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Constitutive $P2Y_2$ receptor activity suppresses lipolysis in human adipocytes

S. Ali[1], J. Turner[2], S. Fountain[1]. [1]Biological Sciences, University of East Anglia, Norwich, United Kingdom, [2]Diabetes and Endocrinology, Norfolk and Norwich University Hospital NHS Foundation Trust, Norwich, United Kingdom.

Introduction: Obesity is a global epidemic that represents a significant health and economical issue. Adipocytes in obese individuals display decreased lipolytic (fat-breakdown) activity[1]. P1 purinergic receptors have an established role in suppressing lipolysis[2,3], but little is known about P2 purinoceptors in adipocytes. This study aims to characterise the functional P2 receptors present in human adipocytes and identify their role in lipolysis.

Methods: Adipose-derived mesenchymal cells were isolated from healthy human subcutaneous adipose tissue and differentiated to adipocytes *in vitro*. Cells were loaded with Fura-2 and incubated with selective P2 receptor antagonists to evaluate effects on basal calcium (Ca^{2+}) and nucleotide-evoked Ca^{2+} responses using a Flexstation-III. Lipolysis was measured by quantification of glycerol liberation in conditioned supernatants. cAMP concentrations were measured by competitive ELISA. Lentiviral transduction of adipocytes was used for shRNA-mediated knockdown $P2Y_2$ receptor.

Results: A robust response to ATP, ADP and UTP was observed (N=6 donors). Antagonists, MRS2500 ($P2Y_1$) and PSB0739 ($P2Y_{12}$), inhibited the ADP-evoked Ca^{2+} responses by 45.1±5% (IC_{50} 77.1±38nM) and 39.7±5% (IC_{50}64.0±7nM) (N=6 donors) respectively. These antagonists also blocked the ATP-evoked responses (N=6 donors). Although AR-C118925XX ($P2Y_2$ antagonist) completely inhibited the UTP-evoked Ca^{2+} responses, IC_{50} 318±399nM (N=6 donors), it only reduced the magnitude of response to ATP by 20.4±3%, IC_{50} 683±116nM (N=6 donors). $P2Y_1$ and $P2Y_{12}$ antagonists and exogenous nucleotide application had no effect on lipolysis, but inhibition of $P2Y_2$, using AR-C118925XX and $P2Y_2$ shRNA, caused an increase in basal glycerol production by 32.2±7.8% and 31.6±8.6% respectively (N=3 donors). Inhibiting $P2Y_2$ decreased intracellular Ca^{2+} (11.3±3.6%, N=6 donors) and elevated cAMP (23.3±5.8%, N=4 donors) in adipocytes. Decreased Ca^{2+} can activate adenylate cyclase, thus increasing cAMP which can subsequently activate the protein kinase A-hormone sensitive lipase lipolytic pathway. $P2Y_2$ knockdown also caused a phenotypic difference in the lipid droplet size in adipocytes, which we hypothesise is due to increased lipolytic activity.

Conclusion: $P2Y_1$, $P2Y_2$ and $P2Y_{12}$ are involved in the nucleotide-evoked Ca^{2+} responses in adipocytes. $P2Y_2$ appears to be tonically active and plays a role in constitutively suppressing basal lipolysis by maintaining intracellular Ca^{2+} to inhibit cAMP production and subsequent downstream lipolysis induction. These findings suggest that $P2Y_2$ may be a novel drug target for controlling lipolysis in human adipocytes.

Reference:

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