Fig. 5. Phosphorylation level of the β isoform of ACC. The phosphorylation level of ACC-β was analyzed by Western blotting. Two bands above 250 kDa are shown in the blot. The thick band corresponds to the α isoform of ACC (≈265 kDa), and the other band corresponds to the β isoform of ACC (≈280 kDa). The ratio of the intensity of the phospho-ACC-β to that of β-actin was calculated to represent the level of phosphorylation. In both Arc and PVN, the levels of pACC-β were not affected by leptin, whereas the levels of pACC-α were decreased in response to leptin (Fig. 1 *A* and *B*). Compared with Fig. 1, the reduction of pACC-α levels may be small as shown in these blots, because longer exposure time, which tends to saturate the pACC-α band, is required to develop clear bands of pACC-β.

Fig. 6. Expression of constitutively active AMPK (CA-AMPK) in the MBH. **(***A*) Cultured A549 cells were infected with adenovirus expressing H150R γ1 subunit of AMPK ($n = 6$) or WT γ1 subunit of AMPK ($n = 6$). The pACC (Ser-79) level was increased in the cells infected with the virus expressing H150R γ1 subunit (*, $P < 0.05$). (*B*) The expression level (the eighth day after adenovirus injection) of the hemagglutinin (HA) tag of H150R γ1 subunit was analyzed by Western blotting. The MBH samples from 10 of 13 rats were positive for the expression of HA (MBH samples mainly included the Arc region). Representative blots from four rats are shown. Rats injected with adenovirus alone were used as the control (null). (*C* and *D*) Adenovirus expressing H150R γ1 subunit of AMPK (CA-AMPK) or WT γ1 subunit (WT-AMPK) was injected into the MBH, and daily food intake and daily body weight change after adenovirus injection were monitored. Food intake and body weight from each rat having a targeted MBH expression of HA were included. WT-AMPK, $n = 11$; CA-AMPK, $n = 10$. * and **, $P < 0.05$.

Fig. 7. Malonyl-CoA recycling assay. Precise and accurate measurement of malonyl-CoA level in small tissue samples has proven to be problematic. To address this problem, a sensitive assay involving a malonyl-CoA recycling technique has been developed (1). We demonstrated its sensitivity in that malonyl-CoA levels from dissected hypothalamic nuclei fell within the linear range of the assay (*A*). We further validated this assay by

showing that refeeding (3 h) elevated malonyl-CoA level in rat liver (*B*). Such an elevation is consistent with a previous report on the liver malonyl-CoA level (2). To quantify the malonyl-CoA level in individual hypothalamic nuclei, dissected nuclei were homogenized in 0.3 M sulfuric acid, and the homogenates were subject to citrate synthase treatment to eliminate the endogenous acetyl-CoA (1). The recycling reaction was performed according to the established protocol by Hu *et al.* (1). The level of malonyl-CoA was normalized to the protein content in the sample. (*A*) A standard curve for the malonyl-CoA recycling assay. (*B*) Three hours of refeeding significantly increased the malonyl-CoA level in the liver ($n = 5$; *, $P = 0.01$). The amount of malonyl-CoA was normalized to liver sample mass. (*C*) Five days after TOFA or DMSO infusion, rats fed ad lib were euthanized at the end of the dark cycle, and the whole hypothalamic malonyl-CoA level was significantly lowered by ICV TOFA infusion ($n = 6$, $P = 0.01$). (*D*) Blood glucose levels were measured in trunk blood by using a glucometer (Bayer) at the time of euthanasia ($n = 24$). (*E*) The malonyl-CoA level in the LHA at 3 h after ICV leptin was measured ($n = 8$). (*F*) The levels of palmitoyl-CoA (16:0) and oleoyl-CoA (18:1) in the LHA at 3 h after ICV leptin were quantified ($n = 8$). * and **, $P < 0.01$.

Fig. 8. Quantitation of the mRNA levels of the neuropeptides in dissected hypothalamic nuclei by quantitative RT-PCR. (*A*) The accuracy of the dissection of hypothalamic nuclei was validated by comparing the mRNA levels of the characteristic neuropeptides in dissected hypothalamic regions by real-time quantitative PCR ($n = 6$ for each nucleus). mRNA level of cyclophilin was used as the loading control. NPY and AgRP have the highest mRNA levels in the Arc located in the mediobasal hypothalamus (MBH); MCH (melanin-concentrating hormone) and orexin have the highest mRNA levels in the lateral hypothalamic area (LHA); PACAP (pituitary adenylate cyclase-activating polypeptide) has the highest mRNA level in the ventromedial nucleus (VMN). RNA isolation, cDNA synthesis, and real-time PCR quantitation were performed based on established protocols (3). The sequences of the primer pairs used in the real-time quantitative PCR are shown in *B*.

Fig. 9. Body weight and food intake in response to ICV infusion of TOFA or DMSO. Rats were undergoing ICV PBS infusion for at least 1 week for habituation to chronic central infusion. The body weight and food intake under PBS infusion were monitored and used as the baseline level. At the end of the PBS infusion, PBS was replaced with either TOFA or DMSO for an additional period of infusion for further habituation (a minimum of 48 h) (n = 15). There was no significant difference of body weight change or food intake from the basal level (PBS infusion) during the first 24 h (day 1) after DMSO or TOFA infusion. Body weight change and food intake from both groups (infusion of DMSO or TOFA) completely returned to the basal levels (PBS infusion) over the next 24 h (day 2).

Fig. 10. A diagram of the potential role for the hypothalamic fatty acid biosynthetic pathway in mediating leptin's anorectic actions. Leptin induces ACC activation in both the Arc and the PVN. In the Arc, ACC activation is mediated through the leptin-induced inhibition of AMPK. Activation of ACC has nuclei-specific effects. In the Arc, ACC activation increases the level of malonyl-CoA. In the PVN, activation of ACC, together with leptin-induced up-regulation of FAS, increases the level of palmitoyl-CoA without increasing the level of malonyl-CoA. The Arc appears to be the primary hypothalamic site for mediating leptin's action. Leptin-mediated alterations of AMPK (inhibition) and ACC (activation) in the PVN depend on leptin inhibition of Arc AMPK.

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