metabolic pathway. Not only will there be a decrease in the pain-producing prostaglandins (by inhibition of cyclooxygenase), but there will be increased production of pain-relieving lipoxygenase metabolites, which are activated by opioids.

Vaughan *et al.* used brain slices containing the periaqueductal grey (PAG) — a central site in the modulation of pain pathways[4]. One of the main effects of opioids in the PAG (and the measure used in this study) is the presynaptic inhibition of GABA (γ-aminobutyric acid)-mediated synaptic currents[5]. Although opioid-mediated presynaptic inhibition, particularly from GABA-releasing terminals, is common in the central nervous system, there is no consensus on the ionic mechanism(s) and second-messenger pathways involved[6]. Possible mechanisms to account for the presynaptic actions of opioids are the inhibition of voltage-dependent calcium currents and the activation of an inwardly rectifying potassium conductance.

The new study indicates that neither of these mechanisms is significant at this synapse in the PAG, but that opioids activate a voltage-dependent potassium current. (Such a mechanism has been described in cell bodies of isolated hippocampal neurons[7].) The presynaptic inhibition by opioids in the PAG was reduced by 4-aminopyridine (4-AP) and dendrotoxin, both of which block voltage-dependent potassium conductances. In the same cells, the presynaptic inhibition caused by activation of $GABA_B$ receptors with baclofen was not sensitive to 4-AP or dendrotoxin. This indicates selectivity of the opioid action. In another study[8], activation of a similar voltage-dependent potassium conductance in sensory neurons of *Aplysia* decreased both the duration of the action potential and the release of transmitter. This mechanism could account for the opioid-mediated inhibition of the GABA release that is induced by action potentials. But the decrease in the frequency of calcium-independent miniature GABA-mediated synaptic currents by opioids was also sensitive to 4-AP and dendrotoxin.

Opioid inhibition of these currents in the PAG was blocked by inhibitors of arachidonic-acid metabolism. This has not been seen before in mammals, although a similar mechanism has been shown to mediate presynaptic inhibition in sensory neurons in *Aplysia*[8]. Many of the enzyme inhibitors that were used in that study were also found by Vaughan *et al.* to be effective in the PAG. In both cases, the 12-lipoxygenase pathway generates compounds that effectively inhibit transmitter release. Identification of the specific metabolites will depend on potent and selective enzyme inhibitors, because exogenous arachidonic acid and metabolites in brain slices are limited by slow diffusion and metabolic instability. A critical remaining question is the connection between opioid receptors and the activation of phospholipase A_2, although an indirect link has been shown in expression systems[9].

There was a striking increase in the sensitivity of brain slices to opioids after they were treated with inhibitors of cyclooxygenase or 5-lipoxygenase. Morphine, for example, caused a weak inhibition when applied alone, but in the presence of indomethacin, aspirin or caffeic acid it produced a near-maximal inhibition. Vaughan *et al.* suggest that the increased sensitivity to opioids resulted from an increased availability of arachidonic acid, such that metabolism through the 12-lipoxygenase pathway was increased. This mechanism may explain the synergism — rather than simple, additive interactions — between aspirin and opioids in the central nervous system.

A synergistic interaction between non-steroidal, anti-inflammatory analgesics and both μ-opioid and α2-adrenoceptor agonists, but not κ-opioid or adenosine-A1 receptor agonists, has already been convincingly shown using intrathecal administration in conscious, active animals[10]. So this interaction is selective but not exclusive. Because activation of both the μ- and α2-receptors causes potent inhibition of transmitter release in the dorsal horn of the spinal cord, studies at the cellular level will be critical to test the hypothesis that has been put forward from this work in the PAG. If this cellular interaction is a common mechanism, identification of arachidonic-acid metabolites, selective enzyme inhibitors and agonists at other receptors (such as the α2-adrenoceptor) may result in safe and effective treatments for pain. □

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Deafness

Sounds from the cochlea

Karen B. Avraham

To be defective in language, for a human being, is one of the most desperate of calamities, for it is only through language that we enter fully into our human estate and culture, communicate freely with our fellows, acquire and share information. (Oliver Sacks; ref. 1)

Language can take on many forms — from signs or gestures, to words produced by vocal cords. The inner ear is fundamental for the latter, enabling us to grasp not only the words but non-verbal communication as well, including laughter and music. Our understanding of the molecular basis of inner-ear function has increased dramatically in past years with advances in gene mapping and cloning. Now, a report by Lynch *et al.*[2] in *Science* describes the identification of a gene that is defective in a family with profound progressive hearing loss. And in this month's issue of *Nature Genetics*, Everett *et al.*[3] report that they have isolated the gene for Pendred syndrome, an inherited disorder of congenital deafness and thyroid disease. These discoveries pave the way for the dissection of two major cellular pathways, actin polymerization and sulphate transport, in the inner ear.

In the Western world, 0.1–0.2 per cent of children are born deaf, with this number reaching a higher proportion in isolated communities throughout the world. Nearly half the population may lose part or all of their ability to hear by the age of 80. Hearing loss can occur prelingually (before speech development) due to genetic defects or childhood diseases, or postlingually due to inherited gene mutations, environmental noise pollution, infection or ageing. Hearing impairment may occur in association with other symptoms, in the form of syndromic deafness, or as an isolated finding (non-syn-

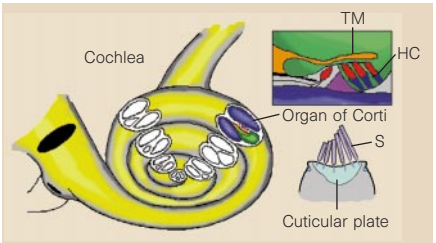

Figure 1 **Cross-section of the cochlea — the portion of the inner ear responsible for hearing. The upper right figure shows an enlargement of the organ of Corti containing the tectorial membrane (TM) and hair cells (HC); the lower right figure shows stereocilia (S) projecting from the cuticular plate on the apical surface of a hair cell.**

dromic deafness, accounting for about 70 per cent of hearing-loss cases).

Scientists in Mary-Claire King's laboratory, with Pedro Leon in Costa Rica, have now found[2] that the deafness locus *DFNA1* in the large Costa Rican family 'M' encodes diaphanous, a member of the formin family of proteins. Diaphanous is involved in cytokinesis and cell polarity, and homologues have been identified in *Drosophila*, yeast and mice. Diaphanous recruits profilin — an actin-monomer binding protein — to the membrane, where it promotes actin polymerization in a GTP-bound, Rho-dependent manner. In family M, diaphanous has a carboxy-terminal truncation and, although the protein is broadly expressed in human tissues, the only site of damage is in the sensory hair cells of the cochlea (Fig. 1). This implies a specialized role for diaphanous in hair cells.

Hair cells possess a rich cytoskeleton meshwork. They also have stereocilia which respond to sound, leading to membrane depolarization and subsequent neurotransmitter release. Mutations in diaphanous may affect the dynamic actin polymerization that occurs during the amplification of sound reception, organization of the cuticular plate (an actin-rich structure into which the stereocilia are inserted), and/or reordering of the paracrystalline array of actin filaments in the stereocilia.

Everett *et al.*[3] isolated the Pendred syndrome gene, *PDS*, taking advantage of the Human Genome Project's systematic sequencing of human chromosome 7 at the Washington University Genome Sequencing Center. (Data are deposited in the database regularly, and are available on the Web at http://genome.wustl.edu/gsc, offering a tremendous short-cut in positional candidate cloning.) Patients with Pendred syndrome suffer from goitre, which is often variable in its expression, and deafness. Their cochleas contain fewer turns than normal, and no (or few) hair cells.

The *PDS* gene encodes a putative sulphate transporter protein called pendrin. The transport of SO_4^{2-} groups is required for sulphation, which is intrinsic to modification of proteoglycan glycosaminoglycan side chains and is one of the major post-translational modifications of tyrosine residues. Pendred syndrome may affect the thyroid because the main secretory product of thyroid follicular cells, thyroglobulin, requires sulphation. And in the inner ear, sulphation is needed for functioning of components involved in the innervation, structural development and/or function of auditory transduction — these include growth factors and the tectorial membrane of the organ of Corti. Alternatively, because thyroid hormones are known to be vital for development of the inner ear, the cochlear defects seen in patients with Pendred syndrome may be secondary to thyroid dysfunction.

This has been a tremendously exciting year for the genetics of hearing loss. Mutations in connexin 26 (*CX26*) were discovered in non-syndromic hearing loss[4], and were subsequently found to be prevalent in deaf populations[5–7]. Given the ease in screening for mutations in *CX26* (the gene is encoded by a single exon), the implications for screening and genetic counselling for the hearing-impaired are dramatic. Myosin VIIA (*MYO7A*), which was originally found to cause deafness and retinitis pigmentosa in Usher's syndrome type IB and deafness in the mouse mutant shaker-1, has now been shown to cause non-syndromic recessive and dominant hearing loss as well[8,9]. Moreover, two genes encoding proteins that are known to interact with each other — IsK (a membrane-spanning glycoprotein) and KvLQT1 (a potassium channel) — are involved in Jervell and Lange–Nielsen syndrome[10–12]. People with this rare form of syndromic deafness also suffer from fainting spells caused by abnormal ventricular repolarization, which may result in early death. But care must be taken when identifying mutations. One of the original *CX26* variants identified in profoundly deaf siblings from a family with dominant hearing loss may represent a simple polymorphism, because this variant has been identified in people with normal hearing[13].

The list of genes involved in deafness that have been discovered in the past year alone points to an amazingly complex repertoire of proteins in the inner ear. Dissecting the functions of these proteins will provide an enormous amount of information regarding their normal roles in the cochlea, and may eventually help to alleviate hearing loss of all forms. □

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Anticancer drugs

Ringing the changes

Andrew Holmes

An increasing problem in cancer chemotherapy is multiple drug resistance. To fight it, medicinal chemists are continually on the lookout for new cytotoxic compounds, and they have allied themselves with specialists in natural products, synthesis and high-throughput screening. The discovery of the powerful anticancer properties of taxol* is a tribute to this multidisciplinary approach to cancer chemotherapy. Taxol has been followed by discodermolide, the epothilones and, in a patent application, eleutherobin. These all work by promoting the formation of stable bundles of microtubules, and so inhibiting cell division. (Indeed, a routine screening method for new compounds is to measure their binding to the 'taxol-binding domains' of microtubule assemblies.) The newest member of this family is eleutherobin, whose isolation and microtubule-stabilizing properties were announced in September[1]. Astonishingly, that has already been followed by the compound's total synthesis and the determination of its absolute stereochemistry, described in two papers from Nicolaou *et al.*[2,3].

The structural diversity of these successive generations of 'taxol mimics' is quite remarkable, but they have all now succumbed to synthesis[2–4]. Eleutherobin and sarcodictyin A (ref. 5) are members of the family of 1,4-oxacladiellanes, including the anti-inflammatory valdivones[6] and the eleuthosides[7]. Embedded in the tricyclic core of these structures (Fig. 1) is a medium-sized ring which can be

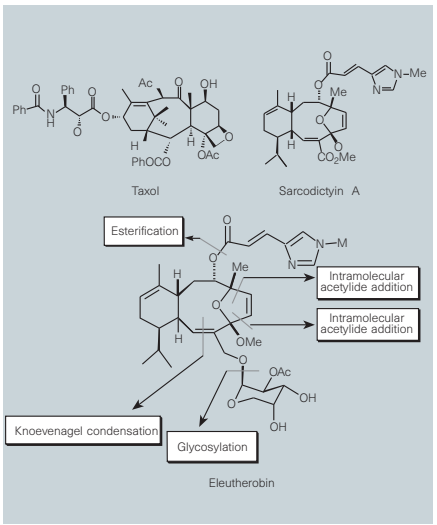

Figure 1 **The anticancer drug taxol and the new taxol mimics eleutherobin and sarcodictyin A. Marked on eleutherobin are points where the tricyclic core of eleutherobin or sarcodictyin A can be easily broken. This hints at strategies for synthesis, using components derived from 'breaking bonds' at these points.**

*Bristol-Myers Squibb has registered Taxol as a trademark and wishes the scientific community to use the name paclitaxel.