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PACAP(6-38) Deletants as Peptide-based PACAP Antagonists

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The receptor for pituitary adenylate cyclase-activating polypeptide (PACAP) is a Gs-coupled GPCR, designated PAC1. Antagonists of this receptor are being pursued for their potential to ameliorate atherosclerogenesis, depression, post-traumatic stress disorder, and migraine; discovery of agonists is also of interest for counteracting the neurodegenerative consequences of stroke and ischemia. However, obtaining small-molecule ligands (SMOLs) for this receptor has been challenging. Peptide-based antagonists include an N-terminally truncated version of PACAP38 (P38) itself, PACAP6-38 (P6-38). The activity of P6-38 as an antagonist is consistent with models of PACAP binding to PAC1 in which the N-terminus of PACAP is required for receptor activation leading to signaling through the Gs protein, while the C-terminus is involved in initial binding (affinity-trapping) to PAC1. P6-38 has been shown repeatedly to block the action of P38 as well as the naturally occurring ligand PACAP1-27 (P27) both in vivo and in cells expressing endogenous PAC1. Several SMOLs reported to be PAC1 antagonists in the literature were tested for antagonist activity in HEK293 cells expressing the human PAC1 receptor and a cAMP biosensor (CBS)-based cAMP detection system; however not only these but also P6 -38 were without inhibitory effect in this assay system. Accordingly, we re-tested the inhibitory activity of P6-38 against both P27 and P38 in cells expressing CBS but with native expression of PAC1: the rat NS-1 pheochromocytoma cell line, and the human SH-SY5Y neuroblastoma cell line. Both CBS-based luminescent read-out, and generation of cAMP provoked by exposure to EC50 concentrations of P27 or P38 (0.2 nM) were blocked by 1 mM P6-38 which also blocked downstream signaling effects (neuritogenesis) of P27 and P38.

Recently, cryo-EM structures of a number of family B (secretin family) GPCR-ligand structures have been obtained, allowing detailed molecular dynamics analysis of ligand-receptor binding, and prediction of residues contributing most strongly to ligand engagement. Molecular dynamics simulations predicted initial receptor engagement with residues 6-30, but not 31-38, of P38. Accordingly, we synthesized a series of deletants of PACAP6-38 and tested their relative inhibitory potency in SH-SY5Y and NS-1 cells. PACAP6-30 (IC50 ~33 nM) was nearly as potent as PACAP6-38 (IC50 ~21 nM) as an inhibitor of cyclic AMP elevation by 0.2 nM P38 (the approximate EC50 for both the CBS-based and the direct cAMP assay, both carried out in the presence of phosphodiesterase inhibition by IBMX). PACAP6-27 was considerably less potent (IC_{50} >10 mM). Thus, the affinity-trap model for PAC1-ligand interaction successfully predicts the behavior of peptide-based inhibitors of P27 and P38 engagement with an endogenously expressed PAC1 receptor. We are currently engaged in peptide modification of P6-30 in order to increase the potency of this PAC1 antagonist.

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