Video Article

A CALCIUM BIOLUMINESCENCE ASSAY FOR FUNCTIONAL STUDIES OF MOSQUITO (Aedes aegypti) and TICK (Rhipicephalus microplus) G PROTEIN-COUPLED RECEPTORS

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Abstract

Arthropod peptide hormone receptors are potential targets for novel insecticides as they regulate many essential physiological and behavioral processes. The majority of them belong to the superfamily of G protein-coupled receptors (GPCRs). We have focused on characterizing arthropod kinin receptors from the tick and mosquito. Arthropod kinins are multifunctional neuropeptides with myotropic, diuretic, and neurotransmitter function. Here, a method for systematic analyses of structure-activity relationships of insect kinins on two heterologous kinin receptor-expressing systems is described. We provide important information relevant to the development of biostable kinin analogs with the potential to disrupt the diuretic, myotropic, and/or digestive processes in ticks and mosquitoes. The kinin receptors from the southern cattle tick, Boophilus microplus (Canestrini), and the mosquito Aedes aegypti (L.), were stably expressed in the mammalian cell line CHO-K1. Functional analyses of these receptors were completed using a calcium bioluminescence plate assay that measures intracellular bioluminescence to determine cytoplasmic calcium levels upon peptide application to these recombinant cells. This method takes advantage of the aequorin protein, a photoprotein isolated from luminescent jellyfish. We transiently transfected the aequorin plasmid (mtAEQ/pcDNA1) in cell lines that stably expressed the kinin receptors. These cells were then treated with the cofactor coelenterazine, which complexes with intracellular aequorin. This bond breaks in the presence of calcium, emitting luminescence levels indicative of the calcium concentration. As the kinin receptor signals through the release of intracellular calcium, the intensity of the signal is related to the potency of the peptide. This protocol is a synthesis of several previously described protocols with modifications; it presents step-by-step instructions for the stable expression of GPCRs in a mammalian cell line through functional plate assays (9, 10). Using this methodology, we were able to establish stable cell lines expressing the mosquito and the tick kinin receptors, compare the potency of three mosquito kinins, identify critical amino acid positions for the ligand-receptor interaction, and perform semi-throughput screening of a peptide library. Because insect kinins are susceptible to fast enzymatic degradation by endogenous peptidases, they are severely limited in use as tools for pest control or endocrinological studies. Therefore, we also tested kinin analogs containing amino isobutyric acid (Aib) to enhance their potency and biostability. This peptidase-resistant analog represents an important lead in the development of biostable insect kinin analogs and may aid in the development of neuropeptide-based arthropod control strategies.

Disclosures

No conflicts of interest declared.