Metabolic inhibition increases feeding and brain Fos-like immunoreactivity as a function of diet


Whether specific brain sites are involved in triggering the eating responses to these metabolic inhibitors, we measured food intake and Fos-like immunoreactivity (Fos-li) in rats maintained on either a low-fat/high-carbohydrate (LF/HC) or high-fat/low-carbohydrate (HF/LC) diet. Rats fed the LF/HC diet increased food intake after administration of 2,5-AM (200 mg/kg ip) but not after treatment with MP (10 mg/kg po), whereas rats maintained on the HF/LC diet increased food intake in response to MP administration but not after 2.5-AM injection. The effects of these inhibitors on brain Fos-li in several specific brain nuclei paralleled those on feeding behavior; that is, the number of cells showing Fos-li increased only under dietary conditions in which 2,5-AM or MP stimulated eating. These results suggest that the eating response to metabolic inhibition is tied to increased neuronal activity in brain regions that process vagal afferent signals. 2,5-anhydro-D-mannitol; methyl palmoxirate; hunger; rat

EATING BEHAVIOR IN RATS is stimulated by administration of a variety of metabolic inhibitors that interfere with glucose use, fatty acid oxidation, and ATP production (e.g., Refs. 6, 19, 29). In addition to providing information about the nature of metabolic events that control food intake, these agents have been used to elucidate neural mechanisms of the metabolic control of eating behavior. One approach has attempted to relate the effect these metabolic inhibitors have on food intake to neuronal activity in specific brain regions using immunohistochemistry for Fos (11, 12, 22, 23), an immediate early gene product that is expressed in response to neuronal stimulation (26). The pattern of Fos-like immunoreactivity (Fos-li) in brain stem, hypothalamus, and forebrain seen after administration of these metabolic inhibitors is consistent with the activation of neural pathways that are part of a system for receiving and integrating visceral and, in particular, vagal sensory information (3, 15). Despite these observations, however, there is relatively little evidence for a functional link between increased neuronal activity in these brain areas and the eating responses to metabolic inhibition.

Two lines of evidence tie the increase in neuronal activity induced by treatment with metabolic inhibitors to the eating behavior elicited by these same treatments. Dose-response analysis suggests that the eating response after administration of the fructose analog, 2,5-anhydro-D-mannitol (2,5-AM), is seen only at a dose level that reliably increases neural activity in specific brain nuclei (11). It is not known whether similar dose-response relationships are seen with other inhibitors that stimulate eating behavior. More evidence linking the neural and behavioral responses to metabolic blockade stems from experiments in rats with visceral denervation (24). Thus vagotomy, which prevents the eating response to administration of 2,5-AM, largely eliminates the induction of Fos-li after treatment with this fructose analog (23, 30). Similarly, vagotomy or visceral deafferentation blocks both the eating response and induction of brain Fos-li seen after treatment with mercaptoacetate (MA), an inhibitor of fatty acid oxidation (22, 25). These findings are consistent with the hypothesis that a visceral and, perhaps, vagal afferent signal triggers both the eating response and induction of Fos-li after treatment with metabolic inhibitors.

To assess the relationship between neuronal activity and food intake in animals with an intact nervous system, we have examined the effects of dietary composition on the neural and behavioral responses to administration of metabolic inhibitors. Whether rats increase food intake after administration of metabolic inhibitors can depend on the content of fat and carbohydrate in the diet (6, 7, 18, 28). This effect of dietary macronutrient composition is seen clearly with 2,5-AM and methyl palmoxirate (MP), an inhibitor of fatty acid oxidation. Rats fed a low-fat/high-carbohydrate (LF/HC) diet increase food intake in response to 2,5-AM treatment, whereas those fed a high-fat/low-carbohydrate (HF/LC) diet do not (18). Administration of 2,5-AM is thought to stimulate eating behavior by reducing the content of ATP in liver (see Refs. 4, 14), and feeding an HF/LC diet apparently prevents the eating response because it attenuates the decline in liver ATP produced by 2,5-AM (13). MP suppresses fatty acid oxidation by inhibiting carnitine palmitoyltransferase I (CPT I), which transports long-chain fatty acids into mitochondria for oxidation (31). Inhibition of CPT I appears to underlie the eating response to administration of MP (5), a response that is seen in rats fed an HF/LC diet (5), but not LF/HC, diet (6, 7). This effect of diet composition is opposite to that seen with 2,5-AM-treated rats and appears to be related to the dependence on fat fuels as a source of metabolic energy in rats fed the HF/LC diet (20). Rats fed an LF/HC diet apparently do not increase food intake after MP treatment because they can readily use carbohydrates to offset the inhibition of fatty acid oxidation (6, 7, 28).
In the present experiments, we took advantage of these clear-cut effects of diet composition on the eating responses to administration of 2,5-AM and MP to further assess the relationship between neuronal activity and stimulation of eating behavior. The results showed that, although the induction of brain Fos-li and stimulation of food intake produced by treatment with 2,5-AM and MP were affected differentially by diets differing in fat and carbohydrate content, the neural and behavioral responses to these inhibitors nevertheless varied in parallel.

**METHODS**

**Subjects and Diet**

Adult male CD Sprague-Dawley rats (Charles River; Kingston, NY) were used for all experiments and were housed individually in a temperature-controlled (22°C) vivarium maintained on a 12:12-h light-dark cycle (lights on at 0700). Rats initially weighed 225–250 g when received from the supplier and 300–500 g at the time of testing. Animals were fed either an LF/HC or HF/LC diet of equal caloric density (see Ref. 20) (ICN Biochemicals, Aurora, OH) in pelleted form placed in glass food cups. The LF/HC diet contained (in %kcal) 65 carbohydrate, 22 protein, and 14 fat, whereas the HF/LC diet contained 67 fat, 12 carbohydrate, and 21 protein. All rats were maintained on a given diet for at least 2 wk before testing. Food and tap water were available ad libitum throughout the experiment, unless indicated otherwise. Rats were weighed frequently before testing to habituate them to handling. Rats were also given at least two mock trials before testing, in which the injection needle or gavage tube was inserted with no injection to adapt them to the test procedures.

**Food Intake Tests**

On 2 separate days starting between 1200 and 1230, rats fed the LF/HC or HF/LC diet (n = 10, each condition) were each given an intraperitoneal injection of either 2,5-AM (200 mg/kg in distilled water) or an equivalent volume of vehicle (3 ml/kg).

**Tissue Collection and Preparation**

At least 3 days after completion of the 2,5-AM-intake tests, rats were injected as above with either 2,5-AM (n = 5 each for LF/HC and HF/LC diets) or saline (n = 5 each for LF/HC and HF/LC diets), and killed 2 h later for analysis of brain Fos-li. At least 1 wk after the MP intake tests, rats were gavaged as above with either MP (n = 5 each for LF/HC and HF/LC diets) or vehicle (n = 5 each for LF/HC and HF/LC diets) and killed at 4 h. Animals were treated identically for analysis of brain Fos-li as they were in the behavioral tests, except that water bottles and food cups were removed after treatment to eliminate nonspecific activation of Fos-li by drinking and eating behaviors.
Rats were deeply anesthetized by injection of 1 ml of 65 mg/ml ip pentobarbital sodium. The thoracic cavity was opened, and both the descending aorta and posterior vena cava were clamped to assure a thorough fixation of the brain. Animals were given 0.3 ml heparin (1,000 IU/ml) intracardially and then perfused transcardially with 300 ml of 0.2 M PBS (pH 7.4) followed by 250 ml of 2% acrolein-4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). It has been shown that acrolein-paraformaldehyde mixtures are superior for preservation of Fos immunoreactivity compared with paraformaldehyde alone (10). Rats were perfused with an additional 150 ml of PBS to remove acrolein from the tissue. Brains were removed and blocked into forebrain and medulla, and pieces were placed in 10% sucrose-PBS followed by 20 and 30% sucrose-PBS, each for 24 h. After cryoprotection in sucrose, brains were quickly frozen on dry ice and cut at 30 µm on a cryostat.

Sections were collected from three locations based on previous work (11, 12, 22, 23): from caudal hindbrain (approximately −14.5 to −12.5 mm bregma) (17), rostral hindbrain (approximately −10 to −9 mm bregma), and forebrain (−0.2 to −3.6 mm bregma). Sections were placed serially into six culture plate wells containing Watson’s cryoprotectant (33) and stored at −20°C for 1–2 wk followed by immunohistochemical processing. Length of time sections were stored in cryoprotectant made no detectable

Fig. 3. Photomicrographs showing Fos-like immunoreactivity in the NTSm/ AP, NTSt, and PBN of rats fed an HF/LC or LF/HC diet and injected intraperitoneally with saline or 200 mg/kg 2,5-AM. cl, Central lateral; el, external lateral. Calibration bar = 100 µm.
difference in the level of Fos-li. Pilot work showed that brain sections processed several days after sectioning were essentially the same as those processed several months later in terms of the level of Fos-li.

Immunohistochemistry

Brain sections were initially rinsed in PBS to remove cryoprotectant. A sequence of incubation steps was done in 1% sodium borohydride in PBS (20 min), 0.3% hydrogen peroxide in PBS (30 min), and 5% normal goat serum (NGS) in PBS containing 0.2% Triton X-100 (PBS-TX), with rinses between each step. Sections were then incubated at room temperature with gentle agitation in 1:40,000 polyclonal anti-Fos (Santa Cruz Biotechnology; Santa Cruz, CA; lot 1283) containing 1% NGS in PBS-TX for 24 h. After rinses in PBS-TX, sections were placed in 1:400 biotinylated rabbit anti-Fos (Santa Cruz Biotechnology; Santa Cruz, CA; lot 1283) containing 1% NGS in PBS-TX for 2 h. After rinses in PBS-TX, sections were placed in 1:400 biotinylated rabbit anti-rat (Elite kit, Vector Laboratories) for 3 h at room temperature. This was followed by rinses in PBS and a 3-h incubation in avidin-biotin complex solution (4.5 µl of avidin and biotin/ml PBS; Elite kit, Vector Laboratories). Sections were then rinsed twice in PBS and twice in 175 mM acetate-10 mM imidazole buffer (A-I buffer, pH 7.4). Sections were placed in 3,3′-diaminobenzidine (DAB; 5 mg/ml in A-I buffer) with nickel sulfate (25 mg/ml) for 2–3 min for the chromogen reaction. Finally, sections were rinsed twice in A-I buffer and twice in PBS. Tissue sections were then mounted on gelatin-coated slides and placed under a coverslip with DPX mountant (Fluka). The specificity of the Fos antiserum was tested by preabsorption with Fos protein (Santa Cruz; lot G294), which completely blocked all staining.

Quantification of Fos-li

Fos-li staining was determined by the presence of a blue-black reaction product in cell nuclei. Tissue sections were viewed with a Nikon Microphot-FXA microscope, and the number of cells expressing Fos-li were quantified using a computer imaging system (MetaMorph 2.0; Universal Imaging, Westchester, PA). The number of cells expressing Fos-li was quantified at ×10 magnification. A threshold for Fos-li staining was set relative to background gray levels for each image analyzed. Average pixel areas were computed for stained cells in a given brain region. This ideal pixel area was used to adjust cell counts of overlapping cells in selected regions of interest. This procedure was routinely validated by comparison with cell counts obtained manually. Such comparisons showed a close agreement between manual and automated methods for cell counting.

On the basis of previous examination of brain sections from rats treated with 2,5-AM or MP (11, 12), cell counts were made in areas that consistently showed Fos-li. To standardize the analysis of Fos-li, cells from each area were counted in coronal sections of brain from each animal at approximately the same level relative to bregma according to Paxinos and Watson (17). The areas analyzed and level relative to bregma used (in parentheses) were the nucleus of the solitary tract (NTSm; −13.8 mm); area postrema (AP; −13.8 mm); NTS rostral (NTSr; −13.3 mm); parabrachial nucleus, external lateral subnucleus (PBNel; −9.2 mm); paraventricular nucleus of the hypothalamus, parvocellular (PVNm; −1.8 mm); PVN magnocellular (PVNm; −1.8 mm); paraventricular nucleus of the thalamus (PVA; −1.8 mm); supraoptic nucleus (SON; −1.4 mm); and subfornical organ (SFO; −1.0 mm). Cell counts were obtained from one section for each brain area, and, because Fos-li was not lateralized in any of the bilateral structures examined, cell counts reflect the totals for both sides in these areas.

Data Analysis

Food intake and cell counts were analyzed by ANOVA. Comparisons between pairs of means were made using one-tailed t-tests (a significant increase in food intake or in the number of cells expressing Fos-li compared with baseline/control conditions was always predicted). For all analyses, a level of P < 0.05 was used to indicate statistical significance.

RESULTS

Effects of 2,5-AM

Feeding behavior. Rats fed the LF/HC diet increased food intake after injection of 2,5-AM, compared with injection of saline, whereas rats fed the HF/LC diet did not [F(1,18) = 8.85, P < 0.05, injection × diet interaction effect at each time point; Fig. 1]. Twenty-four-hour food intakes were lower after 2,5-AM (27.0 ± 1.1 g) compared with saline (31.3 ± 1.0 g) only when rats were maintained on an LF/HC diet [t(9) = 3.75, P < 0.05]. Food intake returned to normal by 2 days after treatment.

Immunohistochemistry. Cell counts from and photomicrographs of hindbrain nuclei are shown in Figs. 2
and 3. Compared with saline injection, 2,5-AM treatment increased Fos-li in the NTSm, NTSr, and AP only in rats fed the LF/HC diet \([F(1,16) = 6.43, P < 0.05, \text{for \text{injection} \times \text{diet interaction}}]\). In the NTS, increased Fos-li was observed mainly in the dorsomedial and medial subnuclei. Compared with saline treatment, 2,5-AM injection increased Fos-li in the PBNel in rats fed either diet \([t(8) = 2.67, P < 0.05]\), but had a greater effect in those fed the LF/HC diet \([F(1,16) = 7.81, P < 0.05, \text{injection} \times \text{diet interaction}}]\). PBNcl Fos-li was not affected by 2,5–AM treatment.

Results of the analysis of forebrain nuclei are shown in Figs. 4–6. Compared with saline injection, administration of 2,5-AM increased the number of cells expressing Fos-li in the PVNp, PVNm, SON, CeA, and SFO more in rats fed the LF/HC diet than in rats fed the HF/LC diet \([F(1,16) = 4.59, P < 0.05, \text{for \text{injection} \times \text{diet interaction}}]\). Rats fed the HF/LC diet also showed greater Fos-li in the CeA after injection of 2,5-AM compared with saline \([t(8) = 2.56, P < 0.05]\). Injection of 2,5-AM increased Fos-li in the BNSTdl similarly in both diet groups \([F(1,16) = 17.31, P < 0.05, \text{for main}]\).

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**Fig. 5.** Photomicrographs showing Fos-like immunoreactivity in the PVN (parvocellular, p; magnocellular, m), SON, and PVA of rats fed an LF/HC or HF/LC diet and injected intraperitoneally with saline or 200 mg/kg 2,5-AM. p, Parvocellular; m, Magnocellular. Calibration bar = 100 µm.
Effect of injection; t(8) = 2.31, P < 0.05 for 2,5-AM vs. saline in both diet conditions. Although Fos-positive cell counts in the PVA were greater in the 2,5-AM-injected animals than in saline-injected animals fed the LF/HC diet [t(8) = 3.93, P < 0.05], there were no overall statistically significant effects of injection or diet.

Effects of MP

Feeding behavior. Administration of MP, compared with vehicle, increased food intake in rats fed the HF/LC, but not the LF/HC, diet over the 8-h test period [F(7,126) = 3.14, P < 0.05, time × treatment × diet interaction; Fig. 7]. This effect was apparent at each time point starting 3 h after MP administration [F(1,18) = 4.61, P < 0.05, for diet × treatment interaction]. Twenty-four hour food intakes were not affected by MP treatment.

Immunohistochemistry. Cell counts of and photomicrographs from hindbrain nuclei are shown in Figs. 8 and 9. Compared with vehicle treatment, MP treatment increased Fos-li in the NTSm only in rats fed the HF/LC diet [F(1,16) = 4.84, P < 0.05, for diet × treatment interaction]. There were very strong trends for Fos-li to be greater in the NTSr and AP after MP, as opposed to vehicle, treatment only in rats fed the HF/LC diet [F(1,16) = 3.83 and 4.13, P = 0.07 and 0.06, respectively, for diet × treatment interaction; t(8) = 2.13, P < 0.05, for MP vs. vehicle for HF/LC diet condition]. MP treatment increased NTS Fos-li mainly in the dorsomedial and medial subnuclei. MP administration increased Fos-li in the PBNel from levels seen...
after vehicle treatment in rats fed either diet, but Fos-li increased more in rats fed the HF/LC diet than in those fed the LF/HC food \(F(1,16) = 13.08, P < 0.05\), for diet \(\times\) treatment interaction; \(t(8) = 3.47, P < 0.05\), for MP vs. vehicle for both diet conditions\]. MP treatment did not affect PBNcl Fos-li.

Results of the analysis of forebrain nuclei are shown in Figs. 10–12. Compared with vehicle treatment, MP treatment increased Fos-li in the CeA in animals fed either diet, but increased Fos-li more in the CeA in rats fed the HF/LC diet than in rats fed the LF/HC diet \(F(1,16) = 6.02, P < 0.05\), for diet \(\times\) treatment interaction; \(t(8) = 2.65, P < 0.05\), for MP vs. vehicle for both diet conditions\]. There were strong trends for MP treatment to increase Fos-li in the PVNp, PVA, and BNSTdl more than vehicle treatment in rats fed HF/LC diet, but not in those fed the LF/HC diet \(F(1,16) = 2.57, 3.77, \) and 3.98, \(P = 0.13, 0.07, \) and 0.06, respectively, for diet \(\times\) treatment interaction; \(t(8) = 2.47, P < 0.05\), for PVA and BNSTdl for MP vs. vehicle in rats fed
the HF/LC diet; for PVNp, \( P = 0.07 \). Only in the BNSTdl did MP treatment of rats fed the LF/HC diet increase Fos-li compared with vehicle treatment \([t(8) = 4.84, P < 0.05]\). Rats fed the HF/LC diet had more Fos-positive cells in the PVNm than those fed the LF/HC diet \([F(1,16) = 6.29, P < 0.05\), for main effect of diet]; however, MP treatment had no effect on Fos-li in these subnuclei. There was a strong trend for an effect of MP treatment on Fos-li in the SON \([F(1,16) = 4.08, P = 0.06\), for main effect of drug treatment, there was also no other significant effect for this analysis]. There were no overall effects of MP and/or diet on Fos-li in the SFO; however, rats fed the LF/HC diet showed more Fos-positive cells in this area after MP as opposed to vehicle treatment \([t(8) = 2.05, P < 0.05]\).

**DISCUSSION**

The results provide strong evidence for a link between increased neuronal activity in certain brain nuclei and the initiation of eating behavior triggered by changes in peripheral fuel metabolism. The effects of two metabolic inhibitors, 2,5-AM and MP, on brain Fos-li and food intake were studied in rats maintained on one of two diets varying in the proportion of fat and carbohydrate. Administration of either 2,5-AM or MP increased Fos-li in specific brain areas and stimulated eating behavior, but did so under different dietary conditions. That is, injection of 2,5-AM elicited both neural and behavioral responses under the dietary condition in which MP treatment was largely ineffective, whereas administration of MP produced similar effects, but under the dietary condition in which 2,5-AM treatment was with little or no effect. Thus the induction of Fos-li, particularly in certain brain nuclei, tracked the eating response across experimental conditions independently of the diet that was fed or the metabolic inhibitor that was administered to rats. Because Fos-li was measured under conditions in which rats were not allowed to eat, these findings suggest that the increase in neural activity in certain brain areas produced by 2,5-AM and MP treatment underlies the eating responses to administration of these metabolic inhibitors.

Rats were maintained on one of two diets before and during testing. One diet was high in carbohydrate and low in fat content (LF/HC) whereas the other was high in fat and low in carbohydrate (HF/LC). Previous work (18) has shown that injection of 2,5-AM stimulates eating in rats maintained on the LF/HC, but not HF/LC, diet; and the present experiments confirmed this result. Results from several different experiments have indicated that the proportion of fat and carbohydrate in the maintenance diet also affects the eating response to MP (6, 7), although in an opposite manner to that seen with 2,5-AM. The results found in the present study confirm this by showing for the first time in a direct comparison that MP treatment increased food intake of rats fed the HF/LC diet, but had no effect on food consumption of rats maintained on the LF/HC diet.

Previous studies have shown that administration of 2,5-AM and MP increases Fos-li in specific brain nuclei of rats fed, respectively, the LF/HC and HF/LC diets (11, 12). The work shown here largely confirms these findings and extends them by showing that the induction of Fos-li, similar to the eating response, depends in large part on the composition of the maintenance diet. Injection of 2,5-AM produced a clear cut increase in Fos-li in rats fed the LF/HC, but not HF/LC, diet in several brain areas, including the NTS, AP, PVN, SON, and PVA. In keeping with its effect on food intake, MP treatment produced a similar pattern of Fos-li in rats fed the HF/LC, but not LF/HC, diet. In contrast to the present experiments, we found no change in Fos-li in the PVA of rats given MP in our earlier work (12), and the source of this discrepancy is unknown. However, in agreement with our previous findings, we observed a clear cut trend for increased Fos-li only in the parvocellular division of the PVN in rats given MP and fed the HF/LC diet. Rats treated with 2,5-AM and fed the HF/LC diet had increased numbers of cells showing Fos-li in both the parvo- and magnocellular divisions. These results raise the possibility that activation of neurons in the parvocellular portion of the PVN is especially crucial for triggering the eating response to metabolic inhibition. The most profound effects of the diet manipulation on 2,5-AM or MP-induced Fos-li occurred in the NTS, AP, and PVN.

Although 2,5-AM and MP at the doses used produced a comparable increase in food intake (~2 g, subtracting the control intakes), there was consistently less induc-
tion of Fos-li by MP treatment (HF/LC diet condition) than by 2,5-AM treatment (LF/HC diet condition). The NTSm, NTSr, PBNel, PVA, CeA, and BNSTdl areas showed about the same amount or slightly less Fos expression after MP treatment compared with 2,5-AM treatment under diet conditions in which responses to metabolic inhibition were observed. The AP and PVNp showed ~50% less Fos expression after MP treatment (HF/LC diet) than 2,5-AM treatment (LF/HC diet). However, the PVNm, SON, and SFO showed the most remarkable differences of ~90% less Fos expression after MP treatment (HF/LC diet) compared with 2,5-AM treatment (LF/HC diet). It is likely that only a subset of cells showing Fos-li in any given nucleus after treatment with 2,5-AM or MP is involved in the stimulation of food intake by these inhibitors. This may account for the similarity in food intake despite differences in the expression of Fos-li produced by 2,5-AM and MP treatment. It is also possible the difference in the induction of Fos-li can be traced in part to differences in the nature of the metabolic perturbations produced by the two inhibitors; for example, MP treatment may be less
and given by mouth vehicle or 10 mg/kg MP. Values are means ± SE. 

Fig. 12. Number of cells positive for Fos-like immunoreactivity in the CeA (A), BNSTdl (B), and SFO (C) of rats fed an HF/LC or LF/HC diet and given by mouth vehicle or 10 mg/kg MP. Values are means ± SE. *P < 0.05 vs. saline.

effective than 2,5-AM because its metabolic effects develop much more slowly.

The two inhibitors induced Fos-li in brain areas that are part of a neural system that receives and processes vagal sensory information (3, 15). Vagal afferents from the visceral organs innervate the NTS (for review, see Ref. 2), which in turn sends projections to all of the sites that showed enhanced Fos-li in the present study (e.g., Ref. 21). The pattern of Fos expression seen here in rats treated with the metabolic inhibitors is thus consistent with an increase in vagal sensory input from visceral organs (see Ref. 4). Earlier studies showing that subdiaphragmatic vagotomy prevents the increase in brain Fos-li and eating behavior after injection of 2,5-AM or treatment with MA, another inhibitor of fatty acid oxidation (22, 23), suggested that changes in peripheral metabolism that influence feeding behavior are communicated via vagal afferents. The correspondence between neuronal activation in certain brain nuclei and eating behavior found in neurologically intact rats in the present experiments provides additional support for this hypothesis.

Some of the neuronal activation observed after 2,5-AM or MP treatment was apparently not associated with the eating responses to metabolic inhibition, because increased numbers of Fos-positive cells in some nuclei were found after 2,5-AM or MP treatment under dietary conditions in which feeding behavior was unaffected. Such an increase in Fos-li was observed in the PBNel, CeA, and BNSTdl, where Fos-li increased after treatment with the inhibitors regardless of which diet rats were fed. The neuronal response was somewhat consistent with the behavioral response; that is, the number of cells showing Fos-li in these areas was greater after 2,5-AM injection in rats fed the LF/HC diet, whereas, after MP treatment, the response was larger in rats fed the HF/LC diet. However, because the eating response to inhibitor treatment was all-or-none, some of the neuronal activation was clearly unrelated to the behavior. The CeA and BNST (1) have been implicated in the control of the autonomic nervous system (see Ref. 8). It is possible that the neuronal activation observed in these two nuclei may be involved in an autonomic response to metabolic inhibition. Furthermore, because both the CeA and BNST project directly to the PBN (32), it is conceivable that some of the cells expressing Fos in this hindbrain structure after inhibitor treatment were activated by descending inputs from these forebrain nuclei rather than by ascending fibers from the NTS/AP.

Both 2,5-AM and MP treatment induced Fos-li in the SFO in rats fed the LF/HC diet, a response that was also unrelated to the effects of these inhibitors on food intake. Increased Fos-li in the SFO has been observed previously in rats injected with a higher dose of 2,5-AM (400 mg/kg; Ref. 11), although the basis for this response is unknown. The SFO is associated with angiotensin II-induced drinking behavior (27), and angiotensin II as well as hypertonic saline are known to induce Fos-li in SFO neurons (9, 16). It is unlikely that 2,5-AM or MP treatment caused the induction of Fos-li in the SFO by inducing an osmotic load, because the effect was seen only in rats fed the LF/HC diet. In addition, injection of fructose equal to or twice the osmolality of the dose of 2,5-AM used in the present study does not induce Fos-li in the SFO (11). However, recent data show that 2,5-AM injection increases plasma renin activity (Ji, Tordoff, and Friedman, unpublished data), which may in turn increase angiotensin II production.

Ritter et al. (23) observed increased brain Fos-li and food intake after 2,5-AM treatment in rats maintained on a high-fat diet. These results seem at odds with the present findings showing clear-cut effects of diet on Fos expression and food intake (see also Ref. 18); one would expect little if any effect of 2,5-AM in rats fed a high-fat diet. Unlike the HF/LC diet used in the present experiments, however, the high-fat diet used by Ritter et al. (23) contained substantial amounts of carbohydrate (42% of energy) as well as fat (39% of energy), which may have left animals sensitive to the effects of 2,5-AM. It is also possible that the discrepancy is based on differences in 2,5-AM dose, because larger doses of 2,5-AM were used in the earlier studies (300 and 500 mg/kg for immunohistochemical experiments and 500 mg/kg for behavioral experiments). It is also conceivable that the use of a high-fat diet in the Ritter et al. experiments may have depressed the neuronal response to 2,5-AM because little or no Fos-li was found in forebrain structures. In this regard, the use of an
acrolein-paraformaldehyde fixative in the present studies may have preserved Fos antigenicity better (10) than the paraformaldehyde fixative used in the earlier experiments (23).

The diets used in the present experiments are thought to influence the eating responses to 2,5-AM and MP by their effects on peripheral metabolism, that is, by affecting the metabolic stimulus that initiates feeding behavior (6, 7, 18, 28). It is possible that the effects of diet on neuronal activation were also mediated by changes in the metabolic actions or effects of the inhibitors. Alternatively, changes in the dietary macronutrient composition may have directly altered neuronal function in the NTS or other brain sites, thereby affecting the neuronal activation and, in turn, eating responses to the metabolic inhibitors. It is unclear to what extent the eating responses to 2,5-AM and MP reflect feeding behavior under normal conditions or whether eating behavior normally depends on the activation of neurons that are activated by treatment with these inhibitors. The present findings do not speak directly to these issues. However, the observation that differences in diet composition can determine whether or not brain Fos expression is induced by metabolic inhibition suggests that relatively benign changes in metabolism due to diet can alterafferent input to or responsibility of neural mechanisms that control eating behavior.

The authors thank Alemayehu Addis for technical assistance and Drs. James C. Mitchell and Mark L. Weiss for invaluable discussions. The present work was supported by grants from the National Institutes of Health (DK-36339 and DC-00014) and the Howard Heinz Endowment. Address for reprint requests: C. C. Horn, Center for Neurobiology and Behavior, Columbia Univ., 722 W. 168th St., Research Annex Box 25, New York, NY 10032.

Received 22 January 1998; accepted in final form 8 April 1998.

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