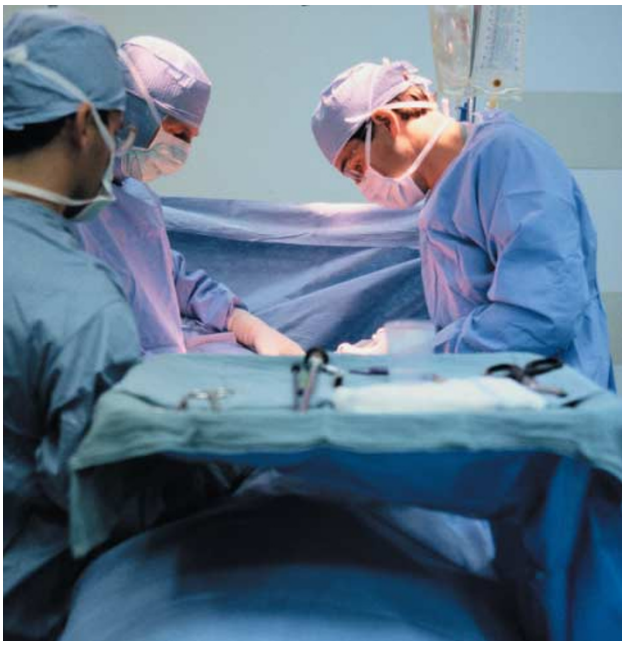
PAIN

Dissecting anaesthetics



Hailed as a revolutionary advance more than 150 years ago, pain-free surgery has become central to the practice of modern medicine. Dependence on general anaesthetics — which induce both loss of consciousness (sedation) and insensitivity to pain (anaesthesia) — has developed despite only rudimentary knowledge of the mechanisms by which these drugs act on the central nervous system. We do know that many anaesthetics target ion channels that control neuronal excitability; Keith Wafford and colleagues have now shown that the anaesthetic and sedative effects of etomidate are mediated by distinct receptor subtypes of the major inhibitory neurotransmitter system.

Etomidate potentiates the action of GABA (γ-aminobutyric acid) at the $GABA_A$ receptor, greatly enhancing inhibitory synaptic transmission. More specifically, the drug targets receptors that contain β2 and β3 subunits, with the asparagine residue at position 265 regulating this selectivity. To distinguish between the action of etomidate at the two subtypes, Wafford *et al.* used a gene-targeting approach to create mice in which the crucial asparagine residue of β2 subunits was substituted with a serine. As such, the anaesthetic could act only on β3-containing receptors.

Mutant mice were phenotypically normal, and distinguishable from their wild-type counterparts only during pharmacological challenge. When treated with intravenous etomidate, both wild-type and mutant subjects lost their righting reflex and became insensitive to pain, indicating that β2 subunits are not necessary *per se* for induction of the anaesthetic effect. However, the period during which wild-type mice were insensitive to noxious stimuli and incapable of righting themselves was much longer than that of the mutants, indicating that the presence of β2 subunits influences the duration of anaesthesia.

The authors used the rotarod test to show that mice lacking etomidate-responsive β2 subunits recover motor

NEURULATION

What's cooking with *cordon-bleu*?

Neurulation entails a coordinated sequence of cell movements and rearrangements, culminating in fusion of the neural folds at the dorsal-most point of the neural tube. The molecular pathways that regulate these morphogenetic events are gradually being elucidated, and in *Developmental Biology*, Carroll and colleagues report on the characterization of *cordon-bleu* (*Cobl*), a gene that seems to be required for neural tube closure in the midbrain.

The *Cobl* locus was originally identified in the mid-1990s in a mouse gene trap screen. The mutant allele, which contained a *lacZ* gene insertion, was named $Cobl^{C101}$. Analysis of the mutant embryos provided few clues as to the function of *Cobl*, as neither heterozygous nor homozygous $Cobl^{C101}$ mutants showed an abnormal phenotype. The $Cobl^{C101}$ locus can produce wild-type *Cobl* mRNA, although Carroll *et al.* showed that the level of transcription is reduced in homozygous mutants, indicating that the $Cobl^{C101}$ allele is hypomorphic rather than null.

The *Cobl* gene is expressed in the ventral neural tube during neurulation, and Carroll *et al.* investigated whether it has a role in this process. They examined the effects of the $Cobl^{C101}$ mutation in embryos that were also mutant for the *loop-tail* (*Lp*) gene. Homozygous *Lp* mutants show failure of neural tube closure from the hindbrain to the caudal end of the embryo, whereas the heterozygotes show the milder looped tail phenotype that gave the mutant its name. Carroll *et al.* found that in around 20% of embryos that were homozygous for $Cobl^{C101}$ and heterozygous for the *Lp* mutation ($Cobl^{C101}/Cobl^{C101}$;*Lp*/+), the neural tube also remained open in the midbrain region.

Although *Cobl* is expressed in the dorsal midbrain from around embryonic day (E) 11.5, this is too late to account for the mutant phenotype, which is evident by E9.5. So, it is probable that *Cobl* acts more ventrally to influence neural tube closure. The product of the *Lp* locus, Vangl2, acts as an antagonist of the ventrally-derived signalling molecule sonic hedgehog (Shh), and the authors postulate that Cobl provides additional inhibition of Shh activity. Shh inhibits the development of the dorsolateral hinge point, which generates inward curvature of the neural plate, and the morphology of the $Cobl^{C101}/Cobl^{C101}$;*Lp*/+ midbrain was consistent with a defect in this process.

Now that the *Cobl* locus has been fully characterized, it should be possible to generate a null mutant so that its role in neurulation can be investigated in more detail. Interestingly, the structure of the *Cobl* gene indicates that it belongs to an entirely new family of genes. A related gene — *Coblr1* — has already been identified in mice, and there are also *Cobl* homologues in many other vertebrate species. The finding of Carroll *et al.* should stimulate further research into this intriguing gene family.

Heather Wood

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performance much sooner than wild-type mice following administration of intravenous etomidate. The reason for this was revealed by analysis of EEG patterns, which showed that slow-wave sleep was prolonged in wild-type mice during recovery from anaesthesia, whereas mutant mice had normal sleep rhythms.

Taken together, these data indicate that β2-containing $GABA_A$ receptors mediate the sedative properties of etomidate, while its anaesthetic effects are exerted through β3 subunits. This insight could lead to the development of effective anaesthetics that do not induce the 'hypnotic hangover' with which so many of us are familiar.

Suzanne Farley

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WEB SITES

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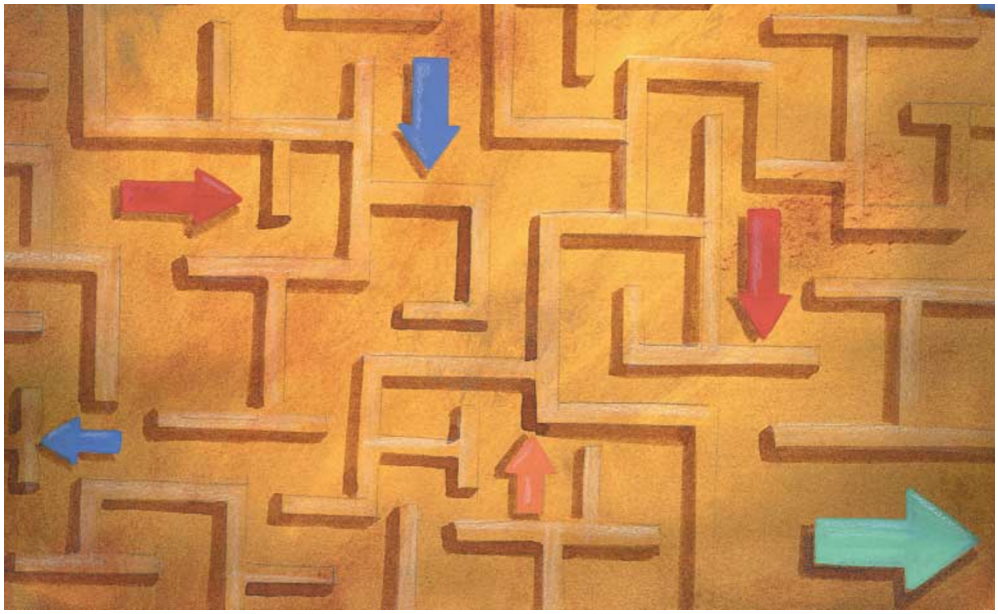
Anaesthesia: modern approaches



DEVELOPMENT

Finding the way

In the mouse visual system, most axons of retinal ganglion cells cross at the optic chiasm to project to the contralateral side of the brain. But a small proportion — about 3% — project ipsilaterally instead, allowing the development of binocular vision. In humans, the ipsilateral projection is larger, but the problem is the same. What controls this divergence of growing axons? A new study provides evidence that the ephrins and Eph receptors are involved.

Previous evidence has implicated the Eph receptors in retinal axon guidance. The two classes of Eph receptor are expressed in gradients across the retina, with EphAs being highest in the temporal retina and EphBs in the ventral retina. In addition, dorsal retinal axons that express ephrin-Bs can use EphBs as a guidance cue. But Williams *et al.* have uncovered more details of the role of Eph receptors and ephrins in retinal axon guidance.

The ganglion cells whose axons fail to cross at the midline are found in the most ventrotemporal part of the retina. Williams and colleagues found that these cells specifically express a particular Eph receptor, EphB1. They also showed that ephrin-B2, a ligand for EphB1, is expressed at the optic chiasm, and that although ephrin-B2 inhibits axon outgrowth from all ventral retinal ganglion cells *in vitro*, it is most inhibitory to the axons of cells from the ventrotemporal retina. These findings tie in with previous findings in the *Xenopus* retina, indicating that the system might be conserved.

When the researchers blocked ephrin-B2 in a coculture of retinal ganglion cells and optic chiasm cells, or in a semi-intact preparation, the treatment abolished the ability of chiasm cells to inhibit outgrowth from ventrotemporal ganglion cells. And in mice lacking the EphB1 receptor, the ipsilateral projection from the retina was greatly reduced, with more axons crossing the midline instead of being repelled by the chiasm.

These results strongly support the idea that a subset of retinal ganglion cells — those that are destined to project ipsilaterally — expresses a specific Eph receptor, EphB1, and that this receptor interacts with ephrin-B2 at the optic chiasm to prevent the axons from crossing. But the fact that the ipsilateral projection in EphB1-null mice was reduced rather than abolished indicates that other signals are also involved.

In a related study, Herrera *et al.* found that the transcription factor Zic2 is selectively expressed in the ventrotemporal subset of retinal ganglion cells that project ipsilaterally. Mice with reduced levels of Zic2 had smaller than normal ipsilateral projections, and ectopic expression of Zic2 in dorsal retinal ganglion cells *in vitro* caused the axons of those cells to become abnormally sensitive to inhibition by chiasm cells. These results raise the tantalizing possibility that Zic2 might regulate the expression of EphB1 in ventrotemporal retinal ganglion cells, and so mediate the formation of the ipsilateral visual projection.

Rachel Jones

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