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MINIREVIEW

Hypothalamic Malonyl-Coenzyme A and the Control of Energy Balance

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An intermediate in the fatty acid biosynthetic pathway, malonyl-coenzyme A (CoA), has emerged as a major regulator of energy homeostasis not only in peripheral metabolic tissues but also in regions of the central nervous system that control satiety and energy expenditure. Fluctuations in hypothalamic malonyl-CoA lead to changes in food intake and peripheral energy expenditure in a manner consistent with an anorexigenic signaling intermediate. Hypothalamic malonyl-CoA is regulated by nutritional and endocrine cues including glucose and leptin, respectively. That malonyl-CoA is an essential component in the energy homeostatic signaling system of the hypothalamus is supported by convergence of physiological, pharmacological, and genetic evidence. This review will focus on evidence implicating malonyl-CoA as a central player in the control of body weight and adiposity as well as clues to the molecular mechanism by which carbon flux through the fatty acid biosynthetic pathway is linked to the neural control of energy balance. (*Molecular Endocrinology* 22: 2012–2020, 2008)

THE CENTRAL NERVOUS system (CNS) plays a vital role in regulating energy balance in higher animals. Diverse regions of the CNS, such as hypothalamic nuclei, the ventral tegmentum, nucleus accumbens, and the nucleus of the solitary tract among others, have been shown to monitor energy status and produce signals to adjust food intake and energy expenditure accordingly. First-order neurons of the ventral-medial hypothalamus that express orexigenic [neuropeptide Y (NPY) and agouti-related protein (AgRP)] and anorexigenic [proopiomelanocortin (POMC)/αMSH and cocaine- and amphetamine-regulated transcript (CART)] neuropeptides have been the most heavily studied and have clearly been shown to play a role in responding to and mediating these signals (1, 2). Expression of these neuropeptides fluctuates with alterations in both physiological and nutritional states including changes in the levels of circulating nutrients (*e.g.* glucose) and hormones (*e.g.* leptin and insulin) that reflect current nutritional-physiological states. Hence, food deprivation and the associated decrease in blood glucose leads to increased expression of NPY and AgRP and reduced expression of POMC/αMSH and CART. Conversely, the increased delivery of blood glucose to the CNS after a carbohydrate meal has the inverse effects, provoking decreased NPY and AgRP and increased POMC/αMSH and CART expression, which produces satiety in the short term. Leptin, a hormone expressed and secreted by adipocytes, also inversely affects the expression of these neuropeptides. Thus, as adipose tissue mass increases during energy surplus, blood leptin increases and interacts with its receptors in the CNS leading to lower NPY and AgRP expression and higher POMC/αMSH and CART production (3). Likewise, increased blood insulin after a carbohydrate meal interacts with its hypothalamic receptors giving rise to a similar pattern of hypothalamic neuropeptide expression (4). These signals are transmitted from the hypothalamus via second-order neurons to higher brain centers where feeding behavior is modulated (2, 5) and via sympathetic projections to skeletal muscle where energy expenditure is altered (6, 7), the net effect being a decrease in food intake and an increase in energy expenditure. Involvement of the sympathetic neural transmission of the malonyl-CoA signal from the CNS/hypothalamus to skeletal muscle is indicated by inhibition of fatty acid oxidation and up-regulation of UCP3 in muscle by α-blockers, *e.g.* phentolamine (6, 7).

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Abbreviations: ACC, Acetyl-CoA carboxylase; AgRP, agouti-related protein; AMPK, AMP kinase; Arc, arcuate nucleus; CART, cocaine- and amphetamine-regulated transcript; CNS, central nervous system; CoA, coenzyme A; CPT1b, carnitine palmitoyltransferase 1b; 2-DG, 2-deoxyglucose; FAS, fatty acid synthase; MCD, malonyl-CoA decarboxylase; NPY, neuropeptide Y; POMC, proopiomelanocortin; PPARα, peroxisome proliferator-activated receptor-α; PVN, paraventricular nucleus; VMN, ventromedial nucleus; WT, wild type.

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Although the molecules that link energy status to the expression of orexigenic and anorexigenic neuropeptides have long been sought, only relatively recently have intermediates in the signaling pathways been identified (8–12). A key intermediate in this pathway, malonyl-coenzyme A (CoA), was serendipitously identified through the use of inhibitors of fatty acid synthase (FAS) (13). It was shown that inhibitors of FAS, notably cerulenin and C75 [C75 refers to 3-carboxy-4-octyl-2-methylenebutyrolactone and cerulenin refers to (2S, 3R) 2,3-epoxy-4-oxo-7E, 10E-dodecadienamide], acting in the CNS suppress food intake and increase energy expenditure, leading to profound weight loss and decreased adiposity (13). This class of inhibitors targets the domain in FAS that catalyzes the condensation of malonyl-CoA with the elongating fatty acyl chain (14). Blocking the FAS reaction would be expected to cause the build-up of its substrate, malonyl-CoA. That these inhibitors do in fact increase the level of malonyl-CoA in the hypothalamus is now well documented (8, 15). Compelling evidence indicates that malonyl-CoA functions as a key intermediate in the hypothalamic energy status-sensing system that regulates feeding behavior and peripheral energy expenditure. The evidence supporting this assertion is the subject of this review.

INHIBITION OF FAS IN THE CNS LOWERS FOOD INTAKE AND INCREASES HYPOTHALAMIC MALONYL-CoA

Food intake by lean and obese (*ob/ob, db/db*, and dietary-induced obese) mice is rapidly (within 2 h) suppressed after the ip or intracerebroventricular administration of FAS inhibitors (8, 13, 16–20). A variety of structurally related synthetic (21) and naturally occurring FAS inhibitors, *e.g.* C75 and cerulenin, have been investigated, C75 being among the most effective and extensively investigated. Of particular interest, the response of lean and obese (*ob/ob*, leptin-deficient, or dietary-induced obese) mice to repetitive administration of FAS inhibitors differs markedly (16). C75 reduces food intake and adiposity of both wild-type (WT)/lean and obese mice during the first few days of treatment. After several days of repeated injections, however, WT mice become refractory to the further treatment with FAS inhibitors, exhibiting no further reduction of food intake or body weight. In contrast, obese mice continue to lose body weight and adiposity with repeated injections of the inhibitor, becoming resistant only after extensive loss of body fat as the mice approach the body weight of lean controls. Repetitive treatment of obese mice over a 4-wk period profoundly reduces adiposity (20) (Fig. 1).

The possibility was considered that substrate (malonyl-CoA) build-up provoked by FAS inhibitors might mediate their effect on feeding behavior. Thus, it became important to quantify the malonyl-CoA content of the hypothalamus. Because of the minute size of the mouse hypothalamus, 10 mg, and its low (submicromolar) malonyl-CoA content, a recycling assay method was developed that increased sensitivity by approximately 1000-fold (8). With this method, it was determined that the central administration of the FAS inhibitor to fasted mice rapidly (~2 h) increased the hypothalamic malonyl-CoA concentration by 4- to 5-fold to 0.8–1.0 μM and blocked food intake when a carbohydrate meal was offered (8). Moreover, the central administration of an acetyl-CoA carboxylase (ACC) inhibitor [5-(tetradecyloxy)-2-furoic acid] to block malonyl-CoA formation just before administration of the FAS inhibitor prevented the C75-induced increase in hypothalamic malonyl-CoA level and restored food intake (8, 13).

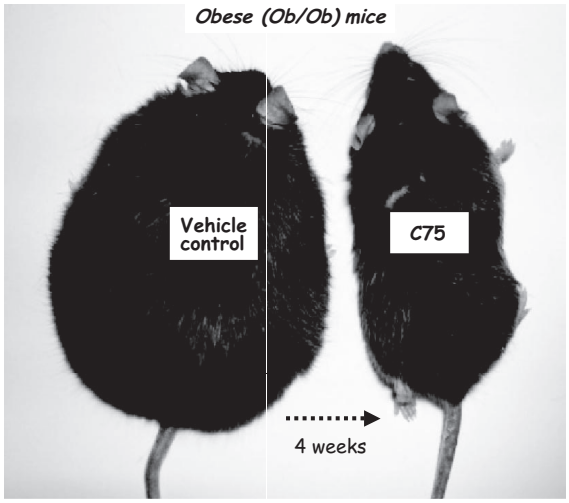

Fig. 1. Reduction in Adiposity by Treatment of Obese Mice with a FAS Inhibitor

Obese (*Ob/Ob*) mice were given an ip injection of C75 or vehicle once every 3 d for 4 wk.

The patterns of expression of the orexigenic and anorexigenic neuropeptides were found to be consistent with the effect of the FAS inhibitors on feeding behavior (8, 17). Thus, the FAS inhibitors rapidly (~2 h) decrease expression of the orexigenic neuropeptides, NPY and AgRP, and increase expression of the anorexigenic neuropeptides, POMC and CART, in the hypothalamus. Together these early findings supported the hypothesis that malonyl-CoA serves as a hypothalamic mediator that regulates food intake.

GENETIC DISRUPTION OF FAS IN THE HYPOTHALAMUS INCREASES MALONYL-CoA AND REDUCES BODY WEIGHT AND ADIPOSITY

Semenkovich's laboratory has crossed floxed FAS mice (22) with RIP-Cre mice, Cre expression being driven by the rat insulin promoter (23). In addition to

driving Cre expression in pancreatic β-cells, this promoter also activates Cre-mediated gene excision in the hypothalamus. Although these mice do not exhibit altered insulin expression/secretion by pancreatic β-cells or metabolic parameters characteristic of insulin deficiency, they do exhibit a hypothalamic FAS knockout phenotype, *i.e.* decreased food intake, body weight gain, and adiposity (23). Consistent with this phenotype, disruption of FAS gene expression in the hypothalamus induced a marked increase in hypothalamic malonyl-CoA concentration and altered neuropeptide expression. This finding markedly strengthens the hypothesis that hypothalamic malonyl-CoA functions in the signaling pathway that regulates feeding behavior and energy expenditure and, thus, body weight and adiposity.

FOOD DEPRIVATION LOWERS AND FEEDING INCREASES HYPOTHALAMIC MALONYL-CoA

Hypothalamic malonyl-CoA levels correlate closely with nutritional state. Thus, when energy expenditure exceeds intake, as in the food-deprived state, the level of malonyl-CoA concentration in the hypothalamus falls to approximately 0.1–0.2 μM. When food intake resumes, malonyl-CoA levels rise by 4- to 5-fold, reaching 0.8–1.0 μM in the re-fed state. This increase in malonyl-CoA level is rapid, the response to refeeding occurring within 2 h (8, 24).

These effects also correlate closely with the expression of the hypothalamic neuropeptides that regulate feeding behavior. In the fasted state, the orexigenic neuropeptides, NPY and AgRP, are expressed at high levels and the anorexigenic neuropeptides, POMC and CART, are expressed at low levels (8, 17). Upon refeeding, the neuropeptide expression pattern rapidly reverses.

The changes in hypothalamic malonyl-CoA concentration are consistent with the state of activation of the orexigenic NPY/AgRP neurons and anorexigenic POMC/CART neurons as indicated by the expression of c-Fos, a documented indicator of neuronal activity (18). Hence, FAS inhibitors prevent the normal activation of hypothalamic neurons that express orexigenic and anorexigenic neuropeptides. After a 24-h period of food deprivation, neuronal activity, as indicated by c-Fos expression, is high in the arcuate, ventromedial, and paraventricular nuclei (Arc, VMN, and PVN), regions in the hypothalamus that regulate feeding behavior. This reflects a state of hunger and is associated with the up-regulation of NPY and AgRP expression and the down-regulation of POMC/αMSH and CART expression. Upon resuming feeding, however, c-Fos expression is rapidly and dramatically suppressed, leading to down-regulation of NPY and AgRP and up-regulation of POMC/αMSH and CART (17, 18).

Consistent with the inactivation of hypothalamic neurons after the central administration of the FAS inhibitor C75, the fasting-induced increase in c-Fos expression in the Arc and PVN is also blocked (18). This inhibitor-induced suppression of neuronal activation (*i.e.* prevention of c-Fos expression) occurs in parallel with the blockade of the reciprocal expression of the hypothalamic neuropeptides that suppress food intake, *i.e.* a reduced expression of NPY and AgRP and increased expression of POMC/αMSH and CART as measured by immunochemical staining (18). Taken together with recent results by Gao *et al.* (25), these findings provide compelling evidence that the level of malonyl-CoA in specific hypothalamic nuclei (Arc, VMN, and PVN) responds to nutritional, hormonal, and pharmacological stimuli that alter feeding behavior.

INHIBITION OF FAS IN THE CNS ACTIVATES ENERGY EXPENDITURE IN SKELETAL MUSCLE

Weight loss caused by agents that alter the hypothalamic malonyl-CoA level such as FAS inhibitors and leptin is not due entirely to the suppression of food intake but also to increased energy expenditure. The connection between the CNS and peripheral tissues has remained enigmatic; however, the sympathetic nervous system (SNS) is clearly involved. Even *Caenorhabditis elegans* exhibit neural regulation over peripheral metabolism (26). Thus, pair-fed (*i.e.* isocaloric food intake) lean and obese mice lose less body weight than inhibitor-treated mice, indicating that, in addition to suppressing food intake, FAS inhibitors increase energy expenditure (16). Definitive proof that energy expenditure is increased was obtained by indirect calorimetry (27) and by the finding that the inhibitors cause increased fatty acid oxidation both *in vivo* and *in vitro* with muscle from mice treated centrally with FAS inhibitor (6, 7). Because these effects are triggered by the central administration of inhibitor, it is evident that the signal is transmitted to skeletal muscle from the CNS, presumably from the hypothalamus.

Closely correlated with the increase of fatty acid oxidation in muscle is the rapid (~2 h) phosphorylation/inactivation of ACC2 and, thereby, reduction of malonyl-CoA level in skeletal muscle. Lowering muscle malonyl-CoA, a potent allosteric inhibitor of muscle carnitine palmitoyltransferase 1b (CPT1b), releases CPT1b from inhibitory constraint to facilitate the entry of fatty acids into mitochondria for β-oxidation (6). Closely correlated with these events are rapid increases in the expression of skeletal muscle peroxisome proliferator-activated receptor-α (PPARα), a transcriptional activator of fatty acid oxidizing enzymes, and uncoupling protein 3 (UCP3), a putative thermogenic mitochondrial uncoupling protein (6, 20).

Consistent with signal transmission from the hypothalamus via the SNS, centrally administered C75 rapidly (~2 h) up-regulates the expression (in skeletal muscle) of the β-adrenergic signaling mole-

cules, *i.e.* norepinephrine, β3-adrenergic receptor, and cAMP; the transcriptional regulators PPARγ coactivator-1α and estrogen-related receptor α; and key oxidative mitochondrial enzymes including pyruvate dehydrogenase kinase, medium-chain length fatty acyl-CoA dehydrogenase, ubiquinone: cytochrome-C reductase, and cytochrome oxidase as well as ATP synthase and UCP3 (6).

Also consistent with these rapid changes in mitochondrial enzyme levels is a somewhat slower increase in mitochondrial biogenesis. Thus, daily centrally administered FAS inhibitor over a 3-d period leads to an increase in the number of mitochondria in white and red (soleus) skeletal muscle (6). Furthermore, both α- and β-adrenergic blockers prevent these effects. Thus, the SNS is implicated in the rapid transmission of the malonyl-CoA signal from brain to skeletal muscle. In line with the up-regulation of UCP3 and PPARα is the concomitant increase in the expression of PGC1α, a transcriptional coactivator of the UCP3 and PPARα genes.

Hence, the malonyl-CoA signal initiated by a centrally administered FAS inhibitor or by physiological changes, such as fasting and feeding (above) is rapidly communicated from the hypothalamus to skeletal muscle where fatty acid oxidation and the expression of mitochondrial oxidative genes relevant to this process are up-regulated (Fig. 2).

FORCED EXPRESSION OF MALONYL-CoA DECARBOXYLASE (MCD) IN THE VENTRAL HYPOTHALAMUS INCREASES FOOD INTAKE AND ADIPOSITY

Independently, two laboratories have employed viral expression vectors to lower the level of malonyl-CoA in the ventral hypothalamus (28, 29). Either an adenoviral (Ad-cMCD) or adenoviral-associated (AAV-cMCD) expression vector encoding cytosolic MCD were introduced into ventral hypothalamus by bilateral stereotaxic injection. Delivery into this site was verified by introducing control β-galactosidase-expressing viral vectors into the ventral hypothalamus by bilateral stereotaxic injection. This region of the hypothalamus encompasses the Arc and VMN, both of which function in the control of feeding behavior. The effectiveness of Ad-cMCD vector was initially tested *in vitro* with hypothalamic neuronal cell lines and shown to lower malonyl-CoA concentration by approximately 80% (28).

Expression of these viral cMCD vectors in the ventral hypothalamus induced increases in both food intake and body weight (28, 29) and in longer-term experiments a marked increase in adiposity (29). Furthermore, introduction of the Ad-cMCD vector into the ventral hypothalamus dramatically increased food intake in mice given the FAS inhibitor C75 by

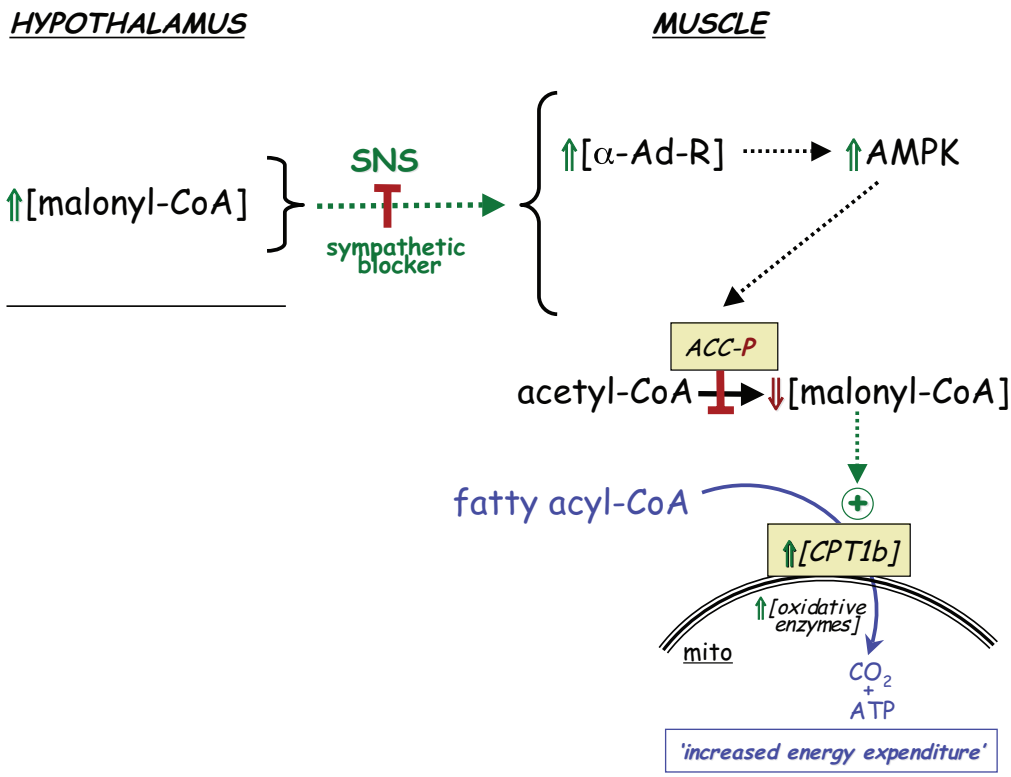

Fig. 2. Model: Role of Hypothalamic Malonyl-CoA in the Control of Energy Expenditure in Muscle
α-Ad-R, α-Adrenergic receptor; mito, mitochondrion.

central administration (28). Thus, intracerebroventricular C75, which increases hypothalamic malonyl-CoA, completely suppressed food intake in control (Ad-LacZ) mice, whereas Ad-cMCD completely reversed the C75-induced blockade of food intake. That fact that the anorexic effect of the FAS inhibitor was completely abrogated lends further support to the concept that hypothalamic malonyl-CoA serves as an indicator of energy status and participates in the regulation of feeding behavior. Also, it suggests that malonyl-CoA, and not fatty acids, is the key to energy homeostasis. Inhibition of FAS leads to an increase in malonyl-CoA and a decrease in *de novo* synthesis of fatty acids, which leads to a lean phenotype. Addition of MCD leads to a reduction in both malonyl-CoA and *de novo* fatty acid synthesis but leads to obesity. These two lines of evidence exclude a role of fatty acids or fatty acyl-CoAs and support a role for malonyl-CoA as the effector.

THE CENTRAL METABOLISM OF GLUCOSE AND LEPTIN ALTERS HYPOTHALAMIC MALONYL-CoA

Although malonyl-CoA has been identified as a putative intermediate in the hypothalamic signaling pathway that controls feeding behavior and energy expenditure, the upstream molecular events that regulate its formation are less well understood. Recent evidence (10, 24, 25, 30) suggests that the changes in hypothalamic malonyl-CoA during feeding and fasting cycles result from changes in the phosphorylation state and activity of ACC mediated by AMP kinase (AMPK). Both ACC1 and ACC2 are found in hypothalamic neurons and known to be substrate target for phosphorylation (thus, inhibition) catalyzed by AMPK. Several lines of evidence indicate that hypothalamic ACC, and thereby the formation of malonyl-CoA, is regulated by AMPK. 1) Conditions that activate AMPK in neurons *ex vivo* (28) and in the hypothalamus *in vivo* (24) lead to the phosphorylation/inactivation of ACC (24, 25). 2) Leptin, an anorexigenic hormone produced by adipocytes, suppresses AMPK activity in the hypothalamus (10), notably the Arc (25). This action derepresses ACC and thereby increases malonyl-CoA (24, 25). 3) The central administration of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), an AMPK activator, lowers hypothalamic malonyl-CoA and promotes food intake (28, 31). AICAR also activates the phosphorylation/ inhibition of ACC and lowers malonyl-CoA concentration in a hypothalamic cell line in culture (28). These findings have led to the notion that during fasting the [AMP]/[ATP] ratio increases in neurons in critical hypothalamic nuclei leading to the activation of AMPK, inactivation of ACC, and a decrease in [malonyl-CoA].

Recent studies (24) have linked the response of these intermediates in the malonyl-CoA pathway to metabolic and endocrine cues, most notably those provoked by glucose and leptin. Thus, hypothalamic malonyl-CoA concentration rises linearly with the carbohydrate content of the diet consumed after food deprivation (24). Peak hypothalamic malonyl-CoA concentration is achieved about 1 h after refeeding or after ip glucose administration. This response is proportional to the dose of glucose administered and is blocked by the central administration of 2-deoxyglucose (2-DG), a classic inhibitor of glucose metabolism, thus indicating that the malonyl-CoA response is dependent on CNS/hypothalamic glucose metabolism. Furthermore, the kinetics of change in hypothalamic malonyl-CoA level after glucose administration coincides with the suppression of phosphorylation of AMPK and ACC. Moreover, the inhibition of glucose metabolism by central 2-DG administration disrupts the effect of glucose on the activation of AMPK, the increase in malonyl-CoA level and reciprocal changes in orexigenic and anorexigenic neuropeptide expression in the hypothalamus, and the suppression of food intake.

Of interest and consistent with the known effect of leptin on AMPK phosphorylation, the central administration of leptin increases hypothalamic malonyl-CoA (24, 25). Moreover, this increase is additive with the effect of glucose administration. However, leptin-deficient *ob/ob* mice do not exhibit defects in the glucose- or refeeding-induced rise in hypothalamic malonyl-CoA after food deprivation. This finding indicates that leptin is not required for the glucose effect (24). Taken together, these observations show that hypothalamic malonyl-CoA responds to the level of circulating glucose and leptin, both of which affect energy homeostasis.

BRAIN-SPECIFIC CPT1C AS A POSSIBLE TARGET OF MALONYL-CoA

Over three decades ago, McGarry and colleagues (32–34) described the major regulatory mechanism by which fatty acid oxidation, and thereby ketogenesis, is controlled in the liver. They showed that malonyl-CoA, a cellular measure of the rate of fatty acid synthesis, is an allosteric inhibitor of CPT1, the rate-limiting enzyme in fatty acid oxidation. CPT1 enzymes catalyze fatty acyl transfer to carnitine. The fatty acyl-carnitine can then traverse cation transporters in the mitochondrial membrane and enter the matrix. Within the matrix. CPT2, which is malonyl-CoA insensitive, transfers the acyl chain to CoA where it can be catabolized by the fatty acid oxidation machinery. Skeletal muscle has little to no FAS, so malonyl-CoA cannot be used for fatty acid synthesis but is used primarily for the regulation of CPT1b, the muscle-specific CPT1, in combination with the outer-mitochondrial tethered ACC2. Therefore, there is precedent for malonyl-CoA as a signaling intermediate.

The hypothalamus expresses FAS (19) and ACC (18), which are localized to specific populations of

neurons. One possible molecular mechanism for a malonyl-CoA signal may be as an allosteric modifier of a neural enzyme. CPT1a, the liver isoform, is found in the CNS and has been implicated as a possible player in the malonyl-CoA signal (35). Intriguingly, however, the CNS expresses a unique brain-specific CPT1 gene, CPT1c (36, 38). CPT1c is expressed in neurons throughout the CNS with concentrated localization in hypothalamic nuclei that are involved in energy homeostasis such as the Arc and VMN (37). CPT1c binds malonyl-CoA (K_d ~0.3 μM) within the physiological range of malonyl-CoA in the hypothalamus (38). Furthermore, a mouse knockout of CPT1c results in decreased body weight resulting from decreased food intake, a phenotype consistent with a malonyl-CoA target enzyme that controls body weight (38). This phenotype is also consistent with the hypothalamic deletion of FAS, a condition in which mice are lean and exhibit lowered food intake and increased malonyl-CoA (23).

We believe this is compelling genetic evidence for a role of CPT1c in the malonyl-CoA signaling pathway. However, what is the link between fatty acid oxidation and the control of body weight? The CNS uses mainly glucose during the fed state but will switch to the utilization of ketones (acetoacetate and β-hydroxybutyrate) during food deprivation. It has been widely appreciated that the CNS does not efficiently use long-chain fatty acids for cellular energy purposes. The hypothalamus is unique in this regard because the regions involved in energy homeostasis have more access to circulating blood constituents than other regions of the CNS (39, 40). The hypothesis that fatty acids (41), or amino acids (42, 43), act as satiety factors is contrary to animal physiology because their concentrations in the blood are high during food deprivation, a time of hunger. Consistent with the rather small contribution of fatty acid oxidation to CNS metabolism in adult animals, CPT1c does not catalyze fatty acyl transfer from fatty acyl-CoAs to carnitine nor does it promote fatty acid oxidation (36, 38).

The inability to catalyze fatty acyl transfer to carnitine is unexpected given the high degree of amino acid sequence similarity/identity between CPT1c and CPT1a and CPT1b or even to *Drosophila* CPT1. CPT1c retains virtually all of the amino acid residues that have been shown to be important for catalysis or malonyl-CoA sensitivity (44). Therefore, we are left with a conundrum, a molecule of unknown catalytic function but which retains its allosteric interaction with malonyl-CoA. Indeed, CPT1c may not act as an enzyme but may function through a regulatory protein-protein interaction that is altered by malonyl-CoA binding.

One interesting phenotype of the CPT1c knockout mouse is that when fed a diet with low to intermediate fat content (10–15% of calories from fat), the mice eat less and are lean; however, when fed a high-fat/ketogenic diet (45–60% of calories from fat) the mice eat less but gain much more weight compared with WT littermates (38). This phenotype is clearly linked to the CNS effect on energy expenditure (see above) because CPT1c knckout mice do not efficiently use fatty acids in skeletal muscle after food deprivation. This may portend a differential role for the substrate or product in the CPT1c reaction/interaction. Whatever the biochemical role of CPT1c is within the CNS, we believe that it is key to understanding nutrient signaling. Clearly, much more research will be necessary to definitively resolve this issue.

HYPOTHALAMIC MALONYL-CoA AND FATTY ACID OXIDATION

An alternative hypothesis for the effect of malonyl-CoA has been proposed whereby malonyl-CoA acts as an inhibitor of CPT1a and therefore, fatty acid oxidation (35). It follows that inhibition of fatty acid oxidation might promote an increase in the cellular long-chain acyl-CoA concentration to activate ATP-sensitive potassium channels independent from the sulfonylurea receptor-1 subunit (45, 46). Thereby, malonyl-CoA could integrate cellular nutrient signals provided by glucose flux and AMPK activity as well as the influx of free fatty acid from the cerebral spinal fluid (41). Neuronal fatty acyl-CoAs acting on ATP-sensitive potassium channels might play a role in hypothalamic nutrient sensing.

A complicating issue, however, is integrating this proposal into animal metabolism. The notion that long-chain fatty acids signal satiety in the hypothalamus is not consistent with the following facts: 1) fatty acids do not readily enter the brain via the circulation because of the blood-brain barrier, although neurons of the arcuate nucleus, a major player in the regulation of food intake, have direct access to the bloodstream (39, 40); 2) plasma free fatty acids are elevated during fasting, conditions under which animals display hunger rather than satiety; 3) inhibition of *de novo* hypothalamic fatty acid synthesis inhibits food intake and, thus, could not be the source of fatty acids to produce satiety (13, 23); 4) lowering hypothalamic malonyl-CoA and *de novo* fatty acid synthesis by expressing MCD results in hyperphagia and obesity (28, 29); and 5) CPT1a is expressed in low abundance in hypothalamic neurons. Rather, CPT1c, which does not facilitate fatty acid oxidation (47), is the predominant isoform in the hypothalamus. Thus, it is difficult to reconcile a physiological rationale or paradigm by which fatty acids would induce satiety. Further research will be required to determine whether hypothalamic fatty acids *per se* induce satiety under physiological conditions and what the source of these fatty acids might be.

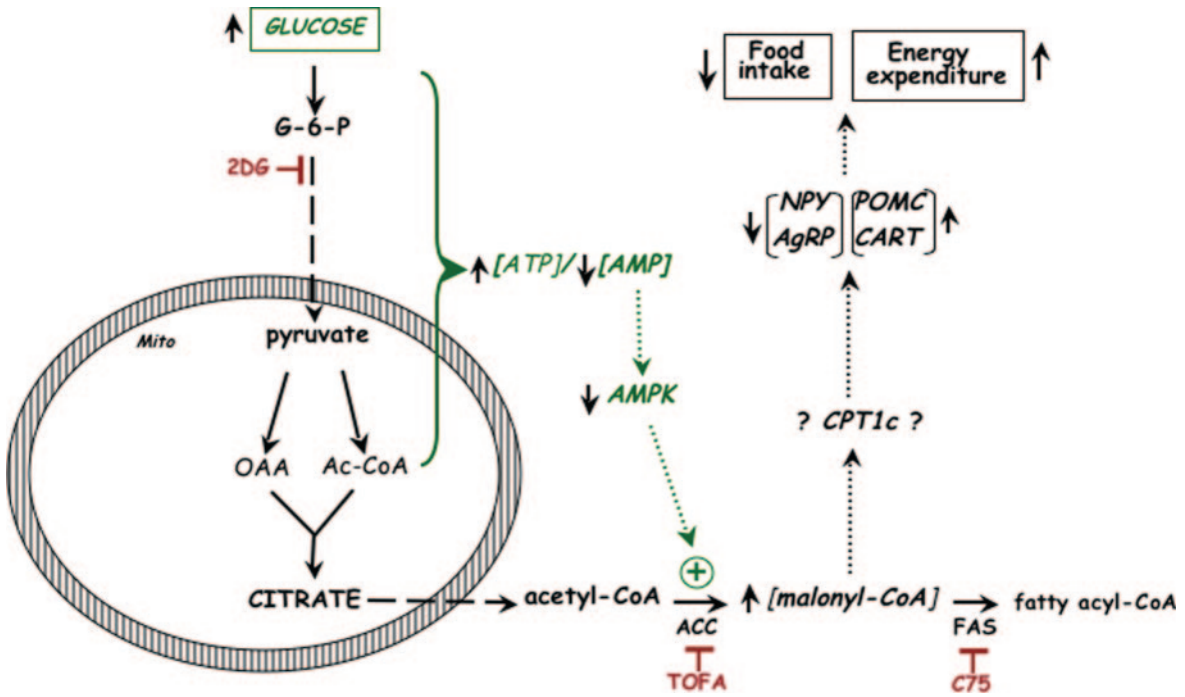

Fig. 3. Model: Role of Malonyl-CoA in the Hypothalamic Control of Feeding Behavior

Glucose delivered to the brain is metabolized to pyruvate, which is translocated into the mitochondrion where pyruvate dehydrogenase and pyruvate carboxylase catalyze the balanced synthesis of acetyl-CoA and oxaloacetate for the net synthesis of citrate. Because isocitrate dehydrogenase activity is suppressed during energy surplus (and the *cis*-aconitase equilibrium lies in the direction of citrate), citrate accumulates and is translocated from the mitochondrion into the cytoplasm by a regulated anion transporter. Citrate then undergoes cleavage by ATP:ctrate lyase to produce cytoplasmic acetyl-CoA for fatty acid synthesis. Acetyl-CoA is poised at a metabolic branch point with multiple options, one being reductive chain elongation to form long-chain fatty acids catalyzed by FAS, a pathway initiated by the ACC-catalyzed conversion of acetyl-CoA to malonyl-CoA. This option is prominent during periods of energy surplus when ACC is maintained in the active state. During periods of energy surplus, ACC is held in the catalytically active state by 1) dephosphorylation and 2) feed-forward allosteric activation by citrate. 1) As discussed above, when the cellular [ATP] is elevated, [5′-AMP] is low. Because 5′-AMP is an activator of AMPK and ACC is an AMPK substrate, when [5′-AMP] is reduced, AMPK activity is also low. Under these conditions, hypothalamic ACC exists in the dephosphorylated/catalytically active state. It should be noted that phospho-ACC can be dephosphorylated by a protein phosphatase and although it is likely that this enzyme is regulated, its regulatory mechanism has not been elucidated. Importantly, leptin also promotes dephosphorylation, thus, inactivation AMPK and thereby default activation of hypothalamic ACC. Although the molecular mechanism by which leptin promotes dephosphorylation and inactivation of AMPK remains obscure, it has been shown that active glucose metabolism in the CNS must occur concomitantly. 2) As illustrated, cytoplasmic [citrate] would be expected to rise in the hypothalamus as it does in other tissues (48) in response to increased glucose flux. Citrate is a *bona fide* allosteric activator of ACC (49, 50). Malonyl-CoA can also undergo decarboxylation catalyzed by MCD, an enzyme known to be present in the hypothalamus. How MCD is regulated, however, has not been elucidated. In physiological states of energy surplus, such as feeding after fasting, the level of malonyl-CoA in the hypothalamus, notably in the Arc, increases concurrently with marked perturbation in the state of activation of hypothalamic neurons as measured by changes in c-Fos expression. These changes rapidly lead to increased expression of the orexigenic (NPY and AgRP) neuropeptides and decreased expression of the anorexigenic (POMC/αMSH and CART) neuropeptides, which suppress food intake and increase energy expenditure (Fig. 2). The brain-specific CPT1c, a malonyl-CoA binding protein of unknown catalytic activity, may serve as the link between increased malonyl-CoA during energy surplus and the changes in expression of the hypothalamic neuropeptides. The molecular mechanism by which this occurs awaits elucidation of the reaction catalyzed by CPT1c. C75 refers to 3-carboxy-4-octyl-2-methylenebutyrolactone. Ac-CoA, Acetyl-CoA; G-6-P, glucose 6-phosphate; OAA, oxaloacetate; TOFA, 5-(tetradecyloxy)-2-furoic acid.

Proposed Schema for the Malonyl-CoA Signaling Pathway in the Hypothalamus

Based on the evidence described above, we have formulated a model signaling pathway for the regulation of feeding behavior and energy expenditure in which hypothalamic malonyl-CoA participates as a key intermediate. The model illustrated in Fig. 3 visualizes a physiological scenario in which food deprivation followed by feeding a carbohydrate meal gives rise to elevated blood glucose, an indicator of energy surplus. The intervening events shown are believed to produce changes in the expression/secretion of hypothalamic orexigenic and anorexigenic

neuropeptides that are known alter food intake and peripheral energy expenditure (see also Fig. 2).

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