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Molecular diversity of a family of pain receptors

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Abstract

A large family of G-protein-coupled receptors, with sequence homology to the oncoprotein MAS1, has been shown to be specifically expressed in nociceptive sensory neurons.

Significance and context

Vertebrate peripheral chemosensory neurons express large families of G-protein-coupled receptors (GPCRs), reflecting the diversity of ligands that these sensory systems detect. In contrast, peripheral somatosensory neurons within the body are thought not to discriminate specifically between different chemical ligands, but rather to respond to polymodal stimuli using broadly tuned receptors such as the vanilloid receptor I (VRI). Dorsal root ganglia (DRGs) contain diverse subpopulations of primary sensory neurons. One category comprises the nociceptors, which respond to a variety of noxious thermal, mechanical and chemical stimuli that cause acute pain. These receptors also mediate the chronic pain associated with inflammatory responses or nerve injury (neuropathic pain). Dong *et al.* describe a gene family consisting of nearly 50 MAS1-related GPCR genes - *Mas-related genes* (*mrgs*) - whose expression indicates an unanticipated degree of molecular diversity among DRG sensory neurons.

Key results

The authors used a subtractive cDNA method to clone genes expressed specifically in nociceptive neurons. The material used was cDNA from wild-type mice and the *Ngn1* knockout mice. The *Ngn1* knockout mice lack a transcription factor (neurogenin 1) needed for the generation of a subclass (TrkA+) of nociceptive neurons. Therefore, genes that are only found in the wild-type mouse cDNA are thought to be specifically expressed in nociceptive neurons.The screen identified several previously unknown genes, among which was one - *mrgA1* - encoding a GPCR with the highest homology to MAS1. Further screening of murine cDNA and bacterial artificial chromosome (BAC) clones, as well as computer searches, revealed a family of close to 50 related GPCRs. The *mrg* genes are divided into three families, *mrgA*, *mrgB* and *mrgC*, plus some additional *Mas1*-related genes, referred to as *mrgD-mrgG*. Database searches also identified four human *mrg* genes (*Hs.mrgX*). Curiously, several of the identified *mrg*genes are pseudogenes.

Using *in situ* hybridization, Dong *et al*. showed that the *mrgA* genes were all expressed in sensory neurons in wild-type neonatal DRGs. They were virtually absent from DRGs of *Ngn1*$^{-/-}$ mice, consistent with the results of the subtractive hybridization screen. The broadest expression pattern was that of *mrgA1*, and the rarer *mrgA* genes (*mrgA2-mrgA8*) were shown to be expressed in a subset of those neurons that express *mrgA1*. The expression of *mrgA* genes in sensory neurons appears highly specific, in that *in situ*hybridization signals have not been detected in any other tissue except for trigeminal ganglia.

Using double-labeled *in situ* hybridization, Dong *et al*. were able to pinpoint the exact subpopulation of sensory neurons expressing *mrgA1* and *mrgD*. Expression of these *mrg* genes was shown to be restricted to nonpeptidergic nociceptive neurons that are positive for isolectin B4 and negative for the vanilloid receptor. As confirmation, the cell-body diameters of the neurons expressing the *mrg* genes were measured and shown to fall within the size range characteristic of small-diameter nociceptive sensory neurons. The localization of *mrg*expression led the authors to conclude that the receptors they encode may be involved in pain perception or pain regulation.

To determine whether mrgAs can function as receptors for neuropeptides, *mrgA1* and *mrgA4* were cloned into a eukaryotic expression vector and transfected into human embryonic kidney (HEK) cells. Candidate peptides (45) were screened for their ability to activate the mrgAs using an intracellular Ca^{2+} -release assay. Several neuropeptides, including adrenocorticotropin (ACTH), CGRP-I and II, NPY and somatostatin, produced some level of activation at 1 μM concentration. The most efficient response was elicited by RFamide peptides. Some RFamides could activate the receptors at nanomolar concentrations, ranging from 20 to 200 nM. RFamides have been shown to be involved in nociception, in accord with the suggested action of mrgAs in pain perception and pain regulation. The fact that ACTH activates MRGs as effectively as do the RFamides raises the question of whether the authentic ligand is an RFamide peptide or some unrelated peptide.

Reporter's comments

Dong *et al*. have combined a set of straightforward methods in an interesting way to obtain a wide variety of information about a gene family. The same strategy could easily be adapted to other fields of molecular biology in order to identify important genes.

Table of links

Cell

References

1. Dong X, Han S, Zylka MJ, Simon MI, Anderson DJ: A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. Cell. 2001, 106: 619-632. 0092-1903