UNIVERSITY OF CALGARY

The Mechanisms of the Attenuated Febrile Response to Bacterial Endotoxin Near the Term of Pregnancy

by

Anita E.A. Fofie

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF MEDICAL SCIENCE

CALGARY, ALBERTA
JUNE, 2003

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studied for acceptance, a dissertation entitle "The Mechanisms of the Attenuated Febrile Response to Bacterial Endotoxin Near the Term of Pregnancy" submitted by Anita E.A. Fofie in partial fulfillment of the requirements of the degree Master of Science.

Supervisor, Dr. James E. Fewell,
Department of Medical Science

Dr. Keith E. Cooper,
Department of Medical Science

Dr. Marc J. Poulin,
Department of Cardiovascular & Respiratory Sciences

Dr. Greg T. Connors,
Department of Medical Science

"Internal" External Examiner, Dr. Sheilagh M. Martin,
Department of Biology, Mount St. Vincent University

June 17, 2003

ABSTRACT

The adaptation to pregnancy involves numerous alterations in maternal physiology. Reversible changes in thermoregulation have been documented in several species. In the rat, these changes include a decrease in core temperature near the term of pregnancy and a dramatic increase in core temperature immediately after parturition. Furthermore, fever in response to exogenous and endogenous pyrogens, as well as to proximal mediators of fever, prostaglandins of the E series, is attenuated near term of pregnancy. These studies were carried out to investigate mechanisms of this febrile response. We found that fever is indeed attenuated in response to intraperitoneal administration of bacterial endotoxin in a gestation-dependent manner. I also found that the attenuated response to E. coli lipopolysaccharide is not due to changes in basal cytokine production throughout pregnancy. Finally, investigation of the cytokine response following challenge with lipopolysaccharide revealed that systemic release of PGEs was not affected by pregnancy. However, I found that release of endogenous pyrogens (IL-1β, IL-6) is suppressed near term, and that the production of endogenous antipyretic IL-1ra is not exaggerated, but rather its effects are sustained longer in comparison to nonpregnant rats, and rats in early to mid gestation. Therefore, there appear to be several mechanisms acting at the level of the periphery, which serve to limit the increase in core temperature in response to bacterial endotoxin near term of pregnancy.

ACKNOWLEDGEMENTS

I would like to take this occasion to acknowledge all of those who have supported my educational endeavors over the past two years. Dr. Fewell, my supervisor: I am deeply grateful for your guidance. You have been instrumental in helping me achieve my goals. To my supervisory committee members, Dr. Marc Poulin, Dr. Greg Connors, thank you for your contributions to my research. Thank you also to Dr. Francine Smith for your kindness during my graduate studies. I would like to recognize Dr. Sheilagh Martin for serving on my examination committee. A very special thank-you to Dr. Keith Cooper for your mentorship. You have gone above and beyond expectation in ensuring my success, and I will remember you always.

I cannot miss the opportunity to include my family in this accomplishment. Thank-you mom and dad for your tireless dedication to my dreams, and for teaching me to value higher learning. To my sisters for pursuing excellence: without you, Monica and Desiree, my motivation to succeed would not be nearly so high. Sean, your constant companionship and emotional support throughout this journey has been invaluable. Words cannot express how glad I am to have you all in my life. Lastly, without God my achievements would have no meaning, in Him all things are possible.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

In 1865 Claude Bernard reflected that the "constancy of the internal milieu was the essential condition to a free and independent life." This extraordinary property of the body has intrigued many physiologists. By 1929, impressed by "the wisdom of the body" capable of guaranteeing with such efficiency the control of the physiological equilibrium, physiologist Walter B. Cannon coined the term homeostasis.[26] Homeostasis is one of the most remarkable and most typical properties of highly complex organisms. A homeostatic system is any open system that maintains its structure and function by means of dynamic equilibriums rigorously controlled by interdependent regulation mechanisms. Such a system reacts to every change in the environment, or to every random disturbance, through a series of modifications of equal size and opposite direction to those that created the disturbance. The goal of these modifications is to maintain the internal balances. One challenge with this view, however, is that homeostasis implies a single optimal condition which must be defended while in reality, optimal function does not always necessitate a

fixed internal milieu. Mrosovsky's concept of rheostasis addresses the changes in regulated levels. Rather than being failures of homeostasis, controlled changes in defended set-point are often adaptive.[158] Successful temperature regulation, is one such example of a system operating as a sliding steady state.

1.1 Regulation of Core Temperature

Homeothermy is a "pattern of temperature regulation in which the cyclic variation in core temperature is maintained within arbitrarily defined limits (±2°C) despite much larger variations in ambient temperature" (IUPS Thermal Commission, 1987). In essence it describes the condition of homeostasis in the thermoregulatory system.[78]

In mammals, control of core temperature is achieved reflexively, and believed to be primarily mediated by the hypothalamus.[80] The preoptic area is a region of the rostral hypothalamus that is critical to internal thermoregulation in response to external changes. This region of the hypothalamus is also an important center for the determination of an organism's basal core temperature as it is the site at which thermoreceptor afferents terminate.[79] The preoptic region contains a high density of thermosensitive neurons whose firing rates can be integrated to obtain a set-point temperature. Additionally, the preoptic area integrates afferent signals from peripheral thermoceptors. As such, the preoptic region is sensitive to both central and peripheral body temperature and actively seeks to regulate these at a constant level. This is essentially the basis for Hammel's set-point theory.[85] If preoptic temperature rises above the set-point, various heat loss responses are initiated to lower body temperature and return the preoptic region back to

the set-point. Conversely, if the integrated signal at the preoptic region indicates a drop in temperature below the set-point, then various heat retention and heat production responses are reflexively evoked to raise body temperature and return the preoptic region to its set-point. As with all reflex arcs, the thermoregulatory system can be modeled as a feedback loop with five distinct components. The integrative and control neurons of the central nervous system which act as a comparator; thermoregulatory effectors, which serve as the controlling elements that bring about alterations in body temperature; body temperature which is the controlled system; set-point which is the reference temperature to which the organism regulates its body temperature; and peripheral and deep body thermoreceptors, which provide feedback to the central nervous system.[38] The following figure adapted from Gordon, illustrates this thermoregulatory control system.[80]

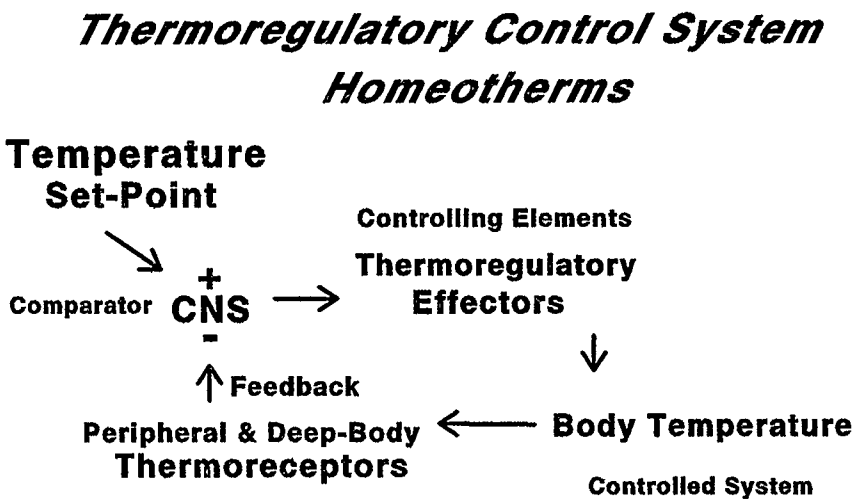


Figure 1.1 Generalized diagram of the thermoregulatory control system of homeotherms as a feedback model consisting of five components. Overall core temperature (the summation of signals from thermoreceptors) is compared with a central set point temperature in the central nervous system. When there is a difference, the appropriate thermoregulatory effect mechanisms are engaged, returning core body temperature toward the set point.

The feedback loop is activated only when the comparator detects a discrepancy between the temperature set-point and the input from peripheral and deep-body thermoreceptors. The resulting error signal triggers the controlling elements or thermoregulatory effectors to adjust activity in order to eliminate any difference these two temperatures. In normal physiology, the temperature set-point remains constant, however, there are many conditions that can alter the hypothalamic set-point. Fever is one such example.

1.2 Fever

Most simply, fever is a state in which body temperature is actively maintained at a higher level than normal. In other words, the febrile organism experiences a regulated hyperthermia or a defended increase in core temperature as the body thermostat is set at a higher level in response to the presence of pyrogenic substances.[115,189] Electrophysiological studies show that endogenously derived pyrogens inhibit warm-sensitive neurons,[101,102]and excite cold-sensitive neurons. Inhibition of warm-sensitive neurons would suppress heat loss responses, while excitation of cold-sensitive neurons would potentiate heat generation responses. According to Hammel's theory, these changes in the hypothalamus mean that, in essence, there has been a shift in set-point, invoking events within the preoptic region that are consistent with fever. Thus, at the onset of the febrile episode, the subject recruits thermogenic mechanisms to increase core temperature to match the new, higher set point.[40] Specifically, these mechanisms include limiting heat dissipation by vasoconstriction of the skin vasculature, activating non–

shivering thermogenesis in brown adipose tissue, and recruiting skeletal muscle for shivering thermogenesis. The acute phase of fever is an excellent example of neuroimmunological modulation as it is an element of the host defense response that requires interaction between the immune system and thermoregulatory processes occurring in the brain.

Evidence supports the assertion that the increased core temperature that characterizes fever also enhances the ability of the host immune system to deal with infection. Leukocyte activity is enhanced, thereby activating T-lymphocytes, stimulating interferon production and decreasing plasma concentration of iron, which has been demonstrated to reduce the replicative capacity of various bacterial strains. Furthermore, the hyperthermia associated with the febrile episode creates a thermal environment that is inhospitable to microbial proliferation. [114-116]

1.2.1 Mechanisms of Fever

When infectious agents invade the body, a cascade of events is launched to mitigate the effects of the invading pathogen. Collectively, these events represent the natural host defense response to infection or the "acute phase reaction".[15] Fever is the hallmark of infection, and the conventional view of febrigenesis is that it is a highly regulated process, involving the synthesis and release of both pyrogenic and antipyrogenic cytokines.[38,189] Following exposure to a blood-borne exogenous pyrogen, such as the cell wall of Gram-negative bacteria (e.g. *E. coli* lipopolysaccharide), immunocompetent cells (e.g. peripheral blood mononucleocytes) synthesize and secrete

endogenous pyrogens (e.g. IL-1β, IL-6).[46] Classically, these cytokines interact with elements in the organum vasculosum lamina terminalis (OVLT), a circumventricular organ in the preoptic anterior hypothalamic area (POA) that lacks a blood-brain barrier. In this way, blood-borne pyrogenic signals are transduced to the POA, resulting in the elaboration of E series prostaglandins. PGE_2 acts centrally on thermosensitive neurons, activating heat producing and heat conserving mechanisms that increase core temperature by binding to receptors in neuroanatomical sites along the ventromedial aspect of the POA just anterior to the OVLT.[60,139,205] More recently however, and depending upon concentration and perhaps species, IL-β and other cytokines have been thought to evoke the central nervous system synthesis and release of E-series prostaglandins via alternate routes including active transport mechanisms[9] and by stimulation of vagal afferents.[206] Barrier cell-mediated pathways have also been suggested such that perivascular phagocytic cells and microvascular endothelial cells in the brain may be the direct targets of circulating cytokines, inducing COX-2, consequently producing PGE_2, and invoking fever.[28,200] These mediators will be discussed extensively in subsequent sections.

1.2.2 Mediators of Fever

In 1871, Liebermeister stated that it is "part of the character of fever that the heat regulation is set for a higher temperature level". Further, any substance that induces this regulated elevation in body temperature through the activity of heat-generating and/or heat-conserving mechanisms can be classified as a pyrogen.[38]

There are two general classes of pyrogen: exogenous and endogenous. Additionally, there is a third class of fever mediators, the E series prostaglandins, acting directly on the hypothalamus to affect changes in set point.

1.2.2.1 Exogenous Pyrogens

Exogenous pyrogens generally originate outside of the host from an external source and may include viruses, fungi, bacteria, and other microorganisms. When these agents invade the internal milieu they set off a chain of events that culminates in an elevation of core temperature. One class of exogenous pyrogen that has been used extensively in fever research is bacterial endotoxin or lipopolysaccharide (LPS). These large (10^3 kDa) lipopolysaccharides are a cell-wall component of Gram-negative bacteria. The O-specific side chain confers specific antigenic properties of the lipopolysaccharide while the lipid-A portion of LPS is responsible for its pyrogenicity.[127] LPS is a valuable research tool for studying fever and related host responses in human and other mammals due to the fact that it induces similar physiological sequelae as Gram-negative sepsis without the concomitant bacterial infection.

The mammalian immune system uses a family of Toll-like receptors to generate a response to molecular patterns present on invading microorganisms. In particular, TLR4 is part of a recognition complex for bacterial lipopolysaccharide, and is likely an important component in the inflammatory response to bacterial infection. Toll-like receptors are transmembrane proteins that are involved in the innate immune recognition of microbial constituents. Among them, Toll-like receptor 4 (TLR4) is a crucial signal

transducer for LPS, the pyrogenic component of Gram-negative bacteria in the outer cell membrane.[211] Recognition of LPS leads to the rapid activation of an intracellular signaling pathway, highly homologous to the signaling pathway of interleukin-1, which results in the release of pro-inflammatory mediators.[213]

Despite the fact the exogenous pyrogens are able to induce fever they do not cross the blood-brain barrier to act directly on the thermoregulatory center. Thus, if the development of fever can be thought of as the end result of a sequence of events, then exogenous pyrogens are the upstream mediators that initiate the synthesis of downstream mediators: endogenous pyrogens.

1.2.2.2 Endogenous Pyrogens

The precise definition of an endogenous pyrogen is any substance synthesized within the tissues of the host and subsequently transported to the central nervous system where it ultimately leads to increases in temperature.[61] Kluger has gone on to describe endogenous pyrogen as those intermediate agents involved in the pathway between pathogen exposure and elevation of thermoregulatory set-point.[117] Many substances have been identified as endogenous pyrogens, including cytokines released by activated monocytes and macrophages.[45] Results support the claim that splanchnic tissue macrophages, especially hepatic Kupffer cells are a major source of circulating cytokines during endotoxemia.[82]

Cytokines are a class of heat-labile, soluble secretory proteins produced by non-lymphoid nucleated cells in response to signaling events at the cell surface.[14,117,129] Evidence points most strongly to a trio of cytokines primarily responsible for the acute phase response of fever IL-1β, TNF-α, and IL-6.[47] The demonstration of a temperature response does not necessarily merit the conclusion that all cytokines are endogenous pyrogens. Instead, it is necessary that the cytokine is detectable in the brain and/or periphery during the time course of fever, and more conclusively that fever in inhibited by antibodies or specific inhibitors.[126] To date, this has only been reported for IL-1 and IL-6. Traditionally, IL-β is thought to act with the OVLT, a circumventricular organ in close proximity to the preoptic area of the anterior hypothalamus to evoke the synthesis and release of humoral mediators like prostaglandins of the E series, particularly PGE_2.[94]

1.2.2.3 Prostaglandins

By definition, the release of endogenous pyrogen constitutes a necessary step in the pathogenesis of fever but it is unlikely that they are the final agents in the pathway.[44] Rather, endogenous pyrogens cause the synthesis of yet another group of fever mediators. Prostaglandins are one such example of an end mediator that can be upregulated by pyrogenic cytokines. Of particular relevance to the development of fever are the E series prostaglandins. Milton and Wendlandt were the first to show that upon injection into the cerebral ventricles, prostaglandins of the E series (PGEs) produced fever symptoms.[147,148] Furthermore, the increase in core temperature occurred very rapidly. Since initial experiments with PGEs, much evidence confirming it as a centrally acting mediator of

fever has been collected. Support for the role of PGEs in fever is the finding that pharmacological agents that block the synthesis of prostaglandin act as efficient antipyretics. The formation of PGEs depends on the activity of cyclooxygenase (COX), which exists in one of two isoforms, the constitutively expressed COX-1 and the inducible form COX-2. In the rat, induction of COX-2 as measured by mRNA or protein expression in response to peripheral injection of a febrigenic dose of LPS can be demonstrated in brain endothelial cells, perivascular microglia and meningeal macrophages.[27,60] Hence, the production of PGE_2 via induction of COX-2 may be an important step in the manifestation of the febrile response to LPS. We may also point to the following: levels of PGE_2 in the cerebrospinal fluid from the third ventricle,[108] and in microdialysis samples from the preoptic area/anterior hypothalamus (POAH)[11] rise in parallel to the LPS-induced changes in body temperature and fall during defervescence. Genetically altered mice lacking one of the four PGE receptors, the EP_3 subtype, show an impaired fever response following peripheral injection of LPS, intracerebroventricular injection of prostaglandin E_1, or intravenous administration of interleukin-1β.[199]In rats, this response is mediated by the EP_1 type prostanoid receptor.[163]

Prostaglandins are relatively small lipophilic molecules that pass readily into the POAH where they act directly on hypothalamic cell groups to alter firing rates of thermosensitive neurons.[102,138] As a result, the activities of warm and cold-sensitive neurons in the preoptic areas are altered leading to an elevation in the thermoregulatory set-point.[190]

1.3 Endogenous Antipyresis

Fever is a highly regulated process that involves both the release of pyrogens and antipyretics. Endogenous antipyretic agents do not affect body temperature in healthy, afebrile subjects.[162] In contrast with cryogens, those agents capable of lowering body temperature irrespective of the presence or absence of fever, endogenous antipyretics are compounds released by the host only during a febrile episode that limit the magnitude of the hyperthermia resulting from fever.[198] Moreover, interfering with the action or release of an antipyretic during fever prolongs the core temperature increase. Hormones such as corticosteroids (e.g. glucocorticoid, melanocortins. ACTH and α-MSH), neuropeptides (e.g. AVP), and cytokines (e.g. IL-10 and IL-1ra) have all been purported to have fever blocking effects.[198] Of these, arginine vasopressin, and interleukin-1 receptor antagonist have been implicated in thermoregulatory responses of pregnancy, and thus will be discussed in some detail in later sections.

1.3.1 Arginine Vasopressin

Arginine vasopressin (AVP) is a posterior pituitary hormone most commonly known for its peripheral actions on V_2-type receptors resulting in anti-diuretic effects on the kidney as well as pressor effects on the cardiovascular system. However, acting through the V_1-type receptor, AVP functions as a centrally acting neuropeptide, responsible for defervescence.[41] An abundance of literature suggests that AVP has

physiological antipyretic activity. Direct microinjection of exogenous AVP into a specific brain region close to the diagonal band of Broca known as the ventral septal area, suppressed fever induced by certain agents in several species studied thus far.[110,168] Neurons with AVP containing projections to the ventral septal area (VSA), the paraventricular nucleus (PVN), and the amygdala, have been mapped in rats and in guinea pigs via immunohistochemical methods,[19,145]and it has been reported that hypothalamic and septal staining density of vasopressinergic neurons increases in fever. As shown by push pull perfusion of the VSA, release of AVP from vasopressinergic neurons increases in response to central administration of IL-1β and the degree to which AVP is released is proportional to the magnitude of the fever. Conditions such as hemorrhage, which increase peripheral as well as central AVP release, are associated with decreased febrile responses.[41] Perhaps, most importantly, neutralization of endogenous vasopressin activity during experimentally induced fever either by specific AVP antibodies or V_1-type receptor antagonists increases the time course and magnitude of the febrile episode. Interestingly, however, there is evidence that because vasopressinergic innervation of the ventral septal area is less well developed in female versus male rats, the release of endogenous AVP in response to centrally administered PGE_2 occurs in males only.[34] This suggests that there are sex-related differences in the response to PGE_2 fever and that other mediators may be more integral to limiting the magnitude and duration of fever in the female rat. Still, as will be discussed later, arginine vasopressin is purported to play a role in certain physiological states in which normal fever responses are suppressed, including those in experimental LPS tolerance, neonates, and periparturient mammals.

1.3.2 Interleukin-1 receptor antagonist

"IL-1inhibitor", as it was originally termed, is a 23-25 kDa protein that occurs naturally in humans and other mammals.[6] Now commonly known as interleukin-1 receptor antagonist, this peptide was first isolated and purified from the urine of human subjects with monocytic leukemia.[6,53] Not unlike most cytokines, IL-1ra is produced by monocytes and released into plasma in response to lipopolysaccharide and other pyrogens (IL-1, IL-6).[5,7] IL-1ra is a structurally related gene product of IL-1α and IL-1β. As such, all three members of the interleukin-1 family of cytokines bind to the same cell surface receptor IL-1R, albeit with differing affinities. However, unlike IL-1α and IL-1β, IL-1ra lacks a secondary binding site necessary for intracellular signal transduction.[133] As such, IL-1ra can act as a competitive inhibitor of pyrogenic cytokines at type I and type II IL-1R receptors without agonist activity. This has been demonstrated both *in vitro* and *in vivo*.[86,183]

In vivo studies with rats have shown a general decrease in eicosanoid (i.e. PGE_2) in response to intravenous IL-1ra during a febrile episode as well as increased survival rate after a heat stroke.[8] Mice over-expressing IL-1ra show greater survival rates upon challenge with LPS as compared to wild-type controls.[99] Similarly, IL-1ra deficient mice show increased lethality following LPS challenge.[99] This mirrors results for studies in which exogenous IL-1ra was administered to mice and rats prior to a potentially lethal exposure of LPS.[2,3] Further, it has been shown that mice overexpressing IL-1ra lack a

febrile response to I.C.V. administration of IL-1β further confirming that the IL-1 family of receptors are functionally inactivated by IL-ra.[131]

1.4 Normal Thermoregulation in Pregnancy

There is evidence that thermoregulatory control is altered near term of pregnancy. In 1949, Stewart reported that body temperature decreases after the third month of gestation and stabilizes by the end of the 4th month.[188] Similar decreases in core temperature have been observed in rabbits,[160] sheep,[122] and in rats.[64] It appears that during gestation, the ability to thermoregulate is impaired. Vaughn, Veale, and Cooper have demonstrated that at both exceedingly warm and cold ambient temperatures (33°C and 3°C), rabbits near term (~3 days prior to parturition) are less able to sustain core body temperatures.[202] Imai-Matsumura *et al* showed that as compared to nonpregnant controls, rats late in gestation are less able to defend core temperature against a cold challenge.[104] Furthermore, rats studied just prior to parturition (day 20) are less able to maintain core body temperature at low ambient temperatures (14-16°C) as compared to nonpregnant controls, and those animals studied earlier in gestation, and during lactation.[57] Regulatory thermogenesis or post-prandial heat production is significantly reduced in human subjects nearing term of pregnancy.[37] In a study describing basal core temperature responses to pregnancy and lactation, Fewell found that in Sprague-Dawley rats, mean 24-hour core temperature is decreased from day 15 to parturition (day 21-22).[64] It has been proposed that the fall in core temperature on day 15 results primarily from a loss of normal circadian fluctuation in body temperature, as the typical nocturnal increase in T_C is

abolished at this gestational age.[57] Further decreases in core temperature near term of pregnancy in rats is a result of a "regulated" thermoregulatory response or regulated hypothermia, whereby core temperature is actively maintained at a lower level following a decrease in the central set point for thermoregulation.[55,78] Programmed rheostasis involves rhythmic alterations of homeostasis that occur during phases of the life cycle (e.g. circadian or menstrual cycles).[157] It is quite probable that pregnancy represents a period of such rheostatic control over core body temperature.

During lactation in rats, steep increases in core temperature have been reported.[57] This increase appears to be the result of a "forced" thermoregulatory response (i.e. forced hyperthermia). That is, core temperature is unnaturally maintained above the CNS thermoregulatory set point. This may be due in part to the highly exothermic processes of parturition and milk production.

There is an adaptive value to alterations in thermoregulatory physiology during pregnancy. In mammals, metabolic rate decreases by 2.3 times for every 10°C decrease in core temperature.[100] In terms of this so called "Q_{10} effect" lowering core temperature by even a modest amount in pregnancy may have a significant effect on metabolic rate, and a downregulation of thermogenesis shifts the balance of blood flow from heat generating organs such as skeletal muscle and brown adipose tissue to the utero-placental interface.[152] Additionally, human studies have revealed fetal temperatures to be 0.5-0.9°C higher than maternal temperatures.[132] Parturition increases both maternal and fetal metabolic demands.[121] Considering that during hyperthermia, fetal temperatures have been shown to increase in parallel[1,] or exceed[122] the rise in maternal core temperature any rise in core temperature in the intrapartum period would tend to exaggerate the fetus'

oxygen demand in utero, and the potential for neuronal loss.[132,152] This is significant, as the inability of the mother to protect against increases in temperature can result in low APGAR scores and morbidity for the hypoxic neonate.[32] Increasing the overall oxygen demands of the mother and fetus in the case of fever can increase the risk of fetal brain ischemias, seizures, cerebral palsy, bradycardias, respiratory distress, and death.[32,161,166,179]

1.5 Fever in Pregnancy

In addition to changes in basal temperature during pregnancy, core temperature responses to a stressor (e.g. exposure to a novel environment) are altered in the pregnant rat.[67] One of the most salient thermoregulatory changes during the maternal adaptation to pregnancy is the impaired febrile response near term. However, this finding is somewhat dependent on both species and gestational stage. Despite the apparent normal functioning of thermoregulatory effector responses there is an attenuated fever response to endotoxin, and certain pyrogenic cytokines, as well as prostaglandins near the time of parturition.[111] This line of investigation was pioneered in 1978 by Kasting, Veale, and Cooper who found that upon intravenous injection of either bacterial endotoxin (i.e. LPS from *Salmonella abortus equi*), or endogenous pyrogen (i.e. IL-1β) late in gestation (143-145 days), pregnant ewes exhibit an attenuated fever response.[112] Moreover, the response is completely abolished closer to term (146 days), while a vigorous fever is observed earlier

in gestation.[1,12,95,122] Comparable findings exist for near-term guinea pigs, which do not develop fever following LPS exposure.[210] Similarly, fever responses are muted after I.V. LPS, and I.C.V. PGE_1 in rabbits near term as compared to nonpregnant animals[120,160] but this result has been disputed by other studies.[13,103] Morishima *et al* found that the pregnant baboon develops a normal fever in response to LPS.[151]

Pregnant rats experience a febrile response of reduced magnitude to exogenous pyrogen and develop no fever to endogenous pyrogen (i.e. IL-1β) in the latter phase of gestation (day 17 to term).[134,135,185] Also, compared to nonpregnant females, near term pregnant rats show significantly attenuated fevers following I.C.V. administration of PGE_1.[136,192] Evidence from rats injected with LPS reveals reduced PGE_2 within the cerebrospinal fluid[105] in the latter part of gestation in rats. Similarly, there is an attenuated PGE response to endogenous pyrogen near term.[66] Given that the induction of COX-2 is critically important in the formation of E series prostaglandins and the subsequent manifestation of a febrile response,[125] the finding that hypothalamic expression of cyclooygenase-2[155] is blunted in pregnancy, may explain why near-term pregnant animals do not have a normal end-mediator response to exogenous and endogenous pyrogen.

Non-shivering thermogenesis in brown adipose tissue is an important autonomic thermoregulatory effector for heat production during the development of fever in rats,[72] and has been reported to be impaired near term of pregnancy.[4,203,204] However, Eliason and Fewell did not find that the attenuated febrile response to pyrogen near the term of pregnancy is a result of impaired efferent limb or effector organ response, but rather an abbreviated one.[56] It is probable that a pregnancy activated endogenous antipyretic

system is engaged during pyrogen exposure. Though the development of fever has physiological advantages, one might imagine that under certain conditions, fever may not be desirable. As maternal fever and infection has been associated with increased risk for early rupture of membranes, preterm labor, and premature birth,[74,178,180] we may speculate that refractoriness to pyrogen-induced fever near term could be a rheostatic response in mammals that has adaptive value.[158]

1.6 Pregnancy Induced Antipyresis

1.6.1 Arginine Vasopressin in Pregnancy

Several lines of evidence suggest that arginine vasopressin (AVP) may be abrogating the rise in core temperature following pyrogen exposure in the latter part of gestation.[111] First, AVP levels in several hypothalamic loci including the ventral septal area and the paraventricular nucleus are higher near the term of pregnancy.[23,123] Furthermore, AVP functions as a endogenous antipyretic in the rat neonate,[113] and parturient rats have been shown to a five-fold elevation in AVP levels in the dorsal hippocampus as compared to nonparturient pregnant animals.[123] Intracerebroventricular administration of PGE_1 after AVP receptor blockade produced increases in core temperature that were similar in magnitude and duration for both nonpregnant rats and pregnant rats close to parturition.[58] That near term pregnant rats mount a normal fever

response to centrally administered PGE_1 following an I.C.V injection of a vasopressin V_1-receptor antagonist, remains strong evidence for AVP as putative mediator of attenuated fever near term. However, when rats are pretreated with a vasopressin antagonist and subsequently given an I.V. injection of rrIL-1β near the term of pregnancy, the response is not "normalized" and the attenuated core temperature response remains unaffected by AVP blockade.[59] Considering this, if the end-mediator response to endogenous pyrogen was abrogated close to parturition, this would argue in favor of a second endogenous antipyretic agent that is exerting some functional effect upstream in the febrigenic pathway in near-term animals. Indeed experiments designed to determine the influence of pregnancy on the synthesis and release of PGEs into the OVLT bear this out. Systemic administration of IL-1β results in significant increases in peri-OVLT PGEs in nonpregnant controls that are not observed in near term pregnant rats.[65] This study did not explore the mechanisms by which this apparent PGE suppression occurs but we may note that while basal core temperatures were found to be higher in nonpregnant animals as compared to the pregnant group, basal PGE levels were similar in both groups. In light of this evidence, it seems likely that whatever mechanism is responsible for the central prostaglandin response to endogenous pyrogen near parturition, it is exerting its antipyretic effect at a point prior to the action of AVP in the central nervous system.[43] This leads to speculation that antagonism of pyrogenic cytokines such as IL-1β, which act upstream of PGEs synthesis and action in the febrigenic pathway, may explain the limited magnitude and duration of fever in late pregnancy. As such, interleukin-1 receptor antagonist emerges as a promising mediator of this response, which remains a very interesting component of the maternal adaptation to pregnancy.

1.6.2 Interleukin-1 Receptor Antagonist in Pregnancy

Interleukin-1 receptor antagonist may play a role in complete absence of fever in response to I.V. IL-1β. This hypothesis is promising in light of evidence that AVP does not mediate the blunted response to IL-1β,[59] and both the IL-1 receptor and IL-1ra are present in vasopressin containing neurons of the paraventricular nuclei in the rat hypothalamus.[43] Along with studies indicating that central IL-1β leads to vasopressin release,[209] co-localization of the IL-1R, IL-1ra, and AVP invites speculation that the interleukin-1 receptor antagonist may also have a stimulatory effect on vasopressinergic fibers, which would potentiate its action as an endogenous antipyretic. Further pointing to the antipyretic properties of IL-1ra, are experiments by Tan and others who have found that the neutralizing antibody to interleukin-1 receptor antagonist alters LPS induced fever in adult female rats.[196]

It has been demonstrated that afebrile pregnant mice produce IL-1ra in concentrations between 5 and 270 fold higher than IL-1α or IL-1β in all tissues analyzed including serum, uterus, fetal membranes, and placenta.[98] It is possible that blood-borne rr-IL-1β does not elicit a normal end-mediator response because of a pregnancy-related increase in the circulating levels of IL-1ra. Furthermore, in humans, interleukin-1 receptor antagonist is detectable in the amniotic fluid compartment in the presence of intrauterine infection.[68,176] Pillay *et al* measured basal plasma levels of IL-1ra in late term women (32 weeks to term) and observed that its concentrations were significantly higher than in nonpregnant women (~7fold).[167] A second pivotal finding of this study was that

monocytes of pregnant subjects stimulated with either IL-1α or LPS *in vitro* produce approximately 3 times more IL-1ra as compared to stimulated monocytes of nonpregnant adults. Glomus cells of paraganglia associated with the subdiaphragmatic vagus express binding sites for biotin labeled IL-1ra,[76] and it is possible that local increases in antipyretic cytokines can mediate fever attenuation despite a lack of significant increases in the general circulation. It seems likely that IL-1ra binding to the IL-1 receptor *in vivo* may be sufficient to suppress the febrile episode following Gram-negative infection in pregnant subjects.

1.7 Rationale

Although rats may develop similar fevers in response to I.V. or I.P. administration of pyrogenic stimuli, there is, in fact much evidence that route of administration has a profound influence on the fever profile (i.e. latency, magnitude, and time course). It is apparent that a regulated change in the response to intravenous LPS occurs in the peripartal period.[135] However, no data have been reported for the pregnant rat regarding core temperature responses to varying doses of lipopolysaccharide administered intraperitoneally.

Within the last decade, experimental evidence has accumulated suggesting the existence of neural communication of immune signals from the periphery of the body to the brain. This evidence derives from the observation that subdiaphragmatic vagotomy attenuates or eliminates a number of the illness responses to I.P. or I.V. exogenous and endogenous pyrogen.[130,206] Attenuation of LPS induced fever occurs after vagotomy in rats and other species,[175] but this appears to depend upon the dose of LPS as well as on

the route of administration. Goldbach, Roth, and Zeisberger have demonstrated that the febrile response to intraperitoneal injection of LPS is abrogated in vagotomized guinea pigs as compared to sham-operated controls, while intramuscular injection results in identical fever in vagotomy and sham groups.[77] Thus, we speculate that the mechanisms involved in the development of fever, and particularly the vagal influence on the febrile response, may be different following I.P. vs. I.V. pyrogen exposure.

The rat is chosen as the species to investigate in this series of experiments for several reasons. The temperature response to prostaglandin, exogenous and endogenous pyrogen in pregnant rats has been previously characterized.[185,192] Additionally, the rat has been a useful model for studying the involvement of endogenous antipyretics in pregnancy.[58,197] The role of behavioral and autonomic thermoregulatory effectors in rats during fever challenge has been well investigated as well as these roles in the normal thermoregulation of pregnancy.[55,57] From a practical standpoint, the rat is small, easy to instrument and care for, and the short gestational period facilitates the investigation of pregnancy related alterations in thermoregulation.

This thesis describes several studies, which, taken together further describe the mechanisms underlying the attenuated febrile response to pyrogen near the term of pregnancy. Selecting an appropriate dose of any substance given to an animal is an essential component of any experimental design. This basic information had to be established from preliminary experiments before any in depth study of underlying mechanisms and putative mediators could be initiated. Thus, I attempted to define the dose response relationship between core temperature and LPS in nonpregnant animals and animals at days 10, 15, and 20 days. This is described in Chapter 3. From this

information I was able to optimize experiments by selecting a dose of LPS that produced a measurable, but submaximal physiological response. A dose-response study is particularly important in the experiments described here where a physiological process is being compared between groups and under conditions where the febrile response may be influenced by variables such as ambient temperature, gender, route of administration, and most importantly pregnancy. The EC_{50}, as well as the EC_{100} were used in subsequent experiments. These represent the concentrations of pyrogen that result in half-maximal (EC_{50}) and maximal (EC_{100}) core temperature responses.

Given the possibility of vagally-mediated mechanisms that are specific for fever following I.P. but not following I.V. LPS administration, the first study was carried out to determine if fever is in fact attenuated in rats near the term of pregnancy following I.P. administration of LPS as it is following I.V. administration of LPS (Chapter 4). Once the febrile response to I.P. administration of exogenous pyrogen was characterized, I began to consider how changes in specific cytokines could alter the febrile response in gestation. Chapter 5 describes the pregnancy-induced changes in basal interleukin-1β and interleukin-1 receptor antagonist. This study prompted me to investigate the influence of pregnancy on the production of endogenous mediators, IL-1β, IL-6, IL-1ra, and PGEs, following stimulation with exogenous pyrogen (Chapter 6). The specific hypotheses and aims of each study are described more fully in their respective chapters. These studies, I hope, will provide a basis upon which to better understand fever responses following bacterial infection in the mammalian pregnancy.

1.8 Specific Aims and Hypotheses

The specific aims of the proposed research were to determine if the febrile response to pyrogen near term of pregnancy is attenuated following intraperitoneal administration of *E. coli* lipopolysaccharide, and to investigate, in particular, interleukin 1-receptor antagonist as a putative mediator of the altered maternal thermoregulatory responses in pregnancy following pyrogen exposure. Specifically, I tested the hypotheses that (i) fever is attenuated near term of pregnancy in response to I.P. bacterial endotoxin, (ii) baseline secretion of IL-1ra is elevated in the plasma of near term (days 17- 21) pregnant rats compared to nonpregnant females and females in early to mid gestation (days 10-15) (iii) that during the febrile episode following exposure to pyrogen, plasma levels of IL-1ra and other cytokines are altered in females near term of pregnancy as compared to non-pregnant females and females early in gestation. The experiments described here have generated a baseline cytokine profile for IL-1β, and IL-1ra levels during the progression of pregnancy in rats (NP, days 10, 15, and 20). Dose-response studies have described the temperature response to varying doses of endotoxin in nonpregnant female Sprague- Dawley rats and throughout pregnancy on days 10, 15, 20. Underlying mechanisms were then explored by describing the cytokine profile during experimentally induced fever. This helped to uncover correlations between post-LPS cytokine release patterns (IL-1β, IL-6, IL-1ra, and PGE) and the attenuated core temperature response to pyrogen near term of pregnancy.

CHAPTER TWO

METHODS & MATERIALS

2.1 Animal Housing and Handling

Experiments were conducted on two groups of Sprague Dawley rats obtained from Charles River Laboratories: 97 virgin nonpregnant females and 253 time-bred females undergoing their first pregnancy. All experiments were carried out on rats aged between 9 and 12 weeks of age weighing between 175-200 g upon arrival. Rats were housed individually in standard Plexiglas caging containing Aspen-Chip Laboratory Bedding (Northeastern Products Corporation), which were kept in a humidity-controlled environmental chamber at an ambient temperature of 25±1°C. The chamber was operated on a 12-hour alternating light/dark cycle. Lights were turned on at 0700 hours and were

shut off at 1900 hours. Each rat was provided with enrichment and had continuous access to a standard lab diet (Lab Diet 5001) and tap water. Pregnant rats arrived between days 4 and 6 of gestation and were studied at either day 10-11, day 15-16, or day 20-21 of gestation, with term being approximately 22 days. Prior to any experiment, rats were handled at least five times to acquaint the animals with the investigator. Handling consisted of picking up the animal, and holding it in the same manner it was to be handled during any experimental procedures. All experiments commenced between 1000 and 1100 hours to preserve uniformity in circadian influences and each animal was studied only once to avoid tolerance to stimuli.

2.2 Surgical Procedures

Animals that were to receive I.P. injection underwent surgery 3 days prior to experiments in order to implant catheters and transmitters. Rats were anaesthetized with 2% halothane in oxygen (for induction and maintenance). In preparation for surgery, the surgical field was shaved and wiped with an iodine tincture solution to disinfect the skin (Triad Medical Solution, H&P Industries), followed by dilute ethanol. During surgery, core temperature was maintained with a heating pad and monitored via a rectal thermistor probe. Animals remained on the heating pad until they awoke from anesthesia and were then allowed to recover in the home cage. All surgical procedures were carried out under aseptic conditions in accordance with the "Guide to the Care and Use of Experimental

Animals" provided by the Canadian Council on Animal Care, and with the approval of the Animal Care Committee of the University of Calgary.

2.2.1 Implantation of Biotelemetry Device and Catheterization

A paramedian laparotomy was performed and a free-floating battery-operated biotelemetry device was inserted into the peritoneal cavity (TA10TA-F20; Data Sciences International) for later measurement of core temperature. In addition, a sterile catheter of silicone tubing (Dow Corning Silastic®; Helix Medical Inc.) was implanted into the peritoneum for administration of injectate. The area between the scapulae was shaved and a small incision was made. A portion of the catheter was inserted into the abdominal cavity and the remainder tunneled under the skin and exteriorized at the dorsal scapular area and sealed with a ligature. Muscle and skin layers were sutured to close the abdominal incision, and the catheter was secured in place with a purse-string suture and tissue adhesive (Vetbond™; 3M). Topical antibiotic sprays (Topazone™; Austin) and spray adhesive bandage (OpSite™; Smith+Nephew), were applied to all wounds.

2.3 Intraperitoneal Injections

Animals with a catheter surgically inserted into the peritoneum, as described above, received I.P. injections according to the following protocol. An injection cannula was constructed by attaching a 21 gauge, 1-inch blunt needle (Monoject™) to the outlet end of a three-way stopcock. A 3.0-mL syringe containing 1.5 mL of sterile normal saline

was placed in the position directly opposite the needle. A second 1.0 mL syringe containing the injectate solution was attached to the port on the stopcock perpendicular to the needle. For an injection, the animals were removed from the home cage, wrapped in a surgical towel, and held in such a way that only the free end of the catheter was exposed. The end of catheter was trimmed and the injection cannula inserted. The syringe containing the injectate was emptied into the catheter. Following this, the stopcock was rotated and the syringe containing saline was emptied into the catheter to flush it as well as to ensure that the entire injectate reaches the peritoneal space. The animal was then returned to the home cage.

2.3.1 Verification of Catheter Placement

In this study, injections were made via catheter into the peritoneum. As this was a novel method of intraperitoneal injection, we wanted to know the accuracy of the technique prior to beginning experiments. To confirm catheter placement, we injected one animal with Evan's Blue and opened the abdomen at necropsy. Correct placement of the catheter was verified by the presence of the dye localized to the peritoneal cavity.

2.4 Experimental Apparatus

Animals used in studies that included measurement of core temperature were studied in a chamber (homecage) (Figure 2.1), which was positioned on a platform receiver designed to interpret signals emitted from the free-floating telemetry device. Receivers were connected to a computer outside of the chamber, thereby allowing core temperature to be continuously monitored in conscious chronically instrumented rats without the investigator being present in the chamber. See section 2.5.1 for more a complete explanation of T_C measurement.

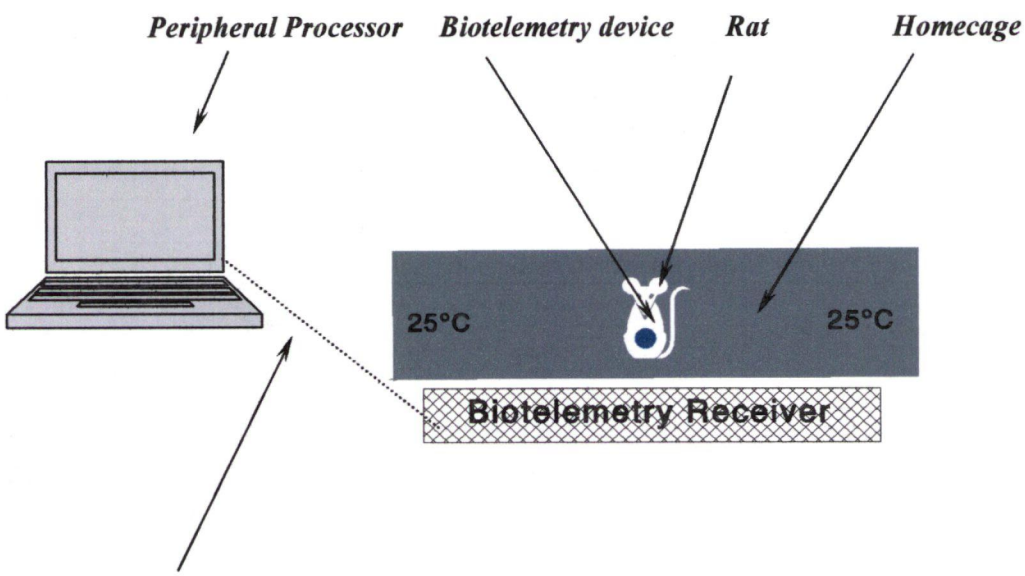


Figure 2.1. Diagram illustrating the chamber, free-floating biotelemetry device, platform receiver, and peripheral processor used in experiments in which core temperature was monitored.

2.5 Plasma Cytokine Analysis

No less than 5 days after arrival, animals that were to be used for cytokine analysis were removed from the home cage and brought into the laboratory for rapid decapitation and trunk blood collection. Trunk blood was collected over ice in centrifuge tubes containing 0.5mL indomethacin (400 μg/mL), 0.5mL EDTA (30 mg/mL), and 1.0mL aprotinin (3.5 TIU/mg protein) (Sigma, St Louis, MO).[25] Plasma samples collected in this fashion avoid coagulation and give a better determination of free circulating cytokines *in vivo* than serum samples which may contain IL-1β and IL-1ra secreted from monocytes *in vitro* after the collection process.[24] Blood was centrifuged at 4°C for 10 minutes. Plasma was then aspirated and stored at -70°C until the volume corrected samples were analyzed for IL-1β, IL-1ra, IL-6, and PGEs by ELISA assay (BioSource).

2.6 Calculated and Measured Variables

2.6.1 Core Temperature

Each animal's core temperature was obtained from signals emitted by the battery operated biotelemetry device in its peritoneal cavity. This device emitted a radio frequency (Hz), which is proportional to the animal's core temperature. The animal, in its cage, was placed over the platform antenna (Physio Tel CTR 86; Data Sciences

International), which received the output signal from the activated biotelemetry device. The platform also interfaced with a peripheral processor (Dataquest ART, Data Sciences International) connected to a PC, which digitized the 10-second signal at a sampling rate of 250Hz and displayed the averaged value as a temperature. Core temperature was sampled and displayed at two-minute intervals. Specific calibrations for each biotelemetry device were obtained from the supplier (DSI), and were entered into the processor prior to the start of an experiment.

2.6.2 Fever Index & Peak Change from Control Temperature

In order to gain information about the overall febrile response of several groups of subjects, the maximal change from control core temperature and fever indices were calculated rather than comparing the response between groups at discrete time points. Conceptually, fever index represents the integrated area described by temperature versus time curve. Fever index was a useful summary statistic for making comparisons regarding the overall magnitude of the febrile response in a given period, because it eliminates apparent differences in the response at certain time points due to the pattern of the curve. By removing time as a factor, fever index instead allowed us to compare the comprehensive response. Fever index was calculated by taking the sum of the change in core temperature from control at each time point in the experiment, and then dividing that value by the total number of data points to obtain a value in $^{\circ}C$ x hours.

2.7 Drugs & Injectates

2.7.1 Normal Saline Vehicle

Normal saline (0.9% Sodium chloride USP) was used as vehicle in experiments in which intraperitoneal injections were made. Saline was purchased from Baxter Corporation Canada®, and was stored in the refrigerator at 4°C until use.

2.7.2 *Escherichia coli* Lipopolysaccharide

Lipopolysaccharide from E. coli 0111:B4 was purchased as a lyophilized powder prepared by phenol extraction from Sigma-Aldrich Canada, and mixed with normal saline (0.9% NaCl) to make solutions from 1 to 1280 μg/mL. LPS solutions were stored in sterile plastic vials and kept in the refrigerator at 4°C until use.

CHAPTER THREE

THE EFFECT OF DOSE OF I.P. E. COLI LIPOPOLYSACCHARIDE ON THE FEBRILE RESPONSE IN FEMALE SPRAGUE-DAWLEY RATS

As has been discussed previously, there are alterations in the thermoregulatory response to pyrogen in female mammals nearing the term of pregnancy. It is apparent that the febrile response to interleukin-1β[185] and prostaglandin E_1[136] are significantly attenuated in pregnant rats in the latter part of gestation. Furthermore, there is a regulated change in the response to bacterial endotoxin near term, such that an equivalent dose of I.V. *E. coli* LPS does not have as great an influence on resetting hypothalamic set point.[135] To date, however, no data have been published regarding the core temperature response to varying doses I.P. of *E. coli* lipopolysaccharide in female rats, and this basic information should be established before any investigation of underlying mechanisms can be reliably undertaken. A thorough understanding of the relationship between dose of exogenous pyrogen and fever in nonpregnant animals is essential before any meaningful conclusions can be made as to how it is altered during pregnancy.

3.1 INTRODUCTION

Evaluation of the dose-response or dose-effect relationship is crucially important when studying complex physiological phenomena such as fever. Classical pharmacology assumes that a biological response increases as the dose is increased. However, there are many predictable factors which may influence the response for a given drug including, age, species and strain, route of administration, sex, oestrus, and hormonal status, pregnancy and lactation. As such, we cannot expect that every hormone-responsive system will produce identical dose-response curves. In the experiments described here, the responding variable (core temperature) was dependent on the concentration of exogenous pyrogen being administered, so it was important to determine the dose-response relation for this particular drug-effector combination.

Although many investigators have used Gram-negative bacterial endotoxin to induce fever, many studies which have determined the dose-effect of LPS on core temperature have administered pyrogen intravenously.[173,195] Those studies which have induced fever via the intraperitoneal route have been conducted in male rats of varying strains.[48,87,172,191] Even within the same strain, there is much evidence that gender has a considerable influence on fever. Murakami and Ono found that female rats showed an attenuated febrile reaction to both lipopolysaccharide and endogenous pyrogen as

measured by both the maximum increase in rectal temperature and the thermal response index.[159] Fevers in response to prostaglandins are also influenced by sex, endocrine status, and gestational stage.[136] Thus we may safely conclude that there is gender dimorphism in the central mechanisms responsible for febrigenesis and antipyresis. Such sex-related differences are most likely due to the influence of steroid hormones on thermoregulatory control. In light of this evidence, it seems reasonable to construct complete dose-response curves for the I.P. administration of bacterial endotoxin in female Sprague-Dawley rats throughout gestation. The experiments presented in this chapter were designed to define the relationship between dose of *E. coli* LPS and core temperature in female rats, and to test the hypothesis that this relationship is altered during pregnancy.

3.2 METHODS

3.2.1 Animals

Experiments were carried out on conscious chronically instrumented animals allocated to one of four groups: 48 nonpregnant and 132 pregnant rats on days 10 (n=44), 15 (n=44), and 20 (n=44), of gestation (term 21-22days). All animals were obtained from Charles River Laboratories. Pregnant animals were undergoing their first pregnancy, and arrived between day 4 and 6 of gestation. All animals were fed, housed, and handled as described in section 2.1.

3.2.2 Surgical Preparation

No less than three days prior to an experiment, each rat was anaesthetized by inhalation of halothane (2% for induction and maintenance) in oxygen. A paramedian laparotomy was performed and a sterile catheter was implanted into the peritoneum for delivery of injectate, also a free-floating, battery-operated telemetry device (TA10TA-F20, Data Sciences International) was inserted into the peritoneal cavity for measurement of core temperature, as described in section 2.21.

3.2.3 Conditions of Observations

During an experiment, each animal was studied in her cage, which was kept in the environmental chamber maintained at an ambient temperature of 25±1°C for the duration of the experiments. Core temperature was obtained from the signal emitted from the previously implanted biotelemetry device, and was recorded at two-minute intervals throughout the experiment (Section 2.5.1). Overall change in core temperature was later calculated, as described in Section 2.5.2.

3.2.4 Experimental Protocol

The day before an experiment, each animal was removed from its cage, weighed and then returned to the cage in the environmental chamber. Rats were randomly divided into ten groups based on injectate received, and each animal was studied only once. On the day of the experiment, following an acceptable control period defined as five two-minute measurements of core temperature that did not vary by more than ±0.2°C, the rat was removed from its cage and given an intraperitoneal injection of either vehicle (normal phosphate buffered saline) or one of nine doses of *E. coli* LPS (*Escherichia coli*, 0111:B4) (1, 5, 20, 40, 80, 160, 320, 640, or 1280 μg per kg body weight) via the indwelling catheter (Section 2.3). After the injection, the rat was returned to its cage and core temperature was measured at two-minute intervals for a period of six hours.

3.2.5 Data & Statistical Analysis

Intraperitoneal LPS dose-maximal increase in core temperature, and LPS dose-overall change in T_C curves were constructed for determination of the approximate EC_{50} and EC_{100}. Overall mean changes in core temperature per hour are presented as fever indices, expressed as area under the core temperature-time curve for the six-hour period following injection. Data analysis for all core temperature experiments was limited to the first six hours following injection to exclude any thermoregulatory changes that may be associated with normal circadian fluctuations of temperature in the rat.[81] Fever indices were determined as described in Section 2.5.2. A two-factor analysis of variance followed by a Newman-Keul's multiple comparison test was done to determine if

pregnancy or injectate influenced the fever index. All results are reported as means ± one standard deviation.

3.3 RESULTS

3.3.1 Maximal Increase in Core Temperature

There was a consistent, dose-dependent increase in the maximum increase in core temperature from control in nonpregnant animals when LPS was administered directly into the peritoneum at doses ranging from 1 up to 1280 μg/kg (Figure 3.1). On day 20 of pregnancy, however, administration of *E. coli* LPS in doses covering the same range elicited negligible increases in core temperature as compared to vehicle. At all stages of gestation, and at all points on the curve the response was less for any given dose of pyrogen, as compared to nonpregnant rats. The EC_{100} was reduced in pregnancy, and furthermore, the dose required to achieve a maximum response is slightly increased on days 10 and 15.

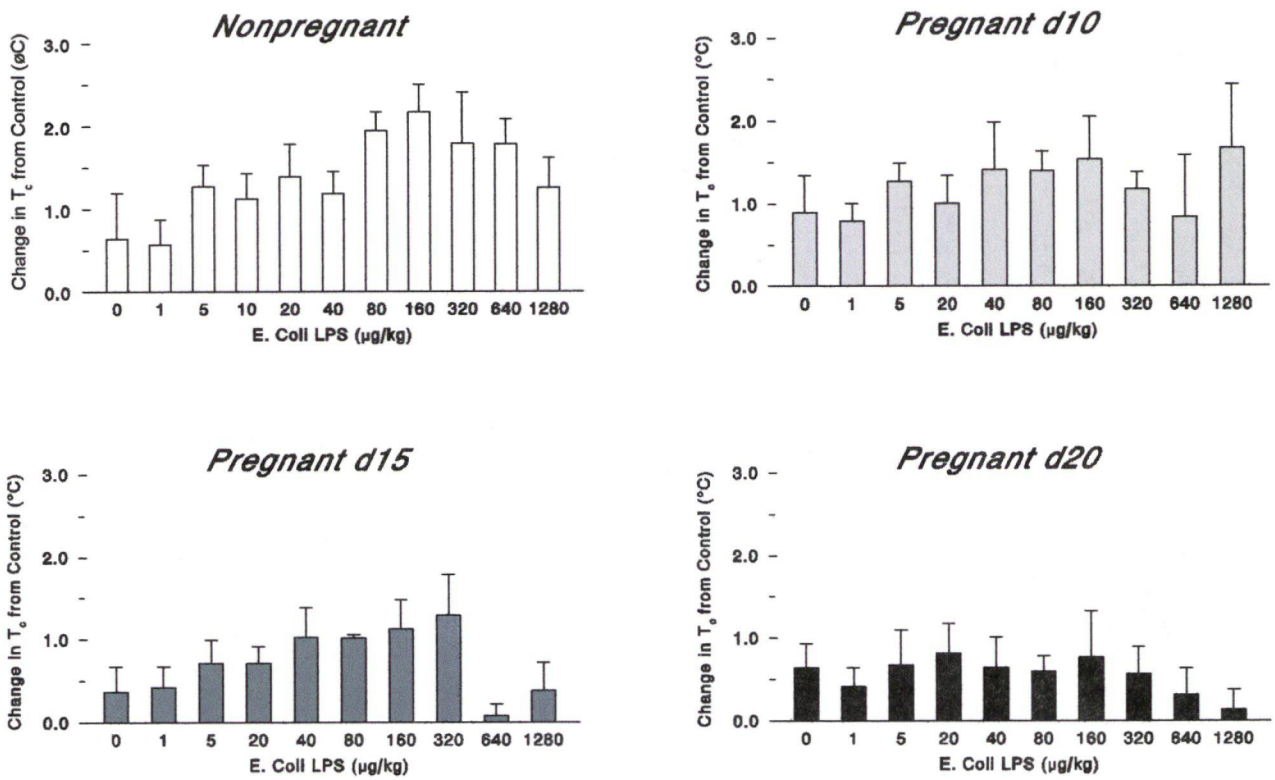


Figure 3.1 Relationship between dose of *E. coli* lipopolysaccharide and the maximal increase in core temperature in nonpregnant rats (n=48) and in pregnant rats on days 10 (n=44), 15 (n=44), and 20 (n=44) gestation for the six-hour period after intraperitoneal administration of E. coli LPS in doses ranging from 0 to 1280 µg/kg. Data are presented as means ± standard deviation for an n = 4 for each dose in each group.

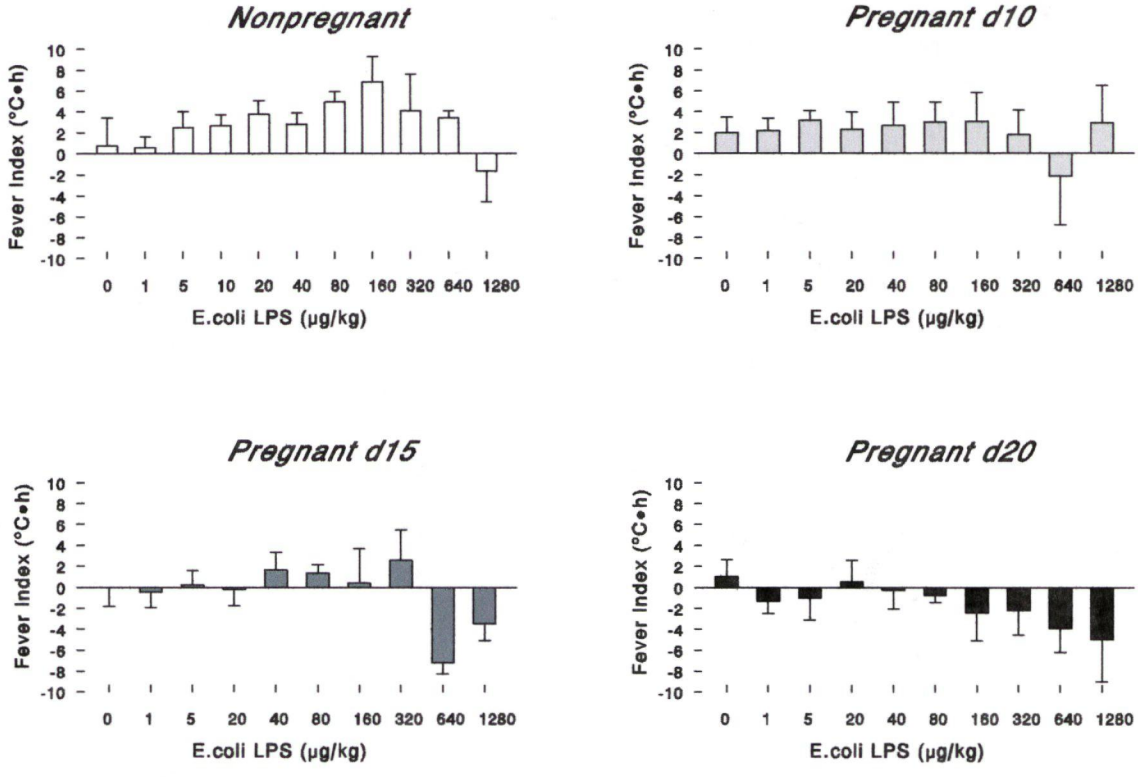


Figure 3.2 Relationship between dose of *E. coli* lipopolysaccharide and the fever index in nonpregnant rats (n=48) and in pregnant rats on days 10 (n=44), 15 (n=44), and 20 (n=44) gestation for the six-hour period after intraperitoneal administration of E. coli LPS in doses ranging from 0 to 1280 µg/kg. Data are presented as means ± standard deviation for an n of no less than 4 for each dose in each group.

3.3.2 Mean Fever Indices

In nonpregnant animals, intraperitoneal administration of *E. coli* LPS at varying concentrations elicited an dose-dependent increase in the fever index (Figure 3.2) as compared to vehicle with an EC_{50} of ~20 μg/kg and an EC_{100} of ~160 μg/kg. However, from a comparison of the dose-response curves it is apparent that the maximum heat gain was lower in pregnant animals on day 15, and more so in those animals near-term. In fact, in the latter part of gestation, animals lost heat with higher doses of endotoxin. It appears as though the pyrogenic activity of lipopolysaccharide is limited in pregnancy, and that animals on gestation day 20 are notably less responsive to LPS. Given that fever indices were gradually, but consistently attenuated as gestation advanced, it is likely that the suppressed core temperature response is not a concentration-related phenomenon, but rather a generalized modification in the animal's response to the drug.

3.4 SUMMARY & CONCLUSIONS

Based on evidence that the EP_3 prostaglandin receptor subtype plays an important role in the influencing of set-point and the development of fever,[199] one possibility is that the suppressed febrile response to varying doses of pyrogen is due to a decrease in receptor number in pregnancy. However, recent evidence showing that the expression of the EP_3 receptor is not impaired near term would seem to refute this claim.

Alternatively, it has been suggested that endogenous pyrogens (e.g. IL-1β) triggered by exposure to bacterial lipopolysaccharide may directly affect preoptic neurons independently of prostaglandin.[102] Electrophysiological studies of anterior hypothalamic neurons have shown that endogenous pyrogens produce activity changes that are consistent with fever production (i.e. inhibition of warm-sensitive and excitation of cold-sensitive afferents). Therefore, the decreased ability of endotoxin to induce febrile changes during pregnancy could be due to a reduced ability of LPS-induced IL-1β to alter thermoregulatory set-point. This may be possibly due to an increased production of endogenous antipyretics. This hypothesis was tested, and will be discussed in Chapter 5.

CHAPTER FOUR

INFLUENCE OF PREGNANCY ON THE FEBRILE RESPONSE TO INTRAPERITONEAL ADMINISTRATION OF BACTERIAL ENDOTOXIN IN FEMALE RATS

4.1 INTRODUCTION

The experiments reported in Chapter 3 define the dose-response relationship between I.P. administration of *E. coli* lipopolysaccharide (bacterial endotoxin) and the febrile response in nonpregnant and pregnant female Sprague-Dawley rats, with core temperature used as the parameter of fever. The primary goal of these experiments was to allow the selection of an optimal dose of LPS to be used in the present study.

There has been considerable emphasis on the involvement of the abdominal vagus nerve in the systemic cytokine-to-brain communication required for febrigenesis.[89,207] This, along with reports that subdiaphragmatic vagotomy inhibits a variety of endocrine, autonomic, and behavioral processes associated with the acute phase response[17,90]resulting from intraperitoneal exposure to LPS, lead me to wonder if pregnancy attenuates the core temperature response to I.P. injection of pyrogen as it does

after I.V. injection of exogenous pyrogen. This chapter describes experiments designed to investigate the febrile response to low, and high doses of I.P. *E. coli* LPS in rats at varying points in gestation, and to test the hypothesis that fever is attenuated near term when pyrogen is administered intraperitoneally.

4.2 METHODS

4.2.1 Animals

Experiments were carried out on conscious chronically instrumented animals allocated to one of four groups: 61 nonpregnant and 151 pregnant rats on days 10 (n=48), 15 (n=48), and 20 (n=55), of gestation (term 21-22 days). All animals were obtained from Charles River Laboratories. Pregnant animals were undergoing their first pregnancy, and arrived between day 4 and 6 of gestation. All animals were fed, housed, and handled as described in section 2.1.

4.2.2 Surgical Preparation

No less than three days prior to an experiment, each rat was anaesthetized by inhalation of halothane (2% for induction and maintenance) in oxygen. A paramedian laparotomy was performed and a sterile catheter was implanted into the peritoneum for delivery of injectate, also a free-floating, battery-operated telemetry device (TA10TA-

F20, Data Sciences International) was inserted into the peritoneal cavity for measurement of core temperature, as described in section 2.21.

4.2.3 Conditions of Observations

During an experiment, each animal was studied in her cage, which was kept in the environmental chamber maintained at an ambient temperature of 25±1°C for the duration of the experiments. Core temperature was obtained from the signal emitted from the previously implanted biotelemetry device, and was recorded at two-minute intervals throughout the experiment (Section 2.5.1). Overall change in core temperature was later calculated, as described in Section 2.5.2.

4.2.4 Experimental Protocol

The day before an experiment, each animal was removed from its cage, weighed and then returned to the cage in the environmental chamber. Rats were randomly divided into three groups based on injectate, and each animal was studied only once. On the day of the experiment, following an acceptable control period defined as five two-minute measurements of core temperature that did not vary by more than ±0.2°C, the rat was removed from its cage and given an intraperitoneal injection of either vehicle (normal phosphate buffered saline), 20μg/kg or 160μg/kg *E. coli* LPS (*Escherichia coli*, 0111:B4): the EC_{50} and EC_{100} concentrations, respectively. Pyrogen was administered via

the indwelling catheter (Section 2.3). After the injection, the rat was returned to its cage and core temperature was measured at two-minute intervals for a period of six hours.

4.2.5 Data & Statistical Analysis

Core temperature-versus-time curves were constructed for the six-hour period following injection. Statistical analysis for the present experiments was carried out using a three-factor analysis of variance for repeated measures followed by a Newman-Keuls multiple comparison test in order to determine if pregnancy, injectate, or time influenced core temperature. All results are reported as means ± one standard deviation; $p<0.05$ was considered to be of statistical significance.

4.3 RESULTS

4.3.1 Intraperitoneal Administration of Vehicle

As Fewell has previously reported,[64] basal core temperatures were significantly lower ($p<0.05$) in near-term pregnant rats (36.7 ± 0.3°C) compared to nonpregnant rats (37.5 ± 0.3C°). This is indicated in Figure 4.1. Intraperitoneal administration of vehicle via surgically implanted catheter did not elicit "stress induced hyperthermia" in nonpregnant or pregnant animals as normally occurs when one pierces the abdominal wall with a needle for I.P. drug administration. Indeed, there was no change in core

temperature in response to vehicle at any point during the experimental period, regardless of state (Figure 4.1). Thus, in the current experiments, any changes in core temperature following *E. coli* LPS can be safely attributed to the effects of the pyrogen, and not to an artifact of the injection process.

4.3.2 Administration of Bacterial Endotoxin

Core temperature time plots, which denote the latency, magnitude, and duration of the thermoregulatory response of nonpregnant and pregnant animals following intraperitoneal administration of low and high doses of *E. coli* LPS are shown in Figures 4.2, and 4.3.

Nonpregnant and pregnant animals developed complex core temperature time curves after administration of exogenous pyrogen. Latency, magnitude, and duration all appeared to depend upon state of pregnancy (nonpregnant versus pregnant), gestation (day 10 versus day 15 versus day 20), and dose of pyrogen (20 versus 160 μg/kg). Following administration of 20μg/kg *E. coli* LPS, nonpregnant rats mounted a core temperature response with a latency, duration, and magnitude of 2.5 hours, at least 3.5 hours, and 0.7°C. On days 15 and 20 of gestation, a distinct period of hypothermia preceded a modest increase in core temperature. The overall heat-gain following administration of 20μg/kg *E. coli* LPS was the same in nonpregnant and pregnant animals early in gestation on day 10 (Figure 4.2). Following intraperitoneal injection of 160μg/kg E. coli LPS, nonpregnant rats invoked a cored temperature response with latency, duration and magnitude of 1.5 hours, at least 4.5 hours, and 1.9°C, respectively. In

pregnant animals there was a moderate febrile response to I.P. administration of high dose LPS only after a profound hypothermia, the magnitude and duration of which appeared to increase with advancing gestation. Figure 4.4. indicates the fever indices for the six hour period following LPS administration. There was no significant influence of vehicle on the fever indices in nonpregnant animals and in pregnant animals at any age of gestation. Overall, animals on days15 and 20 of gestation injected with 20μg/kg LPS were significantly less febrile than nonpregnant animals that received the same dose. Pregnant animals in early, mid and late gestation receiving high dose LPS were significantly less febrile than nonpregnant animals. On gestation day 20, the profound hypothermia observed translated to an overall loss in heat, as evidenced by a negative fever index value.

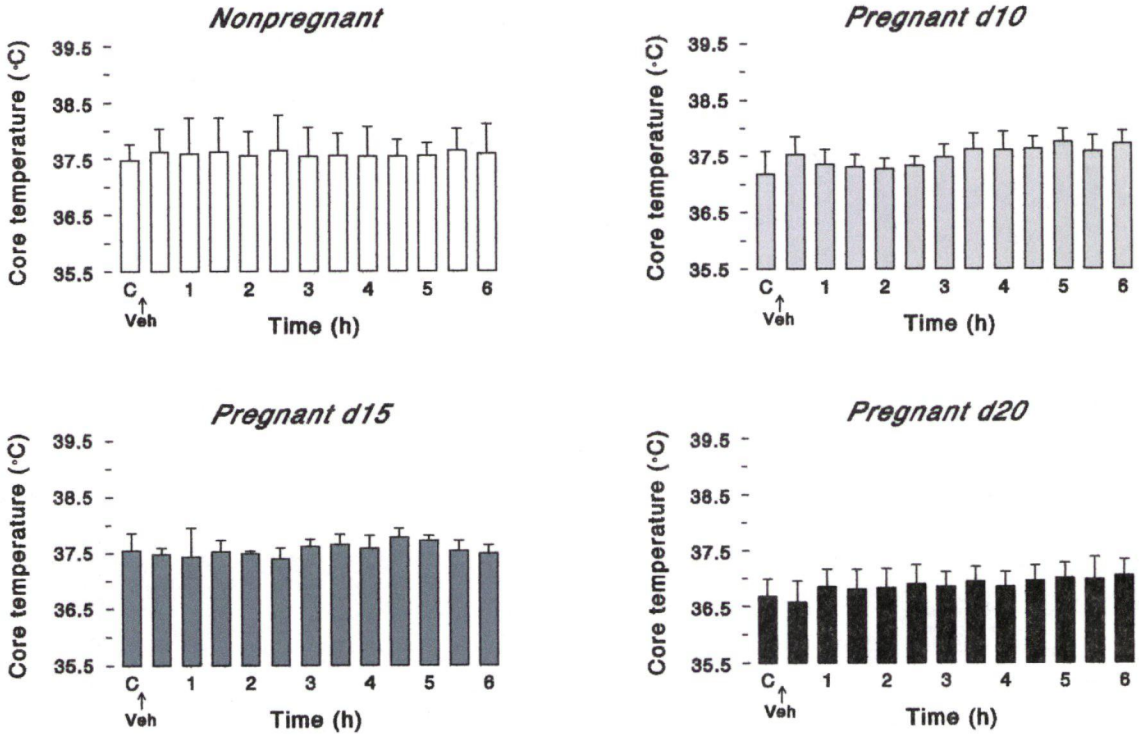


Figure 4.1 Core temperatures measured before and after intraperitoneal injection (↑) of vehicle in nonpregnant rats (n=21) and in pregnant rats on days 10 (n=16), 15 (n=16), and 20 (n=21) of gestation. Data are presented as means ± one standard deviation; * $p<0.05$ versus control temperature.

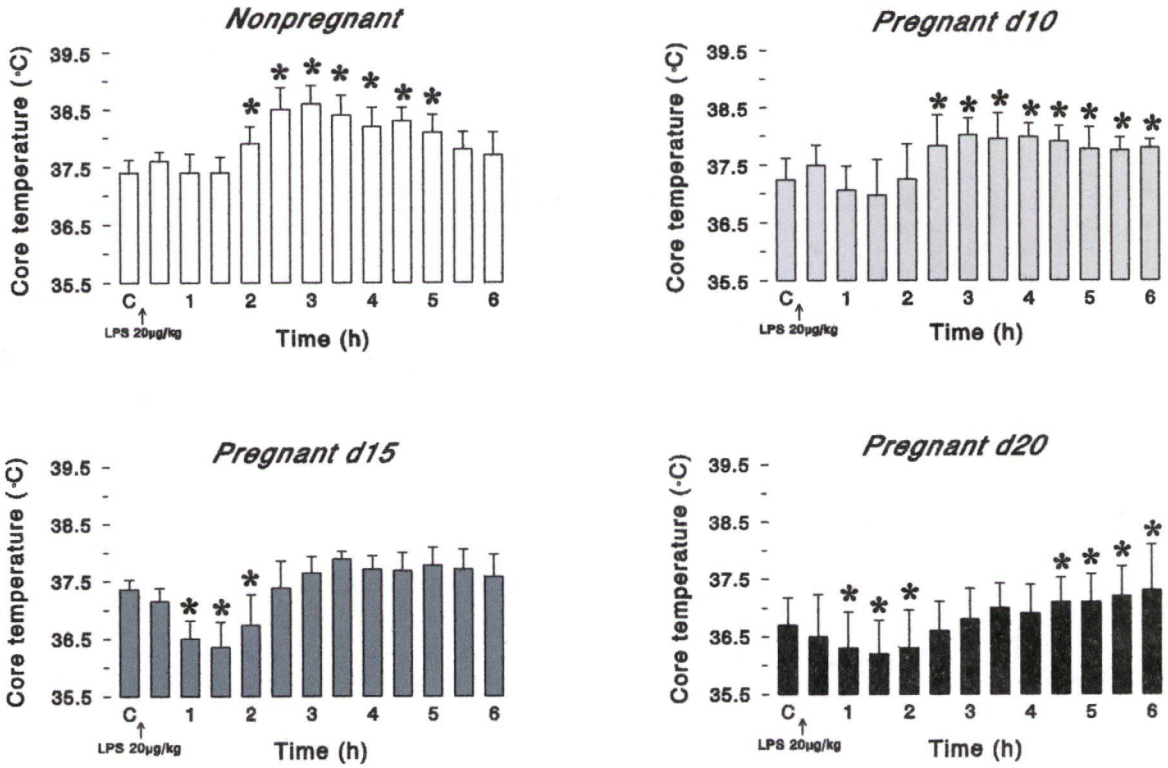


Figure 4.2 Core temperatures measured before and after intraperitoneal injection (↑) of 20 µg/kg *E. coli* LPS in nonpregnant rats (n=20) and in pregnant rats on days 10 (n=16), 15 (n=16), and 20 (n=17) of gestation. Data are presented as means ± one standard deviation; * $p<0.05$ versus control temperature.

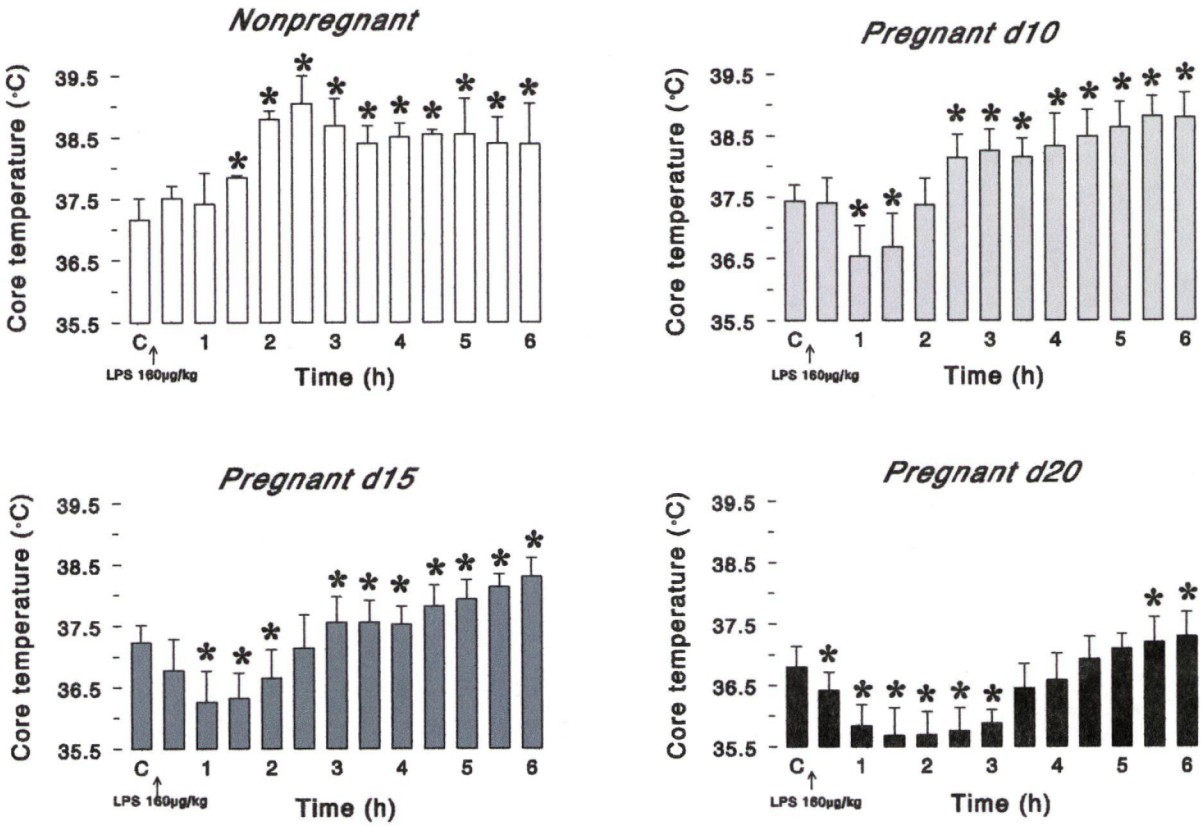


Figure 4.3 Core temperatures measured before and after intraperitoneal injection (↑) of 160 µg/kg *E. coli* LPS in nonpregnant rats (n=20) and in pregnant rats on days 10 (n=16), 15 (n=16), and 20 (n=17) of gestation. Data are presented as means ± one standard deviation; * $p<0.05$ versus control temperature.

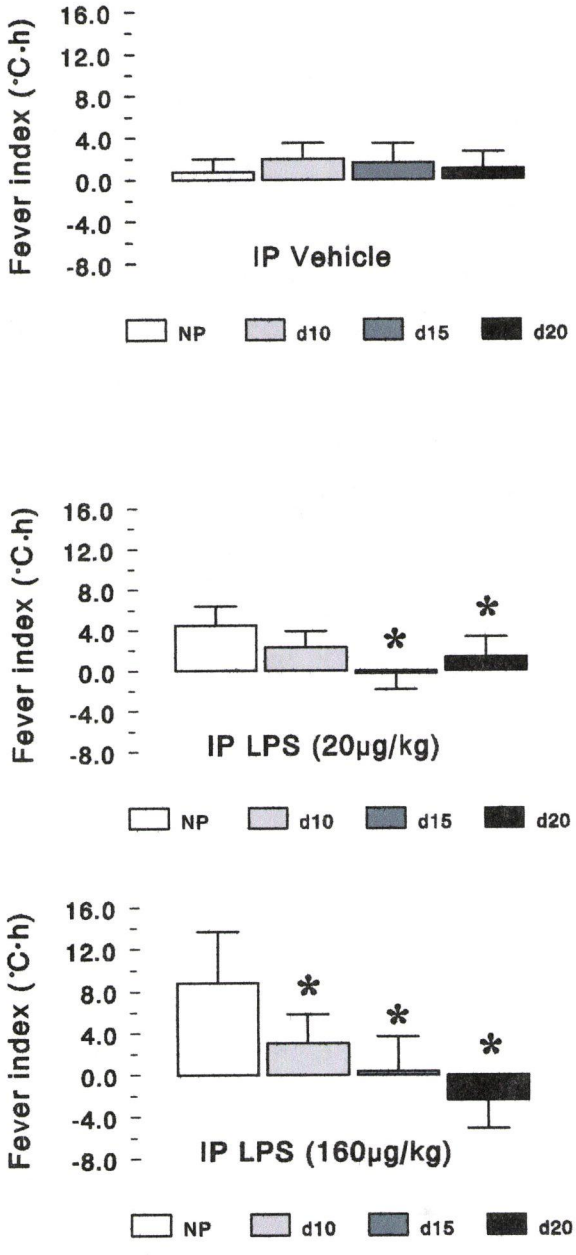


Figure 4.4 Mean fever indices in nonpregnant rats and in pregnant rats on days 10, 15, and 20 of gestation for the six-hour period after intraperitoneal administration of vehicle, 20 µg/kg *E. coli* LPS and 160 µg/kg LPS. Data are presented as means ± one standard deviation for an n of at least 7 for each dose in each group; * $p<005$ versus NP for a given dose of *E. coli* LPS

4.4 SUMMARY & CONCLUSIONS

The results of the present study complement prior studies which have examined the febrile response to bacterial endotoxin in near-term females. It has been shown previously that fever is suppressed in rats close to parturition following intravenous injection of 25 μg/kg *E. coli* LPS.[135] Taking the route of administration into consideration, this dose is analogous to those used here—evidence is the fact that in their study, Martin *et al* saw fever magnitudes of 1.9 ±0.16°C, 2.0 ±0.18°C and 1.8 ±0.7°C, respectively, in nonpregnant rats by I.V. doses of 10, 25, and 50 μg/kg. These correlate closely with the values reported here. Thus, their study provides an excellent basis for comparison, and the present experiments extend their observations as follows. First, our experiments provide evidence that there is an attenuated febrile response to I.P. administration of both a maximal and submaximal dose of *E. coli* lipopolysaccharide near the term of pregnancy. Thus, the results confirm the hypothesis that the mechanisms responsible for attenuation of the fever in rats in the latter part of gestation are operative when exogenous pyrogen is administered into the peritoneal cavity as well as when it is administered directly into the vascular space. Secondly, these data suggest that the attenuated febrile response near the term of pregnancy is not resulting from a simple rightward shift of the *E. coli* LPS dose—core temperature response curve, but rather a dampening of the overall drug-effector relationship. In other words, there appears to be a decreased ability of LPS to induce fever in near-term pregnant rats, which is not dependent on dose.

Next, the present work provides evidence that there is some characteristic of pregnancy that evokes a hypothermic response to endotoxin that is not seen in nonpregnant females in response to either an EC_{50} or an EC_{100} dose of *E. coli* LPS. Two independent hypotheses may explain the initial hypothermic response to endotoxin. The first postulate is that endotoxin exerts a direct toxic effect on peripheral vascular smooth muscle causing vasodilation in the cutaneous vessels.[62] Vasodilation precipitates the heat loss leading to hypothermia. Secondly, it has been proposed that upon initial exposure to endotoxin, rats recruit the detoxifying properties of the reticuloendothelial cell system.[69,187] Presumably, endotoxin is so rapidly removed from the circulation that the stimulus for the synthesis of endogenous pyrogen is considerably weakened. However, this scheme is at odds with the theory that endotoxin remains in the circulation as a vasoactive agent. Nor can it be reconciled with the fact that in the present work, animals displaying an initial hypothermia go on to mount significant fevers. The hypothermia which seems limited to the latter part of gestation may indeed represent a "forced" hypothermia mediated by a pregnancy-induced alteration of the passive active properties of the vasculature. Alternatively it may be a "regulated" hypothermia which Romanovsky *et al* have shown to occur following the administration of high dose *E. coli* LPS in adult male rats.[174] Further investigation is needed to determine the exact mechanism of this thermoregulatory response.

Lastly, we have introduced an alternative method for intraperitoneal administration of pyrogen, which avoids piercing the abdominal wall, as well as blind administration of drug. Intraperitoneal delivery of pyrogen via a chronically implanted catheter has the advantage of eliminating the often confounding thermoregulatory effects

resulting from the stress associated with the injection procedure.[50] Furthermore, this novel technique ensured that the entire volume of injectate was administered into the peritoneal space and not into the bowel, bladder, or other visceral structures. This was an important consideration in these experiments, as there are several lines of evidence to suggest that improper injection can influence the route and duration of systemic uptake of pyrogen and the resulting fevers.[73,87,90]

CHAPTER FIVE

THE INFLUENCE OF PREGNANCY ON BASAL PRODUCTION OF IL-1β AND IL-1 RECEPTOR ANTAGONIST

5.1 INTRODUCTION

In considering efferent mechanisms of the attenuated febrile response to pyrogen near term, experiments performed by other investigators have established that this maternal adaptation is not due to an impairment of the thermoregulatory effectors during pregnancy. This assertion comes in light of a reduced febrile response to pyrogen in pregnant rats despite activation of behavioral and autonomic effector mechanisms.[56] Moreover, intravenous administration of recombinant rat interleukin-1β produces significant increases in peri-OVLT PGEs and core temperature in nonpregnant rats but not in near-term pregnant rats. It is probable that a pregnancy-related antipyresis is responsible.[111] Arginine vasopressin has been extensively investigated in this capacity, and indeed AVP appears to mediate the attenuated febrile response to centrally administered prostaglandin near term of pregnancy.[58] However, there is reason to believe

that other antipyretic agents may be important in abrogating the febrile response in rats. In studies comparing AVP deficient (i.e. Brattleboro) and wild type rats (i.e. Sprague-Dawley), a marked divergence in the core temperature response to LPS endotoxin was observed. AVP deficient rats became febrile while wild type controls became hypothermic.[191] If one were to accept the hypothesis that AVP is the sole endogenous antipyretic substance involved in controlling fever, then we might expect that in animals that are devoid of this peptide, the febrile response would also be accentuated in response to pyrogenic cytokines. However, the results from Stitt *et al* [191] did not bear this out. In response to a downstream mediator of fever, endogenous pyrogen (i.e. interleukin-1), fevers in both AVP deficient and wild type animals were qualitatively and quantitative indistinguishable. Perhaps more compelling is evidence from Eliason and Fewell that AVP does not mediate the suppressed febrile response following I.V. administration of interleukin-1β in near-term pregnant animals.[59]

From these lines of investigation, we may conclude the following. Other upstream antipyretic mediators may be acting to control the magnitude and duration of interleukin 1-induced fever prior to the action of AVP. It is most likely that such a mediator would antagonize the action of pyrogenic cytokines. Interleukin-1 receptor antagonist is such an agent, and a pregnancy-related increase in circulating levels of IL-1ra is a feasible mechanism by which fevers might be attenuated near term. The experiments described in this chapter were carried out to measure basal plasma levels of IL-1β and IL-1ra in nonpregnant and pregnant rats throughout gestation. Specifically, I wanted to test the hypothesis that basal plasma levels of IL-1ra are increased in rats near the term of pregnancy compared to those in nonpregnant and pregnant rats early in gestation.

5.2 METHODS

5.2.1 Animals

Experiments were carried out on 40 conscious animals allocated to one of four groups: 10 nonpregnant and 30 pregnant rats on days 10 (n=10), 15 (n=10), and 20 (n=10), of gestation (term 21-22days). All animals were obtained from Charles River Laboratories. Pregnant animals were undergoing their first pregnancy, and arrived between day 4 and 6 of gestation. All animals were fed, housed, and handled as described in section 2.1.

5.2.2 Experimental Protocol

On the day of an experiment, the rats were removed from their cages and decapitated between 1000 and 1100 hours. Trunk blood was collected on ice in centrifuge tubes containing EDTA, aprotinin, and indomethacin.[25] The blood was centrifuged at 4°C for 10 minutes and the resulting plasma was stored at -70°C until IL-1β and IL-1ra were determined by ELISA (BioSource; IL-1β sensitivity <3 pg/mL; IL-1ra sensitivity <12 pg/mL).

5.2.3 Data & Statistical Analysis

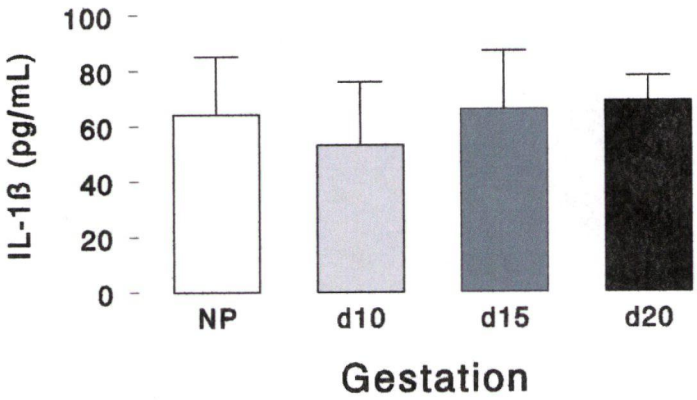
Cytokine data were presented as means ± one standard deviation. Statistical analysis was carried out using a one-factor analysis of variance followed by a Newman-Keuls multiple comparison test to determine if pregnancy influenced basal plasma IL-1β or IL-1ra; $p<0.05$ was considered to be of statistical significance.

5.3 RESULTS

5.3.1 Basal plasma IL-1β and IL-1ra levels in nonpregnant and pregnant rats

Basal plasma interleukin-1 beta concentrations did not change during pregnancy (Figure 5.1A). However, basal interleukin-1 receptor antagonist levels were increased significantly in pregnant rats on day 15 of gestation compared to nonpregnant, day 10, and day 20 gestation animals (Figure 5.1B).

A



B

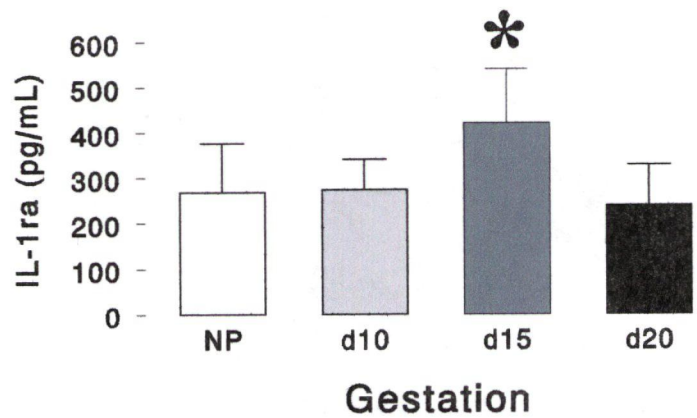


Figure 5.1 Plasma levels of IL-1β (A) and IL-1ra (B) in unstimulated nonpregnant rats (n=10), and pregnant rats on days 10 (n=10), 15 (n=10), and 20 (n=10) gestation. Data presented as means ± one standard deviation; * $p<0.05$ versus NP, for a given cytokine.

5.4 SUMMARY & CONCLUSIONS

While it has been demonstrated in humans that late term pregnancy (>80% of gestation) influences the overall cytokine profile,[167] the present experiments did not confirm the hypothesis that circulating levels of IL-1ra are increased in near-term pregnant Sprague Dawley rats. Contrary to my expectation that interleukin-1 receptor antagonist is elevated near term in rats, these data show that basal serum IL-1ra is elevated at day 15 and returns to nonpregnant levels by day 20 of gestation. It remains possible that local increases in IL-1ra on day 20 can still induce centrally mediated illness responses despite the lack of elevated levels in the general circulation. Presently, we can speculate that IL-1ra is mediating the antipyretic response on day 15 (~70% of gestation), but perhaps not closer to term.

However, the results of this study do not preclude the possibility that upon stimulation with pyrogen, interleukin-1ra may be accentuated in late gestation and thus still mediate the suppressed febrile response to infection near term of pregnancy. So, while baseline levels of antipyretic cytokines do not appear to be elevated near term on day 20 in unstimulated animals, patterns in their release during the course of the acute phase response to pyrogen may still mediate the observed lack of fever. In fact, a recent study investigating intracellular cytokine expression patterns in the peripartum period, reported that after LPS exposure, cord blood monocytes produced less TNF-α--an important proinflammatory cytokine in the host defense response to infection—relative to

nonpregnant adults.[96] Similarly, Pillay *et al* have shown that following stimulation with either IL-1α, or LPS, monocytes of women nearing parturition produce significantly higher levels of IL-1ra, as compared to nonpregnant controls.[167]It also remains possible that reduced production of pyrogenic cytokines (e.g. IL-1β, IL-6) in response to endotoxin challenge may be a contributing factor responsible for the abrogated fevers observed in near term rats. Experiments outlined in the next chapter were conducted to explore these possibilities.

CHAPTER SIX

THE INFLUENCE OF PREGNANCY ON THE CYTOKINE RESPONSE TO INTRAPERTIONEAL LIPOPOLYSACCHARIDE IN FEMALE SPRAGUE-DAWLEY RATS

6.1 INTRODUCTION

Modulations of maternal immune cell function are critical for successful growth and development of the fetus.[208] The utero-placental interface is likely to be the site of the most prominent immune adaptations to pregnancy,[182] and it has been suggested that systemic immunity is also suppressed during gestation. Matthiesen *et al* have shown that the ratio of pro-inflammatory to anti-inflammatory cytokine in peripheral mononuclear cells (PBMCs) is altered during pregnancy.[140] Specifically, pregnancy is associated with suppression of important regulators of the acute phase response, NFκB, TNF-α, and macrophage inflammatory protein alpha (MIP-1α).[143,169]

Interleukin-1β is a blood-borne multifunctional cytokine which induces the production of interleukin-6 and other mediators of infection and fever. Furthermore, circulating prostaglandin (PGE_2) is involved in the development of fever.[171] As part of the coordinated host defense response, interleukin-1 receptor antagonist is also released

into plasma by monocytes and PBMCs following exposure to exogenous pyrogen. IL-1ra serves to modulate the activity of IL-1β and may alter the end-organ responses such as pyrogen-induced fever. Although the present results confirm that there are pregnancy-specific changes in immunomodulation that lead to raised plasma concentrations of interleukin-1 receptor antagonist on day 15 gestation in the Sprague-Dawley rat, the experiments reported in Chapter 5 do not support the notion that differential regulation in basal IL-1β and IL-1ra levels near term is responsible for the suppressed febrile response observed on day 20. However, evidence exists, at least in mice, that following LPS exposure there are gestation-dependent changes in IL-1β mediated processes.[170]

That during pregnancy inflammatory conditions are suppressed is well established.[144] For example, it has been hypothesized that the endocrine environment of pregnancy is associated with IL-1β deficiency, and reduced LPS-induced cytokine responses. Considering that LPS-induced fever is attenuated in rats near term, the experiments described in this chapter were carried out to test the hypothesis that pregnancy influences plasma IL-1ra relative to IL-1β, IL-6, and systemic prostaglandin E after administration of *E. coli* lipopolysaccharide in female rats. The assay used was not specific for PGE_2, as such, total plasma PGE was quantified (i.e. PGE_1 and PGE_2) and these will hereafter be referred to as PGEs.

6.2 METHODS

6.2.1 Animals

Experiments were carried out on 98 conscious chronically instrumented animals allocated to one of four groups: 26 nonpregnant and 72 pregnant rats on days 10 (n=24), 15 (n=24), and 20 (n=24), of gestation (term 21-22days). All animals were obtained from Charles River Laboratories. Pregnant animals were undergoing their first pregnancy, and arrived between day 4 and 6 of gestation. All animals were fed, housed, and handled as described in section 2.1.

6.2.2 Surgical Preparation

No less than three days prior to an experiment, each rat was anaesthetized by inhalation of halothane (2% for induction and maintenance) in oxygen. A paramedian laparotomy was performed and a sterile catheter was implanted into the peritoneum for delivery of injectate, also a free-floating, battery-operated telemetry device (TA10TA-F20, Data Sciences International) was inserted into the peritoneal cavity for measurement of core temperature, as described in section 2.21.

6.2.3 Conditions of Observations

During an experiment, each animal was studied in her cage, which was kept in the environmental chamber maintained at an ambient temperature of 25±1°C for the duration of the experiments. Core temperature was obtained from the signal emitted from the previously implanted biotelemetry device, and was recorded at two-minute intervals throughout the experiment, as described in Section 2.5.1 (temperature data shown in Chapter 4).

6.2.4 Experimental Protocol

The day before an experiment, each animal was removed from its cage, weighed and then returned to the cage in the environmental chamber. Rats were randomly divided into six groups based on injectate and time of blood collection. Each animal was studied only once. On the day of the experiment between 1000 and 1100 hours, following an acceptable control period defined as five two-minute measurements of core temperature that did not vary by more than ±0.2°C, the rat was removed from its cage and given an intraperitoneal injection of either vehicle (normal phosphate buffered saline), 20μg/kg or 160μg/kg *E. coli* LPS (*Escherichia coli*, 0111:B4): the EC_{50} and EC_{100} concentrations, respectively. Pyrogen was administered via the indwelling catheter (Section 2.3). After the injection, the rat was returned to its cage and core temperature was measured at two-

minute intervals for the duration of the experiment. At either 2 or 4 hours after injection, rats were removed from their cage and decapitated for plasma cytokine analysis. The typical biphasic febrile response to LPS in rats indicates peaks in core temperature at 2.5 and 4.5 hours.[172] Assuming that circulating cytokine levels increase prior to the elevation in core temperature, I chose to assay cytokines at 2 and 4 hours following LPS challenge. Trunk blood was obtained from nonpregnant and pregnant rats collected on ice in centrifuge tubes containing EDTA, aprotinin, and indomethacin.[25] The blood was centrifuged at 4°C for 10 minutes and the resulting plasma was stored at -70°C until plasma IL-1β, IL-6, IL-1ra, and PGEs were determined by ELISA (BioSource: IL-1β sensitivity <3 pg/mL; IL-1ra sensitivity <12 pg/mL; IL-6 sensitivity <10pg/mL; PGE sensitivity <36pg/mL).

6.2.5 Data & Statistical Analysis

Cytokine data are presented as raw values for individual animals. Statistical analysis was carried out using two-way analyses of variance to determine if there were any significant influences of gestation or time on the plasma levels of IL-1β, IL-6, IL-1ra, or PGEs, for a given dose of LPS (i.e. statistics performed analyzed effects within injectate groups). Gestation term indicated variation on any day of gestation versus nonpregnant. Time term represented variation at 2 versus 4 hours. 'Gestation x Time' represented the interaction term between state of pregnancy and time of assay. Finally, a p value of less than 0.05 was considered to be of statistical significance.

6.3 RESULTS

6.3.1 Plasma Levels of Interleukin-1 Beta Following I.P. *E. coli* LPS

Figure 6.1C illustrates that following I.P. administration of high dose pyrogen (160 μg/kg *E. coli* LPS), there were notable increases in plasma IL-1β concentrations at both 2 and 4 hours noted in nonpregnant animals. Similarly robust increases in IL-1β occurred both early and late (2 and 4 hours respectively) on days 10 and 15 of gestation (Figure 6.1C). This is in contrast with the attenuated cytokine response observed on day 20. Administration of vehicle did not elicit a significant rise in plasma IL-1β regardless of gestational age (Figure 6.1A). By and large, the IL-β response to I.P. LPS was not distinguishably different when low dose pyrogen (20 μg/kg) was given, as compared to vehicle (Figures 6.1A and 6.1B).

6.3.2 Plasma Levels of Interleukin-6 Following I.P. *E. coli* LPS

Plasma levels of IL-6 did not change following I.P. administration of vehicle at any point in gestation (Figure 6.2A). Likewise, low dose *E. coli* LPS challenge (20

μg/kg) did not elicit significant increases in IL-6 in nonpregnant or pregnant animals on days 10, 15, or 20 (Figure 6.2B). In nonpregnant animals and animals on days 10 and 15 of pregnancy, following I.P. administration of 160 mg/kg LPS, there was an early peak in IL-6 at 2 hours after injection, thereafter plasma IL-6 levels dropped (Figure 6.2C). However, animals on day 20 of gestation did not display this early rise in circulating IL-6; furthermore, cytokine levels remained low at 4 hours after injection.

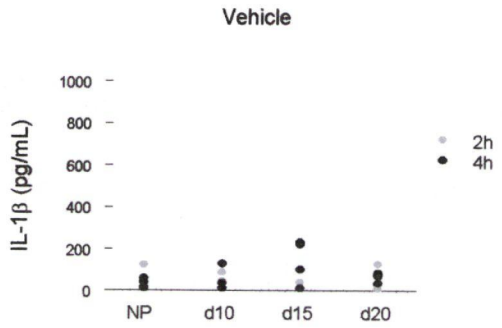
6.3.3 Plasma Levels of PGE Following I.P. *E. coli* LPS

In general, circulating levels of PGEs were not increased early or late in response to I.P. administration of vehicle (6.3A). Furthermore, there were no differences observed between exposure to either 20 or 160 μg/kg *E. coli* LPS on plasma concentrations of PGE in nonpregnant animals or at any point during gestation (Figures 6.3B and 6.3C). Overall, there were no significant changes in PGEs at 2 versus 4 hours after injection for any dose of pyrogen given.

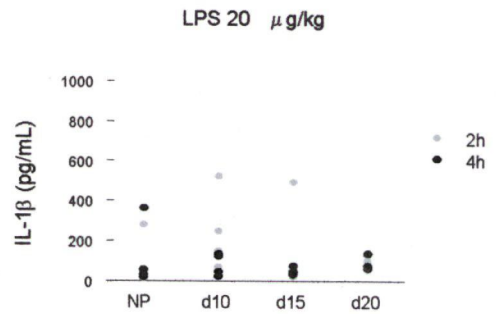
6.3.4 Plasma Levels of Inteleukin-1 Receptor Antagonist Following I.P. *E coli* LPS

Figure 6.4A indicates that there was no effect of vehicle on the concentration of IL-1ra detected at either 2 or 4 hours in the plasma of nonpregnant animals. Nor did administration of vehicle evoke increases in IL-1ra levels in pregnant animals at any stage of gestation (Figure 6.4A). Circulating IL-1ra was only modestly increased in response to I.P. administration of 20 μg/kg *E. coli* LPS in nonpregnant animals and on days 10,15, 20 of pregnancy (Figure 6.4B). Nonpregnant rats and rats on days 10, 15 and 20 of gestation showed notable elevations in plasma IL-1ra 2 hours following injection of 160 μg/kg *E. coli.* IL-1ra levels had fallen by 4 hours in all the animals except those near term, on day 20 (Figure 6.4 C).

A



B



C

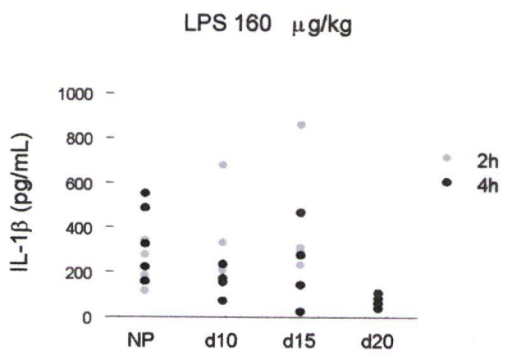
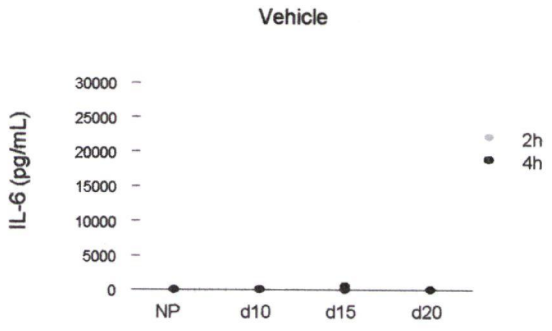
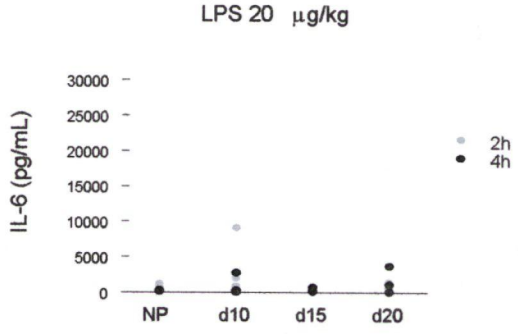


Figure 6.1 Plasma levels of IL-1β in nonpregnant (n=26), and pregnant rats on days 10 (n=24), 15 (n=24), and 20 (n=24) of gestation collected at 2 and 4 hours post injection of vehicle (A) Gestation 0.424 - Time 0.133 - Gestation x Time 0.038, 20 μg/kg *E. coli* LPS (B) Gestation 0.541 – Time 0.138 – Gestation x Time 0.378, or 160 μg/kg *E. coli* LPS (C) Gestation 0.016 – Time 0.255 – Gestation x Time 0.037. ANOVA; $p<0.05$.

A



B



C

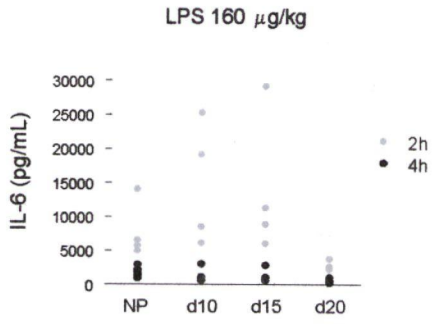
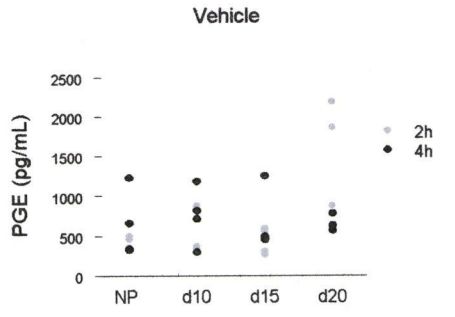
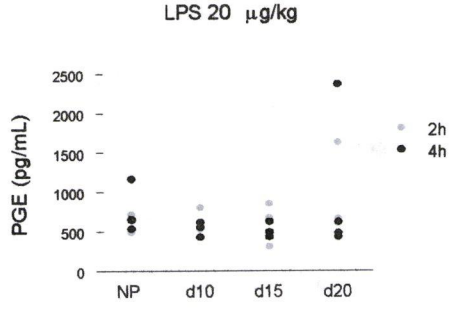


Figure 6.2 Plasma levels of IL-6 in nonpregnant (n=26) and pregnant rats on days 10 (n=24), 15 (n=24), and 20 (n=24) of gestation collected at 2 and 4 hours post injection of vehicle (A) Gestation 0.249 - Time 0.470 - Gestation x Time 0.278, 20 µg/kg *E. coli* LPS (B) Gestation 0.201 – Time 0.213 – Gestation x Time 0.467, or 160 µg/kg *E. coli* LPS (C) Gestation 0.056 – Time 0.001 – Gestation x Time 0.091. ANOVA; $p<0.05$.

A



B



C

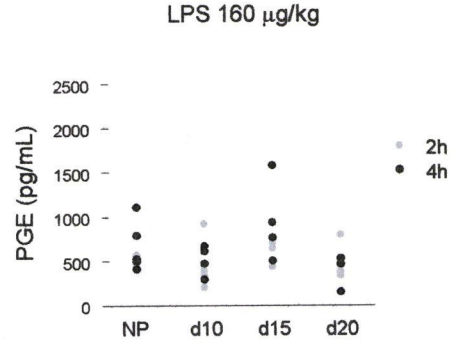
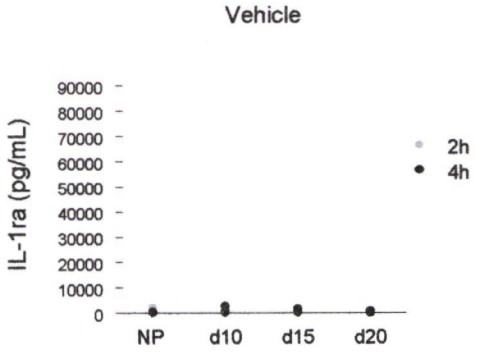
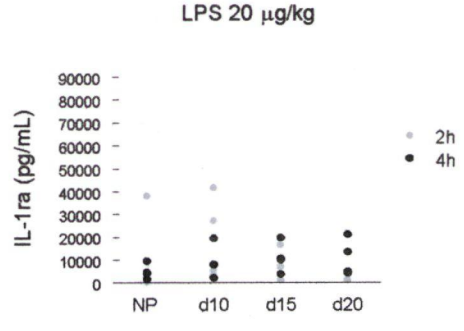


Figure 6.3 Plasma levels of total PGE in nonpregnant (n=26) and pregnant rats on days 10 (n=24), 15 (n=24), and 20 (n=24) of gestation collected at 2 and 4 hours post injection of vehicle (A) Gestation 0.084 - Time 0.689 - Gestation x Time 0.062, 20 µg/kg *E. coli* LPS (B) Gestation 0.436 – Time 0.891 – Gestation x Time 0.910, or 160 µg/kg *E. coli* LPS (C) Gestation 0.142 – Time 0.329 – Gestation x Time 0.392. ANOVA; $p<0.05$.

A



B



C

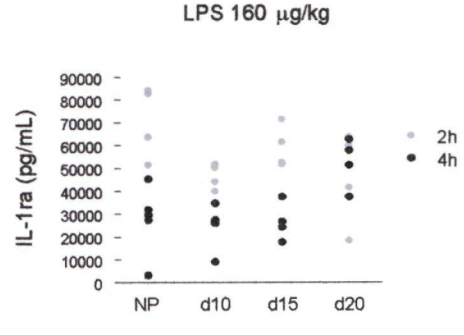


Figure 6.4 Plasma levels of IL-1ra in nonpregnant (n=26) and pregnant rats on days 10 (n=24), 15 (n=24), and 20 (n=24) of gestation collected at 2 and 4 hours post injection of vehicle (A) Gestation 0.413 - Time 0.316 - Gestation x Time 0.062, 20 µg/kg *E. coli* LPS (B) Gestation 0.701 – Time 0.431 – Gestation x Time 0.584, or 160 µg/kg *E. coli* LPS (C) Gestation 0.034 – Time 0.601 – Gestation x Time 0.006. ANOVA; $p<0.05$.

6.4 SUMMARY AND CONCLUSIONS

6.4.1 The Effect of I.P. *E. coli* LPS on Plasma Interleukin-1β

Although the results described in Chapter 4 indicate that nonpregnant and pregnant rats studied on days 10, and 15 are mounting significant increases in core temperature in response to both 20 and 160 μg/kg *E. coli* LPS, it does not appear that animals elicit increases in IL-1β following the lower EC_{50} dose of endotoxin. In sharp contrast, when the higher EC_{100} dose is given, nonpregnant and early gestation rats have a vigorous rise in plasma IL-β. As we would expect, circulating levels of IL-1β are attenuated in rats nearing term of pregnancy after I.P. administration of high dose lipopolysaccharide. We may speculate that since during febrigenesis cytokines communicate to the brain via a neural pathway involving activation of vagal afferents by interleukin-1β, in addition to blood-borne routes, and that several effects of vagotomy have been shown to be differentially determined by dose,[87-89,109] that neural signaling might be expected to be especially important at low doses of cytokine, when local activation could occur, but when only very small quantities of cytokine would become systemic. It has thus been suggested that IL-1β-induced, brain-mediated responses such as fever may be mechanistically distinct depending on dose,[91] and this likely explains the present lack of plasma IL-1β involvement in fever induced experimentally via intraperitoneal exposure to low-dose LPS.

6.4.2 The Effect of I.P. *E. coli* LPS on Plasma Interleukin-6

Again, dose of intraperitoneal LPS appears to be an important factor in determining the role for IL-6 in fever. Administration of 20 μg/kg LPS did not elicit an apparent increase in this pyrogenic cytokine in any of the animals, though from previous studies it is clear that nonpregnant rats and rats in early to mid gestation do develop fever with this dose (Chapter 4). Therefore, it seems reasonable to assume that blood-borne IL-6 does not mediate fever in response to low dose I.P. pyrogen. Following the higher concentration of lipopolysaccharide, 160 μg/kg, robust increases in maternal IL-6 occurred early in all animals except those near term of pregnancy, this is consistent with the core temperature increases induced with this dose of LPS (Chapter 4). The mechanisms that initiate fever to low dose pyrogen without a concomitant increase in circulating pyrogenic cytokines are still unclear.

That glucocorticoids, especially cortisol, play an important role in the preparation for birth in humans, including involvement in lung and cardiac development has long been known.[75] Furthermore, Dupouy *et al* have previously reported that in rats, plasma corticosterone levels reach a peak on day 18 of gestation and remain high until parturition.[49] Considering that glucocorticoids alter fever responses to lipopolysaccharide,[153] one might hypothesize that near term of pregnancy-- which is characterized by an elevated glucocorticoid profile-- may exert inhibitory feedback on IL-6 plasma activity, and thus reduce LPS-induced fever. Taken together, these findings support the notion that reduced synthesis of pyretic agents close to parturition may be a mechanism underlying the attenuated febrile response in periparturient mammals.

The data reported in this chapter provide evidence that a pregnancy-related alteration in the cytokine response to LPS may mediate the attenuated febrile response in Sprague-Dawley rats near the term of gestation. As with all previous experiments described in this thesis, several control groups were studied here in order to ascertain that the observed cytokine expression responses were truly physiological influences of pregnancy and not secondary effects of the injection or plasma collection procedures. In general, nonpregnant animals showed no apparent increases in any cytokines following administration of vehicle. Additionally, the conclusion may be made that basal levels of IL-1β, IL-6, IL-1ra, and PGE are not significantly influenced by pregnancy, as evidenced by the lack of response to vehicle. As such, these data are largely in agreement with the studies described in Chapter 5.

6.4.3 The Effect of I.P. *E. coli* LPS on Plasma Interleukin-1 Receptor Antagonist

As one might expect, there were no early or late increases in plasma IL-1ra in response to vehicle at any point in gestation. In response to low dose lipopolysaccharide, there were slight and insignificant increases in circulating receptor antagonist levels, however the response did not appear to be influenced by pregnancy. However, an interesting temporal pattern emerged following administration of 160 μg/kg LPS. Nonpregnant animals and animals on days 10 and 15 of gestation mounted a very strong interleukin-1 receptor antagonist response within 2 hours of injection. Thereafter IL-1ra levels dropped considerably. In contrast, near-term animals mounted a vigorous IL-1ra

response very soon after injection (2 hours), but unlike animals in early to mid gestation IL-1ra levels remains high throughout the entire 4 hours following injection.

The absence of fever in response to pyrogenic stimulation can be due to a depressed induction of endogenous pyrogens or perhaps the enhanced formation of endogenous antipyretics. Ten years ago, Pillay *et al* reported an exaggerated interleukin-1 receptor antagonist response in near term human women between (32-40 weeks gestation) following stimulation of monocytes *in vitro* with both endogenous and exogenous pyrogen.[167] It appears as though data presented in this chapter did not confirm the original hypothesis that near-term Sprague Dawley rats elicit an accentuated IL-1ra response to bacterial endotoxin as compared to nonpregnant rats and rats early in gestation. Rather, the data provide indirect evidence that pregnancy does influence plasma IL-1ra concentrations relative to IL-1β after I.P. administration of *E. coli* LPS. Specifically, there is a sustained antipyretic response in periparturient animals that may be one potential mechanism accounting for the attenuated febrile response observed near term.

In animal models of *E. coli* LPS induced peritonitis, IL-1ra significantly reduces the severity of disease.[63] In human studies of endotoxemia, plasma concentrations of IL-1ra remain elevated for several hours after IL-1β has begun to decline, indicating a possible blockade of IL-1 receptors during the immune response to infection.[82] Under most circumstances however, this mechanism is most likely not sufficient to attenuate the fever and acute phase responses to pathogenic stimuli. First, a biological response can be induced by occupancy of less than 5% of IL-1 receptors making the system extremely sensitive. Second, IL-1ra must exceed IL-1 by fifteen-fold in order to block its binding to

neutrophils by 50%.[82] Lastly, after IL-1/IL-1 receptor complexes are internalized, recycled receptors are free to return to the cell surface.[86] However, the results presented here indicate that in pregnancy, following stimulation with pyrogen, there is ~100 fold plasma excess of IL-1ra to IL-1β. This ratio far exceeds the requisite levels of IL-1ra needed to effectively antagonize receptor binding and cellular actions of interleukin-1β. Thus, we may speculate that at least in the uniquely immunosuppressed milieu of pregnancy, that IL-1 receptor antagonism is playing a physiologically significant role in antipyresis. Since guinea pigs,[39] and sheep[112] and rabbits[160] have been found to remain afebrile to pyrogen near term, it may be beneficial to establish the plasma IL-1ra response to LPS in other species in an attempt to determine if this strategy is specific to rats.

6.4.4 The Effect of I.P. *E. coli* LPS on Plasma Prostaglandin E

It is known that centrally acting PGEs are the ultimate mediators of fever.[147] However, several lines of evidence suggest a role for peripherally synthesized PGE. For example, I.V. administration of PGE_2 has been shown to cross the blood-barrier and cause fever in a dose dependent fashion.[52] Moreover, the onset of fever in rabbits and sheep is associated with an increased PGE_2 concentration in the venous circulation.[149,181] This provided the rationale for the present experiments which sought to investigate any changes in systemic PGEs that may have been influenced by pregnancy. Yet the data shown here indicate that stimulation with *E. coli* lipopolysaccharide does not have an apparent effect on circulating levels of E series prostaglandins.

Plasma PGE responses to I.P. vehicle were not different from the PGE response to low or high dose of endotoxin, and lipopolysaccharide did not elicit an increase in PGEs in nonpregnant animals. It is true that prostaglandin dehydrogenase (PGDH) — the enzyme responsible for degradation of PGE_2 — is upregulated in reproductive organs near-term in rats.[119,164] Ivanov and Romanovsky have hypothesized that this may potentially accelerate clearance of PGEs from the circulation thereby increasing the gradient between systemic and central PGE concentrations, and in turn facilitating catabolism of PGE_2 in pregnancy.[107] This has been speculated as a putative mechanism for suppressed fever near-term, however until the present experiments, no studies had been done which compared circulating levels of PGE_2 in nonpregnant and pregnant LPS-challenged animals.[106] Indeed, we did not observe an influence of pregnancy on systemic production and release of PGE with pyrogen stimulation which would argue against enhanced prostaglandin metabolism as a means for pregnant mammals to maintain febrile refractoriness to pyrogen. Further consideration would lead to the conclusion that blood-derived PGEs may be associated with but perhaps not critical to febrigenesis. Still, these were important observations as they were consistent with previous investigations in male Sprague Dawley rats reporting that while PGE_2 levels in the cerebral spinal fluid rose during experimentally-induced fever, plasma concentrations of prostaglandin were unchanged after intraperitoneal LPS injection.[128] This is easily explained given that alternative routes of communication between the periphery and the CNS involve cytokine and LPS receptors on the surface of cerebral endothelium of the blood-brain barrier.[10,28,200] Furthermore, there are as yet no reports in the literature which compare circulating PGE levels in nonpregnant and pregnant rats following LPS challenge. The

results reported here would suggest that peripherally derived PGEs are not physiologically relevant in mediating fever responses to bacterial endotoxin in female Sprague Dawley rats, nor is their pattern of release altered in pregnancy. The present study adds to the understanding of gestation-dependent reception, clearance, and now production of systemic PGEs.

CHAPTER SEVEN

DISCUSSION

7.1 SUMMARY AND CONCLUSIONS

Pregnancy is accompanied by many alterations in physiological responses. The immediate pre and post-partum period represents a unique time where female mammals, otherwise capable of evoking fever, are unable to mount such a response. In recent years there has been considerable interest in the mechanisms governing the attenuated febrile response to pyrogen near the term of pregnancy. This interest was first stimulated by the work of Kasting *et al* who reported that pregnant ewes were refractory to fever around the time of parturition, and that this refractoriness disappeared very soon after birth.[112]

Overall, the data presented in this thesis provide some new insight into the mechanisms underlying the altered febrile response to peripheral pyrogens near the term of pregnancy. The first series of experiments which involved the construction of dose-response curves for the effect of I.P. *E. coli* LPS on maximal change in core temperature

and fever index revealed that febrile responses were gradually attenuated with advancing gestation. Also noteworthy is that near-term pregnant animals had a decreased responsiveness to pyrogen rather than a less sensitive response to pyrogen as evidenced by a dampened curve rather than a curve that was merely right-shifted. Regardless of dose, animals studied on day 10, 15, and 20 of gestation never developed a fever equivalent to that of nonpregnant rats (~2°C). However, the doses that produced an approximate maximal (160 μg/kg) and half-maximal (20 μg/kg) response in pregnant rats on days 10, 15, and 20 were similar to nonpregnant controls. Thus, I was confident that the appropriate doses were used to further investigate core temperature and cytokine responses to LPS fever in pregnant rats. From this we may infer that fever suppression at term is due to intrinsic mechanisms not related to dose. I have determined that this may be due to both enhanced endogenous antipyresis and depressed endogenous pyresis.

LPS fevers induced via the intravenous route are attenuated in periparturient animals. By administering lipopolysaccharide into the peritoneal cavity in the next series of experiments I was able to simulate bacterial peritonitis, possible sepsis, fever, and robust cytokine production in rodents. I conclude first, that neural mechanisms responsible for generating fever in response to intraperitoneal pyrogen exposure, namely vagal afferent signaling, are suppressed near term, as has been previously reported for intravascular pyrogen signaling. This was a significant finding because stimuli presented via the intravenous route are likely to activate receptors humorally in the lung and the lymphatics, while substances introduced by the intraperitoneal route may activate peritoneal macrophages, liver macrophages, and Kupffer cells.[22] Thus, while there is good evidence that the febrile response to intravenous LPS is attenuated near term, it

could not be assumed that the mechanisms of clearance would be identical for intraperitoneal LPS. It is known that vagal paraganglia bind interleukin-1 receptor antagonist. Goehler *et al* have reported that centrally mediated inflammatory processes (including fever) caused by endotoxin and IL-1β could be blocked by intraperitoneal administration of recombinant human interleukin-1 receptor antagonist.[76] This is compelling evidence pointing to the importance of peripheral IL-1 receptors and further strengthening evidence for immune to brain communication unique to the I.P. route.

Secondly, I conclude that the attenuated febrile response to I.P. *E coli* lipopolysaccharide in late gestation is not due to changes in constitutive production of cytokines (IL-1β, IL-1ra) in the plasma. Instead, when stimulated with LPS, pregnant rats, particularly those studied on day 20 gestation, appeared to have a suppressed endogenous pyrogen response (IL-1β, IL-6) coupled with a prolonged antipyretic (IL-1ra) response in comparison with nonpregnant controls, and as compared to early (day 10), and mid (day 15) gestation pregnant animals. Therefore, while the studies conducted as part of this thesis represent a meaningful addition to the understanding of the febrile response to pyrogens during pregnancy, there are no doubt questions which have arisen in the course of this research.

A novel finding described here was that LPS-induced hypothermia seems to be prolonged and more profound with advancing gestation. Peripheral vasoconstriction is an important autonomic mechanism used to achieve the increase in core temperature associated with fever and so it is likely that the combined vasodilatory effect of LPS and the reduced contractile ability of cutaneous vasculature in rats close to term,[146] may account for the inability of pregnant animals in our study to mitigate heat loss following

LPS exposure, and further explains the hypothermia we observed in animals late in gestation.

Though it is unclear why the hypothermic response becomes more accentuated as pregnancy progresses, our results are consistent the work of Martin *et al* who reported that in the days preceding parturition, following a dose of 25 μg/kg LPS, virgin females responded initially with hypothermia followed by fevers reaching nearly 2°C in magnitude.[135] Animals within a day of delivery received the same dose but suffered hypothermia from which most did not recover. As the aforementioned study did not include a comprehensive temperature dose response experiment, one may speculate that the concentration of LPS administered was in the upper range of doses eliciting shock. This may explain the hypothermia reported in virgin females, a phenomenon not observed in this study, as well as the high percentage of mortality in parturient animals. It also stresses the importance of the present work, which avoids this complication by establishing the core temperature response in early, mid, and late gestation to endotoxin across a wide range of concentrations.

7.2 LIMITATIONS OF THE EXPERIMENTAL DESIGN

Prior to initiating any of the investigations described in the above sections, careful consideration was made regarding the research question being posed and the methodology with which the hypothesis would be tested. As such, all of the experiments described were carried out in what was thought to be the most systematic and appropriate fashion. Nevertheless, for experiments to be realistically undertaken, there must be

limitations to the scope of the investigation. Furthermore, there are undoubtedly issues that emerge, which in retrospect might have been dealt with differently.

In the experiments described in Chapter 4, the primary focus was to evaluate the influence of pregnancy on the febrile response to exogenous pyrogen. The effect of gestation on LPS-induced hypothermia was somewhat unexpected, and suggests that certain heat conservation mechanisms may have been altered in pregnancy. There is general agreement that pregnancy is characterized by an increased circulating blood volume, cardiac output, and heart rate, with a marked fall in peripheral vascular resistance.[51] It is possible that one of the maternal adaptations to pregnancy is alteration in skin blood flow, which would account for changes in heat conservation ability. Furthermore, the pathophysiological sequelae of Gram-negative bacterial endotoxin includes not only fever but also hypotension.[141] The telemetry system employed in this investigation was equipped to monitor cardiovascular variables, and blood pressure could have been simultaneously recorded in animals that were chronically instrumented for core temperature determination. As such, there might have been some probative value in monitoring both the cutaneous blood flow and the hypotensive responses in this study to determine if these variables changed in an exaggerated way during pregnancy in response to LPS.

The study described in Chapter 5 was designed to evaluate the influence of pregnancy on basal changes in cytokines. In these experiments, however, plasma was only assayed for IL-1β and IL-1ra. Contrarily, in the experimental series investigating cytokine responses in pregnancy following LPS challenge (Chapter 6), plasma IL-1β and IL-1ra were assayed along with IL-6 and PGEs. In retrospect, it would have been

appropriate to also determine basal levels of IL-6 and PGE in pregnancy in order for reliable comparisons to be made. One might argue however, that plasma from those animals receiving vehicle injection served as suitable controls against which to compare cytokine responses in animals that received LPS. Lastly, to more fully understand the mechanism of the hypothermia we observed in mid and late term pregnancy, it might have been beneficial to have assayed cytokines at 6 hours, when core temperatures had returned to control values in animals studied on gestation day 20.

7.3 ALTERNATE MECHANISMS OF THE ATTENUATED FEBRILE RESPONSE TO PYROGEN NEAR TERM OF PREGNANCY

The results presented in Chapters 5 and 6 suggest that the attenuated febrile response to exogenous pyrogen may be mediated by different mechanisms on day 15 and day 20 gestation in rats. Conceivably, these differences may be attributable to changes in hormonal status occurring in the female as pregnancy progresses. The most recent reports suggest that immune responses are influenced by circulating sex steroids in the female. Mouihate and Pittman have found that hormone replacement, namely estrogen in combination with progesterone, in ovariectomized rats attenuated fevers induced by intraperitoneal administration of LPS, as compared with animals receiving estrogen alone.[156] Additionally, this study found that treatment with estrogen and progesterone attenuated COX-2 expression in the hypothalamus as well as circulating plasma IL-1β induced by I. P. LPS. Knowing that progesterone levels in ovarian venous blood peak on day 14 to 15 of gestation in the rat,[93] it seems possible that the attenuated fever observed

on day 15 in the present experiments is mediated by progesterone while alternate mechanisms are responsible for fever suppression observed on day 20.

Corticosterone, which is known to modulate fever in rats following exposure to lipopolysaccharide,[142,153]increases markedly between days 18 and 19 of gestation and stays elevated for the duration of pregnancy.[49] The antipyretic actions of corticosterone and other glucocorticoids are well documented.[36] Furthermore, glucocorticoids stimulate the production of lipocortin-1, a binding protein that inhibits phospholipase A2.[30,194] This is pertinent because phospholipase A2 is responsible for the mobilization of arachadonic acid, a precursor to the formation of prostaglandins—the central mediators of fever.[71] There is particularly persuasive evidence that inflammatory processes are down-regulated near-term. For example, Mouihate and colleagues have found that both basal and LPS induced hypothalamic expression of cyclooxygenase–2 (COX-2), the rate limiting enzyme in prostaglandin production, as revealed by Western blot analysis, is reduced in the latter part of gestation in Sprague-Dawley rats.[155] Likewise, experiments by Imai-Matsumura *et al* reveal that as compared to nonpregnant controls, LPS induced COX-2, as well as PGE_2 in brain vasculature and hypothalamus are attenuated in near-term pregnant animals.[105] It is still speculative as to whether the increased glucocorticoid levels characteristic of late term pregnancy are responsible for these observations, however this hypothesis warrants further investigation.

To exert its biological effects, endotoxin binds to a circulating protein known as lipopolysaccharide binding protein (LBP) and presents endotoxin monomers to CD14, which may be a membrane-bound receptor or a soluble molecule.[213] The endotoxin-LBP-CD14 complex interacts with Toll-like receptor 4 and other regulatory proteins leading to

cellular activation and an inflammatory response.[211] As such, endotoxin has been implicated in the mechanism responsible for the setting of infection in preterm labor of infectious origin. Placentas from normal term pregnancies have been analyzed with immunohistochemistry, and show a strong immunoreactivity for TLR2 and TLR4 in the villous and the intermediate trophoblasts. Furthermore, the expression of CD14 has been shown to increase over gestation.[212] The relationship of TLRs to the development of fever near term has yet to determined, however the presence and upregulation of Toll-like receptors-2 and -4 (TLR2 and TLR4) in the human placenta,[214] suggest that these receptors may be important for immune responses against pathogens during pregnancy.

Alternatively, pregnancy specific anti-inflammatory agents have been postulated to mediate inflammatory events. Uromodulin is a 90 kilo Dalton glycoprotein which has been recently isolated from the urine of pregnant women and has not been detected in males or in nonpregnant females.[18] Uromodulin, also known as "urinary IL-1 inhibitor" has important physiological functions including cytokine binding and catabolism. Uromodulin is of particular interest here, because of all the pro-inflammatory cytokines it has been shown to most potently inhibit interleukin-1 induced thymocyte proliferation.[186] Given that IL-1 is not only pro-inflammatory, but also pyrogenic, uromodulin's inhibition of IL-1 may prove to be important to the development of fever during pregnancy. Moreover, recent investigations looking at glycosylation patterns in the first, second, and third trimesters, have revealed gradual changes in the structure of uromodulin as pregnancy progresses.[201] These changes may confer additional immunosuppression as parturition approaches. To date, none of these postulates have been explored, but they provide interesting directions for future research.

7.4 PHYSIOLOGICAL IMPLICTIONS OF LIMITING FEVER NEAR TERM OF PREGNANCY

Regardless of the mechanism of the altered febrile response to pyrogen near term of pregnancy in rats, there are unquestionably important physiological implications for both the mother and the fetus. For the most part, the fever response per se poses no threat to survival. However, in pregnancy fever affects the thermal status of the developing baby, as it is thermally clamped to its mother. In terms of oxygen supply and demand, it may be of some advantage for the mother not to develop fever near term for several reasons. Fetal oxygen requirement increases secondary to the coefficient of metabolism (i.e. Q_{10}). In light of the fact that in humans, the Q_{10} is ~2.3, metabolic rate increases ~10% for each 1°C in core temperature.[100] Therefore, even a moderate increase in temperature occurring in the latter part of gestation may have deleterious effects for the fetus. It is probable that such a relationship exists between fetal and maternal temperatures in the rat. Namely, higher oxygen demand coupled with lower oxygen affinity and oxygen saturation pursuant to a rightward shift of the oxyhemoglobin dissociation curve. In primates, maternal hyperthermia in the absence of infection is directly correlated to fetal hypoxia, metabolic acidosis, and hypotension.[150] Though disruption of normal tissue development is more frequent and more severe if fever occurs early in gestation there are reports of teratogenic effects in humans exposed to fever in mid-gestation.[137] In mice, suppressed neuronal migration and increased neuronal apoptosis was found in late term fetuses briefly exposed to heat.[97] Lastly, in situations where fetal oxygen supply is severely limited such as would occur in maternal fever, an

increase in body temperature may lead to birth injuries including neonatal sepsis, seizures, asphyxia during birth.[20,124]

Maternal febrigenesis may result in circulatory adjustments resulting in a shift in blood flow from organs crucial to fetal development (e.g. uterus and placenta), toward organs responsible for thermogenesis (e.g. brown adipose tissue).[13] The ensuing decrease in utero-placental perfusion could compromise placental gas exchange and reduce the amount of oxygen supplied to the fetus.[35] Additionally, during a febrile episode, fetal core temperature, which is between 0.5 to 0.9°C higher than maternal temperature,[1]not only increases in parallel,[92] but may actually exceed the rise in maternal core temperature.[122] Laburn *et al* have shown in several studies that during mild,[122]and severe hyper and hypo-thermia,[121]strategies employed by pregnant ewes to maintain homeostasis in response to thermal stress affect heat exchange between mother and fetus. If we accept that uterine arteries form part of the mother's peripheral vasculature, then for a given umbilical flow to the placenta vasodilation of the peripheral vasculature in response to an increased heat load (e.g. fever) will lead to the increased uterine blood flow. This in turn, decreases heat transfer from fetal to maternal circulation. To summarize then, during intrapartum fever, the threat to the fetus is threefold. First, fetal normal core temperature is relatively high. Second, the fetus' core temperature rises along with maternal temperature, and lastly the major route of fetal heat loss (i.e. utero-placental circulation) is compromised in the febrile episode. So, from an evolutionary standpoint, it is reasonable for fever to be avoided during the gestational period.

As has been discussed in previous sections, fever is part of the acute phase response to infection involving both increases in proinflammatory cytokines and in core

temperature. There is much evidence that cytokines released in fever cause perinatal morbidities independent of the associated hyperthermia. For example, Cai *et al* reported recently that prenatal LPS exposure induced IL-1β and TNF-α which caused abnormalities in the neonatal rat brain, particularly glia cells.[21] While Romero *et al* did not find that endotoxin could cross chorioamniotic membranes,[177] it is now clear that lipopolysaccharide released during Gram-negative bacterial infection can indeed permeate the placenta.[118] Eklind *et al* have demonstrated that low dose of endotoxin in neonatal rats dramatically sensitizes the immature brain to injury and induces cerebral infarction in response to short periods of hypoxia, this may occur via synthesis of maternally-derived endogenous pyrogens.[54] This has been hypothesized as the underlying mechanism explaining the increased risk of cerebral palsy in cases of intrapartum fever and chorioamnionitis.[184]

Clinically, it may be beneficial to suppress fever in the latter part of gestation so as not to set off a cascade of events leading to preterm labor. It is known that preterm labor is associated with elevated levels of proinflammatory cytokines in the amniotic fluid in humans.[180] For example, the risk of premature delivery is highest in women with increased plasma or amniotic IL-6, as compared to non-infected controls.[84] Following maternal systemic exposure to LPS, the endometrium may secrete proinflammatory cytokines which then act on the placenta to increase prostaglandin secretion from the decidual and amnion cells.[31] Elevated prostaglandins ultimately stimulate uterine contraction and parturition.[33,83] The health risks to the neonate born prematurely, especially when preterm labor is of infectious origin, are many and include pulmonary complications, (i.e. apneas, respiratory distress syndromes), and abnormal cardiovascular

function.[42,70,165,193] From a clinical perspective, limiting infectious processes, including fever, near the term of pregnancy is critical to avoiding perinatal morbidity and mortality associated with prematurity.

In closing, there is evidence for fever attenuation during pregnancy in many species, however the functional significance and the causal mechanisms have yet to be determined. For this reason, it would be beneficial to repeat the experiments described here in other species to establish whether the present findings are specific to altricial species like the rat, or if the conclusions are more broadly applicable. The fact that the pregnant mammal recruits several hormonal, immune, metabolic, and behavioral mechanisms in order to achieve antipyresis near term strongly suggest that this is an protective response of primary importance to the health and survival of offspring. The experiments outlined in this thesis have contributed to the understanding of the maternal adaptation to pregnancy, but it is clear that many questions remain unanswered.

CHAPTER EIGHT

BIBLIOGRAPHY

1. Abrams, R., D. Caton, L. B. Curet, C. Crenshaw, L. Mann, and D. H. Barron. Fetal brain-maternal aorta temperature differences in sheep. *American Journal of Physiology* 217: 1619-1622, 1969.

2. Alexander, H. R., G. M. Doherty, D. J. Venzon, M. J. Merino, D. L. Fraker, and J. A. Norton. Recombinant interleukin-1 receptor antagonist (IL-1ra): effective therapy against Gram-negative sepsis in rats. *Surgery* 112: 188-194, 1992.

3. Alexander, H. R., G. M. Doherty, D. J. Venzon, and J. A. Norton. A recombinant human receptor antagonist to interleukin-1 improves survival after lethal endotoxemia in mice. *Journal of Experimental Medicine* 173: 1029-1032, 1991.

4. Andrews, J. F., D. Richard, G. Jennings, and P. Trayhurn. Brown adipose tissue thermogenesis during pregnancy in mice. *Annals of Nutrition & Metabolism* 30: 87-93, 1986.

5. Arend, W. P. Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. *Journal of Clinical Investigation* 88: 1445-1451, 1991.

6. Arend, W. P., F. G. Joslin, R. C. Thompson, and C. H. Hannum. An IL-1 inhibitor form human monocytes. Production and characterization of biologic properties. *Journal of Immunology.* 143: 1851-1859, 1989.

7. Arend, W. P., M. Malyak, C. F. Bigler, M. F. Smith, Jr., and R. W. Janson. The biological role of naturally-occurring cytokine inhibitors. *British Journal of Rheumatology* 30 Suppl 2: 49-52, 1991.

8. Arend, W. P., M. Malyak, C. J. Guthridge, and C. Gabay. Interleukin-1 receptor antagonist: role in biology. *Annual Review of Immunology* 16: 27-55, 1998.

9. Banks, W. A. and A. J. Kastin. Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sci.* 48: PL117-PL121, 1991.

10. Bierhaus, A., J. Chen, B. Liliensiek, and P. P. Nawroth. LPS and cytokine-activated endothelium. *Seminars in Thrombosis and Hemostasis* 26: 571-587, 2003.

11. Blatteis and E. Sehic. Fever: How may circulating pyrogens signal the brain? *News in the Physiological Sciences* 12: 1-8, 1997.

12. Blatteis, C. M., J. R. Hales, A. A. Fawcett, and T. A. Mashburn, Jr. Fever and regional blood flows in wethers and parturient ewes. *Journal of Applied Physiology* 65: 165-172, 1988.

13. Blatteis, C. M., W. S. Hunter, D. W. Busija, Q. Llanos, R. A. Ahokas, and T. A. Mashburn, Jr. Thermoregulatory and acute-phase responses to endotoxin of full-term-pregnant rabbits. *Journal of Applied Physiology* 60: 1578-1583, 1986.

14. Blatteis, C. M. and E. Sehic. Cytokines and fever. [Review] [45 refs]. *Annals of the New York Academy of Sciences* 840: 608-618, 1998.

15. Blatteis, C. M., E. Sehic, and S. Li. Pyrogen sensing and signaling: Old view and new concepts. *Clinical Infectious Diseases* 31: 168-177, 2000.

16. Bluthe, R. M., R. Dantzer, and K. W. Kelly. Effects of interleukin-1 receptor antagonist on behavioral effects of lipopolysaccaride in the rat. *Brain Research* 573: 318-320, 1992.

17. Borovikova, L. V., S. Ivanova, M. Zhang, H. Yang, G. I. Botchkina, L. R. Watkins, H. Wang, N. Abumrad, J. W. Eaton, and K. J. Tracey. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405: 458-462, 2000.

18. Brown, K. M., A. V. Muchmore, and D. L. Rosenstreich. Uromodulin, an immunosuppressive glycoprotein derived from pregnant urine, is an inhibitor of interleukin-1. *Proceedings of the National Academy of Science* 83: 9119-9125, 1986.

19. Buijs, R. M. Immunocytochemical demonstration of vasopressin and oxytocin in the rat brain by light and electron microscopy. *Journal of Histochemical Cytochemistry* 28: 357-360, 1980.

20. Busto, R., W. D. Dietrich, M. Y. T. Glubus, I. Valdes, P. Scheinberg, and M. D. Ginsberg. Small differences in intraischaemic brain temperature critically determine the extent of ischaemic neuronal injury. *Journal of Cerebral Blood Flow & Metabolism* 7: 729-738, 1978.

21. Cai, Z., Z. R. Pan, Y. Pang, O. B. Evans, and P. G. Rhodes. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. *Pediatric Research* 47: 64-72, 2000.

22. Caldwell, F. T., Jr., D. B. Graves, and B. H. Wallace. Humoral versus neural pathways for fever production in rats after administration of lipopolysaccharide. *Journal of Trauma-Injury Infection & Critical Care* 47: 120-129, 1999.

23. Caldwell, J. D., E. R. Greer, M. F. Johnson, A. J. Prange, and C. A. Pedersen. Oxytocin and vasopressin immunoreactivity in hypothalamic and extrahypothalamic sites in late pregnancy and postpartum rats. *Neuroendocrinology* 46: 39-47, 1987.

24. Cannon, J. G., L. W. Abad, E. Vannier, and E. A. Lynch. Menstrual and gender-dependent variations in circulating IL-1 agonists, antagonists, and binding proteins. *Journal of Leukocyte Biology* 63: 117-123, 1998.

25. Cannon, J. G., Kwiatkowski, D, Endres, S, Lonnemann, G, Burke, J. F., and Dinarello, C. A. Interleukin-1 beta in human plasma: optomization of blood collection, plasma collection, and radioimmunoassay methods. *Lymphokine Research* 7(4), 457-467. 1988.

26. Cannon, W. B. Organization for physiological processes. *Physiology Reviews* 9: 399-431, 1929.

27. Cao, C., K. Matsumura, K. Yamagata, and Y. Watanabe. Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in rat brain; its possible role in the febrile response. *Brain Research* 697: 187-196, 1995.

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

29. Cao, C., K. Matsumura, K. Yamagata, Y. Watanabe. Involvement of cyclooxygenase-2 in LPS-induced fever and regulation of its mRNA in the rat brain. *American Journal of Physiology* 272: R1712-R1725, 1997

30. Carey, F., R. Forder, M. D. Edge, A. R. Greene, M. A. Horan, P. J. L. M. Strijbos, and N. J. Rothwell. Lipocortin 1 fragment modifies the pyrogenic actions of cytokines in the rat. *American Journal of Physiology* 259: R266-R269, 1990.

31. Casey, B. M. and S. M. Cox. Chorioamnionitis and endometritis. *Infectious Disease Clinics of North America* 11: 203-222, 1997.

32. Cefalo, R. C. and A. E. Hellegers. The effects of maternal hyperthermia on maternal and fetal cardiovascular and respiratory function. *Obstetrics & Gynecology* 131: 687-694, 1978.

33. Challis, J. R. G. Mechanism of parturition and preterm labor. *Obstetrical & Gynecological Survey* 55: 650-660, 2000.

34. Chen, X., R. Landgraf, and Q. J. Pittman. Differential ventral septal vasopressin release is associated with sexual dimorphism in PGE_2 fever. *American Journal of Physiology* 272: R1664-R1669, 1997.

35. Clark, K. E., M. Durnwald, and J. E. Austin. A model for studying chronic reduction in uterine blood flow in pregnant sheep. *American Journal of Physiology* 242: H297-H301, 1982.

36. Coelho, M. M., G. E. P. Souza, and I. R. Pela. Endotoxin-induced fever is modulated by endogenous glucocorticoids in rats. *American Journal of Physiology.* 263: R423-R427, 1992.

37. Contaldo F, Scalfi L, Coltorti A, Di Palo MR, Martinelli P, and Guerritore T. Reduced regulatory thermogenesis in pregnant and ovariectomized women. *International Journal of Vitamin & Nutrition Research* 57: 299-304, 1987.

38. Cooper, K. E. Fever and Antipyresis. Cambridge, Cambridge University Press. 1995.

39. Cooper, K. E., S. Blahser, T. J. Malkinson, G. Merker, J. Roth, and E. Zeisberger. Changes in body temperature and vasopressin content of brain neurons, in pregnant and non-pregnant guinea pigs, during fevers produced by Poly I:Poly C. *Pflugers Archiv - European Journal of Physiology* 412: 292-296, 1988.

40. Cooper, K. E., W. I. Cranston, and E. S. Snell. Temperature regulation during fever in man. *Clinical Science* 27: 345-356, 1964.

41. Cooper, K. E., A. M. Naylor, and W. L. Veale. Evidence supporting a role for endogenous vasopressin in fever suppression in the rat. *Journal of Physiology* 387: 163-172, 1987.

42. Curzi-Dascalova, L. and E. Christova-Gueoguieva. Respiratory pauses in normal prematurely born infants. A comparison with full-term newborns. *Biology of the Neonate* 44: 325-332, 1983.

43. Diana, A., A. M. Van Dam, B. Windblad, and M. Schultzberg. Co-localization of interleukin-1 receptor type I and interleukin-1 receptor antagonist with vasopressin in magnocellular neurons of the paraventricular and supraoptic nuclei of the rat hypothalamus. *Neuroscience* 89: 137-147, 1999.

44. Dinarello, C. A. Interleukin-1. *Reviews in Infectious Disease* 6: 51, 1984.

45. Dinarello, C. A. Endogenous pyrogens. The role of cytokines in the pathogenesis of fever. In Mackowiak, P. A., ed. Fever: Basic Mechanisms and Management. New York, Raven Press. 1991, 23-47.

46. Dinarello, C. A. Cytokines as endogenous pyrogens. *Journal of Infectious Disease* 179: S294-S304, 1999.

47. Dinarello, C. A. and Krueger, J. M. Induction of interleukin 1 by synthetic and naturally occurring muramyl peptides. *Federation Proceedings* 45, 2545-2548. 1986.

48. Dogan, M. D., H. Ataoglu, and E. S. Akarsu. Effects of different serotypes of *Escherichia coli* lipopolysaccharide on body temperature in rats. *Life Science* 67: 2319-2329, 2000.

49. Dupouy, J. P., H. Coffigny, and S. Magre. Maternal and fetal corticosterone levels during late pregnancy in rats. *Journal of Endocrinology.* 65: 347-352, 1975.

50. Dymond, K. E. and J. E. Fewell. Coordination of autonomic and behavioral thermoregulatory responses during exposure to a novel stimulus in rats. *American Journal of Physiology* 275: R673-R676, 1998.

51. Edouard, D. A., B. M. Pannier, G. M. London, J. L. Cuche, and M. E. Safar. Venous and arterial behavior during normal pregnancy. *American Journal of Physiology - Heart & Circulatory Physiology* 274: H1605-H1612, 1998.

52. Eguchi, N., H. Hayashi, Y. Urade, S. Ito, and O. Hayaishi. Central action of prostaglandin E_2 and its methyl ester in the induction of hyperthermia after systemic administration in urethane-anesthetized rats. *Journal of Pharmacology & Experimental Therapeutics* 247: 671-679, 1988.

53. Eisenberg, S. P., R. J. Evans, W. P. Arend, E. Verderber, M. T. Brewer, C. H. Hannum, and R. C. Thompson. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature* 343: 341-346, 1990.

54. Eklind, S., C. Mallard, A. Leverin, E. Gilland, K. Blomgren, I. Mattsby-Baltzer, and H. Hagberg. Bacterial endotoxin sensitizes the immature brain to hypoxic-ischaemic injury. *European Journal of Neuroscience* 13: 1101-1106, 2001.

55. Eliason, H. L. and Fewell, J. E. The decrease in body temperature near term of pregnancy in Sprague-Dawley rats is a regulated thermoregulatory response. *FASEB Journal* 10, A357. 1995.

56. Eliason, H. L. and J. E. Fewell. Influence of pregnancy on the febrile response to ICV administration of PGE_1 in rats studied in a thermocline. *Journal of Applied Physiology* 82: 1453-1458, 1997.

57. Eliason, H. L. and J. E. Fewell. Thermoregulatory control during pregnancy and lactation in rats. *Journal of Applied Physiology* 83: 837-844, 1997.

58. Eliason, H. L. and J. E. Fewell. AVP mediates the attenuated febrile response to administration of PGE_1 in rats near term of pregnancy. *American Journal of Physiology* 275: R691-R696, 1998.

59. Eliason, H. L. and J. E. Fewell. Arginine vasopressin does not mediate the attenuated febrile response to intravenous IL-1beta in pregnant rats. *American Journal of Physiology* 276: R450-R454, 1999.

60. Elmquist, J. K., T. E. Scammel, and C. B. Saper. Mechanisms of CNS response to systemic immune challenge: the febrile response. *TINS* 20: 565-570, 1997.

61. Endres, S., W. Vander Meer, and C. A. Dinarello. Interleukin-1 in the pathogenesis of fever. *European Journal of Clinical Investigation* 17: 469-474, 1987.

62. Feldberg, W. and Saxena, P. N. Prostaglandins, endotoxin, and lipid A on body temperature in the rat. *Journal of Physiology* 249, 601-615. 1975.

63. Ferretti, M., V. Casini-Raggi, T. T. Pizarro, S. P. Eisenberg, C. C. Nast, and F. Cominelli. Neutralization of endogenous IL-1 receptor antagonist exacerbates and prolongs inflammation in rabbit immune colitis. *Journal of Clinical Investigation* 94: 449-453, 1994.

64. Fewell, J. E. Body temperature regulation in rats near term of pregnancy. *Canadian Journal of Physiology & Pharmacology* 73: 364-368, 1995.

65. Fewell, J. E. and Eliason, H. L. Pregnancy impairs central prostaglandin release and the febrile response to intravenous IL-1beta in rats. *FASEB* Journal 15(A1105). 2001.

66. Fewell, J. E., H. L. Eliason, and R. N. Auer. Peri-OVLT E-series prostaglandins and core temperature do not increase following intravenous IL-1β in pregnant rats. *Journal of Applied Physiology* 93: 531-536, 2002.

67. Fewell, J. E. and P. A. Tang. Pregnancy alters body-core temperature response to a simulated open field in rats. *Journal of Applied Physiology* 82: 1406-1410, 1997.

68. Fidel, P. L., R. Romero, M. Ramirez, J. Cutright, S. S. Edwin, S. LaMarche, D. B. Cotton, and M. D. Mitchell. Interleukin-1 receptor antagonist (IL-1ra) production by human amnion, chorion, and decidua. *American Journal of Reproductive Immunology (Copenhagen)* 32: 1-7, 1994.

69. Filkins, J. P. Blood endotoxin activation after endotoxicosis. *Proceedings of the Society for Experimental Biology and Medicine* 151, 89-92. 1976.

70. Fleming, P. J., A. C. Bryan, and M. H. Bryan. Functional immaturity of pulmonary irritant receptors and apnea in newborn preterm infants. *Pediatrics* 61: 515-518, 1978.

71. Flower, R. J. Lipocortin and the mechanism of action of the glucocorticoids. *British Journal of Pharmacology* 65: 897-1015, 1988.

72. Fyda, D. M., K. E. Cooper, and W. L. Veale. Contribution of brown adipose tissue to central PGE_1-evoked hyperthermia in rats. *American Journal of Physiology* 260: R59-R66, 1991.

73. Gaykema, R. P., L. E. Goehler, M. K. Hansen, S. F. Maier, and L. R. Watkins. Subdiaphragmatic vagotomy blocks interleukin-1beta-induced fever but does not reduce IL-1beta levels in the circulation. *Autonomic Neuroscience-Basic & Clinical* 85: 72-77, 2000.

74. Gibbs, R. S., R. Romero, S. L. Hiller, D. A. Eschenbach, and R. L. Sweet. A review of premature birth and subclinical infection. *American Journal of Obstetrics & Gynecology* 166: 1515-1528, 1992.

75. Gluckman, P. D., S. V. Sizonenko, and N. S. Bassett. The transition from fetus to neonate--an endocrine perspective. *Acta Paediatrica* Supplement. 88: 7-11, 1999.

76. Goehler, L. E., J. K. Relton, D. Dripps, R. Kiechle, N. Tartaglia, S. F. Maier, and L. R. Watkins. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Research Bulletin* 43: 357-364, 1997.

77. Goldbach, J., J. Roth, and E. Zeisberger. Fever suppression by subdiaphragmatic vagotomy in guinea pigs depends on the route of pyrogen administration. *American Journal of Physiology* 272: R675-R681, 1997.

78. Gordon, C. J. A review of terms for regulated vs. forced neurochemical-induced changes in body temperature. *Life Science* 32: 1285-1295, 1983.

79. Gordon, C. J. Thermal biology of the laboratory rat. *Physiology of Behavior* 47: 963-991, 1990.

80. Gordon, C. J. Temperature Regulation in Laboratory Rodents. Cambridge, Cambridge University Press. 1993, 1-275.

81. Gordon, C. J. Twenty-four hour rhythms of selected ambient temperature in rat and hamster. *Physiology of Behavior* 53: 257-263, 1993.

82. Granowitz, E. V., A. A. Santos, D. D. Poutsiaka, J. G. Cannon, D. W. Wilmore, S. M. Wolff, and C. A. Dinarello. Production of interleukin-1 receptor antagonist during experimental endotoxaemia. *Lancet* 338: 1423-1424, 1991.

83. Gravett, M. G., S. S. Witkin, G. J. Haluska, J. L. Edwards, M. J. Cook, and M. J. Novy. An experimental model for intraamniotic infection and preterm labor in rhesus monkeys. *American Journal of Obstetrics & Gynecology* 171: 1660-1667, 1994.

84. Greig, P. C., A. P. Murtha, C. J. Jimmerson, W. N. P. Herbert, B. Roitman-Johnson, and J. Allen. Maternal serum interleukin-6 during pregnancy and during term and preterm labor. *Obstetrics & Gynecology* 90: 465-469, 1997.

85. Hammel, H. T. Regulation of internal body temperature. *Annual Reviews in Physiology* 30: 641-710, 1968.

86. Hannum, C. H., C. J. Wilcox, W. P. Arend, F. G. Joslin, D. J. Dripps, P. L. Heimdal, L. G. Armes, A. Sommer, S. P. Eisenberg, and R. C. Thompson. Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 343: 336-340, 1990.

87. Hansen, M. K., S. Daniels, L. E. Goehler, R. P. Gaykema, S. F. Maier, and L. R. Watkins. Subdiaphragmatic vagotomy does not block intraperitoneal lipopolysaccharide-induced fever. *Autonomic Neuroscience-Basic & Clinical* 85: 83-87, 2000.

88. Hansen, M. K. and J. M. Krueger. Subdiaphragmatic vagotomy blocks the sleep- and fever-promoting effects of interleukin-1beta. *American Journal of Physiology* 273: R1246-R1253, 1997.

89. Hansen, M. K., K. T. Nguyen, M. Fleshner, L. E. Goehler, Gaykema, RP, S. F. Maier, and L. R. Watkins. Effects of vagotomy on serum endotoxin, cytokines, and corticosterone after intraperitoneal lipopolysaccharide. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 278: R331-R336, 2000.

90. Hansen, M. K., K. T. Nguyen, L. E. Goehler, R. P. Gaykema, Fleshner, M, S. F. Maier, and L. R. Watkins. Effects of vagotomy on lipopolysaccharide-induced brain interleukin-1beta protein in rats. *Autonomic Neuroscience-Basic & Clinical* 85: 119-126, 2000.

91. Hansen, M. K., K. A. O'Connor, L. E. Goehler, L. R. Watkins, Maier, and SF. The contribution of the vagus nerve in interleukin-1beta-induced fever is dependent on dose. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 280: R929-R934, 2001.

92. Harris, W. H., Q. J. Pittman, W. L. Veale, K. E. Cooper, and G. R. van Petten. Cardiovascular effects of fever in the ewe and fetal lamb. *American Journal of Obstetrics & Gynecology* 128: 262-265, 1977.

93. Hashimoto, I., D. M. Henricks, L. L. Anderson, and R. M. Melampy. Progesterone and pregn-4-en-20 alpha-ol-3-one in ovarian venous blood during various reproductive states in the rat. *Endocrinology* 82: 333-341, 1968.

94. Hashimoto, M., T. Ueno, and M. Iriki. What roles does the organum vasculosum laminae terminalis play in fever in rabbits? *Pflugers Archives-European Journal of Physiology* 429: 50-57, 1994.

95. Heap, R. B., A. Silver, and D. E. Walters. Effects of pregnancy on the febrile response in sheep. *Quarterly Journal in Experimental Physiology* 66: 129-144, 1981.

96. Hebra, A., P. Strange, J. M. Egbert, M. Ali, A. Mullinax, and E. Buchanan. Intracellular cytokine production by fetal and adult monocytes. *Journal of Pediatric Surgery* 36: 1321-1326, 2001.

97. Hinoue, A., S. Fushiki, Y. Nishimura, and K. Shiota. In utero exposure to brief hyperthermia inteferes with the production and migration of neocortical neurons and induces apoptotic neuronal death in the fetal mouse brain. *Developmental Brain Research* 132: 59-67, 2001.

98. Hirsch, E., R. Blanchard, and S. P. Mehta. Differential fetal and maternal contributions to the cytokine milieu in a murine model of infection-induced preterm birth. *American Journal of Obstetrics & Gynecology* 180: 429-434, 1999.

99. Hirsch, E., V. M. Irikura, S. M. Paul, and D. Hirsh. Functions of interleukin 1 receptor antagonist in gene knockout and overproducing mice. *Proceedings of the National Academy of Science* 93: 11008-11013, 1996.

100. Hockaday, T. D. R., W. I. Cranston, K. E. Cooper, and R. F. Mottram. Temperature regulation in chronic hypothermia. *Lancet* 428-432, 1962.

101. Hori, T., T. Kiyohara, T. Nakashima, K. Mizuno, H. Muratani, and T. Katafuchi. Effects of temperature and neuroactive substances on hypothalamic neurones in vitro: Possible implications for the induction of fever. *Physiology Research* 41: 77-81, 1992.

102. Hori, T., M. Shibata, T. Nakashima, M. Yamasaki, and etal. Effects of interleukin-1 and arachidonate on the preoptic and anterior hypothalamic neurons. *Brain Research Bulletin* 20: 75-82, 1988.

103. Hull, D., J. McIntyre, and J. Vinter. Maternal fever at parturition and its effects on the newborn rabbit. *Journal of Developmental Physiology* 18: 87-92, 1992.

104. Imai-Matsumara, K., K. Matsumura, A. Morimoto, and T. Nakayama. Suppression of cold-induced thermogenesis in full-term pregnant rats. *Journal of Physiology* 425: 271-281, 1990.

105. Imai-Matsumara, K., K. Matsumura, A. Terao, and Y. Watanabe. Attenuated fever in pregnant rats is associated with blunted syntheses of brain cyclooxygenase-2 and PGE_2. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 283: R1346-R1353, 2002.

106. Imai-Matsumara, K., K. Matsumura, A. Terao, Y. Watanabe, A. Mouihate, K. Clerget-Froideaux, M. Nakamura, J. L. Wallace, and Q. J. Pittman. Near term suppression of fever: inhibited synthesis of accelerated catabolism of

prostaglandin E_2? *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 284: R860-R865, 2003.

107. Ivanov, A. I., A. C. Scheck, and A. A. Romanovsky. Expression of genes controlling transport and catabolism of prostaglandin E_2 in lipopolysaccharide fever. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 284: R698-R706, 2002.

108. Jones, S. A., Adamson, S. L., Bishai, I., Lees, J., Engelberts, D., and Coceani, F. Eicosanoids in third ventricular cerebrospinal fluid of fetal and newborn sheep. *American Journal of Physiology* 264, R135-R142. 1993.

109. Kapas, L., M. K. Hansen, H. Y. Chang, and J. M. Krueger. Vagotomy attenuates but does not prevent the somnogenic and febrile effects of lipopolysaccharide in rats. *American Journal of Physiology* 274: R406-R411, 1998.

110. Kasting, N. W. Criteria for establishing a physiological role for brain peptides. A case in point: The role of vasopressin in thermoregulation during fever and antipyresis. *Brain Research Reviews* 14: 143-153, 1989.

111. Kasting, N. W., W. L. Veale, and K. E. Cooper. Evidence for a centrally active, endogenous antipyretic near parturition. Current Studies of Hypothalamic Function. Part II. *Metabolism and Behavior* 63-71, 1978.

112. Kasting, N. W., W. L. Veale, and K. E. Cooper. Suppression of fever at term of pregnancy. *Nature* 271: 245-246, 1978.

113. Kasting, N. W. and M. F. Wilkinson. Vasopressin functions as an endogenous antipyretic in the newborn. *Biology of the Neonate* 51: 249-254, 1987.

114. Kluger, M. J. Fever: Its Biology, Evolution and Function. 1979. Princeton, Princeton University Press.

115. Kluger, M. J. Is fever beneficial? *Yale Journal of Biology & Medicine* 59: 89-95, 1986.

116. Kluger, M. J., W. Kozak, C. A. Conn, L. R. Leon, and D. Soszynski. The adaptive value of fever. *Infectious Disease Clinics of North America* 10: 1-20, 1996.

117. Kluger, M. J., W. Kozak, L. R. Leon, D. Soszynski, and C. A. Conn. Cytokines and fever. [*Neuroimmunomodulation* 2: 216-223, 1995.

118. Kohmura, Y., T. Kirikae, M. Nakano, and T. Sato. Lipopolysaccharide induced intrauterine fetal death (IUFD) in mice is principally due to maternal cause but not fetal sensitivity to LPS. *Microbiology and Immunology* 44: 897-904, 2000.

119. Krunic, N., S. L. Adamson, C. Ackerley, R. T. Okita, and F. Coceani. Perinatal changes in choroidal 15-hydroxyprostaglandin dehydrogenase: implications for prostaglandin removal from brain. *Brain Research- Developmental Brain Research.* 121: 145-155, 2000.

120. Kullander, S. Fever and parturition. An experimental study in rabbits. *Acta Obstetricia et Gynecologica Scandinavica - Supplement* 77-85, 1977.

121. Laburn, H. P., K. Goelst, and D. Mitchell. Body temperature of lambs and their mothers measured by radio-telemetry during parturition. *Experientia* 50: 708-711, 1994.

122. Laburn, H. P., D. Mitchell, and K. Goelst. Fetal and maternal body temperatures measured by radiotelemetry in near-term sheep during thermal stress. *Journal of Applied Physiology* 72: 894-900, 1992.

123. Landgraf, R., I. Neumann, and Q. J. Pittman. Septal and hippocampal release of vasopressin and oxytocin during late pregnancy and parturition in the rat. *Neuroendocrinology* 54: 378-383, 1991.

124. Laptook, A. R., R. J. T. Corbett, R. Sterett, D. K. Burns, G. Tollefsbol, and D. Garcia. Modest hypothermia provides partial neuroprotection for ischemic neonatal brain. *Pediatric Research* 35: 436-442, 1994.

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

126. Long, N. C., A. J. Vander, S. L. Kunkel, and M. J. Kluger. Antiserum against tumor necrosis factor increases stress hyperthermia in rats. *American Journal of Physiology* 258: R591-R595, 1990.

127. Luderitz, O., C. Galanos, V. Lehmann, K. Nurminen, E. T. Reitschel, G. Rosenfelder, Simon M., and O. Westphal. Lipid A: chemical structure and biological activity. *Investigations in Infectious Disease* 128: 17-29, 1973.

128. Lugarini, F., B. J. Hrupka, G. J. Schwartz, C. R. Plata-Salaman, and W. Langhans. A role for COX-2 in LPS-induced anorexia in rats. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 283: R862-R868, 2002.

129. Luheshi, G. N. Cytokines and fever. Mechanisms and sites of action. *Annals of the New York Academy of Sciences* 856: 83-89, 1998.

130. Luheshi, G. N., R. M. Bluthe, D. Rushforth, N. Mulcahy, J. P. Konsman, M. Goldbach, and R. Dantzer. Vagotomy attenuates the behavioural but not the pyrogenic effects of interleukin-1 in rats. *Autonomic Neuroscience-Basic & Clinical* 85: 127-132, 2000.

131. Lundkvist, J., A. K. Sundgren-Anderson, S. Tingsborg, P. Ostlund, C. Engfors, K. Alheim, and et al. Acute-phase responses in transgenic mice with CNS overexpression of IL-1 receptor antagonist. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 276: R644-R651, 1999.

132. Macaulay, J. H., N. R. Randall, K. Bond, and P. J. Steer. Continuous monitoring of fetal temperature by noninvasive probe and its relationship to maternal temperature, fetal heart rate, and cord arterial oxygen and pH. *Obstetrics & Gynecology* 79: 469-474, 1992.

133. Mantovani, A., C. A. Dinarello, and P. Ghezzi. Pharmacology of Cytokines. New York, Oxford University Press. 2000.

134. Martin, S. M., T. J. Malkinson, W. L. Veale, and Q. J. Pittman. Changes in the pyrogen induced febrile response in pre and post parturient rats. *Physiology Canada* 70: AXVI, 1992.

135. Martin, S. M., T. J. Malkinson, W. L. Veale, and Q. J. Pittman. Fever in pregnant, parturient, and lactating rats. *American Journal of Physiology* 268: R919-R923, 1995.

136. Martin, S. M., T. J. Malkinson, W. L. Veale, and Q. J. Pittman. Prostaglandin fever in rats throughout the estrous cycle late pregnancy and post parturition. *Journal of Neuroendocrinology* 8: 145-151, 1996.

137. Martinez-Frias, M. L., M. J. G. Mazario, C. F. Caldas, M. P. C. Gallego, E. Bermejo, and E. Roderiguez-Pinilla. High maternal fever during gestation and severe congenital limb disruptions. *American Journal of Medical Genetics* 98: 201-203, 2001.

138. Matsuda, T., T. Hori, and T. Nakashima. Thermal and PGE_2 sensitivity of the organum vasculosum lamina terminalis region and preoptic area in rat brain slices. *Journal of Physiology* 454: 197-212, 1992.

139. Matsumura, K., Y. Watanabe, H. Onoe, Y. Watanabe, and O. Hayaishi. High density of prostaglandin E_2 binding sites in the anterior wall of the 3rd ventricle: a possible site of its hyperthermic action. *Brain Research* 533: 147-151, 1990.

140. Matthiesen, L., M. Khademi, C. Ekerfelt, G. Berg, S. Sharma, T. Olsson, and J. Ernerudh. In situ detection of both inflammatory and anti-inflammatory cytokines in resting peripheral blood mononuclear cells during pregnancy. *Journal of Reproductive Immunology* 58: 49-59, 2003.

141. Mayeux, P. R. Pathobiology of lipopolysaccharide. *Journal of Toxicology & Environmental Health* 51: 415-435, 1997.

142. McClellan, J. L., J. J. Klir, L. E. Morrow, and M. J. Kluger. Central effects of glucocorticoid receptor antagonist RU-38486 on lipopolysaccharide and stress-induced fever. *American Journal of Physiology* 267: R705-R711, 1994.

143. McCracken, S. A., C. L. Drury, H.-S. Lee, and J. M. Morris. Pregnancy is associated with suppression of the nuclear factor kappa B/I kappa B activation pathway in peripheral blood mononuclear cells. *Journal of Reproductive Immunology* 58: 27-47, 2003.

144. Medawar, P. B. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. *Symposia of the Society for Experimental Biology* 7: 320-328, 1953.

145. Merker, G., S. Blahser, and E. Zeisberger. Reactivity pattern of vasopressin-containing neurons and its relation to the antipyretic reaction in the pregnant guinea pig. *Cell & Tissue Research* 212: 47-61, 1980.

146. Meyer, M. C., J. E. Brayden, and M. K. McLaughlin. Characteristics of vascular smooth muscle in the maternal resistance circulation during pregnancy in the rat. *American Journal Obstetrics & Gynecology* 169: 1510-1516, 1993.

147. Milton, A. S. and S. Wendlandt. A possible role for prostaglandin E_1 as a modulator for temperature regulation in the central nervous system of the cat. *Journal of Physiology* 207: 76P-77P, 1970.

148. Milton, A. S. and S. Wendlant. Effect on body temperature of prostaglandins of the A, E and F series on injection into the third ventricle of anesthetized cats and rabbits. *Journal of Physiology* 218: 325-336, 1971.

149. Morimoto, A., K. Morimoto, Y. Watanabe, Y. Sakata, and N. Murakami. Does an increase in prostaglandin E_2 in the blood circulation contribute to a febrile response in rabbits? *Brain Research Bulletin* 29: 189-192, 1992.

150. Morishima, H. O., B. Glaser, W. H. Niemann, and L. S. James. Increased uterine activity and fetal deteriorization during maternal hyperthermia. *American Journal of Obstetrics & Gynecology* 121: 531-538, 1975.

151. Morishima, H. O., W. H. Niemann, and L. S. James. Effects of endotoxin on the pregnant baboon fetus. *American Journal of Obstetrics & Gynecology* 131: 899-902, 1978.

152. Morishima, H. O., M.-N. Yeh, W. H. Niemann, and L. S. James. Temperature gradient between fetus and mother as an index for assessing intrauterine fetal condition. *American Journal of Obstetrics & Gynecology* 129: 443-448, 1977.

153. Morrow, L. E., J. L. McClellan, C. A. Conn, and M. J. Kluger. Glucocorticoids alter fever and IL-6 responses to psychological stress and to lipopolysaccharide. *American Journal of Physiology* 264: R1010-R1016, 1993.

154. Mouihate, A., Chen, X. and Q. J. Pittman. Interleukin-1beta fever in rats: gender difference and estrous cycle difference. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 275: R1450-R1454, 1998.

155. Mouihate, A., M. S. Clerget-Froidevaux, K. Nakamura, M. Negeshi, J. L. Wallace, and Q. J. Pittman. Suppression of fever at near term is associated with reduced COX-2 protein expression in rat hypothalamus. *American Journal of Physiology* 283: R800-R805, 2002.

156. Mouihate, A. and Q. J. Pittman. Neuromimmune response to endogenous and exogenous pyrogens is differently modulated by sex steroids. *Endocrinology* 144: 2454-2460, 2003.

157. Mrosovsky, N. Programmed rheostasis. Rheostasis: The Physiology of Change. New York, Oxford University Press. 1990, 33-76.

158. Mrosovsky, N. Reactive rheostasis. Rheostasis. Oxford, Oxford University Press. 1990, 77-116.

159. Murakami, N. and T. Ono. Sex related dfferences in fever development of rats. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 252: 284-289, 1987.

160. Naccarato, E. F. and W. S. Hunter. Brain and deep abdominal temperatures during induced fever in pregnant rabbits. *American Journal of Physiology* 245: R421-R425, 1983.

161. Naeye, R. L. Underlying disorders responsible for the neonatal deaths associated with low APGAR scores. *Biology of the Neonate* 35: 150-155, 1979.

162. Naylor, A. M., K. E. Cooper, and W. L. Veale. Vasopressin and fever: evidence supporting the existence of an endogenous antipyretic system in the brain. *Canadian Journal of Physiology & Pharmacology* 65: 1333-1338, 1987.

163. Oka, T. and T. Hori. EP1-receptor mediation of prostaglandin E2-induced hyperthermia in rats. *American Journal of Physiology* 36: R289-R294, 1994.

164. Okita, R. T. and J. R. Okita. Prostaglandin-metabolizing enzymes during pregnancy: characterization of NAD(+) dependent prostaglandin dehydrogenase,

carbonyl reductase, and cytochrome P450-dependent prostaglandin omega-hydroxylase. *Critical Review of Biochemistry and Molecular Biology* 31: 101-126, 1996.

165. Periman, J. M. and J. J. Volpe. Episodes of apnea and bradycardia in the preterm newborn: Impact on cerebral circulation. *Pediatrics* 76(3): 333-338, 1985.

166. Petrova, A., K. Demissie, G. G. Rhoads, J. C. Smulian, S. Marcella, and C. V. Ananth. Association of maternal fever during labor with neonatal and infant morbidity and mortality. *Obstetrics & Gynecology* 98: 20-27, 2001.

167. Pillay, V., N. Savage, and H. Laburn. Interleukin-1 receptor antagonist in newborn babies and pregnant women. *Pflugers Archives - European Journal of Physiology* 424: 549-551, 1993.

168. Pittman, Q. J., Chen.X., A. Mouihate, M. Hirasawa, and S. Martin. Arginine vasopressin, fever and temperature regulation. *Prog.Brain Res.* 119: 383-392, 1998.

169. Reznikov, L. L., G. A. Fantuzzi, C. H. Selzman, B. D. Shames, H. A. Barton, J. A. McGregor, H. Bell, and C. A. Dinarello. Utilization of endoscopic inoculation in a mouse model of intrauterine infection-induced preterm birth: role of interleukin-1beta. *Biology of Reproduction* 60: 1231-1238, 1999.

170. Reznikov, L. L., B. D. Shames, H. A. Barton, C. H. Selzman, G. A. Fantuzzi, S.-H. Kim, S. M. Johnson, and C. A. Dinarello. Interleukin-1β deficiency results in reduced NF-kB levels in pregnant mice. *American Journal of Physiology - Regulatory Integrative & Comparative Physiology* 278: 263-270, 2000.

171. Romanovsky, A. A., A. I. Ivanov, and E. K. Karman. Blood-borne, albumin-bound prostaglandin E2 may be involved in fever. *American Journal of Physiology* 276: R1840-R1844, 1999.

172. Romanovsky, A. A., A. I. Ivanov, M. J. Lenczowski, V. A. Kulchitsky, A. M. Van Dam, S. Poole, L. D. Homer, and F. J. Tilders. Lipopolysaccharide transport from the peritoneal cavity to the blood: is it controlled by the vagus nerve? *Autonomic Neuroscience-Basic & Clinical* 85: 133-140, 2000.

173. Romanovsky, A. A., O. Shido, S. Sakurada, N. Sugimoto, and T. Nagasaka. Endotoxin shock: thermoregulatory mechanisms. *American Journal of Physiology* 270: R693-R703, 1996.

174. Romanovsky, A. A., O. Shido, S. Sakurada, N. Sugimoto, and T. Nagasaka. Endotoxin shock hypothermia. How and why does it occur? *Annals of the New York Academy of Sciences* 15: 733-737, 1997.

175. Romanovsky, A. A., C. T. Simons, M. Szekely, and V. A. Kulchitsky. The vagus nerve in the thermoregulatory response to systemic inflammation. *American Journal of Physiology* 273: R407-R413, 1997.

176. Romero, R., R. Gomez, M. Galasso, M. Mazor, S. M. Berry, R. A. Quintero, and D. B. Cotton. The natural interleukin-1 receptor antagonist in the fetal, maternal, and amniotic fluid compartments: the effect of gestational age, fetal gender, and intrauterine infection. *American Journal of Obstetrics & Gynecology* 171: 912-921, 1994.

177. Romero, R., D. Lafrenier, G. W. Duff, N. Kadar, S. Durum, and J. C. Hobbins. Failure of endotoxin to cross the chorioamniotic membranes in vitro. *American Journal of Perinatology* 4: 360-362, 1987.

178. Romero, R., M. Mazor, Y. K. Wu, M. Sirtori, E. Oyarzun, D. M. Murray, and J. C. Hobbins. Infection in the pathogenesis of preterm labor. *Seminars in Perinatology* 12: 262-279, 1988.

179. Romero, R., J. Nores, M. Mazor, and et al. Microbial invasion of the amniotic cavity during term labor, prevalence and clinical significance. *Journal of Reproductive Medicine* 38: 543-548, 1993.

180. Romero, R., R. Quintero, E. Oyarzun, Y. K. Wu, V. Sabo, M. Mazor, and J. C. Hobbins. Intraamniotic infection and the onset of labor in preterm premature rupture of the membranes. *American Journal of Obstetrics &.Gynecology* 159: 6661-666, 1988.

181. Rotondo, D., H. T. Abul, A. S. Milton, and J. Davidson. Pyrogenic immunomodulators increase the level of prostaglandin E_2 in the blood simultaneously with the onset of fever. *European Journal of Pharmacology* 154: 145-152, 1988.

182. Saito, S. Cytokine network at the feto-maternal interface. *Journal of Reproductive Immunology* 47: 87-103, 2000.

183. Seckinger, P., J. W. Lowenthal, K. Williamson, J.-M. Dayer, and H. R. MacDonald. A urine inhibitor of interleukin-1 activity that blocks ligand binding. *Journal of Immunology* 139: 1546-1549, 1987.

184. Shalak, L. F., A. R. Laptook, H. S. Jafri, O. Ramilo, and J. M. Perlman. Clinical chorioamnionitis, elevated cytokines, and brain injury in term infants. *Pediatrics* 110: 673-680, 2002.

185. Simrose, R. L. and J. E. Fewell. Body temperature response to IL-1 beta in pregnant rats. *American Journal of Physiology* 269: R1179-R1182, 1995.

186. Smagula, R. M., H. van Halbeek, J. M. Decker, A. V. Muchmore, C. E. Moody, and A. P. Sherblom. Pregnancy associated changes in oligomannose oligosaccharides of human and bovine uromodulin. *Glycoconjugate Journal* 7: 609-624, 1990.

187. Splawinski, J. A., Zacny, E., and Gorka, Z. Fevers in rats after intravenous *E. coli* endotoxin administration. *Pflugers Archive* - European Journal of Physiology 368, 125-128. 1977.

188. Stewart, H. L. Oral basal temperatures and diagnosis of pregnancy. *West.J.Surg.Gynecol.Obstet.* 57: 192-200, 1949.

189. Stitt, J. T. Fever vs. hyperthermia. *Federation Proceedings* 38: 39-43, 1979.

190. Stitt, J. T. Prostaglandin E as the neural mediator of the febrile response. *Yale J.Biol.Med.* 59: 137-149, 1986.

191. Stitt, J. T. and S. G. Shimada. A comparison of the febrile response of the Brattleboro and Sprague-Dawley strains of rats to endotoxin and endogenous pyrogens. *Canadian Journal of Physiology & Pharmacology.* 65: 1377-1381, 1987.

192. Stobie-Hayes, K. M. and J. E. Fewell. Influence of pregnancy on the febrile response to intracerebroventricular administration of PGE_1 in rats. *Journal of Applied Physiology* 81: 1312-1315, 1996.

193. Storrs, C. N. Cardiovascular effects of apnoea in preterm infants. *Arch.Dis.Child.* 52: 534-540, 1977.

194. Strijbos, P. J., A. J. Hardwick, J. K. Relton, F. Carey, and N. J. Rotherwell. Inhibition of central actions of cytokines on fever and thermogenesis by lipocortin-1 involves CRF. *American Journal of Physiology* 263: E632-E636, 1992.

195. Szekely, M. and Z. Szelenyi. Endotoxin fever in the rat. *Acta Physiol.Acad.Sci.Hung.* 53: 265, 1979.

196. Tan, S. W. and Fewell, J. E. Interleukin-1 receptor antagonist neutralizing antibody alters lipopolysaccharide induced fever in adult female rats. *FASEB Journal* 13, A1060. 1999.

197. Tang, P. A., J. E. Fewell, and H. L. Eliason. Role of AVP in mediating the altered core temperature response to a simulated open field in pregnant rats. *Journal of Applied Physiology* 87: 170-174, 1999.

198. Tatro, J. B. Endogenous antipyretics. [Review] [105 refs]. *Clinical Infectious Diseases* 31 Suppl 5: S190-S201, 2000.

199. Ushikubi, F., E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ichikawa, T. Tanaka, N. Yoshida, and S. Narumiya. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP_3. *Nature* 395: 281-284, 1998.

200. van Dam, A. M., H. E. de Vries, J. Kuiper, F. J. Zijlstra, A. G. de Boer, F. J. H. Tilders, and F. Berkenbosch. Interleukin-1 receptors on rat brain endothelial cells: a role in neuroimmune interaction? *FASEB Journal* 10: 351-356, 1996.

201. van Rooijen, J. J. M., P. Hermentin, J. P. Kamerling, and J. F. G. Vliegenthart. The patterns of complex and oligosaccharide type glycans of uromodulin (Tamm-Horsefall glycoprotein) in the course of pregnancy. *Glycoconjugate Journal* 18: 539-546, 2001.

202. Vaughn, L. K., W. L. Veale, and K. E. Cooper. Impaired thermoregulation in pregnant rabbits at term. *Pflugers Archives - European Journal of Physiology* 378: 185-187, 1978.

203. Villarroya, F., A. Felipe, and T. Mampel. Sequential changes in brown adipose tissue composition, cytochrome oxidase activity and GDP binding throughout pregnancy and lactation in the rat. *Biomed.Biochim.Acta* 993: 187-191, 1986.

204. Villarroya, F. and T. Mampel. Changes in brown adipose tissue lipoprotein lipase activity and lipogenesis rate during pregnancy and lactation in the rat. *Biochem.Int.* 13: 511-519, 1986.

205. Watanabe, Y., Y. Watanabe, and O. Hayaishi. Quantitative autoradiographic localization of prostaglandin E2 binding sites in monkey diencephalon. *Journal of Neuroscience* 8: 2003-2010, 1988.

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

207. Watkins, L. R., S. F. Maier, and L. E. Goehler. Cytokine-to-brain communication: a review and analysis of alternative mechanisms. *Life Science* 57: 1011-1026, 1995.

208. Wegmann, T., H. Lin, L. Guilbert, and T. Mosmann. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunology Today* 14: 353-356, 1993.

209. Wilkinson, M. F., T. F. Horn, N. W. Kasting, and Q. J. Pittman. Central interleukin-1 beta stimulation of vasopressin release into the rat brain: activation of an antipyretic pathway. *Journal of Physiology* 481: 641-646, 1994.

210. Zeisberger, E., G. Mercker, and S. Blahser. Fever response in the guinea pig before and after parturition. *Brain Research* 212: 379, 1981.

211. Armant, M.A. Fenton M.J. Toll-like receptors: a family of pattern recognition in mammals (Review). *Genome Biology* 29:3-8, 2002.

212. Espinoza J, Chaiworapongsa T, Romero R, Gomez R, Kim JC, Yoshimatsu J, Edwin S, Rathnasabapathy C, Yoon BH. Evidence of participation of soluble CD14 in the response to microbial invasion of the amniotic cavity and intra-amniotic inflammation in term and preterm gestation. *Journal of Maternal Fetal and Neonatal Medicine* 12: 304-312, 2002.

213. Heumann, D. Roger T. Initial responses to endotoxins and G ram Negative bacteria. *Clin Chim Acta* 323: 59-72, 2002.

214. Holmlund U, Cebers G, Dahlfors AR, Sandstedt B, Bremme K, Ekstrom ES, Scheynius A. Expression and regulation of the pattern recognition receptors Toll-like receptor-2 and Toll-like receptor-4 in the human placenta. *Immunology* 107: 145-151, 2002.