Oxytocic plant cyclotides as templates for peptide G protein-coupled receptor ligand design

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Abstract

Cyclotides are plant peptides comprising a circular backbone and three conserved disulfide bonds that confer them with exceptional stability. They were originally discovered in Oldenlandia affinis based on their use in traditional African medicine to accelerate labor. Recently, cyclotides have been identified in numerous plant species of the coffee, violet, cucurbit, pea, potato, and grass families. Their unique structural topology, high stability, and tolerance to sequence variation make them promising templates for the development of peptide-based pharmaceuticals. However, the mechanisms underlying their biological activities remain largely unknown; specifically, a receptor for a native cyclotide has not been reported hitherto. Using bioactivity-guided fractionation of an herbal extract known to indigenous healers as “kalata-kalata”, the cyclotide kalata B7 was found to induce strong contractility on human uterine smooth muscle cells. Radioligand displacement and second messenger-based reporter assays confirmed the oxytocin and vasopressin V1a receptors, members of the G protein-coupled receptor family, as molecular targets for this cyclotide. Furthermore, we show that cyclotides can serve as templates for the design of selective G protein-coupled receptor ligands by generating an oxytocin-like nonapeptide with enhanced receptor selectivity. This naturally-occurring peptide served as a template for the design of an oxytocin-like nonapeptide with enhanced receptor selectivity, highlighting the potential of cyclotides for the discovery of peptide-based GPCR ligands.

Significance

G protein-coupled receptors (GPCRs) are promising drug targets: >30% of the currently marketed drugs elicit their actions by binding to these transmembrane receptors. However, only ~10% of all GPCRs are targeted by approved drugs. Resorting to plant-derived compounds catalogued by ethnopharmacological analyses may increase this repertoire. We provide a proof of concept by analyzing the uterotonic action of an herbal remedy used in traditional African medicine. We identified cyclic peptides, investigated the molecular mechanisms underlying their uterotonic activity, and report an oxytocic plant peptide that modulates the human oxytocin/vasopressin receptors. This naturally-occurring peptide served as a template for the design of an oxytocin-like nonapeptide with enhanced receptor selectivity, highlighting the potential of cyclotides for the discovery of peptide-based GPCR ligands.