Vagal CCK and 5-HT$_3$ receptors are unlikely to mediate LPS or IL-1β-induced fever

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Vagal CCK and 5-HT$_3$ receptors are unlikely to mediate LPS or IL-1β-induced fever. Am J Physiol Regulatory Integrative Comp Physiol 279: R960–R965, 2000.—Previous studies suggested that peripheral immune mediators may involve intermediates acting on the vagus nerve, such as CCK or serotonin (5-HT). We have therefore investigated a possible role for vagal CCK-A and 5-HT$_3$ receptors in the febrile response after intraperitoneal human recombinant interleukin-1β (IL-1β) or lipopolysaccharide (LPS). Unanesthetized, adult male rats instrumented with abdominal thermistors were given intraperitoneal CCK-8 sulfate (100 or 150 μg/kg) or 2-methyl-5-hydroxytryptamine maleate (4 mg/kg). In other experiments, rats were treated with either antagonists to the 5-HT$_3$ receptor (ondansetron HCl; 100 μg/kg) or the CCK-A receptor (L-364,718, 100 or 200 μg/kg) in combination with LPS or IL-1β. CCK administration caused a short-lived hypothermia, but interference with the action of endogenous CCK at CCK-A receptors was without effect on IL-1β- or LPS-induced fever. Neither activation of 5-HT$_3$ receptors nor blockade of 5-HT$_3$ receptors affected body temperature or LPS fever. Taken together, our data support the idea that vagal afferents responsive to pyrogenic cytokines may be different from those responsive to CCK or 5-HT.

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tized, unrestrained rats. In addition, we pretreated rats with antagonists to the 5-HT₃ receptor and the CCK-A receptor to identify a possible participation of endogenous CCK or 5-HT in the temperature response to LPS and IL-1β.

**METHODS**

**Animals.** Fifty-eight male, Sprague-Dawley rats (228–352 g) obtained from the University of Calgary Animal Breeding Colony were used in the experiments. Rats were housed in the vivarium at an ambient temperature of 20–22°C under a 12:12-h light-dark cycle (lights on at 0700) and given food and water ad libitum. All experimental procedures were approved by the University of Calgary Animal Care Committee and were carried out in accordance with the Canadian Council of Animal Care guidelines.

**Surgery.** Rats were anesthetized with pentobarbital sodium (50–60 mg/kg ip). Under aseptic conditions, a telemetry thermistor (Minimitter, Sun River, OR) was inserted into the abdominal cavity of rats housed in high cages, were allowed a minimum of 3 days recovery from surgery before the start of the experiments.

**Experimental procedures.** All experiments were conducted in a temperature-controlled room (22°C) during the light phase. Rats were conditioned to the room before the time of the experiment and were provided with food and water during the experiments. Body temperatures were recorded using antennas under each rat’s home cage. These picked up the signal from the telemetry device and directed that signal to a computer for continuous online recording of body temperature. Online data acquisition and analysis were done with the software Nextlab (Data Sciences, St Paul, MN) on an IBM AT computer. Body temperature was measured for 1 h before and 6 h postinjection. All injections were given intraperitoneally in a 0.3-ml saline volume for each compound (except for the CCK-A receptor antagonist, which was given in 0.5% BSA in saline) and were administered between 1130 and 1330. Only one injection of LPS was given to any animal. Because ondansetron (5-HT₃ receptor antagonist) and L-364,718 (CCK-A receptor antagonist) are short-acting blockers, in some experiments a second injection of each was given ~2.25–2.5 h after the initial injections to ensure correct concentration of the antagonist during the endotoxin-induced fever experiments. Animals participated in anywhere from one to four experiments, including control studies; intervals of 3–9 days separated any given set of experiments, and experiments were carried out using a cross-over design to control for order effects.

**Four sets of experiments were conducted to investigate 1) the effect of CCK on body temperature; 2) the effect of CCK-A receptor antagonist (L-364,718) on body temperature, LPS fever, and IL-1β fever; 3) the effect of the 5-HT₃ receptor agonist (2-methyl-5-hydroxytryptamine maleate) on body temperature; and 4) the effect of the 5-HT₃ receptor antagonist ondansetron on LPS fever.

**Drugs.** We used the following compounds: CCK (CCK-8 5-sulfated; 100 or 150 μg/kg, Bachem, Torrance, CA); CCK-A receptor antagonist, L-364,718 (100 or 200 μg/kg dissolved in 10 μl DMSO, Merck, Rarhway, NJ); 5-HT₃ receptor antagonist (ondansetron HCl; 100 μg/kg, Glaxo, obtained from Foot hills Hospital pharmacy); 5-HT₃ receptor agonist (2-methyl-5-hydroxytryptamine maleate); 4 mg/kg, Research Biochem International, Natick, MA); LPS (derived from *Escherichia coli*; 50 μg/kg, Sigma, St Louis, MO); human recombinant IL-1β (1.0 μg/kg, 10⁶ U/mg, Immunex, Seattle WA).

**RESULTS**

**Effect of CCK on body temperature.** The body temperatures in the vehicle and CCK-treated groups were similar at the time of injection (36.7 ± 0.1 and 36.9 ± 0.2°C; respectively, P ≥0.5). Intraperitoneal injection of vehicle (n = 7) led to a transient increase in body temperature that returned to the pretreatment value by the next hour (Fig. 1). This initial slight elevation of body temperature was observed in some but not all experimental groups and is thought to be associated with the behavioral response to the injection procedure. In contrast to the control injections, when these same rats were injected with 100 μg/kg of CCK, they displayed a significant drop in body temperature (P₁₁₂ = 8.089, P = 0.016) during the first hour postinjection without initial elevation in body temperature. The hypothermia reached its minimum (36.2 ± 0.3°C; a drop of approximately −0.7°C) 30–45 min after the injection and returned to the pretreatment value by the second hour after the injection (Fig. 1). This experiment was repeated in seven other rats using a higher dose of CCK (150 μg/kg ip), and similar hypothermic values were obtained (data not shown) in the hour after CCK injection.

**Effect of CCK-A receptor antagonist (L-364,718) on body temperature.** The initial values of body temperature in the vehicle and CCK-A receptor antagonist-treated groups were similar (37.3 ± 0.2 and 37.4 ± 0.2°C, respectively, P ≥0.5; n = 5). Intraperitoneal injection of vehicle or 200 μg/kg CCK-A receptor antagonist led to similar small transient increases in body temperature that returned to the pretreatment value by the next hour. Overall temperature responses were identical between the two groups, indicating that the antagonist was without effect on normal body temperature (Fig. 2A).
observed similar fever development between rats receiving LPS and vehicle (n = 6) and other rats receiving LPS with the antagonist (n = 6; data not shown). Thus, under all conditions tested, the CCK-A receptor antagonist was without effect on LPS fever.

Effect of CCK-A receptor antagonist on IL-1β fever. The initial values of body temperature in the IL-1β and IL-1β + CCK-A receptor antagonist-treated groups were similar (37.2 ± 0.1 and 37.1 ± 0.9°C, respectively, P<0.5). The administration of IL-1β (1 μg/kg ip) resulted in a fever that peaked ~135 min after the injection at a body temperature of 38.2 ± 0.2°C and defervescence proceeded for the next 3 h. (Fig. 2C). Treatment with the CCK-A receptor antagonist (200 μg/kg ip) in these same animals on a different occasion in conjunction with IL-1β (1 μg/kg ip) resulted in a fever almost identical (P=0.05; n = 10) to that induced by IL-1β alone. Thus the CCK-A receptor antagonist was also without effect on IL-1β fever.

Effect of 5-HT3 receptor agonist (2-methyl-5-HT) on body temperature. The initial values of body temperature in the vehicle or the 2-methyl-5-HT-treated groups were similar (37.3 ± 0.1 and 37.1 ± 0.1°C, respectively, P<0.5). Intrapерitoneal injection of either vehicle or the 2-methyl-5-HT (4 mg/kg ip) into the same animals (n = 10) on different occasions caused no significant changes in body temperature (Fig. 3).

5-HT3 receptor antagonist (ondansetron) and fever. The initial values of body temperature in the vehicle or ondansetron-treated groups were identical (37.0 ± 0.1 and 37.0 ± 0.1°C, respectively, P<0.5). The administration of LPS (50 μg/kg ip) resulted in a fever similar to that observed in the experiments reported above. The fever peaked ~165 min after the injection, with body temperature reaching 38.6 ± 0.2°C; the hyperthermic body temperature values were observed during the next 4–5 h (Fig. 4). In the presence of the 5-HT3 receptor antagonist ondansetron (100 μg/kg; n = 6), LPS-induced fever was identical to that seen in the control group (P<0.05, ANOVA).

Effect of CCK-A receptor antagonist on LPS fever. The initial values of body temperature in the LPS- and LPS + CCK-A receptor antagonist-treated groups were similar (37.1 ± 0.1; n = 5 and 37.1 ± 0.1°C; n = 5, respectively, P<0.5). The administration of LPS (50 μg/kg ip) resulted in a fever that began to rise ~90 min after injection. The fever reached a peak of 38.7 ± 0.2°C ~150 min after the injection; the body temperature remained elevated over the duration of the 6 h postinjection recording period (Fig. 2B). In the other group of animals receiving an identical dose of LPS, along with the CCK-A receptor antagonist (200 μg/kg), a similar fever profile developed with no significant differences between the two groups (P>0.5). This experiment was repeated in an additional 12 rats, using a dose of 100 μg/kg of the CCK-A receptor antagonist (or vehicle) and 50 μg/kg LPS; the antagonist was given twice, both at the time of LPS administration and again 2.5 h later. At this dose and treatment regimen, we also observed similar fever development between rats receiving LPS and vehicle (n = 6) and other rats receiving LPS with the antagonist (n = 6; data not shown). Thus, under all conditions tested, the CCK-A receptor antagonist was without effect on LPS fever.

Effect of CCK-A receptor antagonist on IL-1β fever. The initial values of body temperature in the IL-1β and IL-1β + CCK-A receptor antagonist-treated groups were similar (37.2 ± 0.1 and 37.1 ± 0.9°C, respectively, P<0.5). The administration of IL-1β (1 μg/kg ip) resulted in a fever that peaked ~135 min after the injection at a body temperature of 38.2 ± 0.2°C and defervescence proceeded for the next 3 h. (Fig. 2C). Treatment with the CCK-A receptor antagonist (200 μg/kg ip) in these same animals on a different occasion in conjunction with IL-1β (1 μg/kg ip) resulted in a fever almost identical (P=0.05; n = 10) to that induced by IL-1β alone. Thus the CCK-A receptor antagonist was also without effect on IL-1β fever.

Effect of 5-HT3 receptor agonist (2-methyl-5-HT) on body temperature. The initial values of body temperature in the vehicle or the 2-methyl-5-HT-treated groups were similar (37.3 ± 0.1 and 37.1 ± 0.1°C, respectively, P<0.5). Intrapерitoneal injection of either vehicle or the 2-methyl-5-HT (4 mg/kg ip) into the same animals (n = 10) on different occasions caused no significant changes in body temperature (Fig. 3).

5-HT3 receptor antagonist (ondansetron) and fever. The initial values of body temperature in the vehicle or ondansetron-treated groups were identical (37.0 ± 0.1 and 37.0 ± 0.1°C, respectively, P<0.5). The administration of LPS (50 μg/kg ip) resulted in a fever similar to that observed in the experiments reported above. The fever peaked ~165 min after the injection, with body temperature reaching 38.6 ± 0.2°C; the hyperthermic body temperature values were observed during the next 4–5 h (Fig. 4). In the presence of the 5-HT3 receptor antagonist ondansetron (100 μg/kg; n = 6), LPS-induced fever was identical to that seen in the control group (P<0.05, ANOVA).
DISCUSSION

Our results show that direct activation of the peripheral serotoninergic system with a 5-HT$_3$ receptor agonist is without effect on body temperature. Furthermore, interference with the action of endogenous 5-HT with a specific 5-HT$_3$ receptor antagonist did not interfere with generation of an LPS fever, indicating that endogenous, peripheral 5-HT (at least acting at this receptor) is not likely to be involved to a significant degree in the generation of the febrile response. Our data concerning the possible involvement of endogenous CCK in body temperature regulation and febrile response are more equivocal: whereas CCK administration caused a short-lived hypothermia, interference with the action of endogenous CCK at CCK-A receptors was without effect on body temperature. Although CCK may play a role in body temperature regulation, no evidence was found to suggest that intraperitoneal injection of LPS or IL-1$\beta$ induced fever development via the activation of the CCK-A receptor system. Thus our data support the idea that vagal afferents responsive to pyrogenic cytokines may be different from those responsive to CCK or 5-HT$_3$ agonists.

CCK and body temperature. There is good evidence to consider the participation of CCK in the febrile response to pyrogens. Both CCK and LPS administration result in a similar activation of Fos protein or of its mRNA in central autonomic nuclei (11, 25, 28). In particular, there is activation by both CCK and LPS of cells within the nucleus of the solitary tract, the site of vagal afferent termination in the brain, and the paraventricular nucleus, a site implicated in fever (19). Furthermore, CCK can activate both gastric afferents (1, 8) and the hepatic branch of the vagus (7), the branch thought to be the most relevant for mediating the effects of LPS (32), but possibly not IL-1$\beta$ (36) effects on body temperature. On the basis of a report describing similar electrophysiological responses of vagal afferents to both CCK and IL-1$\beta$ and the reduction in the magnitude of an IL-1$\beta$-induced increase in vagal nerve activity by a CCK-A antagonist (24), we predicted that the CCK antagonist would interfere with the febrile response. To investigate the possible role of endogenous CCK in the febrile response to modest doses of LPS and IL-1$\beta$, we gave a CCK-A antagonist with these pyrogens. Contrary to our expectations, we did not see any effect of the antagonist on the febrile response to either LPS or IL-1$\beta$, even in replicate experiments, despite the fact that the doses we employed were found previously to interfere with the action of both endogenous CCK (6, 26) and exogenously administered CCK (34). Our observations of the lack of involvement of CCK in the febrile response to LPS and IL-1$\beta$ are in agreement with previous reports that peripheral CCK receptors are not involved in the anorexic, behavioral (3), or hypothalamic-pituitary-adrenal response (9) to pyrogens. However, further experiments using a broader range of doses of LPS may yet uncover a subtle interaction of peripheral cytokines and CCK.

The potential involvement of CCK in body temperature regulation is still a possibility given that we observed a transient hypothermia after exogenous CCK. This action of CCK has been reported previously (23, 33, 34), but temperature measurements in previous studies had not been extended long enough to rule out a subsequent hyperthermia. Although the intraperitoneal route of administration of CCK in our experiments does not allow us to specify the site of this action, the previous demonstration that this hypothermia-inducing action is capsaicin sensitive (33) makes it likely that it is acting on afferent fibers. The presence of CCK-A receptors on the vagus makes this nerve a likely site for the hypothermic action of CCK, although capsaicin could also interfere with a possible action on spinal afferents. If indeed peripheral CCK plays a role in thermoregulation, one possibility is that it may be involved in the initial hypothermia sometimes seen after a large dose of LPS. In fact, there is some evidence that this hypothermia is a regulated drop in body temperature important in the body’s response to LPS (31).

On the basis of our observations of a hypothermic effect of exogenous CCK, one might anticipate that the CCK antagonist would have caused a bigger fever in response to pyrogen. This we did not see, possibly because the doses of pyrogens used in our experiments did not activate endogenous CCK release. However, Kurosawa and colleagues (24) showed, in anesthetized rats, that plasma CCK was elevated after doses similar to those used in our experiments in conscious rats. Thus, although our experiments do not provide evidence for a role of endogenous plasma CCK in fever generation, CCK may participate in some other aspect of the host-defense response. For example, there is evidence that brain CCK may participate in fever, but this is thought to be via a CCK-B receptor (34).

5-HT and body temperature. 5-HT has been known for many years to influence body temperature (20), but this effect is thought to be mediated by 5-HT$_1$ receptors (22). However, as the receptors on vagal afferents are most likely the 5-HT$_3$ subtype (18), we investigated the
possible involvement of 5-HT3 receptors in body temperature regulation using a 5-HT3 agonist. It was without effect on body temperature, indicating that 5-HT-sensitive vagal afferents do not influence normal body temperature. It was interesting to note that, although both 5-HT3 and CCK receptors have been described on vagal afferents, their activation resulted in different responses in body temperature. This possibly reflects the fact that 5-HT3 agonist and CCK are thought to activate different populations of vagal afferents (17).

In keeping with a lack of effect of 5-HT3 activation on body temperature, the 5-HT3 antagonist ondansetron was also without effect on fever. Nonetheless, the possibility that LPS could activate some population of vagal afferents via a serotonergic mechanism remains, given that gut mast cells, a source of 5-HT (35), are innervated by vagal afferents (38). Furthermore, these cells also contain CD14 receptors that are responsive to LPS (13).

**Perspectives**

Although our findings are not supportive of a role for either endogenous CCK or 5-HT in fever, it is important to note that the conditions under which the participation of the vagus in fever has been implicated are critical and have been the subject of considerable controversy and experimentation. For example, vagotomy is effective in reducing or abolishing LPS or IL-1β fever only when the doses of these compounds used are low. When more pronounced fevers were seen, vagotomy was not effective in interfering with fever development (16, 30). Compared with values reported in the literature, our intraperitoneal dose of LPS would be considered fairly low. Nonetheless, under the appropriate conditions, it is still possible that either CCK or 5-HT could contribute to the body’s responses to pyrogen.

Of perhaps most interest is the fact that there may be a specific subset of vagal afferents that are sensitive to cytokines, but not to either CCK or 5-HT. To the best of our knowledge, the physiological function of this class of afferents has not been determined. It is also interesting that lack of responses to intraperitoneal CCK is often used as a test for completeness of vagotomy in studies investigating the role of the vagus in communicating peripheral immune responses to the brain. The findings that vagal afferents responsive to CCK are apparently not involved in immune responses relevant for fever or food-motivated behavior (4) suggest that this may not be entirely appropriate.

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