Oestrogen on the brain

Lisa Melton looks at work to extend the beneficial effects of the female hormone oestrogen to men as well as women

As Christian Behl cycled to work that morning, he was blissfully unaware of the commotion brewing atop the hill. At the Salk Institute laboratories in San Diego, California, a media gathering awaited. It was Behl they were after. But why the fuss? That very morning Behl had published a paper in the journal *Cell* showing that free radicals played a heavy hand in Alzheimer's disease.[1] Apparently, the electron bachelors could be triggering the abnormal protein tangles that are the disease's hallmark. The implications were enormous. Would antioxidants such as vitamin E prevent or cure this devastating disease? It was 1994, and there were no ready answers.

Only a year later, Behl hit on a winning formula: the female hormone oestrogen. In addition to its role as a sex hormone, oestrogen has the unsuspected talent of acting as an antioxidant. Behl demonstrated that in high concentrations, oestrogen reduces the neurone-killing effects of oxidising free radicals. In essence, the hormone is a neuroprotectant.[2] At the same time, evidence from other laboratories revealing oestrogen's role in cognition, motor coordination and memory added to the mounting interest. In the space of only a few years, oestrogen had become the neuroscientists' darling.

For Behl, turning oestrogen into a 'drug for the brain' to prevent Alzheimer's disease was an exciting prospect. But there was no escaping the fact that the hormone has unacceptable sexual side-effects. If used as therapy, men might grow breasts and women increase their risk of uterine and breast cancer. As a biochemist, Behl believed that it should be possible to clip the molecule into an innocuous core, while retaining its neuroprotective qualities. 'Since the molecule has only a few chemical moieties, it may be possible to modify it', he explains. To pursue this idea, Behl returned to Germany and set up his own group at the Max Planck Institute of Psychiatry in Munich.

Brain activity

In the past decade, oestrogen's image has been turned on its head. For years, studies on this hormone were relegated to the arena of sexual development, conception, contraception and the menopause. 'We now know that oestrogens have effects outside their traditional reproductive function', declared Bruce McEwen in 1999, at a Novartis Foundation symposium in London. 'One of the most striking and intriguing aspects in this emerging area is that oestrogen has effects on cognition and protection from Alzheimer's disease', points out McEwen, a pioneer in the field based at Rockefeller University in New York City, US.

It was McEwen who first showed that oestrogen had the potential to improve mental function by enhancing neuronal survival and shaping neuronal populations in the hippocampus - the area involved in laying down memories.[3] His team found that, in rodents, the female hormone helps to build and maintain new synapses, strengthening neuronal connections.

Oestrogen also holds sway over the human brain. Women who are deprived of the hormone suffer from memory lapses that vanish once the hormone is replaced. Psychologist Barbara Sherwin of McGill University in Montreal, Canada, convincingly demonstrated that young women who underwent surgery to remove their ovaries and uterus, recovered their verbal memory skills when put on oestrogen replacement therapy, while those on placebo did not.[4]

Oestrogen can even prevent dementia. 'Oestrogen therapy after menopause can halve the risk of developing Alzheimer's disease', says Victor Henderson, a clinical researcher at the University of Southern California, who drew his conclusions from a large, randomised, placebo controlled trial.[5]

But by what mechanisms does the female sex hormone protect neurones? Scientists

agree there are multiple mechanisms but that its ability to act as antioxidant may be an important contribution. The brain is particularly vulnerable to excessive oxidation - what is known as 'oxidative stress'. In Alzheimer's disease, Parkinson's disease and stroke, the cellular damage that ultimately leads to nerve cell death is perpetrated, at least in part, by reactive oxygen species (ROS).

Antioxidant effects

These ROS are normal byproducts of cellular physiology but can be highly destructive when in excess. ROS species include superoxide radicals ($OX^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^{\bullet}$), nitric oxide ($NO^{\bullet}$) and other metabolites. They may cause oxidative damage that nicks or mutates the DNA, ultimately leading to errors during cellular replication. But ROS can also affect vital cellular proteins and membrane lipids resulting in nerve cell death.

Oestrogen can shield neurones from such damage because it scavenges for free radicals and 'neutralises' them.[6] But if antioxidants are vital, why not use α-tocopherol (vitamin E), (*Chem. Br.*, February 2003, p25)? After all, it is a well-known free radical buster with no untoward feminising effects and can be bought in any self-respecting health food shop. 'Vitamin E is a wonderful compound but it does not cross the blood-brain barrier', Behl points out. The blood-brain barrier is a lipid bilayer rich in fatty compounds; as a lipophilic molecule,
α-tocopherol ought to get through. But in practice it is too large and unwieldy. 'We wanted a smaller molecule, a natural compound that could easily enter the brain', Behl recalls.

Oestrogen turned up trumps. The biologically active oestrogen molecule, 17β-oestradiol, is remarkably similar in structure to vitamin E: both are aromatic alcohols with phenol groups in ring A. Behl found that the presence of the hydroxyl group in the C3 position on the A ring of the steroid molecule endowed oestrogen with antioxidant abilities.[7] 'The free radical scavenging group is present in oestrogen as well as in vitamin E. The difference between these structures is the length of the hydrophobic tail. Vitamin E is much larger,' Behl notes.

Unlike vitamin E, 17β-oestradiol has no difficulty getting into the brain. Once there, it acts on various target areas expressing oestrogen receptors. If present in sufficient amounts it also shields neurones against a wide range of oxidative insults. The only structural requirement for scavenging highly reactive free radicals is the chemical phenolic structure of the molecule. The hydroxyl group on ring A of the monophenolic compound 17β-oestradiol donates a hydrogen that pairs up with rogue electrons, thus detoxifying free oxygen radicals (such as the hydroxyl radical, $HO^{\bullet}$).

But the niggling worry is that oestrogen's scope far exceeds that of an antioxidant for the brain. In fact its physiological functions are widespread and are mediated mainly by cytoplasmic receptors α and β (ERα and ERβ). These receptors are expressed throughout the body and in the brain they often overlap. Oestrogen activates both these intracellular receptors and initiates cross-talk with other intracellular signalling pathways in a highly promiscuous fashion. In addition to the classical cytoplasmic ERα and ERβ receptors, 'fast-acting' membrane-bound receptors have also been proposed, although nobody has yet succeeded in purifying or cloning them.

Shared chemistry

Yet despite the myriad receptors scattered around the body, oestrogen acts as an antioxidant independently of these or any other signaling pathways.[8] This split activity is good news because, in theory, oestrogen's free radical quenching properties could be salvaged while eliminating the sexual side-effects.

The downside is that both activities share the same location in the 17β-oestradiol molecule. The very same phenolic group that extinguishes highly destructive free radicals also binds to oestrogen receptors to induce sex-related changes. To remove the hormonal effects, Behl tinkered with the oestrogen molecule. He added huge methyl groups on either side of the phenolic ring to stop the hormone from binding to its receptors. At the same time, he clipped some parts of the molecule to make it even shorter and boost the chances of crossing the blood-brain barrier. The result is 2,4,6-trimethyl phenol (TMP). 'In TMP, the phenol can no longer bind to the oestrogen receptors, but it still acts as a powerful free radical scavenger. That's the trick', he says.

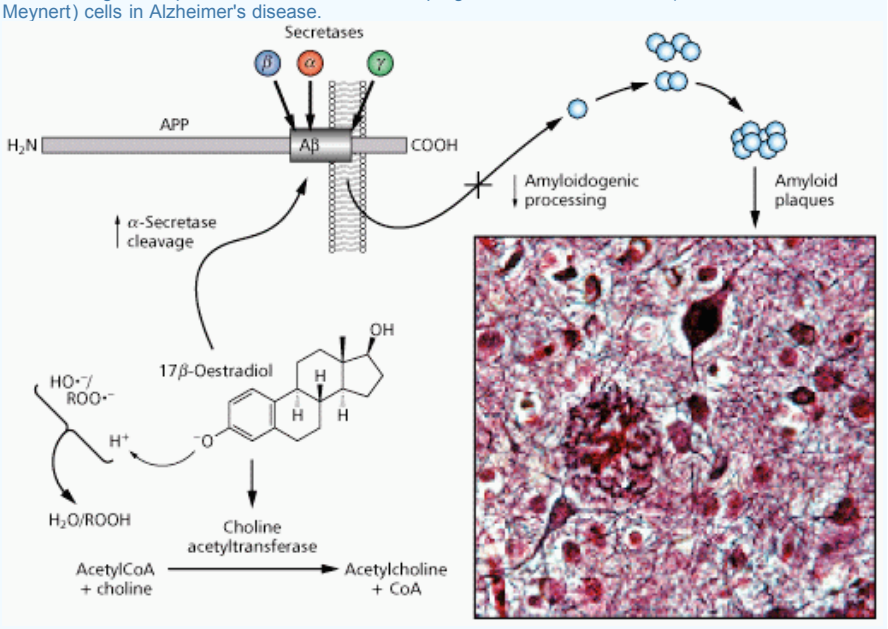
Thus far, TMP is measuring up well as an antioxidant. *In vitro* experiments performed by Behl's team subjecting nerve cells to oxidative stress showed that TMP is much more potent than 17β-oestradiol. Animal experiments have confirmed that the compound does enter the brain and Behl is now testing it in an animal models of global ischaemia where there is acute oxidative damage, as well as models of

Alzheimer's disease where there is amyloid deposition and neurodegeneration (*see Box*). 'Of course, we have to wait for the *in vivo* results first, but we hope that with this compound we can relieve some amount of oxidation-related cell death', he remarks.

Oxidative damage in Alzheimer's disease

The formation of amyloid plaques in the brain is one of the hallmarks of Alzheimer's disease. These are produced by the processing of amyloid precursor protein (APP) by a group of enzymes called secretases. APP may be processed by two routes: amyloidogenic and non-amyloidogenic, which involve the enzymes β- and α-secretase respectively.

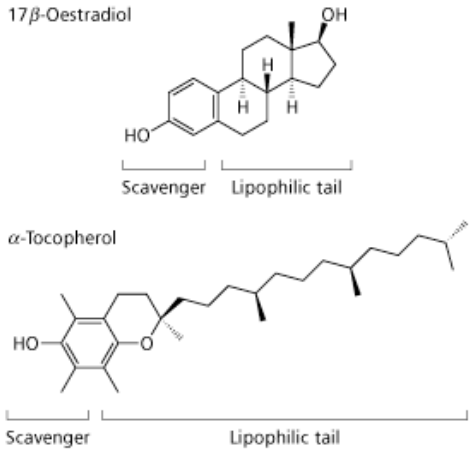
Oestrogen promotes the cleavage of APP via the non-amyloidogenic route, in this way preventing the formation of the toxic fibrils that make up the amyloid plaque. In addition, oestrogen is known to upregulate the activity of the enzyme choline acetyltransferase in rodent brains. This suggests that the memory enhancing effects of oestrogen may be due to the stabilisation of acetylcholine levels. In fact, oestrogen receptors have been seen to be upregulated in certain human (nucleus basalis of Meynert) cells in Alzheimer's disease.



Were TMP to make it as a therapeutic drug, Behl envisages few complications. 'Since the molecule itself has few chemical moieties and side-groups and chains that can be modified into toxic compounds in the liver, I do not expect many side-effects,' he comments. In fact, the body is awash with natural phenolic compounds, mostly derived from the diet, with no untoward consequences. A person's intake ranges from 1 to 100mg per day, depending on the intake of vegetable oils, soya and onions, for example.[9]

Behl has high hopes that TMP might one day be packaged into an antioxidant pill to fend off Alzheimer's disease, Parkinson's disease and stroke, for which there is currently no cure. But getting a new molecule to be approved for human use is a long and expensive process. 'We would like to try to find a compound that is already available as a drug for other conditions and which contains TMP. This would hasten the whole process of approval and getting it to the clinic'.

Oestradiol and vitamin E (α-tocopherol) consist of a free radical scavenging part (ring OH group) and a lipophilic tail



'Perfect pharmacology'

Behl is certain there must be ready-made compounds 'out there' that already carry

these groups. Unfortunately his laboratory lacks the tools to carry out such a search, but he still hopes to find them. 'I'm sure that in the libraries of pharmaceutical companies, there are a lot of phenolic compounds with perfect pharmacology and also information on *in vivo* toxicity, that could be tested for additional antioxidant activities.'

At the moment, Behl is carrying out various pharmacological and toxicological tests for TMP. The animals have been assigned and treated in a blind experiment, and the results are still to be analysed. Although he admits there is no apparent damage, Behl insists it is still too early to comment.

Meanwhile, another team led by Jim Simpkins of the University of Florida at Gainsville, US, is also attempting to exploit oestrogen's structure for its neuroprotective effects. Simpkins believes that the phenolic A ring and at least two other rings prevent nerve cell death (apoptosis) not only by inhibiting oxidative stress but through a plethora of possible cellular actions. These include stimulating anti-apoptotic proteins, and activating various cell signaling pathways. It is these signaling pathways that either individually or collectively endow oestrogen with its protective properties, Simpkins proposes.

But there are those who argue that even a designer oestradiol molecule will have lingering hormonal side-effects. This is because oestrogen seems able to act directly on tissues, bypassing the receptors. To get round this, Behl is pursuing new compounds - certain imines - that have a hydrogen atom coupled to nitrogen instead of oxygen.[10] 'They are about a thousand times better than oestradiol. It's very exciting', says Behl, although he admits that it's hardly surprising because the basic chemistry is the same. 'The oxygen or the nitrogen kicks away the hydrogen atom to detoxify the free radical.'

These small, scavenging wonders are at this moment being tested in animal models of acute neurodegeneration such as ischaemia where rapid oxidative damage to the brain is induced by injecting kainic acid, which leads to over-excitation. 'For the moment, all we can really say is it's entering the brain', comments Behl. 'But we don't know what to expect because it's a new molecule and a new model.'

But Behl is not about to abandon oestrogen. 'Although we may be looking to find new drugs, new compounds with improved activities in the brain, oestrogen still does all these other weird and wonderful things in the blood, protecting from heart disease and in the bones, protecting from osteoporosis. It all shows that oestrogen maintains a basic level of protection.' Behl and his team who have recently moved to the University of Mainz are now using chip technology to identify which genes are switched on by oestrogen. These genes and their products could become novel drug targets.

As knowledge of oestrogen's prowess grows, the pressure to find suitable alternatives without sexual side-effects is mounting. 'If I were a woman, I would count on oestrogen, but what would you say to male patients?', he ponders. With a few chemical nips and tucks to his favourite molecule, Behl himself may supply the answer.

Lisa Melton is science writer at the Novartis Foundation, London.

References

1. C. Behl *et al*, *Cell*, 1994, **77**, 817.
2. C. Behl *et al*, *Biochemical and Biophysical research communications*, 1995, **216**, 473.
3. C. S. Woolley and B. S. McEwen, *J. Neurosci.*, 1994, **14**, 7680.
4. B. B. Sherwin in *Neuronal and cognitive effects of oestrogens*. Chichester: Wiley, 2000.
5. V. W. Henderson *et al*, *Neurology*, 2000, **54**, 295.
6. C. Behl, *Nature Rev. Neuroscience*, 2002, **3**, 433.
7. C. Behl *et al*, *Mol. Pharmacol.*, 1997, **51,** 535.
8. B. Moosmann and C. Behl, *Proc. Natl Acad. Sci. USA*, 1999, **96**, 8867.
9. C. Behl in *Estrogen - mystery drug for the brain?* Wien/New York: Springer-Verlag, 2001.
10. C. Behl and B. Moosmann, *Biol. Chem.*, 2002, **383**, 521.

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