Abstract

Signal transduction in most olfactory sensory neurons of vertebrates is initiated by the binding of odorants to G-protein-coupled receptors in the ciliary membrane. The activation of the odorant receptor initiates a signal transduction cascade leading to an increase of intracellular cAMP. In a small subpopulation of olfactory sensory neurons, a cGMP-dependent pathway seems to be involved, since none of the components of the cAMP pathway were found in these cells. These neurons express a membrane bound guanylate cyclase, the GC-D. The physiological ligand of the GC-D has not been identified yet. The GC-D may be activated by pheromone-like odorants that control social behavior and suckling.

In the present study the cDNA of the GC-D from mouse (mGC-D) was cloned from genomic DNA. Specific antibodies against the GC-D were used to localize the protein in the subpopulation of olfactory sensory neurons in the mouse olfactory epithelium. The mGC-D protein was expressed in eucaryontic cell lines. Two assays were developed to measure the protein activity of GC-D with the goal of detecting the physiological ligand of the protein. The first assay is an *in vitro* system. Here, the activation of the mGC-D protein is measured by the amount of cGMP formed by the protein. A small GC-D activity, the basal activity, could be measured in the absence of ligand. In order to increase the sensitivity of the assay, the recombinant mGC-D protein can be solubilised and purified by affinity chromatography. A purification protocol was tested and has to be optimized. The second assay is a cellular system. Here, the GC-D and a cGMP-sensitive ion channel are coexpressed in an eucaryontic cell line. The binding of the ligand leads to the activation of GC-D. The increase of formed cGMP inside the cell opens the ion channels. Ca^{2+} ions from the extracellular medium flow into the cell. The intracellular Ca^{2+} concentration can be monitored by Ca^{2+}-sensitive fluorescence dyes. This assay is very sensitive due to signal amplification by the Ca^{2+} influx through opened ion channels.

In further experiments the ligand of the GC-D may be identified using the described assays. Various body fluids of mice should be divided in their single components and applicated in the assays, in order to detect a possible activation of the GC-D.