Inhibition of malonyl-CoA decarboxylase reduces the inflammatory response associated with insulin resistance


Cardiovascular Research Centre, Mazankowski Alberta Heart Institute, University of Alberta, Edmonton, Alberta, Canada

Submitted 9 January 2012; accepted in final form 9 October 2012

Inhibition of malonyl-CoA decarboxylase reduces the inflammatory response associated with insulin resistance. Am J Physiol Endocrinol Metab 303: E1459–E1468, 2012. First published October 16, 2012; doi:10.1152/ajpendo.00018.2012.—We previously showed that genetic inactivation of malonyl-CoA decarboxylase (MCD), which regulates fatty acid oxidation, protects mice against high-fat diet-induced insulin resistance. Development of insulin resistance has been associated with activation of the inflammatory response. Therefore, we hypothesized that the protective effect of MCD inhibition might be caused by a favorable effect on the inflammatory response. We examined if pharmacological inhibition of MCD protects neonatal cardiomyocytes and peritoneal macrophages against inflammatory-induced metabolic perturbations. Cardiomyocytes and macrophages were treated with LPS to induce an inflammatory response, in the presence or absence of an MCD inhibitor (CBM-301106, 10 μM). Inhibition of MCD attenuated the LPS-induced inflammatory response in cardiomyocytes and macrophages. MCD inhibition also prevented LPS impairment of insulin-stimulated glucose uptake in cardiomyocytes and increased phosphorylation of Akt. Additionally, inhibition of MCD strongly diminished LPS-induced activation of palmitate oxidation. We also found that treatment with an MCD inhibitor prevented LPS-induced collapse of total cellular antioxidant capacity. Interestingly, treatment with LPS or an MCD inhibitor did not alter intracellular triacylglycerol content. Furthermore, inhibition of MCD prevented LPS-induced increases in the level of ceramide in cardiomyocytes and macrophages while also ameliorating LPS-initiated decreases in PPAR binding. This suggests that the anti-inflammatory effect of MCD inhibition is mediated via accumulation of long-chain acyl-CoA, which in turn stimulates PPAR binding. Our results also demonstrate that pharmacological inhibition of MCD is a novel and promising approach to treat insulin resistance and its associated metabolic complications.

Inflammation can impede insulin signaling and contribute to insulin resistance. Inhibition of insulin signaling is usually mediated by stress kinases involved in the inflammatory response that phosphorylate and inhibit different components of the insulin-signaling pathway (23). For instance, serine phosphorylation of IRS-1 by JNK and IKK plays a crucial role in the suppression of insulin action (22). Activation of these stress kinases, in turn, can be triggered by various cytokines such as tumor necrosis factor (TNF)α and monocyte chemoattractant protein (MCP)-1 (49). These cytokines, best known as being immune mediators, participate in the regulation of metabolic homeostasis (11, 19, 55). This link is reinforced by epidemiological studies showing a rise in acute-phase inflammatory response proteins in the serum of patients with type 2 diabetes (10, 40). Also, during infections, patients can demonstrate a state of metabolic disorder, including insulin resistance (56). Intriguingly, a very tight functional link has been shown among inflammation, macrophages, and cardiomyocytes (5). Furthermore, metabolically compromised cardiomyocytes release various proinflammatory cytokines, which in turn further instigate cardiac pathologies (2).

While inflammatory mediators can alter metabolism, there is also a growing body of evidence showing that classically described metabolic pathways can also modulate the inflammatory response. For instance, fatty acid-binding protein (FABP), peroxisome proliferator-activated receptors (PPARs), and the liver X receptor (LXR) all have been shown to affect the inflammatory response (15, 21). In addition, lipin 1, a protein responsible for biosynthesis of phospholipids and triacylglycerol, can regulate the inflammatory response in adipose tissue (26). The catabolite activator protein (CAP) is a known positive regulator of glucose uptake in adipocytes (6) and also has a significant role in the regulation of the inflammatory response in macrophages (32). The metabolic stress kinase AMP-activated protein kinase (AMPK) can also significantly modulate the inflammatory response (43). It is important to mention that inflammatory reactions play an important role in cardiac repair (24).

Unfortunately, molecular mechanism(s) linking metabolic and inflammatory responses has yet to be identified. Studies focusing on the pathogenesis of obesity demonstrate that lipid metabolites (ceramide and diacylglycerol) can mediate the cross talk between the metabolic and inflammatory pathways (16, 42). The link between inflammation and metabolism has been referred to as immunometabolism (48). This potentially links alterations in fatty acid metabolism to the regulation of inflammatory pathways. In support of this, we (30) previously showed that mice deficient in malonyl-CoA decarboxylase (MCD; a key enzyme regulating fatty acid oxidation secondarily to modifying malonyl-CoA levels) were protected against high-fat diet-induced insulin resistance. Finding new mechanisms involved in regulation of inflammatory response in the heart appears to be one of the most promising directions in the treatment of cardiovascular diseases in the near future (8). On the basis of the reasons above, we investigated whether the inactivation of MCD could attenuate an inflammatory response induced by treatment with LPS in neonatal cardiomyocytes and peritoneal macrophages. Furthermore, we explored the molecular mechanisms linking metabolic and inflammatory pathways.

Address for reprint requests and other correspondence: G. Lopaschuk, 423 Heritage Medical Research Center, Univ. of Alberta, Edmonton, Alberta T6G 2S2, Canada (e-mail: gary.lopaschuk@ualberta.ca).
MATERIALS AND METHODS

Cell cultures. Rat neonatal cardiomyocytes were isolated and cultivated in DMEM supplemented with 15% horse serum, 5% fetal bovine serum, and 1% penicillin and streptomycin, as previously described (29). Peritoneal macrophages were isolated as previously described (46). Briefly, mice were injected with thioglycolate (5% in saline), and 5 days later, peritoneal macrophages were isolated from the abdominal cavities of euthanized mice and cultivated in DMEM supplemented with 10% fetal bovine serum.

Treatment of neonatal cardiomyocytes and peritoneal macrophages with LPS in the presence or absence of an MCD inhibitor. Neonatal cardiomyocytes were cultured for 12 h in DMEM (10 ml per 10-cm-diameter dish) in the presence or absence of the MCD inhibitor CBM-301106 (10 μM). After pretreatment with the MCD inhibitor, the medium was replaced with either 1) fresh medium, 2) 10 μM CBM-301106, 3) 1 μg/ml LPS, or 4) 10 μM CBM-301106 + 1 μg/ml LPS. After 24 h of incubation, cardiomyocytes were harvested and lysed. Additional groups of neonatal cardiomyocytes were also treated in a similar manner, except that 1 mM oxfenicine (a carnitine palmitoyltransferase I inhibitor) was substituted for the MCD inhibitor.

Peritoneal macrophages were treated similarly to neonatal cardiomyocytes, except for LPS, where it was added as 10 ng/ml.

Macrophage treatment with LPS and preparation of conditioned media. Peritoneal macrophages were cultured for 24 h in DMEM (10 ml per 10-cm-diameter dish) in the presence or absence of 10 ng/ml LPS with or without addition of 10 μM CBM-301106 for 24 h. Macrophages were then washed several times with PBS and fresh medium was added. After 12 h, the medium was collected, centrifuged, and used to incubate neonatal cardiomyocytes. No LPS or CBM-301106 was expected to be present in the conditioned medium from macrophages due to the intermediate washes.

Cell lysis. Confluent neonatal cardiomyocytes in 10-cm-diameter dishes were treated as indicated and then lysed as described previously (39).

Intracellular metabolite assessment. The levels of long-chain acyl-CoA’s, ceramide, and malonyl-CoA were assayed by high-performance liquid chromatography as previously described (17). Triacylglycerols were extracted from cell lysates using standard procedure (14). Briefly, 1.2 ml chloroform-methanol was added, hexanes was used to flip phases, and the top phase was dried. Triacylglycerol levels were quantified in the solubilized dried sample with a commercial kit (Wako Pure Chemical Industries). Values were normalized against protein levels.

2-deoxy-[3H]glucose uptake. Neonatal cardiomyocytes grown in 24-well plates and serum starved for 4–6 h were treated without or with insulin (100 nM) for 20 min. 2-Deoxyglucose uptake was measured as described (38), with a minor modification. Briefly, 2-deoxyglucose uptake was measured in the presence of 1 mM glucose.

Fatty acid oxidation. Palmitate oxidation was measured in rat neonatal cardiomyocytes grown in T25 flasks following cell medium being switched to Krebs-Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄·H₂O, 2.5 mM CaCl₂·2H₂O, 1.01 mM KHCO₃, 25 mM NaHCO₃, 11 mM glucose).
Inhibition of MCD abrogates LPS-induced increase palmitate oxidation on neonatal cardiomyocytes. Supernatants were analyzed by ELISA for mouse TNFα and MCP-1 (ALPCO Immunoassays, Salem, NH).

**Table 1. Catalytic activity of MCD in isolated neonatal cardiomyocytes**

<table>
<thead>
<tr>
<th>Activity of MCD</th>
<th>Control</th>
<th>LPS</th>
<th>CBM-301106</th>
<th>LPS + CBM-301106</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.2 ± 0.36</td>
<td>4.8 ± 0.41</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values represent means ± SE; n = 4. MCD, malonyl CoA decarboxylase; LPS, lipopolysaccharide; CBM-301106 is a specific inhibitor of MCD; ND, not detectable. MCD activity is defined as the amount of acetyl-CoA (in nmol) converted by the endogenous MCD enzyme from exogenous malonyl-CoA per minute per milligram of protein from the cell lysate. CBM-301106 reduces MCD activity, whereas LPS has no significant effect on the enzyme. *P < 0.05, significantly different from the control group.

Pharmacological inhibition of MCD prevents LPS-induced activation of the inflammatory response and enhances total antioxidant capacity in neonatal cardiomyocytes. As expected, LPS induced a marked proinflammatory response in neonatal cardiomyocytes. Incubation of cardiomyocytes with LPS robustly enhanced the release of proinflammatory cytokines such as TNFα and MCP-1 (Fig. 1, A and B). This observation is consistent with previously reported findings showing that treatment with LPS caused an inflammatory response in cardiomyocytes (3). Furthermore, treatment with LPS upregulated NF-κB DNA binding activity in these cells (Fig. 1C). Interestingly, if the MCD inhibitor CBM-301196 (10 μM) was present, even in the absence of LPS, a trend toward a reduction in release of TNFα, MCP-1, and NF-κB DNA binding activity was seen in the cardiomyocytes. In the presence of LPS, the MCD inhibitor markedly decreased TNFα and MCP-1 release (Fig. 1, A and B) and also diminished NF-κB DNA binding activity (Fig. 1C). These data show that MCD inhibition significantly reduced the LPS-induced proinflammatory response in cardiomyocytes. We also found that treatment with LPS significantly lowered total antioxidant capacity. This deleterious effect of LPS was abrogated by addition of the MCD inhibitor. Furthermore, treatment with the MCD inhibitor alone robustly enhanced total antioxidant capacity in neonatal cardiomyocytes (Fig. 1D).

Fig. 3. Inhibition of MCD attenuates LPS-induced decrease in PPARα binding and increases levels of long-chain acyl-CoA in neonatal cardiomyocytes. Inhibition of MCD abrogates LPS-induced increase palmitate oxidation on neonatal cardiomyocytes. A: PPARα binding; B: levels of long chain-acyl CoA; C: palmitate oxidation. Cardiomyocytes were treated for 24 h with 1 μg/ml LPS in the presence of absence of 10 μM CBM-301106. Values represent means ± SE; n = 5. Differences were determined by two-way ANOVA followed by Bonferroni post hoc analysis. *P < 0.05, significantly different from control group; #P < 0.05, significantly different from LPS-treated group.
Effect of pharmacological inhibition of MCD on metabolism of neonatal cardiomyocytes. Treatment with either LPS or the MCD inhibitor did not change intracellular triacylglycerol content, which indirectly suggests that there were not any alterations in fatty acid storage (Fig. 2A). Although LPS did not significantly alter the levels of malonyl-CoA in cardiomyocytes (Fig. 2B), it did dramatically increase the levels of intracellular ceramide (Fig. 2C). As expected, inhibition of MCD significantly elevated the levels of cardiomyocyte malonyl-CoA in both the absence and presence of LPS. Interestingly, treatment with the MCD inhibitor not only decreased the level of ceramide in untreated cardiomyocytes, but also diminished the LPS-induced elevation in ceramide levels. In accord with these results, we also found that exposure to LPS robustly upregulated the activity of SPT-1, the first and rate-limiting enzyme in de novo ceramide synthesis. Conversely, inhibition of MCD not only decreased the activity of SPT-1 but also significantly prevented LPS-induced elevation in SPT-1 activity (Fig. 2D). Our data suggest that inactivation of MCD can act as a negative regulator of ceramide synthesis. We also examined whether LPS has any direct effect on MCD activity in neonatal cardiomyocytes. Addition of LPS did not alter MCD activity (Table 1). This suggests that the inflammatory and metabolic effects of LPS are not due to the direct actions of LPS on MCD activity.

Inactivation of MCD prevents the LPS-induced decline in PPARα binding activity and the intracellular pool of long-chain acyl-CoA in neonatal cardiomyocytes. To determine whether PPARα activation contributes to the anti-inflammatory effect of inhibiting MCD, we assayed PPARα DNA binding activity in neonatal cardiomyocytes. Treatment with an MCD inhibitor CBM-301106 and LPS separately demonstrated opposing effects on PPARα DNA binding activity (Fig. 3A). MCD inhibition strongly activated PPARα binding, whereas exposure to LPS caused a pronounced suppression of PPARα binding activity. Of interest is that pharmacological inactivation of MCD prior to addition of LPS dramatically prevented LPS-induced reduction in PPARα binding. Exposure to LPS resulted in a significant depletion of the intracellular pool of long-chain acyl-CoA (Fig. 3B). MCD inhibition itself robustly enhanced the content of long-chain acyl-CoA in neonatal cardiomyocytes, which would be expected as a result of malonyl-CoA inhibiting carnitine palmitoyltransferase I (CPT I). The addition of an MCD inhibitor to LPS-treated cardiomyocytes significantly reversed the LPS-induced decline in long-chain acyl-CoA content. The decrease in ceramide and increase in long-chain acyl-CoA caused by MCD inhibition suggests that these metabolites are important in mediating the anti-inflammatory effect of MCD inhibition.

Inactivation of MCD significantly lowers LPS-induced increase of palmitate oxidation in neonatal cardiomyocytes. In our experiments we found that treatment with LPS induced a strong increase in palmitate oxidation in neonatal cardiomyocytes. This finding was unexpected, as it conflicts with previously published studies showing that LPS decreases fatty acid oxidation (12, 13). As expected, treatment with an MCD inhibitor strongly reduced palmitate oxidation. Furthermore, treatment with the MCD inhibitor also significantly reduced the LPS-induced increase in palmitate oxidation in neonatal cardiomyocytes (Fig. 3C). Prompted by this observation, we suggest that the protective function of MCD inhibition might be realized, at least in part, through inhibition of fatty acid oxidation.

Inhibition of MCD attenuates LPS-induced decreases in insulin-stimulated glucose uptake and phosphorylation of Akt in neonatal cardiomyocytes. As expected, treatment with LPS for 24 h induced a marked alteration in glucose uptake in neonatal cardiomyocytes. This was evident as a significant decrease in the ratio between basal and insulin-stimulated glucose uptake, as well as a diminished phosphorylation of Akt in response to insulin (Fig. 4, A and B). However, addition of the MCD inhibitor CBM-301106 significantly preserved both insulin-stimulated glucose uptake and insulin-stimulated Akt phosphorylation (Fig. 4, A and B) in LPS-treated neonatal cardiomyocytes.

Pharmacological inactivation of MCD in peritoneal macrophages reduces the LPS-induced inflammatory response. Peritoneal macrophages were isolated from mice and treated with LPS. As expected, LPS caused a robust activation of the inflammatory effect of MCD inhibition.

Fig. 4. Inhibition of MCD ameliorates the LPS-induced decrease in glucose uptake and phosphorylation of Akt in neonatal cardiomyocytes. A: glucose uptake. B: phosphorylation of Akt Ser473. Values represent means ± SE; n = 5–8. Differences were determined by two-way ANOVA followed by Bonferroni post hoc analysis. *P < 0.05, significantly different from control group; #P < 0.05, significantly different from the LPS-treated group.
Inflammatory response in macrophages. As shown in Fig. 5, A and B, release of the proinflammatory cytokines TNFα and MCP-1 was robustly increased. Furthermore, LPS also upregulated NF-κB DNA binding activity in macrophages (Fig. 5C). Intriguingly, treatment with the MCD inhibitor CBM-301106 significantly depressed the LPS-induced macrophage inflammatory response.

**Inactivation of MCD in peritoneal macrophages alters intracellular lipid metabolite levels and PPARγ binding activity, similar to what was found in neonatal cardiomyocytes.** Peritoneal macrophages were treated with LPS in the presence or absence of the MCD inhibitor CBM-301106. Similar to what was seen in cardiomyocytes, treatment of peritoneal macrophages with LPS did not increase the level of malonyl-CoA but did induce ceramide accumulation in peritoneal macrophages (Fig. 6, A and B). In contrast, LPS decreased long-chain acyl-CoA content, which was significantly attenuated in the presence of CBM-01106 (Fig. 6C). Peritoneal macrophages treated with LPS also showed lower PPARγ binding, which was significantly improved after concomitant treatment with the MCD inhibitor (Fig. 6D).

**Inactivation of MCD in mouse peritoneal macrophages treated with LPS ameliorates the effect of conditioned medium on glucose uptake in neonatal cardiomyocytes.** Conditioned medium from inflammatory stimulated macrophages has been shown to cause insulin resistance in various cells (47). Furthermore, macrophages are a major culprit in propagating inflammation in the whole body in addition to initiating insulin resistance (41). Therefore, we were interested in determining whether inactivation of MCD in peritoneal macrophages could prevent the macrophages from secreting proinflammatory factors capable of inhibiting insulin sensitivity in cardiomyocytes. Conditioned medium from LPS-treated macrophages, treated in the presence or absence of the MCD inhibitor CBM-301106, was transferred onto cultivated neonatal cardiomyocytes. After 24 h of incubation, we assessed insulin-stimulated glucose uptake in cardiomyocytes. Conditioned medium collected from untreated macrophages did not alter glucose uptake in cardiomyocytes. In contrast, conditioned medium collected from macrophages treated with LPS produced a significant decrease in the ratio between basal and insulin-stimulated glucose uptake in neonatal cardiomyocytes (Fig. 7). In particular, treatment with LPS significantly enhanced basal, insulin-independent glucose uptake in neonatal cardiomyocytes while inhibiting insulin-stimulated glucose uptake. Of interest, pharmacological inactivation of MCD in macrophages treated with LPS significantly diminished the capacity of the conditioned medium to cause an inflammatory response in neonatal cardiomyocytes.

---

**Fig. 5.** Inhibition of MCD diminishes the inflammatory response in peritoneal macrophages. A: release of TNFα from peritoneal macrophages. B: release of MCP-1 from peritoneal macrophages. C: NF-κB DNA binding activity on peritoneal macrophages. Macrophages were incubated for 24 h with 10 ng/ml LPS in the presence of absence of 10 μM CBM-301106. Values represent means ± SE; n = 7. Differences were determined by two-way ANOVA followed by Bonferroni post hoc analysis. *P < 0.05, significantly different from control group; #P < 0.05, significantly different from LPS-treated group.

**Fig. 6.** Effect of an MCD inhibitor on accumulation of intracellular lipid metabolites and on PPARγ binding in peritoneal macrophages. A: accumulation of malonyl-CoA. B: accumulation of ceramide. C: accumulation of long-chain acyl-CoA. D: PPARγ binding. Macrophages were incubated for 24 h with 10 ng/ml LPS in the presence of absence of 10 μM CBM-301106. Values represent means ± SE; n = 6. Differences were determined by two-way ANOVA followed by Bonferroni post hoc analysis. *P < 0.05, significantly different from control group. #P < 0.05, significantly different from LPS-treated group.
Pharmacological inactivation of CPT I with oxfenicine recapitulates the effects of MCD inhibition. As previously shown, inactivation of MCD reversed the deleterious effects of LPS on insulin resistance in cardiomyocytes. However, it remains unclear whether the anti-inflammatory effects of MCD inactivation was caused by a direct interaction between MCD and the inflammatory pathways or if it was a consequence of altered malonyl-CoA signaling. To examine this, we treated cardiomyocytes with the CPT I inhibitor oxfenicine (1 mM). CPT I is involved in transportation of long-chain fatty acids across the mitochondrial membrane (by converting long-chain acyl-CoA to long-chain acylcarnitine) and is inhibited by malonyl-CoA (53). Since MCD degrades malonyl-CoA, MCD inhibition increases malonyl-CoA and decreases CPT I activity in cardiomyocytes. Treatment of cardiomyocytes with oxfenicine markedly prevented LPS-induced liberation of TNFα from neonatal cardiomyocytes and ameliorated the LPS-initiated decrease in PPARα binding (Fig. 8). These results suggest a key role for alterations in the activity of CPT I in the mediation of the anti-inflammatory effects of MCD inactivation rather than direct involvement of MCD in the regulation of proinflammatory pathways.

DISCUSSION

In our experiments, we used LPS as a tool to evoke the intrinsic proinflammatory response in neonatal cardiomyocytes. As expected, treatment with LPS caused a massive release of proinflammatory cytokines from cardiomyocytes. Furthermore, we also demonstrate that exposure to LPS elicits activation of NF-κB DNA binding activity. Activation of the proinflammatory response is a major culprit in the pathogenesis of insulin resistance (42). Indeed, in our experiments we demonstrate that treatment with LPS impairs insulin-stimulated glucose uptake and phosphorylation of Akt in cardiomyocytes. We also demonstrate that treatment with LPS strongly enhances palmitate oxidation, an effect that can cause abnormally high production of ROS by mitochondria. Oxidation of phospholipids by ROS further triggers the inflammatory response, resulting in a perpetual “vicious cycle” (18). In addition, we observed that treatment with LPS robustly diminishes PPARα DNA binding activity in neonatal cardiomyocytes. This finding appears to be very important, as PPAR nuclear receptors are known as powerful suppressers of the inflammatory response (4).

The LPS-induced alterations in insulin action in neonatal cardiomyocytes are associated with a marked increase in the intracellular levels of ceramide. The role of ceramide accumulation in the development of insulin resistance is well described (20, 44, 51). Furthermore, LPS has been shown to stimulate ceramide synthesis by increasing mRNA expression and activ-
Fig. 9. Hypothetical explanation of the anti-inflammatory effect of MCD inhibition. A: TLR receptors, when activated by LPS, activate IKK. IKK phosphorylates IκBα, causing it to dissociate from NF-κB. A possible mechanism through which LPS can upregulate activity of SPT-1 is to increase long-chain acyl-CoA supply for ceramide biosynthesis. Noteworthy, activation of PPARα has been shown to lower the activity of SPT-1 (50, 57). This finding correlates with our own observations and suggests that activation of PPARα binding activity due to inhibition of MCD could be an essential mechanism in decreasing SPT-1 activity and lowering the levels of intracellular ceramide. In our preliminary results (data not shown), we found that treatment with a PPARα antagonist diminishes the protective effect of MCD inhibition.

We also examined the effects of MCD inhibition on the LPS-induced inflammatory response in peritoneal macrophages. Macrophages are important initiators and propagators of the inflammatory response, and inactivation of the inflammatory cascade in macrophages is effective in protecting mice from high-fat diet-induced insulin resistance (36, 41). Our results show that pharmacological inhibition of MCD decreases the inflammatory activation of macrophages caused by treatment with LPS. We show that pharmacological inhibition of MCD...
strongly reduces LPS-stimulated release of proinflammatory cytokines and activation of NF-κB DNA binding activity in macrophages. Inhibition of MCD also robustly ameliorates LPS-induced accumulation of ceramide in peritoneal macrophages. Moreover, treatment with the MCD inhibitor preserved the pool of intracellular long-chain CoA in macrophages, lowered after treatment with LPS. PPARγ is highly expressed in macrophages, where it negatively regulates the inflammatory response. Therefore, inactivation of PPARγ would be expected to increase the magnitude of the inflammatory response in macrophages. We demonstrate that treatment with LPS causes a significant reduction in PPARγ binding in macrophages, which was strongly preserved by cotreatment with the MCD inhibitor.

Studies of conditioned medium harvested from inflammatory activated macrophages demonstrate that it causes insulin resistance in various target cells (35). In another set of experiments, we examined whether inhibition of MCD would affect the ability of macrophage conditioned medium to cause insulin resistance in neonatal cardiomyocytes. As expected, we found that conditioned medium collected from LPS-treated macrophages induced insulin resistance in cultured neonatal cardiomyocytes. This is likely due to LPS causing macrophages to release a broad spectrum of proinflammatory cytokines capable of decreasing insulin sensitivity in target cells. However, cotreatment of macrophages with the MCD inhibitor greatly diminished the ability of the conditioned medium to cause insulin resistance in cardiomyocytes. We presume that treatment with the MCD inhibitor evoked the above-described anti-inflammatory reactions in macrophages, which resulted in a lowered ability of the conditioned medium to cause insulin resistance.

A crucial finding of our study is that pharmacological inhibition of MCD induces the accumulation of long-chain acyl-CoA, which may activate PPAR nuclear receptors. It is important to mention, however, that accumulation of long-chain acyl-CoA has also been suggested to cause insulin resistance (27, 28). On the contrary, overexpression of MCD in the liver enhances hepatic fatty acid oxidation rates, decreasing plasma free fatty acid levels, which reversed whole body insulin resistance and was associated with an elevation in intramuscular long-chain acyl-CoA content (1). Thus, the role of long-chain acyl-CoA in the pathogenesis of insulin resistance remains unclear. Furthermore, in elegant experiments performed by Kanter et al. (25), it was demonstrated that increased expression of long-chain acyl-CoA synthetase is associated with a proinflammatory phenotype in macrophages. The results obtained in the above-mentioned study are important yet conflict with our data. Our study supports a positive effect for long-chain acyl-CoA accumulation on the inflammatory response and insulin sensitivity. Thus, we propose that malonyl-CoA inhibition of CPT I causes the accumulation of long-chain acyl-CoA, which can act as activating ligands for the PPAR family of nuclear receptors. Activated PPARs are known to be effective downregulators of the inflammatory response. This suggested complex mechanism is illustrated in Fig. 9, and understanding its intrinsic pathways is a major objective of our future studies. The proposed mechanism is further reinforced by our results with an inhibitor of CPT I. Pharmacological inhibition of CPT I also decreased release of proinflammatory cytokines and evoked activation of PPARα binding in cardiomyocytes. Thus, accumulation of long-chain acyl-CoA and subsequent activation of PPARs appears to be a key mechanism for the anti-inflammatory phenomenon of MCD inhibition.

In summary, our findings add to a growing body of evidence supporting numerous metabolic health benefits of MCD inhibition that include protection against insulin resistance (30), ischemic heart disease (9, 54), and body weight gain (34). Indeed, these new findings highlight an important link between inflammatory and energy metabolic pathways and suggest that targeting MCD may be a novel treatment against diseases where inflammation plays a pivotal role.

ACKNOWLEDGMENTS

We thank Cory Wagg for technical assistance on this project. V. Samokhvalov, J. R. Ussher, and W. Keung are postdoctoral fellows of Alberta Innovates Health Solutions. G. D. Lopaschuk is an Alberta Heritage Foundation for Medical Research Scientist. J. Seubert is an Alberta Heritage Foundation for Medical Research Health Scholar.

GRANTS

This research was funded by grants to G. D. Lopaschuk and J. Seubert from the Canadian Institutes of Health Research.

DISCLOSURES


REFERENCES


AJP-Endocrinol Metab doi:10.1152/ajpendo.00018.2012 www.ajpendo.org


