Pharmacology Biochemistry and Behavior, Vol. 63, No. 4, pp. 629–637, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00036-2

Endotoxin- and Interleukin-1–Induced Hypophagia Are Not Affected by Adrenergic, Dopaminergic, Histaminergic, or Muscarinic Antagonists

ARTUR H. SWIERGIEL, TINASHE BURUNDA, BRIGETTE PATTERSON AND ADRIAN J. DUNN

Department of Pharmacology and Therapeutics, Louisiana State University Medical Center, Shreveport, LA 71130

Received 14 August 1998; Revised 11 January 1999; Accepted 29 January 1999

SWIERGIEL, A. H., T. BURUNDA, B. PATTERSON AND A. J. DUNN. *Endotoxin- and interleukin-1–induced hypophagia are not affected by adrenergic, dopaminergic, histaminergic, or muscarinic antagonists*. PHARMACOL BIOCHEM BEHAV **63**(4) 629–637, 1999.—Endotoxin (lipopolysaccharide, LPS) and interleukin-1 (IL-1) administration induce hypophagia in rodents. Both IL-1 and LPS are known to activate cerebral norepinephrine and serotonin metabolism, and IL-1 affects that of acetylcholine and histamine. Each of these neurotransmitters has been implicated in feeding behavior. Therefore, the ability of specific antagonists of the above neurotransmitter systems to counteract feeding responses to peripherally injected mIL-1β and LPS was studied. Feeding was assessed in nondeprived mice by measuring the intake of sweetened milk in a 30-min period, as well as daily food pellet intake. LPS and mIL-1β reliably reduced milk intake, and often reduced food pellet intake and body weight. Treatment of the mice with peripherally administered α-adrenergic (phentolamine or prazosin) or β-adrenergic antagonists (propranolol), either alone or in combination, did not significantly alter the hypophagic responses to mIL-1β or LPS. Mice in which cerebral norepinephrine was depleted with DSP-4 or 6-hydroxydopamine also displayed the usual hypophagia in response to mIL-1β and LPS. The hypophagic responses to mIL-1β and LPS were not affected by the histaminergic antagonists, pyrilamine (H_1), cimetidine (H_2), thioperamide (H_3), or the histamine-depleting agent, α-fluoromethylhistidine, nor by the muscarinic cholinergic antagonist, scopolamine. The responses to mIL-1β were also unaffected by the dopamine receptor antagonist, haloperidol, the opioid receptor antagonist, naloxone, and the NO synthase inhibitor, L-NAME. These results suggest that adrenergic, dopaminergic, histaminergic, cholinergic, opioid or nitric oxide systems are not essential for the hypophagia induced by IL-1, and that multiple redundant pathways may be involved in illness-related hypophagia. © 1999 Elsevier Science Inc.

Feeding LPS Anorexia Norepinephrine Dopamine Histamine Acetylcholine Naloxone NO

ACUTE administration of bacterial endotoxin (lipopolysaccharide, LPS) or interleukin-1 (IL-1) has been shown to induce illness-like behaviors in rats and mice (18). One of the more prominent sickness behaviors is an inhibition of food intake (23,46,51). Both LPS and IL-1 affect chemical neurotransmission in the CNS (10,17).

A pronounced and consistent response is an increased activity of cerebral noradrenergic systems most evident in the hypothalamus (7). Secretion of norepinephrine (NE) from the medial hypothalamus is significantly elevated by peripheral administration of either LPS (24) or IL-1 (43). The noradrenergic system has been implicated in the modulation of many behaviors, including various aspects of feeding behavior (4,27). Thus, LPS and IL-1 might result in modulation of feeding by affecting α-adrenergic receptors in the paraventricular nucleus (PVN) (52) or β-adrenergic receptors in the perifornical area (28).

Requests for reprints should be addressed to Dr. Adrian J. Dunn, Department of Pharmacology, LSU Medical Center, P.O. Box 33932, Shreveport, LA 71130-3932.

IL-1β has been reported to increase the turnover of histamine in the hypothalamus (21). Histamine has been implicated in feeding behavior in rats (40). Central administration of dopaminergic agonists has been shown to depress feeding (29). Peripheral administration of IL-1β decreases extracellular acetylcholine secretion in the hippocampus (39), and classical studies indicated that intracerebral injection of carbamylcholine affects feeding (15). Thus, LPS- and IL-1–induced hypophagia may be related to altered noradrenergic, dopaminergic, cholinergic, or histaminergic activity. This possibility has been tested by investigating the ability of various pharmacologic antagonists to attenuate LPS- or IL-1–induced hypophagia in mice.

METHOD

Animals

Six-week-old CD-1 male mice were purchased from Charles River (VAF Plus Colony R16 from the Raleigh-Durham facility). They were housed at 22–23°C in individual plastic cages with wood shaving bedding under a 12-h light–dark cycle with lights on at 0700 h. Mice were given free access to water and Teklad® chow. The animals were rotated within the groups and none of the individuals received more than one injection of LPS or two injections of IL-1. The experiments were conducted in accordance with the NIH Guide on the care and use of animals for research, and the protocol pertaining was approved by the LSUMCS Animal Care and Use Committee.

Feeding Behavior

Food pellet intake and intake of sweetened condensed milk diluted with three parts of water were assessed as described previously (46,47). Mice were habituated for at least 3 days to drink milk from 20-ml glass bottles fitted with metal spouts. The weighed bottles were placed in the cages at around 1100 h for 30 min, then removed and reweighed. Only the animals that drank at least 1.5 g of milk in the session on the final day of habituation were used for the experiments. To estimate food intake, two fresh and firm food pellets were weighed and placed in the cage at around 1200 h. On the following morning at 0800 h the pellets were recovered and weighed. If an individual mouse crumbled the pellets, these data were not included in the analysis. Water intake was not measured.

LPS, IL-1, and Other Drugs

E. coli LPS was purchased from Sigma Co. (St. Louis, MO: L3755, serotype 026:B6) and recombinant mouse IL-1β from R&D Systems (Minneapolis, MN). mIL-1β (100 ng/mouse) and LPS (1 μg/mouse) were dissolved in sterile pyrogen-free isotonic saline such that the total dose for each mouse was contained in 0.1 ml and injected intraperitoneally (IP) 2 h before presenting the milk bottle. LPS and mIL-1β were administered between 0900 and 1000 h. Previous studies indicated that these doses of mIL-1β and LPS reliably produced hypophagia 1–2 h later without causing complete anorexia (46).

Prazosin, phentolamine, *S*-propranolol, haloperidol, pyrilamine, cimetidine, scopolamine and N^G-nitro-L-arginine monomethyl ester (L-NAME) were purchased from Sigma Chemical Co. (St. Louis, MO), naloxone from Endo Laboratories, Inc. (Garden City, NY), and (N-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), 6-hydroxydopamine hydrobromide (6-OHDA), thioperamide and α-fluoromethylhistidine (α-FMH) from Research Biochemicals International (RBI, Natick, MA). The drugs were either dissolved or suspended in sterile isotonic saline (acidified with acetic acid for haloperidol) and injected IP or subcutaneously (SC) in a volume of 0.1 ml (0.2 ml for α-FMH).

We chose to use the highest doses of the drugs that maintained their relative specificity and did not elicit apparent nonspecific effects on the behavior. Prazosin was used at a dose of 0.5 mg/kg, whereas 0.05–0.2 mg/kg was previously found to inhibit restraint-induced changes in mouse behavior in the multicompartment chamber (MCC) (2). Phentolamine was used at a dose of 2 or 4 mg/kg, because 3 mg/kg was reported to block desmethylimipramine-induced aggressive behavior in mice (33). *S*-Propranolol was used at doses of 1.25 and 2.5 mg/kg, doses that had previously been shown to reverse restraint-induced changes in the behavior of mice in the elevated plus-maze and in the MCC (13). The dose of scopolamine used was 0.1 mg/kg IP, because scopolamine had previously been shown to cause a dose-dependent reduction in ACTH-induced grooming in mice with an ED_{50} of 0.03 mg/kg (9). Haloperidol was used at a dose of 0.1 mg/kg, because this dose had been shown to inhibit ACTH- and novelty-induced grooming behavior in mice (16). Naloxone was used at a dose of 2 mg/kg, because it had previously been shown to prevent ACTH- and novelty-induced grooming at doses of 0.02–2.0 mg/kg (14). L-NAME has been widely used as a nonselective inhibitor of NOS at doses in the range 30–50 mg/kg. We observed previously that 30 mg/kg prevented the IL-1- and LPS-induced increases in brain tryptophan and 5-hydroxyindoleacetic acid in mice (8). Pyrilamine (10 mg/kg) was reported to inhibit nociception (32), and cimetidine (50 mg/kg) blocked conditioned taste aversion (30), both in mice. Thioperamide at a dose of 2 mg/kg increased brain histamine metabolism (11) and decreased feeding (25). α-FMH was used at a dose (50 mg/kg) that had been shown previously to deplete brain histamine almost completely and to decrease histidine decarboxylase activity by 90% in mice (31).

Noradrenergic Lesions

DSP-4 was freshly dissolved in saline and injected IP at a dose of 50 mg/kg. This treatment has been shown previously to effectively deplete brain NE in mice (3). 6-OHDA was dissolved in 0.1% ascorbic acid and injected intracerebroventricularly under pentobarbital anesthesia (50 mg/kg). After exposing the skull around bregma, 50 μg in 2 μl was injected free hand into each cerebral lateral ventricle (total dose 100 μg) using a microsyringe. This treatment has previously been shown to effectively deplete NE, with much lesser effects on DA (45). Upon completion of the experiments, 15 days after DSP-4 treatment or 14 days after 6-OHDA treatment, the mice were decapitated and frontal cortex and hypothalamus excised, weighed, and frozen on dry ice. After thawing, the samples were homogenized, centrifuged, and supernatants analyzed by HPLC for the contents of norepinephrine (NE), dopamine (DA), serotonin (5-HT), and their metabolites as previously described (7).

Data Analysis

One- or two-way analysis of variance (ANOVA) was performed using SuperAnova (Abacus Concepts, Inc.). If ANOVA indicated significant effects, Student's *t*-test was used for pair-wise comparisons. All data are reported as mean ± standard error of the mean.

RESULTS

The Effects of Adrenergic Receptor Antagonists on mIL-1β-Induced Changes in Feeding Behavior

In a series of experiments, the effects of adrenergic receptor antagonists on the changes in feeding behavior evoked by injection of IL-1β were studied. We tested the nonspecific α-adrenergic antagonist, phentolamine (4.0 mg/kg), the α_1-adrenergic antagonist, prazosin (0.5 mg/kg), and the nonselective β-adrenergic antagonist, *S*-propranolol (1.25 mg/kg). The antagonists were given 90 min after injection of 100 ng of mIL-1β and 30 min before access to milk, because the peak response to IL-1 occurred 1.5 to 2 h after administration (46). In all cases, milk intake was significantly decreased by mIL-1β (Fig. 1). Food pellet intake (Fig. 1) and body weight 24 h later (data not shown) were also decreased. ANOVA indicated no statistically significant interactions between the mIL-1β and any of the antagonist treatments for milk [prazosin: $F(1, 25) = 2.7$; phentolamine: $F(1, 21) = 1.6$; propranolol: $F(1, 21) = 0$] or food intake [prazosin: $F(1, 25) = 0.1$; phentolamine: $F(1, 21) = 0.1$; propranolol: $F(1, 21) = 0.3$]. None of the drugs had effects of their own, except for a marginal effect of phentolamine on milk intake, $F(1, 21) = 4.3$, $p = 0.06$. Similarly, ANOVA indicated no statistically significant interactions between the IL-1β and antagonist treatments when phentolamine and propranolol were both administered to provide a combined α/β-adrenergic blockade, $F(1, 21) = 3.1$; $F(1, 21) = 0.0$, milk, and food intake, respectively.

In the above experiments, the antagonist treatments were given after mIL-1β, and it is possible that the effect of the noradrenergic activation on feeding was initiated before adrenoreceptors were blocked. Therefore, the effects of a combined dose of phentolamine (2.0 mg/kg) and *S*-propranolol (2.5 mg/kg) given 10 min before injection of mIL-1β were also tested. Milk intake 2 h later and food pellet intake over the next 22 h were depressed by mIL-1β, $F(1, 27) = 31$, $p < 0.0001$; $F(1, 25) = 7.6$, $p < 0.05$, respectively, but neither response was affected by the drug treatment (Fig. 2). ANOVA showed no statisti-

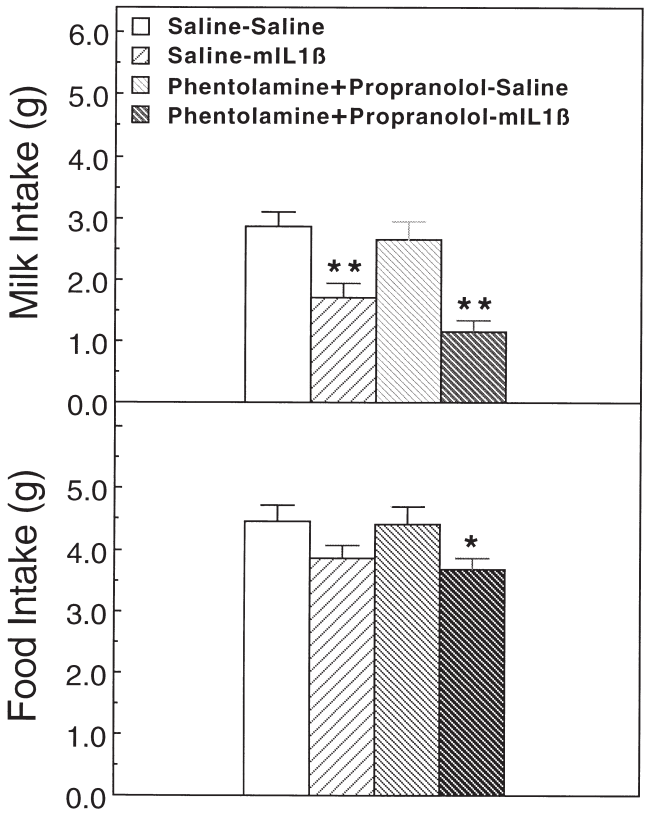

FIG. 2. Milk intake and food pellet intake in mice injected IP with phentolamine (2 mg/kg) plus S-propranolol (2.5 mg/kg) 10 min before mIL-1β. The milk bottle was placed in the cage 2 h after mIL-1β injection. $n = 8$. Significantly different from the corresponding control group (*$p < 0.05$, or **$p < 0.01$).

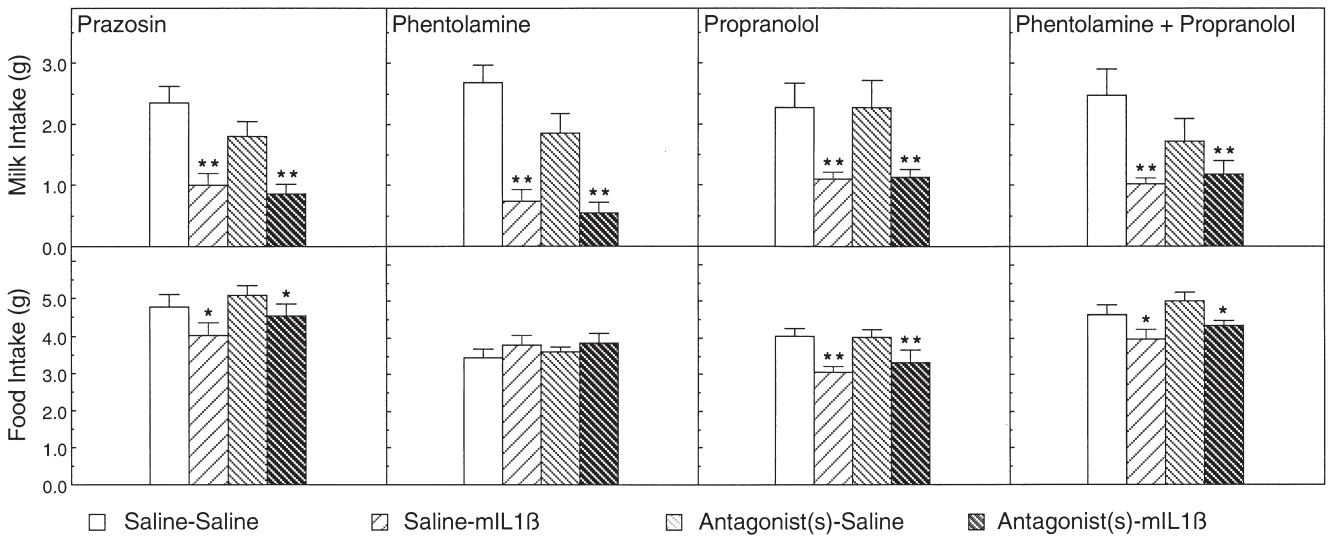

FIG. 1. Milk intake and food pellet intake in mice injected with mIL-1β (100 ng, IP) 2 h before, and prazosin (0.5 mg/kg, SC), phentolamine (4 mg/kg, IP), S-propranolol (1.25 mg/kg, IP) or phentolamine plus propranolol 30 min before the milk bottle was placed in the cage. $n = 7$. Significantly different from the corresponding control group (*$p < 0.05$ or **$p < 0.01$).

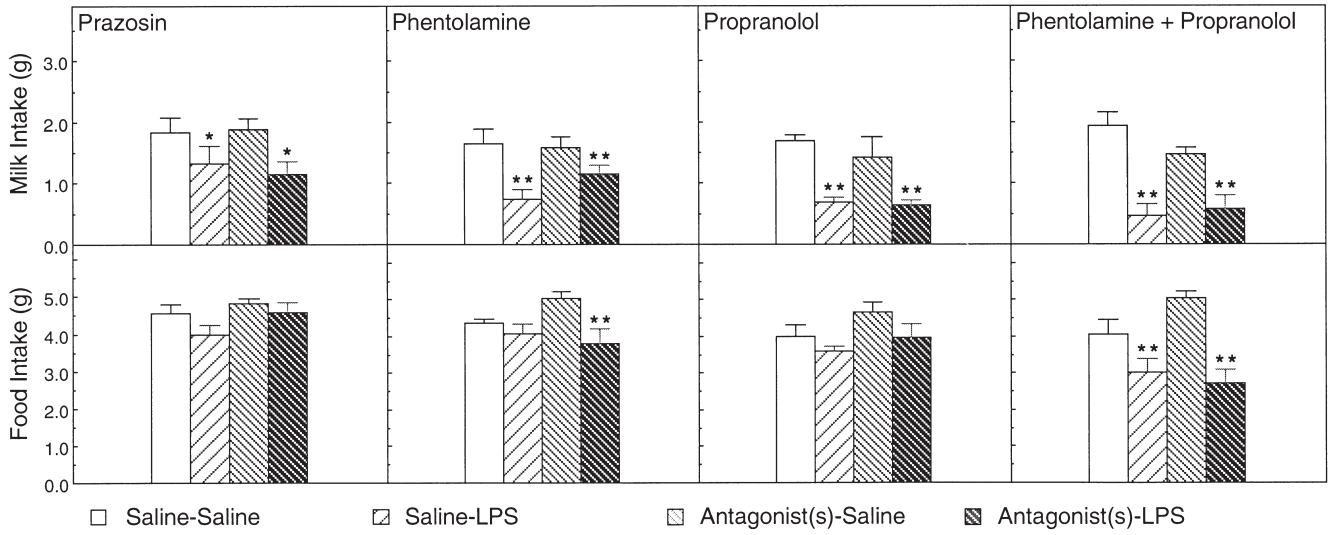

FIG. 3. Milk intake and food pellet intake in mice injected with LPS (1 μg/mouse) 2 h before, and prazosin (0.5 mg/kg), phentolamine (4 mg/kg), S-propranolol (1.25 mg/kg) or phentolamine plus propranolol 30 min before the milk bottle was placed in the cage. $n = 7$. Significantly different from the corresponding control group (*$p < 0.05$ or **$p < 0.01$).

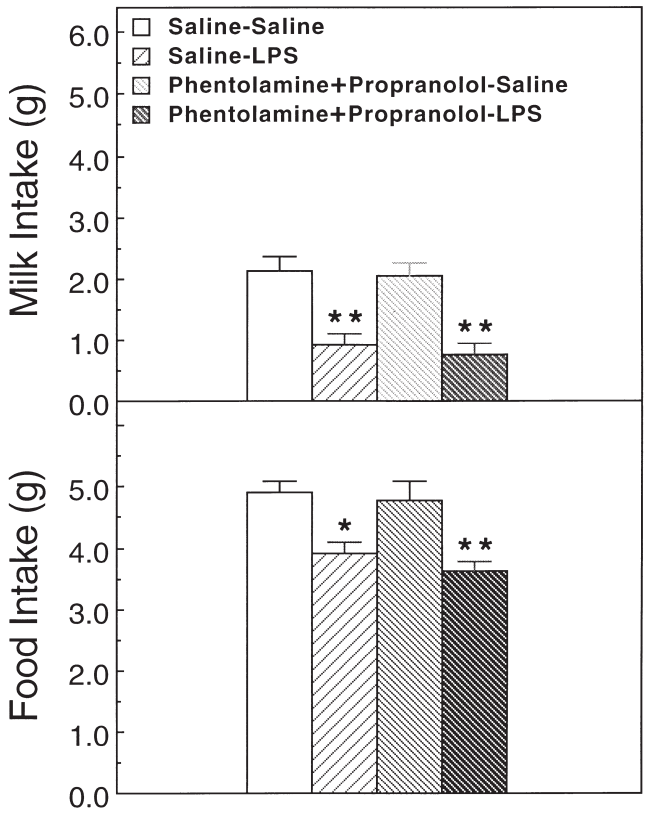

FIG. 4. Milk intake and food pellet intake in mice injected with phentolamine (2 mg/kg, IP) and propranolol (2.5 mg/kg) 10 min before LPS. The milk bottle was placed in the cage 2 h after LPS injection. $n = 8$. Significantly different from the corresponding control group (*$p < 0.05$ or **$p < 0.01$).

cally significant interactions between the mIL-1β and the antagonist treatments, $F(1, 27) = 0.1$; $F(1, 25) = 0$, respectively.

The Effects of Adrenergic Antagonists on LPS-Induced Changes in Feeding Behavior

In a similar series of experiments, the effects of the same adrenoreceptor antagonists on the feeding responses induced by LPS were studied. Prazosin, phentolamine, and propranolol were administered 90 min after injection of 1 μg of LPS and 30 min before access to milk, because the nadir of milk intake was previously shown to occur 2 h after this dose of LPS (46). Milk and food pellet intake and body weight were consistently decreased by LPS (Fig. 3). ANOVA indicated no statistically significant interactions between the LPS and antagonist treatments for milk [prazosin: $F(1, 24) = 0.2$; phentolamine: $F(1, 24) = 0$; propranolol: $F(1, 24) = 0.3$] or food intake [prazosin: $F(1, 24) = 0.5$; phentolamine: $F(1, 24) = 3.1$; propranolol: $F(1, 24) = 0.2$]. Comparable results were obtained using combined treatment with phentolamine and propranolol (Fig. 3). ANOVA failed to show significant interactions between the LPS and the antagonist treatments for milk, $F(1, 23) = 0.2$, or food intake, $F(1, 23) = 3.0$.

Similar results were obtained when phentolamine and propranolol were given 10 min before injection of 1 μg of LPS and milk intake assessed 2 h later (Fig. 4). Milk and food pellet intake were depressed by LPS, $F(1, 28) = 37$, $p < 0.0001$; $F(1, 28) = 25$, $p < 0.0001$, respectively, but neither was affected by the drug treatment, $F(1, 28) = 0.4$; $F(1, 28) = 1.1$, respectively. ANOVA showed no statistically significant interactions between the LPS and antagonist treatments [milk: $F(1, 28) = 0.0$; food pellet: $F(1, 28) = 0.1$].

Effect of DSP-4 Treatment on mIL-1β– and LPS-Induced Hypophagia

By the fourth day after DSP-4 treatment, the mice ingested their pretreatment amounts of milk and food pellets, and their body weights had returned to those of sham-treated mice. The DSP-4 treatment decreased the NE content by

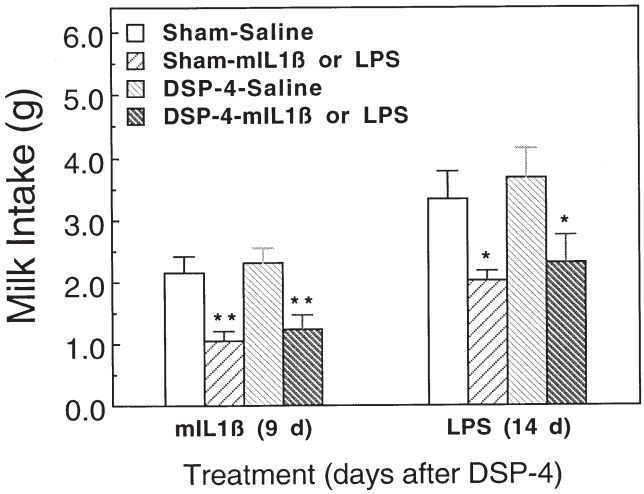

FIG. 5. Effect of DSP-4 pretreatment on mIL-1β– and LPS-induced hypophagia. Mice were pretreated with DSP-4 and injected 9 days later with mIL-1β (100 ng, IP), or 14 days later with LPS (1 μg, IP). Mice injected with mIL-1β on day 9 received saline on day 14, and mice injected with saline on day 9 were injected with LPS on day 14. Milk intake was assessed 2 h after mIL-1β or LPS. $n = 8$. Significantly different from the corresponding control group (*$p < 0.05$ or **$p < 0.01$).

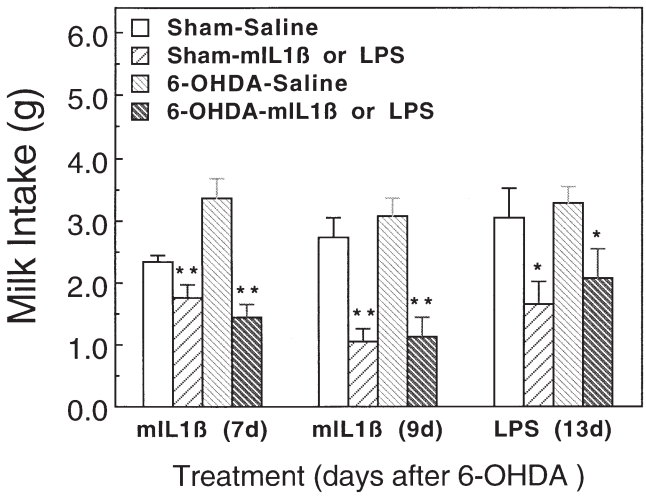

FIG. 6. Effect of 6-OHDA pretreatment on mIL-1β– and LPS-induced hypophagia. Mice were pretreated with 6-OHDA and injected 7 or 9 days later with mIL-1β, and 13 days later with LPS. Milk intake was assessed 2 h after mIL-1β or LPS. $n = 8$. Significantly different from the corresponding control group (*$p < 0.05$ or **$p < 0.01$, respectively).

67% ($p < 0.001$) in the cortex and by 21% ($p < 0.01$) in the hypothalamus measured 15 days later. There were no statistically significant effects on the content of DA or 5-HT. mIL-1β (100 ng/mouse) was injected 9 days after the DSP-4 treatment and mice given access to milk 2 h later. Milk intake was depressed by mIL-1β, $F(1, 19) = 28$, $p < 0.0001$, but was not affected by DSP-4 (Fig. 5). There was no statistically significant interaction between the mIL-1β and the DSP-4 treatments, $F(1, 19) = 1.1$. Food pellet intake and body weight were only slightly affected in this experiment.

LPS (1 μg/mouse) was injected 14 days after the DSP-4 treatment and mice were given access to milk 2 h later. Milk intake was depressed by LPS, $F(1,28) = 10.6$, $p < 0.01$, but was not affected by DSP-4 (Fig. 5). Food pellet intake was marginally decreased, $F(1, 28) = 3.8$, $p < 0.06$, and body weight was significantly depressed by the LPS injections, $F(1, 28) = 5.3$, $p < 0.05$. There were no statistically significant interactions between the LPS and DSP-4 treatments, $F(1, 28) = 0.0$; $F(1, 28) = 0.3$; $F(1,28) = 0.3$, milk and food intake and body weight, respectively.

Effect of 6-OHDA Treatment on mIL-1β- and LPS-Induced Hypophagia

By the seventh day, the animals regained their pretreatment body weight and ingested the pretreatment amounts of food pellets and milk. Upon termination of the experiment 14 days after the 6-OHDA, the NE content was decreased by 93% in the cortex and by 77% in the hypothalamus. DA was significantly depleted by 25 and 26% in the cortex and hypothalamus, respectively. Cortical 5-HT was not affected, but hypothalamic 5-HT was decreased significantly by 17%.

The effects of mIL-1β (100 ng/mouse, IP) were tested on the 7th and 9th days after the 6-OHDA treatment, each animal receiving mIL-1β only once (Fig. 6). Injections of mIL-1β depressed milk intake in both the first and second trial, $F(1, 25) = 31$, $p < 0.0001$; $F(1, 25) = 41$, $p < 0.0001$. There was a statistically significant mIL-1β × 6-OHDA interaction in the first trial, $F(1, 25) = 9.1$, $p < 0.01$, but not in the second, $F(1, 25) = 0.1$. The interaction in the first trial may be attributed to an increase in milk intake in the 6-OHDA treated group rather than to a change in the mIL-1β-induced hypophagia. Body weight and food pellet intake were significantly depressed by the first mIL-1β injection, $F(1, 25) = 11$, $p < 0.01$, and $F(1, 25) = 6.2$, $p < 0.05$, respectively, but there was no significant difference between the sham- and 6-OHDA–treated animals, nor any statistically significant mIL-1β × 6-OHDA treatment interactions. LPS (1 μg/mouse), injected 13 days after the 6-OHDA treatment, depressed milk intake and body weight, $F(1, 25) = 10$, $p < 0.01$; $F(1, 25) = 6.2$, $p < 0.05$, respectively, but there were no interactions between LPS and 6-OHDA with respect to milk or food intake or body weight, $F(1, 25) = 0.0$.

The Effects of Histaminergic Antagonists on mIL-1β- and LPS-Induced Changes in Feeding Behavior

The effects of histaminergic antagonists on the feeding responses induced by mIL-1β and LPS were examined. The H_1 antagonist, pyrilamine (10 mg/kg), the H_2 antagonist, cimetidine (50 mg/kg), and the H_3 antagonist, thioperamide (2 mg/kg) were administered IP 10 min before injection of 100 ng of IL-1β and 100 min before access to milk. The suicide inhibitor of histidine decarboxylase, α-fluoromethylhistidine (50 mg/kg) was administered 4.5 h before IL-1β. In each case, milk and food pellet intake was significantly decreased by IL-1β, but was unaffected by the antagonist (Fig. 7). In no case was there a statistically significant interaction between treatment with mIL-1β and any of the antagonists.

Pyrilamine, cimetidine, thioperamide, and α-FMH were also tested for their effects on LPS-induced hypophagia. Milk and food pellet intake were significantly decreased by LPS but unaffected by the antagonists (Fig. 8). In no case was there a statistically significant interaction between LPS and the antagonist treatments.

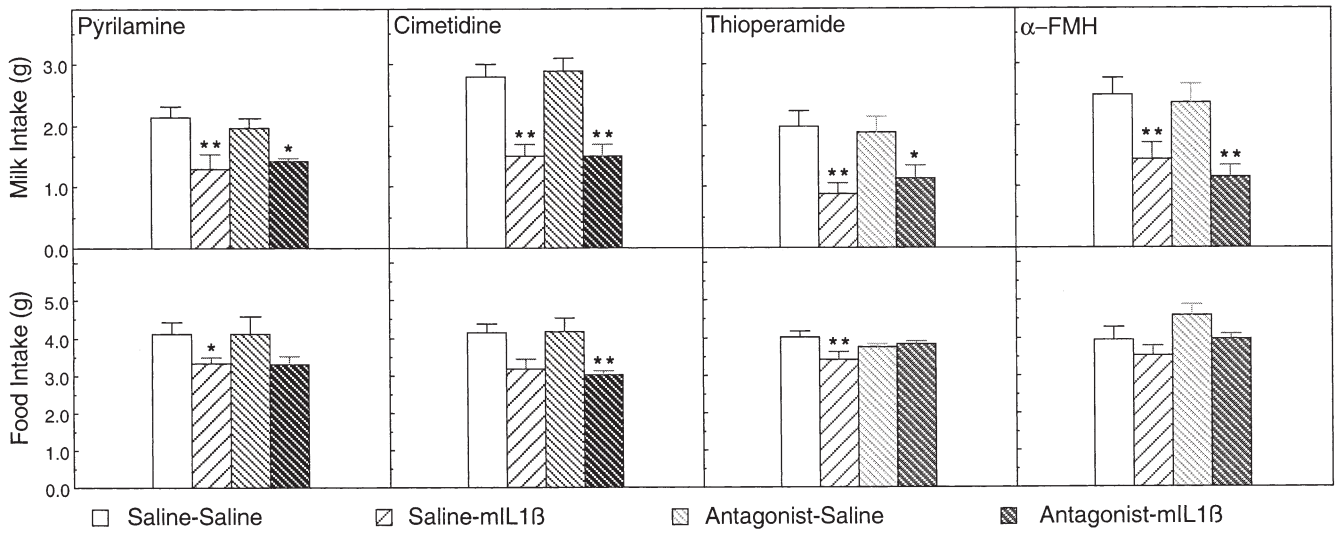

FIG. 7. Effects of pretreatment with H_1, H_2, H_3 antagonists or α-FMH on mIL-1β-induced hypophagia. Pyrilamine (10 mg/kg, IP), cimetidine (50 mg/kg, IP), thioperamide (2 mg/kg, IP), or saline was injected followed by mIL-1β 10 min later, 90 min before access to milk. α-FMH (50 mg/kg, IP) was injected 4.5 h before mIL-1β. $n = 6$–8. Significantly different from the corresponding control group ($*p < 0.05$, $**p < 0.01$).

The Effects of Muscarinic and Dopaminergic Antagonists on mIL-1β- and LPS-Induced Changes in Feeding Behavior

The muscarinic receptor antagonist, scopolamine (0.1 mg/kg) or the dopamine (D_1/D_2) antagonist, haloperidol (0.1 mg/kg) was injected 10 min before mIL-1β or LPS. Although IL-1 and LPS induced the normal decreases in milk and food pellet intake (Fig. 9), there was no effect of scopolamine or haloperidol alone, nor any significant interactions. Similarly, a lack of attenuation of the responses to mIL-1β was observed with the opiate antagonist, naloxone (2 mg/kg) or the NOS inhibitor L-NAME (30 mg/kg) (data not shown).

DISCUSSION

The reductions in milk and food pellet intakes following acute peripheral administration of LPS or mIL-1β closely resemble those observed in our previous experiments (46,47). They are also consistent with a substantial literature indicating that both LPS and IL-1 depress feeding in animals (23,34,36,51).

The focus of the present studies was the mechanism by which LPS and/or IL-1 induced hypophagia. Within the CNS, the most likely candidates for neurotransmitter mediators of the responses are cerebral NE, 5-HT, histamine, and acetyl-

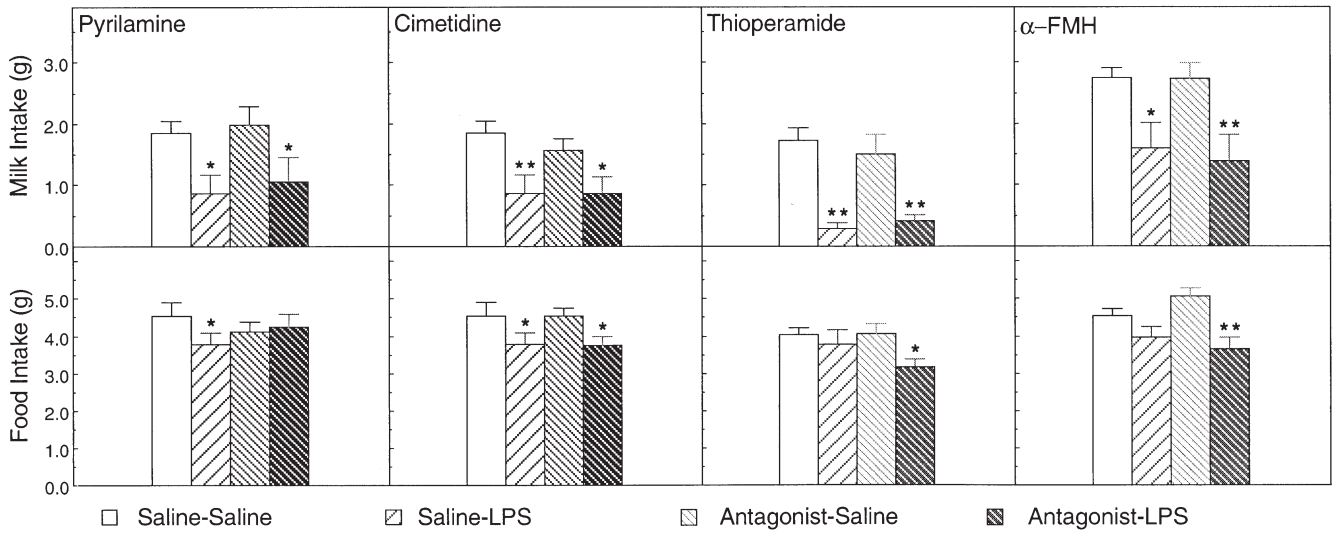

FIG. 8. Effects of pretreatment with H_1, H_2, H_3 antagonists or α-FMH on LPS-induced hypophagia. Pyrilamine, cimetidine, thioperamide, α-FMH, or saline was injected as in the experiments of Fig. 7, followed by LPS 10 min later, 120 min before access to milk. $n = 6$–8. Significantly different from the corresponding control group ($*p < 0.05$ or $**p < 0.01$).

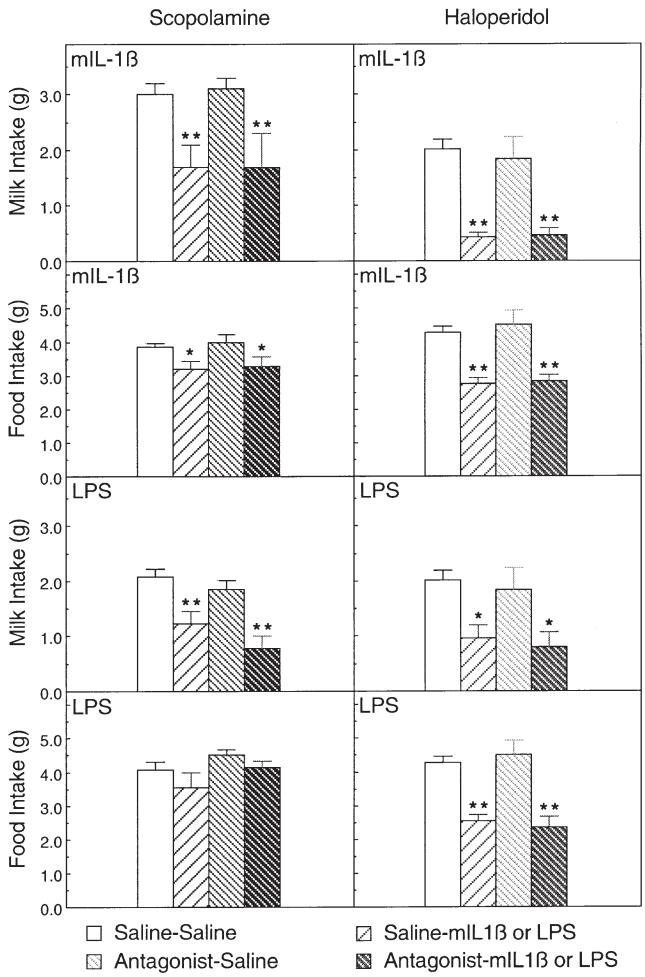

FIG. 9. Effects of pretreatment with muscarinic and dopaminergic antagonists on mIL-1β– or LPS-induced hypophagia. Scopolamine (0.1 mg/kg, IP) or haloperidol (0.1 mg/kg, IP) or saline was injected followed by mIL-1β or LPS 10 min later, 90 or 120 min, respectively, before access to milk. $n = 8$. Significantly different from the corresponding control group (*$p < 0.05$, **$p < 0.01$).

choline. Our earlier studies indicated that the maximal reduction in milk intake following IL-1 and LPS occurred around 1.5 to 2 h after administration (46). This correlates well with the maximal noradrenergic response in the hypothalamus at around 2 h following either IL-1 (7,43) or LPS (7,24). NE or clonidine injected directly into the PVN induces feeding in satiated animals, and this effect appeared to be mediated by postsynaptic α_2-adrenoreceptors, but not α_1-adrenoreceptors (12). Selective depletion of NE with RO 4-1284 and FLA63 reduced deprivation-induced food intake (53). These results suggest that hypothalamic NE increases feeding. On the other hand, microinjections of α_1-adrenergic agonists into the PVN induced a hypophagia that was prevented by pretreatment with α_1-adrenergic antagonists (52). These results suggest that whereas α_2-adrenergic receptors in the PVN may increase feeding, α_1-adrenergic receptors decrease feeding. Thus, IL-1- or LPS-induced NE release might act on α_1-receptors to reduce feeding. However, our results with prazosin and phentolamine appear to exclude this as a mechanism for the hypophagic effects of mIL-1β and LPS. The observation that the combination of phentolamine and propranolol failed to alter the hypophagic responses to IL-1 and LPS appears to exclude any role for α- or β-adrenergic receptors. Our results are consistent with those of Langhans et al., who observed that although the anorexic effect of peripherally administered epinephrine could be blocked by phentolamine plus propranolol, combined α- and β-blockade failed to alter the anorexic effect of LPS (22).

NE does not appear to be essential for feeding because mice lacking the gene for dopamine-β-hydroxylase, and therefore unable to synthesize NE, exhibit normal increases in food intake and metabolic rate when exposed to cold (49). This observation is consistent with our findings that depletion of NE with either DSP-4 or 6-OHDA did not alter the reduction in sweetened milk intake in response to LPS or mIL-1β. Thus, the present studies provide no support whatsoever for the involvement of cerebral adrenergic or noradrenergic systems in the IL-1- or LPS-induced decreases in feeding.

Central (21) and peripheral (20) administration of IL-1β increases hypothalamic histamine turnover. Histamine has been shown to decrease feeding in rats, most likely through cerebral H_1 receptors (35,38,40). Both ad lib (35,38,41) and short-term feeding (35) have been shown to be suppressed. However, the results of the present experiments indicate that H_1- and H_2-receptor antagonists did not affect IL-1β- or LPS-induced hypophagia. Histamine H_3-receptors on presynaptic terminals exert autoinhibitory control on both histamine synthesis and release. Thioperamide is a potent and specific H_3-receptor antagonist that has been shown to increase the activity of histaminergic neurons in vivo (1) lasting from 30 min to 16 h after injection (11). Thioperamide has been reported to inhibit intake of solids and liquids (41), but in the present experiments, thioperamide did not alter milk intake or the responses to mIL-1β or LPS. Kang et al. found that α-FMH inhibited the production of neuronal histamine in the hypothalamus almost completely for 2 to 12 h, and attenuated the depressing effects of peripherally administered IL-1β on the intake of solid food in free-feeding rats. However, α-FMH facilitated the suppressive effect of IL-1β on water drinking (20). Nevertheless, in our experiments, α-FMH failed to alter the IL-1β- or LPS-induced reductions in milk intake. Perhaps intake of solid food is linked more closely to drinking water in rats than in mice, or an effect of α-FMH masked any effect on the responses to IL-1β or LPS on the consumption of sweetened milk. The reasons for the apparent conflict between our data and those of Sakata et al. are not readily apparent. Most likely, the role of histamine in feeding depends on the specific feeding paradigm used and possibly the animal species.

The muscarinic cholinergic receptor antagonist, scopolamine at doses known to block central muscarinic receptors (9), failed to attenuate or prevent LPS- or IL-1–induced reduction in milk intake. Thus, muscarinic cholinergic receptors do not appear to be involved in LPS or IL-1–induced hypophagia. Dopamine agonists have long been associated with reduced feeding (48). Central administration of amphetamine, apomorphine, and dopamine (29) have been shown to depress feeding. The effects of amphetamine were blocked by the DA antagonists, haloperidol and pimozide, at doses that did not themselves reduce spontaneous food intake (28). Nevertheless, in the present experiments, haloperidol failed to alter the IL-1β–induced reduction in sweetened milk intake. Opioid peptides have also been postulated to be involved in ingestive behavior (37). Nevertheless, the nonselective opiate receptor antagonist, naloxone, failed to alter basal sweetened

milk intake in mice, or the responses to mIL-1β or LPS, providing no support for an involvement of endorphins in these responses.

Similarly, although a recent report indicated that NOS inhibitors attenuated glucoprivic feeding and deprivation-induced drinking (5), we observed no effect of the nonselective NOS inhibitor, L-NAME, on the mIL-1β–induced hypophagia.

Several investigators have demonstrated that cyclo-oxygenase inhibitors, such as indomethacin, can antagonize the hypophagic effects of IL-1β (19) and LPS (22), suggesting that prostaglandins are involved. Using the same mouse feeding paradigm as in the present study, we found that indomethacin pretreatment significantly attenuated the effects of IL-1 and LPS on milk intake, but did not block them (46). The responses to IL-1α and IL-1β were attenuated to a greater extent than LPS, but neither response was completely blocked. These findings suggest that whereas cyclooxygenase products appear to be involved in the hypophagic responses, other mediators may also play a role.

The present results failed to associate the hypophagic activity of IL-1 and LPS with several of the most likely neurotransmitter candidates. Neither NE nor epinephrine appears to be involved, and our data provide no support for a role of DA, histamine, acetylcholine, or endorphins. Other potential candidates include 5-HT, and peptides, such as corticotropin-releasing factor (CRF), urocortin, neuropeptide Y, and cholecystokinin (CCK) (6,26,42,44,50). It is likely that anorexia is mediated by multiply redundant pathways, so that the simultaneous blockade of several pathways may be necessary to prevent LPS/IL-1–induced hypophagia.

ACKNOWLEDGEMENTS

This research was supported by grants from the U.S. National Institute of Mental Health (MH46261) and the U.S. National Institute of Neurological Diseases and Stroke (NS25370). Tinashe Burunda and Brigette Patterson were participants in the NIH Jumpstart Summer Enrichment Program (5R25 RR111468). The technical assistance of Charles Dempsey and Galina Mikhaylova is greatly appreciated.

REFERENCES

1. Arang, J. M.; Garbarg, M.; Lancelot, J. C.; Lecomte, J. M.; Pollard, H.; Robba, M.; Schunack, W.; Schwartz, J. C.: Highly potent and selective ligands for histamine H_3-receptors. Nature 327:117–123; 1987.
2. Berridge, C. W.; Dunn, A. J.: Restraint-stress-induced changes in exploratory behavior appear to be mediated by norepinephrine-stimulated release of CRF. J. Neurosci. 9:3513–3521; 1989.
3. Berridge, C. W.; Dunn, A. J.: DSP-4-induced depletions of mouse brain norepinephrine produces opposite effects on exploratory behavior 3 and 14 days after treatment. Psychopharmacology (Berlin) 100:504–508; 1990.
4. Cooper, S. J.; Clifton, P. G.: Drug receptors subtypes and ingestive behavior. London: Academic Press; 1996.
5. Czech, D. A.: A nitric oxide synthase inhibitor N^G-nitro-L-arginine, attenuates glucoprivic feeding and deprivation-induced drinking in the mouse. Pharmacol. Biochem. Behav. 60:601–607; 1998.
6. Daun, J. M.; McCarthy, D. O.: The role of cholecystokinin in interleukin-1-induced anorexia. Physiol. Behav. 54:237–241; 1993.
7. Dunn, A. J.: Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: Comparison with interleukin-1. J. Pharmacol. Exp. Therap. 261:964–969; 1992.
8. Dunn, A. J.: Nitric oxide synthase inhibitors prevent the cerebral tryptophan and serotonergic responses to endotoxin and interleukin-1. Neurosci. Res. Commun. 13:149–156; 1993.
9. Dunn, A. J.; Vigle, G.: Grooming behavior induced by ACTH involves cerebral cholinergic neurons and muscarinic receptors. Neuropharmacology 24:329–331; 1985.
10. Dunn, A. J.; Wang, J.: Cytokine effects on CNS biogenic amines. Neuroimmunol. Modulat. 2:319–328; 1996.
11. Garbarg, M.; Trung Tuong, M. D.; Gros, C.; Schwartz, J.-C.: Effects of histamine H_3-receptor ligands on various biochemical indices of histaminergic neuron activity in rat brain. Eur. J. Pharmacol. 164:1–11; 1989.
12. Goldman, C. K.; Marino, L.; Leibowitz, S. F.: Postsynaptic α_2-noradrenergic receptors mediate feeding induced by paraventricular nucleus injection of norepinephrine and clonidine. Eur. J. Pharmacol. 115:11–19; 1985.
13. Gorman, A. L.; Dunn, A. J.: β-Adrenergic receptors are involved in stress-related behavioral changes. Pharmacol. Biochem. Behav. 45:1–7; 1993.
14. Green, E. J.; Isaacson, R. L.; Dunn, A. J.; Lanthorn, T.: Naloxone and haloperidol reduce grooming occurring as an after effect of novelty. Behav. Neural Biol. 27:546–551; 1979.
15. Grossman, S. P.: Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. Science 132:301–302; 1960.
16. Guild, A. L.; Dunn, A. J.: Dopamine involvement in ACTH-induced grooming behavior. Pharmacol. Biochem. Behav. 17:31–36; 1982.
17. Haas, H. S.; Schauenstein, K.: Neuroimmunomodulation via limbic structures—The neuroanatomy of psychoimmunology. Prog. Brain Res. 51:195–222; 1997.
18. Hart, B. L.: Biological basis of the behavior of sick animals. Neurosci. Biobehav. Rev. 12:123–137; 1988.
19. Hellerstein, M. K.; Meydani, S. N.; Meydani, M.; Wu, K.; Dinarello, C. A.: Interleukin-1-induced anorexia in the rat. Influence of prostaglandins. J. Clin. Invest. 84:228–235; 1989.
20. Kang, M.; Yoshimatsu, H.; Chiba, S.; Kurokawa, M.; Ogawa, R.; Tamari, Y.; Tatsukawa, M.; Sakata, T.: Hypothalamic neuronal histamine modulates physiological responses induced by interleukin-1β. Am. J. Physiol. 269:R1308–R1313; 1995.
21. Kang, M.; Yoshimatsu, H.; Ogawa, R.; Kurokawa, M.; Oohara, A.; Tamari, Y.; Sakata, T.: Thermoregulation and hypothalamic histamine turnover modulated by interleukin-1 beta in rats. Brain Res. Bull. 35:299–301; 1994.
22. Langhans, W.; Harlacher, R.; Scharrer, E.: Verapamil and indomethacin attenuate endotoxin-induced anorexia. Physiol. Behav. 46:535–539; 1989.
23. Langhans, W.; Savoldelli, D.; Weingarten, S.: Comparison of the feeding responses to bacterial lipopolysaccharide and interleukin-1β. Physiol. Behav. 53:643–649; 1993.
24. Lavicky, J.; Dunn, A. J.: Endotoxin administration stimulates cerebral catecholamine release in freely moving rats as assessed by microdialysis. J. Neurosci. Res. 40:407–413; 1995.
25. Lecklin, A.; Tuomisto, L.: The blockade of H1 receptors attenuates the suppression of feeding and diuresis induced by inhibition of histamine catabolism. Pharmacol. Biochem. Behav. 59:753–758; 1998.
26. Le Feuvre, R. A.; Aisenthal, L.; Rothwell, N. J.: Involvement of corticotrophin releasing factor (CRF) in the thermogenic and anorexic actions of serotonin (5-HT) and related compounds. Brain Res. 555:245–250; 1991.
27. Legg, C. R.; Booth, D.: Appetite: Neural and behavioural bases. Oxford: Oxford University Press; 1994.
28. Leibowitz, S. F.: Brain monoamines and peptides: Role in the control of eating behavior. Fed. Proc. Fed. Am. Soc. Exp. Biol. 45:1396–1403; 1986.
29. Leibowitz, S. F.; Rossakis, C.: Pharmacological characterization of perifornical hypothalamic dopamine receptors mediating feeding inhibition in the rat. Brain Res. 172:115–130; 1979.
30. Liu, W.; Hu, N.; Beaton, J.: Behavioral toxicological assessment

of oral pralidoxime methanesulfonate in the rat. Neurobehav. Toxicol. Teratol. 6:121–127; 1984.
31. Maeyama, K.; Watanabe, T.; Taguchi, Y.; Yamatodani, A.; Wada, H.: Effect of α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, on histamine levels in mouse tissues. Biochem. Pharmacol. 31:2367–2370; 1982.
32. Malmberg-Aiello, P.; Lamberti, C.; Ipponi, A.; Bartolini, A.; Schunack, W.: Evidence for hypernociception induction following histamine H1 receptor activation in rodents. Life Sci. 63:463–476; 1998.
33. Matsumoto, K.; Cai, B.; Satoh, T.; Ohta, H.; Watanabe, H.: Desipramine enhances isolation-induced aggressive behavior in mice. Pharmacol. Biochem. Behav. 39:167–170; 1991.
34. McCarthy, D. O.; Kluger, M. J.; Vander, A. J.: Effect of centrally administered interleukin-1 and endotoxin on food intake of fasted rats. Physiol. Behav. 36:745–749; 1986.
35. Mercer, L. P.; Kelley, D. S.; Humphries, L. L.; Dunn, J. D.: Manipulation of central nervous system histamine or histaminergic receptors (H1) affects food intake in rats. J. Nutr. 124:1029–1036; 1994.
36. Mrosovsky, N.; Molony, L. A.; Conn, C. A.; Kluger, M. J.: Anorexic effects of interleukin 1 in the rat. Am. J. Physiol. 257:R1315–R1321; 1989.
37. Nencini, P.: Sensitization to the ingestive effects of opioids. In: Cooper, S. J.; Clifton, P. G., eds. Drug receptors subtypes and ingestive behavior. London: Academic Press; 1996:193–218.
38. Ookuma, K.; Sakata, T.; Fukugawa, K.; Yoshimatsu, H.; Kurokawa, M.; Machidori, H.; Fujimoto, K.: Neuronal histamine in the hypothalamus suppresses food intake in rats. Brain Res. 628:235–242; 1993.
39. Rada, P.; Mark, G. P.; Vitek, M. P.; Mangano, R. M.; Blume, A. J.; Beer, B.; Hoebel, B. G.: Interleukin-1β decreases acetylcholine measured by microdialysis in the hippocampus of freely moving rats. Brain Res. 550:287–290; 1991.
40. Sakata, T.; Fukagawa, K.; Fujimoto, K.; Yoshimatsu, H.; Shiraishi, T.; Wada, H.: Feeding induced by blockade of histamine H1-receptor in rat brain. Experientia 44:216–218; 1988.
41. Sakata, T.; Fukugawa, K.; Ookuma, S.; Fujimoto, K.; Yoshimatsu, H.; Yamatodani, A.; Wada, H.: Hypothalamic neuronal histamine modulates ad libitum feeding by rats. Brain Res. 537:303–306; 1990.
42. Shibasaki, T.; Yamauchi, N.; Kato, Y.; Masuda, A.; Imaki, T.; Hotta, M.; Demura, H.; Oono, H.; Ling, N.; Shizume, K.: Involvement of corticotropin-releasing factor in restraint stress-induced anorexia and reversion of the anorexia by somatostatin in the rat. Life Sci. 43:1103–1110; 1988.
43. Smagin, G. N.; Swiergiel, A. H.; Dunn, A. J.: Peripheral administration of interleukin-1 increases extracellular concentrations of norepinephrine in rat hypothalamus: Comparison with plasma corticosterone. Psychoneuroendocrinology 21:83–93; 1996.
44. Spina, M.; Merlo-Pich, E.; Chan, R. K. W.; Basso, A. M.; Rivier, J.; Vale, W.; Koob, G. F.: Appetite-suppressing effects of urocortin, a CRF-related neuropeptide. Science 273:1561–1564; 1996.
45. Swiergiel, A. H.; Dunn, A. J.; Stone, E. A.: The role of cerebral noradrenergic systems in the Fos response to interleukin-1. Brain Res. Bull. 41:61–64; 1996.
46. Swiergiel, A. H.; Smagin, G. N.; Dunn, A. J.: Influenza virus infection of mice induces anorexia: Comparison with endotoxin and interleukin-1 and the effects of indomethacin. Pharmacol. Biochem. Behav. 57:389–396; 1997.
47. Swiergiel, A. H.; Smagin, G. N.; Johnson, L. J.; Dunn, A. J.: The role of cytokines in the behavioral responses to endotoxin and influenza virus infection in mice: Effects of acute and chronic administration of the interleukin-1-receptor antagonist (IL-1ra). Brain Res. 776:96–104; 1997.
48. Terry, P.: Dopamine receptor subtypes and ingestive behaviour. In: Cooper, S. J.; Clifton, P. G., eds. Drug receptors subtypes and ingestive behavior. London: Academic Press; 1996:233–236.
49. Thomas, S. A.; Palmiter, R. D.: Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline. Nature 387:94–97; 1997.
50. Uehara, A.; Sekiya, C.; Takasugi, Y.; Namiki, M.; Arimura, A.: Anorexia induced by interleukin-1: Involvement of corticotropin-releasing factor. Am. J. Physiol. 257:R613–R617; 1989.
51. Weingarten, H. P.: Cytokines and food intake: The relevance of the immune system to the student of ingestive behavior. Neurosci. Biobehav. Rev. 20:163–170; 1996.
52. Wellman, P. J.; Davies, B. T.; Morien, A.; McMahon, L.: Modulation of feeding by hypothalamic paraventricular nucleus α1- and α2-adrenergic receptors. Life Sci. 53:669–679; 1993.
53. Zolovick, A. J.; Rossi, J.; Davies, R. F.; Panksepp, J.: An improved pharmacological procedure for depletion of noradrenaline: Pharmacology and assessment of noradrenaline-associated behaviors. Eur. J. Pharmacol. 77:265–271; 1982.