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A Nitric Oxide Synthase Inhibitor N^G-Nitro-L-Arginine, Attenuates Glucoprivic Feeding and Deprivation-Induced Drinking in the Mouse

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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**(3) 601–607, 1998.— Possible involvement of nitric oxide (NO) in glucoprivic hyperphagia was investigated in nondeprived male ICR mice in independent groups designs. One pair of experiments demonstrated dose-related reductions in 2-deoxy-D-glucose (2DG)- and insulin-induced solid food intake with increasing dose (10, 25, and 50 mg/kg SC) of the NO-synthase (NOS) inhibitor, N^G-nitro-L-arginine (L-NOARG), reaching statistical significance at 10 mg/kg L-NOARG when compared to vehicle controls. In a second pair of experiments, initial pretreatment with L-arginine (500 and 1000 mg/kg IP) partially or completely restored the feeding inhibitory action of an effective challenge dose (25 mg/kg) of L-NOARG; D-arginine (500 mg/kg IP) was ineffective, thus supporting a stereospecific action of arginine. A third set of experiments demonstrated dose-related reduction in glucoprivic feeding under delayed access (4 or 6 h) to food. These findings suggest involvement of NO in glucoprivic hyperphagia; they are consistent with and extend research linking NO and ingestive behaviors through use of NOS inhibitors. Deprivation-induced drinking was attenuated by these doses of L-NOARG as well. © 1998 Elsevier Science Inc.

2-Deoxy-D-glucose 2DG D-Arginine Delayed feeding Drinking Glucoprivation Food intake Feeding behavior Insulin L-Arginine L-NOARG Mice N^G-Nitro-L-arginine Nitric oxide Nitric oxide synthase inhibitor Water intake

IT has recently been recognized that nitric oxide (NO), a naturally occurring vasodilating gas, might play an important role in mechanisms regulating feeding behaviors in animals under a number of conditions. There is evidence that NO functions as a neurotransmitter and intracellular messenger in both central and peripheral nervous systems (8,18). Pharmacologic manipulations purportedly interfering with production of endogenous NO have been reported to reduce food intake in food deprived mice (20,21,23), rats (31), and chickens (5), and in genetically obese strains of mice (22) and rats (32). Feeding stimulated by morphine (3), neuropeptide Y (21) and chlordiazepoxide (7) was similarly attenuated in mice. In these studies, NO production was restricted through inhibition of the catalytic enzyme, NO-synthase (NOS). Feeding deficits could be restored to varying degree with L-arginine (L-arg), the natural substrate for NOS and NO precursor. Further, it has been reported that pretreatment with a NOS inhibitor can alter various parameters of feeding behavior, including time spent feeding and number and duration of meals in the rat (35). In further probing the extent to and conditions under which inhibition of NO generation might influence feeding behaviors, we now report that pretreatment with the NOS inhibitor, N^G-Nitro-L-arginine (L-NOARG) attenuates feeding induced by glucoprivic challenge in mice [see (26,29) for reviews of glucoprivic hyperphagia]. L-NOARG has been reported to be highly selective for inhibiting brain NO synthesis (15); L-NOARG can compete with L-arg for active sites on the NOS and thus inhibit generation of NO from L-arg. In an

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initial set of experiments, food intake in mice pretreated with L-NOARG was monitored from time of insulin administration or after 2-h delayed access to food following 2-deoxy-D-glucose (2DG) injection. A second set of experiments explored a possible effect of coadministering arginine isomers with an effective challenge dose of L-NOARG in both the insulin and 2DG paradigms. A final set of experiments, prompted by the findings of Ritter's group (28), asked if mice would exhibit glucoprivic feeding after extended delay in access to food and, if so, possible influence of a NOS inhibitor.

METHOD

Animals

Male ICR mice, weighing 31-49 g, were individually housed in standard opaque polypropylene tub-type cages and maintained on a 12 L:12 D cycle (lights on 0700–1900 h) in a temperature- and humidity-controlled colony room, with continuous access to tap water and pelleted food (Teklad rodent diet 8604) except as noted. They were held in the colony room for at least 7 days before testing. Animals were tested individually, and all testing was carried out during the light period between 0900 and 1500 h.

Drugs

Regular insulin (Iletin® I, Lilly) was obtained from a local pharmaceutical distributor; 2-deoxy-D-glucose (2DG), N^G-nitro-L-arginine (L-NOARG), and L- and D-arginine (arg) were purchased from Sigma Chemical Co. (St. Louis, MO). All drugs were freshly prepared in sterile 0.9% NaCl vehicle on the morning of testing. Insulin and L- or D-arg (or vehicle) were injected intraperitoneally (IP), L-NOARG (or vehicle) was injected subcutaneously (SC), and 2DG (or vehicle) SC according to experimental protocols indicated below. All drug doses were selected based on prior research using the mouse as an experimental subject. Drugs were injected in a volume of 0.1 m1/10 g body weight.

Behavioral Testing Procedures

Mice were adapted to the experimental test environment, with both food and water (Experiments 1–3) or with water only (Experiment 4) present, prior to testing. On the day of testing, mice in Experiments 1–3 were weighed to the nearest 0.5 g, placed into a living cage with fresh bedding and water, but without food, and returned to the colony room. Mice in Experiment 4 were water deprived overnight from 1530 h, at which time they were weighed; they were placed into a living cage on day of testing without food or water. Approximately 30 min later, drug administration was started in all experiments. Procedures specific to particular experiments were as follows.

Experiment 1A—Insulin and L-NOARG dose–response series. Mice (n = 16–17/group) were first pretreated with 10, 25, or 50 mg/kg of L-NOARG or 0.9% NaCl vehicle SC. Forty-five minutes later, 5 U/kg insulin or vehicle was administered IP. Within several minutes mice were then placed in a bare polypropylene test cage, identical to the living cage, with a preweighed food pellet (same as provided for maintenance diet) secured in a removable "in-wall-mounted" holder, The pellet was weighed at 30, 60, 120, and 240 min on a balance accurate to 0.001 g (Mettler model PC-180). Water was available during the test period. Uneaten crumbs (generally minimal when occurring) dropped into a collector bin mounted below the food pellet, and were incorporated into pellet weighing at 240 min.

Experiment 1B—2DG and L-NOARG dose-response series. Mice (n = 12–14/group) were first administered 500 mg/kg of 2DG or vehicle SC. Seventy-five minutes later, they were injected SC with 10, 25, or 50 mg/kg of L-NOARG or vehicle. Forty-five minutes after this last injection, mice were transferred to test cages with food and water. Food intake was measured at 30, 60, 120, and 180 min. Measurement procedures were again followed here and below as in Experiment 1A.

Experiment 2A—Insulin, L-NOARG, and L- and D-arginine interaction series. Mice (n = 13–16/group) were initially pretreated with L-arg (500 or 1000 mg/kg), D-arg (500 mg/kg) or vehicle control IP, along with 25 mg/kg of L-NOARG SC, or with double vehicle only (SC or IP). Forty-five minutes later, 5 U/kg insulin was administered IP. Following insulin injection, mice were transferred to test cages with food and water. Food intake was measured at 30, 60, 120, and 240 min.

Experiment 2B—2DG, L-NOARG, and L- and D-arginine interaction series. Mice (n = 12/group) were first administered 500 mg/kg of 2DG SC. Seventy-five minutes later, they were injected IP with L-arg (500 or 1000 mg/kg), D-arg (500 mg/kg), or vehicle along with 25 mg/kg of L-NOARG SC, or with double vehicle only (SC or IP). Forty-five minutes after this last pair of injections, mice were transferred to test cages with food and water. Food intake was measured at 30, 60, 120, and 180 min.

Experiment 3A—Insulin/4-h delay and L-NOARG dose response series. Mice (n = 13–14/group) were first administered 5 U/kg of insulin or vehicle IP, and returned to a holding/living cage with fresh bedding and water only for 4 h. Forty-five minutes prior to the end of the 4-h delay, mice were injected SC with 10, 25, or 50 mg/kg of L-NOARG or vehicle. The shorter delay with insulin was selected after initially observing that several animals exhibited varying degrees of lethargy by the fifth hour, and ate little or no food. Given its higher metabolism, the mouse is likely to be at greater risk under extended energy deficit in this protocol. At the end of 4 h, they were transferred to test cages with food and water. Food intake was measured at 30, 60, 120, and 180 min.

Experiment 3B—2DG/6-h delay and L-NOARG dose-response series. Mice (n = 13–15/group) were first administered 500 mg/kg of 2DG or vehicle SC, and returned to a holding cage with fresh bedding and water only for 6 h. Forty-five minutes prior to the end of the 6-h delay, mice were injected SC with 10, 25, or 50 mg/kg of L-NOARG or vehicle. At the end of 6 h, they were transferred to test cages with food and water. Food intake was measured at 30, 60, and 120 min.

Experiment 4—Water deprivation and L-NOARG dose–response series. Mice (n = 15–16/group) were injected with 10, 25, or 50 mg/kg of L-NOARG or 0.9% NaCl vehicle SC; 45 min later, they were placed in a bare test cage with water dispenser only. Duration of water deprivation was approximately 20 h. The water tube was weighed to 0.001 g accuracy at 30, 60, and 120 min. Spillage collected in a drip cup mounted inside the unit directly under the sipper tube was incorporated into final weighing.

All research protocols were reviewed and approved by Marquette University's Institutional Animal Care and Use Committee (IACUC) and are in compliance with the USDA Animal Welfare Act.

Statistical Analyses

Cumulative food or water intake data at each measurement period were evaluated separately with independent one-way ANOVAs along with Dunnett's t-tests (Experiments 1, 3,

and 4), or with Bonferonni protected Student's *t*-tests (Experiments 1–3), (one tail). Minimally acceptable alpha level was set at $p < 0.05$.

RESULTS

Experiment 1A—Insulin and L-NOARG Dose–Response Series

Cumulative food intake data for insulin-treated animals are summarized in Fig. 1 (upper panel). Insulin produced a robust feeding response, as expected, and which was maintained across all time periods [Veh/Veh vs. Veh/insulin; t (32) = 4.22, 4.75, 4.64, and 3.77, respectively, at 30, 60, 120, and 240 min, all $p < 0.01$, Student's *t*-test]. The one-way ANOVAs (not including double vehicle baseline control) yielded $F(3,62)$ = 7.09, 8.54, 6.17, and 4.38, respectively, at these time periods (all $p < 0.01$). When compared to L-NOARG vehicle condition/group, cumulative food intake in mice administered 5 U/kg insulin was significantly lower at all doses of L-NOARG used ($p < 0.05$ or $p < 0.01$, Dunnett's *t*-test) at all measurement periods, with the exception of the 10 mg/kg L-NOARG dose at 120 and 240 min.

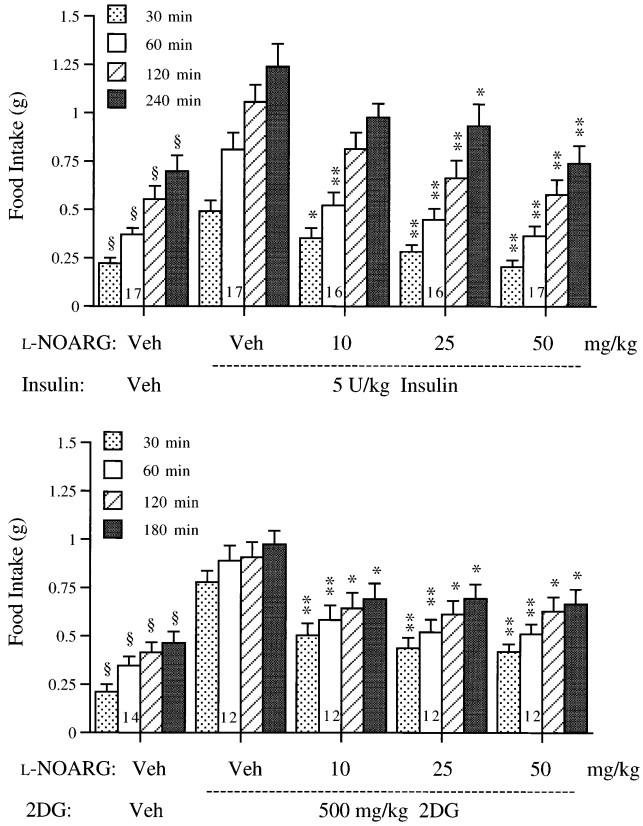

FIG. 1. Mean (±SEM) cumulative food intake in insulin-treated (upper panel) or 2DG-treated (lower panel) mice following pretreatment with L-NOARG or 0.9% NaCl vehicle at 30, 60, 120, and 180 (or 240) min. §$p < 0.01$ compared to Veh/insulin or to Veh/2DG groups, Student's *t*-test (one-tail). *$p < 0.05$, **$p < 0.01$ compared to Veh/insulin or to Veh/DG, Dunnett's *t*-test (one-tail). All comparisons are at same measurement period. For each group, sample size (n) is indicated in base of open bar for that group. Veh/Veh group is baseline control.

Experiment 1B—2DG and L-NOARG Dose–Response Series

Cumulative food intake data for 2DG-treated animals are shown in Fig. 1 (lower panel). A robust feeding response was produced by 2DG across all time periods [Veh/Veh vs. Veh/2DG; t (24) = 8.43, 6.15, 5.38, and 5.75, respectively, at 30, 60, 120, and 180 min, all $p < 0.01$, Student's *t*-test]. The ANOVAs yielded $F(3,44)$ = 9.68, 6.94, 3.41, and 3.79, respectively, at these time periods ($p < 0.01$, $p < 0.01$, $p < 0.02$, and $p < 0.02$). When compared to L-NOARG vehicle condition, cumulative food intake in mice administered 500 mg/kg 2DG was significantly lower at all doses of L-NOARG used ($p < 0.05$ or $p < 0.01$, Dunnett's *t*-test) at all measurement periods.

Experiment 2A—Insulin, L-NOARG, and L- and D-Arginine Interaction Series

Cumulative intake data for insulin-treated mice pretreated with arginine isomers in conjunction with 25 mg/kg of L-NOARG are summarized in Fig. 2 (upper panel). Pretreatment with 500 or 1000 mg/kg of L-arg partially reversed an attenuating effect of L-NOARG at 30 and 60 min (both $p < 0.05$, Student's *t*-test); mean intakes were 46.8 and 52.8% higher at 30 min, and 46.0 and 53.0% higher at 60 min, under the two doses of L-arg than under L-arg vehicle. In contrast, 500 mg/kg of the inactive isomer, D-arg, failed to significantly alter food intake.

Experiment 2B—2DG, L-NOARG, and L- and D-Arginine Interaction Series

Cumulative food intake data for 2DG-treated mice pretreated with arginine isomers in conjunction with 25 mg/kg of L-NOARG are summarized in Fig. 2 (lower panel). Pretreatment with 500 or 1000 mg//kg of L-arg partially or completely reversed an attenuating effect of L-NOARG at all time periods ($p < 0.05$ or $p < 0.01$, Student's *t*-test); mean intakes were increased by 48.3, 65.8, 65.6, and 63.2% and by 53.3, 67.1, 81.8, and 84.6%, respectively, at 30, 60, 120, and 180 min under the two doses of L-arg, when compared to L-arg vehicle. In contrast, 500 mg/kg of the inactive isomer, D-arg, failed to significantly alter food intake at any time point.

Experiment 3A—Insulin/4-h Delay and L-NOARG Dose–Response Series

Cumulative food intake data under 4-h delayed access to food for insulin-treated animals are summarized in Fig. 3 (upper panel). Insulin produced a robust feeding response, which was maintained across all time periods [Veh/Veh vs. Veh/insulin; $t(25)$ = 2.06, 3.06, 3.17, and 3.11, respectively, at 30, 60, 120, and 180 min, $p < 0.05$ or $p < 0.01$, Student's *t*-test]. The one-way ANOVAs yielded $F(3,49)$ = 3.67, 8.18, 9.44, and 7.48, respectively, at these time periods ($p < 0.02$ or $p < 0.01$). When compared to L-NOARG vehicle condition/group, cumulative food intake in mice administered 5 U/kg insulin was significantly lower at a dose of 10 mg/kg of L-NOARG at 60, 120, and 180 min ($p < 0.05$ or $p < 0.01$) and at all measurement periods under 25 and 50 mg/kg of L-NOARG ($p < 0.05$ or $p < 0.01$), Dunnett's *t*-test.

Experiment 3B—2DG/6-h Delay and L-NOARG Dose–Response Series

Cumulative food intake data under 6-h delayed access for 2DG-treated animals are shown in Fig. 3 (lower panel). An increase in feeding was again produced by 2DG across all time

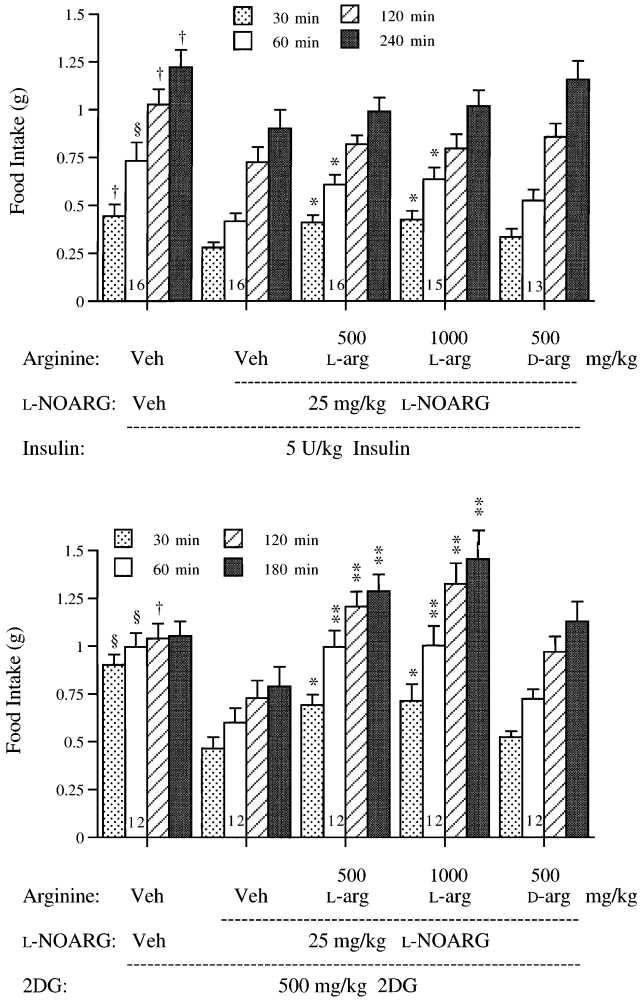

FIG. 2. Mean (±SEM) cumulative food intake in insulin-treated (upper panel) or 2DG-treated (lower panel) mice following pretreatment with 25 mg/kg of L-NOARG along with L- or D-arg or 0.9% NaCl vehicle, or with double vehicle control, at 30, 60, 120, and 180 (or 240) min. †$p < 0.05$, §$p < 0.01$ compared to Veh/L-NOARG/insulin or to Veh/L-NOARG/2DG groups, Student's *t*-test (one tail). *$p < 0.05$, **$p < 0.01$ compared to Veh/L-NOARG/insulin or to Veh/L-NOARG/2DG, Student's *t*-test (one tail). All comparisons are at same measurement period. For each group, sample size (*n*) is indicated in base of open bar for that group.

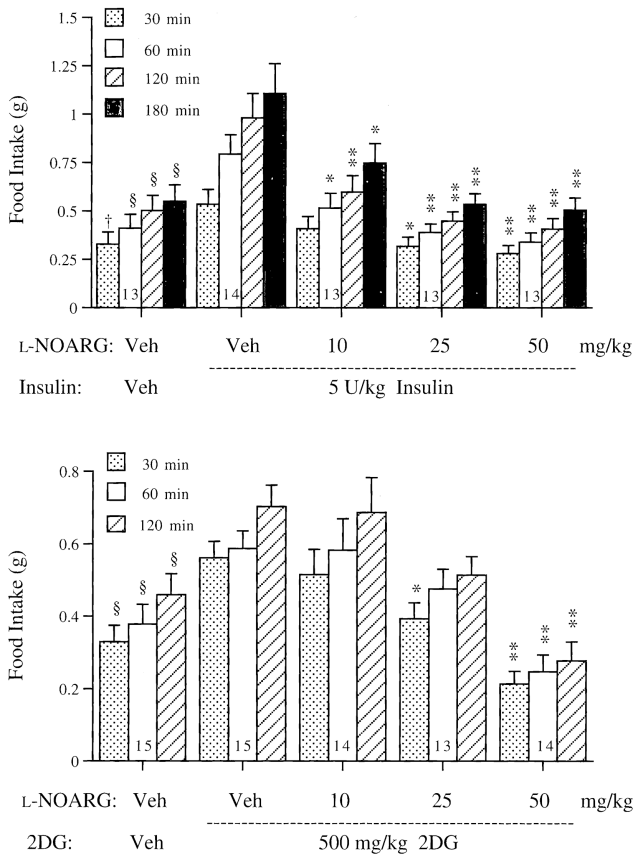

FIG. 3. Mean (±SEM) cumulative food intake in insulin-treated (upper panel) or 2DG-treated (lower panel) mice following L-NOARG or 0.9% NaCl vehicle injections at 30, 60, 120, and 180 min, with delayed access to food for 4 h (insulin) or 6 h (2DG). †$p < 0.05$, §$p < 0.01$, compared to Veh/insulin or to Veh/2DG groups, Student's *t*-test (one tail). *$p < 0.05$, **$p < 0.01$ compared to Veh/insulin or to Veh/2DG, Dunnett's *t*-test (one tail). All comparisons are at same measurement period. For each group, sample size (*n*) is indicated in base of open bar for that group. Veh/Veh group is baseline control.

periods [Veh/Veh vs. Veh/2DG; $t(28) = 3.60$, 2.87, and 2.94, respectively, at 30, 60, and 120 min, all $p < 0.01$, Student's *t*-test]. The ANOVAs yielded $F(3,52) = 9.82$, 6.92, and 8.79, respectively, at these time periods (all $p < 0.01$). When compared to L-NOARG vehicle condition/group, cumulative food intake in mice administered 500 mg/kg 2DG was significantly lower at 25 mg/kg of L-NOARG at 30 min ($p < 0.05$) and at 50 mg/kg of L-NOARG at all measurement periods (all $p < 0.01$), Dunnett's *t*-test.

Experiment 4—Water Deprivation and L-NOARG Dose–Response Series

Cumulative water intake data in 20-h water-deprived animals are shown in Table 1. The ANOVAs yielded $F(3, 59) =$ 8.70 and 3.64, respectively, at 30 and 60 min ($p < 0.05$ and $p < 0.02$). When compared to L-NOARG vehicle condition, cumulative water intake was significantly lower at 25 mg/kg of L-NOARG at 30 min only ($p < 0.05$) and at 50 mg/kg of L-NOARG at 30 and 60 min ($p < 0.01$ and $p < 0.05$), Dunnett's *t*-test. No other comparisons were statistically significant.

DISCUSSION

The principal focus of the present study was to systematically investigate a possible role of nitric oxide in the feeding stimulatory effect of two glucoprivic agents—insulin and 2DG (26,29). Endogenous NO production was purportedly blocked by the neuronal NOS inhibitor, L-NOARG, and cumulative food intake was measured under different protocols at several posttreatment time points. Three major sets of findings emerged. First, peripheral administration of L-NOARG attenuated both insulin- and 2DG-induced feeding in a dose-related manner under immediate or short delay access to food. A second was that L-arg, the natural substrate for synthesis of NO and NO precursor, restored feeding blocked by an effective

TABLE 1

CUMULATIVE WATER INTAKE (g) FOLLOWING SALINE VEHICLE OR L-NOARG INJECTION IN 20-h WATER-DEPRIVED MICE

	Dose of L-NOARG (mg/kg)			
Time (min)	Veh (0)	10	25	50
30	0.86 (±0.08)	0.79 (±0.05)	0.63* (±0.07)	0.40† (±0.07)
60	0.96 (±0.08)	0.95 (±0.05)	0.73 (±0.08)	0.67 (±0.09)*
120	1.10 (±0.10)	1.03 (±0.06)	0.91 (±0.12)	0.93 (±0.13)
n/group	16	16	15	16

Results are shown as mean (±SEM)

*$p < 0.05$, †$p < 0.01$ compared to corresponding vehicle group (Dunnett's *t*-test, one-tail).

attenuating challenge dose of L-NOARG. Further, this action of L-arg was stereospecific, i.e., the inactive D-isomer was ineffective—thereby providing strong suggestion of NO involvement. These observations are in general agreement with the recent literature implicating NO in regulation of feeding, extending previous research with morphine-, neuropeptide Y- and chlordiazepoxide-induced feeding, as well as for deprivation-induced feeding, in animals administered NOS inhibitors. Third, it was further shown that both insulin and 2DG stimulated a vigorous feeding response in mice even when access to food was delayed for 4 (insulin) or 6 (2DG) h. These findings are consistent with those first reported for the rat by Ritter and his colleagues (28). These investigators demonstrated glucoprivic feeding induced by insulin and 2DG in the rat when food was withheld until physiological indication of glucoprivation had abated, i.e., altered blood glucose concentrations had returned to normal levels. The delayed feeding currently observed in mice was also attenuated in a dose-related manner by NOS inhibition. The only apparent difference, in contrast to the current Experiment 1 findings, was a weaker attenuating effect on food intake in 2DG-treated mice at the lower doses of L-NOARG.

In considering a putative role for NO in feeding mechanisms, it is important to provide evidence that observed shifts in food intake are not attributable to a nonspecific effect of inhibiting NOS activity, as, for example, a shift in general arousal or responsiveness—perhaps suggesting reduced sensory or motor function. Such nonspecific influence would thus, of course, be a concern in evaluating the shifts in food intake currently observed under glucoprivic challenge. While reduced horizontal locomotion in rats (30) and mice (19,33) in unfamiliar or novel environments and altered exploratory patterns (19,30) have been linked to systemic injection of NOS inhibitors, these were observed only at relatively high doses (e.g., ≥100 mg/kg). Control data from our laboratory are in general accord. Both horizontal and vertical (rears) locomotion in an open field were significantly attenuated at 50 and 100 mg/kg of L-NOARG in ICR mice, while lower doses (10 and 25 mg/kg) were without effect (7). Most recently, Prendergast et al. reported no apparent diminution in motor (swim speed) or visuosensory (visual location of "safe/dry" platform) functioning in a water maze at or below, respectively, 50 mg/kg of N^G-nitro-L-arginine methyl ester (L-NAME) in rats (25). Thus, while relatively high doses of a NOS inhibitor might contribute to some blunting of these behaviors/functions, a significant drop in food intake was observed in the current study (in all but Experiment 3B) at the low dose of 10 mg/kg of L-NOARG in acclimated animals in a familiar environment using familiar protocols.

A further consideration is whether a general malaise or other debilitating circumstance might have led to reduced food intake; indeed, this is generally a concern whenever an activated state/condition is attenuated following drug administration. A less clear picture emerges here. The related work of Morley's group would seem to argue against such interpretation. These authors report that while the classical "illness-inducing" agent, lithium chloride (LiCl), reduced lever pressing for milk reinforcement equally in partially sated and in 18-h fasted mice (11), L-NAME affected lever pressing differentially under these conditions (20). Further, unpublished data (not shown) from our laboratory failed to show a conditioned taste aversion (CTA) to a 0.1% saccharin solution in rats under L-NAME at the same doses as were used in the present study; a LiCl control, as expected, did induce a robust CTA. In contrast, Prendergast et al. reported evidence of a CTA to a 10% sucrose solution at 20 mg/kg of L-NAME in the rat (25). Overall, however, the lower effective dose(s) of NOS inhibitors found to be effective here and in much of the previous feeding work and the dose-related behavior patterns shown, coupled with the stereospecific reversing action of L-arg, would argue against the nonspecific actions herein considered.

A related consideration is a potential confounding influence of introducing a drug known to attenuate behavioral responsiveness into a system already under the considerable physiological stress of pronounced glucoprivation. In the hope of providing some insight, this issue was addressed by also probing a potential effect of NOS inhibition on behavior linked to a challenge less stressful than glucoprivation, as, for example, water deprivation. As has been reported for other species, L-NOARG dose relatedly attenuated deprivation-induced drinking in the mouse. The effect, however, was no longer significant at the second hour.

While mechanism(s) and site(s) of action is/are currently unknown, the literature points to altered NO activity both peripherally and centrally that could contribute to observed shifts in feeding behavior. NOS inhibitors have been shown to abolish reflexive relaxation of the stomach to accommodate intake of liquid or solid food in guinea pig (10), and to antagonize the lower esophageal sphincter muscle relaxation response to swallowing and to vagal stimulation in opossum (38). Both were reversed only by the active isomer, L-arg. In the dog, NOS inhibition delayed gastric emptying of a solid food meal, also reversed by L-arg; the inactive D-isomer was not evaluated (24). Reduced ingestion is arguably consistent with inhibition of any of these gastrointestinal responses. Glucoprivic agents, such as insulin and 2DG, are known to accelerate gastric emptying as well as to increase gastric acid secretion (6,12,17,34); NOS inhibitors would be expected to antagonize these responses. Involvement of NO in digestive systems/processes has been the focus of a recent review (14). Squadrito et al. (31,32) suggest brain NO involvement in a central serotonergic system regulating food intake, reporting that depressed levels of serotonin (5-HT) and 5-hydroxyindoleacetic acid in diencephalon following 24-h food deprivation were reversed/increased by systemic injection of L-NOARG, and that selective antagonism of 5-HT receptor subtypes abolished L-NOARG–induced hypophagia in deprived animals. Involvement of serotonin in feeding behavior is well docu-

mented [for reviews, see citations (2,16)]. Recent reports have pointed to interactions of 5-HT receptor subtypes and opioid mechanisms in modulating insulin and 2DG hyperphagia in the rat (1,13). De Luca et al. (9) most recently reported opposing shifts in oxygen consumption, and in activity of sympathetic nerves innervating and temperature of brown adipose tissue in 24-h fasted rats following central or peripheral administration of L-NAME, while both resulted in depressed food intake—suggesting multiple mechanisms of NOS-linked inhibition of feeding. There are currently no research data available to implicate central nervous system (CNS) circuitry/sites linking NO and glucoprivic feeding. At the same time, it is interesting to note several recent observations. While central neural circuitry mediating glucoprivic feeding is largely unknown, there is evidence to indicate that important CNS regions involved in 2DG-induced feeding are located in caudal hindbrain and in central nucleus of the amygdala (CeAmy) in the rat (26,36). Using Fos immunohistochemistry, Ritter and Dinh (27) recently reported that systemic injection of 2DG induced Fos-like immunoreactivity (Fos-li) in area postrema (AP), nucleus of the solitary tract (NTS), and parabrachial nucleus (PBN), as well as in the CeAmy. Other areas linked to feeding behavior, most notably the paraventricular nucleus of the hypothalamus (PVN), also exhibited Fos-li. Total destruction of the PVN, however, failed to impair 2DG-induced feeding in the rat (4)—perhaps reflecting multiple and redundant pathways and/or mediation of other physiological responses to 2DG (27). Further, Vincent and Kimura recently identified presence of NOS in neurons in some of these same areas, i.e., in AP, NTS, and CeAmy (37). It will be important to carry out discrete central administration of NOS inhibitors into these and other sites in conjunction with glucoprivic feeding challenges.

A final note—in contrast to a growing literature linking NOS inhibition to systematic dose-related decreases in food intake under a variety of experimental conditions at low to moderate dosing, several investigators have failed to observe a similar significant depressant effect of NOS inhibition on normal/spontaneous feeding in both mice (3,7) and rats (31). Thus far, there have been no suggestive data to account for this difference. This and other issues will need to be pursued in future investigations.

In summary, these experiments provide further evidence for involvement of NO in the regulation of feeding behaviors, extending the work of others in this area. To our knowledge, this is also the first report of delayed glucoprivic feeding and of an NOS inhibitor influence on drinking behavior in the mouse. It will be important to carry out CNS mapping research, and to systematically probe possible contributions of change(s) in factors such as taste, and in meal parameters, as well as interactions with other neurochemical systems in both rat and mouse models.

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