Neural Route of Pyrogen Signaling to the Brain

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In the pathogenesis of systemic inflammation and fever, peripheral inflammatory and pyrogenic signals gain access to the brain via humoral and neural routes. One of the neural routes is represented by chemosensitive afferent fibers of the abdominal vagus. We summarize our recent studies of the role of the abdominal vagus in fever. We conclude that capsaicin-sensitive fibers traveling within the hepatic vagal branch constitute a necessary component of the afferent mechanism of the febrile response to low, but not high, doses of circulating pyrogens. We speculate that this mechanism is triggered by blood-borne prostaglandins of the E series.

The mechanisms of fever are complex. Exogenous (typically, microbial) pyrogens, such as lipopolysaccharide (LPS), are known to act through immunocompetent cells in the circulation and peripheral tissues to trigger a cascade of pyrogenic cytokines, such as TNF–α, IL–1β, IL-6, and IFN-γ [1]. However, it is still not known how peripheral pyrogenic signals reach the central nervous system. It has been proposed that cytokines and/or other pyrogenic molecules gain access to the brain not only via circulation (humoral routes) but also via neural routes (reviewed in Watkins et al. [2] and Maier et al. [3]). The humoral routes were the first to be discovered, and their significance is now accepted. These routes are beyond the focus of the present review. Our focus will be on a more recent addition to the concept of febrile pathogenesis: the neural routes.

The idea of neural afferentation playing a role in fever and inflammation emerged more than a decade ago. In 1987, Morimoto et al. [4] speculated that peripheral nerves are involved in fever. In 1990, the possibility of neural signaling from the immune system to the brain was addressed at an international conference on neuroimmunomodulation [5]. At this meeting, many questions were posed, including "What afferent messages are transmitted to the central nervous system … by means of hard-wired (neuronal) fibers? … Are these of equal importance to chemical messages in the circulation?" [5, p. 444]. Another year elapsed before experimental evidence of a role of chemosensitive neural fibers in fever and inflammation was reported by Cooper and Rothwell [6], and another 2 years elapsed before Niijima [7] described activation of vagal afferent fibers by IL-1β. In 1994, Bluthé et al. [8] and Watkins et al. [9] demonstrated that the abdominal vagus nerve has an essential role in triggering behavioral manifestations of systemic inflammation. In 1995, Watkins et al. [10] reported that rats with subdiaphragmatic vagotomy do not develop fever in response to ip administration of IL-1, and Székely et al. [11] reported that desensitization of intra-abdominal chemosensitive afferents with ip capsaicin greatly decreases febrile responsiveness of rats to iv LPS. These initial communications demonstrating the capacity of both surgical (transection) and chemical (capsaicin) vagotomy to attenuate experimental fever have now been confirmed by several laboratories [12–14], including our own [15, 16].

The purpose of this review is to summarize our recent data on the role of the neural (vagal) pathway in fever, specifically, in the initial stage of the febrile response, which we term "early febrile phase."

Fever: A Complex, Multistage Response

Experimental models of fever have shown that the febrile response depends strongly on the pyrogen dose and the route of its administration. Moreover, this response is characterized by a nonlinear time dynamics of body temperature and is highly sensitive to internal (e.g., nutritional status) and external (e.g., environmental temperature) factors. When LPS is administered iv to rats at a neutral (30°C) ambient temperature, a low, near-threshold (1 μg/kg) dose induces the first febrile phase only (monophasic fever), which is characterized by a relatively long latency and a temperature peak at ~80 min. If the dose is somewhat higher (10–100 μg/kg), the first phase peaks earlier (50–70 min) and is followed by another temperature increase (second febrile phase), peaking at ~140 min. The response to very low pyrogen doses (monophasic fever) can be considered a pure first (early) febrile phase, whereas, at higher pyrogen doses, the early phase becomes "contaminated" with a later, second phase [17]. This concept is central to the interpretation of the results we present below.

Each of the febrile phases is characterized by a different thermoregulatory pattern. The first phase is associated with an

Financial support: National Institutes of Health (1 R01 NS41233-01), Collins Medical Trust, Good Samaritan Foundation, Oregon Health Sciences Foundation (Medical Research Foundation of Oregon), Bayer AG, Dr. Temple Fay's Memorial Account (to A.A.R.), and Hungarian Scientific Research Grant Agency (OTKA T026511; to M.S.).

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Clinical Infectious Diseases 2000; 31(Suppl 5):S162–7

1058-4838/2000/3104S5-0004$03.00

upward shift in the threshold body temperature for activation of the heat-defense effector mechanisms and an equal shift in the threshold body temperature for activation of the cold-defense responses [18]. As a result, body temperature is precisely regulated at a new, elevated level. The mechanism of the second febrile phase is threshold dissociation: the threshold body temperature for activation of the heat-defense responses remains elevated, whereas that for activation of the cold-defense mechanisms decreases [18]. Consequently, poikilothermy occurs, and body temperature becomes the result of passive heat exchange between the body and the environment. It has also been proposed [17, 19] that each febrile phase is characterized by its own complex of associated "sickness symptoms": hyperalgesia, motor hyperexcitability, arterial hypertension, and an increase in vigilance (the early phase) versus hypoalgesia, low motor activity, normotension or hypotension, and sleepiness (the late febrile phase). It is reasonable to assume that each febrile phase has its own triggering mechanism (reviewed in Romanovsky et al. [20]) and involves the vagus nerve in its own unique fashion [21]. To complete the description of the febrile response, we should note that moderate-to-high pyrogenic doses actually cause >2 febrile phases (for a description of the third phase, see Romanovsky et al. [22, 23]).

Depending on the pyrogen dose and ambient temperature, iv LPS may also induce hypothermia [24], which will not be addressed further in this review; nor will thermoregulatory responses to pyrogens administered by routes other than iv. For information on vagal involvement in the febrile response to ip pyrogen administration, we refer readers to 2 other articles [3, 25].

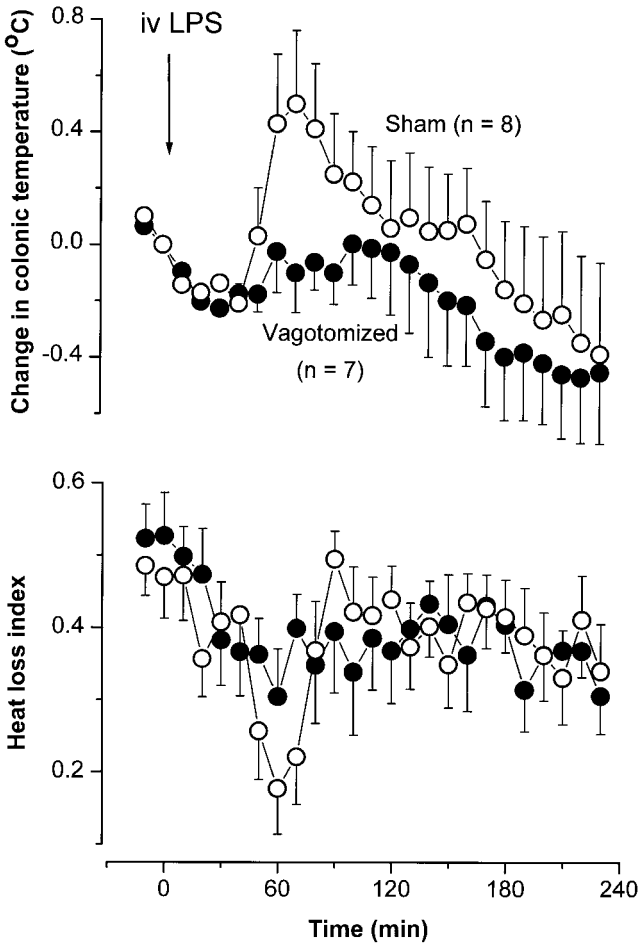

Figure 1. Thermoregulatory effects of the iv administration (the arrow indicates the time of injection) of lipopolysaccharide (LPS; 1 μg/kg) in sham-operated rats and vagotomized rats. Heat loss index (a ratio between 2 temperature gradients: skin-ambient and deep body–ambient; for details, see Romanovsky and Blatteis [51]) is used to assess changes in skin vasomotor tone, which varies from 0 (full vasoconstriction) to 1 (full vasodilation). Modified from Romanovsky et al. [15], with the permission of the American Physiological Society.

Vagus Nerve in Fever: Recent Results

Is the vagal route essential for febrile pathogenesis? In our recent experiments, we monitored the thermal response of vagotomized rats to iv LPS in doses ranging from subpyrogenic to ones inducing shock. It appeared that the integrity of the vagus is required for the response to minimal pyrogenic doses of LPS (figure 1)—that is, for the development of the early febrile phase [16, 26]. However, when higher doses of LPS were administered, leading to "contamination" of the first phase with the second phase, vagotomy had no effect on the genesis of fever [16]. Analysis of these data suggests that some hypothetical nonvagal routes, whether neural or humoral, may compensate for the interrupted vagal route during the later febrile phase. Interestingly, our observations are in concert with those of Morimoto et al. [4] and Cooper and Rothwell [6]; these authors suggested the involvement of neurally conveyed messages in the early, but not the late, febrile phase. Teleologically, "such messages, by means of nerve fibers, would have the advantage of much greater speed of transmission, and would not be subject to impedance by the blood-brain barrier" [5, p. 444]. Thus the rapidity of neural (vagal) communication between the immune system and the brain seems crucial for the onset of fever (the early febrile phase) and less important or unimportant for the later febrile phases.

Afferents or efferents: which fibers are involved? Initially, the decreased febrile responsiveness of vagotomized animals was assumed to be due to interruption of afferent vagal signals. However, the abdominal vagus is not exclusively an afferent nerve, since it consists of motor (efferent) and sensory (afferent) fibers. Therefore, we asked whether the mechanisms of vagal involvement in the early febrile response require integrity of afferent or efferent fibers. We found that, in rats, desensitization of intra-abdominal chemosensitive afferents with ip capsaicin (which does not affect efferent fibers) greatly decreases the early febrile responsiveness to iv LPS [11]. We concluded that the

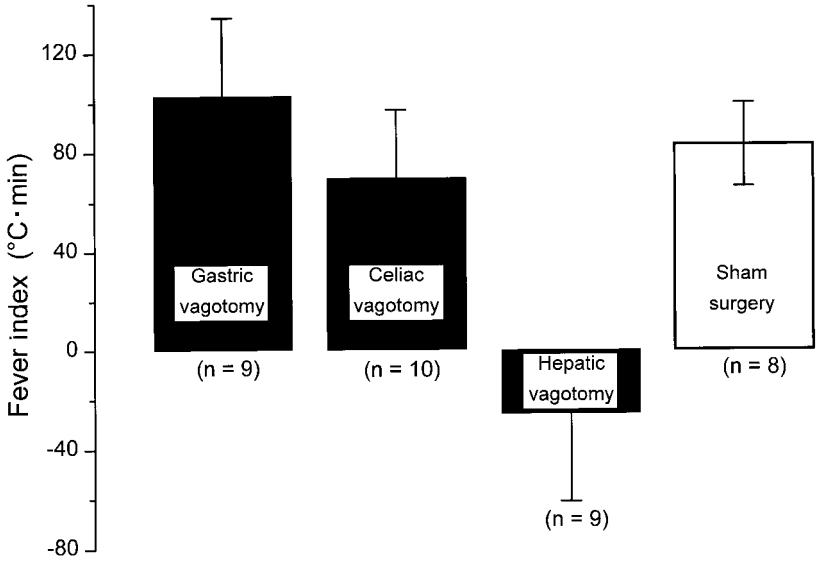

Figure 2. Febrile response to iv lipopolysaccharide (1 μg/kg) is compared among groups of rats subjected to 4 different types of surgery. Fever index, an integral of the deviation of body temperature from its preinjection level, was calculated over 0–4 h after injection and was used as a response measure. Modified from Simons et al. [26], with the permission of the American Physiological Society.

early febrile response probably depends on neural, perhaps vagal, afferentation to the brain. Pyrogen (IL-1)–induced enhancement of afferent vagal activity had previously been demonstrated by Niijima [7, 27]. Others demonstrated that IL-1 induces expression of Fos protein [28–30], type 1 IL-1 receptor [30], and EP3 subtype prostaglandin E (PGE) receptor [30] in the bodies of the primary afferent vagal neurons located in the nodose ganglion. When these data are taken together, they indicate that vagal afferent fibers are responsible, at least partially, for pyrogenic signaling the brain after peripheral inflammatory stimulation.

Why does vagotomy abrogate the early febrile phase? While answering this question, we first considered the possibility that both vagotomy and ip capsaicin desensitization lead to a thermoeffector deficiency—either secondary to malnutrition (a common side effect of vagotomy) or independent of malnutrition. We rejected both hypotheses after finding that fever is attenuated in vagotomized rats, even when malnutrition is prevented by special measures [15], and that well-nourished vagotomized [31] and well-nourished capsaicin-desensitized [32] rats exhibit no thermoeffector dysfunction.

We then examined the possibility that vagotomy activates the endogenous antipyretic system. If this were the case, vagotomized animals would not develop fever, even if a pyrogenic signal did reach the brain. This hypothesis was also rejected after we and others showed that vagotomized rats exhibit a normal thermal response to intracerebral administration of pyrogenic mediators, such as PGE_2 [33, 34] and cholecystokinin octapeptide [34].

Others suggested yet another possibility that vagotomy induces translocation of intestinal bacteria, resulting in the development of pyrogen tolerance [35]. We have argued elsewhere [36] that this explanation can be rejected for 2 reasons: tolerance is reported to have no effect on the first febrile phase, even when the second phase is prevented; and our work with selective subdiaphragmatic vagotomies *(vide infra)* indicates that the febrile nonresponsiveness of vagotomized animals is caused by denervation of the liver, portal vein, or both, but not of the gut.

Lacking an alternative explanation, we then concluded that vagotomy most likely alters transduction of propyretic signals from the blood to the brain. This conclusion is consistent with findings based on a wide array of data from other laboratories that demonstrated failure of propyretic signals to reach the brain after peripheral pyrogen administration in vagotomized animals. Such data concern LPS-induced IL-1β mRNA synthesis in the brain of mice [37] and rats [38], *c-fos* expression in the brain [39] and nodose ganglion [29] of rats, and the increase in PGE_2 concentration in the brain of guinea pigs [14], all of which are attenuated or even prevented by vagotomy.

Which abdominal vagal branch is involved in fever? We have also tried to identify the specific abdominal vagal branch involved in the genesis of fever [26]. In the rat—the species most commonly studied in vagotomy experiments—the anterior and posterior vagal trunks divide just below the diaphragm into 5 primary branches: the anterior (ventral) gastric, posterior (dorsal) gastric, anterior (accessory) celiac, posterior celiac, and hepatic [40]. In experiments involving rats with selective abdominal vagotomies (celiac, gastric, and hepatic), we have shown that only the hepatic branch is crucial for the early febrile

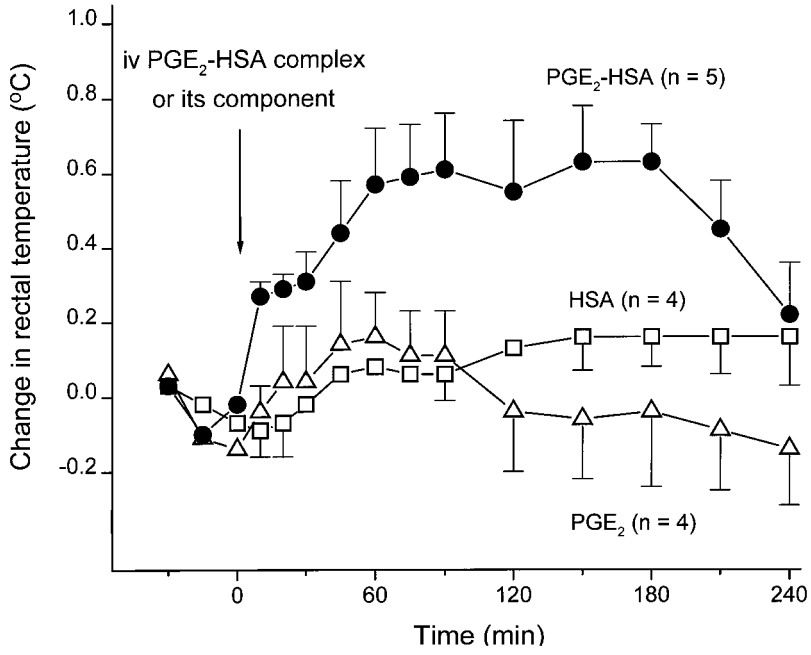

Figure 3. Thermal responses of rabbits to the iv administration (the arrow indicates the time of injection) of one of the following: the preformed complex of prostaglandin E_2 (PGE_2) with human serum albumin (HSA; 70 μg/kg and 30 mg/kg, respectively); its component, free PGE_2 (70 μg/kg); or HSA alone (30 mg/kg). Modified from Romanovsky et al. [47], with the permission of the American Physiological Society.

response (figure 2). The hepatic branch is the second smallest of the primary branches of the abdominal vagus and has the lowest content of efferent fibers [41]. Selective hepatic vagotomy is arguably the least traumatic method of vagal deafferentation that has thus far been demonstrated to successfully alter febrile responsiveness. Hence, it is reasonable to suggest that the effect observed in the animals with selective hepatic vagotomy was not due to complications of the surgery but rather reflected the interruption of afferentation from the anatomic region serviced by the hepatic branch—that is, the liver and its portal vein.

The liver has long been suspected of having a role in febrile pathogenesis [42]. This suspicion has been reinforced by reports of hepatic clearance of peripherally injected pyrogens [43, 44]. Additionally, we have recently shown that, in rats with liver congestion, the first phase of the febrile response to LPS is markedly attenuated [45]. Moreover, others have shown that infusion of IL-1 into the portal vein increases the discharge rate of hepatic vagal afferents [7, 27]; that IL-1 receptors are present on vagal paraganglia associated with the hepatic branch [46]; and, most importantly, that hepatic vagotomy abolishes not only fever [26] but also other responses to LPS, such as hyperalgesia [9].

What triggers vagal afferentation in fever? In 1987, Morimoto et al. [4] showed that the first phase of the febrile response to iv LPS can be blocked by a peripheral, but not central, injection of the cyclooxygenase (COX) inhibitor, indomethacin; the authors also speculated that the mechanism of the first phase of fever involves peripheral nerves. Similarly, Cooper and Rothwell [6] have found that the initial febrile phase of the response to intramuscular turpentine is mediated by prostaglandin synthesis outside, but not inside, the blood-brain barrier, and that the same febrile phase also depends on integrity of afferent neural fibers. Analysis of these data taken together suggests that neural afferentation in fever is coupled with blood-borne prostaglandins. However, several attempts to induce fever by an iv or intracarotid injection of PGE_2 or PGE_1 have failed, as have attempts to detect PGE_2 in the cerebrospinal fluid or preoptic microdialysate after PGE_2 infusion into the carotid artery (reviewed in [47]). As a result, the idea of blood-derived PGE_2 playing an important role in fever was rejected [48]. Nevertheless, we have cautioned the reader [47] that such a rejection might have been premature and that the reported inability of peripheral PGE_2 to enter the brain and to trigger the febrile response might have had a methodological explanation.

Prostaglandins are amphipathic, poorly water-soluble substances. As such, they readily self-aggregate in aqueous solutions. Such aggregation can alter tissue distribution and diminish the biological activity of amphipaths [49]. One way to prevent aggregation of prostaglandins is by binding to serum albumin, a principal transport protein for amphipathic molecules in the blood [50]. In a recent study [47], we compared the pyrogenic effects of free PGE_2 and its preformed complex with human serum albumin injected iv into rabbits. Neither free PGE_2 nor human serum albumin alone was pyrogenic, whereas

the PGE_2–human serum albumin complex produced fever characterized by a short latency and a substantial increase in body temperature (figure 3). Analysis of these data demonstrates the pyrogenicity of PGE_2 administered iv in a physiologically relevant form (as an albumin complex) and thus eliminates the most important objection against the involvement of peripheral PGE_2 in fever. We believe that blood-derived PGE_2 mediates the early febrile phase and that its likely targets are the vagal afferent fibers, perhaps those within the hepatic branch. Indeed, expression of prostaglandin receptors on vagal sensory neurons and effects of peripheral PGE_2 on vagal afferent transmission have been demonstrated (reviewed in Ek et al. [30]). Moreover, activation of vagal afferents by cytokines has recently been shown to be sensitive to COX inhibitors, thus suggesting that vagal afferentation during fever is triggered, at least partially, by prostaglandins [27, 30].

Conclusions

On the basis of the foregoing data, we tentatively conclude that capsaicin-sensitive neural fibers traveling within the hepatic vagus constitute a necessary component of the afferent mechanism of the first (early) febrile phase, but not the later febrile phases. We also speculate that the vagal afferent pathway is activated by blood-borne PGE_2. If the latter speculation is valid, it might explain the robust antipyretic effects of peripherally administered COX inhibitors that do not cross the blood-brain barrier. Examples of such effects of COX inhibitors can be found elsewhere in these proceedings.

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