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Lack of Evidence for a Role of Serotonin in Interleukin-1–Induced Hypophagia

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SWIERGIEL, A. H. AND A. J. DUNN. *Lack of evidence for a role of serotonin in interleukin-1–induced hypophagia.* PHARMACOL BIOCHEM BEHAV **65**(3) 531–537, 2000.—Interleukin-1 (IL-1) administration depresses food intake in rodents. IL-1 is known to increase the metabolism of serotonin, which is known to affect feeding behavior. Thus, serotonin is an obvious candidate for a mediator of the hypophagic response to IL-1. Therefore, we tested the ability of serotonergic agonists and antagonists to alter the hypophagic responses to IL-1 and bacterial lipopolysaccharide (LPS). Hypophagia was assessed in ad lib-fed mice by recording the intake of sweetened milk in a 30-min period. Acute intraperitoneal administration of mouse IL-1β reliably decreased milk intake. This hypophagic response was not affected by any of the serotonin antagonists tested, including 5-HT_{1A} (WAY100135 and propranolol), 5-HT_{1B} (GR127935), 5-HT_2 (ritanserin, ketanserin, SB206553, and RS102221), mixed 5-$HT_{1/2}$ (methysergide and metergoline), and 5-HT_3 (tropisetron) receptor antagonists. The 5-HT_{1A} agonists (8-OH-DPAT and ipsapirone) and a 5-HT_{1B} agonist (CGS12066B) known to decrease the activity of serotonergic neurons, also had no effect. Mice pretreated with 5,7-dihydroxytryptamine to deplete brain serotonin ate less, but, nevertheless, displayed similar hypophagic responses to mIL-1β or LPS. The results suggest that serotonin is not involved in the decrease in short-term milk intake induced by mIL-1β or LPS in mice that have been fed ad lib. © 2000 Elsevier Science Inc.

Food Intake Anorexia Interleukin-1 Serotonin 5-HT-receptor antagonists

INFECTIONS often result in lethargy, hypophagia, and body weight loss, which are also observed after treatment with bacterial endotoxins (lipopolysaccharide, LPS) (21). Infections and LPS administration both result in increased synthesis and secretion of cytokines, such as interleukin-1 (IL-1) (10). Peripheral or central administration of IL-1 elicits sickness behavior in rats and mice; thus, IL-1 may mediate the behavioral responses observed in sickness (21,28). Specifically, acute single injections of IL-1 induce hypophagia (34,50, 51,56).

Infections and administration of endotoxins and cytokines affect CNS neurotransmission (13). Consistent responses include elevations of cerebral catecholamine metabolism and increases of brain tryptophan, and the serotonin (5-hydroxytryptamine, 5-HT) catabolite, 5-hydroxyindoleacetic acid (5-HIAA) (12,33,59). Increased availability of tryptophan may result in increased cerebral serotonin synthesis and metabolism (15). The peak response of tryptophan and 5-HIAA occurs between 2 and 8 h after intraperitoneal (IP) injections of IL-1 and LPS (12).

Serotonergic neurons have been implicated in the initiation, mediation, and modulation of specific behavioral patterns, including many aspects of feeding behavior. Increased serotonergic activity is believed to exert an inhibitory influence upon feeding (3), and numerous pharmacological and behavioral studies have indicated that the administration of direct or indirect 5-HT agonists causes depression of feeding (3,5,8,18).

Because administration of IL-1 and LPS affect brain serotonin which may affect feeding, it has been be postulated that the IL-1- and LPS-induced hypophagia is related to the altered serotonergic activity. This hypothesis has been tested by investigating the ability of various compounds active on serotonergic neurons to attenuate IL-1β-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, and with ad lib access to water and Harlan (Madison, WI) Teklad food pellets. All procedures were approved by the Louisiana State University

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Medical Center–Shreveport Animal Care and Use Committee. Animals were tested a number of times, but care was taken that they received different treatments in consecutive experiments. IL-1 was never injected more than once in any 1 week.

Feeding Behavior

Intake of sweetened condensed milk diluted with three parts of water was assessed as described previously (48–51). Briefly, 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 animals that drank at least 1.5 g of milk in the session on the last day of habituation were included. Food pellet intake over the next 22 h was also measured, but was affected only slightly by IL-1, so the results have not been presented. Water intake was not measured. Many previous experiments have shown that mice fed ad lib consumed significant quantities of sweetened milk in a short period of time during the light phase of the daily light–dark cycle with no statistically significant effect on the daily intake of food pellets.

IL-1 and Serotonergic Agents

Recombinant mouse IL-1β (mIL-1β) was purchased from R&D Systems (Minneapolis, MN.), and *E. coli* lipopolysaccharide (LPS, serotype 026:B6; L-3755) and desmethylimipramine HCl salt (DMI) from Sigma Chemical Co. (St. Louis, MO). IL-1β and LPS were dissolved in sterile pyrogen-free isotonic saline such that the total dose for each mouse (100 ng or 1 μg/mouse, respectively) was contained in 0.1 ml and injected IP 90 (mIL-1β) or 120 min (LPS) before presenting the milk bottle.

Sources of serotonin agonists and antagonists were as follows: ipsapirone (TVX Q7821) from Miles Inc., Pharmaceutical Division (West Haven, CT); metergoline, tropisetron (ICS205930), SB206553, (+)-WAY100135 and 5,7-dihydroxytryptamine creatinine salt (5,7-DHT) from Research Biochemicals International (RBI, Natick, MA); methysergide and RS102221 from Tocris Cookson Inc. (Ballwin, MO); R(+)-8-hydroxy-2-di-*n*-propylaminotetralin hydrobromide (8-OH-DPAT), ritanserin and ketanserin from Janssen Pharmaceutica (Beerse, Belgium), and GR127935 from Glaxo Wellcome Research and Development (Stevenage, UK). The drugs were dissolved in saline, except for metergoline, RS102221, and ritanserin, which were dispersed in saline using ultrasound and injected as suspensions. 5,7-DHT was dissolved in saline containing 0.1% ascorbic acid. The doses used of the serotonergic drugs were determined based on appropriate literature reports, specifically: ipsapirone (57), WAY100135 (35), GR127935 (32,46), SB206553 (19,26), RS102221 (4), ketanserin, ritanserin, methysergide and metergoline (16,37). Wide dose ranges of 8-OH-DPAT and tropisetron were tested (11,17,24).

Serotonin Depletion

Mice were pretreated with DMI (25 mg/kg, IP) 1 h prior to intracerebral infusion of 5,7-DHT to prevent damage to noradrenergic neurons (2). They were anesthetized with modified Hypnorm (2.5 mg Fentanyl (Abbot Laboratories, Chicago, IL), 175 mg droperidol (American Regent Laboratories, Shirley, NY), and 125 mg midazolam (Roche Laboratories, Nutley, NJ) made up to 145 ml with sterile saline) administered IP at a dose of 10 μl/g body weight. After exposing the skull around bregma, a hole was drilled in the skull on each side of the midline 0.6 mm caudal to bregma and 1.6 mm lateral. Five microliters of artificial CSF or 5,7-DHT (40 μg in 5 μl artificial CSF) was infused into each cerebral lateral ventricle over 6 min using a pump-driven microsyringe inserted to a depth of 2 mm. Upon completion of the experiments, 22 days after the 5,7-DHT treatment, the mice were decapitated, and frontal cortex, hypothalamus, hippocampus, striatum, and brain stem excised, weighed, and frozen on dry ice. After thawing, the samples were homogenized, centrifuged, and supernatants analyzed by HPLC for the contents of serotonin and its metabolites as previously described (12).

Data Analysis

Two-way analysis of variance (ANOVA) was performed to determine effects of mIL-1β and the agonists and antagonists. If ANOVA revealed a significant effect, Fishers' Least-Significant Difference test was used for pairwise comparisons. All data are reported as mean ± standard error of the mean.

RESULTS

The Effects of Serotonin Receptor Antagonists on mIL-1–induced Reductions in Milk Intake

In a series of experiments, the effects of different serotonergic antagonists on the mIL-1β–induced reductions in milk intake were studied. The compounds were injected shortly before ip injection of mIL–1β (100 ng), which in turn, was given 90 min before access to milk. In all experiments, mIL-1β reliably decreased milk intake in control (saline-treated) animals.

5-HT_{1A} Receptor Antagonists

Initially, we tested the effects of the 5-HT_{1A} antagonist, (+)-WAY100135. WAY100135 (3 mg/kg) was administered subcutaneously (SC) 15 min before mIL-1β. Milk intake was decreased by mIL-1β, $F(1, 26) = 26$, $p < 0.0001$, but not by WAY100135 ($F = 0.62$) (Table 1). WAY100135 treatment did not affect the reductions in milk intake in response to mIL-1β; ANOVA indicated no statistically significant interactions between mIL-1β and the antagonist ($F = 0.53$). A second experiment using 6 mg/kg WAY100135 produced very similar results. Likewise, a 9 mg/kg dose of WAY100135 did not attenuate the reduction of milk intake by IP administration of 1 μg of LPS [interaction: $F(1, 27) = 0.13$].

In an experiment reported previously, another 5-HT_{1A} antagonist, S(−)-propranolol, injected (2.5 mg/kg, IP) 10 min before mIL-1β, failed to alter the reduction in milk intake induced by mIL-1β (48).

5-HT_{1B} Receptor Antagonists

The 5-HT_{1B} antagonist, GR127935 (10 mg/kg, SC), injected 15 min before IL-1B had no effect on the IL-1β–induced hypophagia (Table 1). There was no interaction between the mIL-1β and the antagonist treatments, $F(1, 24) = 0.00$.

5-HT_2 Receptor Antagonists

The 5-$HT_{2A}/_{2C}$ receptor antagonist, ketanserin, was injected IP at doses of 0.5 and 1.0 mg/kg 15 min before mIL-1β. Administration of mIL-1β profoundly depressed milk intake, $F(1, 19) = 16$, $F(1, 20) = 143$, respectively, $p < 0.001$, but milk intake was not significantly affected by either dose of ketanserin (Fig. 1). ANOVA indicated no statistically significant

TABLE 1

EFFECT OF mIL-1β ON MILK INTAKE IN MICE TREATED WITH SEROTONIN ANTAGONISTS

Antagonist (Dose (mg/kg) Route)	Milk Intake % Vehicle Control	*df*	*F*-Value (Interaction)
5-HT_{1A}			
Vehicle	56.3†	1,26	0.54
WAY100135 (3.0, SC)	64.3†		
Vehicle	27.6†	1,26	3.45
WAY100135 (6.0, SC)	56.5*		
5-HT_{1B}			
Vehicle	52.0*	1,24	0.01
GR127935 (10.0, SC)	57.1†		
Mixed 5-$HT_{1/2}$			
Vehicle	59.1*	1,26	0.54
Methysergide (4.0, IP)	48.0*		
Mixed 5-$HT_{1/2}$			
Vehicle	50.0†	1,33	0.02
Metergoline (1.0, IP)	25.0*		
5-HT_3			
Vehicle	78.3	2,25	1.99
Tropisetron			
(0.003, SC)	76.2		
(0.300, SC)	37.5†		

Mice were injected with the serotonin antagonists indicated 15 min before mIL-β (100 ng, IP) and milk intake (g/30 min) assessed 90 min later.

n = 6–9. *mIL-1β injected groups differed significantly from the saline-injected groups (*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$). Degrees of freedom (*df*) and *F*-values (two-way ANOVA) are provided for the mIL-1β × antagonist interaction.

interactions between mIL-1β and the antagonist (F = 0.31 and 2.34, respectively). Ketanserin, at doses of 2 and 5 mg/kg, significantly depressed milk intake, so that its effect on the response to IL-1β was difficult to assess. Nevertheless, there was no indication that the hypophagia induced by IL-1β was attenuated. The 5-HT_{2A} antagonist, ritanserin, was tested IP at doses of 1 and 2 mg/kg, but did not attenuate the hypophagia (Fig. 1). The interaction between the mIL-1β and antagonist treatments was not statistically significant at either dose, $F(1, 19) = 0.100$, $F(1, 24) = 0.42$, respectively.

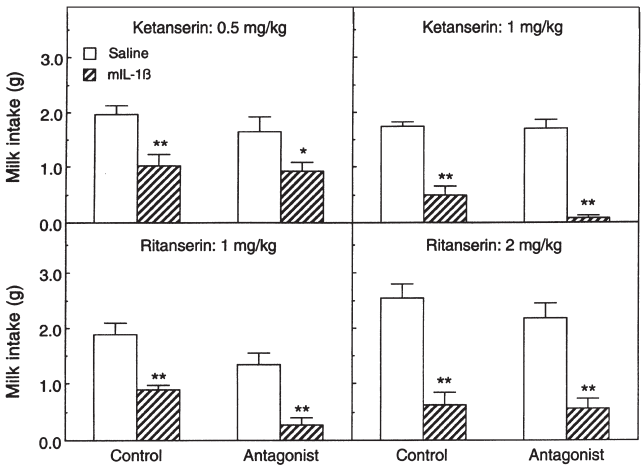

FIG. 1. Effect of mIL-1β on milk intake of mice pretreated with ketanserin or ritanserin. Mice were pretreated with ketanserin (0.5 and 1 mg/kg, IP, upper panel) or ritanserin (1 and 2 mg/kg, IP, lower panel) 15 min before mIL-1β injection. n = 7. Significantly different from the corresponding saline-injected group (*$p < 0.05$ or **$p < 0.01$).

The highly selective 5-HT_{2C} antagonist, RS102221, was tested at a dose of 2 mg/kg IP, and the 5-$HT_{2B/2C}$ antagonist, SB206553, at a dose of 5 mg/kg IP (Fig. 2). Neither drug attenuated the mIL-1β induced hypophagia (interaction between the mIL-1β and the antagonist treatments, $F(1, 24) = 1.59$, and $F(1, 26) = 0.25$, respectively. RS102221 was also ineffective at 2 and 5 mg/kg SC.

5-$HT_{1/2}$ Receptor Antagonists

Similar results were obtained with 5-$HT_{1/2}$ mixed antagonists. Methysergide (4 mg/kg, IP) and metergoline (1 mg/kg, IP) affected neither the milk intake nor the mIL-1β induced hypophagia (Fig. 3). The interactions between the mIL-1β and the methysergide or metergoline treatments were not statistically significant, $F(1, 26) = 0.53$, and 0.02, respectively.

5-HT_3 Receptor Antagonists

The 5-HT_3 antagonist, tropisetron (0.003 and 0.3 mg/kg, SC) was administered 10–20 min before injection of mIL-1β. It did not affect the mIL-1β induced decrease in milk intake (Table 1).

Effects of 5-HT_{1A} Receptor Agonists on mIL-1–Induced Changes in Milk Intake

Because 5-HT_{1A} agonists have been shown to decrease the activity of serotonergic cells, resulting in decreased 5-HT_{1A} secretion, such agonists can be regarded as functional 5-HT antagonists. Therefore, we also tested 5-HT_{1A} agonists for their effects on IL-1–induced hypophagia. Because the 5-HT_{1A} receptor agonist, 8-OH-DPAT, has been reported to increase food intake, we first tested it over a wide range of doses from

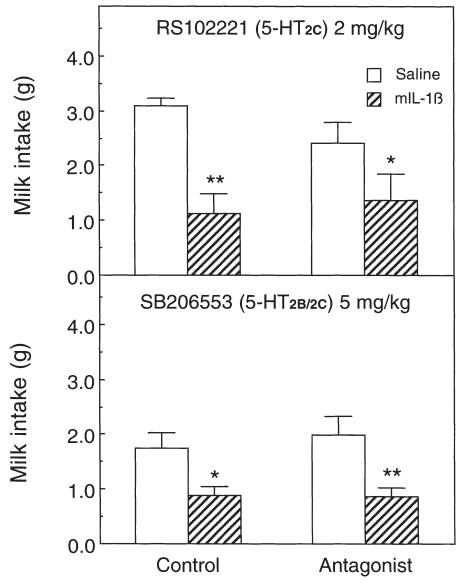

FIG. 2. Effect of mIL-1β on milk intake of mice pretreated with RS102221 or SB206553. Mice were treated with RS102221 (2 mg/kg, IP, upper panel) or SB206553 (5 mg/kg, IP, lower panel) 15 min before mIL-1β injection. n = 7. Significantly different from the corresponding saline-injected group (*$p < 0.05$ or **$p < 0.01$).

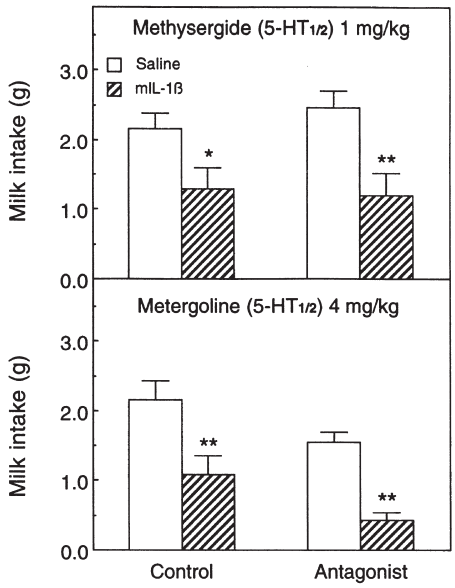

FIG. 3. Effect of milL-1β on milk intake of mice pretreated with methysergide or metergoline. Mice were treated with methysergide (4 mg/kg, IP, upper panel) or metergoline (1 mg/kg, IP, lower panel) 15 min before mIL-1β injection. $n = 7$. Significantly different from the corresponding saline-injected group (*$p < 0.05$ or **$p < 0.01$).

0.001 to 1 mg/kg SC 30 min before feeding (Fig. 4). Doses of 0.030 mg/kg or less did not affect milk intake, whereas doses of 0.10 mg/kg and higher depressed it, $F(7, 85) = 13$, $p < 0.0001$.

8-OH-DPAT was then injected at a dose of 10 μg/kg SC 15 min before injection of mIL-1β. This dose had previously been shown to decrease the firing of serotonergic cells in the raphe nucleus (47). Milk intake was depressed by mIL-1β, $F(1, 15) = 4.61$, $p < 0.05$, but not affected by 8-OH-DPAT, $F(1, 15) = 0.72$. The interaction was not statistically significant, $F(1, 15) = 0.01$. A second 5-HT_{1A} agonist, ipsapirone, was injected at doses 2.5 or 5 mg/kg IP 15 min before injection of mIL-1β (Fig. 5). Milk intake was depressed by IL-1β, $F(1, 25) = 35$, $p < 0.0001$, but was not affected by ipsapirone administered at either dose, $F(2, 25) = 0.31$. There was no significant interaction between the mIL-1β and the agonist treatments, $F(1, 25) = 0.13$. Thus, 5-HT_{1A} agonists do not appear to alter the IL-1–induced reductions in milk intake. Similar results were obtained with the 5-HT_{1B} agonist CGS12066B (1, 5 and 10 mg/kg SC).

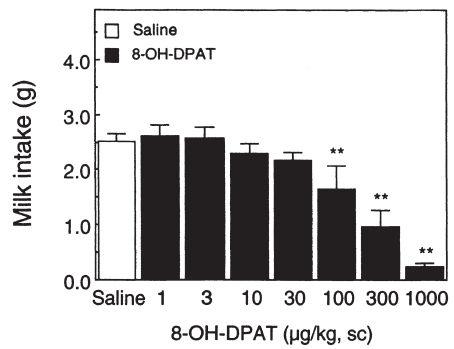

FIG. 4. Effect of different doses of 8-OH-DPAT on milk intake of mice. Mice were injected with 8-OH-DPAT at doses from 0.001 to 1 mg/kg, SC) and milk presented 30 min later for 30 min. $n = 7$–8 **Significantly different from the saline-injected group ($p < 0.01$).

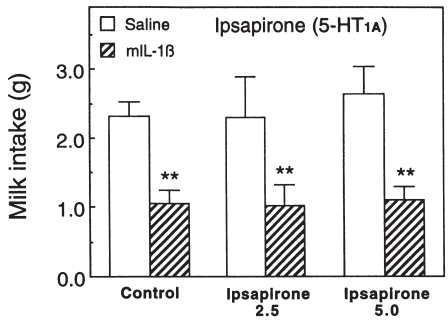

FIG. 5. Effect of ipsapirone pretreatment on mIL-1β induced changes in milk intake of mice. Ipsapirone (2.5 and 5.0 mg/kg, IP) was injected 15 min before the mIL-1β injection. The milk bottle was placed in the cage 90 min after the mIL-1β injection and milk intake over the next 30 min measured. $n = 7$. **Significantly different from the corresponding saline-injected group ($p < 0.01$).

Effect of 5,7-DHT Treatment on mIL-1β and LPS-Induced Hypophagia

5,7-DHT treatment depleted the serotonin content in the cortex by 94%, in the hypothalamus by 82%, in the hippocampus by 89%, in the striatum by 97%, and in the brain stem by 73% compared to control mice (all $p < 0.001$), with no significant effects on norepinephrine or dopamine. After 5,7-DHT, the mice decreased their milk intake, but nevertheless maintained a stable rate. mIL-1β was injected 20 days after the 5,7-DHT treatment. Milk intake was decreased in the 5,7-DHT-treated animals, $F(1, 26) = 22$, $p < 0.0001$, and depressed by mIL-1β, $F(1, 26) = 86$, $p < 0.0001$ (Fig. 6, upper panel). There

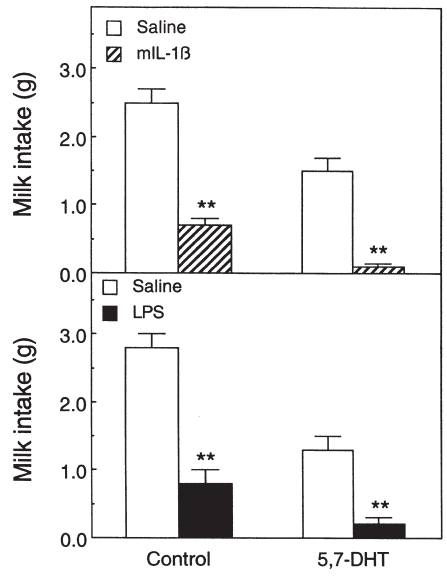

FIG. 6. Effect of mIL-1β and LPS on milk intake in 5,7-DHT treated mice. Mice were pretreated with 5,7-DHT (80 μg, ICV) and injected 20 days later with mIL-1β or 22 days later with LPS (1 μg, IP). Milk intake was assessed 90 min after mIL-1β or 2 h after LPS. $n = 8$. **Significantly different from the corresponding control group ($p < 0.001$).

was no statistically significant interaction between the mIL-1β 5,7-DHT treatments, $F(1, 26) = 1.51$. LPS was injected 22 days after the 5,7-DHT treatment (Fig. 6, lower panel). Milk intake was depressed by LPS, $F(1, 26) = 110$, $p < 0.0001$, and 5,7-DHT, $F(1, 26) = 46$, $p < 0.0001$. A significant interaction between LPS and 5,7-DHT, $F(1, 26) = 8.50$, $p < 0.01$, was observed, but inspection of the data indicated that the 5,7-DHT treatment intensified the hypophagia rather than diminishing it. Thus, the results obtained in 5,7-DHT–treated mice provide no support for a role of 5-HT in IL-1β–induced reductions in milk intake. Very similar results were obtained in a second experiment.

DISCUSSION

The reductions in milk intake following mIL-1β and LPS treatments were robust, and very similar to those observed in our previous experiments (50,51). These results are consistent with the existing literature indicating that both LPS and IL-1 depress feeding in animals (28,30,34,56).

LPS and IL-1 administration both increase brain tryptophan and 5-HIAA (12,23). The increase in tryptophan may consequently elevate 5-HT synthesis (15), although we have not observed increases in brain 5-HT content in mice. The increased synthesis may result in increased 5-HT secretion, which has the potential to decrease food intake (20,36,40). Thus, administration of antagonists of serotonin release or its receptors might be expected to reverse the hypophagia. Numerous studies have demonstrated that serotonin antagonists attenuate the hypophagia elicited by the *d*-fenfluramine, known to increase serotonin secretion, and have suggested the involvement of 5-HT_1 and 5-HT_2 receptors (8).

In the present experiments, we investigated the role of various 5-HT receptor subtypes in the hypophagic action of mIL-1β, using a variety of different receptor antagonists. In none of the experiments did we observe attenuation of the IL-1–induced hypophagia. There were no statistically significant mIL-1β × serotonin receptor antagonist interactions, providing no evidence for the involvement of 5-HT receptors in the mIL-1β-induced depression in milk intake. Although in several cases (ritanserin, ketanserin, metergoline, tropisetron, and 5,7-DHT), the hypophagia induced by LPS and IL-1 seemed to be exacerbated by serotonergic treatment; however, these augmentations were not statistically significant, except in the single case of LPS in 5,7-DHT-treated mice (Fig. 6).

Involvement of 5-HT_1 Receptors

The mixed 5-$HT_{1/2}$ receptor antagonists, methysergide and metergoline, have been observed to enhance food intake in some models (16). Importantly, it has been shown in overnight food-deprived rats that the depression of 30 min milk intake by peripherally administered 5-HT was completely blocked by methysergide, and attenuated by ketanserin, suggesting that 5-HT_1 and 5-HT_2 receptors play a role in serotonin-induced hypophagia (45). However, in our experiments, neither methysergide nor metergoline altered the IL-1β-induced decrease in milk intake (Fig. 3).

The 5-HT_{1A} antagonists, WAY100135 (Table 1) and *S*-propranolol (48), were also ineffective in attenuating the IL-1–induced decrease in milk intake. GR127935, a 5-HT_{1B} receptor antagonist (46) at the same dose used in the present study, has been shown to block the effects of the 5-HT_{1B} agonist, RU24969 and cocaine on c-*fos* and locomotion (9,32), and of a 5-HT_1 agonist (GR46611) to decrease 5-HT release in frontal cortex (46), but failed to alter the IL-1–induced reduction in milk intake (Table 1). Thus, 5-HT_{1A} or 5-HT_{1B} receptors do not appear to be involved in the hypophagic effects of IL-1.

Involvement of 5-HT_2 Receptors

The mixed 5-$HT_{1/2}$ receptor antagonists, methysergide and metergoline, failed to attenuate the decreased milk intake response to IL-1. Moreover, the 5-HT_{2A} receptor antagonist, ritanserin, and the 5-$HT_{2A/C}$ receptor antagonist, ketanserin, failed to alter the decreased milk intake induced by IL-1 (Fig. 1). Ample evidence suggests that 5-HT_{2C} receptors are involved in feeding (8). 1-(*m*-Chlorophenyl)piperazine (*m*CPP), an agonist with relatively high affinity for 5-HT_{2C} receptors, induced hypophagia that was reversed by selective 5-HT_{2C} antagonists (27). *m*CPP failed to reduce food intake in 5-HT_{2C} receptor knockout mice (52). We tested RS102221, a highly selective 5-HT_{2C} receptor antagonist that increased food intake and body weight gain in rats (4), and SB206553, a potent and selective 5-$HT_{2B/2C}$ antagonist that displays anxiolytic properties in both rats and mice (19,26). SB206553 attenuated the hyperphagia induced in satiated rats by the 5-HT_{2B} receptor agonist, BW723C86, in the dose range we used (25). However, neither RS102221 nor SB206553 attenuated the IL-1–induced reduction in milk intake.

Involvement of 5-HT_3 Receptors

We failed to observe any effects of tropisetron on feeding over a wide range of doses. The 5-HT_3 antagonist, GR38032F, was reported to enhance feeding in mice (42). The quaternary form of tropisetron (which does not cross the blood–brain barrier) was shown to attenuate the anorectic responses of the rat to amino acid-imbalanced diets (24). Another 5-HT_3 antagonist (Y25130) was shown to attenuate the impairment of meal time-associated anticipatory activity in aging rats (44). However, there have been many reports that 5-HT_3 agonists do not affect feeding. Tropisetron did not affect either palatable food consumption, nor did it antagonize the anorectic effect of *d*-fenfluramine (37). A study with another 5-HT_3 antagonist, ondansetron, indicated depressed intake of palatable food by decreasing the mean duration of feeding bouts (55). Parrott et al. (38) reported that ondansetron failed to modify the behavioral inhibition and nausogenic actions of cholecystokinin (CCK).

Effects of 5-HT_1 Receptor Agonists

5-HT_{1A} and 5-HT_{1B} agonists were tested because of their reported ability to decrease the activity of serotonergic neurons via autoreceptors. Systemic administration of 8-OH-DPAT decreased cerebellar 5-HT release, and this effect was reversed by the selective 5-HT_{1A} antagonist, WAY100635 (a more potent analog of WAY100135) (35). Neither 8-OH-DPAT or ipsapirone nor CGS12066B altered the reduction in milk intake induced by IL-1 (Fig. 5).

It is of some interest that neither ipsapirone nor 8-OH-DPAT evoked hyperphagia or attenuated mIL-1β induced hypophagia at any dose tested, because both agonists have been reported to stimulate long-term food intake in nonfasted rats at the same doses used in the present study (11,57). However, in the report by Dourish, 8-OH-DPAT increased intake of sweetened condensed milk in nonfasted rats tested in their home cages at only one dose (30 μg/kg), while at 1 mg/kg, intake was suppressed, as in our studies. In the same study,

there were small increases in milk intake at doses of 10 and 30 μg/kg when the 8-OH-DPAT was given 10 min after feeding commenced. Higher doses induced a dose-dependent reduction in milk intake, consistent with our results. 8-OH-DPAT also increased the duration of eating familiar food in a novel environment, but had no effect on eating familiar food in familiar environment (17). The lack of hyperphagic effects may reflect the specific paradigm used in our experiments. There may also be interspecies differences, because 8-OH-DPAT had no apparent effect on feeding in hamsters (1).

The Effects of 5,7-Dihydroxytryptamine

Microdialysis studies have indicated that although 5,7-DHT lesions did not change basal 5-HT release in the ventral striatum, the secretion of 5-HT in response to fenfluramine was significantly diminished (29). In our experiments, 5,7-DHT treatment decreased daily milk drinking, but the mechanism of this effect is not known. Scant attention has been paid to the possibility that 5-HT may play a role in determining the palatability of ingested foods. However, there are some grounds to suspect such a role (7), and it is possible that the 5,7-DHT treatment reduced milk intake by decreasing its rewarding properties (14). However, even though the 5,7-DHT treatment decreased daily milk intake, the failure to alter the milk intake response to mIL-1β or LPS further supports the lack of involvement of 5-HT in cytokine-induced hypophagia.

Thus, despite the well-documented roles of 5-HT in feeding, none of the manipulations of the serotonergic system examined in the present study resulted in significant changes in the reduction of milk intake induced by mIL-1β or LPS in mice. This appears to conflict with an extensive literature in which 5-HT has been implicated in feeding behavior (8). However, our results clearly show that none of the serotonergic agonists or antagonists tested altered the hypophagic responses to IL-1 (or LPS). We must conclude that either serotonin is not involved in these hypophagic responses, or that there are redundant mechanisms (pathways) by which IL-1 exerts its hypophagic effects.

Changes in total food intake may fail to reveal alterations of the feeding microstructure, and it is possible that IL-1 selectively affects specific components of feeding behavior. Many other chemical messengers affect food intake (58), and it is possible that IL-1 directly or indirectly interacts with certain neuropeptides known to affect feeding, such as, cholecystokinin (CCK), leptin, neuropeptide Y (NPY), substance P, corticotropin-releasing factor (CRF), or urocortin. In one study, the hypophagic effects of fenfluramine, usually associated with increased serotonergic activity, were reversed by the selective CCK receptor antagonist MK-329 (6). Other likely possibilities are CRF or urocortin (31,43,53). There is a complicated modulation of CRF neurons involving different serotonin receptors in the hypothalamus (54). Hypothalamic CRF modulates feeding induced by NPY (22), and it has been suggested that attenuation of NPY-induced hyperphagia by DOI involves activation of hypothalamic CRF (41). However, our recent study showed unaltered reductions in milk intake in response to mIL-1β and LPS in mice lacking the gene for CRF (49). Urocortin, acting on CRF receptors, remains a possibility. Cytokines and serotonin acting via different receptor subtypes and interacting with different neuropeptides have multiple possibilities to reduce food intake, constituting a regulatory cytokine–peptide–neurotransmitter triangle as suggested by Plata-Salamán (39). One interpretation of our results is that multiple neurotransmitter pathways mediate the behavioral responses to LPS and IL-1, so that simultaneous blockade of several pathways may be necessary to reveal the neural mechanisms underlying LPS/IL-1–induced hypophagia.

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