Metabolic inhibitors synergistically decrease hepatic energy status and increase food intake

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Food intake is controlled in part by the metabolism of energy-yielding substrates and involves a mechanism that integrates information about the metabolism of different fuels (1, 2, 4, 15). This integrative mechanism is thought to underlie various phenomena related to the control of food intake, including circadian rhythms of eating, coordination of short- and long-term controls, and the maintenance of energy intake (see Ref. 1). Perhaps the most direct evidence for an integrated metabolic control of food intake is the common triggering signal for eating behavior induced by lowering hepatic energy status. Rats treated with both inhibitors increased feeding behavior compared with the controls, whereas those treated with 2,5-AM or MP alone did not. Although 2,5-AM alone lowered hepatic ATP content regardless of MP treatment, only the combination resulted in decreases in hepatic ATP/ADP ratio and phosphorylation potential. MP treatment did not affect the uptake of 2,5-AM into liver. These results suggest that a reduction in hepatic energy status is the common triggering signal for eating behavior induced by 2,5-AM and MP and provide additional evidence for an integrated metabolic control of food intake.

Eating behavior; liver; metabolism; rats

Metabolic inhibitors synergistically decrease hepatic energy status and increase food intake. Am J Physiol Regulatory Integrative Comp Physiol 278: R1579–R1582, 2000.—Previous studies indicate that administration of the metabolic inhibitor, 2,5-anhydro-D-mannitol (2,5-AM) or methyl palmoxirate (MP), induces feeding behavior in rats by lowering hepatic energy status. Combined treatment with these agents synergistically increases food intake. The present study was designed to investigate whether combined treatment also has a synergistic effect on hepatic energy status. Rats treated with both inhibitors increased feeding behavior compared with the controls, whereas those treated with 2,5-AM or MP alone did not. Although 2,5-AM alone lowered hepatic ATP content regardless of MP treatment, only the combination resulted in decreases in hepatic ATP/ADP ratio and phosphorylation potential. MP treatment did not affect the uptake of 2,5-AM into liver. These results suggest that a reduction in hepatic energy status is the common triggering signal for eating behavior induced by 2,5-AM and MP and provide additional evidence for an integrated metabolic control of food intake.

It is not clear how information about the metabolism of different fuels is integrated. Integration could occur neurally with different sensors monitoring the processing of different fuels, or it could take place at a metabolic level whereby a metabolic event common to the metabolism of different fuels generates a signal. Recent studies suggest that a reduction in liver ATP content or a related event triggers the eating response to some metabolic inhibitors (3, 7, 13, 14). Because ATP production is a final common pathway for the metabolism of different fuels, it has been suggested that an aspect of this process could generate an integrated signal for feeding behavior (8). Despite differences in their mechanisms of action, the metabolic inhibitors, 2,5-anhydro-D-mannitol (2,5-AM) and methyl palmoxirate (MP), both stimulate feeding behavior in rats evidently by decreasing ATP availability in liver (3, 7, 13, 14). Co-administration of 2,5-AM and MP increases food intake in a synergistic fashion (4, 15). If these two inhibitors increase feeding by reducing hepatic energy status and this reduction constitutes a common, integrated stimulus for the behavioral response, then one would expect that combined treatment would also produce a synergistic decrease in liver energy status. The present experiment was designed to test this hypothesis.

Material and Methods

Animals. Male Sprague-Dawley rats (Crl:CDBR; Charles River Breeding Laboratories, Wilmington, MA), weighing 225–250 g upon arrival, were kept individually in hanging wire-bottom cages at 21°C under a 12:12-h lighting schedule with lights on at 0900. Rats were fed a custom-made diet (Dyets, Bethlehem, PA) containing (wt/wt) 20% casein, 31.2% corn starch, and 13.9% corn oil, yielding 38% of energy each from carbohydrate and fat (12). Water was provided ad libitum. Rats were allowed to acclimate to this diet and the laboratory environment for 2 wk prior to testing. To adapt them to the procedure used to administer one of the inhibitors (MP), rats underwent mock gavage (insertion of tube with injection of 1 ml of water) three times during the aclimation period.

Experiment 1. Twenty-eight rats were divided into four groups of seven animals each, matched for body weights. At 0900, food was removed from the rats’ cages. Two groups of rats were given by gavage 5 mg/kg MP suspended in 0.5% methyl cellulose (vehicle), whereas the other two groups were given by gavage an equivalent volume (3 ml/kg) of the methyl cellulose vehicle. Three hours later, one group of MP-treated and one group of vehicle-treated rats were injected intraperitoneally with 150 mg/kg 2,5-AM dissolved in water, whereas the remaining two groups were injected with an equivalent volume (3 ml/kg) of isotonic saline. Food was returned immediately after the injection, and food consumption was measured (to the nearest 0.1 g, corrected for spillage) during the following 1-h period. Each rat was then anesthetized by an intramuscular injection (0.4 ml) of ketamine HCl (100 mg/ml) plus acepromazine maleate (1 mg/ml). A piece of liver (2–3 g) was excised through a midline abdominal incision and immediately freeze-clamped using a pair of aluminum tongs.
prechilled in liquid nitrogen. The samples were stored at -80°C and analyzed for ATP, ADP, and inorganic phosphate within a week using procedures described elsewhere (7).

Experiment 2. Twelve rats were divided into two groups of six animals, matched for body weight. One group was given MP by gavage and the other was given vehicle, then 3 h later both groups received an intraperitoneal injection of 150 mg/kg of 2,5-AM containing [U-14C]2,5-AM (0.75 µCi/kg). Thirty minutes after the injection, rats were anesthetized, and a piece of liver (~1 g) was excised, rinsed with saline, blotted on a paper towel, weighed, and frozen. The rest of the liver was excised and weighed as well. The frozen liver sample was later homogenized in 4.5 ml of 6% HClO4. Radioactivity in 0.5 ml of the homogenate was counted and used to estimate the uptake of 2,5-AM into liver.

Reagents. [U-14C]2,5-AM was synthesized by converting [U-14C]glucosamine HCl to 2,5-anhydro-α-mannose (11), which was then reduced to [U-14C]2,5-AM (5). 2,5-AM was a gift from the Ajinomoto Company. All other reagents were purchased from Sigma (St. Louis, MO).

Data analysis. Statistical analyses of the data were performed using a two-way ANOVA to compare the effects of 2,5-AM and MP on food intake and liver energy status. Tukey test was used as the post hoc test to determine the difference between individual groups. A Student’s t-test was used to compare the effect of MP on the liver uptake of 2,5-AM. Results are presented as means ± SE. Phosphorylation potential was calculated as [ATP]/([ADP] × [Pi]).

RESULTS

Experiment 1. Combined treatment with MP and 2,5-AM increased food intake in a synergistic fashion [F(1,23) = 4.6, P = 0.04, for interaction] (Fig. 1). Thus, whereas rats given MP or 2,5-AM alone ate the same amount as those given control treatments (vehicle/saline), rats treated with both inhibitors ate significantly more than control-treated rats.

Coadministration of MP and 2,5-AM synergistically decreased two of three measures of hepatic energy status (Fig. 2). Liver ATP was reduced by 2,5-AM regardless of MP treatment [F(1,23) = 22.6, P < 0.001 for the main effect of 2,5-AM]; however, compared with control treatment, MP given alone had no effect on ATP content, and combined treatment did not affect ATP any differently than did 2,5-AM alone. Neither inhibitor given alone affected liver ATP/ADP ratio or phosphorylation potential compared with control treatment, whereas combined administration of the two inhibitors significantly reduced both parameters by 41 and 51%, respectively [F(1,23) = 10.2 and 5.7, P = 0.004 and 0.03, respectively, for interaction]. Coadministration of MP and 2,5-AM also had a synergistic effect on liver ADP content [F(1,23) = 13.4, P = 0.001 for the interaction]. The ADP content in the group treated with both agents (0.72 ± 0.04 µmol/g liver) was significantly higher than that of control-treated rats or those given MP or 2,5-AM alone (0.57 ± 0.04, 0.55 ± 0.04, and 0.46 ± 0.03 µmol/g liver, respectively). The liver contents of inorganic phosphate did not differ significantly among the groups of rats (2.57 ± 0.32, 2.50 ± 0.14, 2.42 ± 0.25, and 2.94 ± 0.16 µmol/g liver for the groups of control treatment, MP, 2,5-AM, and combined treatment, respectively).

Experiment 2. MP treatment had no effect on the hepatic uptake of 2,5-AM. Thirty minutes after the injection of [14C]2,5-AM, livers from rats given MP or vehicle had, respectively, 12.2 ± 0.7% and 13.4 ± 1.1% of the amount of radioactivity injected [t(10) = 0.99, P = 0.34].
DISCUSSION

A combined treatment with 2,5-AM and MP synergistically increased food intake and decreased liver energy status in rats. The effect on food intake observed in the present experiment confirmed previous results (4, 15) showing that these two inhibitors stimulate feeding when given together even at doses that are ineffective when given alone. The behavioral response to combined treatment was accompanied by a decrease in hepatic energy status as reflected in reductions in liver ATP/ADP ratio and phosphorylation potential. These results suggest that MP and 2,5-AM stimulate eating via the same mechanism of action, namely, by decreasing liver energy status, and that the synergistic increase in food intake seen after a combined treatment with these inhibitors is due to their combined effect of reducing liver energy status.

Both MP and 2,5-AM affect metabolic pathways in liver that are involved in the processing of metabolic fuels. For example, MP inhibits the oxidation of long-chain fatty acids (18), and 2,5-AM limits hepatic gluconeogenesis and glycogenolysis (16), and as a result of these actions both inhibitors produce a modest decrease in blood glucose concentration (15). However, these and other actions of the inhibitors at the substrate level of metabolism do not appear to account for their effects on food intake or for the synergistic eating response to the combined treatment (15). That 2,5-AM and MP both reduce liver energy status (3, 14) and, as shown here, can act synergistically to do so indicates that the effect of these inhibitors on food intake depends on their actions at the level of energy production, beyond those pathways for the metabolism of specific fuels.

Events at the substrate level of metabolism may affect food intake, however, by altering hepatic energy production. Indeed, the decrease in hepatic energy status produced by MP treatment appears to result from its combined effects on both glucose and fatty acid oxidation. Recent studies show that MP stimulates eating at doses that inhibit both liver and muscle fatty acid oxidation and, as a result of a compensatory increase in glucose utilization in muscle, depletes liver glycogen (3). The increased demand for glucose in muscle that is derived from liver glycogen may limit the supply of glucose to hepatocytes. It has been suggested that MP treatment stimulates eating because the limited availability of glucose derived from glycogen stores combined with a reduction in fatty acid oxidation curbs hepatic fuel oxidation, which in turn decreases hepatic energy status (3). An earlier study has shown that 2,5-AM partially prevents the depletion of liver glycogen caused by MP (15). Given that 2,5-AM inhibits glycogenolysis (16), the present findings showing that MP did not decrease liver energy status unless given in combination with 2,5-AM suggest that inaccessibility of glycogen, not its reduced level, contributes to a decline in liver energy status when the capacity to oxidize fatty acids is restricted.

The effect of 2,5-AM on hepatic energy status also appears to depend on fuel metabolism, specifically on fatty acid oxidation. Injection of 2,5-AM increases fat oxidation, as evidenced by the increases in plasma free fatty acid and ketone body levels and a decrease in whole body respiratory quotient (10). Inducing relatively high rates of fat oxidation by feeding rats a high-fat, low-carbohydrate diet attenuates the effect of 2,5-AM on food intake (15), suggesting that the increased fatty acid oxidation can offset the decrease in liver energy status. The present findings support this hypothesis by showing that administration of an inhibitor of fatty acid oxidation exacerbates the decrease in liver energy status produced by injection of 2,5-AM. Treatment with MP did not appear to amplify the effect of 2,5-AM on hepatic energy status simply by increasing its availability to liver cells, because MP treatment did not alter the uptake of 2,5-AM.

Injection of 2,5-AM reduced liver ATP content to the same extent regardless of whether it was combined with MP treatment, but it increased food intake only when it was given along with MP. In contrast, only the combined treatment with the two inhibitors caused significant reductions in ATP/ADP and phosphorylation potential and induced eating behavior. In previous experiments, the eating response to 2,5-AM was found to be associated with a reduction in liver ATP content, a finding that suggested that a decrease in hepatic ATP generates a signal which triggers eating behavior (7, 13, 14). The present findings argue against this hypothesis and suggest that other features of liver energy status might be more directly involved in the production of a hepatic signal for feeding. Because adenine nucleotide metabolism is a dynamic process, a change in ATP level, unlike the other two parameters, does not necessarily reflect the availability or turnover rate of ATP, which may be a more critical parameter. Recently, the eating response to MP treatment was found to be more closely tied to a decrease in hepatic phosphorylation potential than to a decline in liver ATP content (3). Like the present findings, this may simply reflect the fact that phosphorylation potential is a more sensitive index of the dynamic state of cellular energy status than is ATP content. Indeed, changes in ATP do not always track similar changes in phosphorylation potential (e.g., Ref. 9), which takes other components (ADP and Pi) of ATP metabolism into account. One might expect that a very large reduction in ATP content is more indicative of significant drop in ATP availability and would therefore more likely parallel a fall in phosphorylation potential. In earlier studies showing a relationship between liver ATP content and eating behavior, the decrease in ATP was larger than that reported here. It is possible that under such conditions ATP content more accurately reflected the availability of ATP.

Because the animals in the present study had access to food after the injection of the inhibitors, it may raise the concern that the differences in hepatic energy status among the groups were due to the differences in the amount of food consumed. However, both inhibitors
decrease hepatic energy status (ATP/ADP and phosphor-ylation potential) when given alone at higher doses, and food consumption restores hepatic energy status after a fast (6). Therefore, one would expect that the food intake induced by the combined treatment of MP and 2,5-AM would only lead to an underestimate of the reduction in hepatic energy status, if it had any effect at all.

The synergistic eating response to combined treat-ment with inhibitors that act on different metabolic pathways constitutes perhaps the most direct evidence that control of food intake involves mechanisms that integrate different metabolic events and translate them into a change in feeding behavior (1, 4, 8). Despite this and other evidence for an integrated metabolic control of feeding behavior, the mechanism of integration has not been elucidated. It is possible that changes in the availability or utilization of different substrates generate separate signals that are integrated by the nervous system, and this may be the case when different metabolic signals are detected in the brain and in peripheral tissues (17). Alternatively, the processing of different metabolic fuels may produce a single signal generated by a metabolic event that is common to the utilization of these different fuels. Recent studies show that the synergistic eating response to combined treat-ment with MP and 2,5-AM is associated with a synergis-tic increase in neuronal activity in the nucleus of the solitary tract (NTS) as measured using fos immunohistochemistry (4). Because the NTS is the first relay in the processing of vagal afferent signals, it was sug-gested that the effects of MP and 2,5-AM were inte-grated at this first relay or earlier in the periphery. The present findings showing a relationship between the behavioral and hepatic responses to the combined treatment raise the possibility that integration may occur in the periphery before a neural signal is generated, specifically through a decrease in energy produc-tion, the final common path for the use of different metabolic fuels.

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