Neural substrate for an integrated metabolic control of feeding behavior

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Horn, Charles C., Aleymayehu Addis, and Mark I. Friedman. Neural substrate for an integrated metabolic control of feeding behavior. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R113–R119, 1999.—Evidence indicates that feeding behavior in rats is controlled by a mechanism that integrates information about different aspects of fuel metabolism. We investigated the neural substrate for this integrated control by measuring the effect of metabolic inhibitors given alone and in combination on food intake and neuronal activity as reflected by the expression of c-Fos protein. Combined administration of methyl palmitoxirate (5 mg/kg po), an inhibitor of fatty acid oxidation, and 2,5-anhydro-o-mannitol (150 mg/kg ip), which decreases liver ATP content, increased feeding in rats more than expected on the basis of eating responses after treatment with either inhibitor given alone. Combined treatment also produced a synergistic increase in Fos-like immunoreactivity in several brain areas, including the nucleus of the solitary tract, area postrema, and parvocellular portion of the hypothalamic paraventricular nucleus. These findings provide strong evidence for the involvement of selected brain regions in the metabolic control of food intake and suggest that metabolic information used to control feeding behavior is integrated in the periphery or at the level of the brain stem.

c-fos; methyl palmitoxirate; 2,5-anhydro-o-mannitol; food intake; metabolism

SUBSTANTIAL EVIDENCE indicates that the intracellular utilization of metabolic fuels generates signals that are used by the central nervous system to control feeding behavior (7, 25, 26, 28). Although the stimuli have not been specified, several metabolic processes have been implicated in the control of food intake on the basis of experiments showing that administration of metabolic inhibitors that act on different metabolic pathways stimulate eating in rats. These studies suggest that an acute decrease in glucose utilization, fatty acid oxidation, or hepatic ATP content is sufficient to trigger feeding behavior (6, 19, 28).

The brain is thought to integrate information about different aspects of fuel metabolism to control food intake and maintain energy homeostasis (e.g., Ref. 5). Direct evidence for such an integrative function stems from studies showing that simultaneous inhibition of different metabolic pathways can produce a synergistic increase in food intake. Thus combined treatment with 2-deoxy-D-glucose (2-DG), an inhibitor of glucose utilization, and methyl palmitoxirate (MP), which suppresses fatty acid oxidation, in doses that alone have no effect stimulates food intake (6, 7). Similarly, administration of MP combined with injection of 2,5-anhydro-o-mannitol (2,5-AM), which reduces liver ATP, also produces a synergistic increase in food intake (20), as does coadministration of 2,5-AM and mercaptoacetate (MA), another inhibitor of fatty acid oxidation.

The mechanisms that integrate information about different metabolic processes and translate them into changes in feeding behavior are not well understood. Studies using expression of c-Fos protein as a marker for neuronal activity have identified brain regions that may play a role in the eating response to administration of 2-DG, MP, 2,5-AM, and MA (8, 10, 22, 23). These different inhibitors, each with a different mode of action, induce Fos-like immunoreactivity (Fos-li) in many of the same brain areas, including the nucleus of the solitary tract (NTS), area postrema (AP), lateral parabrachial nucleus (PBN), and central lateral nucleus of the amygdala (CeA). It is possible, therefore, that neurons located in these regions of the central nervous system are involved in integrating different metabolic signals that control feeding. In the experiments described here, we addressed this possibility by examining food intake and brain Fos-li after separate and combined treatment with MP and 2,5-AM to determine whether a synergistic induction of Fos-li parallels the synergistic eating response to combined metabolic inhibitor treatment.

METHODS

Subjects and environment. Adult male CD Sprague-Dawley rats (Charles River, Kingston, NY) weighing 300–500 g at the time of testing were used for all experiments. Rats were housed individually in a temperature-controlled (22°C) vivarium that was maintained on a 12:12-h light-dark cycle (lights on at 0700). Rats were fed a medium fat–medium carbohydrate diet in pelleted form, providing 40% of energy as fat, 40% as carbohydrate, and 20% as protein (ICN Biochemicals; see Ref. 18). All animals were given food and tap water ad libitum for at least 2 wk before testing. Rats were weighed frequently before testing to habituate them to handling. Before the testing sessions, rats were given at least two mock trials in which a gavage tube and a hypodermic needle were inserted with no injection to adapt them to the test procedures.

Experiment 1: Food intake. Different groups of rats (n = 8) were given either vehicle + saline (control), MP + saline (MP alone), vehicle + 2,5-AM (2,5-AM alone), or MP + 2,5-AM (combined treatment). MP (5 mg/kg) was suspended in a 0.05% methyl cellulose vehicle, and both MP and vehicle were administered (po) in a volume of 3 ml/kg at 0900. MP suspensions were sonicated before gavage. Saline (0.9% NaCl) or 2,5-AM (150 mg/kg) were given (intraperitoneally) in volumes of 2 ml/kg 3 h after administration of MP or vehicle.
(i.e., at 1200). Food intakes were measured to the nearest 0.1 g (corrected for spillage). Because there were no differences in food intake during the 3 h between vehicle or MP treatment and saline or 2,5-AM injection, only food intakes after injection of saline or 2,5-AM are reported.

Experiment 2: Brain Fos expression. An additional 32 rats were divided into four treatment groups as in the previous experiment (n = 8). Food cups and water bottles were removed after gavage of vehicle or MP to eliminate the nonspecific activation of Fos by eating and drinking behaviors (2–4). On the basis of pilot work showing that a 2-h survival time was optimal for expression of Fos-li, rats were anesthetized and perfused as described below 2 h after injection of saline or 2,5-AM.

Experiment 3: Role of plasma glucose. Eating behavior (26) and brain Fos-li (14, 16) are increased during insulin-induced hypoglycemia. Because combined MP and 2,5-AM treatment decreases plasma glucose (20), control experiments were performed to determine whether a reduction in plasma glucose can account for the behavioral and neuronal responses seen after combined treatment.

The first control experiment determined the effect of combined treatment on blood glucose and food intake. n = 16 rats were given either control or combined treatment at 0900 on 2 different days 3 days apart. Food cups and water bottles were removed from 0900 to 1400, blood (100 ml) was collected from the tip of the tail at 1200, 1300, and 1400, and plasma was assayed for glucose (glucose oxidase method, Sigma kit no. 510; Sigma, St. Louis, MO). After the last blood sample was collected, food and water were returned and food intakes were measured 1 and 2 h later.

In a second control experiment, 12 additional rats were treated identically to those in the first control experiment, except that they were injected with either saline or 1 U/kg insulin (1 ml/kg sc regular Iletin I; Eli Lilly, Indianapolis, IN) on the 2 test days. At least 6 days after the second (last) food intake test, food and water were removed from the rats’ cages at 0900 and rats were re-injected with 1 U/kg insulin (n = 6) or saline (n = 6) at 1200 and killed at 1400 for analysis of brain Fos-li.

Tissue collection. Brain tissue was collected, processed, and analyzed for Fos-li as described previously (8). Rats were deeply anesthetized with 1 ml of 65 mg/ml pentobarbital sodium (intraperitoneally) and perfused transcardially with 300 ml of 0.2 M phosphate buffered saline (PBS; pH 7.4) followed by 250 ml of 2% acrolein-4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed, divided into forebrain and brain stem, and 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. On the basis of previous observations (8–10) of brain sections from rats treated with MP and 2,5-AM, 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 middle NTS (NTSm; −13.8 mm), AP (−13.8 mm), rostral NTS (NTSr; −13.3 mm), external lateral subnucleus of the PBN (PBNel; −9.2 mm), central lateral subnucleus of the PBN (PBNcl; −9.2 mm), CeA (−3.0 mm), dorsal lateral bed nucleus of the stria terminalis (BNSTdl; −0.3 mm), parvocellular division of the paraventricular nucleus of the hypothalamus, (PVNp; −1.8 mm), magnocellular division of the PVN (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. Although there were small differences between animals in the level of sections used for analysis in each brain area, the sections selected were distributed similarly in the different treatment groups.

Data analysis. Food intake and cell count data were analyzed by ANOVA. Planned comparisons were conducted using least significant difference tests (a significant increase/decrease in food intake, number of cells expressing Fos-li, or blood glucose compared with control treatment was always predicted). Insulin-induced Fos expression was analyzed by using t-tests. For all analyses, a level of P < 0.05 was used as the criterion for statistical significance.

RESULTS

Experiment 1: Feeding behavior. Combined treatment with MP and 2,5-AM produced a synergistic increase in food intake [F(2,28) = 5.43, P < 0.05, for interaction at 2 h; Fig. 1]. Rats given combined treatment increased food intake more in the first 2 h after 2,5-AM injection than did those given control treatment or either inhibitor alone (P < 0.05). Administration of either MP or 2,5-AM alone produced a small but statistically significant increase in food intake at 2 h relative to control treatment (P < 0.05). There were no significant effects of metabolic inhibitor treatments on
food intake at 1 h after intraperitoneal injection compared with control treatment ($P > 0.05$). Although food intakes were greater at 3 and 4 h in rats given MP + 2,5-AM than those given vehicle + saline or either inhibitor alone ($P < 0.05$), they were not synergistically increased ($P = 0.07$ and $P = 0.45$ for interaction, respectively).

Experiment 2: Brain fos-li. Combined treatment produced a clear synergistic increase in fos-li in several areas of the hindbrain. Only administration of MP and 2,5-AM together increased fos-li in the NTSr and AP ($F(2,28) = 5.32, P < 0.05$, for interaction; Figs. 2 and 3). Combined treatment increased fos-li relative to that seen after control treatment or after administration of either inhibitor alone ($P < 0.05$). Neither MP nor 2,5-AM treatment alone affected fos-li in these areas compared with control treatment ($P > 0.05$). Combined treatment increased fos-li mainly in the dorsomedial and medial subnuclei of the NTS. Although ANOVA indicated that fos-li in the NTSm was not increased synergistically after combined treatment ($F(2,28) = 2.38, P = 0.13$, for interaction), there was a strong trend in that regard (Fig. 2); only rats given both inhibitors had significantly more fos-li in the NTSm than did those given control treatment ($P < 0.05$). Injection of 2,5-AM increased fos-li in the PBNel ($F(2,28) = 31.6, P < 0.05$, for main effect of 2,5-AM), and both 2,5-AM alone and combined treatment increased fos-li in this nucleus compared with control treatment ($P < 0.05$). Furthermore, injection of MP also increased fos-li in the PBNel ($F(2,28) = 12.43, P < 0.05$, for main effect of MP). There were no significant changes in fos-li in the PBNcl after metabolic inhibitor treatments relative to control treatment ($P > 0.05$).

In the forebrain, combined treatment produced a synergistic increase in fos-li in the PVNp ($F(2,28) = 5.35, P < 0.05$, for interaction; Figs. 4 and 5). Adminis-
tration of MP plus 2,5-AM increased cell counts in the PVNp more than did the other three treatments ($P < 0.05$), which had similar effects on Fos-li in this nuclear region ($P > 0.05$). There was some tendency for Fos-li in the PVNm to be higher after combined treatment; however, this effect was not statistically reliable ($F(2,28) = 1.78, P = 0.19$, for interaction; Fig. 4). ANOVA showed no statistically significant interactive effect of MP and 2,5-AM on Fos-li in the other forebrain structures examined. However, more cells showed Fos-li in the PVA, SON, and SFO (Figs. 4–6) after combined treatment compared with the control treatment or either inhibitor treatment alone ($P < 0.05$), which produced similar effects on Fos-li in these brain areas. Administration of 2,5-AM increased cell counts in the CeA whether or not animals also received MP ($F(2,28) = 16.7, P < 0.05$, for main effect of 2,5-AM; Fig. 6). Administration of the inhibitors either separately or together increased the number of cells expressing Fos-li in the BNSTdl compared with the control treatment ($F(2,28) = 13.9, P < 0.05$, for main effects; $P < 0.05$ for all comparisons to control condition).

Experiment 3: Role of blood glucose. As shown in Fig. 7A, administration of both MP and 2,5-AM decreased blood glucose by 30%. Treatment with MP, which was given 3 h before 2,5-AM, decreased blood glucose slightly as seen at the 0-h time point ($P < 0.05$); blood glucose decreased further 1 and 2 h after injection of 2,5-AM ($P < 0.05$). When rats were allowed to eat after blood sampling, they ate more 3 and 4 h after combined treatment than after control treatment ($P < 0.05$).

Also shown in Fig. 7B, plasma glucose decreased ($P < 0.05$) after injection of insulin in a manner similar to that seen after combined inhibitor treatment (decrease of 33% at 1 h). However, insulin injection neither stimulated food intake (Fig. 7B; $P > 0.05$) nor induced Fos-li in the brain (Fig. 8), with the exception of the lateral division of the CeA ($P < 0.05$).

**DISCUSSION**

In keeping with results from previous experiments (20), the present findings show that combined treatment with the metabolic inhibitors MP and 2,5-AM produces an increase in food intake that is greater than that expected from administration of either inhibitor alone. The present experiments extend this observation to show that this effect of combined inhibitor treatment on food intake is paralleled by increased neuronal activity in specific brain nuclei in brain stem and forebrain. This result suggests that MP and 2,5-AM elicit food intake via activation of a common neural

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**Fig. 4.** Number of cells positive for Fos-like immunoreactivity in the parvocellular division of the paraventricular nucleus of the hypothalamus (PVNp), magnocellular PVN (PVNm), supraoptic nucleus (SON), and paraventricular nucleus of the thalamus (PVA) of rats given vehicle or MP (5 mg/kg po) and, 3 h later, saline or 2,5-AM (150 mg/kg ip). Values are means ± SE. *$P < 0.05$ vs. saline.

**Fig. 5.** Fos-like immunoreactivity in the PVN, SON, and PVA of rats given vehicle or MP (5 mg/kg po) and, 3 h later, saline or 2,5-AM (150 mg/kg ip). Note that labeling of PVNm in MP + 2,5-AM-treated animal was not typical of most sections in this condition. p, Parvocellular; m, magnocellular. Calibration bar equal to 100 µm.
mechanism that integrates information about different aspects of fuel metabolism to control food intake.

Fos-li was increased in a synergistic manner most clearly in the NTSr, AP, and PVNp after combined treatment with MP and 2,5-AM. Combined treatment also consistently produced marked increases in Fos-li in the NTSm, PBNel, SON, PVA, and SFO, whereas little or no effect was observed after administration with MP or 2,5-AM treatments alone. Vagal afferents from the viscera terminate in the NTS (e.g., Ref. 15), and the NTS sends projections either directly or through the PBN to all of the forebrain sites expressing Fos in the present study (e.g., Refs. 21, 24, and 31). The pattern of Fos expression observed after combined inhibitor treatment is therefore consistent with activation of vagal afferents.

The clear association between activation of NTS/AP and PVN neurons and the eating response to combined inhibitor treatment is particularly intriguing, because the NTS has direct neural projections to the PVN (1, 27) and the PVNp has been implicated in numerous experiments as a brain site involved in the control of feeding behavior (e.g., Ref. 13). Administration of MP or 2,5-AM alone in doses higher than those used in the present experiment also increases Fos-li in the PVNp (8, 10).

Recent experiments with chronic decerebrate rats (11) indicate that activation of neurons in the PVNp after a high dose of 2,5-AM is dependent on intact ascending pathways from the brain stem. This pathway from hindbrain to forebrain may play a role in stimulating feeding behavior after MP and 2,5-AM treatments.

Induction of Fos-li in the CeA and BNST did not show a clear relationship to eating responses after metabolic inhibitor treatment, suggesting that these structures do not play a major role in the effects of MP and 2,5-AM on food intake. This conclusion is consistent with recent.

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Fig. 6. Number of cells positive for Fos-like immunoreactivity in central lateral nucleus of the amygdala (CeA), dorsal lateral bed nucleus of the stria terminalis (BNSTdl), and subfornical organ (SFO) of rats given vehicle or MP (5 mg/kg po) and, 3 h later, saline or 2,5-AM (150 mg/kg ip). Values are means ± SE. *P < 0.05 vs. saline.

Fig. 7. Plasma glucose and cumulative food intake of rats treated (A) with MP (5 mg/kg po) + 2,5-AM (150 mg/kg ip), or vehicle (Veh) (po) + saline (ip) or injected (subcutaneously) (B) with saline or insulin (1 U/kg). No food was available from 0–3 h. Values are means ± SE.

Fig. 8. Number of cells positive for Fos-like immunoreactivity in brain nuclei after injection (subcutaneously) with saline or insulin (1 U/kg). Values are means ± SE. *P < 0.05 vs. saline.
The pattern of Fos expression after MP + 2,5-AM treatment was similar to that observed after injection of 2-DG (22), MA (22), or higher doses of 2,5-AM or MP alone (8, 10, 23), which also stimulate eating behavior in rats. Brain areas expressing Fos after administration of these metabolic inhibitors include the NTS, AP, PBN, and CeA. However, MA, unlike 2-DG, 2,5-AM, and MP, apparently does not induce Fos expression in the PVN (22). This discrepancy may reflect differences in sites and mechanisms of action, metabolic or physiological effects, or immunohistochemical procedures (see Refs. 8, 9, and 10). 2-DG, a glucose analog, is thought to increase food intake by inhibition of glucose utilization in the brain (26), whereas 2,5-AM, a fructose analog, apparently triggers eating behavior by decreasing ATP in liver (18). Co-administration of MP and either 2-DG or 2,5-AM increases food intake in a synergistic fashion, suggesting that a common mechanism underlies the eating response to these different inhibitors (6, 7, 20). The similarities in the pattern of Fos-li induced by 2-DG, MP, and 2,5-AM suggest that these treatments may activate some of the same neural pathways to eliciting feeding. Whether MA, which differs in its effect on PVN, also activates these pathways is not determined. It is also possible that the metabolic perturbations induced by these different agents are mildly stressful and that at least some of the induction of Fos-li results from this relatively nonspecific effect of the inhibitors. 2,5-AM and MP elicit feeding behavior by their action in peripheral tissues, and evidence indicates that these peripheral effects are communicated to the brain via vagal afferents (e.g., Ref. 29). Because neurons in the NTS receive direct innervation from first-order vagal afferents, the synergistic increase in Fos-li observed in the NTS after combined inhibitor treatment suggests that information about the metabolic consequences of 2,5-AM and MP treatment is integrated at the level of the brain stem or earlier in the periphery. Injection of 2,5-AM appears to elicit feeding behavior by decreasing liver ATP (18), whereas the eating response to administration of MP is associated with a decrease in fatty acid oxidation (6). It is possible that different vagal sensory neurons carry information about liver ATP content and fatty acid oxidation, and these afferents converge on NTS neurons, which then integrate these signals. Alternatively, integration may take place before afferent signals reach the NTS. In this scenario, integration could occur neurally with different metabolic stimuli stimulating different branches of single vagal afferents, which then transmit a relatively strong signal to NTS neurons. Integration could also occur metabolically, whereby 2,5-AM and MP generate a common metabolic stimulus (e.g., decreased ATP), albeit by different modes of action, that is detected by a set of vagal afferents that send a magnified signal to the NTS. Further research is required to determine the specific mechanism(s) by which information about the metabolic effects of 2,5-AM and MP is integrated to control food intake. The present results suggest, however, that this integration occurs at a relatively early step in the generation or processing of vagal afferent signals.

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