *J Leukoc Biol* 65, 863-866 (1999)

133. Paul-Clark M. J, T. Van Cao, N. Moradi-Bidhendi, D. Cooper & D. W. Gilroy: 15-epi-lipoxin A_4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. *J Exp Med* 200, 69-78 (2004)

134. Ueno R, S. Narumiya, T. Ogorochi, T. Nakayama, Y. Ishikawa & O. Hayaishi: Role of prostaglandin D_2 in the hypothermia of rats caused by bacterial lipopolysaccharide. *Proc Natl Acad Sci USA* 79, 6093-6097 (1982)

135. Derijk R. H, M. Van Kampen, N. Van Rooijen & F. Berkenbosch: Hypothermia to endotoxin involves reduced thermogenesis, macrophage-dependent mechanisms, and prostaglandins. *Am J Physiol* 266, R1-R8 (1994)

136. Gilroy D. W, P. R. Colville-Nash, D. Willis, J. Chivers, M. J. Paul-Clark & D. A. Willoughby: Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 5, 698-701 (1999)

137. Mouihate A, L. Boisse & Q. J. Pittman: A novel antipyretic action of 15-deoxy-Delta-^{12,14}-prostaglandin J_2 in the rat brain. *J Neurosci* 24, 1312-1318 (2004)

138. Murakami M. & I. Kudo: Recent advances in molecular biology and physiology of the prostaglandin E_2-biosynthetic pathway. *Prog Lipid Res* 43, 3-35 (2004)

139. Piper P. J, J. R. Vane & J. H. Wyllie: Inactivation of prostaglandins by the lungs. *Nature* 225, 600-604 (1970)

140. Coggins K. G, A. Latour, M. S. Nguyen, L. Audoly, T. M. Coffman & B. H. Koller: Metabolism of PGE_2 by prostaglandin dehydrogenase is essential for remodeling the ductus arteriosus. *Nat Med* 8, 91-92 (2002)

141. Ivanov A. I, A. C. Scheck & A. A. Romanovsky: Expression of genes controlling transport and catabolism of prostaglandin E_2 in lipopolysaccharide fever. *Am J Physiol Regul Integr Comp Physiol* 284, R698-R706 (2003b)

142. Davidson J, H. T. Abul, A. S. Milton & D. Rotondo: Cytokines and cytokine inducers stimulate prostaglandin E_2 entry into the brain. *Pflügers Arch* 442, 526-533 (2001)

143. Nakamura K, T. Kaneko, Y. Yamashita, H. Hasegawa, H. Katoh, A. Ichikawa & M. Negishi: Immunocytochemical localization of prostaglandin EP3 receptor in the rat hypothalamus. *Neurosci Lett* 260, 117-120 (1999)

144. Yoshida K, K. Nakamura, K. Matsumura, K. Kanosue, M. Konig, H. J. Thiel, Z. Boldogkoi, I. Toth, J.

Roth, R. Gerstberger & T. Hubschle: Neurons of the rat preoptic area and the raphe pallidus nucleus innervating the brown adipose tissue express the prostaglandin E receptor subtype EP3. *Eur J Neurosci* 18, 1848-1860 (2003)

145. Nakamura K, K. Matsumura, T. Kaneko, S. Kobayashi, H. Katoh & M. Negishi: The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. *J Neurosci* 22, 4600-4610 (2002)

146. Morrison S. F, A. F. Sved & A. M. Passerin: GABA-mediated inhibition of raphe pallidus neurons regulates sympathetic outflow to brown adipose tissue. *Am J Physiol* 276, R290-R297 (1999)

147. Morrison S. F: Raphe pallidus neurons mediate prostaglandin E_2-evoked increases in brown adipose tissue thermogenesis. *Neuroscience* 121, 17-24 (2003)

148. Rathner J. A. & R. M. McAllen: Differential control of sympathetic drive to the rat tail artery and kidney by medullary premotor cell groups. *Brain Res* 834, 196-199 (1999)

149. Tanaka M, K. Nagashima, R. M. McAllen & K. Kanosue: Role of the medullary raphé in thermoregulatory vasomotor control in rats. *J Physiol* 540, 657-664 (2002)

150. Hatae N, Y. Sugimoto & A. Ichikawa: Prostaglandin receptors: advances in the study of EP3 receptor signaling. *J Biochem* 131, 781-784 (2002)

151. Nakamura K: Fever-inducing sympathetic neural pathways. *J Therm Biol* 29, 339-344 (2004)

152. Oka T, K. Oka & C. B. Saper: Contrasting effects of E type prostaglandin (EP) receptor agonists on core body temperature in rats. *Brain Res* 968, 256-262 (2003b)

153. Bligh J: Temperature regulation: a theoretical consideration incorporating Sherringtonian principles of central neurology. *J Therm Biol* 9, 3-6 (1984)

154. Zhang Y. H, M. Yanase-Fujiwara, T. Hosono & K. Kanosue: Warm and cold signals from the preoptic area: which contribute more to the control of shivering in rats? *J Physiol* 485, 195-202 (1995)

155. Chen X. -M, T. Hosono, T. Yoda, Y. Fukuda & K. Kanosue: Efferent projection from the preoptic area for the control of non-shivering thermogenesis in rats. *J Physiol* 512, 883-892 (1998)

156. Griffin J. D, C. B. Saper & J. A. Boulant: Synaptic and morphological characteristics of temperature-sensitive and -insensitive rat hypothalamic neurons. *J Physiol* 537, 521-535 (2001)

157. Cliffer K. D, R. Burstein & G. J. Giesler Jr: Distributions of spinothalamic, spinohypothalamic, and spinotelencephalic fibers revealed by anterograde transport of PHA-L in rats. *J Neurosci* 11, 852-868 (1991)

158. Morrison S. F: Central pathways controlling brown adipose tissue thermogenesis. *News Physiol Sci* 19, 67-74 (2004)

159. Bamshad M, C. K. Song & T. J. Bartness: CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *Am J Physiol* 276, R1569-R1578 (1999)

160. Oldfield B. J, M. E. Giles, A. Watson, C. Anderson, L. M. Colvill & M. J. McKinley: The neurochemical characterization of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. *Neuroscience* 110, 515-526 (2002)

161. Cano G, A. M. Passerin, J. C. Schiltz, J. P. Card, S. F. Morrison & A. F. Sved: Anatomical substrates for the central control of sympathetic outflow to interscapular adipose tissue during cold exposure. *J Comp Neurol* 460, 303-326 (2003)

162. McAllen R. M: Preoptic thermoregulatory mechanisms in detail. *Am J Physiol Regul Integr Comp Physiol* 287, R272-R273 (2004)

163. Horn T, M. F. Wilkinson, R. Landgraf & Q. J. Pittman: Reduced febrile responses to pyrogens after lesions of the hypothalamic paraventricular nucleus. *Am J Physiol* 267, R323-R328 (1994)

164. Almeida M. C, A. A. Steiner, N. C. Coimbra & L. G. S. Branco: Thermoeffector neuronal pathways in fever: a study in rats showing a new role of the locus coeruleus. *J Physiol* 558, 283-294 (2004)

165. Sugimoto N, O. Shido, S. Sakurada & T. Nagasaka: Day-night variations of behavioral and autonomic thermoregulatory responses to lipopolysaccharide in rats. *Jpn J Physiol* 46, 451-456 (1996)

166. Florez-Duquet M, E. Peloso, E. Satinoff: Fever and behavioral thermoregulation in young and old rats. *Am J Physiol* 280, R1457-R1461 (2001)

167. Turek V. F, D. H. Olster, A. Ettenberg & H. J. Carlisle: The behavioral thermoregulatory response of febrile female rats is not attenuated by vagotomy. *Pharmacol Biochem Behav* 80, 115-121 (2005)

168. Maruyama M, M. Nishi, M. Konishi, Y. Takashige, K. Nagashima, T. Kiyohara & K. Kanosue: Brain regions expressing Fos during thermoregulatory behavior in rats. *Am J Physiol Regul Integr Comp Physiol* 285, R1116-R1123 (2003)

169. Grunfeld C, C. Zhao, J. Fuller, A. Pollack, A. Moser, J. Friedman & K. R. Feingold: Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. *J Clin Invest* 97, 2152-2157 (1996)

170. Janik J. E, B. D. Curti, R. V. Considine, H. C. Rager, G. C. Powers, W. G. Alvord, J. W. Smith II, B. L. Gause &

W. C. Kopp: Interleukin-1 alpha increases serum leptin concentrations in humans. *J Clin Endocrinol Metab* 82, 3084-3086 (1997)

171. Sarraf P, R. C. Frederich, E. M. Turner, G. Ma, N. T. Jaskowiak, D. J. Rivet III, J. S. Flier, B. B. Lowell, D. L. Fraker & H. R. Alexander: Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J Exp Med* 185, 171-175 (1997)

172. Loffreda S, S. Q. Yang, H. A. Lin, C. L. Karp, M. L. Brengman, D. J. Wang, A. S. Klein, G. B. Bulkely, C. Bao, P. W. Noble, M. D. Lane & A. M. Diehl: Leptin regulates proinflammatory immune response. *FASEB J* 12, 57-65 (1998)

173. Lord G. M, G. Matarese, J. K. Howard, R. J. Baker, S. R. Bloom & R. I. Lechler: Leptin modulates T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 394, 897-901 (1998)

174. Scarpace P. J, M. Nicolson & M. Matheny: UCP2, UCP3 and leptin gene expression: Modulation by food restriction and leptin. *J Endocrinol* 159, 349-357 (1998)

175. Pelleymounter M. A, M. J. Cullen, M. B. Baker, R. Hechy, D. Winters, T. Boone & F. Collins: Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269, 540-543 (1995)

176. Haynes W. G, D. A. Morgan, S. A. Walsh, A. L. Mark & W. I. Sivitz: Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest* 100, 270-278 (1997)

177. Luheshi G. N, J. D. Gardner, D. A. Rushforth, A. S. Loudon & N. J. Rothwell: Leptin actions on food intake and body temperature are mediated by IL-1. *Proc Natl Acad Sci, USA* 96, 7047-7052 (1999)

178. Turek V. F, D. H. Olster, K. R. Gililland, M. Sheehy, A. Ettenberg & H. J. Carlisle: The effects of melanocortin agonists and antagonists on leptin-induced fever in rats. *J Therm Biol* 29, 423-430 (2004)

179. Ivanov, A. I. & A. A. Romanovsky: Fever responses of Zucker rats with and without fatty mutation of the leptin receptor. *Am J Physiol Regul Integr Comp Physiol* 282, R311-R316 (2002)

180. Dascombe M. J, A. Hardwick, R. A. Lefeuvre & N. J. Rothwell: Impaired effects of interleukin-1 beta on fever and thermogenesis in genetically obese rats. *Int J Obes* 13, 367-373 (1989)

181. Jászberényi M, E. Bujdosó, E. Kiss, I. Pataki & G. Telegdy: The role of NPY in the mediation of orexin-induced hypothermia. *Regul Pept* 104, 55-59 (2002)

182. Székely M, E. Pétervári, M. Balaskó, I. Hernándi & B. Uzsoki: Effects of orexins on energy balance and thermoregulation. *Regul Pept* 104, 47-53 (2002)

183. Monda M, A. Viggiano & V. De Luca: Paradoxical effect of orexin A: hypophagia induced by hyperthermia. *Brain Res* 961, 220-228 (2003)

184. Balaskó M, Z. Szelényi & M. Székely: Central thermoregulatory effects of neuropeptide Y and orexin A in rats. *Acta Physiol Hung* 86, 219-222 (1999)

185. McMahon C. D, D. F. Buxton, T. H. Elsasser, D. R. Gunter, L. G. Sanders, B. P. Steele & J. L. Sartin: Neuropeptide Y restores appetite and alters concentrations of GH after central administration to endotoxic sheep. *J Endocrinol* 161, 333-339 (1999)

186. Linthorst A. C, C. Flachskamm, S. J. Hopkins, M. S. Hoadley, M. S. Labeur, F. Holsboer & J. M. Reul: Long term intracerebroventricular infusion of corticotropin-releasing hormone alters neuroendocrine, neurochemical, autonomic, behavioral, and cytokine responses to a systemic inflammatory challenge. *J Neurosci* 17, 4448-4460 (1997)

187. Drolet G. & S. Rivest: Corticotropin-releasing hormone and its receptors; an evaluation at the transcription level *in vivo*. *Peptides* 22, 761-767 (2001)

188. Turnbull A. V. & C. L. Rivier: Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* 79, 1-71 (1999)

189. Coelho M. M, G. E. Souza & I. R. Pela: Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. *Am J Physiol* 263, R423-R427 (1992)

190. De Fanti B. A. & J. A. Martinez: Central urocortin activation of sympathetic-regulated energy metabolism in Wistar rats. *Brain Res* 930, 37-41 (2002)

191. Asakawa A, A. Inui, N. Ueno, S. Makino, M. Fujimiya, M. A. Fujino & M. Kasuga: Urocortin reduces oxygen consumption in lean and ob/ob mice. *Int J Mol Med* 7: 539-541 (2001)

192. Bachtell R. K, A. Z. Weitemier & A. E. Ryabinin: Lesions of the Edinger-Westphal nucleus in C57BL/6J mice disrupt ethanol-induced hypothermia and ethanol consumption. *Eur J Neurosci* 20, 1613-1623 (2004)

193. Shimuzu H, M. Miyoshi, K. Matsumoto, O. Goto, T. Imoto & T. Watanabe: The effect of central injection of angiotensin-converting enzyme inhibitor and the angiotensin type 1 receptor antagonist on the induction by lipopolysaccharide of fever and brain interleukin-1beta response in rats. *J Pharmacol Exp Ther* 308, 865-73 (2004)

194. Szelényi Z, L. Barthó, M. Székely & A. A. Romanovsky: Cholecystokinin octapeptide (CCK-8) injected into a cerebral ventricle induces a fever-like thermoregulatory response mediated by type B CCK-receptors in the rat. *Brain Res* 638, 69-77 (1994)

195. Székely M, Z. Szelényi & M. Balaskó: Cholecystokinin participates in the mediation of fever. *Pflügers Arch* 428, 671-673 (1994)

196. Martin S. M, B. C. Wilson, X. Chen, Y. Takahashi, P. Poulin & Q. J. Pittman: Vagal CCK and 5-HT(3) receptors are unlikely to mediate LPS or IL-1beta-induced fever. *Am J Physiol Regul Integr Comp Physiol* 279, R960-R965 (2000)

197. Ivanov A. I, V. A. Kulchitsky & A. A. Romanovsky: Role for the cholecystokinin-A receptor in fever: a study of a mutant rat strain and a pharmacological analysis. *J Physiol* 547, 941-949 (2003c)

198. Huang Q. H, V. J. Hruby & J. B. Tatro: Systemic alpha-MSH suppresses LPS fever via central melanocortin receptors independently of its suppression of corticosterone and IL-6 release. *Am J Physiol Regul Integr Comp Physiol* 44, R524-R530 (1998)

199. Huang Q. H, V. J. Hruby & J. B. Tatro: Role of central melanocortins in endotoxin-induced anorexia. *Am J Physiol Regul Integr Comp Physiol* 45, R864-R871 (1999)

200. Sinha P. S, H. B. Schioth & J. B. Tatro: Activation of central melanocortin-4 receptors suppresses lipopolysaccharide-induced fever in rats. *Am J Physiol Regul Integr Comp Physiol* 284, R1595-R1603 (2003)

201. Pittman Q. J & M. F. Wilkinson: Central arginine vasopressin and endogenous antipyresis. *Can J Physiol Pharmacol* 70, 786-790 (1992).

202. Romanovsky A. A, A. A. Steiner, L. G. S. Branco, L. Jansky & V. N. Gourine: Arginine vasopressin in fever: a still unsolved puzzle. *J Therm Biol* 29, 407-412 (2004)

203. Bernardini, G. L, J. M. Lipton & W. G. Clark: Intracerebroventricular and septal injections of arginine vasopressin are not antipyretic in the rabbit. *Peptides* 4, 195-198 (1983)

204. Clerget-Froidevaux M. S & Q. J. Pittman: AVP V_{1a}-R expression in the rat hypothalamus around parturition: relevance to antipyresis at term. *Exp Neurol* 183, 338-345 (2003)

205. Imai-Matsumura K, K. Matsumura, A. Terao & Y. Watanabe: Attenuated fever in pregnant rats is associated with blunted syntheses of brain cyclooxygenase-2 and PGE_2. *Am J Physiol Regul Integr Comp Physiol* 283, R1346-R1353 (2002)

206. Ivanov A. I & A. A. Romanovsky: Near-term suppression of fever: inhibited synthesis or accelerated catabolism of prostaglandin E_2? *Am J Physiol Regul Integr Comp Physiol* 284, R860-R861 (2003)

207. Fofie A. E & J. E. Fewell: Influence of pregnancy on plasma cytokines and the febrile response to intraperitoneal administration of bacterial endotoxin in rats. *Exp Physiol* 88, 747-754 (2003)

208. Romanovsky A. A. & M. Székely: Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Med Hypotheses* 50, 219-226 (1998)

209. Aronoff D. M. & E. G. Neilson: Antipyretics: mechanisms of action and clinical use in fever suppression. *Am J Med* 111, 304-315 (2001)

210. Diringer M. N, N. L. Reaven, S. E. Funk & G. C. Uman: Elevated body temperature independently contributes to increased length of stay in neurologic intensive care unit patients. *Crit Care Med* 32, 1489-1495 (2004)

211. Zaremba J: Hyperthermia in ischemic stroke. *Med Sci Monit* 10, RA148-RA153 (2004)

212. Xie W. L, J. G. Chipman, D. L. Robertson, R. L. Erikson & D. L. Simmons: Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci USA* 88, 2692-2696 (1991)

213. Kujubu D. A, B. S. Fletcher, B. C. Varnum, R. W. Lim & H. R. Herschman: TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem* 266, 12866-12872 (1991)

214. O'Banion M. K, H. B. Sadowski, V. Winn & D. A. Young: A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. *J Biol Chem* 266, 23261-23267 (1991)

215. Mitchell J. A, P. Akarasereenont, C. Thiemermann, R. J. Flower & J. R. Vane: Selectivity of nonsteroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci USA* 90, 11693-11697 (1994)

216. Futaki N, S. Takahashi, M. Yokoyama, I. Arai, S. Higuchi & S. Otomo: NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro. *Prostaglandins* 47, 55-59 (1994)

217. Copeland R. A, J. M. Williams, J. Giannaras, S. Nurnberg, M. Covington, D. Pinto, S. Pick & J. M. Trzaskos: Mechanism of selective inhibition of the inducible isoform of prostaglandin G/H synthase. *Proc Natl Acad Sci USA* 91, 11202-11206 (1994)

218. Gans K. R, W. Galbraith, R. J. Roman, S. B. Haber, J. S. Kerr, W. K. Schmidt, C. Smith, W. E. Hewes & N. R. Ackerman: Anti-inflammatory and safety profile of DuP 697, a novel orally effective prostaglandin synthesis inhibitor. *J Pharmacol Exp Ther* 254, 180-187 (1990)

219. Futaki N, K. Yoshikawa, Y. Hamasaka, I. Arai, S. Higuchi, H. Iizuka & S. Otomo: NS-398, a novel non-steroidal anti-inflammatory drug with potent analgesic and antipyretic effects, which causes minimal stomach lesions. *Gen Pharmacol* 24, 105-110 (1993)

220. Riendeau D, M. D. Percival, C. Brideau, S. Charleson, D. Dube, D. Ethier, J. P. Falgueyret, R. W. Friesen, R. Gordon, G. Greig, J. Guay, J. Mancini, M. Ouellet, E. Wong, L. Xu, S. Boyce, D. Visco, Y. Girard, P. Prasit, R. Zamboni, I. W. Rodger, M. Gresser, A. W. Ford-Hutchinson, R. N. Young & C. C. Chan: Etoricoxib (MK-0663): preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. *J Pharmacol Exp Ther* 296, 558-566 (2001)

221. Tacconelli S, M. L. Capone, M. G. Sciulli, E. Ricciotti & P. Patrignani: The biochemical selectivity of novel COX-2 inhibitors in whole blood assays of COX-isozyme activity. *Curr Med Res Opin* 18, 503-511 (2002)

222. Crofford L. J: Specific cyclooxygenase-2 inhibitors: what have we learned since they came into widespread clinical use? *Curr Opin Rheumatol* 14, 225-230 (2002)

223. Solomon D. H, S. Schneeweiss, R. J. Glynn, Y. Kiyota, R. Levin, H. Mogun & J. Avorn. Relationship between selective cyclooxygenase-2 inhibitors and acute myocardial infarction in older adults. *Circulation* 109, 2068-2073 (2004)

224. Boutaud O, D. M. Aronoff, J. H. Richardson, L. J. Marnett & J. A. Oates: Determinants of the cellular specificity of acetaminophen as an inhibitor of prostaglandin H_2 synthases. *Proc Natl Acad Sci USA* 99, 7130-7135 (2002)

225. Flower R. J. & J. R. Vane: Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). *Nature* 240, 410-411 (1972)

226. Botting R. M: Mechanism of action of acetaminophen: is there a cyclooxygenase 3? *Clin Infect Dis* 31, Suppl 5, S202-S210 (2000)

227. Chandrasekharan N. V, H. Dai, K. L. Roos, N. K. Evanson, J. Tomsik, T. S. Elton & D. L. Simmons: COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci USA* 99, 13926-13931 (2002)

228. Davies N. M, R. L. Good, K. A. Roupe & J. A. Yanez: Cyclooxygenase-3: axiom, dogma, anomaly, enigma or splice error? – not as easy as 1, 2, 3. *J Pharm Pharmaceut Sci* 7, 217-226 (2004)

229. Dou W, Y. Jiao, S. Goorha, R. Raghow & L. R. Ballou: Nociception and the differential expression of cyclooxygenase-1 (COX-1), the COX-1 variant retaining intron-1 (COX-1v), and COX-2 in mouse dorsal root ganglia (DRG). *Prostaglandins Other Lipid Mediat* 74, 29-43 (2004)

230. Snipes J. A, B. Kis, G. S. Shelness, J. A. Hewett & D. W. Busija: Cloning and characterization of cyclooxygenase-1b (putative COX-3) in rat. *J Pharmacol Exp Ther* DOI:10.1124/jpet.104.079533 (2005)

231. Shaftel S. S, J. A. Olschowka, S. D. Hurley, A. H. Moore & M. K. O'Banion: COX-3: a splice variant of cyclooxygenase-1 in mouse neural tissue and cells. *Brain Res Mol Brain Res* 119, 213-215 (2003); Erratum in: *Brain Res Mol Brain Res* 123, 1362004 (2004)

232. Dinchuk J. E, R. Q. Liu & J. M. Trzaskos: COX-3: in the wrong frame in mind. *Immunol Lett* 86, 121 (2003)

233. Kis B, J. A. Snipes, T. Isse, K. Nagy & D. W. Busija: Putative cyclooxygenase-3 expression in rat brain cells. *J Cereb Blood Flow Metab* 23, 1287-1292 (2003)

234. Warner T. D, I. Vojnovic, F. Giuliano, R. Jimenez, D. Bishop-Bailey & J. A. Mitchell. Cyclooxygenases 1, 2, and 3 and the production of prostaglandin I_2: investigating the activities of acetaminophen and cyclooxygenase-2-selective inhibitors in rat tissues. *J Pharmacol Exp Ther* 310, 642-647 (2004)

235. Cui J. G, H. Kuroda, N. V. Chandrasekharan, R. P. Pelaez, D. L. Simmons, N. G. Bazan & W. J. Lukiw: Cyclooxygenase-3 gene expression in Alzheimer hippocampus and in stressed human neural cells. *Neurochem Res* 29, 1731-1737 (2004)

Abbreviations: In addition to the commonly accepted (*e.g.*, mRNA), the following abbreviations are used in this paper (all introduced at first mentioning): ANG, angiotensin, AVP, arginine vasopressin, BBB, blood-brain barrier, c, cytosolic (as in $cPLA_2$ or cPGES), CCK, cholecystokinin, COX, cyclooxygenase(s), $COX\text{-}1V_1$, COX-1 variant retaining intron 1, CR, carbonyl reductase, CRF, corticotropin-releasing factor, IL, interleukin(s), LPS, lipopolysaccharide(s), m, microsomal (as in mPGES), MOAT, multispecific organic anion transporter, MSH, melanocyte stimulating hormone, NPY, neuropeptide Y, OVLT, the *organum vasculosum laminae terminalis*, PAF, platelet-activating factor, PG, prostaglandin, 15-PGDH, 15-hydroxy-PG dehydrogenase, PGES, PGE synthase(s), PGT, PG transporter, PL, phospholipase(s), s, secretory (as in $sPLA_2$), T_b, body temperature, TLR, Toll-like receptor(s), TNF, tumor necrosis factor, VSA, ventral septal area.

Key Words: Body temperature, Thermoregulation, Set point, Balance point, Fever, Febrile response, Hypothermic response, Anapyrexia, Leptin, Lipopolysaccharide, LPS, Toll-like receptors, Cytokines, IL-1beta, IL-6, TNF-alpha, Prostaglandins, PGE_2, Blood-brain barrier, *Organum vasculosum laminae terminalis*, OVLT, Vagus Nerve, PGE_2-synthesizing enzymes, Phospholipases, PLA_2,

Cyclooxygenases, COX-1, COX-2, Prostaglandin synthases, PGES, EP3 receptor, Thermoeffectors, Skin vasoconstriction, Thermogenesis, Brown adipose tissue, Neuropeptides, Orexins, Neuropeptide Y, Arginine vasopressin, Angiotensin II, Cholecystokinin, Alpha-MSH, Corticotropin-releasing factor, Urocortins, Selective COX-2 inhibitors, Antipyretic therapy, Review

Send correspondence to: Andrej A. Romanovsky, M.D., Ph.D., Director, Systemic Inflammation Laboratory, Trauma Research, St. Joseph's Hospital and Medical Center, 350 West Thomas Road, Phoenix, Arizona 85013, USA, Tel: 602-406-5059, Fax: 602-406-4113, E-mail: aromano@chw.edu, URL: http://www.FeverLab.net, http://www.IChoseStJoes.com/Fever

http://www.bioscience.org/current/vol10.htm