Opposite effects of saturated and unsaturated free fatty acids on intracellular signaling and metabolism in neuronal cells

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Elevated levels of plasma saturated free fatty acids are a major pathogenic factor in diabetes, cardiovascular and liver diseases. Growing evidence suggests a link between sFFA-induced metabolic impairments and neurodegeneration. Excessive sFFAs in the brain circulation may trigger neuroinflammation and insulin resistance, however the underlying signaling changes have not been clarified in neuronal cells. We recently reported the effects of FFAs on intracellular signaling and metabolism in neuronal cells. We found that palmitate induced both insulin resistance and mitochondrial dysfunction while promoting phosphorylation and nuclear translocation of NF-κB p65 in neuronal cells. The latter is a key event in the inflammatory cascade. Oleate pre-exposure and then removal was sufficient to completely block subsequent palmitate-induced intracellular signaling and metabolic derangements. Interestingly, oleate also prevented ceramide-induced insulin resistance and cytotoxicity. Protein kinase A and triglyceride accumulation were implicated in the mechanism of oleate action. This is the first demonstration showing that oleate has beneficial properties against sFFA and ceramide models of neuronal injury. The lasting effect following oleate removal on preventing palmitate- or ceramide-induced cell damage was striking.

Keywords: palmitate; oleate, insulin resistance; mitochondrial dysfunction; inflammation


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Elevated levels of plasma saturated free fatty acids (sFFAs, e.g. palmitate) are a major pathogenic factor in diabetes, cardiovascular and liver diseases [1, 2]. They provoke inflammation and insulin resistance which can result in cellular degeneration. Although most research on FFAs and biologic effects has focused on peripheral tissue (e.g. muscle, liver and adipocytes), recent evidence suggests a possible link between sFFA-induced metabolic impairments and neurodegeneration, for instance in mild cognitive impairment (MCI) and Alzheimer’s disease (AD) [3, 4]. An excess of sFFAs in the brain circulation could also trigger neuroinflammation and insulin resistance [5, 6], however the underlying signaling changes have not been clarified in neuronal cells.
Table 1. Summary of the physiological effects of palmitate and/or oleate on neurons.

<table>
<thead>
<tr>
<th></th>
<th>Palmitate</th>
<th>Oleate</th>
<th>Pre-oleate then palmitate</th>
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<tbody>
<tr>
<td>pERK</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>pJNK</td>
<td>†</td>
<td>‡</td>
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<tr>
<td>Total IkBα</td>
<td>†</td>
<td>‡</td>
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<tr>
<td>pNF-κB</td>
<td>†</td>
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<td>-</td>
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<tr>
<td>Nuclear translocation of (p)NF-κB</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>Total PGC-1α</td>
<td>†</td>
<td>‡</td>
<td>-</td>
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<tr>
<td>pAkt</td>
<td>†</td>
<td>‡</td>
<td>-</td>
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<tr>
<td>insulin-stimulated pAkt</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>Cleaved caspase-3</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>Cleaved caspase-9</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>Cell viability</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>Cellular ATP</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>Mitochondrial superoxide</td>
<td>†</td>
<td>‡</td>
<td>-</td>
</tr>
<tr>
<td>Diacylglycerol</td>
<td>†</td>
<td>‡</td>
<td>-</td>
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<tr>
<td>Triglyceride</td>
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N2a cells or primary rat cortical neurons were incubated with oleate (300 μM) or BSA for 24 h and then incubated with palmitate (300 μM) or BSA for another 24 h in the absence of oleate. Intracellular inflammatory and insulin signaling as well as mitochondrial function and cell viability were measured. BSA was used as control. (†) increase compared with BSA, (‡) decrease compared with BSA.

It has been reported that sFFAs induce intracellular inflammatory signaling, mitochondrial dysfunction and insulin resistance in animal [6, 7] or cell models [8-11]. In contrast, polyunsaturated n-3 fatty acids (PUFAs; e.g. DHA and EPA) prevent or alleviate inflammation and insulin resistance [12, 13]. Interestingly, several recent studies in cultured myotubes reported that coincubation of palmitate with oleate, a monounsaturated n-9 fatty acid, prevents palmitate-induced cytotoxicity, insulin resistance and mitochondrial dysfunction [8, 9, 14, 15]. In our recent publication entitled “Oleate prevents palmitate-induced mitochondrial dysfunction, insulin resistance and inflammatory signaling in neuronal cells” [16], we examined the effects of palmitate on mitochondrial function and viability as well as on intracellular insulin and inflammatory signaling pathways in neuronal cells. We also tested whether oleate preconditioning can protect neurons from palmitate-induced toxicity. We found that oleate pre-exposure and then removal was sufficient to completely block subsequent palmitate-induced intracellular signaling and metabolic derangements in neuronal cells.

Cytotoxicity

We first observed that oleate coincubation prevented palmitate-induced cytotoxicity in a concentration dependent manner. Ultimately, preconditioning proved sufficient to produce the same effect. Palmitate (300 μM, 24 h) induced neurite degeneration in both differentiated Neuro-2a (N2a) and primary rat cortical neurons. The toxic effect of palmitate was most severe to N2a cells in the absence of tropic support (>95% cell death). Both oleate (300 μM, 24 h) precondition and coincubation successfully blocked the palmitate-induced neuronal degeneration even under serum-deprived conditions. Palmitate-induced cell death was correlated to caspase activation [10, 17], while the induction of autophagy following palmitate treatment was associated with cell survival function [18, 19]. We detected the increase in cleavage of caspases -3 and -9 following palmitate treatment, as observed in non-neuronal cells [10, 17]. Oleate completely reversed this. It even decreased basal caspase cleavage in our neuronal cells. Oleate preconditioning also attenuated ceramide-induced cytotoxicity in neuronal cells. Moreover, oleate preconditioning was superior to DHA (n-3) or linoleate (n-6) in the protection of neuronal cells against palmitate- or ceramide- induced cytotoxicity. Further, unlike DHA and linoleate, oleate had no toxicity to cells at high concentrations (200-300 μM). The protective effect of oleate also extended to ceramide toxicity. However, oleate preconditioning showed no effect on other cytotoxic conditions (e.g. H2O2, tumor necrosis factor (TNF)-α/interferon-γ or serum deprivation).

Intracellular inflammatory signaling

sFFAs induce inflammatory signaling while PUFAs have cellular protective and anti-inflammatory effects [12, 20]. We found that palmitate increased nuclear factor-κB (NF-κB) p65 phosphorylation at serine 536 and inhibitory κBα (IκBα) degradation in neuronal cells. Palmitate also induced nuclear translocation of both phosphorylated and total NF-κB p65. Contrary, oleate preconditioning decreased the basal phosphorylation of NF-κB p65 and
increased total IκBα. Oleate preconditioning also completely blocked the palmitate-induced NF-κB p65 signaling and nuclear translocation. The palmitate-induced NF-κB signaling is possibly mediated by mitogen-activated protein kinase signaling pathway [21, 22]. We also detected that palmitate induced the phosphorylation of the extracellular signal-regulated kinase (ERK) 1/2 and c-Jun N-terminal kinase (JNK) in neuronal cells. However, contrary to other reports in muscle cells [22, 23], our results showed that inhibition of ERK1/2 had no effect on palmitate-induced NF-κB p65 signaling in neuronal cells. In addition, inhibition of TNF-α-induced ERK1/2 activation by oleate preconditioning or by inhibitor U0126 showed no effect on the TNF-α-induced phosphorylation of NF-κB p65 and degradation of IκBα. Thus, it appears that ERK1/2 is not directly upstream of palmitate- or TNF-α- induced increases in NF-κB p65 signaling in neuronal cells.

Mitochondrial dysfunction

Palmitate causes mitochondrial dysfunction in skeletal muscle cells [9, 10] and oleate coinubcation blocks it [9]. Consistent with these reports, we found that palmitate decreased cellular ATP generation and increased mitochondrial superoxide production in neuronal cells. Oleate preconditioning decreased the basal level of mitochondrial superoxide production and completely prevented the palmitate-induced mitochondrial dysfunction. We believe that this effect is possibly due to an increase in peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α following oleate preconditioning. PGC-1α acts as a transcriptional factor for gene expression involved in energy metabolism and mitochondrial biogenesis [24] and also plays an important role to induce several reactive oxygen species (ROS) scavenging enzymes [25]. Since the NF-κB signaling pathway may be involved in both the regulation of PGC-1α [23] and mitochondrial gene expression [26], our finding that oleate reduced NF-κB p65 signaling, may be connected to the observed increase in PGC-1α. Thus, increased PGC-1α may be crucial in the protective effect of oleate against palmitate-induced mitochondrial dysfunction in neurons as shown in muscle [9].

Insulin resistance

Palmitate-induced insulin resistance is a well-known phenomenon in muscle [8, 11] and other non-neuronal cells [27-29]. However, the few existing data on neuronal cells is discordant [30, 31]. We found that palmitate decreased the levels of basal and insulin-stimulated pAkt in neuronal cells. Oleate preconditioning completely rescued these palmitate-induced decreases in both basal and insulin-stimulated pAkt, as similarly demonstrated in myotube coinubcation studies [8, 9]. Interestingly, we found that oleate also reversed ceramide-induced insulin resistance in neuronal cells. Palmitate-induced insulin resistance appears to be mediated in part by diacylglycerol (DAG), ROS, and/or ceramide [8, 9]. However, treatment with myriocin, an inhibitor of ceramide synthesis, showed no effect on blocking palmitate-induced insulin resistance in our neuronal cells, indicating that de novo synthesis of ceramide was not necessary for palmitate-induced insulin resistance. Thus, it is postulated that oleate can directly antagonize already-formed ceramide’s action in addition to ceramide-independent palmitate-induced insulin resistance in neuronal cells. A few recent studies have demonstrated that oleate prevented palmitate-induced insulin resistance via AMP-activated protein kinase (AMPK) activation [32] and preventing abnormal DAG synthesis and protein kinase C/NF-κB activations [8]. There are likely several protective mechanisms involved in the action of oleate to mitigate palmitate-induced insulin resistance as discussed below.

Mechanism of oleate action

We found involvement of protein kinase A (PKA) in the protective effect of oleate against palmitate. This mechanism was similarly shown in skeletal muscle cells [8]. Thus, PKI (50 μM), an inhibitor of PKA, significantly attenuated the protective effect of oleate against palmitate in our neuronal cells. PKA activation induces downstream proteins such as PGC-1α and peroxisome proliferator-activated receptor α (PPARα) [8]. Thus, it is possible that oleate, via the PKA/PGC-1α signaling pathway, contributes to protect neuronal cells from palmitate-induced cytotoxicity. Another possible mechanism of oleate protective action not explored here involves the AMPK pathway, as shown in muscle cells [32]. Finally, we demonstrated that oleate increased cellular triglyceride (TG) accumulation while palmitate increased DAG synthesis in neuronal cells. Moreover, both oleate preconditioning and coinubcation conditions blocked palmitate-induced DAG synthesis. The changes in the levels of TG and DAG are consistent with previous reports showing that oleate may protect palmitate-induced cytotoxicity by increasing the sequestration of TG droplets in non-neuronal cells [8, 33].

Conclusions and future research directions

We have demonstrated for the f rst time that oleate has beneﬁcial properties against sFFAs and ceramide models of neuronal cell injury. Moreover, the protective effect of oleate outlasted its removal. Similar to the results in neuronal cells, oleate preconditioning signiﬁcantly alleviated or prevented subsequent palmitate-induced inflammatory
signaling and insulin resistance in cultured C2C12 myotubes (unpublished data). Skeletal muscle is a major organ that can utilize blood FFAs as an energy source. sFFA-induced insulin resistance and inflammation in muscle are closely associated with the risk factors for the metabolic syndrome (e.g., abdominal obesity, hypertension, hyperglycemia and hyperlipidemia) [34]. Cellular protective and anti-inflammatory effects of olate have been demonstrated in experimental human and animal models. For example, an olate-enriched diet improved skeletal muscle insulin sensitivity and limited inflammation in rats [35]. It also decreased the risk of cardiovascular diseases in humans [36]. A possible association between the consumption of mono- or polyunsaturated FFAs and a reduced risk of MCI and AD has been recently reviewed [37]. To date, the protective effect of olate against lipotoxicity-induced neurodegeneration in the brain has not been clarified using animal models. Our in vitro neuronal cell findings highly recommend in vivo studies to determine effects of systemic olate supplementation on neuronal protection against excessive sFFAs and ceramide circulation in various models of neurodegeneration including high-fat diet-induced obesity [5, 6] and alcoholic steatosis [37].

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Conflict of interest

The authors have nothing to disclose.

References


