Prostaglandin in the ventromedial hypothalamus regulates peripheral glucose metabolism

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The hypothalamus plays a central role in monitoring and regulating systemic glucose metabolism. The brain is enriched with phospholipids containing poly-unsaturated fatty acids, which are biologically active in physiological regulation. Here, we show that intraperitoneal glucose injection induces changes in hypothalamic distribution and amounts of phospholipids, especially arachidonic-acid-containing phospholipids, that are then metabolized to produce prostaglandins. Knockdown of cytosolic phospholipase A2 (cPLA2), a key enzyme for generating arachidonic acid from phospholipids, in the hypothalamic ventromedial nucleus (VMH), lowers insulin sensitivity in muscles during regular chow diet (RCD) feeding. Conversely, the down-regulation of glucose metabolism by high fat diet (HFD) feeding is improved by knockdown of cPLA2 in the VMH through changing hepatic insulin sensitivity and hypothalamic inflammation. Our data suggest that cPLA2-mediated hypothalamic phospholipid metabolism is critical for controlling systemic glucose metabolism during RCD, while continuous activation of the same pathway to produce prostaglandins during HFD deteriorates glucose metabolism.
Recent evidence from animal models indicates that the brain plays a critical role in the systemic regulation of glucose metabolism. Neurons in the hypothalamus integrate hormonal and nutritional information and maintain glucose homeostasis by controlling metabolism in peripheral tissues. Numerous brain regions have been reported to maintain whole-body glucose homeostasis. In particular, the ventromedial nucleus of the hypothalamus (VMH) and arcuate nucleus of the hypothalamus (ARC) are critical nuclei for the glucose metabolism. Obesity can attenuate the function of these nuclei and promotes type II diabetes via hypothalamic inflammation.

However, the hypothalamic mechanism that regulates systemic glucose metabolism is not fully understood. The VMH has important roles in regulating glucose metabolism in peripheral tissues, and the majority of neurons in the VMH express steroidogenic factor 1 (SF1). Photoactivation of SF1 neurons increases hepatic glucose production (HGP) and simultaneously enhances insulin sensitivities in the liver, muscle, and brown adipose tissue (BAT). Leptin regulates the neuronal activity of SF1 neurons, and increases glucose utilization and insulin sensitivity in peripheral tissues. There are two main neuronal populations in the ARC, the orexigenic agouti-related peptide (AgRP) neurons, which co-express neuropeptide Y (NPY), and the anorexigenic proopiomelanocortin (POMC) neurons. POMC and AgRP neurons control HGP in opposite ways, i.e., activation of POMC increases insulin sensitivity in the liver, while AgRP activation decreases liver insulin sensitivity. Hormones, including insulin, leptin, and ghrelin, regulate glucose metabolism by changing the activities of these neurons and their gene expression. In addition, subpopulations of SF1, POMC, and AgRP neurons are also activated (glucose excited neurons) or inhibited (glucose inhibited neurons) by glucose.

Fatty acids regulate the activities of hypothalamic neurons and the lipid metabolism within the hypothalamus plays important roles in energy balance and glucose metabolism. Phospholipids with biologically active polyunsaturated fatty acids (PUFAs), including phosphatidyl-inositol (PI), phosphatidyl-ethanolamine (PE), and phosphatidyl-serine (PS), are abundantly found in the brain. Some membrane phospholipids generate free PUFAs to regulate physiological functions of the brain. For example, phospholipase A2 (PLA2) preferentially mediates AA release and production of prostaglandins by COX1/2, also impaired glucose tolerance (Fig. 2c,d) and increased blood glucose levels after refeeding (Fig. 2e), suggesting that prostaglandins regulate hypothalamic function to decrease blood glucose levels. However, intra-hypothalamic injection of phospholipase C (PLC) inhibitor, MAFP-injected mice showed decreased glucose tolerance compared to vehicle-injected mice and no changes in circulating insulin levels were observed (Fig. 2a,b). Hypothalamic injection of indomethacin, an inhibitor of COX1/2, also impaired glucose tolerance (Fig. 2c,d) and increased blood glucose levels after refeeding (Fig. 2e), suggesting that prostaglandins regulate hypothalamic function to decrease blood glucose levels. However, intra-hypothalamic injection of phospholipase C (PLC) inhibitor, U73122, or IP3 receptor antagonist, xestospondin 2, did not affect glucose tolerance (Supplementary Fig. 3). Thus, our results suggest that the PLA2-mediated AA release and production of prostaglandins by COX1/2 in the hypothalamus, but not PLC-IP3 pathway, play a role in regulating glucose metabolism during acute hyperglycemia.
Fig. 1 Hyperglycemia increases prostaglandin production derived from phospholipids. a, b Representative results of imaging mass spectrometry (IMS) showing distributions of hypothalamic fatty acids (a) and phospholipids (b) from untreated RCD-fed mice. The dashed black line shows the position of the VMH. Scale bar: 500 µm. c–h Distributions of phospholipids and fatty acids in the hypothalamus 30 min after injection of saline (Sal) or glucose (Glc; 2 g/kg). c, f Representative results of IMS on hypothalamic phosphatidyl-inositol (PI; 18:1/20:4) (c) and arachidonic acid (AA) (f) from mice i.p. injected with saline (left half) or glucose (right half). Scale bar: 500 µm. d, e Relative intensities of phospholipids in the VMH (d) and ARC (e) after injection with saline (n = 4) or glucose (n = 4). (two-tailed t test for each molecule, VMH: p = 0.0268 in PI (18:0/20:4), p = 0.0005 in PI (18:1/20:4), and p = 0.0491 in PE (18:0/20:4); ARC: p = 0.0073 in PI (18:0/20:4), p = 0.0347 in PI (18:1/20:4), p = 0.0106 in PE (18:0/20:4), and p = 0.0331 in PS (18:0/16:0), Glc vs Sal). g, h Relative intensities of fatty acids in the VMH (g) and ARC (h) after injection with saline (n = 4) or glucose (n = 4). i–m LC-MS results showing the effects of glucose injection on AA metabolites in the whole hypothalamus. j, k Relative amounts of hypothalamic prostaglandins mediated by cyclooxygenase. Major prostaglandins were underlined. j 6-keto-PGF1α, k PGD2, l 13,14-dihydro-15-keto-PGF2α and m PGE2 were increased by glucose injection (two-tailed t test, p = 0.0009 in j, p = 0.0244 in k, p = 0.0011 in l, p = 0.0099 in m, n = 5/each, Glc vs Sal). d–h and j–m represent the mean ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001. i represents the mean fold change in color. PA palmitic acid, SA stearic acid, AA arachidonic acid, DHA docosahexaenoic acid, PE phosphatidyl-ethanolamine, PI phosphatidyl-inositol, PS phosphatidyl-serine.
PLA2-mediated production of prostaglandin is necessary for the responsiveness of the VMH to glucose. To understand the role of PLA2 in controlling glucose metabolism, we examined the effect of PLA2 inhibitors on hypothalamic neuronal activation by cFos expression. Vehicle, MAFP, or indomethacin were injected intracerebroventricularly (i.c.v.) 30 min prior to i.p. injection of either saline or glucose in fasted mice. In i.c.v. vehicle-injected mice, glucose administration increased cFos-positive cells in the dorsomedial (dm) and ventrolateral (vl) regions of the VMH and in the ARC (Fig. 2f–h). In i.c.v. MAFP-injected mice, glucose did not alter the number of cFos-positive neurons in the dmVMH (Fig. 2g). An increase in cFos-positive neurons after glucose injection was still detected in the vlVMH and ARC compared with saline injected mice (Fig. 2g, h). Similar results were observed in i.c.v. indomethacin-injected mice after an i.p. injection of glucose (Fig. 2g, h). Taken together, these data showing that both MAFP and indomethacin block neuronal activation during acute hyperglycemia in the dmVMH, indicates that metabolites of phospholipid-derived prostaglandins regulate glucose responsiveness of neurons in the dmVMH, while glucose activates neurons in the vlVMH and ARC independently of PLA2 and COX1/2.

The role of PLA2 in the leptin-induced neuronal activation was also assessed in the i.c.v. MAFP-injected mice. In i.c.v. vehicle-injected mice, leptin injection increased cFos-positive cells in the dmVMH (Supplementary Fig. 4a–c) and it also increased...
Knockdown of \textit{pla2g4a} in Sf1 neurons impairs glucose metabolism in regular chow diet feeding. Next, to explore the role of \textit{PLA2} in VMH neurons, short hairpin RNA (shRNA) against \textit{pla2g4a}, a gene encoding cytosolic PLA2 (c\textit{PLA2}), which has specificity for sn-2 arachidonic acid and a role in eicosanoid production, was transfected to the VMH through an adenovirus-associated virus (AAV) cre-recombinase (cre)-dependent in Sf1-cre mice (Supplementary Fig. 5a, b). Expression of \textit{pla2g4a} mRNA was significantly decreased in the VMH of Sf1-cre mice injected with AAV-DIO-shRNA (c\textit{PLA2KD} SN1) compared with AAV-DIO-GFP (GFP \textit{SN1})-injected mice (Supplementary Fig. 5c). The hypothalamic c\textit{PLA2} activity was not changed by glufosfate injection in C57BL/6J mice (Supplementary Fig. 5d). The activity was measured by the tissue homogenate of the hypothalamus, in which the cellular calcium concentration was not conserved. Thus, the hypothalamic c\textit{PLA2} activity in response to glucose may be regulated by cytosolic calcium concentration. However, the decrease of AA-contained-phospholipids in the VMH by glucose injection was blocked by shRNA transfection in c\textit{PLA2KD} SN1 mice (Supplementary Fig. 5e, f). In the ARC, these phospholipids were still decreased by glucose (Supplementary Fig. 5g, h), suggesting that the AAV-induced shRNA expression only affects neurons in the VMH rather than in the ARC. We further confirmed that the cre-dependent shRNA expression is restricted in the VMH without leaking to the ARC. The AAV-DIO-GFP was injected into the VMH of Sf1-cre mice and a-melanocyte stimulating hormone (a-MSH) was stained as an indicator of POMC neurons. In the image (Supplementary Fig. 5h), POMC neurons appear to be distributed in the main part of the ARC, but fewer in the dorsal part. GFP-positive neurons were not merged with POMC neurons (Supplementary Fig. 5i, j), suggesting that the AAV-mediated gene expression occurred in the VMH, but not in the ARC.

After 8 weeks of viral injection, knockdown of c\textit{PLA2} in the Sf1 neurons decreased glucose tolerance and insulin sensitivity compared to GFP \textit{SN1} mice (Fig. 3a, b), although it did not change body weight or weights of adipose tissues, muscle, and liver (Supplementary Fig. 6a). These changes in the glucose metabolism depend on the accuracy of the AAV injection, since the mice with missed injection site of the virus had no effects on glucose and insulin tolerance (Supplementary Fig. 6b–d). c\textit{PLA2KD} SN1 mice also increased blood glucose levels after refeeding compared with GFP \textit{SN1} mice (Fig. 3c). 2-deoxy-glucose (2DG)-induced hyperglycemia, which represents a glucose deprivation-induced counter-regulatory responses, was comparable between groups (Fig. 3d). To rule out the involvement of astrocytic c\textit{PLA2}, AAV-GFAP-Cre, and AAV-DIO-shRNA against \textit{pla2g4a} were co-injected into the hypothalamus of wild type mice to knockdown the expression of \textit{pla2g4a} in hypothythic astrocytes (Supplementary Fig. 7a, b). The knockdown of c\textit{PLA2} in astrocytes did not alter glucose metabolism, insulin sensitivity, or body weight compared with control mice (Supplementary Fig. 7c–e). Thus, our data suggest that c\textit{PLA2} in Sf1 neurons, not astrocytes, regulates peripheral glucose metabolism.

To further investigate the role of c\textit{PLA2} in Sf1 neurons in glucose metabolism, we next performed hyperinsulinemic–euglycemic clamp studies. c\textit{PLA2KD} SN1 mice showed a lower glucose infusion rate (GIR) to maintain euglycemia compared with GFP \textit{SN1} mice (Fig. 3e–g). The rate of disappearance (Rd) and glycolysis were also lower in c\textit{PLA2KD} SN1 mice compared with GFP \textit{SN1} mice (Fig. 3h, i). However, endogenous glucose production (EGP) was not different between the two groups (Fig. 3j, k), suggesting that glucose utilization, rather than EGP, was impaired in c\textit{PLA2KD} SN1 mice. In agreement with this, c\textit{PLA2KD} SN1 mice showed decreased 2DG uptake in the red part of gastrocnemius muscle (GR) compared with control mice (Fig. 3i). 2DG uptake in white adipose tissue (WAT) and the brain (cortex) were similar between groups (Fig. 3m, n).

To assess changes in neuronal activation, we next analyzed cFos expression in c\textit{PLA2KD} SN1 mice compared with controls. Glucose-induced cFos expression in the dmVMH of c\textit{PLA2KD} SN1 mice was blunted compared with glucose-injected control mice (Fig. 3o, p). The glucose-induced cFos expression in either vVMH or ARC was not changed after the knockdown of c\textit{PLA2} (Fig. 3p, q).

Taken together, our data suggest that c\textit{PLA2}-mediated prostaglandin production regulates glucose-induced activation of dmVMH neurons to control insulin sensitivity in muscle.

High-fat diet decreases AA-containing phospholipids and produces prostaglandins in the hypothalamus. High-fat diet (HFD) induces inflammation and impairs hypothalamic functions. Long chain fatty acyl CoAs, a proinflammatory signal, accumulates in the hypothalamus during HFD feeding. Thus, we examined the effect of HFD on lipid distribution in the hypothalamus. In mice fed an HFD for 8 weeks, the signal intensities for FAs, including AA, were greater in the ARC but not the VMH than those observed in control mice fed a RCD (Fig. 4a–c). However, signal intensities for phospholipids in the hypothalamus were lower in HFD-fed mice (Fig. 4d–f). In both VMH and ARC, the signal intensities for PI (18:0/20:4), PI (18:1/20:4), PE (18:0/20:4), PE (p18:0/20:4), and PS (18:0/22:6) were significantly decreased in HFD-fed mice (Fig. 4e, f). The signal intensity for lysophosphatidyl-inositol (LPI; 18:0) was also higher in the VMH and ARC of HFD-fed mice compared to RCD-fed mice (Supplementary Fig. 8). Because c\textit{PLA2} generates AA from these phospholipids to regulate cellular activities, we next analyzed the activity of hypothalamic c\textit{PLA2} and found that c\textit{PLA2} activity was higher in HFD-fed mice compared with RCD-fed mice (Fig. 4i, j). The phosphorylation of c\textit{PLA2} at Ser505, which stimulates its enzymatic activity, was measured by western blotting (Fig. 4i, j). The phosphorylation of c\textit{PLA2} in the hypothalamus was higher in the HFD-fed mice compared with RCD-fed mice, while total amount of c\textit{PLA2} was not changed (Fig. 4i, k).

We next explored the effect of HFD on the production of eicosanoids in the hypothalamus by LC-MS (Fig. 4l and Supplementary Fig. 9). In HFD-fed mice, AA tended to be higher but did not reach significant difference (Fig. 4m). COX-mediated production of prostaglandins, including PGD2, PGE2, PGF2α, PGE2, 11-beta-13,14-dihydro-15-keto-PGF2α, 13,14-dihydro-15-keto-PGD2, and 20-hydroxy-PGF2α, was increased in HFD-fed mice compared to RCD-fed mice (Fig. 4n–s). Among lipoxgenase-mediated eicosanoids, only 12-HETE was significantly increased after HFD feeding (Supplementary Fig. 9).

Knockdown of \textit{pla2g4a} improves HFD-induced impairment of glucose metabolism and recovers glucose responsiveness of the vVMH and ARC to hyperglycemia. To understand the role of HFD-induced activation of hypothalamic c\textit{PLA2}, we fed c\textit{PLA2KD} SN1 mice with HFD for 8 weeks and examined the role...
of cPLA2 on glucose metabolism. Body weight and tissue weight of HFD-fed cPLA2KD^{SI} mice (cPLA2KD^{SI}-HFD) were comparable to those of HFD-fed control GFP^{SI} mice (GFP^{SI}-HFD; Fig. 5a and Supplementary Fig. 10). Unlike RCD-fed mice (Fig. 3a), knockdown of cPLA2 in Sf1 neurons increased glucose tolerance (Fig. 5b). However, insulin tolerance test showed no difference between groups (Fig. 5c). The similar results were also observed in female mice. The body weight was not changed between female GFP^{SI}-HFD and female cPLA2KD^{SI}-HFD mice (Supplementary Fig. 11a). Knockdown of cPLA2 in Sf1 neurons increased glucose tolerance but not insulin sensitivity (Supplementary Fig. 11b, c). The tissue weight was also not changed by knockdown of cPLA2 in Sf1 neurons of female mice (Supplementary Fig. 11d).

In GFP^{SI}-HFD mice, no significant changes in cFos-positive neurons in the VMH or ARC were observed after glucose injection compared with saline injection (Fig. 5d–f), suggesting that HFD inhibits glucose sensing in the VMH and the ARC, which has been already reported in previous studies\(^{26-30}\). However, in cPLA2KD^{SI}-HFD, the number of cFos-positive neurons were significantly higher in the vlVMH and ARC after glucose injection compared with saline injection, suggesting that cPLA2 knockdown improved neuronal responsiveness to glucose under the HFD-fed condition (Fig. 5d–f).

To understand the role of hypothalamic cPLA2 on glucose metabolism in HFD-fed mice, we performed hyperinsulinemic–euglycemic clamping (Fig. 5g–m). To maintain euglycemia, lower GIR was required in GFPSf1-HFD (Fig. 5i, black) than RCD-fed GFPSf1 mice (Fig. 3g, black), suggesting that the HFD-fed mice became insulin resistant and thus lower glucose was needed to maintain euglycemia in the HFD-fed mice than RCD-fed mice. In the HFD-fed groups, significantly higher GIR was required in cPLA2KD^{SI}-HFD than in GFP^{SI}-HFD (Fig. 5g–i). Unlike RCD-fed mice (Fig. 3h, i), glucose utilization (Rd and glycolysis) in HFD-fed mice was not altered by knocking down cPLA2 (Fig. 5j, k). In contrast, insulin inhibition of EGP was stronger during the clamp period in cPLA2KD^{SI}-HFD mice than in GFP^{SI}-HFD (Fig. 5l, m). These results suggest that cPLA2 in the VMH...
Fig. 3 Knockdown of Sf1-neuronal pla2g4a impairs systemic glucose metabolism. a Glucose tolerance test in cPLA2KD Sf1 (n = 6) and GFP Sf1 mice (n = 6; two-way ANOVA followed by Sidak multiple comparison test, p = 0.0126 at time = 60, cPLA2KD Sf1 vs GFPSf1; two-tailed t test in area under the curve (AUC) during GTT, p = 0.0433, cPLA2KD Sf1 vs GFPSf1). b Insulin tolerance test in cPLA2KD Sf1 (n = 6) and GFPSf1 mice (n = 9; two-way ANOVA followed by Sidak multiple comparison test, p = 0.0094 at time = 60, cPLA2KD Sf1 vs GFPSf1; two-tailed t test in AUC during GTT, p = 0.0071, cPLA2KD Sf1 vs GFPSf1). c Blood glucose levels after refeeding in cPLA2KD Sf1 (n = 6) and GFPSf1 mice (n = 9; two-way ANOVA followed by Sidak multiple comparison test, p = 0.0255 at time = 30, p = 0.0003 at time = 60, p = 0.0211 at time = 120, cPLA2KD Sf1 vs GFPSf1). d 2-deoxy-glucose (2DG)-induced hyperglycemia in cPLA2KD Sf1 (n = 6) and GFPSf1 mice (n = 6). n-4 Hyperinsulinemic-euglycemic clamp studies in cPLA2KD Sf1 or GFPSf1 mice. f The glucose infusion rate (GIR) required to maintain euglycemia during the clamp period in cPLA2KD Sf1 (n = 7) or GFPSf1 mice (n = 7). g The average GIR between 75 and 115 min in cPLA2KD Sf1 (n = 7) or GFPSf1 mice (n = 7; two-tailed t test, p = 0.0395, cPLA2KD Sf1 vs GFPSf1). h The rate of glucose disappearance (Rd) during the clamp period, which represents whole-body glucose utilization (two-tailed t test, p = 0.0355, cPLA2KD Sf1 vs GFPSf1). i The rates of whole-body glycolysis in cPLA2KD Sf1 (n = 7) or GFPSf1 mice (n = 7; two-tailed t test, p = 0.0497, cPLA2KD Sf1 vs GFPSf1). j Endogenous glucose production (EGP) during both the basal and clamp periods in cPLA2KD Sf1 (n = 7) or GFPSf1 mice (n = 7). k Insulin-induced suppression of EGP, which represents hepatic insulin sensitivity in cPLA2KD Sf1 (n = 7) or GFPSf1 (n = 7). l-n Graphs showing 2-[14C]-Deoxy-D-Glucose uptake in red portions of the gastrocnemius (GR; l), white adipocyte (EWAT; n; two-tailed uorescent cFos staining in the hypothalamus of cPLA2KD Sf1 and GFPSf1 mice after saline or glucose injection (3 g/kg). Scale bar: 500 μm. p, q Quantification of cFos expression in the dmVMH, cVMH, vVMH, and ARC of cPLA2KD Sf1 or GFPSf1 mice after saline (n = 3) or glucose (n = 3) injection (3 g/kg; VMH: two-way ANOVA followed by Sidak multiple comparison test, for dmVMH: p = 0.0001 GFPSf1 Sal vs GFPSf1 Glc, p = 0.0001 GFPSf1 Glc vs cPLA2KD Sf1 Glc; for vVMH: p = 0.0001 GFPSf1 Sal vs GFPSf1 Glc, p = 0.0001 cPLA2KD Sf1 Sal vs cPLA2KD Sf1 Glc. For ARC: one-way ANOVA followed by Sidak multiple comparison test, p = 0.0425 GFPSf1 Sal vs GFPSf1 Glc, p = 0.0174 cPLA2KD Sf1 Sal vs cPLA2KD Sf1 Glc.) All data represent the mean ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

Discussion

The roles of hypothalamic phospholipids and eicosanoids in the regulation of energy homeostasis is ill-defined. In this study, we found that the composition of phospholipids in the hypothalamus, especially AA attached phospholipids, are dynamically affected by blood glucose levels. cPLA2 in the VMH plays an important role in AA metabolism to produce prostaglandins and increase insulin sensitivity in skeletal muscles during hyperglycemia in RCD-fed mice. cPLA2-mediated phospholipid metabolism also regulates glucose-responsiveness in the dmVMH. HFD feeding, which promotes hyperglycemia, continuously activates cPLA2 and produces prostaglandins, and thus induces inflammation in the hypothalamus and attenuates insulin sensitivity in the liver (Fig. 7). Therefore, cPLA2-mediated phospholipid metabolism in the hypothalamus is critical for the physiological and pathological control of systemic glucose homeostasis.

FAs and PUFA s are believed to be transported from the bloodstream to the hypothalamus, and FA metabolism in the hypothalamus changes food intake and energy expenditure. However, we observed reductions in AA-containing phospholipids, increases in lysoforms and increases in prostaglandins in the hypothalamus after glucose injection. This suggests that AA is produced from intrinsic membrane phospholipids in the hypothalamus to make eicosanoids during hyperglycemia. The produced prostaglandins play important roles in controlling hyperglycemia because the injection of COX inhibitor impaired glucose tolerance. It has been reported that FA oxidation by carnitine palmitoyltransferase I in the VMH plays important roles in food intake and energy homeostasis. However, our data showed that the cPLA2 in Sf1 neurons has a minor effect on changes in body weight and tissue weight. This suggests that cPLA2 in Sf1 neurons controls glucose metabolism, but not body weight regulation, and it is likely that FAs generated from phospholipids are utilized for prostaglandin production.

AA exists in the sn-2 position of phospholipids, and cPLA2 is the rate-limiting enzyme for catalyzing AA by extracellular stimulation. cPLA2 is activated by an increase in the intracellular calcium concentration and by the phosphorylation of 505-serine residue, which is induced by the MAP kinase pathway. The mechanism that activated cPLA2 in our study remains to be elucidated. However, we found that the glucose-induced activation of the dmVMH is dependent on prostaglandin production by Sf1 neurons. Sf1 neurons exist mainly in the dmVMH, and most AA-containing phospholipids were found near the third ventricle in our study. Therefore, the hyperglycemia-induced prostaglandin production occurs in the medial part of the hypothalamus and affects neuronal activity in this region probably via changes in ion channel activities. Similarly, prostaglandins regulate glucose-stimulated insulin secretion (GSIS) from pancreatic beta cells.
GSIS is the most studied mechanism of glucose sensing. Thus, it is possible that a similar mechanism for prostaglandins affecting GSIS may be involved in the hypothalamic glucose sensing. Neurons in the vlVMH and ARC are glucose sensing neurons. In agreement with this, the glucose injection increased cFos-positive cells in the vlVMH and ARC. However, i.c.v. injection of inhibitors for cPLA2 and COX1/2 did not change the glucose-induced increase in cFos, suggesting that the prostaglandin production has a minor role in the glucose sensing by vlVMH and ARC. We also found that cPLA2 regulates neuronal activity of the dmVMH in response to leptin. Therefore, cPLA2 may be required for the activation of dmVMH not only by glucose but also by peripheral hormones.

Sfl neurons are critical for the regulation of whole-body energy homeostasis. Activation of VMH neurons increases glucose uptake in skeletal muscle and BAT, but not in WAT or other organs. Similar results were found in mice with intra-VMH administration of leptin. Leptin receptors are located in the dmVMH and required to maintain normal glucose homeostasis. In agreement with this, glucose sensing by Sfl neurons via UCP2 is also critical for systemic glucose metabolism.
glucose injection regulates insulin sensitivity in skeletal muscle through cPLA2-mediated prostaglandin production.

VMH neurons are also known to evoke counter regulatory responses (CRR) during hypoglycemia. We firstly thought that the fasting may affect the neuronal activity of glucose inhibited neurons in the VMH to evoke CRR. However, blood glucose levels after fasting or 2DG injection were similar between cPLA2KDSf1 and GFPStf1 mice, suggesting that the cPLA2 in the VMH has minor roles in the CRR. Thus, we started to use fasted mice in GTT because it can set the feeding condition similar in each animal.

A HFD feeding causes diet-induced-obesity and a state of chronic, low-grade inflammation occurs in several tissues, including the hypothalamus. This hypothalamic inflammation is accompanied by an activation of microglia, and these changes decrease activities of POMC and AgRP neurons in response to several endocrine signals, such as leptin and insulin. We also found that microglia was activated by the HFD feeding in both VMH and ARC. Additionally, a HFD feeding increases astrogliosis in the ARC, paraventricular hypothalamus and dorsomedial hypothalamus, but not the VMH. Our data also shows that a HFD feeding induces astrogliosis in the ARC but not the VMH.

After the mice in this study were fed with HFD, FAs such as AA, OA, PA and SA accumulated in the hypothalamus, especially in the ARC. However, AA-containing phospholipids decreased because of an increase in hypothalamic cPLA2 activity. The increased cPLA2 activity is regulated by phosphorylation of cPLA2, but not its protein expression. Prostaglandins are proinflammatory signals in the brain and knockdown of cPLA2 in Sf1 neurons attenuates inflammation in the hypothalamus. Knockdown of cPLA2 in Sf1 neurons of female mice also improved glucose metabolism and ameliorated hypothalamic inflammation, suggesting that the effects of cPLA2 in HFD-fed mice do not depend on sex in our study, even though the responses to HFD are reported to be different between male and female mice.

It is possible that the long-term production of prostaglandins, which has a physiological role in glucose metabolism in RCD-fed mice, initiates HFD-induced inflammation which impairs neuronal functions. A limitation of this study is that we do not know how long the prostaglandin−induced neuroinflammation last after the end of HFD feeding. This should be investigated in future because this is important for the clinical intervention to treat type II diabetes after changing the food.

It is notable that the species of PGs produced by the glucose injection in RCD-fed mice are different from PGs after HFD feeding (Figs. 1 and 4). Glucose injection increased 6-keto-PGF1α and 13,14-dihydro-15-keto-PGF2α, while HFD feeding stimulated the production of PGF2α, 11-beta,13,14-dihydro-15-keto-PGF2α, 13,14-dihydro-15-keto-PGD2, and 20-hydroxy-PGF2α in 8 weeks of HFD-fed mice (n = 3) compared with those of RCD-fed mice (n = 3). Major phospholipids were underlined. m–s Bar graphs showing COX-mediated production of AA (m), PGD2 (n), PGF2α (o), PGE2 (p), 11-beta,13,14-dihydro-15-keto-PGF2α (q), 13,14-dihydro-15-keto-PGD2 (r), and 20-hydroxy-PGF2α (s) in 8 weeks of HFD-fed mice (n = 3). Two-tailed t test, p < 0.05; ***p < 0.001; ****p < 0.0001.

VNVMH. The VNVMH is filled with glucose-sensing neurons, both glucose-excited and glucose-inhibited neurons, and regulated systemic glucose balance. Our results show that HFD feeding inhibited neuronal glucose-sensing in the VNVMH. This impairment
of glucose-sensing in vlVMH was recovered in cPLA2KD mice accompanied with ameliorated hypothalamic inflammation. Therefore, the cPLA2 may play a deteriorative role in the glucose responsiveness of the VMH/ARC by inducing hypothalamic inflammation. In the present study, we did not knockdown cPLA2 in neurons of the ARC and accumulations of fatty acids were observed in the ARC after HFD feeding. Thus, the importance of PG production and lipid synthesis in the ARC on inflammation is not clearly defined. Further study is needed to understand the whole mechanism which initiates and deteriorates hypothalamic inflammation.

Our data suggest that inflammation of the hypothalamus contributes to attenuating glucose sensing by the VMH and ARC. POMC and AgRP neurons are reported to regulate hepatic insulin sensitivity, but not muscle glucose metabolism. Therefore, the improvement of the neuronal activity in the ARC contributes to restoring glucose metabolism by changing insulin sensitivity in the liver.
Aspirin, a COX inhibitor, suppresses insulin sensitivity in healthy human subjects, but improves insulin resistance in diabetic patients. Our results were in good agreement with the human studies. The hypothalamic prostaglandin production may be critical for the effects of aspirin on the whole-body insulin sensitivity.

In summary, our study shows that the cPLA2 is fundamental for the function of the hypothalamus in regulating glucose homeostasis. Neuronal cPLA2 is necessary for their own activities in the dmVMH to respond to glucose and control blood glucose levels. However, cPLA2 in the VMH also has the critical role in inducing hypothalamic inflammation during HFD feeding. Therefore, the role of cPLA2-mediated eicosanoid production in the hypothalamus is different between RCD and HFD. Our findings provide evidence that cPLA2-mediated phospholipid metabolism in hypothalamic neurons plays an important role in systemic glucose metabolism.

Methods
Reagents. All the reagents and resources used in this study are listed in the Supplementary Table 1.

Animals. Sf1-cre mice were purchased from the Jackson Laboratory (STOCK Tg(NR5a1-cre)7Lowl/J; Bar Harbor, ME). For IMS and assessing the effects of inhi-...tions: AP: 1.4, L: ± 0.4, DV: − 5.6. Cannules were secured on the skulls with cyanoacrylate glue and the exposed skulls were covered with dental cement.

Stereotaxic surgeries and AAV injection. Male C57BL/6J mice were anesthetized with mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and were put on a stereotaxic instrument (Narishige, Tokyo, Japan). Mice were implanted with cannules for intracerebroventricular (i.c.v.) or intra-hypothalamic injection. The i.c.v. cannules were implanted in the lateral ventricle in an anterior–posterior (AP) direction: −0.3 (0.3 mm posterior to the bregma), lateral (L): 1.0 (1.0 mm lateral to the midline). Brains were collected to check the injection site (see the section of “Immunohistochemistry” below). The mice, in which the AAV injection was not successful, were excluded from the data.

Measurement of blood glucose levels after glucose, insulin, 2DG or refedging. A glucose tolerance test was performed on ad libitum fed or fasted mice. The ad

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Fig. 5 Knockdown of cPLA2 improves HFD-induced impairment of glucose metabolism. a Body weight change in cPLA2KDSf1 mice (n = 12) and GFPSf1 mice (n = 11). b Glucose tolerance test on cPLA2KDSf1 mice (n = 12) and GFPSf1 mice (n = 10; two-way ANOVA followed by Sidak multiple comparison test, p = 0.0415 at time = 15, p = 0.0482 at time = 30, cPLA2KDSf1 vs GFPSf1). c Two-tailed t test in area under the curve (AUC) during GTT, p = 0.0428, cPLA2KDSf1 vs GFPSf1. d Insulin tolerance test on cPLA2KDSf1 (n = 8) mice and GFPSf1 (n = 6) during 8 weeks of HFD feeding. d Representative micrographs showing immunofluorescent cFos staining in the hypothalamus of HFD-fed cPLA2KDSf1 and GFPSf1 mice after saline or glucose injection. Scale bar: 500 μm. f Quantification of cFos expression in the dmVMH, cVMH, vVMH (e) and ARC (f) of HFD-fed cPLA2KDSf1 or GFPSf1 mice after saline or glucose injection (n = 3–4 in each experimental group; VMH: two-way ANOVA followed by Sidak multiple comparison test, for vVMH: p = 0.0231 cPLA2KDSf1 Sal vs GFPSf1 Sal, p = 0.0144 GFPSf1 Glc vs cPLA2KDSf1 Glc. For ARC: one-way ANOVA followed by Sidak multiple comparison test, p = 0.0176 cPLA2KDSf1 Sal vs GFPSf1 Glc, p = 0.0444 GFPSf1 Glc vs cPLA2KDSf1 Glc. g–m Hyperinsulinemic–euglycemic clamp studies in HFD-fed cPLA2KDSf1 (n = 7) or GFPSf1 (n = 7) mice. g The glucose infusion rate (GIR) required to maintain euglycemia during the clamp period in cPLA2KDSf1 (n = 7) or GFPSf1 (n = 6) mice. I Endogenous glucose production (EGP) for the function of the hypothalamus in regulating glucose metabolism. The hypothalamic prostaglandin production may be critical for the effects of...
Fig. 6 Knockdown of cPLA2 prevents HFD-induced microgliosis and astrogliosis. a-c Left: representative micrographs showing immunochemistry GFAP staining in the hypothalamus of RCD-fed GFPSf1 mice (GFPSf1-RCD) (a), HFD-fed GFPSf1 mice (GFPSf1-HFD) (b), and HFD-fed cPLA2KDsf1 (cPLA2KDsf1-HFD) mice (c). Scale bar: 500 μm. Right: magnified areas in the VMH and ARC in the left. Scale bar: 30 μm. d,e, Quantification of GFAP-positive cells in the VMH (d) or ARC (e) of GFPSf1-RCD (n = 4), GFPSf1-HFD (n = 4) and cPLA2KDsf1-HFD (n = 4) mice (one-way ANOVA followed by Sidak multiple comparison test, in e, p = 0.0007 GFPSf1 RCD vs GFPSf1 HFD, p = 0.0045 GFPSf1 HFD vs cPLA2KDsf1 HFD). f,g, Size of GFAP-positive cells in in the VMH (f) or ARC (g) of GFPSf1-RCD (n = 3), GFPSf1-HFD (n = 3), and cPLA2KDsf1-HFD (n = 3) mice (one-way ANOVA followed by Sidak multiple comparison test, in g, p = 0.0002 GFPSf1 RCD vs GFPSf1 HFD, p = 0.0012 GFPSf1 HFD vs cPLA2KDsf1 HFD). h-j Left: representative micrographs showing immunochemistry Iba1 staining in the hypothalamus of GFPSf1-RCD (h), GFPSf1-HFD (i) and cPLA2KDsf1-HFD mice (j). Scale bar: 500 μm. Right: magnified areas in the VMH and ARC from the left photos. Scale bar: 30 μm. k,l Quantification of Iba1-positive cells in the VMH (k) or ARC (l) of GFPSf1-RCD (n = 3), GFPSf1-HFD (n = 3), and cPLA2KDsf1-HFD (n = 3) mice (one-way ANOVA followed by Sidak multiple comparison test, in l, p = 0.0049 GFPSf1 RCD vs GFPSf1 HFD, p = 0.0310 GFPSf1 HFD vs cPLA2KDsf1 HFD). m,n Size of Iba1-positive cells in the VMH (m) or ARC (n) of GFPSf1-RCD (n = 3), GFPSf1-HFD (n = 3), and cPLA2KDsf1-HFD (n = 3) mice (one-way ANOVA followed by Sidak multiple comparison test, in m, p < 0.0001 GFPSf1 RCD vs GFPSf1 HFD, p = 0.0004 GFPSf1 HFD vs cPLA2KDsf1 HFD, in n, p < 0.0001 GFPSf1 RCD vs GFPSf1 HFD, p = 0.0004 GFPSf1 HFD vs cPLA2KDsf1). All data represent the mean ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001.
Fig. 7 Distinct roles of prostaglandin in the regulation of peripheral glucose metabolism. In RCD-fed mice, a glucose injection activates cPLA2 in the VMH, which increases the production of prostaglandins from neurons. The increase in prostaglandins is critical for the activation of dmVMH and glucose metabolism in peripheral tissues. Chronic HFD feeding also increases the cPLA2-mediated prostaglandin production from VMH neurons. The chronic effect of prostaglandins enhances hypothalamic inflammation and thus impairs peripheral glucose metabolism.

Immunohistochemistry. Ad libitum fed mice were i.p. injected with either saline or glucose (3 g/kg), killed using CO2 asphyxiation and perfused with heparinized saline for 4% paraformaldehyde (PFA) transcardially at 30 min after injection. Inhibitors were i.c.v. injected 30 min before glucose injection. To assess the effect of MAFP on leptin signals, leptin was injected i.c.v. 10 min after i.c.v. injection of MAFP. Mice were killed 30 min after leptin injection and perfused with saline and 4% PFA as mentioned above. Brain sections (50 μm each) containing the whole VMH were collected. For cFos and GFP staining, the floating sections were incubated with rabbit-anti-c-Fos antibody (1:200, Santa Cruz Biotechnology, Den- ton, TX) or rabbit-anti-GFP antibody (1:1000, Frontier Institute, Hokkaido, Japan) in staining solution (0.1 M phosphate buffer (PB) containing 4% normal guinea pig serum, 0.1% glycine, and 0.2% Triton X-100) overnight at room temperature. After rinsing with PB, sections were incubated in secondary antibody (1:500, Alexa 488 or 647 Goat Anti-Rabbit (IgG) secondary antibody, Cell Signaling Technologies, Danvers, MA) for 2 h at room temperature. For staining of phosphorylated STAT3 (pSTAT3), antigen was retrieved by incubated with 10 mM citrate sodium at 80 °C for 30 min. Sections were then incubated with Rabbit-anti-pSTAT3 (Tyr 705) antibody (Cell Signaling Technologies) in staining solution overnight at 4 °C. After rinsing with PB, sections were incubated with biotinylated-Goat-Anti-Rabbit (IgG) secondary antibody (1:500, Thermo Fisher Scientific) for 1 h followed by incubated in Alexa 488-streptavidin for 2 h at room temperature. The stained sections were washed with PB three times and mounted on glass slides with vectashield (Vector Laboratories, Burlingame, CA).

To assess the cell population of astrocytes and microglia, sections were treated with 1% H2O2 for 20 mins followed by 20 min treatment of 0.1% Triton-X 100 at room temperature. The sections were washed with PB 3 times and incubated with rabbit-anti-Iba1 antibody (1:3000, Sigma-Aldrich) in staining solution overnight at 4 °C. After rinsing with PB, sections were incubated with biotinylated-Goat-Anti-Rabbit (IgG) secondary antibody (1:500, Thermo Fisher Scientific) for 1 h followed by incubated in Alexa 488-streptavidin for 2 h at room temperature. The stained sections were washed with PB three times and mounted on glass slides with vectashield (Vector Laboratories, Burlingame, CA). Cell size (Iba1- and GFAP-positive cells) in VMH and ARC, a total of 10–13 cells in each nucleus per section, was measured using ImageJ software. Cell size (Iba1- and GFAP-positive cells) in VMH and ARC, a total of 10–13 cells in each nucleus per section, was measured using ImageJ software.

Assessment of cytosolic- or secretory-phospholipase-A2 activity. Mice fed ad libitum were i.p. injected with either glucose (2 g/kg) or saline. Mice were killed using CO2 asphyxiation and mouse hypothalami were collected 30 min after injection and stored at −80 °C until use. Tissues were homogenized and centrifuged at 10,000×g for 15 min at 4 °C and supernatants were collected. Activity of cytosolic- or secretory-phospholipase-A2 was measured following procedures described in the kit manuals (Abcam, Cambridge, UK).
Hyperinsulinemic–euglycemic clamp and measurement of 2-[^14C] deoxy-o-glucose (2DG) uptake. The hyperinsulinemic–euglycemic clamp protocol was followed as described in previous papers.14 The mice were fasted for 4 h and experiments were initiated in a free moving condition. A 115 min clamp period (t = 0–115 min) was following a 90-min basal period (t = 90 to 0 min). A bolus of [3H]-glucose (5 mCi) was injected through the jugular vein at the beginning of the basal period (t = 90 min) and tracer was infused at a rate of 0.05 mCi for 90 min. Blood samples were collected at t = 15 and 5 min to measure the rate of appearance (Ra). The clamp period was initiated with continuous infusion of insulin (2.5 mU/kg/min). During the clamp period, blood was collected and blood glucose levels were measured from arterial blood every 5–10 min. Cold glucose was infused at a variable rate via the jugular vein to maintain a blood glucose level at 110 mg/dL. Erythrocytes in withdrawn blood were suspended in sterile saline and returned to each animal. To assess 2DG uptake, 2-[14C] DG (10 mCi) was infused at t = 70 min and blood samples were collected at t = 75, 85, 95, 105, and 115 min. After collecting the blood sample at t = 115 min, mice were euthanatized by an intravenous infusion of thiopental sodium (Nipro, Osaka, Japan), and small pieces of tissue samples from the soleus, Gastro-R (red portion of gastrocnemius), Gastro-W (white portion of gastrocnemius), BAT, heart, spleen, EWAT (epididymal white adipose tissue), brain (cortex), and liver were rapidly collected. The rate of disappearance (Rd), which reflects whole-body glucose utilization, rate of appearance (Ra), which mainly reflects endogenous glucose production (EGP), and the rates of whole-body glycolysis and glycogen synthesis were determined as described previously.12 In the steady state, the Ra is equal to the Rd. At first, Rd was calculated by the ratio of 3H-glucose infusion rate (dpm/min) to the specific activity of plasma glucose (dpm/mg), which is a ratio of plasma 3H-glucose (dpm/mg) to glucose levels (mg/dL). In the clamp period, Ra is composed of EGP and GIR. Thus, EGP is calculated by subtracting GIR from Rd. Whole-body glycolysis was calculated by using plasma 3H.O.EGP is a value showing glucose production (gluconeogenesis and glycogenolysis) from the liver to the circulation, but it also includes gluconeogenesis from the kidney and other tissues. Insulin can suppress hepatic glucose production. Therefore, the suppression of EGP by insulin (Figs. 3k and 5m) mainly represents the hepatic insulin sensitivity.

Statistical analysis and reproducibility. Two-way or one-way ANOVA were used to determine the effect of inhibitors or knockdown of cPLA2 with the Prism 8 software (GraphPad). For repeated-measures analysis, ANOVA was used when values for different times were analyzed, followed by the Sidak multiple comparisons test. When only two groups were analyzed, statistical significance was determined by the unpaired Student’s t-test (two-tailed p value). A value of p < 0.05 was considered statistically significant. All data are shown as mean ± SEM. Representative image was from at least three independent experiments.

Data availability Source data are provided with this paper. Any data not included in the source data file is available upon request. Received: 12 April 2020; Accepted: 12 March 2021; Published online: 20 April 2021

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Author contributions

C.T. conceived this study and designed the experiments. M.L. performed most of the experiments and C.T. supervised the entire study. H.M. performed the study on astrocytes. Y.S. performed LC-MS measurements. T.H. and T.I. performed imaging mass spectrometry. I.Y. and D.I. performed GTT, ITT, and AAC injections. M.S. N.I., K.K., and D.S. assisted in preparing the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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