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Free fatty acid receptor 3 is a key target of short chain fatty acid

What is the impact on the sympathetic nervous system?

Eduardo Javier López Soto[1,*], Luisina Ongaro Gambino[2], and Emilio Román Mustafá[1]

[1]Laboratory of Electrophysiology; Multidisciplinary Institute of Cell Biology (IMBICE - CICPBA/CONICET); Buenos Aires, Argentina; [2]Laboratory of Neuroendocrinology; Multidisciplinary Institute of Cell Biology (IMBICE - CICPBA/CONICET); Buenos Aires, Argentina

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Abbreviations: NS, nervous system; SCFAs, short chain fatty acids; GPCR, G-protein coupled receptor; FFA2, Free Fatty Acid Receptor 2; FFA3, Free Fatty Acid Receptor 3; NE, norepinephrine; NTCC, N-type calcium channel; BHB, β-hydroxybutyrate; VD, voltage dependent; VI, voltage independent; PTX, pertussis toxin.

*Correspondence to: Eduardo Javier López Soto; Email: jlopezsoto@imbice.gov.ar

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Nervous system (NS) activity participates in metabolic homeostasis by detecting peripheral signal molecules derived from food intake and energy balance. High quality diets are thought to include fiber-rich foods like whole grain rice, breads, cereals, and grains. Several studies have associated high consumption of fiber-enriched diets with a reduced risk of diabetes, obesity, and gastrointestinal disorders. In the lower intestine, anaerobic fermentation of soluble fibers by microbiota produces short chain fatty acids (SCFAs), key energy molecules that have a recently identified leading role in the intestinal gluconeogenesis, promoting beneficial effects on glucose tolerance and insulin resistance[1]. SCFAs are also signaling molecules that bind to specific G-protein coupled receptors (GPCRs) named Free Fatty Acid Receptor 3 (FFA3 or GPR41) and 2 (FFA2 or GPR43). However, how SCFAs impact NS activity through their GPCRs is poorly understood.

Recently, some studies have demonstrated the presence of FFA2 and FFA3 in the sympathetic NS of rat, mouse and human[2, 3]. Two studies have shown that FFA3 activation by SCFAs increases firing and norepinephrine (NE) release from sympathetic neurons[3, 4]. However, the recent study from the Ikeda Laboratory[2] revealed that activation of FFA3 by SCFAs impairs N-type calcium channel (NTCC) activity, this finding contradicts the idea of FFA3 activation leading to increased action potential evoked NE release. Here we will discuss the scope of the latter study and the putative physiological role of SCFAs and FFAs in the sympathetic NS.

FFAs Coupling to N-type Calcium Channels

Taking advantage of their electrophysiological expertise, Won et al. (2013) performed high quality whole-cell voltage clamp recordings in dissociated sympathetic neurons to assess the molecular coupling of FFAs to NTCC. In this setting, that offers extremely sensitive temporal resolution of native G protein mediated modulation of NTCC, they found that native NTCC currents are inhibited by heterologously expressed FFA2 and FFA3. Authors showed that FFAs activation is induced by either SCFAs (acetate or propionate) or the ketone body β-hydroxybutyrate (BHB), which was previously reported as an FFA antagonist.[3] Thereby, they provided the first evidence that SCFAs and BHB negatively modulate NTCC by activating FFAs.

In order to characterize FFA2 and FFA3 mediated inhibition of NTCC, Won et al. (2013) recorded calcium currents evoked by a double-pulse voltage protocol.[5] This challenging protocol allows the physiologist to dissect the 2 GPCR signaling pathways involved in NTCC inhibition: the Gβγ mediated pathway, which is impaired by strong depolarizing voltages, thereby producing a voltage dependent (VD) inhibition; and the Gα mediated pathway, which is resistant to changes in membrane voltage, resulting in a voltage

independent (VI) inhibition (for review, see ref. 6). Interestingly, the authors report that the facilitation ratio of NTCC, a measurement of the degree of VD inhibition, increased when FFA3 was activated and also increased but in a lesser extent when FFA2 was activated. Thus, they described that heterologously expressed FFA3 activated by SCFAs inhibits NTCC currents via a fully VD pathway while FFA2 has a partially VD mechanism of action. The authors could have compared the change in facilitation ratio to the total inhibition values to give a formal quantification of the VD and VI components of inhibition.

Understanding the contribution of VD and VI mechanisms to the total inhibition by FFA2 and FFA3 is important because each mechanism underlies completely different physiological impacts. VD inhibition is a membrane-delimited pathway that could display a transient disinhibition of NTCC during periods of strong neuronal electrical activity. In contrast, VI inhibition involves second messenger signaling and is not affected by changes in electrical activity. In the case of sympathetic neurons, the impact of VD and VI inhibition on presynaptic NTCC will be different depending if neurons belong to pre- or paravertebral ganglia since they have 2 distinct firing patterns.[7] We suggest that whereas NTCC VD inhibition could be impaired to a greater degree during high frequency tonic action potentials in prevertebral neurons, it could persist in paravertebral neurons which display phasic firing properties.

Make Bets: What's FFAs Downstream?

Signaling cascades downstream of GPCR activation define the global cellular effect of each agonist-receptor couple, and the G protein subtype confers the first level of specificity. Won et al. (2013) investigated the particular G proteins involved in the NTCC inhibition by heterologously expressed FFAs. In agreement with previous data, they found that FFA3 effect was completely mediated by Gβγ dimers released from activated pertussis toxin (PTX)-sensitive Gi/o protein, consistent with their finding that FFA3 inhibits NTCC in a VD manner. On the other hand, FFA2 inhibition of NTCC was partially occluded by PTX and the remaining fraction was VI. Based on previous reports, the authors hypothesized that the VI inhibition by FFA2 was mediated by its coupling to Gq, which involves lipid signaling such as PtdIns(4,5)P_2 depletion from plasma membrane or arachidonic acid generation. To explore this pathway further, the authors could have incubated cells with a Gq-selective inhibitor (e.g., YM-254890) or transfected a dominant negative Gq mutant that prevents Gq binding to its targets.

What Happens with Sympathetic FFAs?

Based on the robust result obtained in heterologously expressed FFAs, Won et al. (2013) tested the SCFAs effect on native FFAs from sympathetic neurons. They found no effect of SCFAs on NTCC from superior cervical and stellate ganglia (paravertebral ganglia), but they did find a highly variable intra-assay inhibitory effect on major pelvic and celiac-superior mesenteric ganglia neurons (prevertebral ganglia) that correlates with the FFAs higher expression levels in these ganglia. Although both FFAs are detected in prevertebral ganglia, NTCC inhibition was probably mediated by FFA3 since FFA3 transcript levels are higher compared with FFA2 in prevertebral ganglia and the inhibition was totally VD and PTX sensitive. While this variable response is likely due to differences in receptor expression level, it cannot account for all of the phenomenon since abundant FFA3 expression is detected in superior cervical ganglia.[2,3] Other explanations include the possibility that specific FFAs isoforms could have different signaling pathways or could have modified their agonist induced activity to inhibit NTCC.[8] Moreover, this could reflect a broad portfolio of NTCC isoforms designed by alternative splicing in sympathetic neurons[9] which may have substantially different sensitivity to modulation by G proteins, as seen in sensory neurons (for review, see ref. 10).

Anta and Agonist

The most surprising result from Won et al. (2013) is the finding that BHB is a FFA3 agonist, a ketone body that is produced by the liver to be used as an energy source for peripheral tissues when glucose is not readily available (i.e., fasting, starving condition, diabetes mellitus). In contrast with previous results that postulated BHB to be a FFA3 antagonist,[3] Won et al. (2013) clearly showed that high BHB concentrations inhibit native NTCC from FFA3 expressing neurons by a PTX-sensitive and VD mechanism. To further support their result showing that BHB is a FFA3 agonist, they showed that BHB also activates G protein coupled inward rectifying K+ channels and reduces adenylate cyclase stimulation via FFA3 in a heterologous expression system. While these are solid results, to understand the discrepancies between these 2 studies it will be important to recognize if there are different effects of BHB probably depending on the origin and concentrations of the ketone body, models of study, or effectors studied.

Physiological Remarks

GPCRs are key targets for modulating synaptic transmission at both pre- and postsynaptic membranes by fine control of neurotransmitter release and electrical activity, respectively. Since NTCC rules NE release in the sympathetic NS,[11] the activation of FFA3 could impair peripheral synaptic transmission by a presynaptic inhibitory mechanism.[2] Strong evidence shows that propionate evokes action potentials and increases NE release in a FFA3 dependent manner in sympathetic neurons.[3,4] Although these results seem to be opposite to the effect on NTCC, it is possible that these mechanisms occur in different ganglia. These studies support that FFA3 mediated sympathetic activation occurs in paravertebral sympathetic neurons where no NTCC inhibition by native FFA3 is detected, while FFA3 activation by propionate inhibits NTCCs in prevertebral sympathetic neurons, where

the effect on neuronal firing and/or NE release has not been tested.

Despite the fact that the physiological consequences of FFAs activation are not completely understood, studies such as the Won et al. (2013) contribute to our understanding of SCFAs as signaling molecules that act via FFA3 and modify sympathetic NS activity. Recent metabolic studies highlighted the benefits of soluble fiber by providing SCFAs energy substrates for intestinal gluconeogenesis, contributing to the management of energy balance and glucose metabolism.[1] Additionally, it is well known that fiber-enriched diets promote intestinal motility by butyrate acting on enteric neurons.[12,13] Here, we hypothesize acetate or propionate-induced FFA3 activation and further impact on NTCC activity in sympathetic neurons also contributes to this mechanism. SCFAs could target presynaptic terminals of prevertebral sympathetic neurons that innervate the lower intestine. Thereby, FFA3 activation could impair NE release, decreasing NE-mediated intestinal relaxation and finally contributing to increased intestinal motility.

In conclusion, Wong et al.'s (2013) study of the molecular relevance of FFA3 activation increases the understanding of effects provided by some healthy diets and suggests FFA3 as potential therapeutic targets to improve metabolic disorders such as obesity and diabetes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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