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A new signalling metabolite? Characterizing N-Lactotyl-phenylalanine (Lac-Phe) activation of G protein-coupled receptors (GPCRs)

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Background: Recent reports show that lactoylation of the amino acid phenylalanine (Phe) to produce **N-lactoyl-Phenylalanine (lac-Phe)**, increases during exercise in rodents and humans, and serves as an appetite suppressant, protecting against obesity. Extracellular lac-Phe levels positively correlate with lactate production, and are therefore also of interest in cancer, diabetes, and cardiac disease; where circulating lactate and lac-Phe levels are often elevated. However, nothing is known about the cellular target of lac-Phe bioactivity. This study aims to determine if lac-Phe is a signalling metabolite through activation of GPCRs.

Methods: We monitored lac-Phe production and disappearance in cell culture media using LC-MS (Liquid chromatography–mass spectrometry.) We then quantified and validated the activation of 298 GPCRs treated with two physiologically relevant concentrations of lac-Phe using a high-throughput b-arrestin2 recruitment assay (PRESTO-Tango). GPCRs were classified as a candidate lac-Phe receptor if the activation magnitude exceeded 2-fold and was statistically significant.

Results: Results from the LC-MS monitoring shows that lac-Phe persists in HTLA culture media for sufficient time to perform Tango assay and can be accurately tracked. Validation of the top 10 putative candidate receptors following screening shows that lac-Phe significantly activates 5 of the 298 receptors (R139, R147, R154, R100, R202) at the highest tested concentration (2 mM) with concentration-dependent increases and showed EC_{50} within a micomolar range, consistent with putative paracrine signaling.

Discussion/Conclusion: These findings align with the proposed local/paracrine signalling action of lac-Phe, and suggest that it is a promising signalling metabolite that warrants further pharmacological characterization to determine the mechanism of action behind its exciting role in metabolic regulation.

Keywords: GPCRs, N-lactoyl-Phenylalanine, lac-Phe, Signal Transduction