C75, a Fatty Acid Synthase Inhibitor, Reduces Food Intake via Hypothalamic AMP-activated Protein Kinase*

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Energy homeostasis and feeding are regulated by the central nervous system. C75, a fatty acid synthase (FAS) inhibitor, causes weight loss and anorexia, implying a novel central nervous system pathway(s) for sensing energy balance. AMP-activated protein kinase (AMPK), a sensor of peripheral energy balance, is phosphorylated and activated when energy sources are low. Here, we identify a role for hypothalamic AMPK in the regulation of feeding behavior and in mediating the anorectic effects of C75. 5-Aminomidazole-4-carboxamide-1-$\beta$-D-ribofuranoside (AICAR), an activator of AMPK, increased food intake, whereas compound C, an inhibitor of AMPK, decreased food intake. C75 rapidly reduced the level of the phosphorylated AMPK α subunit (pAMPKα) in the hypothalamus, even in fasted mice that had elevated hypothalamic pAMPKα levels. Furthermore, AICAR reversed both the C75-induced anorexia and the decrease in hypothalamic pAMPKα levels. C75 elevated hypothalamic neuronal ATP levels, which may contribute to the mechanism by which C75 increased AMPK activity. C75 reduced the levels of pAMPKα and phosphorylated cAMP response element-binding protein (pCREB) in the arcuate nucleus neurons of the hypothalamus, suggesting a mechanism for the reduction in NPY expression seen with C75 treatment. These data indicate that modulation of FAS activity in the hypothalamus can alter energy perception via AMPK, which functions as a physiological energy sensor in the hypothalamus.

Despite significant advances in the understanding of appetite and satiety at molecular levels (1–5), practical therapies for weight loss remain elusive. We and others (4–8) have demonstrated that C75, a synthetic fatty acid synthase (FAS) inhibitor, caused profound weight loss and anorexia in lean, diet-induced obese (DIO) and genetically obese (ob/ob) mice. In addition to FAS inhibition, C75 also stimulates carnitine palmitoyltransferase-1 (CPT-1) activity, increasing fatty acid oxidation and ATP levels (8). Since enzymes of the fatty acid metabolic pathways are highly expressed in hypothalamic neurons that regulate feeding behavior (9), we hypothesize that C75-induced alterations in fatty acid metabolism may affect neuronal energy flux, which could signal a change in energy status, leading to changes in feeding behavior.

AMPK (AMP-activated protein kinase) is activated by metabolic stresses such as nutrient starvation (10) and ischemia-hypoxia (11) and by physiological processes such as vigorous exercise (12, 13). Specifically, increases in the AMP/ATP ratio, decreases in cellular pH, and increases in the creatine/phosphocreatine ratio are known to activate AMPK via allosteric activation of AMPK by AMP and by phosphorylation of AMPK by AMPK kinase (14–19). Once activated, AMPK switches off ATP-consuming biosynthetic pathways such as fatty acid synthesis and switches on ATP-generating metabolic pathways such as fatty acid oxidation to preserve ATP levels (20, 21). The central roles of AMPK in both energy sensing and the control of fatty acid metabolism (16, 22) and its regulation by leptin in muscle (23) make it a candidate metabolic sensor in the hypothalamus to relay changes in metabolism. Furthermore, emerging reports (24, 25) that AMPK is involved with the regulation of food intake support our hypothesis. Therefore, we chose to study AMPK as a candidate metabolic sensor in the hypothalamus, which might perceive changes in energy status caused by C75.

**EXPERIMENTAL PROCEDURES**

**Animals**—All animal experiments were done in accordance with guidelines on animal care and use established by the Johns Hopkins University School of Medicine Institutional Animal Care and Use Committee. Male BALB/c mice (7–9 weeks) were purchased from Charles River Laboratories (and housed in a controlled-light (a 12 h light/12 h dark cycle) environment (lights on 0200 to 1400 h) and allowed ad libitum access to standard laboratory chow and water. For fasting, food was withdrawn from cage at the onset of the dark cycle for 24 h, but ad libitum access to water was allowed.

**Measurement of Food Intake**—Mice were implanted with permanent stainless steel cannulae into the lateral ventricle of the brain 0.6 mm caudal to Bregma, 1.2 mm lateral to the midline, and sunk to a depth of 2.2 mm below the surface of the skull. Implanted mice were housed in individual cages and utilized for intracerebroventricular (i.v.) and intraperitoneal (i.p.) injections as indicated. C75 dissolved in RPMI 1640 was administered by i.v. injection (100 μl) or i.p. injection (200 μl) 1 h before sacrifice.

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C75 Reduces Food Intake via Hypothalamic AMPK

19971

1640 (Invitrogen). AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside) (Toronto Research Chemicals Inc.), or compound C (26) (FASgen, Inc.) in saline was injected i.e.c. such that the desired dose could be administered in a volume of 2.5 μl, while control groups received vehicle only. Injections were done immediately preceding dark onset, and food intake measurements were taken at 1 h (0–1-h interval), 3 h (1–3-h interval), and 24 h (2–24-h interval) after dark onset. C75 and AICAR i.c.v. treatment groups were i.p. injected with 15 mg/kg of body weight C75 dissolved in 200 μl of glucose-free RPMI 1 h before the dark onset, followed by 3 μg/25 μl of saline i.e.v. AICAR immediately preceding the dark onset. Control groups received 200 μl of glucose-free RPMI 1 h before lights off and 2.5 μl of saline. Administration of i.p. compound C (10 or 30 mg/kg of body weight) or C75 (10 mg/kg of bodyweight) was followed by food intake measurement at the same time points indicated.

Western Blot Analysis—Hypothalami were dissected using as landmarks the optic chiasm rostrally, and the mammillary bodies caudally to a depth of 2 mm. Dissected hypothalamic and liver tissue were immediately frozen in liquid nitrogen. Tissues were homogenized in 200 μl of lysis buffer (50 mM Tris-HCl, pH 7.5, 250 mM sucrose, 5 mM sodium pyrophosphate, 50 mM NaF, 1 mM EDTA, 1 mM dithiothreitol, 0.5 mM phenethylsulfonyl fluoride, 0.1 mM benzamidine, 50 μg/ml leupeptin, 50 μg/ml soybean trypsin inhibitor). SDS detergent was added to a final 0.2%, and lysates were boiled for 5 min. After the supernatant was harvested, protein concentration was determined by BCA kit (Bio-Rad). Phosphorylation of AMPKα was determined on a 4–15% gradient SDS-polyacrylamide gel using anti-phospho-AMPKα (Thr 172)/β (Cell Signaling). Anti-AMPK antibody (α1 and α2, 1:1000, Cell Signaling). Anti-AMPK antibody (α1 and α2, 1:1000, Cell Signaling) was used as a loading control.

Primary Hypothalamic Neuron Cultures and ATP Measurement—Hypothalami were dissected from E17 Sprague-Dawley rats (Harlan) and dissociated by trypsin (0.125%)/DNA (0.001%) solution and trituration as described previously (27). Cells were plated at 6 × 10^4 on poly-l-lysine-coated 96-well plates (Corning Inc.) in neurobasal medium supplemented with B27, 0.5 mM t-glutamine, 1% penicillin-streptomycin (Invitrogen). To limit non-neuronal cell proliferation, cells were treated with cortisone arabinoside furosinone (1 μM) on day 4 after plating, and 6–8-day-old cells were assayed for ATP. Hypothalamic neurons were lysed in TE (100 mM Tris-HCl, pH 7.4, 4 mM EDTA), and ATP levels were measured within the linear range using the ATP BioLuminescence Kit CLSII (Roche Applied Science) by following the manufacturer’s recommendation. Data were analyzed by a PerkinElmer Life Sciences Victor^2 1420.

RNA Preparation and Northern Blot Analysis—Hypothalamic total RNA was purified using TRIzol reagent (Invitrogen), and Northern blot analysis of total RNA was performed as described previously (9). RNA was hybridized with random primed ^32P-labeled DNA probes made from cloned plasmids of mouse AGRP (GenBank™ accession number U98496), human NPY (GenBank™ accession number XM004941), rat CART (GenBank™ accession number U10071), and mouse POMC (GenBank™ accession number AH005319). As a loading control, the probe for mouse GAPDH gene was used at the same blot.

The signals were quantified using an image analyzer (Amersham Biosciences) and Imagequant software.

Immunohistochemistry—Floating brain sections were prepared as described previously (9) with modifications (28). Free-floating sections were blocked in phosphate-buffered saline containing 5% goat serum, 0.5% bovine serum albumin, 0.05% Triton X-100, 1 mM NaF, pH 7.2 at room temperature and incubated with anti-phospho-AMPKα (α1 and α2) antibody (1:100) or anti-phospho-CREB antibody (1:500, Cell Signaling) in phosphate-buffered saline containing 1% goat serum, 0.1% bovine serum albumin, 0.05% Triton X-100, 1 mM NaF overnight at 4 °C. Signal was visualized by using a Vectastain ABC kit (Vector).

In Situ Hybridization—Antisense digoxigenin-labeled NPY riboprobe was obtained from a plasmid containing the NPY gene (GenBank™ accession number XM004941). Hybridization and washing were performed as described previously (9). For double fluorescent in situ hybridization, digoxigenin-labeled riboprobe was generated from plasmid containing AMPKα2 gene (pEBG2c, a gift from L. A. Witters) for AMPKα2 (FITC), and biotin-labeled riboprobe was used for NPY (Texas Red). Sheep FITC-conjugated antidi-goxigenin antibody (1:50, Roche Applied Science) was incubated in TNB buffer (100 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.5% blocking reagent) for FITC detection. Streptavidin-Texas Red (1:50, Amersham Biosciences), rabbit-anti-Texas Red antibody (1: 50, Molecular Probes), goat biotin-conjugated anti-rabbit IgG antibody (1:50, Santa Cruz Biotechnology), and streptavidin-Texas Red (1:30) were incubated serially in TNB buffer for Texas Red detection.

Analysis and Quantification of Images—Images of in situ hybridization and immunohistochemistry were visualized using an Axioscan HRc digital camera (Carl Zeiss), and images were acquired using Improvision Openlab software and quantified by NIH Image program (Macro).

Statistical Analysis—All values are presented as mean ± S.E. Data were analyzed by one-way analysis of variance or t test.

RESULTS

Feeding Behavior Is Changed by C75, AICAR, or Compound C Treatment—We investigated the relationships among C75-induced alterations in fatty acid metabolism, AMPK activation, or inhibition, and feeding behavior. We utilized mice implanted with i.c.v. cannulae to measure food intake after dark onset administration of C75 (Fig. 1a).

All mice had access to food ad libitum during the 24-h cycle. C75 significantly reduced food intake during the 1–3- and 3–24-h time intervals in a dose-dependent manner (Fig. 1a). Injection of 5 and 10 μg of C75 caused a 20.3% (p < 0.05) and 37.7% (p < 0.01) reduction in food intake over 24 h, respectively. The 10-μg dose also produced a reduction in bodyweight (Fig. 1d).

Consistent with our previous report (4), these results indicate that C75 reduces food intake via central mechanism(s).

AICAR, a compound that stimulates AMPK activity, is taken up into cells and phosphorylated to form ZMP (29), which mimics the effects of AMP on AMPK activation (30). We chose a pharmacological approach to alter AMPK activity, as opposed to a genetic approach, as we wished to be able to reverse the effects of inhibition on a rapid time scale. We determined the time course of action of AICAR, as its effect may be transient, since it is further metabolized (31). In contrast to the feeding inhibition produced by C75, i.c.v. administration of AICAR increased food intake. A dose of 3 μg increased food intake to 230% (p < 0.01) within 1 h and 135% (p < 0.01) at 3–24 h, and total 24-h food intake was increased to 130% of control (p < 0.05) (Fig. 1b). Consistent with the recent finding (25), the i.c.v. injection of AICAR increased food intake. Despite this increase in food intake, this single dose of AICAR has no significant effect on body weight (Fig. 1d).

Body weight does not always change in proportion to food intake (32). A previous report noted that chronic subcutaneous injection of AICAR (1 g/kg body weight) for 4 weeks had no impact on either food intake or body weight (33) but that there was a reduction in fat pad mass and an increase in liver mass. Thus, i.c.v. administration of a single dose of AICAR may have an effect on the mass of these peripheral tissues, such that body weight does not change despite increased food intake. To confirm the effect of AMPK on food intake, we used compound C, which is a selective AMPK inhibitor (34–36). The i.c.v. injection of 5 μg of compound C caused a 36.2, 37.8, and 35.6% reduction in food intake at 0–1, 3–24 h and over 24 h, respectively (Fig. 1c). This dosage of compound C led to a weight loss (Fig. 1d).

Interestingly, with the stimulatory effect of AICAR on feeding, the inhibitory effect of compound C on feeding was profound at 0–1 h and 3–24 h. The i.p. injection of compound C also had a similar reduction in food intake (Fig. 1e), showing that a higher dose (30 mg/kg body weight) decreased food intake during all time intervals (27.4, 36.8, 65.7, and 57.8% of control during 0–1, 1–3, 3–24 h and total, respectively). Even though AICAR or compound C may have additional cellular effects that cannot be excluded, the opposite results on food intake obtained using an AMPK activator and inhibitor supports the hypothesis that AMPK is involved in feeding behavior.

We determined the time course of action of the i.p. C75 administration, with the intention of utilizing this route of administration for C75 in further experiments designed to compare the central and peripheral effects of C75 on the change in
AMPK activation and to combine C75 and AICAR treatments. Administration of C75 i.p. (10 mg/kg of body weight) caused a dramatic decrease in food intake during all intervals measured (8.3, 23.3, and 30.1% of control during 0–1, 1–3, and 3–24 h, respectively) (Fig. 1f). Total 24-h food consumption was significantly reduced to 26.3% of control (*p < 0.001). The effect of C75 on food intake was more pronounced and lasted longer than that of compound C. The greater magnitude of the effect following peripheral administration of C75 on food intake compared with the i.c.v. route of administration may reflect the larger dose that can be administered via this route or an additional peripheral effect of this compound. Collectively, these results demonstrate that C75 and compound C (administered either i.c.v. or i.p.) produce opposite effects on food intake over similar time courses compared with i.c.v. administration of AICAR.

**C75 Decreases the Phosphorylation of Hypothalamic AMPK**—The hypothalamus plays an important role in monitoring energy balance and integrating peripheral signals that affect food intake (1, 2). Although the expression of AMPK in brain has been reported (37, 38), its function in the brain is unknown. C75 inhibits FAS and stimulates CPT-1, the enzyme that imports palmitate into the mitochondrion for β-oxidation (8). Both of these actions may signal a positive energy balance in neurons of the hypothalamus, which may inactivate hypothalamic AMPK. To examine the effect of C75 on hypothalamic AMPK activity, we determined the effect of C75 treatment on the level of phosphorylation of the catalytic subunit of AMPK (pAMPK) in the hypothalamus, which correlates with its activity (Fig. 2).

Mice received vehicle, 5 or 10 μg of C75 i.c.v., and the levels of hypothalamic pAMPKα were determined by Western blot. The level of AMPKα (α1 and α2 subunits) served as a loading control. Compared with levels of pAMPKα in vehicle-treated control animals, C75 reduced the levels of pAMPKα (α1 and α2) in the hypothalamus 3- and 6-fold at 30 min and 3 h, respectively (Fig. 2, a and b). As seen with central administration of C75, i.p. injection of C75 (10 mg/kg of body weight) significantly reduced the levels of pAMPKα in the hypothalamus at 30 min and 3 h (Fig. 2, c and d). In contrast, C75 had little effect on pAMPKα levels in the liver 30 min after administration but increased pAMPKα levels at 3 h (Fig. 2, e and f). These results demonstrated that C75 treatment rapidly de-
increased AMPK activity in the hypothalamus. The decrease in hypothalamic pAMPK\(_\alpha\) levels could result from the metabolic changes that occur as a result of FAS inhibition, which would diminish energy expenditure and signal a favorable energy balance. These results also indicate that the phosphorylation of AMPK is regulated differently in the hypothalamus than in the liver in response to C75. This difference most likely reflects differences between metabolic pathways, or the flux through those pathways, found in neurons and in liver. By 3 h, the decreased food intake seen with C75 treatment may signal an energy poor state in liver (Fig. 2e), leading to AMPK activation, indicating an attempt to preserve energy levels through the stimulation of fatty acid oxidation, for example.

**C75 Decreases the Fasting-induced Phosphorylation of Hypothalamic AMPK**—It has been shown that the activity of AMPK is elevated in fasted rat liver (39). To investigate whether hypothalamic AMPK is responsive to fasting, the level of pAMPK\(_\alpha\) was monitored after withdrawal of food at the onset of dark cycle in mice fed ad libitum. There was no change in pAMPK\(_\alpha\) levels within 3 h of food withdrawal (Fig. 3, a and b). However, fasting for 24 h resulted in a 2-fold stimulation in the level of hypothalamic pAMPK\(_\alpha\) (Fig. 3, a and b). While one report (40) noted no difference in AMPK activity between dark and light cycles in rats fed ad libitum, only one time point (6 h) was investigated, without correlation to feeding profile in the interval before this measurement was made. Our results suggest that the activation of hypothalamic AMPK could be involved in the fasting-induced stimulation of food intake.

We next examined whether C75 could reduce AMPK phosphorylation in the setting of fasting, when AMPK phosphorylation is increased. This is important in establishing a link between C75-induced FAS inhibition and AMPK activity, as C75 does inhibit feeding even in fasted mice (4, 8). After 24 h of fasting, either vehicle (RPMI) or C75 was administrated i.p. and the levels of hypothalamic pAMPK\(_\alpha\) were determined (Fig. 3, a and b). While one report (40) noted no difference in AMPK activity between dark and light cycles in rats fed ad libitum, only one time point (6 h) was investigated, without correlation to feeding profile in the interval before this measurement was made. Our results suggest that the activation of hypothalamic AMPK could be involved in the fasting-induced stimulation of food intake.

**C75 Increases the Hypothalamic Neuronal ATP Level**—It has been shown that C75 increases ATP levels in 3T3-L1 adipocytes (8) and even in primary cortical neurons (27). Since an increase in the ATP/ATP ratio is known to activate AMPK (15, 16), we hypothesized that a C75-induced increase in hypothalamic ATP levels could contribute to a decrease in AMPK activity, resulting in reduced hypothalamic AMPK activity. Treatment of primary cultures of hypothalamic neurons with 40 \(\mu\)g/ml C75 led to a significant increase in neuronal ATP levels to 118 and 128\% of control at 30 min and 2 h, respectively (Fig. 4a). C75 treatment caused a similar change in ATP levels in primary cortical neurons (27), producing a decrease in the ratio of AMP/ATP and inactivation of AMPK. Therefore, it is likely that an increase in ATP caused by C75 also contributed to the decrease in AMPK activity in the hypothalamus.

**AICAR Reverses the Anorexic Effect of C75 and Increases the Phosphorylation of Hypothalamic AMPK**—To determine whether AICAR could reverse the C75-induced decrease in food intake, we treated mice 1 h before the onset of dark cycle with either vehicle or C75 (5 mg/kg of body weight) i.p., followed 1 h later by an i.c.v. injection of vehicle or AICAR (3 \(\mu\)g) (Fig. 4b). C75 reduced food intake at 1 h to 37.5\% of control (RPMI/saline) \((p < 0.01)\). In contrast, AICAR treatment increased food intake at 1 h to 346\% of the amount of C75/saline treatment \((p < 0.001)\). AICAR treatment reversed the C75-induced anorexia, resulting in food intake that was similar to that of control vehicle-treated mice. The effect of AICAR on C75-treated mice was of limited duration, consistent with the metabolism of AICAR (31). The lack of an effect on food intake during the 3–24-h time interval may represent the net effect of the opposing actions of C75 and AICAR.

If the reversal of C75-mediated anorexia by AICAR involves alteration of AMPK activity, AICAR should similarly reverse the decrease in the level of hypothalamic pAMPK\(_\alpha\) that occurs with C75 treatment. Ad libitum fed mice received an i.p. injection followed by an i.c.v. injection 1 h later as follows: i.p. RPMI and i.c.v. saline, intraperitoneal RPMI and i.c.v. AICAR, i.p. C75 and i.c.v. saline, and i.p. C75 and i.c.v. AICAR (Fig. 4c). Hypothalamic tissues were prepared for Western blot 30 min after the i.c.v. injections (Fig. 4, c and d). A low level of pAMPK\(_\alpha\) was detected in vehicle-treated mice, which was in-
creased in AICAR-treated animals (Fig. 4, c and d). Mice that received C75 i.p. and saline i.c.v. displayed a profound decrease in pAMPKα levels. AICAR treatment following C75 treatment completely reversed the C75-induced decrease in hypothalamic pAMPKα levels. Sub-threshold doses would have been used with only behavioral data, but the fact that AICAR prevented the C75-induced changes in both behavior and the status of AMPK phosphorylation supports a common site of action for the effects of C75 and AICAR. These results indicate that AICAR restores both C75-induced anorexia and the C75-induced suppression of AMPK activity.

C75 Alters pAMPK, pCREB, and NPY Expression in Arcuate Nucleus Neurons—AMPK acutely regulates cellular metabolism and chronically regulates gene expression (41). To ascertain whether the changes in the phosphorylation status of AMPK in the hypothalamus reflected the level of pAMPKα in the arcuate nucleus, we performed immunohistochemistry for pAMPKα using coronal brain sections containing the arcuate nucleus (Fig. 5a, panels 1–3). pAMPKα was detected in the arcuate nucleus of mice fed ad libitum (Fig. 5a, panel 1). The specificity of immunostaining was confirmed by preabsorption experiments using the phospho-AMPKα peptide (data not shown). Compared with control, pAMPKα immunoreactivity was increased to 171% of control in the arcuate nucleus of mice fasted for 24 h (Fig. 5a, panel 3). pAMPKα immunoreactivity was reduced in C75-treated mice to 12% of control, even in the setting of reduced food intake (Fig. 5a, panel 2). These changes are consistent with our Western blot data (Fig. 2, a and c) and confirm that C75 reduces pAMPKα levels in the arcuate nucleus.

We have previously demonstrated by Northern blot analysis that C75 decreased hypothalamic NPY expression (4, 9). We next investigated whether the decreases in pAMPKα in the arcuate correlated with changes in NPY that occur with C75 treatment. NPY expression in neurons within the arcuate nucleus was determined in control, C75-treated, and fasted mice...
Consistent with previous Northern blot analysis of hypothalamic tissues (9), NPY mRNA expression was down-regulated in the arcuate nucleus of C75-treated mice to 66% of control (Fig. 5a, panel 5) and up-regulated in fasted mice to 140% of control (Fig. 5a, panel 6). It has been shown that the cAMP-CREB pathway mediates NPY gene expression under fasted conditions (28, 42), suggesting that leptin modulates NPY gene expression through this pathway (28). To elucidate the pathways involved in the down-regulation of NPY that occurs with C75 treatment, we determined the level of pCREB in the arcuate nucleus (Fig. 5a, panels 7–9). As previously reported (28), 24-h fasting increased pCREB immunoreactivity in the arcuate nucleus to 197% of control (Fig. 5a, panel 9). In contrast, C75 decreased the level of pCREB to 39% of control (Fig. 5a, panel 8), consistent with the hypothesis that the decrease in NPY gene expression caused by C75 may be mediated by a decrease in the level of pCREB. To clarify the colocalization of AMPK and NPY in the arcuate nucleus, double fluorescent in situ hybridization was performed (Fig. 5b). A subpopulation of neurons in the arcuate nucleus that expressed AMPK/H92512 mRNA also expressed NPY mRNA (Fig. 5b). It is known that NPY and CREB co-localize to neurons in the arcuate nucleus (28). These results indicate that AMPK, NPY, and CREB are co-expressed in a subpopulation of neurons within the arcuate nucleus and support the hypothesis that AMPK may modulate CREB phosphorylation to affect NPY expression. In contrast to C75, AICAR had the opposite effect (Fig. 5c and d). Thus, consistent with our findings that AICAR stimulated feeding, AICAR significantly increased hypothalamic NPY expression 20 h after i.c.v. administration (Fig. 5c). The increase in NPY expression seen with AICAR treatment may mediate the stimulation of food intake seen at later times (3–24 h) in Fig. 1b. Since no change in NPY expression with AICAR treatment was detected within 5 h (data not shown), it appears that the earlier change in feeding (0–1 h) is mediated by NPY gene expression-independent mechanism. AICAR also increased pCREB level in the arcuate up to 231% of control (Fig. 5d), which supports that AMPK may modulate CREB phosphorylation.

**DISCUSSION**

In this study, we investigated the hypothesis that C75, which alters neuronal energy balance in vitro (27), may mediate at least a part of its anorexigenic effect by altering energy balance and thus affecting neuronal AMPK activity in vivo. Studies support a complex mechanism whereby the central nervous system responds to changes in energy status imparted by fluctuations in fuel levels and circulating hormones (2, 3). AMPK is a known sensor of peripheral energy balance and is activated when cellular energy is low (20, 31, 43); however, its role in the central nervous system has been unclear. It was recently reported (25) that leptin and ghrelin can regulate AMPK, although the mechanisms by which this occurs are still unknown.
Our observations suggest a mechanism by which C75 can affect feeding behavior by modulating AMPK activity (Fig. 6). Through its ability to inhibit FAS and stimulate CPT-1, C75 may increase ATP levels in hypothalamic neurons, as it does in the periphery (8) and in cortical neurons (27). This change would signal a positive energy balance, leading to a decrease in AMPK activity and resulting in a decrease in NPY expression. In fasting, when energy is depleted, AMPK is stimulated, thereby activating the CREB-NPY pathway and food intake. There appears to be relatively little change in the level of phosphorylated hypothalamic AMPK during normal feeding, and a prolonged period of decreased food intake is required before hypothalamic pAMPK levels increase. Since the lateral hypothalamic area and paraventricular nucleus are identified as important subregions to which arcuate nucleus neurons project to regulate feeding (44, 45), it would be of interest to study a change in pAMPK levels in these areas as well. Hypothalamic AMPK seems responsive to changes in energy status due to C75 treatment or fasting. Thus, AMPK may function as a “fuel sensor” in the central nervous system, as it does in peripheral tissues such as muscle (23, 43). Several mechanisms have been proposed by which AMPK could influence gene transcription. AMPK may act through Raf/Erk pathway (46), or may act via Raf-independent mechanisms, as AMPKα2 has been localized to the nucleus (47) and directly regulates proteins involved in gene transcription (48). The contribution of these pathways remains to be elucidated.

Recent studies support the hypothesis that the levels of fatty acids or flux through the fatty acid synthetic pathway are important in the monitoring of energy balance. For example, it has been recently shown that inhibition of hypothalamic CPT-1 causes a reduction in food intake, suggesting that increased long chain fatty acyl-CoAs function as a sensor of nutrient availability in hypothalamic neurons (49). Through its inhibition of FAS and stimulation of CPT-1 activity, C75 may alter energy balance with the same net outcome. C75 is claimed to cause a reduction in food intake, suggesting that increased availability in hypothalamic neurons (49). Through its inhibi-

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