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The behavioral thermoregulatory response of febrile female rats is not attenuated by vagotomy

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Abstract

An oft-overlooked consequence of fever is the occurrence of thermoregulatory heat-seeking/producing behaviors. Subdiaphragmatic vagotomy attenuates fever resulting from low dose, peripherally administered pyrogens, suggesting that the vagus is involved in generating the pathogen-induced rise in core body temperature (T_c). This study was designed to confirm that rats utilize behavioral thermoregulation to augment fever following systemic administration of lipopolysaccharide (LPS), and to test the hypothesis that, in febrile animals, vagotomy would block the preference for a higher ambient temperature (T_a) as T_c is rising. First, female Sprague–Dawley rats received IP injections of either saline or LPS (50 μg/kg), prior to placement inside a thermal gradient that offered subjects T_a values between 7 and 45 °C. LPS injection caused significant increases in T_c and selection of a higher T_a as compared to saline administration. Second, groups of rats were vagotomized, sham-vagotomized or received no surgery, and then underwent the same gradient testing procedure. Vagotomy attenuated LPS-induced fever, but did not influence the concomitant behavioral thermoregulatory response. All groups selected comparable, higher T_a values following LPS vs. saline. These data suggest that the reduction in the febrile response to LPS administration following vagotomy is not due to inhibition of the behavioral thermoregulatory response to the pyrogen. Rather, this behavioral response to LPS appears to be mediated by a nonvagal mechanism.

Keywords: LPS; Fever; Thermoregulation; Rat; Thermal; Gradient

1. Introduction

The development of a fever is one of the first defensive responses of many organisms to invasion by a pathogen. Fever is a phylogenetically ancient and complex response involving both physiological and behavioral components, which has been evolutionarily conserved because of its adaptive value. Almost all animals, both warm- and cold-blooded, can develop fevers in response to invasive organisms, such as bacteria and viruses, or agents, such as lipopolysaccharide (LPS), a component of gram-negative bacterial cell walls (Kluger, 1991).

Behavioral thermoregulation is an important and, for some species, crucial, component of fever development. Indeed, when animals experience fever, they do not seek out cooler ambient temperatures to counteract the change in equilibrium; rather, they facilitate their fever by moving to a warmer environment if given the opportunity. Animals that are unable to autonomically produce a febrile response (e.g., lizards, toads and newborn rabbits) will behaviorally generate a fever when given a toxin, by moving to a warm area of their environment (Vaughn et al., 1974; Satinoff et al., 1976; Bicego and Branco, 2002). Moreover, mammals, such as rats, dogs and rabbits that are able to become febrile at room temperature when

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given a pyrogen, will similarly augment their fevers behaviorally when given a chance to do so (Briese, 1997; Cabanac et al., 1970).

In order for fever to occur, the brain must first detect an exogenous pyrogen. Much research has yielded evidence that both humoral and neural mechanisms signal the brain about the presence of an invader. The humoral mechanism involves the release of cytokines, such as IL-1β, from macrophages and monocytes. Cytokines are believed to circulate in the blood and then cross the blood–brain barrier via the circumventricular organs. After entering the brain, they act on neurons in the preoptic and anterior hypothalamus (PO/AH) to release prostaglandins, such as prostaglandin E_2 (PGE_2). PGE_2, as well as IL-1β, in turn, cause a decrease in the firing rate of warm-sensitive neurons and an increase in the firing rate of cold-sensitive neurons (Shibata and Blatteis, 1991; Hori et al., 1992; Matsuda et al., 1992), which leads to the thermoregulatory set-point being increased. This in turn causes the animal to feel cold, and then if it can, to engage in heat-producing/heat-conserving responses, which facilitate the onset of the febrile temperature rise (Satinoff, 1978). More recently, Blatteis et al. (1998) have proposed a neural mechanism for the febrile response: cytokine stimulation of vagal afferents generates a signal traveling first to the nucleus of the solitary tract, and then to the PO/AH via the ventral noradrenergic bundle. Norepinephrine (NE) released in the PO/AH then prompts the release of PGE_2, thus triggering the aforementioned cascade. These two mechanisms—humoral and neural—are thought to act in concert, with the vagal route being more immediate.

This proposed role of the vagus nerve in the development of fever has been the subject of much debate in the past decade. Some laboratories have reported that vagotomy reduces or blocks development of a fever, supporting a role for the vagus nerve in febrigenesis, but others have disputed this notion (see Romanovsky, 2000 for review). The consensus seems to be that the effectiveness of vagotomy in attenuating fever is dependent upon the dose of the pyrogen and its experimental route of administration, with vagotomy being ineffective against high doses of pyrogens, and less effective when intravenous administration is used as opposed to intraperitoneal administration (Azab and Kaplanski, 2001; Hansen et al., 2001; Szekely et al., 2000).

The present study was undertaken to investigate the role of the vagus nerve in behavioral thermoregulation. Our goals were twofold: in Experiment 1, we wished to confirm prior observations that rats would utilize behavioral thermoregulation to augment their fevers following systemic injection of LPS; and in Experiment 2, we tested the hypothesis that an intact vagus is necessary for both behavioral thermoregulation and development of a fever following administration of a moderate dose of this pyrogen.

2. Methods

2.1. Animals

All animal procedures followed the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1996). Forty female Sprague–Dawley rats (10 for Experiment 1 and 30 for Experiment 2), were obtained from Charles River Laboratories (Hollister, CA), and weighed 200–250 g at the time of experimentation. They were housed individually in hanging wire cages with water and Purina rat chow 5001 (Brentwood, MO) available ad libitum. The vivarium temperature was kept at 22 °C, and the light/dark cycle was 12:12 (lights on at 0700 h PST). All tests were conducted during the light phase. Female rats were used to ensure that the subjects would remain small enough to easily move and turn around inside the thermal gradient apparatus.

2.2. Surgery

The 10 animals used in Experiment 1 did not undergo any surgery. For Experiment 2, 10 rats underwent subdiaphragmatic vagotomies (VGX), following injection of atropine (0.04 mg/kg IM, Phoenix Scientific, St. Joseph, MO) and anesthetization with 2.5% isoflurane (Abbott Laboratories, Chicago, IL). The surgical procedure was modeled after that of Sehic and Blatteis (1996). In brief, a midline laparotomy was performed and the two bundles of the vagus nerve, one on each side of the esophagus, were dissected free and sectioned. The analgesic flunixin meglumine (Banamine, 1.5 mg/kg SC, Schering–Plough Animal Health, Union, NJ) was also given prior to surgery, and the antibiotic enroflaxin (Baytril, 5 mg/kg SC, Bayer, Shawnee Mission, KS) was administered postoperatively to prevent infection. Antibiotic treatment was continued postoperatively by adding Baytril (0.1 ml containing 2.27 mg) to animal crackers every day for 1 week following surgery. Additional highly palatable food and fluids (i.e., soymilk and cookies) were given to the VGX rats after surgery until their body weights reached preoperative levels (approximately 10 days). Ten sham vagotomies (SHM) were performed in exactly the same manner, except that the vagus nerve was merely handled gently with a sterile cotton swab, but was not dissected or cut. An additional 10 animals did not undergo any surgery or anesthetization (NS).

2.3. Gradient apparatus

The temperature gradient consisted of a 7.5-cm-diameter Plexiglas tube, 183 cm long, with copper tubing coiled around it along its length. The tubing was coiled tightly at one end with the coils gradually spaced further apart towards the other end. The apparatus was kept in a room in which the air temperature was maintained at 5 °C. Warm

water (50 °C) was pumped to the tight end and flowed through the copper coils along the length of the gradient. The water flow was controlled so that the temperature inside the gradient varied from 7 °C at the cold end to 45 °C at the warm end. A scale marked in inches on the gradient was visible to an observer seated outside the room. The temperature corresponding to this scale was calibrated by measuring the temperature along the gradient's inner length with a Yellow Springs Instruments meter (no. 46, Yellow Springs, OH) and thermistor probe (no. 402) encased in 25 g of clay. The observer outside the cold room continuously recorded the position of the animal inside the gradient for 2 h. These data were divided into 10-min blocks; for each block, an ambient temperature (T_a) preference was calculated, based on the time spent by the animal at any location in the gradient. The mean of the T_a preferences during these consecutive 10-min blocks was used as a measure of each animal's overall T_a preference during the 2 h it was in the gradient.

2.4. Procedure

The protocols for the two experiments were identical. The animals were fully adapted to the experimental procedures. On days 15–20 following arrival (Expt. 1) or postsurgery (Expt. 2), animals underwent daily rectal temperature measurement followed by placement in the thermal gradient for 1 h. T_c was measured rectally with a Physitemp (Clifton, NJ) BAT-12 meter and a thermocouple probe inserted 7 cm. On the day of testing, each rat received an IP injection of either pyrogen-free saline (1 ml/kg) or 50 μg/kg LPS (*Escherichia coli* serotype 0111:B4, Sigma, St Louis, MO) suspended in pyrogen-free saline at a concentration of 50 μg/ml. Half of the animals received an injection of saline first and then an injection of LPS 5–7 days later, and half received injections in the opposite order. Injections were given at 0800 or 1000 h. To avoid potential estrous-cycle-induced variations in response to LPS, rats were only tested on the day of diestrus, as determined by daily vaginal smears.

After each injection, rats were returned to their home cages for 3 h, after which each was placed in the gradient apparatus for 2 h. Preliminary data had indicated that this time interval was optimal for identifying a febrile response to LPS treatment. Core temperature (T_c) was measured at four time points: before injection of LPS or saline (Time 1), 3 h later (i.e., just before placement into the gradient; Time 2), 1 h after placement in the gradient (Time 3), and upon removal from the gradient (Time 4). T_c measurement at Time 3 required brief removal of the animal from the gradient.

2.5. Data analysis

For all analyses, an alpha level of 0.05 was required for statistical significance.

2.5.1. Core body temperature (T_c)

A two-way analysis of variance with repeated measures was used to evaluate the effects of Drug (LPS vs. saline), Time and Drug×Time interactions on T_c values for animals in Experiment 1. A three-way analysis of variance with repeated measures was used to examine the effects of Drug (LPS vs. saline), Group (VGX vs. SHM vs. NS), Time, and interactions among these factors on T_c for the animals in Experiment 2. One-way analyses of variance were performed on individual time points for post hoc paired comparisons.

2.5.2. Preferred ambient temperature (T_a)

For Experiment 1, a two-tailed paired *t*-test was used to determine whether the animals' overall mean T_a preferences differed following injection of LPS vs. saline. For Experiment 2, a two-way analysis of variance was used to evaluate the effects of Drug (LPS vs. saline), Group (VGX vs. SHM vs. NS), and Drug×Group interactions on overall mean preferred T_a. Fisher's PLSD test was performed post hoc for paired comparisons.

2.5.3. Completeness of vagotomy

Completeness of vagotomy was determined by removal and weighing of the stomachs of animals after they had been euthanized with CO_2. The stomachs of vagotomized animals were expected to weigh more due to the fact that vagal transection causes delayed gastric emptying (Kraly et al., 1986; Romanovsky et al., 1997). A one-way analysis of variance was used to compare VGX, SHM and NS stomach weights. Fisher's PLSD was performed post hoc for paired comparisons.

3. Results

3.1. Experiment 1. Fever and behavioral thermoregulation following injection of LPS

The T_c values of animals prior to and following injection of LPS or saline are shown in Fig. 1. LPS injection was highly effective in triggering febrigenesis, as evidenced by the significant main effect of Drug [$F(1,9)=9.461$, $p<0.01$]. T_c values following LPS injection were higher than those observed following saline injection. There was also a significant main effect of Time [$F(3,27)=48.612$, $p<0.001$], with T_c values increasing over time on both LPS and saline trials from Time 1 to Time 4: by an average of 0.66 °C after saline injection and an average of 1.84 °C after LPS. The time effect seen after saline administration is presumably due to the fact that the rats were sitting at a higher T_a than the vivarium temperature, which is well below the animals' thermoneutral zone (Romanovsky et al., 2002). A significant Drug×Time interaction was also found [$F(3,27)=7.078$, $p<0.001$], with the effects of LPS administration on T_c over time differing from those of saline.

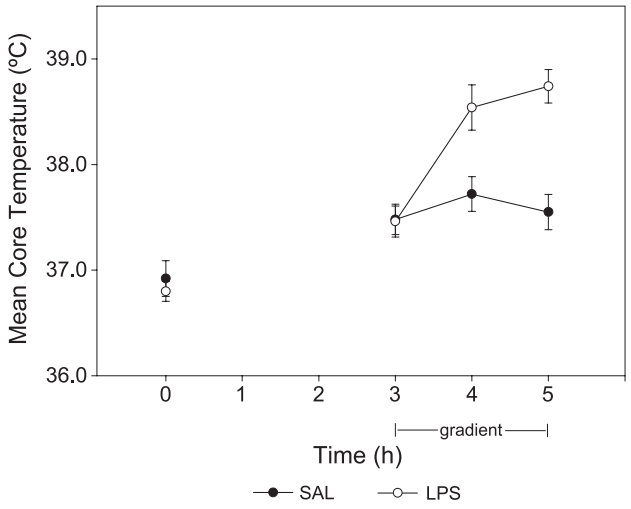

Fig. 1. Mean (±S.E.M.) core temperatures (T_c values) of rats (N=10) following injection of LPS or saline. Time 1, prior to injection; Time 2, 3 h after injection, and just prior to placement in the gradient; Time 3, after 1 h in the gradient; Time 4, after 2 h in the gradient (5 h postinjection).

Animals' T_c values after injection of both LPS and saline increased from Time 1 to Time 2; however, T_c values in those rats that received LPS continued to increase across Time 3 (fever becoming evident at this time point) and Time 4, while those of animals that received saline did not.

Fig. 2 depicts the preferred T_a values while the animals were in the thermal gradient following LPS or saline treatment. Analysis of the data revealed a significant difference between the LPS and saline trials $t(9)=-3.405$, $p<0.01$. Animals chose higher T_a values after LPS than after saline administration (selecting an average of 2 °C higher after LPS), confirming that rats do, in fact, use behavioral thermoregulation to increase fever following injection of LPS.

3.2. Experiment 2. Effect of VGX on LPS-induced fever and behavioral thermoregulation

T_c values of VGX, SHM and NS animals prior to and after LPS or saline injection are depicted in Fig. 3. The pyrogenic action of LPS was evident as a significant main effect of Drug [$F(1,48)=42.269$, $p<0.0001$]; overall, animals in all three groups had higher T_c values following injection of LPS than after saline. As in Experiment 1, there was also a significant main effect of Time [$F(3,144)=20.601$, $p<0.0001$], and a significant Drug×Time interaction [$F(3,144)=12.032$, $p<0.0001$], demonstrating a tendency for T_c to increase overall and that the effects of the LPS differed from those of saline over time. There was no statistically significant main effect of Group (VGX vs. SHM vs. NS), nor a significant Time×Group interaction, indicating that VGX did not alter animals' overall T_c values nor their T_c changes over time. However, VGX rats did respond somewhat differently than SHM and NS animals to LPS injection, the Drug×Group interaction approached significance [$F(2,48)=2.428$, $p<0.09$]. VGX animals showed an attenuated febrile response after LPS administration as compared to the SHM and NS animals (Fig. 3). A slight increase in T_c in all groups after LPS was evident at Time 2. However, the T_c values of both the SHM and the NS animals continued to rise across both Times 3 and 4, reaching their peaks at Time 4; while the T_c values of VGX animals increased very slightly from Time 2 to Time 3 but then did not increase any further. Post hoc tests showed no difference between NS vs. SHM groups after LPS at Time 3 or Time 4, while there were significant differences between NS vs. VGX groups at both Time 3 ($p<0.04$) and Time 4 ($p<0.0049$). The difference between SHM vs. VGX groups at Time 3 approached significance ($p=0.06$), while at Time 4, the difference was significant ($p<0.0079$). There were no differences between any groups at Times 3 or 4 after saline.

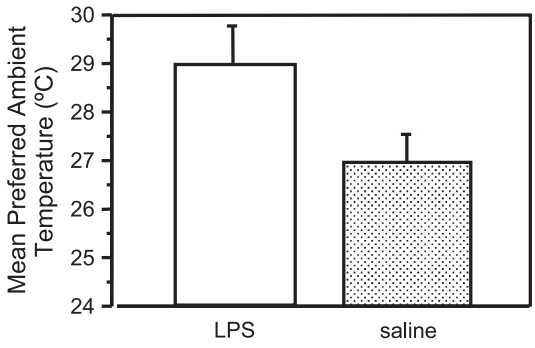

Fig. 2. Mean (±S.E.M.) preferred ambient temperature for rats (N=10) after LPS and saline administration.

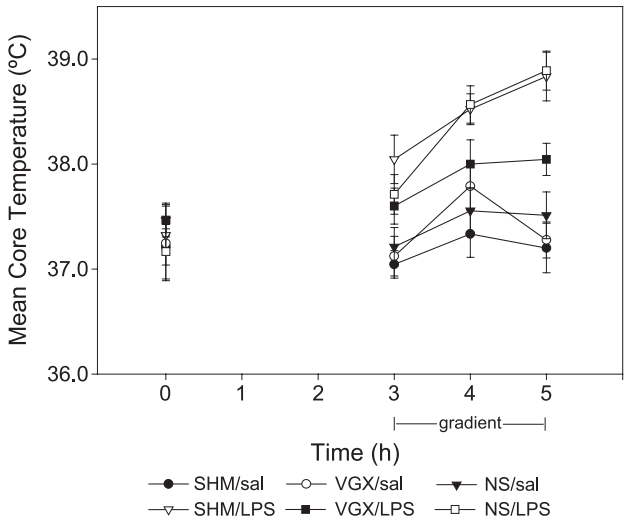

Fig. 3. Mean (±S.E.M.) core temperatures (T_c values) of vagotomized (VGX; N=10), sham-VGX (SHM; N=10) or no-surgery (NS; N=10) rats following injection of LPS or saline. Time 1, prior to injection; Time 2, 3 h after injection, and just prior to placement in the gradient; Time 3, after 1 h in the gradient; Time 4, after 2 h in the gradient (5 h postinjection).

The preferred T_a values while animals were in the thermal gradient following injection of LPS or saline in SHM, NS and VGX rats are shown in Fig. 4. Analysis of the data revealed a significant main effect of Drug

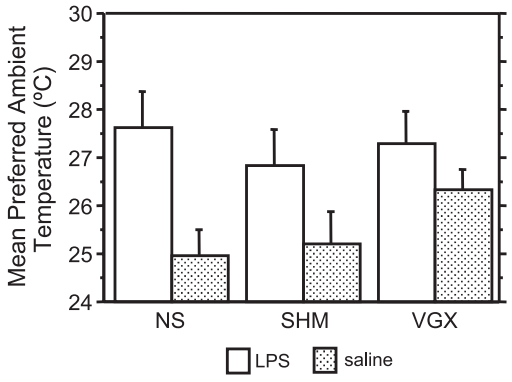

Fig. 4. Mean (±S.E.M.) preferred ambient temperature for vagotomized (VGX; N=10), sham-VGX (SHM; N=10) and no-surgery (NS; N=10) rats following injection of LPS or saline.

[$F(1,48)$=12.151, p<0.001], with VGX, SHM and NS rats choosing higher average T_a values in the gradient after injection of LPS as compared to saline. However, there was no statistically significant main effect of Group, nor a significant Drug×Group interaction, indicating that there were no differences in the behavioral response of VGX, SHM and NS animals to LPS, or in their overall selection of T_a. All groups selected comparable and higher T_a values following injection of LPS as compared to saline. Post hoc tests showed no significant differences between NS vs. SHM, NS vs. VGX or SHM vs. VGX. In addition, when the data from these three groups were compared after saline alone, there was no significant difference among them.

As expected, there was a significant difference in stomach weights between vagotomized and nonvagotomized animals [$F(2,24)$=6.456, p<0.0057]. The mean stomach weight of VGX rats was 12.62±0.35 g, as compared to 3.56±0.31 g for the SHM and 3.58±0.25 g for the NS animals, confirming the effectiveness of the surgery. Post hoc tests showed no difference between NS vs. SHM, but a significant difference between NS vs. VGX (p<0.0048) and between SHM vs. VGX (p<0.0047).

4. Discussion

The present results confirm that rats do utilize behavioral thermoregulation (i.e., select a higher ambient temperature) to enhance the febrile response following injection of the pyrogen, LPS. Moreover, these data demonstrate that although subdiaphragmatic vagotomy somewhat attenuated the fever that results from LPS injection, it did not reduce or change the thermoregulatory behavior that animals exhibit after endotoxin injection. VGX animals still selected a somewhat higher T_a in the gradient after LPS administration than they did after saline, as did their non-VGX counterparts, and the pattern of temperature selection after administration of LPS was not different between VGX and control animals.

Several studies have reported a greater attenuation of LPS-induced fever by VGX in rats that were not allowed to choose an ambient temperature (Kapas et al., 1998; Opp and Toth, 1998; Gordon and Rowsey, 2000; Romanovsky et al., 2000). Although there are numerous methodological differences between the current study and those of the cited reports (dose of LPS, route of administration, time of day or strain of rat), one key difference is the use of a thermal gradient in the present work. It is possible that the VGX animals in the present study were still able to generate a moderate fever because they were given the opportunity to behaviorally increase their T_c values. In a recent study by Hansen et al. (2000) using similar treatment parameters as those used in the current study, excluding the use of a thermal gradient, vagotomy failed to produce a reduction in LPS-induced fever. The reason for this discrepancy remains unexplained.

A study by Konsman et al. (2000) found that vagotomy did not block the fever seen after injection of 250 μg/kg LPS, but did block the behavioral depression (as measured by the social interaction test) that is observed after this dose. Although these results may seem at first to be in opposition to the results found in the present experiments, there are a number of differences between the two studies that preclude such a conclusion from being drawn. First, it is not surprising that vagotomy did not block fever in the Konsman et al. (2000) study, since the dose used is fairly high and fever is only blocked by vagotomy at lower doses (Azab and Kaplanski, 2001; Hansen et al., 2001; Szekely et al., 2000). In the present study, 50 μg/kg LPS was used for precisely this reason. Second, social interaction is not the same as behavioral thermoregulation, in our case, heat-seeking behavior. Konsman et al. (2000) used the lowest possible dose of LPS that would result in behavioral depression, which suggests that if they had used 50 μg/kg, the behavioral depression they were measuring probably would not have been observed. However, we did observe heat-seeking behavior after 50 μg/kg LPS; therefore, different pathways might mediate the two kinds of behavior, or perhaps the differences were due to the disparate time points at which behavior was examined in the two studies. Konsman et al. (2000) measured behavioral depression 2 h after administration of LPS, while we measured heat-seeking behavior from 3 to 5 h postinjection.

The present data suggest that the behavioral response to fever is controlled by a different mechanism than the physiological response that occurs during infection or inflammation. Although we demonstrate that the behavioral response does not require an intact vagus, it is not clear what the mechanism for behavioral thermoregulation might be. It seems unlikely that the behavioral thermoregulation observed in the current experiment is mediated by the PO/AH, since Carlisle (1969) and Satinoff and Rutstein (1970) have shown that rats with lesions of the PO/AH can still behaviorally thermoregulate even with extreme disruption in autonomic function. This stands in contrast to ectotherms,

such as toads, that do not develop a fever in response to LPS following preoptic area lesions (Bicego and Branco, 2002). However, ectotherms are only able to develop a fever by behavioral means; so it follows that if the key area in the brain regulating temperature "set-point" is destroyed, fever would not result after pyrogen administration. The role of prostaglandins in behavioral thermoregulation could be examined by attempting to block the response that animals exhibit after LPS administration with a cyclooxygenase inhibitor. Bernheim and Kluger (1976) have shown that lizards, which develop fevers by behavioral means, will not show a febrile response to bacterial injection following administration of sodium salicylate.

Thus, although the neural signal for febrigenesis may be blocked by vagotomy, cytokines are still circulating and are able to affect other systems. Another possibility is that the pathway for behavioral thermoregulation is a motivational system based on sensory input, as the affective states brought about by perceived thermal comfort are important initiators of thermoregulatory behavior. Recent work by Craig (2002, 2003) has suggested that the motivational aspect of thermal sensation involves the medial lamina I spino–thalamo–cortical pathway to the anterior cingulate cortex (ACC) and the insula. Afferent Aδ and C-fibers from all over the body convey sensory information about pain, as well as noxious and innocuous temperature, through the thalamus to these areas. Insular cortex has been found to be activated by pain and also by temperature in imaging studies of humans (Craig, 2002), and lesions of the posterior insula limit the amount of pain and temperature information that is perceived (Greenspan et al., 1999).

Johansen et al. (2001) found that lesions of the ACC in rats reduced the conditioned place avoidance that normally results from receiving a painful hind-paw injection of formalin. This finding suggests that the affective, motivational component of pain (i.e., what leads an animal to exhibit a certain behavior to avoid pain) may be mediated via the ACC. It is possible that the behavioral thermoregulation that is engaged in to maintain homeostasis and comfort could be regulated via this pathway as well. The roles of the ACC and insular cortex in thermoregulatory behavior have not been studied; however, if circulating cytokines did stimulate this system, the possibility for its involvement is reasonable.

Sensory neuron activity is modulated by cytokines. Studies have shown that TNF-α can evoke discharges in C-fibers when applied to dorsal root ganglion neurons, as well as increase the sensitivity of these neurons to electrical stimulation (Zhang et al., 2002). IL-1β has also been found to modulate the activity of C-fibers, the activation of which appears to be essential in evoking particular inflammatory responses, such as leukocyte recruitment (Ahluwalia and Perretti, 1996; McLean et al., 2000). Moreover, Bret-Dibat et al. (1997) reported that when C-fibers are selectively destroyed by capsaicin, there is no effect on the anorexia induced by IL-1β administration, demonstrating the importance of these sensory fibers to a cytokine-modulated behavior. It would be interesting to explore the role of this pathway in behavioral thermoregulation. Perhaps animals with lesions of the ACC or insular cortex, or those that had been given capsaicin would not seek a higher T_a after LPS injection.

In conclusion, the present study has demonstrated that rats exhibit heat-seeking behavior after administration of LPS, but this behavioral response to pyrogen injection is not dependent on the vagus nerve. Further study is still needed to determine the exact circuit for behavioral thermoregulation.

Acknowledgments

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