[Frontiers in Bioscience 9, 3068-3084, September 1, 2004]

THERMAL HOMEOSTASIS IN SYSTEMIC INFLAMMATION: MODULATION OF NEURONAL MECHANISMS

Ladislav Jansky [1] and Stanislav Vybiral [2]

[1] Faculty of Biology, University of South Bohemia, Budweis, [2] Faculty of Science, Charles University, Prague, Czech Republic

TABLE OF CONTENTS

1. ABSTRACT

Fever can be defined as a specific model of body temperature control, modified by action of humoral substances released due to bacterial infection. Under laboratory conditions exogenous and endogenous pyrogens affect nervous endings in the body periphery, as well as thermosensitive neurons in the hypothalamus, which first manifests as a shock reaction and then as shifts of temperature thresholds for activation of thermoregulatory effectors (cold thermogenesis, vasomotion, sweating or panting) to higher body temperatures. During the later phase of fever, the temperature threshold for cold thermogenesis starts to move downwards, while the thresholds for other thermoregulatory outputs remain elevated, the result being enlargening of the interthreshold zone. This creates conditions for cooling of the body and for termination of the fever. During different phases of fever cytokines, prostaglandins, neuropeptides and catecholamines participate in modulation of mechanisms regulating thermoregulatory functions. This paper aims to specify the role of individual cytokines in induction of fever, as well as in activation of thermoregulatory centers as well as individual thermoregulatory effectors and to define differences in their mode of action. The paper further attempts to summarize our knowledge on humoral modulation of the cytokine release. It is concluded that cytokines are not the primary factors responsible for seting of the body thermostat during fever.

2. INTRODUCTION: FEVER AS A THERMOREGULATORY MODEL

During the early phase of fever the functions of the hypothalamic regulator controling thermal homeostasis are altered and set to work at higher levels of body temperature. This reveals as a shift of temperature thresholds for induction of all thermoregulatory effectors (cold thermogenesis, vasomotion, sweating or panting) to higher body temperatures (Figure 1B) (1). As evident from the unchanged slopes of curves, the change in the set-point is neither due to lowered temperature sensitivity of the controling system, or due to lowered efficiency of individual thermoregulatory effectors (for details see [2] in this volume). Thus, the febrile state is not a sign of a thermoregulatory deficiency, but rather a result of modulation of activity of thermoregulatory centres and functions. Therefore, fever can be characterized as a specific thermoregulatory model, which is modified by action of exogenous and endogenous pyrogens on nervous endings in the body periphery, as well as on thermosensitive neurons in the hypothalamus. Pyrogens appear in the circulation as a result of invasion of microbes and subsequent activation of the immune system.

Cytokines are very important pyrogenic substances found in the blood during endotoxin fever. A complex activation of the immune cascade due to different cytokines creates conditions for a complex modulation of thermoregulatory mechanisms. During the late phase of fever other substances, namely neuropeptides, may also play a role (see 2 and below) in influencing the thresholds. Neuropetides may shift the threshold for cold thermogenesis to lower body temperatures, thus enlarging the interthreshold zone (1, 3, 4). (Figure 1C) Although many humoral substances may modulate activities of the hypothalamic body temperature controller, it is presumed that their effect is not exerted directly, but rather by means of the prostaglandin E_2 (5, 6). Findings showing that LPS, when injected directly into the hypothalamus, induces fever (7, 8 and others), that peripheral IL-1 increases PGE_2 levels in the hypothalamus and in the blood (9, 10) and that LPS induces release of PGE_2 from brain slice preparations (11) support this view. It should be kept in mind, however, that direct injections of LPS into the brain may rather induce brain inflammation than fever (8).

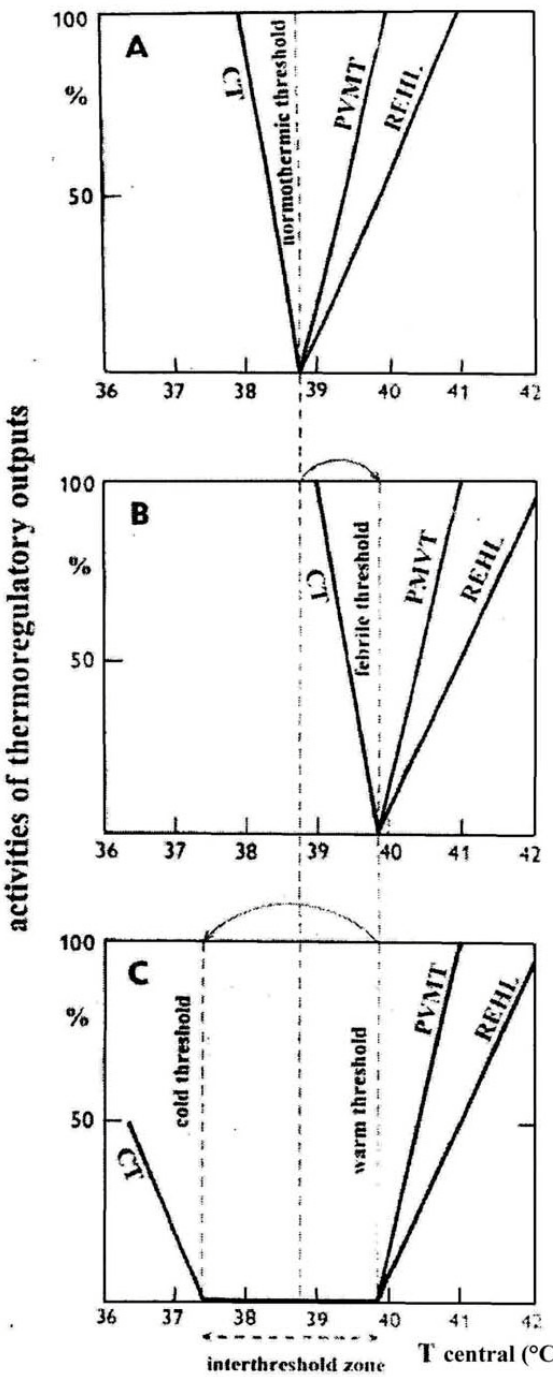

Figure 1. Scheme of activation of thermoregulatory centers and effectors in normal rabbits (above-A), febrile rabbits during the early phase of fever (middle-B) and during the late of fever (below-C), showing an upward shift of the threshold and no changes in hypothalamic sensitivity and activity of individual thermoregulatory outputs (CT=cold thermogenesis, PVMT=peripheral vasomotor tone, REHL=respiratory evaporative heat loss) during the early phase of fever and dissociation of the thresholds during the late phase of fever (1, 3). The slope of the relationship between central body temperature and activities of individual thermoregulatory outputs denotes hypothalamic thermosensitivity.

3. TIME COURSE OF CHANGES IN BODY TEMPERATURE DURING LPS FEVER

Numerous studies on fever came out from the supposition that fever induced experimentally due to application of exogenous pyrogens (LPS) might correspond to the fever induced under natural conditons by microbial infection. This supposition is only partially valid, because under natural conditions the intensity of infection and its duration vary from case to case. Thus, the LPS fever should be considered as a model of bacterial infection, which only simulates the true infection, since it is a nonproliferating bacterial component from gram-negative bacteria, whereas many infections involve both gram-negative and gram-positive bacteria. Nevertheless, a conclusion can be made that under laboratory conditions manifestation of fever depends on the concentration of endotoxin (LPS) applied. While lower doses of LPS usually induce a monophasic fever, higher doses induce a biphasic fever, characterized by partial and temporal lowering of the elevated body temperature 90–120 min after LPS application and by longer lasting time course of the fever (3). Romanovsky *et al*. even suggested that the LPS fever could be polyphasic (12, 13). Thus, it can be expected that during different phases of fever, thermal homeostasis is being controlled differently as a result of action of various humoral substances, namely by action of cytokines, neuropeptides and prostaglandins.

Several proinflammatory cytokines are pyrogenic (namely IL-1β, IL-6, TNF-α, IFNγ) and to specify their role in the febrile process, several questions should be answered:

1. Does a certain cytokine play a dominant role in inducing fever, or are all cytokines equally effective?

2. What is the time sequence of participation of individual cytokines in regulation of the febrile response?

3. Do all cytokine exert the same, or a different mode of action on body temperature control?

4. Can their effect be potentiated by action of different humoral substances, namely by other cytokines?

5. Are individual cytokines the final substances inducing changes in the thermoregulatory set point during fever?

4. CHANGES IN CYTOKINE PRODUCTION DURING LPS FEVER

4.1. *In vivo* studies

Several authors described marked increases in concentration of cytokines in the blood and in the brain of control and LPS- treated animals and humans (for details see 14). Evidence from these earlier works can be summarized as follows: The only cytokine present in plasma of nonfebrile animals was IL-6. During fever, the concentrations of TNF-α increased faster than that of IL-6, but IL-6 concentrations reached higher levels and these high levels persisted for a longer time than levels of TNF-α. No IL-1 β was detectable in the blood of control and LPS-treated animals.

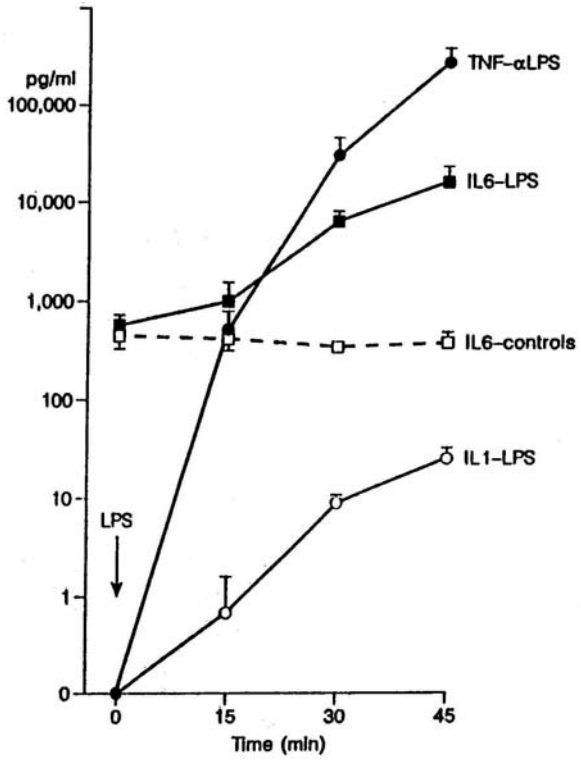

Figure 2. Concentrations of cytokines in plasma of control (interrupted line) and LPS-treated (solid lines) guinea pigs. No TNF-α and IL-1β were detectable in the blood of control animals. LPS was applied at time 0. Mean values of 6 experiments performed on 6 different animals. The vertical bars indicate the standard error. From (24).

In earlier papers description of the detailed time course of changes in cytokine concentrations in the blood and in the brain was not performed. Furthermore, measurements of cytokine concentrations in the brain have been generally performed in the late stage of the fever, when the body termostat was already set up to the higher level of body temperature. Thus, data in the literature did not allow conclusion about the specific role of individual cytokines in induction of the febrile response. Therefore, we attempted to measure cytokine concentrations in the blood and in the cerebrospinal fluid within the first 45-60 min after injection of LPS (14). Results indicated that in guinea pigs, concentration of TNF-α in plasma increased markedly from zero up to around 200 ng/ml. IL-1β concentrations also increased immediately after injection of LPS to reache values corresponding to about 50 pg/ml. IL-6 was present in the blood in nonfebrile animals in a considerable amount (approx.800 pg/ml) and increased about ten times after LPS. (Figure 2) Although the increases in concentrations of IL-1β and TNF-α were detectable 15 min after injection of LPS already, until the 45th min they did not reach the steady state level. The increase in concentration of IL-6 was slower and was found significant 30 min after LPS injection, only.

In the cerebrospinal fluid, TNF-α was present in highest concentrations (50-100 pg were collected during a 15 min perfusion period), both in controls and LPS injected animals. Thirty min after LPS injection concentration of TNF-α increased by about 200%. (Figure 3) IL-6 appeared in the cerebrospinal fluid earlier than TNF-α (15 min after LPS injection), but evidently too late to be responsible for vasoconstriction occuring immediately after LPS injection (see 3 and Figure 9). No IL-1β was detectable in the cerebrospinal fluid of control and LPS-treated animals.

Data on blood levels of cytokines after LPS injection obtained on guinea pigs are in consent with the data obtained on other mammalian species (for literature see 14). On the basis of this evidence we conclude, that the presence of cytokines in the hypothalamus may not be a critical factor for the immediate onset of the febrile response, such as vasomotion and panting (see Figure 9), which occurs within 5-10 min after injection of LPS, already. Cytokines may play a role in increasing the temperature threshold for induction of thermoregulatory effectors during the subsequent phase of the fever (30 min after injection – see Figure 8), however. Furthermore, from the fact that IL-6 is present in the blood of nonfebrile animals we conclude that in guinea pigs the role of IL-6 in induction of changes in thermoregulatory thresholds may not be crucial.

4.2. *In vitro* studies

Cytokine production in response to LPS can be easily studied under *in vitro* conditions using isolated human peripheral blood mononuclear cells (PBMC). This methodical approach has certain advantages, namely that it simulates the *in vivo* situation better than isolated cells or subpopulations of lymphocytes. General characteristics of the cytokine response to LPS in PBMC have been described by several authors already (15-20 and others). The detailed time course of cytokine production has been neglected, however. In consent with the previously published data our recent experiments showed that human PBMC stimulated by LPS increased IL-1β and TNF-α production considerably (up to 220 pg/ milion of cells) (21). Production of IL-6 was about 1/3 of that of other cytokines, only. Production of these cytokines started immediately after LPS stimulation to reach maximum within 4 hours. Production of IL-1β and TNF-α remained increased for at least 24 hod. On the other hand, production of IL-6 seemed to decline after 4 hours already. (Figure 4) IFN-γ production started to increase later that productions of IL-1β, TNF-α and IL-6, while production of IL-10 started to increase after 12 hours and increased steady for at least 24 hours. Production of IL-12 was not influenced (21).

Data suggest that IL-1β and TNF-α could be designated as the most early and the most important inducers of processes leading to induction of febrile responses, while IL-6 might play a smaller role. Evidently, since IL-1β and TNF-α are being produced by monocytes and macrophages predominantly (22, 23), these subpopulations of PBMC appeared to be activated in the first place after LPS. Since IFN-γ is being produced by NK and T cells mostly (24), its slightly delayed release may

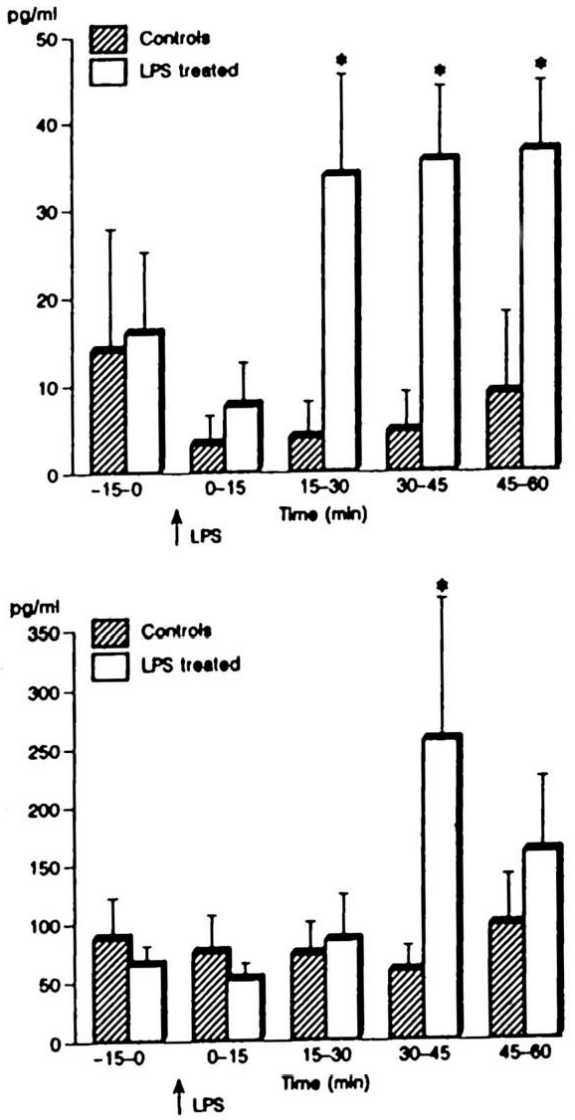

Figure 3. Concentrations of IL-6 (above) and TNF-α (below) in the cerebrospinal fluid of control (hatched columns) and LPS treated (white columns) guinea pigs. Samples were collected by the push-pull technique during 15 min periods. LPS was applied at time 0. Mean values of 6 experiments performed on 6 different animals. The vertical bars indicate the standart error. Asterisks denote significant differences. From (24).

indicate that not only the early, but also the later phases of the immune response are activated under experimental conditions used. IFN -γ may further potentiate the IL-1β release. Since IL-10 is known to act as an antipyretic substance (25, 26), its delayed production could represent a negative feedback mechanism restricting the IL-1β and TNFα releases under *in vivo* conditions.

Increased production of cytokines after LPS stimulation may persist for more than 24 hours. This long lasting effect of LPS may indicate their very strong binding to CD 14 and TLR4 receptors (27-29), or an absence of processes degradating cytokines under *in vitro* conditions.

5. TIME COURSE OF CHANGES IN BODY TEMPERATURE DURING CYTOKINE FEVER

Hyperthermic effects of peripherally and centrally administered cytokines have been demonstrated repeatedly within the last 20 years in many laboratories (for review see 29). In our recent paper (29), the effect of TNF-α, IL-6 and IL-1β on body temperature changes was studied in rabbits. It was found that TNF-α (1 μg.kg $^{-1}$), when applied **intravenously** at thermoneutral conditions, induced a long lasting monophasic fever response. Hypothalamic temperature reached maximal values within 40 min and then got stabilized for at least 4 h. On the other hand, IL-6 when applied i.v. in the same dose (1 μg.kg $^{-1}$) and IL-1β, in a much lower dose (60 ng.kg $^{-1}$), induced monophasic fevers lasting less than two hours, only. (Figure 5) Endotoxin (LPS), administered peripherally induced greater increase in hypothalamic temperature than the cytokines used.

These data support earlier observations that all cytokines studied are pyrogenic. Data may further indicate that IL-1β is the most potent fever inducer, being effective in ng doses, while TNF-α and IL-6 give similar responses in μg doses. On the other hand, since the increased body temperature after IL-1β and 1L-6 persisted for about 2 hours only, while the effect of TNF-α persisted for at least 3 hours, data indicate that TNF-α is also an important pyrogenic substance. Data further show that under *in vivo* conditions IL-1β and 1L-6 get degradated faster than TNF-α.

When injected **intrahypothalamically**, all cytokines induced a long lasting increase in body temperature, similar to that after i.v. injection of a low dose of LPS (1 μg/kg), with the exception that the effect of hypothalamic cytokines was somewhat delayed (Figure 5). IL-1β appeared to be more effective than other cytokines, similarly as after peripheral application. Thus, the direct mode of action of all cytokines studied on the hypothalamic system controlling thermal homeostasis appears to be very similar and, hence, unspecific. The similarity of responses induced by cytokines and by LPS may indicate that the hypothalamic mode of action of all these substances is mediated by similar signalling pathways, as suggested by several authors (25, 26).

Finally, it should be stated that it is presumed that cytokines may substitute, or potentiate mutually each other in their actions on hypothalamic thermoregulatory centers. Because of that the real physiological role of individual cytokines in induction of fever is difficult to assess on the basis of the above experiments.

The long lasting effect of peripheral TNF-α and of central TNF-α, IL-1β and IL-6 is striking. In a separate experiment we even observed that the solitary i.h. application of IL-6 induced a steady increase in body temperature lasting at least 6 hours (Vybíral, unpublished).

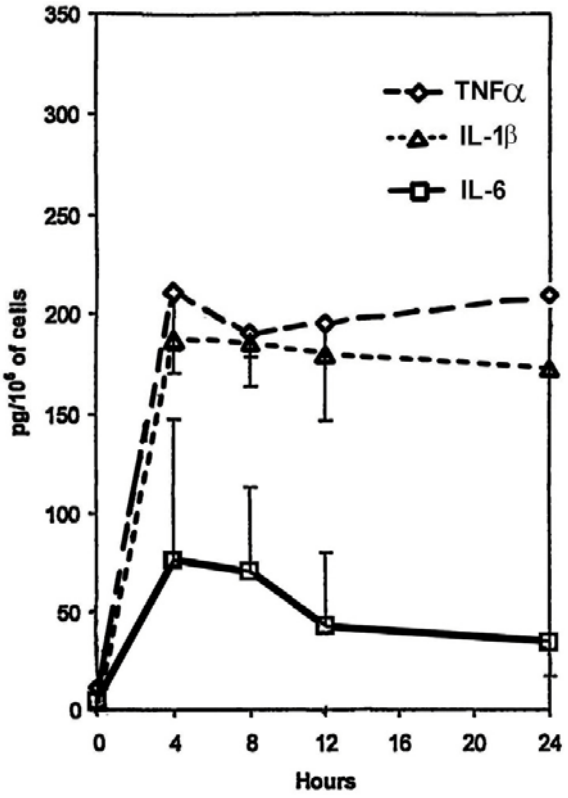

Figure 4. Time courses of releases of TNF-α, IL-6, IL-1β from human PBMC due to application of LPS. The vertical bars indicate the standart error. Redrawn on the basis of the data (31).

Since the half life of cytokines in circulation is considered to be very short (about 5 min in case of IL-6), these observations may either indicate very strong binding of cytokines to their receptors, or, more probably, the absence of processes, which, during the LPS fever, eliminate cytokines from the blood or from the cerebrospinal fluid. Whether the long lasting effect of cytokines is due to absence of natural antipyretic substances (AVP, ACTH, α-MSH) (for review see 2), which, during the LPS fever, can overcome the action of cytokines on hypothalamic control centers, or due to absence of processes eliminating cytokines from the blood during the cytokine fever, is to be clarified.

6. CHANGES IN ACTIVITY OF THERMOREGULATORY EFFECTORS AND CENTERS DURING CYTOKINE FEVER

The method of intestinal cooling and warming, which enables to activate thermoregulatory effectors during fever, can define changes in thermoregulatory thresholds and in functional capacity of thermoregulatory effectors, as well as modifications of apparent hypothalamic thermosensitivity. Thus, this method can also analyze the time course of changes in activities of mechanisms regulating thermal homeostasis after administration of cytokines (for details about the method see 2).

As it was shown above in this paper, TNF-α (1 μg.kg $^{-1}$), when applied **intravenously** to rabbits at thermoneutral conditions, induced a long lasting monophasic fever (Figure 5) (29). Figure 6 shows that this response was induced by intensive and long lasting attenuation of panting and by transient increase in vasoconstriction (skin temperature returned to the preinjection level within 100 min). Metabolic rate tended to increase by about 20 % during the early phase of the fever, only (not shown here). IL-1 administration gave a similar response. On the other hand, i.v. applied IL-6 (1 μg.kg $^{-1}$) induced a short lasting hyperthermia, only (Figure.5). This was achieved by transient attenuation of panting and by transient vasoconstriction, both lasting about 50 min (29). (Figure 6) No significant increase in metabolic rate was observed after IL-6 and no shift of thermoregulatory thresholds could be detected (see below). Thus, peripherally applied IL-6 induced hyperthermia just by preventing heat loss from the body and not by increasing metabolic rate.

The immediate attenuation of panting and induction of vasoconstriction after i.v. administration of cytokines, which manifests within few seconds, rather resembled a reflex (shock) response than specific thermoregulatory reactions. This represents an argument for the view that peripheral cytokines may first induce an immediate fever-like response by activating nervous endings of afferent nervous fibres in the body periphery than by acting on the hypothalamus (see below).

Further it was found that peripherally applied TNF-α and IL-1β modulated activity of thermoregulatory centers within 30 min. after i.v. administration, which revealed as an upward shift of the temperature threshold for cold thermogenesis (29) (Figure 7). In contrast to TNF-α and IL-1β, the i.v. applied IL-6 did not influence the threshold for cold thermogenesis as well as that for heat loss mechanisms neither 30 min, or 180 min after injection. Thus, a slight hyperthermia induced by peripheral administration of IL-6 can be realized without influencing the body temperature controler in the hypothalamus. These data support the view that effect of peripheral IL-6 on thermoregulation is different from that of other cytokines and of LPS.

Three hours after i.v. injection of IL-1β the threshold for cold thermogenesis as well as body temperature returned to the preinjection level, while the effect of TNF-α was still persisting, indicating different ways of degradation of these cytokines.

When injected **intrahypothalamically** all cytokines induced a long lasting increase in body temperature and shifts in temperature thresholds for induction of cold thermogenesis (Figures 5 and 7). Similarly as after peripheral injection, IL-1β induced the hyperthermic effect in lower concentrations than IL-6 and TNF-α. The thresholds remainded elevated at least 3 hours after injection. No changes in hypothalamic thermosensitivity were observed after IL-1β and IL 6, but after TNF-α small attenuation of thermosensitivity seemed to occur (29).

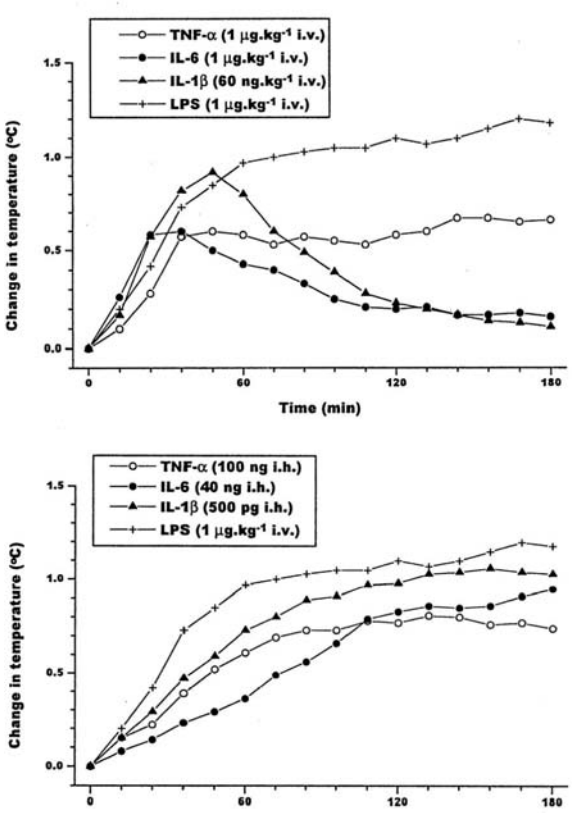

Figure 5. Time course of changes in hypothalamic temperatures after i.v. administration of LPS and after i.v. (above) and i.h. (below) application of TNFα, IL-1β and IL-6. (n= 5-7). From (62).

Since cytokines, when applied i. h. in concentration used, do not induce a biphasic fever response, it is tempting to conclude that hypothalamic cytokines induce a different kind of febrile response than peripherally applied LPS. (LPS induces biphasic fever and widening of the interthreshold zone during the late phase of the fever [1, 3, 7] due to action of antipyretic neuropeptides [30-33 and others]).

Furthermore, since the direct effect of all cytokines studied on central control of thermal homeostasis appears to be very similar, it may be concluded that the effect of these substances is not specific from the thermoregulatory point of view and may be a result of neuronal irritation due to local inflammation.

Because of these limitations the relative role of individual cytokines in modulating neuronal activities under physiological conditions is difficult to assess.

7. CHANGES IN ACTIVITY OF THERMOREGULATORY EFFECTORS AND CENTERS DURING LPS FEVER

It was found that in rabbits peripheral application of LPS first induces instant vasoconstriction and attenuation of panting, while cold thermogenesis is not influenced (3) (Figure 8).Thus, the very early initiation of febrile response is due to attenuation of mechanisms regulating heat loss and not due to activation of heat production. Furthermore, the fact that the immediate febrile responses, which preceed increases in concentrations of cytokines in the brain (see above), may be supportive for the conclusion that initiation of the febrile response is rather mediated by activation of peripheral nervous endings and subsequent activation of vasomotor and respiratory centers in the medulla oblongata than by activation of specific thermoregulatory pathways and centers in the hypothalamus.

The view, that the very rapid onset of the febrile response may involve a neuronal rather than a humoral mechanism of peripheral signaling, was originally proposed by Blatteis and Sehic (34). Their theory is based on the evidence that subdiaphragmatic vagotomy eliminates the increase in body temperature induced by LPS and the concomittant increase in PGE_2 in the hypothalamic area (35), blocks release of IL-1β in the brain after peripheral administration of LPS (36) and prevents IL-1β to induce hyperthermia (37). Furthermore, it was found that injection of the IL-1β into the portal vein increases electrical activity of the vagus and that the IL-1β receptors are localized to hepatic branches of the vagus nerve (34). These findings were interpreted as meaning that LPS, by means of complement activation, increases release of cytokines from Kupffer cells, which, subsequently, activates afferent vagal fibers. This conveys the pyrogenic message to the nucleus tractus solitarius in the brain stem and via noradrenergic bundle affects the preoptic area in the hypothalamus to stimulate the local release of PGE_2 (34). This appears to be a long process, however, which can hardly explain the immediate vasoconstriction and suppression of panting occuring in rabbits within seconds after LPS injection (Figure 8). It seems to be more probable, therefore, that a direct stimulation of afferent vagal fibres by LPS, rather than by cytokines, may be responsible for the „shock reaction“ preceeding the actual febrile response. Findings that injections of LPS into the subcutaneous pouch induce fever responses in absence of any cytokines in the blood or cerebrospinal fluid support this view (38). This theory does not exclude the possibility that peripheral cytokines may also activate the vagal afferents and by means of the hypothalamic PGE_2 , increase temperature thresholds for induction of thermoregulatory effectors, as it typically appears during the first phase of the fever. Neither the direct effect of cytokines on hypothalamic centers can be excluded.

During the late phase of the fever, the increased level of IL-1β in the blood activates the hypothalamo-pituitary-adrenal axis (39-47) and the concentrations of ACTH and other neuropeptides in the blood may increase. IL-6 induces a similar effect, but the detailed mode of action of IL-6 is probably different than that of IL-1β (46). ACTH and other neuropeptides (AVP, α-MSH) may then exert an antipyretic effect (30-33), perhaps by influencing thermosensitive neurons, or by releasing cytokines. There is evidence that administration of α-MSH analogues reduces the release of IL-1β and TNF-α (32). The increased production of antipyretic neuropeptides may induce a downward

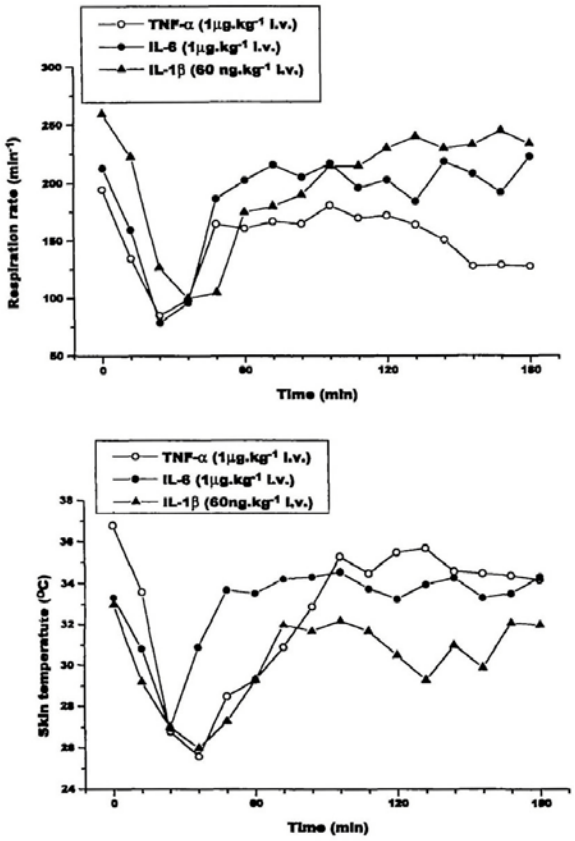

Figure 6. Time course of changes in skin temperature and respiration rate of rabbits after i.v. administration of IL-6, IL-1β and TNF-α. (n=5-7). Redrawn on the basis of the data (62).

shift of the threshold for cold thermogenesis to the prefebrile level, while the thresholds for vasomotion and panting remain temporarily increased, the result being enlargement of the interthreshold zone (48, 49) (for details see 2). Thus, during the late phase of the fever animals become insensitive to relatively large changes in their body temperatures. Body temperature changes within this range are, therefore, a consequence of the passive heat exchange between body and environment. Further it is assumed that when microbial infection disappears and concentrations of endogenous pyrogens in the circulation decrease, the thresholds for heat loss mechanisms also return to the normal level and fever ceases. Mechanisms controling the very late phases of the fever (12, 13) remain unclear, however.

8. EFFECT OF PYRETIC AND ANTIPYRETIC SUBSTANCES ON ACTIVITY OF THERMOSENSITIVE NEURONS

Effect of pyrogenic cytokines on warm sensitive, cold sensitive and temperature insensitive hypothalamic neurons was partially reviewed elsewhere in this volume (27). All data so far published (50-56) seem to indicate that cytokines decrease firing rates of warm sensitive neurons and increase that of temperature insensitive and cold sensitive neurons. A lowered thermosensitivity after IL-1β was also observed. Shibata and Blatteis (57) found that the response of individual neurones treated with different cytokines were not exactly the same, however. They concluded that different cytokines may rather affect distinct neurons functionally connected to common pyrogenic effectors. Thus, different neuronal pathways may be ulitized by each cytokine to exert their pyrogenic effects.

Data also suggests that the action of cytokines requires local synthesis and release of prostaglandins from endothelian and other cells. Hori *et al*. have shown on brain slice preparations that LPS can release PGE_2 (9). Our data on the effect of prostaglandin E_2 on neuronal activity in hypothalamic slices of the rat also documented lowered activity of warm sensitive neurons and increased activity of thermoinsensitive neurons (58), similarly as it was observed by other authors after administration of cytokines (Figure 9). All this may suggest that the effect of cytokines on thermoregulatory centers could be mediated via action of prostaglandins.

In consent with the above data, Moravec and Pierau have found that an antipyretic neuropeptide - arginine vasopressin (AVP) increased activity of warm sensitive neurons and changed their thermosensitivity (59). Our data on the subject were reviewed (60). Recently, it was suggested that the effect of cytokines on hypothalamic neurons could be mediated by purinergic receptors (61).

Modulation of activity of hypothalamic thermoregulatory centers by humoral substances cannot be studied by direct methods on intact animals. Therefore, attempts have been made to clarify the effect of pyrogens on hypothalamic centers using different models of body temperature control. The model of Bligh (62, for details see 63) appears to be the most appropriate for elucidating basic hypothalamic processes controling body temperature in nonfebrile subjects. (Figure 10) This model is based on the presumption that neurons in the preoptic area of the anterior hypothalamus (POAH) receive inputs from warm and cold sensors located in the POAH, or in other parts of the body. The model further presumes that the warm or cold signals are transmitted by separate, but **interconnected pathways**, consisting of interneurones, which are temperature insensitive. The specific feature of this regulatory system is that the „warm“ and „cold“ pathways can **reciprocally inhibit** each other. As deep body temperature increases, activity of „warm“ pathways is elevated and activity of „cold“ pathways is depressed, the final result being activation of heat loss mechanisms. In cold exposed subjects, in contrast, „cold“ pathways are activated, while the „warm“ ones are inhibited, so that the heat production mechanisms are set into action.

In febrile subjects, modulation of thermoregulatory functions should be expected. The following scheme is being suggested to describe the sites of action of pyrogenic substances: During the first phase of the fever, when the set point of the body temperature controler is elevated, an inhibitory effect of endogenous pyrogens both on the warm and the cold pathways behind the point of crossing inhibition (points A) must be anticipated to explain increases the thresholds both for heat loss and heat production mechanisms.

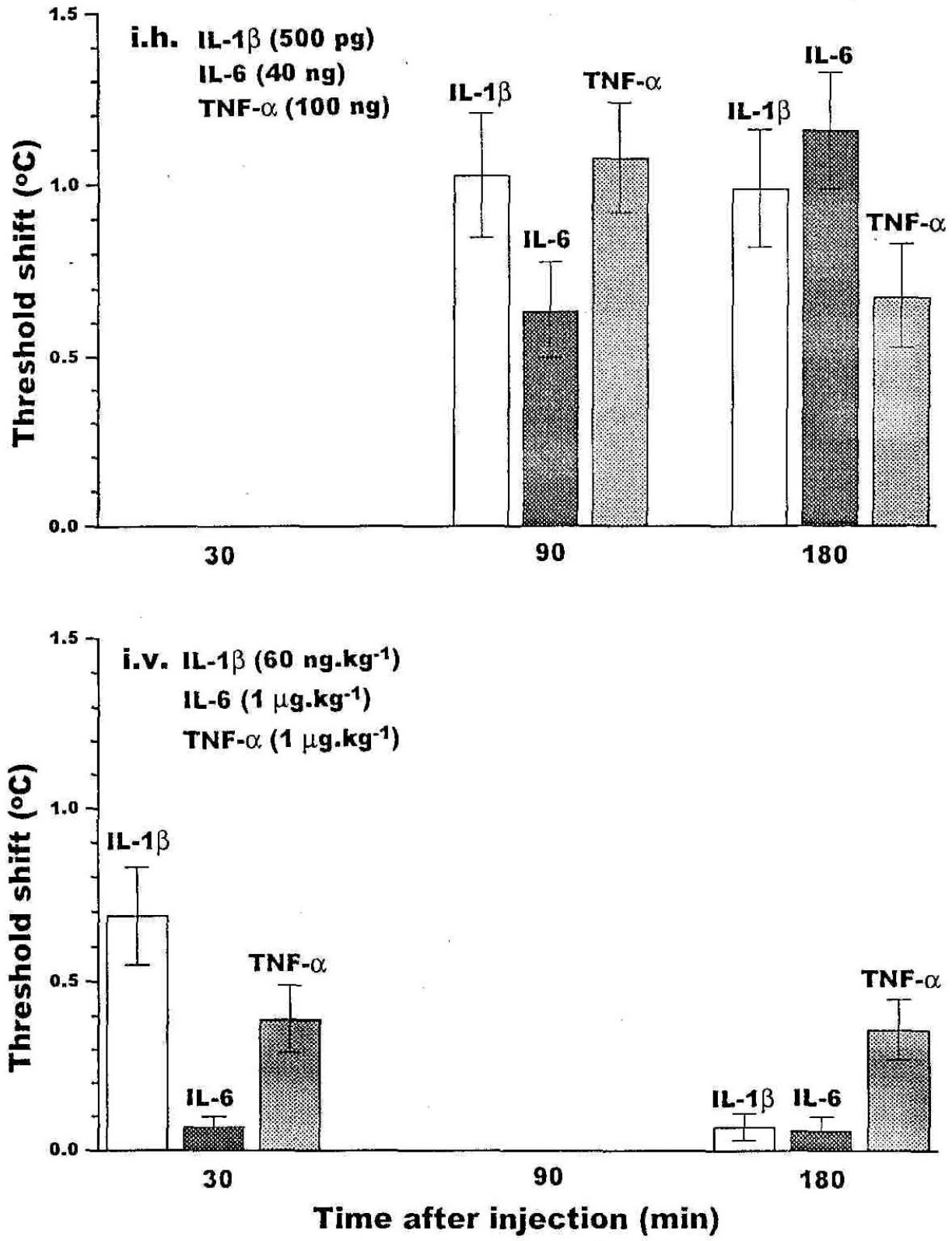

Figure 7. Shifts of the temperature threshold for cold thermogenesis in rabbits 30, 90 and 180 min after i.h. (above), or i.v. (below) injections of different cytokines in concentrations used. The vertical bars indicate the standart error From (62).

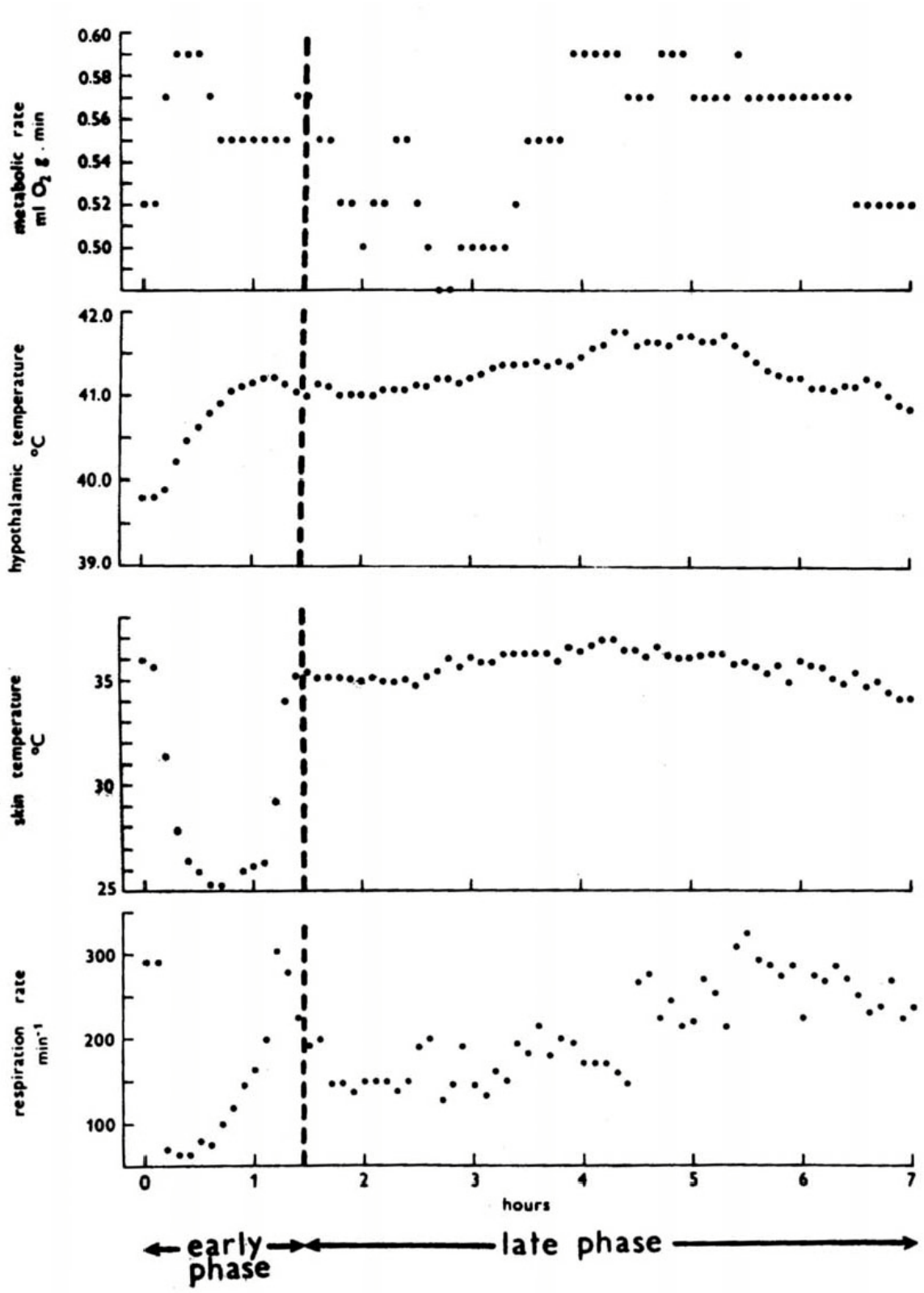

Figure 8. Time course of changes in hypothalamic temperature, respiration rate, skin temperature and metabolic rate of rabbits after i.v. administration of LPS to rabbits. Data show nonsignificant changes in heat production and rapid changes in vasomotion and panting during the early phase of the fever. During the late phase of the fever the increased hypothalamic temperature persisted due to attenuation of panting. A typical experiment. From (3).

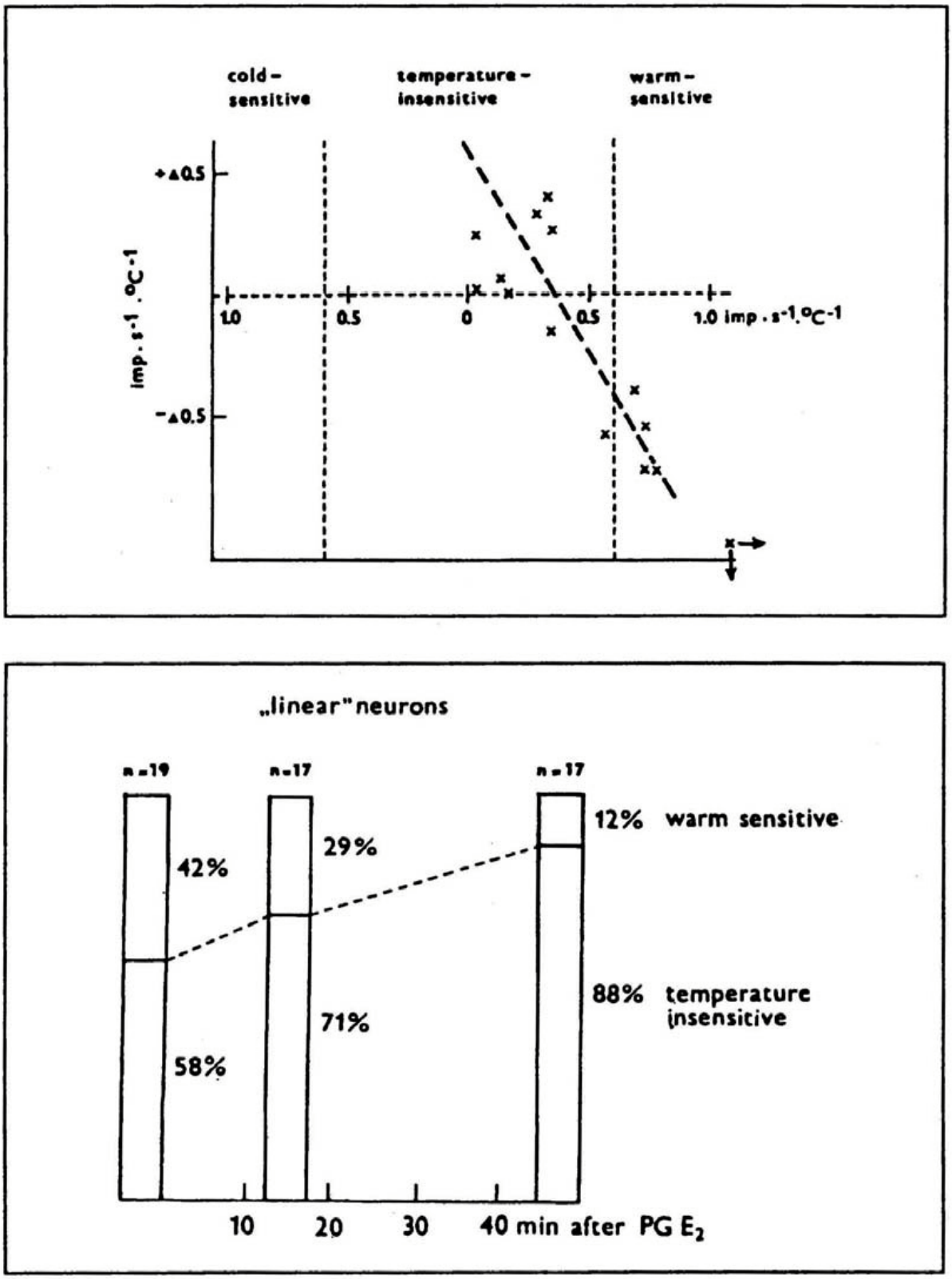

Figure 9. Relative decrease in firing rates of warm sensitive neurones and increase in firing rates of thermoinsensitive neurones in hypothalamic slices from the rat after administration of PGE_2 (above). Lower part of the figure shows relative participation of warm sensitive and temperature insensitive neurones on total neurones studied at different times after administration of PGE_2. From (88).

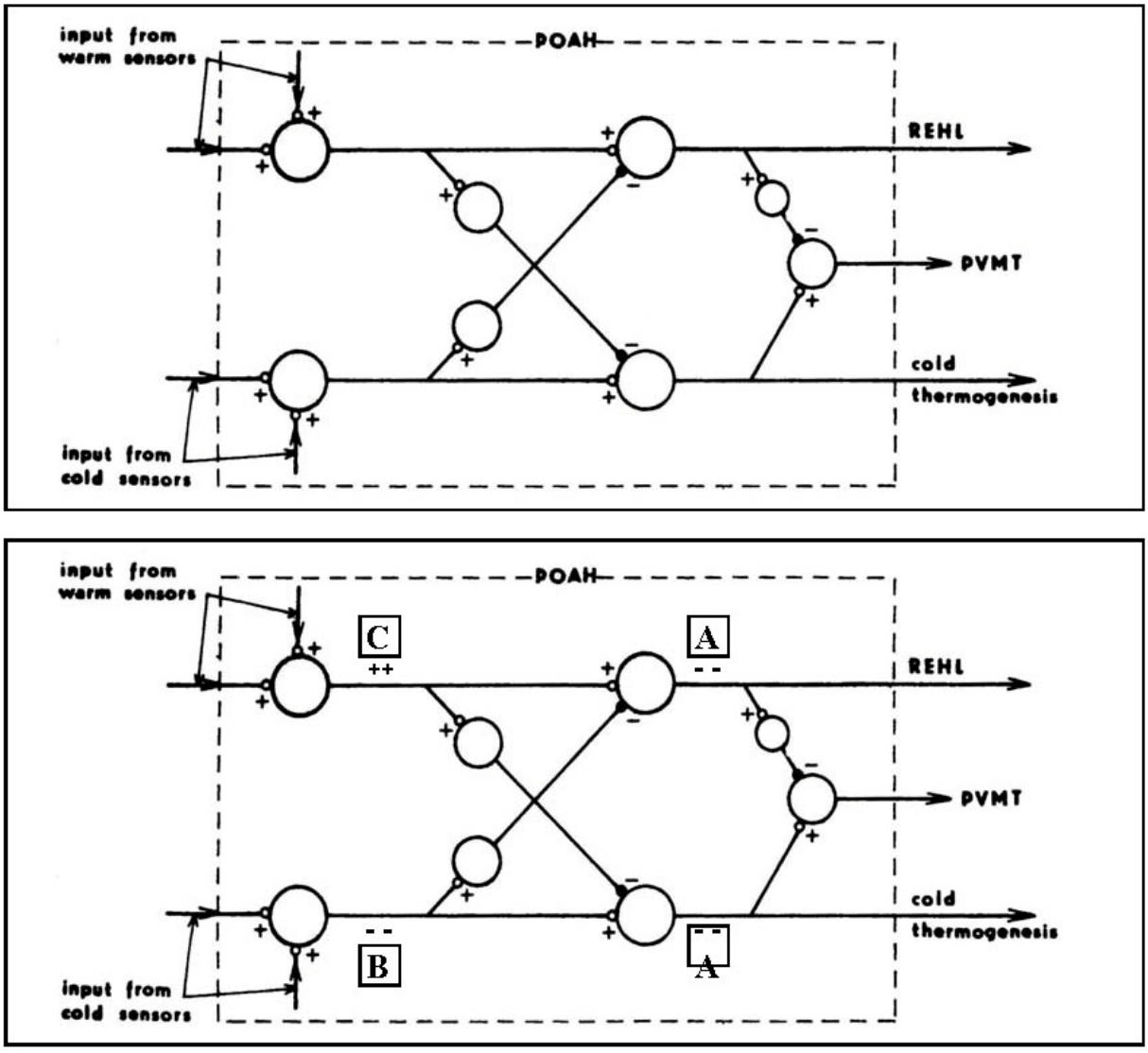

Figure 10. Scheme of thermoregulatory pathways in the preoptic area of the hypothalamus in normal (above) and febrile subjects (below) showing possible sites of pyrogen and neuropeptide action.

In the later phase of the fever, the separate downward shift of the threshold for cold thermogenesis could be either: 1. due to partial cessation of action of the primary pyrogen on the cold pathway, or 2. due to replacement of the action of the primary pyrogen with another one, acting at the same place, or elsewhere on the neural pathways (see the scheme). Thus, due to the described antipyretic action of AVP, α-MSH and ACTH, these substances could either inhibit the cold pathway **behind the point of crossing inhibition** (point B), or reactivate the warm pathway **in front of the point of crossing inhibition** (point C).

Results of the experiments performed on hypothalamic slices, showing lowered activity of warm sensitive neurons during the first phase of the fever and increased activity of warm sensitive neurons during the late phase of the fever, do not fully support this hypothesis, however. According to Bligh s model, changes in activities of warm sensitive neurons in front of the point of crossing inhibition, where the localization of warm sensitive neurons is anticipated, should induce reciprocal changes in the „cold“ pathway and this was not the case.

The presented scheme of action of pyretic and antipyretic substances differs from that we suggested earlier (1). It should be kept in mind, however, that all thermoregulatory models, including that of Bligh, are highly speculative and cannot fully explain all mechanisms participating in the control of thermal homeostasis during fever.

9. ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN CYTOKINE RELEASE

Sympathetic nervous system is being activated during the febrile process, as evident from the increased

heat production due to nonshivering thermogenesis (64-66) and from peripheral vasoconstriction (3, 67). Furthermore, already in 1970s it was suggested, that the prostaglandin fever could be induced due to release of hypothalamic noradrenaline (68). Therefore, the role of noradrenaline in modulating activities of the body temperature controller should be taken into consideration. The works of Zeisberger nad Brück (for review see 69) showed that activation of noradrenergic pathways in the brain stem, or injections of noradrenaline into the brain increased the threshold for cold thermogenesis. This could be the reason for elevated temperature thresholds during fever. It should be mentioned, however, that the evidence that intrahypothalamic injections of noradrenaline did not induce the febrile response (70) is in contrast with the above view.

Since cytokines may play an important role in modulation of thermoregulatory responses, factors which influence their release should be also taken into consideration. During the last 20 years many papers were published showing that the immune reaction (71-77) and production of cytokines (78-82) may be influenced by changes in activity of the autonomic nervous system.. Moderate work, or changes in blood concentrations of catecholamines, increase activity of the immune system, while the strenuous exercise may have a negative effect.

Furthermore, application of catecholamines influence the release of cytokines both under *in vivo* (83-84) and *in vitro* conditions (85-88) in macrophages as well as in astrocytes. Production of cytokines after noradrenaline can be also changed in cells activated by LPS. In consent with that, it was found that acetylcholin decreased release of IL-1β, IL-6 and TNF-α.(89). Serotonin also inhibited TNF-α production, but increased IL-1β production in LPS stimulated PBMC (90).

Our unpublished data show that noradrenaline, when added to isolated human PBMC in doses higher than 10^{-7} M inhibited release of TNF-α by about 20 % . On the other hand, lower doses seemed to increase releases of IL-6 and of IL-1β slightly. Release of cytokines from LPS stimulated cells was not influenced.

10. MODULATION OF THE CYTOKINE RELEASE BY HUMORAL SUBSTANCES

Studies on isolated cell populations revealed that release of cytokines can be modulated due to action of other cytokines. The complicated hierarchy of the immune response and mutual interactions among individual subpopulations of immune cells make it difficult to establish the precise role of individual cytokines in inducing release of other cytokines, however. Interpretation of the data is further complicated by the fact that experiments were mostly performed on isolated subpopulations of lymphocytes or on isolated astrocytes and glial or other cells. Very few experiments were performed using diluted blood or isolated PBMC to imitate situation under *in vivo* conditions.

Since macrophages appear to be the primary target of endotoxin action, effects of cytokines produced by these cells (namely IL-1β) were studied in the first place. Particular attention was payed to the role of IL-1β in inducing the release of IL-6. It was found that IL-1β stimulated the cultured astrocytes, glial and muscle cells to produce IL-6 (91-98 and others). IL-1β in combination with IFN-γ also stimulated astrocytes to produce TNF-α (99). Thus, data in the literature seem to confirm a leading role of IL-1β in modulating releases of IL-6 and TNF-α . These findings were confirmed by *in vivo* experiments (100 - 102). Furthermore, it is known for quite a long time that TNF-α has a stimulatory effect on IL-1β and IL-6 release (103, 104).

On the other hand, other data indicate that IL-6 acts inhibitorily on IL-1β and TNF-α releases from human PBMC or monocytes stimulated by LPS (155, 106). IL-4 has also an inhibitory effect on production of IL-1β , IL-6 and TNF-α in human monocytes stimulated by LPS (107). Similarly, IL-10 is supposed to act as a negative regulator of cytokine production (25, 26), although data from experiments performed under *in vitro* conditions on PBMC are still missing.

Recent experiments performed in our laboratory (108) indicate that in isolated PBMC low doses of IL-1β (up to 100 pg/10^6 of cells) decrease, while higher doses of IL-1β (up to 10000 pg/10^6 of cells) increase production of TNF-α. In contrast, IL-1β exerted no effect on IL-6 production in resting and LPS stimulated PBMC. Thus, the release of IL-6 from isolated PBMC seems to be IL-1β independent. The reason for the discrepancy with data presented above is not clear.

Furthermore, it is known that cytokines stimulate prostaglandin production (109) and that prostaglandins might exert an inhibitory effect on IL-1β and TNF-α productions (110, 111). In our experiments, PGE_2 in concentrations ranging from 300 to 3000 pg/ 10^6 of cells did not influence either the resting, or the LPS stimulated release of IL-1β from PBMC.

11. CONCLUSIONS

Data presented in this review suggest that:

1. The LPS fever appears to be polyphasic.

2. Various humoral substances modulate activities of the hypothalamic body temperature controller during different phasis of the LPS fever in a different way.

3. Fever can be induced by cytokines acting both peripherally on nervous endings and centrally on the hypothalamus. IL-1β is the most potent fever inducer.

4. Peripherally administered IL-6 differs in its mode of action on thermoregulatory centers and effectors from that of IL-1β and TNFα.

5. All centrally administered cytokines induce upward shift of the thresholds for all thermoregulatory outputs.

6. Increase in body temperature after central administration

of cytokines appears to be cytokine unspecific and may be due to local inflammation.

7. Effects of centrally applied cytokines on body temperature are long lasting, indicating absence of mechanisms responsible for degradation of cytokines.

8. *In vivo* administration of LPS increases release of TNF-α by several orders of magnitude. The releases of IL-1β and of IL-6 are also increased.

9. *In vitro* administrations of LPS to PBMC increase mainly the releases of TNF-α and IL-1β. IL-6 is being produced to a smaller extent.

10. ACTH, α-MSH and AVP are the natural antipyretic substances. Their release is induced by cytokines.

11. Cytokines inhibit activities of warm sensitive neurons in the hypothalamus, similarly as does PGE_2, while AVP increases their activities.

12. Catecholamines and cytokines may influence release of other cytokines and this way modulate thermal homeostasis during fever. Effect of cytokines on cytokine release is long lasting, which indicates absence of degradation mechanisms under *in vitro* conditions.

13. It is suggested that initiation of the febrile response may be due to direct action of LPS on peripheral nerves. The upward shift of temperature thresholds for induction of all thermoregulatory effectors during the first phase of the fever may be due to central action of cytokines and /or PGE_2 and the downward shift of the threshold for cold thermogenesis during the second phase of the fever may be due to central action of neuropeptides.

11. REFERENCES

1. Janský, L: Neuropeptides and the central regulation of body temperature during fever and hibernation. *J therm Biol* 15, 329-347 (1990)

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

3. Jansky, L., and S. Vybiral: Neuropeptides and body temperature control during normothermia and fever. *Acta Physiol Pol* 41, 33-44 (1990)

4. Vybíral, S., M. Székely, L. Janský and L. Černý: Thermoregulation of the rabbit during the late phase of endotoxin fever. *Pflügers Arch* 410, 220-222 (1987)

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

6. Netea, M. G., B. J. Kullberg and J. W. M. Van der Meer: Do only circulating pyrogenic cytokines act as mediators in the febrile response? A hypothesis. *Eur J Clin Investig* 29, 351-356 (1999)

7. Macari, M., I. R. Pela, C. A. A.Silva and R. S. Viana: Fever response induced by intravenous and intracerebroventricular injection of pyrogen in thyroidectomised and protein-calorie malnourished rabbits. *Pflügers Arch* 415, 440-443 (1990)

8. Spíšková, K. and B. Štefl: Quantitative aspects of the fever response elicited by intracerebral injection of endotoxin in the guinea pig. *Physiol Res* 41, 63-64, (1992)

9. Rotondo, D., H. T. Abul, A. S. Milton and J. Davidson: Pyrogenic immunomodulators increase the level of prostaglandin E in the blood simultaneously with the onset of fever. *Eur J Pharmacol* 154, 145-153 (1988)

10. Abul, H. T. and A. S. Milton: The effects of dexamethasone on prostaglandin E_2 release into rabbit CSF during fever and on the pyrogen-stimulated release of prostaglandin E_2 from rabbits monocytes. *J Physiol (London)* 409, 60P (1989)

11. Hori, Y., C. M. Blatteis and A. Nasletti: Production of PG E_2 by brain slices stimulated by various thermoactive agents. *Fed Proc* 46, 683 (1987)

12. Romanovsky, A. A., Ch. T. Simons and V. A. Kulchitsky: Biphasic fever often consist of more than two phases. *Am J Physiol* 275, *Regulatory Integrative Comp Physiol* 44, R-323-R331 (1998)

13. Romanovsky, A. A., V. A. Kulchitsky, Ch. T. Simons and N. Sugimoto: Methodology of fever research: why are polyphasic fevers often thought to be biphasic? *Am J Physiol* 275, *Regulatory Integrative Comp Physiol* 44, R332/R338 (1998)

14. Janský, L., S. Vybíral, D. Pospíšilová, J.Roth, J. Dornand, E. Zeisberger, E. and J. Kamínková: Production of systemic and hypothalamic cytokines during the early phase of endotoxin fever. *Neuroendocrinology* 62, 55-61 (1995)

15. Cavaillon, J. M., C. Fitting, N. Haeffner-Cavaillon, S. J. Kirsch and H. S. Warren: Cytokine response by monocytes/macrophages to free and lipoprotein bound lipopolysaccharide. *Infect Immunol* 58, 2375-2382, (1990)

16. De Grotte, D., P. F. Zangerle, Y. Gevaert, M. F. Fassotte, Y. Beguin, F. Noizat-Pirenne, R. Gathy, M. Lopez, I. Dehart, D. Igot, M. Baudrihaye, D. Delacroix and P. Franchimont: Direct stimulation of cytokines (IL-1, TNF-alpha, IL-6, IL-2, INF-gamma, GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. *Cytokine* 4, 239-248 (1992)

17. Flegel, W. A., A. Wolpl, D. N. Mannel, H. Northoff: Inhibition of endotoxin-induced activation of human monocytes by human lipoproteins. *Infect Immunol* 57, 2237-2245 (1989)

18. Van der Meer, J. W. M., S. Endres, G.Lonnemann, J. G.

Cannon, T. Ikejima, S. Okusawa, J. A. Gelfand and Ch. A. Dinarello: Concentrations of immunoreactive human tumor necrosis factor alpha produced by human mononuclear cells *in vitro*. *J Leukocyte Biology* 43: 216-223 (1988)

19. Lonnemann, G., S. Endres, J. W. M. Van der Meer, J. G. Cannon, K. M. Koch and Ch. A. Dinarello: Differences in the synthesis and kinetics of release of interleukin 1α, interleukin 1β and tumor necrosis factor from human mononuclear cells. *Eur J Immunol* 19, 1531-1536 (1989)

20. Matsushima, K., A. Procopio, H. Abe, G. Scala, J. R. Ortaldo and J. J. Oppenheim: Production of interleukin 1 activity by normal human peripheral blood B lymphocytes. *J Immunol* 135, 1132-1136 (1995)

21. Janský, L., P. Reymanová and J. Kopecký: Dynamics of cytokine production in human peripheral blood mononuclear cells stimulated by LPS or infected by Borrelia. *Physiol Res* 52, 593-598 (2003)

22. Häupl, T., S. Landgraf, P. Netusil, N. Biller, C. Capiau, P. Desmonds, P. Hauser and G. R. Burmester: Activation of monocytes by three OspA vaccine candidates: lipoprotein OspA is a potent stimulator of monokines. *FEMS Immunol Med Microbiol* 19, 15-23 (1997)

23. Giambartolomei, G. H., V. A. Dennis, B. L. Lasater and M. T. Phillip: Induction of pro- and anti-inflammatory cytokines by Borrelia burgdorferi lipoproteins in monocytes is mediated by CD14. *Infect Immunol* 67, 140-147 (1999)

24. Ma, Y., K. P. Seiler, K. F. Tai, L. Yang, M. Woods and J. J. Weiss: Outer surface lipoproteins of Borrelia burgdorferi stimulate nitric oxide production by the cytokine-inducible pathway. *Infect Immunol* 62, 3663-3671 (1994)

25.Tobias, P. S., K. Soldau, L. Kline, J. D. Lee, K. Kato, T. P. Martin and R. Ulevitch: Crosslinking of lipopolysaccharide to CD14 on THP-1 cells mediated by lipopolysaccharide binding protein. *J Immunol* 159, 3011-3021 (1993)

26. Leon, L. R: Invited review: Cytokine regulation of fever: studies using gene knockout mice. *J Appl Physiol* 92, 2648-2655 (2002)

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

28. Conti, B., J. Tabarean and C. A. T. Bartfai: Cytokines and fever. *Front Biosci* 9, 1433-1449 (2004)

29. Vybíral, S., Z. Pešanová, L. Barczayová and L. Janský: Pyrogenic effect of cytokines (IL-1β, IL-6, TNFα) and their mode of action on thermoregulatory centers and functions. *J Thermal Biol*, accepted for publication

30. Martin, L. W., A. Catania, M. E. Hiltz and J. M. Lipton: Neuropeptide α-MSH antagonizes IL-6 and TNF-induced fever. *Peptides* 12, 297-299 (1991)

31. Derijk, R. H. and F. Berkenbosch: Hypothermia to endotoxin involves the cytokine tumor necrosis factor and the neuropeptide vasopressin in rats. *Am J Physiol (Regulatory Integrative Comp Physiol* 35) 266, R9-R14 (1994)

32. Gonindard, Ch., C. Goigoux, E. Hollande and L. D. D´Hinterland: The administration of an alpha-MSH analogue reduces the serum release of IL-l alpha and TNF alpha induced by the injection of a sublethal dose of lipopolysaccharides in the balb/c mouse. *Pigment Cell Res* 9, 148-153 (1996)

33. Zeisberger, E., G. Merker, S. Blähser and M. Kranning: Role of vasopressin in fever regulation. In: Homeostasis and thermal stress. S. Karger Publ., Basel, 62-65 (1986)

34. Blatteis, C. M. and E. Sehic: Fever: How may circulating pyrogens teach the brain? *News Physiol Sci* 12, 1-9 (1999)

35. Sehic, S. and C. M. Blatteis: Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea pigs. *Brain Res*726, 160-166 (1996)

36. Layé, S., R.-M. Bluthé, S. Kent, C. Combe, C. Medina, P. Parnet, K. Kelley and R. Dantzer: Subdiaphragmatic vagotomy blocks induction of IL-1mRNA in mice brain in response to peripheral LPS. *Amer J Physiol* 268, R 1327- R1331 (1995)

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

38. Cartmell, J. T., S. Poole, A. V. Turnbull, N. J. Rothwell and G. N. Lukeshi: Circulating interleukin-6 mediates the febrile response to localized inflammation in rats. *Journal of Physiology* 526, 653-661 (2000)

39. Barbanel, G., G. Ixart, A. Szafarczyk, F. Malaval and I. Assenmacher: Intrahypothalamic infusion of interleukin-1beta increases the release of corticotropin-releasing hormone (CRH 41) and adrenocorticotropic hormone (ACTH) in free-moving rats bearing a push-pull cannula in the median eminence. *Brain Research* 516, 31-36 (1990)

40. Spinedi, E., R. Hadid, T. Daneva and R. C. Gaillard: Cytokines stimulate the CRH but not the vasopressin neuronal system: evidence for a median eminence site of interleukin-6 action. *Neuroendocrinology* 56, 46-53 (1992)

41. Harbuz, M. S., A. Stephanou, N. Sarlis and S. L. Lightman: The effects of recombinant human interleukin (IL)-1α, IL-1β or IL-6 on hypothalamo-pituitary-adrenal axis activation. *J Endocrinology* 133, 349-355 (1992)

42. Terao, A. and M. Satto: Possible role of IL-6 in IL-1-induced plasma iron and corticosterone responses in rats. *Biomedical Research* 14, 301-303 (1993)

43. Ebisui, O., J. Fukata, N. Murakami, H. Kobayashi, H. Segava, S. Mura, I. Hanaoaka, Y. Naito, Y. Masui, Y. Ohmoto, H. Imur and K. Nakao: Effect of IL-1 receptor

antagonist and antiserum to TNF-α on LPS-induced plasma ACTH and corticosterone rise in rats. *Am J Physiol (Endocrinol. Metab. 29)* 266, 986-992 (1994)

44. Zhen, C., S. Gatti, D. Malinovski, J. Ludkvist, K. Alheim, J. Brisulf, V. Poli and T. Bartfai: Cytokine induction and glukocorticoides in fever: RT-PCR and knock-out study. Cytokines as communication(s) factors between the immune system and the central nervous system. *Proc of the Int Symp* Arcachon, P.10 (1995)

45. Harbuz, M. S. and S. L. Lightman: Stress and the hypothalamo-pituitary-adrenal axis: acute, chronic and immunological activation. *J Endocrinol134, 327-339 (1992)*

46. Matta, S. G., J. Weatherbee and B. M. Sharp: A central mechanism is involved in the secretion of ACTH in response to IL-6 in rats: comparison to and interaction with IL-1β. *Neuroendocrinology* 56, 516-525 (1992)

47. De Kloet, E. R., M. S. Oitzl and B. Schobitz: Cytokines and the brain corticosteroid receptor balance: relevance to pathophysiology of neuroendocrine-immune communication. *Psychoneuroendocrinology*19, 121-134 (1994)

48. Vybíral, S., L. Černý and L. Janský: Mode of ACTH antipyretic action. *Brain Res Bull* 21, 557-562 (1988)

49. Ehymayed, H. M. and L. Janský: A discrete mode of the antipyretic action of AVP, α-MSH and ACTH. *Physiol Res* 41, 57-61 (1992)

50. Kruk, B., J. Lyszczarz and J. Laszczynska: Reduced hypothalamic thermosensitivity following intraventricular injection of pyrogen in conscious rabbits. *Brain Research* 267, 237-240 (1983)

51. Hori, T., M. Shibata, T. Nakashima, M. Yamasaki, A. Asami, T. Asamiand and H. Koga: Effects of interleukin-1 and sarachidonate on the preoptic and anterior hypothalamic neurons. *Brain Res Bull* 20, 75-82 (1988)

52. Nakashima, T., T. Hori, T. Mori, K. Kuriyama and K. Mizuno: Recombinant human interleukin-1 beta alters the activity of preoptic thermosensitive neurons *in vitro*. *Brain Res Bull* 23, 209-213 (1989)

53. Nakashima, T., T. Kiyohara and T. Hori: Tumor necrosis factor-beta specifically inhibits the activity of preoptic warm-sensitive neurons in tissue slices. *Neurosci Lett* 28, 97-100 (1991)

54. Xin, L. and M. Blatteis: Hypothalamic neuronal responses to interleukin-6 in tissue slices: effects of indomethacin and naloxone. *Brain Res Bull* 29, 27-35 (1992)

55. Katafuchi, T., K. Motomura, S. Baba, K. Ota and T. Hori: Differential effects of tumor necrosis factor-α and -β on rat ventromedial hypothalamic neurons *in vitro*. *Am J Physiol* 272 *(Regulatory Integrative Comp Physiol* 41), R-1966-R1971 (1997)

56. Vasilenko, V. Y., T. A. Petruchuk, V. N. Gourine and F. K. Pierau: Interleukin-1 beta reduces temperature sensitivity but elevates thermal thresholds in different populations of warm-sensitive hypothalamic neurons in rat brain slices. *Neurosci Let* 92, 207-210 (2000)

57. Shibata, M. and C. M. Blatteis: Differential effects of cytokines on thermosensitive neurons in guinea pig preoptic area slices. *Am J Physiol* 261 *(Regulatory Integrative Comp Physiol* 30), R-1096-R1103 (1991)

58. Janský, L., F.- K. Pierau and J. Schenda: The effect of PG E_2 on activity and thermosensitivity of hypothalamic neurones in rat brain slices. *Physiol Res* 41, 85-88 (1992)

59. Moravec, J. and F.-K. Pierau: Arginine vasopressin modifies the firing rate and thermosensitivity of neurons in slices of the rat PO/AH area. Integrative and cellular aspects of autonomic function: temperature and osmoregulation. Eds: K. Pleschka, R. Gerstberger. John Libbey Eurotext, Paris, 143-152 (1994)

60. Janský, L., F.-K.Pierau, E. Simon, W. Riedel, H. Schmid, Ch. Simon-Oppermann, J. Schenda, H. Sann, S. Vybíral, J. Moravec, M. Konrad, H. M. Ehymayed, J. Nacházel and L. Černý: Neuropeptides and body temperature control: integration of *in vivo* and *in vitro* approaches. Integrative and cellular aspects of autonomic functions: temperature and osmoregulation. Eds: K. Pleschka, R. Gerstberger, John Libbey Eurotext, Paris, 17-22, (1994)

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

62. Bligh, J., W. H. Cottle and M. Maskrey: Influence of ambient temperature on the thermoregulatory responses to 5-hydroxytryptamine, noradrenaline and acetylcholine injected into the lateral cerebral ventricles of sheep, goats and rabbits. *J Physiol (London)* 212, 377-392 (1971)

63. Bligh, J: Temperature regulation in mammals and other vertebrates. Frontiers in Biology. North Holland Publ. Comp., Amsterdam (1973)

64. Horwitz, B. A. and G. E. Hanes: Propranolol and pyrogen effects on shivering and nonshivering thermogenesis in rats. *Am J Physiol* 230, 637-642 (1976)

65. Blatteis, C. M: Fever: Exchange of shivering by nonshivering pyrogenesis in cold-acclimated guinea pigs. *J Appl Physiol* 40, 29/34 (1976)

66. Blatteis, C. M: Effect of propranolol on endotoxin-induced pyrogenesis in newborn and adult guinea pigs. *J Appl Physiol* 40, 35-39 (1976)

67. Saigura, T. and M. Iriki: Regional differentiation of sympathetic nerve activity during fever caused by intracerebroventricular injection of

PGE_2. *Pflügers Arch* 411, 121-125 (1989)

68. Myers, R. D. and M. B. Waller: Is prostaglandin fever mediated by the presynaptic release of hypothalamic 5-HT or norepinephrine? *Brain Research* 1, 47-56 (1976)

69. Brück, K. and E. Zeisberger: Adaptive changes in thermoregulation and their neuropharmacological basis. In: E. Schönbaum and P. Lomax: *Thermoregulation Physiology and Biochemistry,* Pergamon Press, New York, London, 131, 255- 308 (1990)

70. Preston, E. and K. E. Cooper: Absence of fever in the rabbit following intrahypothalamic injections of noradrenaline into PGE_1-sensitive sites. *Neuropharmacology* 15, 239-244 (1976)

71. Kappel, M., N. Tvede, H. Galbo, P. M. Haahr, M. Kjaer, M. Linstow, K. Klarlund and B. K. Pedersen: Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. *J Appl Physiol* 70, 2530-2534 (1991)

72. Verde, T. J., S. G. Thomas, R. W. Moore, P. Shek and R. J. Shepard: Immune responses during increased training of the elite athlete. *J Appl Physiol* 74, 1494-1499 (1992)

73. Cook-Mills, J. M., R. L. Cohen, R. L. Perlman and D. A. Chambers: Inhibition of lymphocyte activation by catecholamines: evidence for a non-classical mechanism of catecholamine action. *Immunol* 85, 544-549 (1995)

74. Nagao, F., M. Suzui, K. Takeda, H. Yagita and K. Okumura: Mobilization of NK cells by exercise: downmodulation of adhesion molecules on NK cells by catecholamines. *Am J Physiol (Regulatory Integrative Comp Physiol),* 279, R-1251-1256 (2000)

75. Stevenson, J. R., J. Westermann, P. M. Liebmann, M. Hortner, I. Rinner, P. Felsner, A. Wolfler and K. Schauenstein: Prolonged alpha-adrenergic stimulation causes changes in leukocyte distribution and lymphocyte apoptosis in the rat. *J Neuroimmunol* 120, 50-57 (2000)

76. Wolfler, A. and K. Schauenstein: Prolonged alpha-adrenergic stimulation causes changes in leukocyte distribution and lymphocyte apoptosis in the rat. *J Neuroimmunol* 120, 50-57 (2001)

77. Green, K. J., D. G. Rowbottom and L. T. Mackinnon: Exercise and T-lymphocyte function: a comparison of proliferation in PBMC and NK cell-depleted PBMC culture. *J Appl Physiol* 92, 2390-2395 (2002)

78. Fielding, R. A., T. J. Manfredi, W. Ding, M. A. Fiatarone, W. J. Evans and J. G. Cannon: Acute phase response in exercise. III. Neutrophil and IL-1 beta accumulation in skeletal muscle. *Am J Physiol (Regulatory Integrative Comp Physiol)* 265, R166-R172 (1993)

79. Ullum, H., P. M. Haahr, M. Diamant, J. Palmo, J .Halkjaer-Kristensen and B. K. Pedersen: Bicycle exercise enhanced plasma IL-6 but does not change IL-1α, IL-1β, IL-6 or TNF-α pre-mRNA in BMNC. *J Appl Physiol* 77, 93-97 (1994)

80. Camus, G., M. Nys, J.-R. Poortmans, I. Venneman, T. Monfils, G. Deby-Dupont, A. Juchmes-Ferir, C. Deby, M. Lamy and J. Duchateau: Endotoxaemia, production of tumor necrosis factor alpha and polymorphonuclear neutrophil activation following strenuous exercise in humans. *Eur J Appl Physiol* 79, 62-68 (1998)

81. Suzuki, K., M. Yamada, S. Kurakake, N. Okamura, K. Yamaya, Q. Liu, S. Kudoh, K. Kowatari, S. Nakaji and K. Sugawara: Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. *Eur J Appl Physiol* 81, 281-287 (2000)

82. Van Gool, J., H. Van Vugt, M. Helle and L. A. Aarden: The relation among stress, adrenalin, interleukin-6 and acute phase proteins in the rat. *Clin Immunol Immunopathol* 57, 200-210 (1990)

83. Liao, J., J. A. Keiser, W. E. Scales, S. L. Kunkel and M. J. Kluger. Role of epinephrine in TNF and IL-6 production from isolated perfused rat liver. *Am J Physiol (Regulatory Integrative Comp Physiol)* 268, R896-R901 (1995)

84. Sondergaard, S. R., K. Ostrowski, H. Ullum and K. Pedersen: Changes in plasma concentrations of interleukin-6 and interleukin-1 receptor antagonists in response to adrenaline infusion in humans. *Eur J Appl Physiol* 83, 95-98 (2000)

85. Spengler, R. N., R. M. Allen, D. G. Remick, R. M. Strieter and S. L. Kunkel: Stimulation of α-adrenergic receptor augments the production of macrophage-derived tumor necrosis factor. *J Immunol* 145, 1430-1434 (1990)

86. Maimone, D., Ch. Cioni, S. Rosa, G. Macchia, F. Aloisi and P. Annunziata: Norepinephrine and vasoactive intestinal peptide induce IL-6 secretion by astrocytes: synergism with IL-1β and TNFα. *J Neuroimmunol* 47, 73-82 (1993)

87. Spengler, R. N., S. W. Chensue, D. A. Giacherio, N. Blenk and S. L. Kunkel: Endogenous norepinephrine regulates tumor necrosis factor-α production from macrophages *in vitro*. *J Immunol* 152, 3024-3031 (1994)

88. Baerwald, Ch. G. O., M. Wahle, T. Ulrichs, D. Jonas, A. von Bierbauer, P. Von Wichert, G. R. Burmester and A. Krause: Reduced catecholamine response of lymphocytes from patients with rheumatoid arthritis. *Immunol* 200, 77-91 (1999)

89. Borovikova, L. V., S. Ivanova, M. Zhang, H. Yang. G. Botchkina, L. R. Watkins, Wang, N. Abumrad, J. W. Eaton and K. J. Tracey: Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405, 458-461 (2000)

90. Cloez-Tayarani, I., A. F. Petit-Bertron, H. D. Venters and J. M. Cavaillon: Differential effect of serotonin on cytokine production in lipopolysaccharide-stimulated human peripheral blood mononuclear cells: involvement of

5-hydroxytryptamine (2A) receptors. *International Immunology* 15, 233-240 (2003)

91. Helle, M., J. P. J. Brakenhoff, E. R. DeGroot and L. A. Arden: Interleukin –6 is involved in interleukin-1 induced activities. *Eur J Immunol* 18, 957 (1988)

92. Benveniste, E. N., S. M. Sparacio, J. G, Norris, H. E. Grenett and G. M. Fuller: Induction and regulation of interleukin 6 gene expression in rat astrocytes. *J Neuroimmunol* 30, 201, (1990)

93.Testa, U., G. Levi and C. Peschle: Production of hemolymphopoietic cytokines (IL-6, IL8, colony-stimulating factors) by normal human astrocates in response to IL-1β and tumor necrosis factor-α. *J Immunol* 149, 2358-2366 (1992)

94. Luo, G., D. D. Hershko, B. W. Robb, C. J. Wray and P.-O.Hasselgren: IL-1β stimulates IL-6 production in cultured skeletal muscle cells through activation of MAP kinase signalling pathway and NF-kB. *Am J Physiol (Regul Integr Comp Physiol)* 284, R1249-R1254 (2003)

95. Di Loreto, S., R. Maccarone, L. Corvetti, P. Sebastiani, D. Piancatelli and D. Adorno: Differential modulation of interleukin-6 expression by interleukin-1β in neuronal and glial cultures. *Eur Cytokine Netw* 14, 97-102 (2003)

96. Aloisi, F., A. Care, G. Borsellino, P. Gallo, S. Rosa, A. Bassani, A. Cabibbo, C. Libert, P. Brouckaert, A. Shaw, W. Fiers: Induction of interleukin 6 by human and murine recombinant interleukin 1 in mice. *Eur J Immunol* 20, 691-694, (1992)

97. Tsuzaki, M., G. Guyton, W. Garrett, J. M. Archambault, W. Herzog, L. Almekinders, D. Bynum, X. Yang and A. J. Banes: IL-1β induces COX2, Mmp-1, -3 and –13, ADAMTS-4, IL-1β and IL-6 in human tendon cells. *J Orthop Res* 21, 256-264 (2003)

98. Zhang, Y. H., J. X. Lin and J. Vilcek: Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of nuclear factor binding to a kappa B-like sequence. *Mol Cell Biol* 10, 3818-3823 (I990)

99. Chung, I. Y. and E. N. Benveniste: Tumor necrosis factor-α production by astrocytes. Induction by lipopolysaccharide, IFN-γ, and IL-1β. *J Immunol* 144, 2999-3007 (1990)

100. Desimoni, M. G., M. Sironi, A. Deluigi, A. Manfridi, A. Mantovani and P. Ghezzi: Intracerebroventricular injection of interleukin 1 induces high circulating levels of interleukin 6. *J Exp Med* 171, 1773-1778 (1990)

101. Lemay, L .G., I. G. Otterness, A. J. Vander and M. J. Kluger: *In vivo* evidence that the rise in plasma IL 6 following injection of a fever-inducing dose of LPS is mediated by IL 1beta. *Cytokine* 2(3), 199-204 (1990)

102. Steensberg, A., Ch.. P. Fischer, Ch. Keller, K. Moller and B. K. Pedersen: IL-6 enhanced plasma IL-1ra, IL-10, and cortisol in humans. *Am J Physiol (Endocrinol Metab)* 285, E433-E437 (2003)

103. Dinarello, C. A., J. G. Cannon and S. M. Wolff: Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin–1. *J Exp Med* 163, 1433-1450 (1986)

104. Defilippi, P., P. Poupart, J. Tavernier, W. Fiers and J. Content: Induction and regulation of mRNA encoding 26-kDa protein in human cell lines treated with recombinant human tumor necrosis factor. *Proc Natl Acad Sci USA* 84, 4557 (1987)

105. Aderka, D., J. Le and J. Vilcek: IL 6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. *J Immunol* 143, 3517-3523 (1989)

106. Schindler, R., J. Mancilla, S. Endres, R. Ghorbani, S. C. Clark and Ch. A. Dinarello: Correlation and interactions in the production of Interleukin-6 (IL-6), IL-1, and Tumor Necrosis Factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* 75, 40-47 (1990)

107.Te Velde, A. A., R. J. F. Huijbens, K. Heije, J. A. de Vries and C. G. Figdor: Interleukin-4 (IL-4) inhibits secretion of IL-1β, tumor necrosis factor α, and IL-6 human monocytes. *Blood* 76, 1392-1397 (1990)

108. Stará, A Humoral factors mediating release of cytokines. Diploma work. University of South Bohemia, (in Czech) (2004)

109. Matsell, D. G., L. W. Gaber and K. U. Malik: Cytokine stimulation of prostaglandin production inhibits the proliferation of serum stimulated mesangial cells. *Kidney International* 45, 150-165 (1994)

110. Kunkel, L. S., M. Spengler, M. A.May, R. Sprengler, J. Larrick and D. Remick: Prostaglandin E2 regulates macrophage-derived tumor necrosis factor expression. *J Biol Chem* 263, 5380 (1988)

111. Long, N. C., A. Morimoto, T. Nakamori, O. Yamashiro and N. Murakami: Intraperitoneal injections of prostaglandin E_2 attenuate hyperthermia induced by restraint or interleukin-1 in rats. *J Physiol* 444, 363-373 (1991)

Key Words: Inflammation, Neuron, Nervous System, Body temperature, Cytokine, Fever, Review

Send correspondence to: Dr Ladislav Jansky, Faculty of Biology, University of South Bohemia, Prague, Czech Republic, E-mail: JanskyL@seznam.cz, Jansky@tix.bf.jcu.cz