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G protein-coupled receptors from mosquito *Aedes aegypti* excretory system: characterization, function and structure- activity relationships

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Adult diuresis (urine production) in the mosquito *Aedes aegypti* involves at least two types of hormones: diuresin and kinins. The three *Aedes* kinins had been previously established to have diuretic activity *in vivo* (Cady and Hagedorn, 1999). *In vitro*, however, *Aedes* kinin 1 and kinin 3 but not *Aedes* kinin 2 elicit fluid secretion in the isolated Malpighian tubule (Veenstra et al., 1997). *Aedes* kinin 2, instead, depolarizes the transepithelial voltage in the isolated M. tubule.

We have cloned, expressed and characterized the kinin receptor from females of *A. aegypti*. The receptor was expressed in CHO-K1 cells and an intracellular calcium bioluminescence assay was developed to study receptor function. We have demonstrated that the three *Aedes* kinins indeed activate the receptor, increasing the intracellular concentration of calcium in CHO-K1 cells. The rank order of potency was *Aedes* kinin 3 > *Aedes* kinin 2 > *Aedes* kinin 1, with EC50s being statistically different. The unusual activity of *Aedes* kinin 2 on the isolated M. tubule thus cannot be explained by the lack of activity on the receptor, this being a multiligand receptor for the three *Aedes* kinins (Pietrantonio et al., 2005).

In collaboration with Dr. Ronald Nachman we have recently studied the structure-activity relationships of kinin analogs on this heterologous expression system. Several peptides were tested in order to elucidate the minimum structural requirements for activity.

The results of an alanine scan of the kinin analog **FFSWG-NH₂** revealed that amino acids Phe¹ and Trp⁴ were essential for activity of the insect kinin. It was confirmed that the pentapeptide kinin core is the minimum sequence required for activity and that the C-terminal amide is also essential. This information is useful for the design of novel agonists.

Information about other G protein-coupled receptors affecting excretion will be provided.

Publication:

Pietrantonio, P. V., C. Jagge, S. Taneja-Bageshwar, R. Nachman, and R. Barhoumi. 2005. The mosquito *Aedes aegypti* (L.) leucokinin receptor is a multiligand receptor for the three *Aedes* kinins. *Insect Mol. Biol.* 14: 55-67