SIGNALLING

Orphans find a family

Identifying the ligands of 'orphan' G-protein-coupled receptors (GPCRs) is not an easy task, and potential ligands for such receptors are often predicted on the basis of homology to GPCRs for which the ligand is known. However, as Ling and colleagues now show in *Nature*, such predictions can be inaccurate. GPR91 and GPR99 were predicted to bind nucleotide ligands, but these authors show that they actually bind to citric-acid-cycle intermediates — a result that will create "…renewed interest in a biochemical pathway discovered more than 60 years ago".

They first found that, of the various tissue extracts they tested, pig kidney extract could activate GPR91-expressing cells. When they purified the natural ligand contained in this extract, they were surprised to find that it was succinate. As GPR91 and GPR99 are close relatives, they reasoned that the ligand for GPR99 might also be a citric-acid-cycle intermediate. Indeed, they showed that α-ketoglutarate activates GPR99-expressing cells.

Using various biochemical assays, Ling and colleagues showed that the activation of GPR91 by succinate is linked to at least two signalling pathways that function through different G-protein subunits (a G_i/G_o-mediated pathway and a G_q-mediated pathway). GPR99, on the other hand, seems to function only through a G_q-mediated pathway. These authors also showed that ligand stimulation induces the internalization of GPR91 and GPR99, which is often characteristic of GPCR activation and signal attenuation.

To further understand the GPCR–ligand interaction, the authors created a partial three-dimensional model of GPR91. They identified four, clustered, positively charged residues that are directed towards a central cavity, which might function as the succinate-binding site. Interestingly, when they compared ~300 GPCRs, they found that only GPR91 and GPR99 contain all four of these basic residues. These GPCRs might therefore represent a specialized family of receptors for dicarboxylate ligands.

A previous report showed that succinate-treated kidney cultures release renin (an important enzyme in the regulation of blood pressure). So, in the final part of their study, Ling and colleagues studied the physiological significance of their data. They showed that GPR91 and GPR99 are predominantly expressed in the kidney, and that the intravenous administration of succinate to rats increased the activity of plasma renin and increased blood pressure in a dose-dependent manner. Furthermore, they showed that succinate could not induce hypertension in GPR91-deficient mice.

So, as well as identifying ligands for orphan GPCRs, this work has identified unexpected signalling functions for citric-acid-cycle intermediates. And, as Ling and colleagues conclude, their findings "…should facilitate the understanding of molecular links of the citric acid cycle to metabolic diseases, such as hypertension, atherosclerosis and diabetes, and the design of novel drugs with GPR91 and GPR99 as molecular targets".

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER He, W. *et al.* Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* **429**, 188–193 (2004)

FURTHER READING Hebert, S. C. Physiology: orphan detectors of metabolism. *Nature* **429**, 143–145 (2004)