A Second Site Of Action For The CGRP Antagonist Olcegepant, A Drug For The Acute Treatment Of Migraine

Calcitonin gene-related peptide CGRP is a neuropeptide that plays a central role in the pathophysiology of migraine and hence there is great interest in identifying CGRP antagonists as treatments for this disease. CGRP mediates its effects through the $CGRP_1$ receptor. However the pharmacology of this receptor is complex requiring both a G protein-coupled receptor, the calcitonin receptor-like receptor (CRLR) and a second protein, the receptor activity modifying protein 1 (RAMP1) for functional activity. Furthermore RAMP1 can also form a distinct functional receptor in complex with the closely related Calcitonin receptor. This complex demonstrates high affinity for the peptide amylin and hence has been designated the Amylin receptor.

Identification of CGRP antagonists for migraine has focussed primarily on compounds demonstrating high selectivity for the $CGRP_1$ receptor over the Amylin receptor. However the peptide CGRP can bind and functionally activate both the $CGRP_1$ and Amylin receptors with high affinity/potency. The purpose of this study was to determine if olcegepant, a potent $CGRP_1$ receptor antagonist which has demonstrated clinical efficacy against migraine, was able to antagonise the effects of CGRP at the Amylin receptor.

COS-7 cells were transiently transfected with equal ratios of DNA for CRL/RAMP1 ($CGRP_1$) or Calcitonin/RAMP1 (Amylin receptor). After 24 h cells were seeded at 12,500 cells/well in half-area 96-well plates in DMEM/10 % FBS. 16 h later media was removed and serum free media supplemented with 500μM IBMX and increasing concentrations of olcegepant for 30 min at 37 °C. Agonist EC_{80} challenge with either CGRP or amylin was then performed for a further 30 min at 37 °C. To measure functional responses through $G\alpha_s$ G protein cAMP was measured as per manufacturer's instructions (CisBio). The IC_{50} values were converted to pKB using the Cheng-Prusoff equation.

Table 1 Functional affinity of olcagepant in COS-7 cells expressing the CGRP or amylin receptor, determined against the agonists CGRP and Amylin.

Agonist	**$CGRP_1$ Receptor pKB**	**Amylin Receptor pKB**
CGRP	10.5 ± 0.02	8.2 ± 0.05
Amylin	10.3 ± 0.04	<5

Values are duplicate observations or mean triplicate observations ± s.e.m.

Results demonstrate that olcegepant is able to bind and functionally antagonise the Amylin receptor when CGRP is used as the agonist albeit with a lower potency than at the CGRP receptor. However olcegepant is selective for the Amylin receptor when amylin is used as the agonist. The recent identification of Amylin receptor expression in trigeminal neurons (1), highlights the potential for a second site of action for CGRP in the pathophysiology of migraine. Therefore it is possible that the ability of olcegepant to block the effects of CGRP at the Amylin receptor may contribute to its effectiveness as an anti-migraine treatment. It will therefore be important to determine the contribution each receptor plays in the pathophysiology of migraine as more research is performed in this area.

1. Walker C.S et al.,(2015) *Ann Clin & Trans Neuro* **2(6)**: 595–608.