A ROLE FOR HYPOTHALAMIC MALONYL-CoA IN THE CONTROL OF FOOD INTAKE
Zhiyuan Hu*, Yun Dai*, Marc Prentki#, Shigeru Chohnan and M. Daniel Lane*
From the *Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205; #Department of Bioresource Science, College of Agriculture, Ibaraki University, 3-21-1 Chu-ou, Ami, Ibaraki 300-0393, Japan; □Montreal Diabetes Research Center, 1560 Sherbrooke Est, Montreal PQ H2L4M1, Canada.
(ZH and YD contributed equally to this research)

SUMMARY
The cellular level of malonyl-CoA, an intermediate in fatty acid biosynthesis, depends on its rate of synthesis catalyzed by acetyl-CoA carboxylase relative to its rate of utilization and degradation catalyzed by fatty acid synthase and malonyl-CoA decarboxylase, respectively. Recent evidence suggests that hypothalamic malonyl-CoA functions in the regulation of feeding behavior by altering the expression of key orexigenic and anorexigenic neuropeptides. Here we report that AICAR, a 5'-AMP kinase activator, rapidly lowers malonyl-CoA both in GT1-7 hypothalamic neurons and in the hypothalami of mice. These effects correlate closely with the phosphorylation of acetyl-CoA carboxylase, an established target of AMP kinase. Intracerebroventricular (i.c.v.) administration of AICAR rapidly lowers hypothalamic [malonyl-CoA] and increases food intake. Expression of an adenoviral cytosolic malonyl-CoA decarboxylase vector (Ad-cMCD) in hypothalamic GT1-7 cells decreases malonyl-CoA. When delivered by bilateral stereotaxic injection into the ventral hypothalamus (encompassing the arcuate nucleus) of mice, Ad-MCD increases food intake and body weight. Ad-MCD delivered into the ventral hypothalamus also reverses the rapid suppression of food intake caused by i.c.v.-administered C75, a fatty acid synthase inhibitor that increases hypothalamic [malonyl-CoA]. Taken together these findings implicate malonyl-CoA in the hypothalamic regulation of feeding behavior.

INTRODUCTION
A unique hypothalamic mechanism appears to link fatty acid synthesis to the expression of the key neuropeptides that regulate feeding behavior. The steady-state level of malonyl-CoA, an intermediate in fatty acid synthetic pathway, is thought to have a regulatory role in this system (1). The cellular level of malonyl-CoA is determined by its rate of formation catalyzed by acetyl-CoA carboxylase (ACC), relative to its rate of utilization and degradation catalyzed by fatty acid synthase (FAS) and malonyl-CoA decarboxylase (MCD), respectively. Administration of C75, a potent inhibitor of FAS (2), suppresses food intake causing profound weight loss in both obese and lean mice (3,4). With obese mice this weight loss is due primarily to a reduction of body fat (5). These effects are independent of leptin, since C75 causes weight loss in both leptin-deficient (ob/ob) or leptin receptor-deficient (db/db) mice (3). C75 appears to exert its anorectic effect by disrupting the signaling system that regulates expression of the hypothalamic neuropeptides that control feeding behavior. Thus, C75 blocks the fasting-induced up-regulation of orexigenic (AgRP and NPY) and down-regulation of anorexigenic (CART and POMC) neuropeptides in the hypothalamus (3,6). These and other findings suggested (7) that the accumulation of malonyl-CoA, a substrate of FAS and MCD, may mediate the changes in the expression of these neuropeptides and therefore, food intake. Consistent with this hypothesis the administration of C75 leads to an increase of hypothalamic malonyl-CoA (7).

Recent studies suggest that the changes in hypothalamic malonyl-CoA may depend upon the activity of hypothalamic AMP kinase (8,9). It has long been recognized that AMP kinase in peripheral tissues (e.g. liver, adipose and muscle) acts as a "sensor" of cellular energy charge (10,11). Only recently, however, has evidence...
been obtained for the involvement of AMP kinase sensing in the hypothalamus (8,12,13) where global energy status is monitored and energy intake regulated (1). Conditions under which AMP kinase would be expected to be active and ACC inactive, e.g., in the fasted state, hypothalamic malonyl-CoA is extremely low (7). Consistent with this finding, the anorexigenic hormone leptin lowers AMP kinase activity in the hypothalamus (13). Likewise, the anorexigenic FAS inhibitor, C75, appears to reduce hypothalamic AMP kinase activity (8). These findings suggest that lowering hypothalamic AMP kinase activity is required to elicit the anorexigenic effects of leptin or C75. Lowering hypothalamic AMP kinase activity might be expected to activate ACC and elevate hypothalamic malonyl-CoA. While a direct linkage of the AMP kinase signaling system to changes in malonyl-CoA in the hypothalamus is suspected, it has not been demonstrated.

Moreover, the role of malonyl-CoA as a regulator of feeding behavior in studies with C75 remains controversial because the specificity of the FAS inhibitor has been questioned (14-16) raising the possibility of indirect effects. In the present paper, we describe two independent approaches that provide compelling evidence for the direct involvement of malonyl-CoA in the hypothalamic regulation of feeding behavior. It was found that overexpression of cytosolic MCD (cMCD) or treatment with a 5'-AMP kinase agonist, AICAR, decreases malonyl-CoA in hypothalamic GT1-7 neurons. We also show that intracerebroventricular (i.c.v.) administration of AICAR to mice activates the phosphorylation of ACC, lowers hypothalamic [malonyl-CoA] and increases food intake. Likewise, bilateral stereotaxic injection of adenoviral cMCD into the ventral hypothalamus increases food intake.

**EXPERIMENTAL PROCEDURES**

**Animals.** Male BALB/c mice (20-25g) from Charles River Laboratories were acclimated for 1 week to a 12-h light/12-h dark cycle at 22° C before experimentation. Mice were housed individually and fed standard laboratory chow (PROLAB RMH 1000) *ad libitum*. Animal studies were conducted in accordance with guidelines of the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee.

**Culture and treatment of hypothalamic GT1-7 cells, malonyl-CoA concentration and immunoblotting.** GT1-7 cells were cultured in DMEM containing 25 mM glucose and 10% fetal bovine serum under 90% air/10% CO₂. As the cells approached confluence they were treated or not with 2 mM AICAR for 4 h or were transduced with the appropriate Adenoviral vector (3.5 x 10⁹ pfu/6 cm dish). Malonyl-CoA was quantified using an ultra-sensitive substrate recycling assay (7). Immunoblotting was performed as described (17) with antibodies to MCD (18) obtained from Dr. Steven Gould (Johns Hopkins University School of Medicine) and to ACC2 and phospho (Ser79)-ACC2 (which also recognizes the AMPK phosphorylation sites in ACC1) from Cell Signaling, Beverly, MA.

**Construction of Adenoviral-CMV cytosolic malonyl-CoA decarboxylase (Ad-cMCD) expression vector.** The construction of a recombinant adenovirus allowing constitutive expression of MCD in the cytosol with the use of a rat MCD cDNA devoid of its mitochondrial and peroxisomal targeting sequences has been detailed before (19).

**Stereotaxic microinjection of adenoviral expression vectors.** Male BALB/c mice (20-25g) were placed in a stereotaxic frame (David Kopf Instruments) under ketamine/xylazine anesthesia (80/12 mg/kg body weight). The arcuate nucleus and dorsomedial and ventromedial hypothalamus were targeted bilaterally using a 30-G needle (Hamilton) connected to a Hamilton 5 µl syringe. The injection was directed to stereotaxic coordinates 1.5 mm posterior to bregma, ±0.5 mm lateral to midline and 5.8 mm below the surface of the skull. Adenovirus vectors, either Ad-lacZ (6.3 pfu/ml) or Ad-cMCD (7.8 pfu/ml), were delivered with a syringe injector pump (WPI, Sarasota, FL) at a rate of 100 nl/min for 2.5 min (250 nl/injection site) and the entire injector system was left in place for an additional 10 min after the injections were completed. After the procedure was complete, mice were placed in a heated cage until they recovered from anesthesia after which they were returned to their cages. Food intake and
body weight were measured daily for two weeks starting from day 3 following surgery.

**Intracerebroventricular (i.c.v.) injection of AICAR and C75.** BALB/c mice were either fed ad libitum or were fasted for 23 h, then anesthetized with isoflurane before i.c.v. injection of AICAR (6 µg) or C75 (10 µg) in 2 µl of RPMI 1640 medium, respectively. I.c.v. injections were delivered into the lateral ventricle with a calibrated 10 ml Hamilton syringe 1 h before the start of the dark cycle. After C75 administration, where indicated, mice were refed 30 min later. Two and a half hours after i.c.v. injection, cumulative food intake was measured and hypothalami were removed. Mice receiving C75 i.c.v. exhibited drastically-reduced (>90%) food intake.

**Immunohistochemical Analyses.** On day 17 after adenovirus injection, mice were placed under deep ketamine/xylazine anesthesia and perfused with 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde. Perfused brains were removed, placed in the same fixative for an additional 4 h at room temperature and then cryoprotected in 30% sucrose/0.1 M PBS overnight. Brains were snap-frozen on dry ice and stored at -80º C for further analysis. Coronal brain sections (16 mm) were washed in PBS and incubated in X-gal staining solution (1mg/ml X-Gal, 5 mM K₃Fe(CN)₆, 5mM K₄Fe(CN)₆) overnight to visualize β-galactosidase activity.

**RESULTS AND DISCUSSION**

**Effects of an AMP kinase activator and an adenoviral cMCD vector on hypothalamic GT1-7 neurons.** The phosphorylation of ACC1 and ACC2 by 5'-AMP kinase is known to decrease its catalytic activity (10,11). Preliminary to testing the effect of AICAR, a precursor of a potent AMP kinase activator, on hypothalamic malonyl-CoA and feeding behavior in vivo, AICAR was first tested on hypothalamic GT1-7 neurons in cell culture. As illustrated in Fig. 1A treatment of GT1-7 cells with AICAR rapidly activated the phosphorylation of ACC without altering expression of the enzyme. Closely correlated with phosphorylation, the cellular level of its carboxylation product, malonyl-CoA, decreased by ≥70% (Fig. 1B).

To assess the effect of overexpressing cytosolic cMCD on malonyl-CoA, hypothalamic GT1-7 neurons were transduced with an adenoviral cMCD expression vector (Ad-cMCD). Ad-cMCD harbors the mouse cDNA encoding a cMCD in which both the peroxisome and mitochondrial targeting signals have been deleted to promote expression in the cytoplasm (16). As verified by immunoblotting the expression vector markedly increased the cellular level of cMCD relative to that of endogenous MCD (Fig. 1C and inset). Moreover, overexpression of cMCD leads to a marked decrease in malonyl-CoA. This observation and that described above (Fig. 1A and B), validate two approaches by which malonyl-CoA can be lowered in hypothalamic neurons ex vivo, either by preventing its formation or by accelerating its removal.

**Effect of i.c.v. administration of the AMP kinase activator, AICAR.** To investigate the effect of activating AMP kinase in vivo, AICAR was administered to mice by i.c.v. injection. Following injection, food intake was measured during the next 2h at which time hypothalamic extracts were prepared and subjected to immunoblotting for ACC and phospho-ACC and analysis for malonyl-CoA content. As shown in Fig. 2A the phosphorylation of ACC was rapidly and significantly increased without affecting the level of ACC protein. This increase in phospho-ACC was accompanied by a decrease in [malonyl-CoA] and an increase of food intake (Fig. 2B and C, respectively).

**Effect of stereotaxic delivery of Ad-cMCD into the ventral hypothalamus.** To determine the effect of over-expressing cMCD in the ventral hypothalamus on food intake and weight gain, an Ad-cMCD expression vector was delivered directly into the ventral hypothalamus of mice by bilateral stereotaxic injection. To verify the delivery of the viral construct to the ventral hypothalamus, a control adenoviral β-galactosidase vector (Ad-LacZ) was tested using the same stereotaxic injection procedure. As illustrated in Fig. 3A, three days after bilateral stereotaxic injection β-galactosidase expression was detected in the ventral hypothalamus adjacent to and below the 3rd ventricle. Expression was particularly pronounced in the region...
encompassing the arcuate nucleus, a region that contains sets of neuron known to function in the regulation of feeding behavior (20). This staining pattern is typical of many other stereotaxic injection experiments, thus verifying the appropriate placement of the adenoviral expression vectors in the ventral hypothalamus.

Three days after delivery of the Ad-cMCD vector into the ventral hypothalamus the mice began to exhibit modest, but consistent, increases in food intake and body weight that continued over the next 12-days (Fig. 3B and C). Since the region into which the cMCD expression vector was delivered comprises only a small fraction of the entire hypothalamus, reliable quantification of the malonyl-CoA concentration within this limited region was not possible. Nevertheless, the same Ad-cMCD vector was shown to lower [malonyl-CoA] ex vivo when transduced into hypothalamic GT1-7 neurons (Fig. 1C).

In contrast to the modest effect on food intake of mice chronically exposed to hypothalamic cMCD (Fig. 3B and C), cMCD dramatically altered food intake in mice given C75 (Fig. 3D). Thus, while i.c.v. C75 ~completely suppressed food intake in control (Ad-LacZ) mice, Ad-cMCD completely reversed the C75-induced blockade of food intake (Fig. 3D). It should be noted that in previous studies (7) it was shown that i.c.v. C75 caused a 4-fold increase in hypothalamic malonyl-CoA. Since Ad-cMCD was stereotaxically delivered into the ventral hypothalamus, it is likely that the neurons targeted include those found in the arcuate nucleus, eg. NPY/AgRP and POMC/CART neurons, that function in the regulation of feeding behavior (20).

Why the introduction of Ad-cMCD into the ventral hypothalamus does not produce a greater effect on food intake is unclear. Conceivably, the chronic hypothalamic exposure to cMCD may prompt compensatory changes in the expression of regulatory factors in attempt to normalize food intake. Such compensatory effects occur in gene knockout models, eg. NPY- and AgRP-knockout mice do not exhibit the expected decreases in food intake (21,22). However, when placed in the context of leptin deficiency, as in NPY−/−-ob/ob mice, the expected phenotype of NPY deficiency occurs (23).

Taken together with previous findings (7,17) these results provide compelling evidence that malonyl-CoA plays a major role in the hypothalamic control of food intake. Thus, malonyl-CoA appears to be a key cellular indicator of fuel abundance that controls multiple functions related to body weight and fuel including insulin secretion (24) insulin action (25) and food intake (1).

**FOOTNOTES**

1 Under a licensing agreement between FASgen and the Johns Hopkins University, M. D. L. is entitled to a share of royalty received by the University on products embodying the technology described in this article. Terms of this arrangement are managed by the University in accordance with its conflict of interest policies.

**ACKNOWLEDGEMENTS**

This research was supported by Astellas Pharma Inc., Tsukuba, Japan and by a grant (to MP) from the Canadian Institute of Health Research.

**REFERENCES**

1. Dowell, P., Hu, Z., and Lane, M. D. (2005) Annu Rev Biochem 74, 515-534
2. Kuhajda, F. P., Pizer, E. S., Li, J. N., Mani, N. S., Frehywot, G. L., and Townsend, C. A. (2000) Proc Natl Acad Sci U S A 97, 3450-3454
3. Loftus, T. M., Jaworsky, D. E., Frehywot, G. L., Townsend, C. A., Ronnett, G. V., Lane, M. D., and Kuhajda, F. P. (2000) Science 288, 2379-2381
4. Cha, S. H., Hu, Z., and Lane, M. D. (2004) Biochem Biophys Res Commun 317, 301-308
5. Kumar, M. V., Shimokawa, T., Nagy, T. R., and Lane, M. D. (2002) Proc Natl Acad Sci U S A 99, 1921-1925
6. Shimokawa, T., Kumar, M. V., and Lane, M. D. (2002) Proc Natl Acad Sci U S A 99, 66-71
7. Hu, Z., Cha, S. H., Chohnan, S., and Lane, M. D. (2003) Proc Natl Acad Sci U S A 100, 12624-12629
8. Kim, E. K., Miller, I., Aja, S., Landree, L. E., Pinn, M., McFadden, J., Kuhajda, F. P., Moran, T. H., and Ronnett, G. V. (2004) J Biol Chem 279, 19970-19976
9. Landree, L. E., Hanlon, A. L., Strong, D. W., Rumbaugh, G., Miller, I. M., Thupari, J. N., Connolly, E. C., Huganir, R. L., Richardson, C., Witters, L. A., Kuhajda, F. P., and Ronnett, G. V. (2004) J Biol Chem 279, 3817-3827
10. Yeh, L. A., Lee, K. H., and Kim, K. H. (1980) J Biol Chem 255, 2308-2314
11. Hardie, D. G., and Carling, D. (1997) Eur J Biochem 246, 259-273
12. Minokoshi, Y., and Kahn, B. B. (2003) Biochem Soc Trans 31, 196-201
13. Minokoshi, Y., Alquier, T., Furukawa, N., Kim, Y. B., Lee, A., Xue, B., Mu, J., Foufelle, F., Ferre, P., Birnbaum, M. J., Stuck, B. J., and Kahn, B. B. (2004) Nature 428, 569-574
14. Lawrence, D. S., Zilfou, J. T., and Smith, C. D. (1999) J Med Chem 42, 4932-4941
15. Takahashi, K. A., Smart, J. L., Liu, H., and Cone, R. D. (2004) Endocrinology 145, 184-193
16. Cha, S. H., Hu, Z., Chohnan, S., and Lane, M. D. (2005) Proc Natl Acad Sci U S A, In press
17. Sacksteder, K. A., Morrell, J. C., Wanders, R. J. A., and Ruderman, N., Rhodes, C., Poitout, V., and Prentki, M. (2004) Diabetes 53, 1007-1019
18. Schwartz, M. W., Woods, S. C., and Porte Jr., D., Seeley, R. J., and Baskin, D. G. (2000) Nature 404, 661-671
19. Ruderman, N., and Prentki, M. (2004) Nat Rev Drug Discov 3, 340-351

FIGURE LEGENDS

Figure 1. Effect of AICAR or Ad-cMCD on ACC phosphorylation and malonyl-CoA levels in hypothalamic GT1-7 neurons. GT1-7 hypothalamic neurons were cultured as described in Experimental Procedures. A. Four hours after treating the cells with 2 mM AICAR, cell extracts were subjected to SDS/PAGE and immunoblotting with antibodies directed against ACC2 or the phosphorylation site at position Ser 79 in ACC2. B. Four hours after treating the cells with 2 mM AICAR, the cellular malonyl-CoA was determined. C. Two days after infecting the cells with an Ad-cMCD, cell extracts were subjected to immunoblotting (inset) with antibody directed against MCD and the cellular [malonyl-CoA] was determined. *, p < 0.001 vs. control or Ad-LacZ.

Figure 2. Effect of i.c.v. administration of AICAR on ACC phosphorylation and malonyl-CoA in the hypothalamus. AICAR was administered to BalbC mice by i.c.v. injection. After 2 h hypothalamic tissue was quickly (<60 s) removed and subjected to: A. immunoblotting with anti-phospho (Ser 79) ACC2 or ACC antibodies, and B. quantification of malonyl-CoA content. C. Food intake was measured for 2h after i.c.v. administration of AICAR. *, p < 0.05 or **, p < 0.01 vs. control.

Figure 3. Effect of stereotaxic injection of Ad-cMCD into the ventral hypothalamus on food intake and body weight of mice given i.c.v. C75. Ad-cMCD or Ad-LacZ expression vectors were administered
by bilateral stereotaxic injection into the ventral hypothalamus of BalbC mice. A. After 3 days the brain was sectioned and stained for β-galactosidase expression. Shown is the typical β-galactosidase staining pattern obtained in the ventral hypothalamus with prominent staining in the region of the arcuate nucleus. 3V refers to third ventricle. B. Food intake and C. body weight were measured during the next 12 days. D. Two groups of mice (4 mice) were given stereotaxic injections of the Ad-cMCD or Ad-LacZ expression vectors. Fourteen days later half of the mice in each group were given received i.e.v. injections of 10 mg of the FAS inhibitor, C75, after which food intake was measured during the next 2h. MCD and LacZ refer to mice that received stereotaxic injections (ventral hypothalamus) of the Ad-cMCD or Ad-LacZ expression vectors, respectively. (10 mice in LacZ group and 13 mice in Ad-cMCD group); In B and C: 10 mice in LacZ group and 13 mice in Ad-cMCD group. The differences between groups approached statistical significance at the p = 0.05 and in D: 5 mice /group; *, p < 0.001 vs. Ad-LacZ, MCD and MCD + C75.

Fig. 1

Fig. 2
Fig. 3

A) Arcuate nucleus

B) Cumulative food intake (grams)

C) WEIGHT GAIN, grams

D) Food intake (grams/2h)
A role for hypothalamic malonyl-CoA in the control of food intake
Zhiyuan Hu, Yun Dai, Marc Prentki, Shigeru Chohnan and M. Daniel Lane

J. Biol. Chem. published online October 11, 2005

Access the most updated version of this article at doi: 10.1074/jbc.C500398200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts