Important role of ventromedial hypothalamic carnitine palmitoyltransferase-1a in the control of food intake

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1Department of Pediatrics, Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Alberta, Canada; 2Department of Biochemistry and Molecular Biology, Facultat de Farmàcia, Universitat de Barcelona, Barcelona, Spain; and 3Institut de Biomedicina de la Universitat de Barcelona and CIBER Fisiopatología de la Obesidad y la Nutrición, Instituto de Salud Carlos III, Madrid, Spain

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Gao S, Serra D, Keung W, Hegardt FG, Lopaschuk GD. Important role of ventromedial hypothalamic carnitine palmitoyltransferase-1a in the control of food intake. Am J Physiol Endocrinol Metab 305: E336–E347, 2013.—Carnitine palmitoyltransferase-1a (CPT-1a) liver isoform, or CPT-1a, is implicated in CNS control of food intake. However, the exact brain nucleus site(s) in mediating this action of CPT-1a has not been identified. In this report, we assess the role of CPT-1a in hypothalamic ventromedial nucleus (VMN). We stereotaxically injected an adenoviral vector containing CPT-1a coding sequence into the VMN of rats to induce overexpression and activation of CPT-1a. The VMN-selective activation of CPT-1a induced an orexigenic effect, suggesting CPT-1a in the VMN is involved in the central control of feeding. Intracerebroventricular administration of etomoxir, a CPT-1 inhibitor, decreases food intake. Importantly, in the animals with VMN overexpression of a CPT-1a mutant that antagonizes the CPT-1 inhibition by etomoxir, the anorectic response to etomoxir was attenuated. This suggests that VMN is involved in mediating the anorectic effect of central inhibition of CPT-1a. In contrast, arcuate nucleus (Arc) overexpression of the mutant did not alter etomoxir-induced inhibition of food intake, suggesting that Arc CPT-1a does not play significant roles in this anorectic action. Furthermore, in the VMN, CPT-1a appears to act downstream of hypothalamic malonyl-CoA action on feeding. Finally, we show that in the VMN CPT-1 activity was altered in concert with downstream target in Arc malonyl-CoA action on food intake. Thus, our recent studies have demonstrated that modulation of CPT-1a specifically in the Arc fails to alter food intake (15). Instead, in the Arc, the brain isoform of CPT-1 (CPT-1c), which has a rather weak acyltransferase activity (46, 53, 61) and is not involved in fatty acid oxidation (53), appears to play a role in the control of feeding (17).

In the peripheral tissues, CPT-1 activity is regulated by the inhibitory action of malonyl-CoA, an intermediate in the fatty acid biosynthetic pathway (38, 42). Malonyl-CoA in the hypothalamus acts as an anorectic mediator of food intake (12, 32, 34). The Arc is a site where malonyl-CoA exerts its action on feeding control (16, 17, 20, 34). Since malonyl-CoA inhibits CPT-1 activity, inhibition of CPT-1a in the Arc had been predicted to mediate the downstream effect of Arc malonyl-CoA-mediated control of food intake (34, 42). However, our recent studies have challenged this prediction and suggest that Arc malonyl-CoA control of feeding does not act through CPT-1a (but rather through CPT-1c) (17). Central treatment with leptin or cerululin (a fatty acid synthase inhibitor) increases the malonyl-CoA level in the Arc, which is required for leptin- or cerululin-induced anorectic action (15, 16, 20). Unexpectedly, the blockade of malonyl-CoA-mediated inhibition of CPT-1a in the Arc fails to affect the feeding inhibition by either leptin or cerululin (15). Moreover, our studies have also demonstrated that CPT-1a activity in the Arc does not change under fasting or refeeding conditions, although Arc malonyl-CoA levels are altered (15). Taken together, these data have strongly challenged the role of CPT-1a as an intracellular downstream target in Arc malonyl-CoA action on food intake. Our previous studies have further revealed that, in the Arc, the...
brain isoform of CPT-1, or CPT-1c, may serve as a downstream mediator in malonyl-CoA anorectic signaling action (17).

In addition to the Arc, the hypothalamic ventromedial nucleus (VMN) is another well-known site involved in the central control of feeding behavior. The VMN was historically designated as a brain “satiety center”, as lesions in this area are usually associated with hyperphagia and obesity (28). A large and growing body of evidence has identified important roles of VMN in the central control of food intake and regulation of energy homeostasis (5, 7, 8, 10, 13, 19, 22, 25–31, 37, 44, 45, 55, 57–59, 62, 63). Of relevance, as in the Arc, malonyl-CoA metabolism is implicated in VMN-mediated control of feeding (36). Moreover, to our knowledge, there have been no reports until at least 14 days following the viral injections.

### MATERIALS AND METHODS

#### Animal preparations.

The animal experiments were performed in accordance with the guidelines and instructions of the Canadian Council for Animal Care, and the experimental procedures were approved by the Animal Policy and Welfare Committee of the University of Alberta. Male Sprague-Dawley rats (225–300 g) were purchased from Charles River Laboratories. The rats were housed in a controlled environment (12:12-h light-dark cycle, 25°C). They were allowed ad libitum access to standard laboratory chow and water unless otherwise noted. Before any experimental treatments, the rats were subjected to daily handling and mock injections to minimize the stress from the procedures.

#### Brain cannulation and icv injection.

Cannulas were implanted into the third ventricle as described before (15, 16). The accuracy of cannula placement was verified by the angiotensin II drinking test or by histological analysis (16). After the cannulation surgery, daily body weights were monitored. Once body weights had returned to the presurgical levels and the rats had been fully habituated to the handling and mock injection procedures, the experimental treatments were performed.

#### Adenoviruses.

The recombinant adenoviral vectors having no CPT-1a sequence (null), encoding wild-type CPT-1a, or encoding the mutant CPT-1a were prepared as described previously (15). In brief, the virus encoding wild-type CPT-1a contains the nucleotide sequence covering the coding region of rat CPT-1a (58–2700). This sequence was used to generate the mutant CPT-1a with the methionine residue 593 being mutated to a serine residue (MS93). This MS93 mutant CPT-1a is insensitive to malonyl-CoA inhibition and exhibits reduced response to etomoxir-induced inhibition (15, 39, 40). The adenoviruses were delivered into the hypothalamic VMN or Arc by bilateral stereotaxic injection (1 × 10^7 pfu/µl; 0.4 µl per side). The coordinates of VMN injection were as follows: anterior-posterior, −2.8 mm; dorsal-ventral, −9.1 mm; and medial-lateral, ± 0.6–0.7 mm. The accuracy of the injections was tested elsewhere (addressed in a separate paper). The coordinates of Arc injection were: anterior-posterior, −2.8 mm; dorsal-ventral, −9.5 mm; and medial-lateral, ± 0.4 mm. The accuracy of the Arc injection was verified and documented in our previous report (15). Significantly high levels of CPT-1a protein or enzyme activity were detected starting at around 7 days following the viral injections, and the expression levels lasted until at least 14 days following the viral injections.

### RESULTS

#### Activation of CPT-1a induces orexigenic effects.

In the hypothalamus, the predominant CPT-1 isoform possessing acyltransferase activity is the liver isoform CPT-1a (42). To explore the VMN-specific role of CPT-1a in the control of feeding, we used the stereotaxic injection approach to target the VMN with the adenoviruses encoding CPT-1a protein (15). We first confirmed the accuracy of the injection by examining the expressions of GFP following VMN administration of an adenovirus encoding GFP. The GFP expression is evident in the dissected
VMN region and is not detected in the areas of pooled Arc and lateral hypothalamic area (LHA) (Fig. 1A). The specific GFP expression supports an accurate stereotaxic delivery procedure in our studies. Then, we injected the adenoviral vector encoding wild-type CPT-1a (CPT-1a wt) or the malonyl-CoA-insensitive mutant CPT-1a (CPT-1a mt) into the VMN of rats. The adenoviral vector without the CPT-1a insert (CPT-1a null) was used as the control. One week following the viral injections, the rats were euthanized, and the CPT-1 protein levels in the VMN area were measured by Western blotting. Compared with the rats injected with the null virus, CPT-1a (either wt or mt) viral infections induced increases in CPT-1a protein levels in the VMN (Fig. 1B). In addition to CPT-1a, the brain isoform of CPT-1, or CPT-1c, is also expressed in hypothalamic nuclei (46, 61). Following the viral injection, there were no detectable differences of CPT-1c protein levels in the VMN among the groups (Fig. 1B), which confirmed the isoform-specific overexpression following the viral infection. In parallel, we show that CPT-1a protein levels in the Arc from the same animal were not different among the three groups (null, CPT-1a wt, and CPT-1a mt; Fig. 1C). Taken together, the protein expression patterns support a VMN-specific overexpression of CPT-1a following the stereotaxic injection.

The upregulations of the protein levels of CPT-1a wt and CPT-1a mt were associated with significant increases in the CPT-1 activities in both CPT-1a wt and CPT-1a mt groups (Fig. 2A). Confirming the specificity of VMN expression, we observed no changes in CPT-1 activity in the other hypothalamic sites, including the paraventricular nucleus (PVN) and the LHA (Fig. 2A). In the current study, we did not measure CPT-1 activity in the Arc area, because we could not obtain sufficient Arc tissue lysates following dissecting VMN from the same animal. However, we did measure the levels of long-chain acylcarnitines (LC-ACs) in these two nuclei (VMN and Arc). LC-ACs are direct products of CPT-1a activity; therefore, the measurement of LC-AC levels has been used as

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**Fig. 1.** Administration of carnitine palmitoyltransferase-1a (CPT-1a) adenoviruses induces overexpressions of the CPT-1a protein. A: an adenovirus encoding green fluorescence protein (GFP) was stereotaxically administered into the ventromedial nucleus (VMN). Protein levels of GFP in the dissected VMN and pooled areas of arcuate nucleus (Arc) and lateral hypothalamic area (LHA) were assessed by Western blotting. Actin was used as the loading control. B and C: the adenovirus without the CPT-1a insert (null), encoding wild-type CPT-1a (CPT-1a wt), or M593S mutant CPT-1a (CPT-1a mt) was administered into the hypothalamic VMN. One week after adenovirus administration, rats were euthanized. Hypothalamic nuclei were dissected, and CPT-1a and CPT-1c protein levels were measured by Western blot analysis. Representative blots from dissected VMN (B) and Arc (C) areas of each group are shown. The ratio of the band intensity of CPT-1a or CPT-1c to that of tubulin was quantitated. Levels are presented as %null. *, **wt or mt vs. null, P < 0.05.
a surrogate assay of CPT-1a activity (15, 24). The typical acyltransferase activity of CPT-1 converts palmitoyl-, oleoyl-, and stearoyl-CoA, the major LCFA-CoA species, to palmi-
toyl-, oleoyl-, and stearoylcarnitine, the major LC-AC species, respectively (15, 24). We quantified the levels of these LC-ACs in the VMN and Arc regions. Their levels in the CPT-1a (wt and mt) animals were increased in the VMN but were not altered in the Arc (Fig. 2B), which demonstrates the VMN-selective activation of CPT-1a within the mediobasal hypothal-
amus encompassing the VMN and the Arc.

In our previous study in which the CPT-1a wt and CPT-1a mt adenoviruses were injected into the Arc, we did not detect any significant changes in food intake or body weight in the CPT-1a (wt and mt) groups, compared with the null control (15). To determine whether VMN overexpression of CPT-1a wt or CPT-1a mt would alter feeding behavior, we injected the same amount of the viruses into the VMN but were not altered in the Arc (Fig. 2B), which demonstrates the VMN-selective activation of CPT-1a within the mediobasal hypothal-
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In our previous study in which the CPT-1a wt and CPT-1a mt adenoviruses were injected into the Arc, we did not detect any significant changes in food intake or body weight in the CPT-1a (wt and mt) groups, compared with the null control (15). To determine whether VMN overexpression of CPT-1a wt or CPT-1a mt would alter feeding behavior, we injected the same amount of the viruses into the VMN that we had previously used in the Arc study and then monitored daily food intake and body weight. In contrast with the Arc findings, VMN overexpression of CPT-1a wt or CPT-1a mt induced increases in food intakes starting on the 4th day (day 4) following the viral injections (Fig. 3A). Along with the increases in food intake, body weight gains were also increased starting on the day 4 (Fig. 3B). The increases in food intake and weight gain lasted at least until day 8 (Fig. 3, A and B). Thus, our data demonstrate that VMN-selective activation of CPT-1a induces orexigenic effects. It should be noted that the CPT-1a mt induced a greater increase in food intake than the CPT-1a wt on the 8th day. Since the mutant CPT-1a is insensitive to malonyl-CoA inhibition, overexpression of this mutant should result in a greater in vivo increase in CPT-1a activity (than the overexpression of the wild type), due to a greater resistance to endogenous malonyl-CoA inhibition. This would account for the observed greater orexigenic response in the mutant group. Alteration of CPT-1a activity can affect the levels of LCFA-CoAs that are the substrates of CPT-1a, and the LCFA-CoAs were believed to act as downstream effectors in mediating hypothalamic CPT-1a action on feeding (42, 43). We therefore measured the LCFA-CoA levels in the VMN. Around the time period when the robust CPT-1 activations were detected (i.e., after 7 days following the viral injections), the LCFA-CoA levels were found to decrease in the VMN (Fig. 3C). This finding also further confirmed the activation of CPT-1a in the VMN following the viral injection. It should be noted that the animals had been fasted for 8 h before they were euthanized for the acyl-CoA assay in order to minimize the potential effect of feeding on the levels of these metabolites. Then, the endogenous malonyl-CoA levels would have been reduced by fasting, so that the malonyl-CoA inhibitory effect on CPT-1a activity would have been diminished. It follows that after fasting the animal overexpressing the mutant has a similar level of CPT-1
activity resulting in similar changes of acyl-CoA levels as the animal overexpressing the wild-type CPT-1a.

Activation of CPT-1a in the VMN blocks central etomoxir-induced anorectic effects. Previous studies examining the role of CPT-1a in feeding behavior did not identify the target brain site in mediating the anorectic effect by icv administration of CPT-1a inhibitors (42). Having shown that CPT-1a in the VMN plays a role in feeding control, we explored the potential involvement of VMN in mediating the anorectic effects exerted by central CPT-1a inhibition. To this end, we used etomoxir, a well-known inhibitor of CPT-1 activity (9). Inside the cell, etomoxir is converted to etomoxiry-CoA, and etomoxiry-CoA binds CPT-1 and inhibits its activity, partially mimicking the inhibitory action of malonyl-CoA on CPT-1 activity (39). Importantly, it has been demonstrated that the malonyl-CoA-insensitive mutant CPT-1a is also resistant to etomoxiry-CoA-mediated inhibition (39). We centrally (icv) injected etomoxir to the rats with VMN overexpression of either CPT-1a wt or CPT-1a mt. Eтомoxir treatment reduced LC-AC levels (Fig. 4A) and elevated LCFA-CoA levels (Fig. 4B) in the VMN in both null and CPT-1a wt rats, which demonstrates the inhibitory action of etomoxir on CPT-1a activity in the VMN. Overexpression of CPT-1a mt partially blocked these changes (Fig. 4, A and B), indicating that the inhibitory effect of etomoxir treatment on CPT-1a activity was attenuated.

Next, we monitored food intake and body weigh change in these animals. We showed that central etomoxir reduced food intakes and induced weight losses in the null and CPT-1a wt animals (Fig. 4, C and D). These results are consistent with the previous findings from different laboratories that demonstrated the anorectic effects induced by icv CPT-1 inhibitors (39, 42). Importantly, we found that VMN overexpression of the CPT-1a mt attenuated the anorectic and weight loss effects of central etomoxir administration (Fig. 4, C and D). These results show that CPT-1a in the VMN is involved in mediating the feeding response by icv etomoxir.

Activation of CPT-1a in the Arc does not block central etomoxir-induced anorectic effects. The original report addressing brain CPT-1a feeding effect demonstrated an association of
the inhibition of CPT-1a in the Arc with anorectic response following icv delivery of CPT-1a inhibitors (42). To assess the potential causal role of the Arc in mediating the feeding response, we injected (icv) etomoxir into the rats with Arc overexpression of CPT-1a. The Arc-selective overexpression and the activation of CPT-1a activity had been demonstrated in our previous study (15). In the current study, we show that icv etomoxir decreased LC-ACs and increased LCFA-CoA levels in the Arc in null and CPT-1a wt rats (Fig. 5, A and B). These changes confirm the inhibitory effect of central etomoxir treatment on CPT-1a activity in the Arc. As in the VMN, Arc overexpression of the CPT-1a mt blocked the etomoxir-induced inhibition of CPT-1a activity (Fig. 5, A and B). However, unlike in the VMN, we found that central etomoxir reduced food intakes and body weights to the same extents across the three groups (i.e., null, CPT-1a wt, and CPT-1a mt; Fig. 5, C and D). These data demonstrate that the blockade of CPT-1a inhibition (by etomoxir) in the Arc does not affect the anorectic effects following central etomoxir.

Activation of CPT-1a in the VMN blocks malonyl-CoA-mediated anorectic effects. As in the Arc, malonyl-CoA in the VMN acts as an anorectic mediator in hypothalamic control of feeding (36). In addition, fasting and refeeding states induce VMN-specific changes in the expression levels of fatty acid synthase (FAS), which uses malonyl-CoA as the substrate (36). We then assessed whether the inhibition of CPT-1a activity would act downstream of VMN malonyl-CoA-mediated feeding effect. To this end, we centrally (icv) treated animals with cerulenin, a potent FAS inhibitor (33, 56), to induce an increase in malonyl-CoA levels in the VMN. It has been shown that FAS inhibition in the VMN induces anorectic response and the increase in malonyl-CoA levels is required for the feeding inhibition (36). We first confirmed that icv cerulenin increased malonyl-CoA levels in the VMN in the null rats (%vehicle; vehicle-treated: 168 ± 15, P < 0.05). We then injected cerulenin into the rats overexpressing CPT-1a in the VMN. Cerulenin treatment produced anorectic effects and weight losses in the rats overexpressing null or CPT-1a wt (Fig. 6, A and B). Importantly, VMN overexpression of the CPT-1a mt (which is insensitive to malonyl-CoA inhibition) attenuated these changes (Fig. 6, A and B). Since the feeding action of central cerulenin is attenuated when malonyl-CoA inhibition of CPT-1a is antagonized, the inhibition of CPT-1a is a significant step in the malonyl-CoA signaling action in cerulenin anorectic feeding effect (in the VMN).
mediated effect on feeding. We further addressed the physiological relevance of these changes by examining the activity of CPT-1a in the VMN under fasting and refeeding conditions. Fasting, a negative energy balance condition, reduced the malonyl-CoA level in the VMN, whereas refeeding, a positive energy balance condition, increased malonyl-CoA to the fed level (Fig. 7A). These malonyl-CoA level changes were likely driven by the alterations of the activities of AMPK and ACC. Compared with the fed state, the fasting condition increases the phosphorylation level of AMPK, indicating activation, and increases the phosphorylation level of ACC, indicating inhibition (Fig. 8A). The refeeding condition reverses these changes (Fig. 8B). We also measured the levels of LCFA-CoAs (the substrates of CPT-1a) and acylcarnitines (the products of CPT-1a), to assess the endogenous CPT-1a activity. The levels of long-chain acylcarnitines increased in the fasting state, whereas they decreased in the refeeding state (Fig. 7B). Furthermore, we show that the fasting condition reduced LCFA-CoA levels, whereas the refeeding condition elevated them (Fig. 7C). Taken together, our data show that CPT-1a activity increases in the fasting state when the malonyl-CoA level decreases, whereas CPT-1a activity decreases in the refeeding state when the malonyl-CoA level increases. Thus, in the VMN, CPT-1a activities are altered in concert with malonyl-CoA levels under fasting and refeeding conditions.

**DISCUSSION**

In this report, we show that activation of CPT-1a in the VMN induces an orexigenic effect. The hyperphagia was unlikely due to the VMN lesion resulting from the stereotaxic surgery procedure, because the null animals subjected to the same procedure did not exhibit any changes in food intake or body weight compared with the untreated animals. Furthermore, previous data demonstrate an anorectic effect (opposite to the hyperphagia) following VMN-selective downregulation of FAS, which would inhibit CPT-1a activity by the increasing malonyl-CoA level (36). Thus, the observed hyperphagia following VMN CPT-1a activation is most likely a consequence of the specific effect of the alteration (activation) of CPT-1 acyltransferase activity rather than any potential nonspecific impairment of VMN function.

Our study reproduced the finding (39) that icv administration of etomoxir induces anorectic actions. This result is also in line with the previous observation that central administration of specific CPT-1a inhibitors [CPT-1a-ribozyme (CPT-1a-Ribo) and compound ST-1326] reduces food intake (42). However, these data seem to be inconsistent with the finding that central treatment with compound C89b, a putative CPT-1 activator, inhibits food intake (1). Central administration of C89b had been expected to increase food intake rather than inhibit feeding. In this study, it was concluded that C89b acts as a CPT-1 activator, based entirely on an in vitro assay (1).
However, the actual effects of C89b treatment on brain CPT-1 acyltransferase activity have not been tested, and therefore the in vivo action of C89b on CPT-1 has remained primarily presumptive. As a result, the validity of using compound C89b as an “activator” of CPT-1 in in vivo studies is questionable. In contrast, the molecular actions of either etomoxir or CPT-1a-Ribo and ST-1236 on brain CPT-1a activity have been fully verified using in vivo approaches. Furthermore, in the C89b study, the specificity of C89b-mediated action has not been assessed, and the potential nonspecific effects may have played a predominant role in altering food intake. In our etomoxir study, however, we believe the action on CPT-1a played a significant role in mediating the observed feeding effects. The overexpression of the mutant CPT-1a (insensitive to the etomoxiry-CoA-mediated inhibition) would not block the etomoxir-induced feeding action if etomoxir action on CPT-1a did not play a significant role in the observed change in food intake.
The intracellular downstream mediators of hypothalamic CPT-1a action on feeding are not clear. It has been reported that ivc infusion of oleic acid (a major LCFA) reduces food intake (43, 52). Because fatty acids can be converted into acyl-CoA, oleic acid-derived oleoyl-CoA may mediate the observed anorectic effect (43). Furthermore, since they are the substrates of CPT-1a, LCFA-CoAs such as oleoyl-CoA were proposed to mediate the downstream effect of hypothalamic CPT-1 action on feeding (42). However, some other data do not fully support these hypothesized roles of LCFA-CoAs (15). LCFA-CoAs consist of CoA species with different lengths and saturation degrees, rendering them with distinct properties and functions (49). As a result, the actions of oleoyl-CoA can be different from those of the other LCFA-CoAs. Indeed, unlike central oleic acid, ivc infusion of palmitic acid (another major LCFA), which can increase hypothalamic palmitoyl-CoA level, did not affect feeding (52). We have also shown that intrahypothalamic administration of palmitic acid acutely increases food intake (personal communication, Dr. Timothy H. Moran, Johns Hopkins University School of Medicine, Baltimore, MD). Consistent with this, high-saturated-fat (palmitic acid) diets attenuate the hypothalamic anorectic signaling actions of leptin and insulin (4). Thus, individual LCFA-CoAs exhibit differential actions on the control of feeding, and LCFA-CoAs do not possess one single generic effect on feeding.

In peripheral tissues such as liver, CPT-1 is a rate-limiting enzyme in the mitochondrial fatty acid oxidation (FAO) pathway (60). In addition to affecting substrate levels, modulation of CPT-1 activity can impact FAO and the production of FAO-derived mitochondrial reactive oxygen species (ROS). In turn, ROS levels can affect the activity and/or the level of uncoupling proteins (UCPs) that play a role in regulating ROS homeostasis (3). Of relevance, it has been shown that hypothalamic UCP2 is implicated in ghrelin’s hypothalamic action on feeding (2). Ghrelin treatment activates CPT-1 activity in the hypothalamus (35), which leads to upregulation of hypothalamic UCP2 message level (2). The increased action of UCP2 limits the elevated production of ROS level resulting from the activation of CPT-1-mediated FAO (by ghrelin) (2). As the increase in hypothalamic ROS level can induce anorectic effects (11, 14, 23), UCP2 seems to play a permissive role in ghrelin’s orexigenic effect. Indeed, we found that overexpression of CPT-1a (in the VMN) induces a significant upregulation of UCP2 message level, which may restrict the potential overproduction of ROS associated with CPT-1 activation (to be addressed in another paper). Thus, UCP2 is implicated, and...
might serve as a mediator, in the downstream signaling pathways of CPT-1a action on feeding control.

A previous study (42) demonstrated an association of the reduction of Arc CPT-1a activity with the anorectic effect following icv treatment with CPT-1a-Ribo or ST-1326 (specific CPT-1a inhibitors). Primarily based on this association, the inhibition of CPT-1a in the Arc was predicted to account for the observed anorectic actions (42). Since icv administration targets many brain sites, and association does not indicate causality, the CPT-1a in the Arc may not necessarily mediate the feeding response. Indeed, our current data show that the CPT-1a in the VMN plays an important role in mediating the etomoxir-inhibition and a fellowship from Heart and Stroke Foundation of Canada (to S. Gao). The following grant supports are also acknowledged: Grant SAF2011-30520-C02-01 to D. Serra, and Grant CIBER Fisiopatología de la Obesidad y target in the malonyl-CoA-mediated effect on feeding. The site-specific roles of CPT-1a in malonyl-CoA feeding mechanisms are also relevant under physiological conditions. Our previous study demonstrates that in the Arc CPT-1a activities do not alter, although malonyl-CoA levels change in response to a fasting and refeeding cycle (15). In contrast, the current data show that the CPT-1a activities in the VMN are altered in concert with changes in malonyl-CoA levels under fasting and refeeding. As an anorectic mediator, the hypothalamic malonyl-CoA level decreases during fasting, which would promote feeding. Following refeeding, the malonyl-CoA level increases, which would constrain the rebound feeding. Thus, in the VMN malonyl-CoA likely targets CPT-1a to control feeding and maintain normal energy balance. Concerning the underlying mechanisms of the differential actions, neuronal heterogeneity might play a role. In the Arc, the major enzymes for malonyl-CoA metabolism such as FAS might not be present in the cells that express CPT-1a. On the other hand, they may be coexpressed in the VMN.

The VMN projects to many brain sites involved in the controls of feeding and energy balance, including the other hypothalamic areas and extra-hypothalamic sites such as the nucleus of the solitary tract (NTS) in the hindbrain (41). Although our data strongly suggest that the VMN is (while the Arc is not) a primary site in mediating the feeding effect of central CPT-1a inhibition following icv etomoxir, we cannot exclude the potential involvement of certain other brain site(s), as the icv injection approach did not confine etomoxir treatment to the VMN. For example, the acetylcarinate levels were decreased in the NTS following the icv injection (values are normalized to the PBS-treated; PBS 100 ± 14%, etomoxir 44 ± 15%, *P* < 0.05), which indicates that icv etomoxir inhibited CPT-1 activity in the NTS. This also leaves open the possibility that the NTS is another primary site in mediating the anorectic effect of central CPT-1a inhibition. Thus, the overexpression of the mutant CPT-1a in the VMN might impact the neuronal activity of NTS through the synaptic connection between these two nuclei, which would contribute to the observed mitigation of the anorectic action by icv etomoxir.

In summary, we have identified the VMN as a site in mediating the action of central CPT-1a activity in feeding control. In contrast, our data argue against a significant role of Arc CPT-1a in the hypothalamic control of food intake. Furthermore, in the VMN, but not in the Arc, CPT-1a appears to act downstream of malonyl-CoA’s effect on food intake. Thus, VMN-selective modulation of CPT-1 acyltransferase activity would provide a strategy to control food intake and regulate body weight.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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