CELL BIOLOGY OF THE NEURON

A Piccolo in the presynaptic orchestra

Piccolo is a presynaptic protein that seems to be involved in the assembly of the active zone. Owing to its two C_2 domains (Ca^{2+}- and lipid-binding motifs), it has also been suggested that it might participate in signalling processes at the synapse. However, the functional significance of these domains for Piccolo remains unknown. A new structural study provides an intriguing lead on this problem by reporting that the C_2A domain of Piccolo undergoes alternative splicing, which yields isoforms that have markedly different biochemical properties.

Synaptotagmin 1, another presynaptic protein, also has C_2 domains, which confer on this protein the ability to bind Ca^{2+} and act as its sensor during synaptic exocytosis. Intriguingly, the C_2A domains of Piccolo and synaptotagmin are quite similar; one of the main differences is a stretch of nine amino acids that is only present in Piccolo. In the new study, Garcia *et al*. found evidence that this segment was alternatively spliced. Remarkably, the 'short' version of the protein had a higher affinity for Ca^{2+} than the unspliced variant. In addition, splicing resulted in the abrogation of the previously characterized Ca^{2+}-dependent dimerization of Piccolo.

From the structural point of view, spectroscopic analysis showed that, in the 'long' version of the protein, the nine-amino acid segment displaces a canonical β-strand from a region of the protein known as the β-sandwich. The displaced

NEUROPHYSIOLOGY

Just a wee dram…

Although alcohol is perhaps the oldest and most commonly used psychoactive drug, we still don't understand exactly how it acts on the nervous system. Two new studies take us closer to understanding the effects of alcohol by showing how it might depress neuronal activity. One study shows that, in *Caenorhabditis elegans*, the behavioural effects of ethanol are largely mediated by a BK potassium channel, whereas the other finds that the activity of extrasynaptic GABA (γ-aminobutyric acid) receptors can be enhanced *in vitro* by low concentrations of ethanol.

Davies and colleagues used a behavioural assay to search for *C. elegans* mutants that were resistant to the effects of ethanol. The screen showed that *slo-1* mutants, which have uncoordinated movements, were resistant to the behavioural effects of ethanol. *Slo-1* encodes a calcium-activated, large-conductance BK potassium channel, and electrophysiological analysis showed that the channel is activated by ethanol at concentrations that cause intoxication in humans. The authors also found that a gain-of-function mutation in *slo-1*, which increased the activity of the channel, caused behaviour similar to that caused in wild-type animals by ethanol treatment.

Activation of the BK channel by ethanol would be expected to inhibit neuronal activity. In the second study, by Wallner *et al*., ethanol was found to have another inhibitory effect: enhancement of GABA receptor activation. Ethanol was previously known to enhance activation of GABA receptors, but only at high concentrations, so it was unclear what the physiological relevance of this effect might be. Wallner and colleagues show that rat GABA receptors containing the δ-subunit are sensitive to much lower concentrations of ethanol, similar to those that are achieved in humans by moderate drinking.

GABA receptors that contain the δ-subunit are thought to be exclusively extrasynaptic, and have a high affinity for GABA. They are also slow to desensitize, and probably mediate tonic inhibition of neurons. Activation of these receptors was markedly enhanced by low concentrations of ethanol in a *Xenopus* oocyte expression system. The sensitivity varied depending on the precise subunit composition of the receptors, with receptors containing the β_3-subunit being about 10 times as sensitive to ethanol as those with the β_2-subunit. The β_3-subunit is also thought to mediate the effects of some anaesthetics.

BK channels and δ-subunit-containing GABA receptors might mediate similar behavioural effects of ethanol. BK channels seem to be the primary target for ethanol in *C. elegans*, but extrasynaptic GABA receptors might have an important role in other organisms. However, until the effects of ethanol on these pathways can be measured directly in mammalian neurons, their importance for the effects of alcohol in humans will remain unclear.

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References and links

ORIGINAL RESEARCH PAPERS Davies, A. G. *et al.* A central role of the BK potassium channel in behavioural responses to ethanol in *C. elegans*. *Cell* **115**, 655–666 (2003) | Wallner, M. *et al.* Ethanol enhances $\alpha_4\beta_3\delta$ and $\alpha_6\beta_3\delta$ γ-aminobutyric acid type A receptors at low concentrations known to affect humans. *Proc. Natl Acad. Sci. USA* **100**, 15218–15223 (2003)

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