ability of the kidney to make free water so our point should not only move to the right it should get closer to the diagonal.

With the patient concentrating urine we get the *pièce de résistance*. In contrast to the loop diuretics, the thiazides interfere with reabsorption of salt at a locale removed from the papilla so they do not interfere with maintenance of that 1200 mOsm region surrounding the collecting duct. The kidney does not lose its ability to concentrate; it can still make negative free water. Our point will not move toward the diagonal as we saw happen with the loop agents. Where will it go? Well, the kidney still has the assignment of keeping the urine flow low to maintain water balance. Thus, we would expect the compensation would probably not be perfect so we might expect the point actually to move to the right and slightly upwards. The action of the thiazides can be summarized in Fig. 3c.

Concluding remarks

Note how, starting from the physiology and just a minimal indication regarding the site of action of the drugs, we can make some quantitative deductions as to what the drugs will do. This is a nice illustration of what pharmacology is all about. There is more to it than just that horrible list of drug names all physicians recall from their medical student days. Unfortunately, one cannot really appreciate the conceptual aspect on the first round because it is clouded by all that memorization of names. This story might help to show you some of the pretty stuff that you might have missed first time around.

Neurotrophins and depression

C. Anthony Altar

Exogenous delivery of the neurotrophic factors, brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3), promotes the function, sprouting and regrowth of 5-HT-containing neurones in the brains of adult rats. Similar infusions of BDNF into the dorsal raphe nucleus produce an antidepressant effect, as evaluated by several 'learned helplessness' paradigms. Environmental stressors such as immobilization induce depression and decrease BDNF mRNA. Antidepressants increase BDNF mRNA in the brain, via 5-HT_{2A} and β-adrenoceptor subtypes and prevent the stress-induced decreases in BDNF mRNA. In this article, **Tony Altar** discusses how existing treatments of depression might work by increasing endogenous brain levels of BDNF or NT-3, which in turn could promote monoamine-containing neurone growth and function. Drugs that selectively stimulate the production of neurotrophins could represent a new generation of antidepressants.

Depression is a potentially life-threatening disorder that affects hundreds of millions of people. It can occur at any age from early childhood to late life. Dulling hope, ambition and sometimes even the will to live, depression exerts a tremendous cost upon society. Fortunately, the treatment of depression has advanced in recent years with the advent of drugs that block the inactivation of the brain neurotransmitters 5-HT and noradrenaline. One class of antidepressant drugs, the monoamine oxidase inhibitors, slows the normal enzymatic degradation of these neurotransmitters. Another class, the monoamine reuptake blockers, prevents the normal recapture of 5-HT and noradrenaline by their transport back into the presynaptic nerve-terminal. Selective reuptake blockers such as fluoxetine (Prozac) or desipramine thereby enhance levels of 5-HT or noradrenaline, respectively, in the nerve-terminal synapse. Other treatments for depression include electroconvulsive therapy (ECT), which is reserved for severely depressed patients who do not respond to conventional drug therapy. Yet, it has remained a puzzle as to why these diverse treatments are effective and why several weeks of antidepressant drug therapy or ECT are needed for a positive response.

Recent findings now suggest that antidepressant medications and ECT might work by boosting the production of the brain's own neurotrophic factors. Such actions could implicate deficiencies in endogenous neurotrophin production in depression, and suggest a 'neurotrophin-boosting' pharmacotherapy for depression that requires nerve growth for a clinical response.

Neurotrophins: growth factors for 5-HT-containing neurones

Before the link between neurotrophins and depression was suspected, this family of growth factors had been studied for its role in the adult nervous system. Among these endogenous proteins, BDNF and NT-3 were shown to promote the function and growth of 5-HT-containing neurones in the adult brain. Unlike chronic infusions of nerve growth factor (NGF), chronic infusions of BDNF or NT-3 into the rat midbrain increased the turnover of 5-HT and levels of noradrenaline in many forebrain areas including the neocortex, basal ganglia and hippocampus[1–4]. More strikingly, infusions of BDNF into the adult rat neocortex produced an unprecedented and robust

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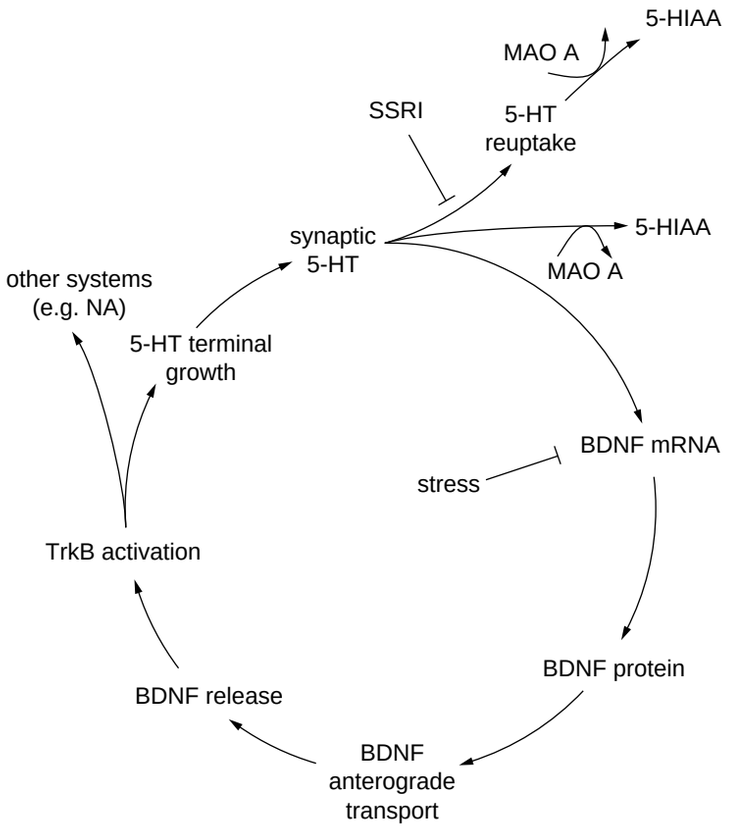

Fig. 1. The interaction between serotonin (5-HT), brain-derived neurotrophic factor (BDNF) production, and the growth of 5-HT-containing nerve fibres in the brain. The antidepressant effects of ECT and selective serotonin reuptake inhibitor (SSRI) drug therapies could be caused by a neurotrophin-enhanced growth of serotonergic and noradrenergic nerve fibres and increased synaptic levels of their respective neurotransmitters. 5-HIAA, 5-hydroxyindoleacetic acid; NA, noradrenaline; MAO A, monoamine oxidase A.

sprouting of 5-HT nerve terminals[5]. Infusions of BDNF or NT-3 into the brain also accelerated the regrowth of serotonergic nerve fibres following their destruction by a serotonergic neurotoxin, parachloroamphetamine[5].

The dramatic effects of BDNF and NT-3 on serotonergic neurone function, growth and regeneration suggested a potential link between neurotrophins and depression. This link was, in part, based on the knowledge that brain tissue and the ventricular fluids of those with major depression revealed decreases in brain 5-HT turnover[6–8].

BDNF ameliorates learned helplessness

To evaluate the potential link between neurotrophins and depression, Suiciak studied the behavioural effects of the same midbrain infusions of BDNF that had elevated 5-HT turnover in the earlier studies. Chronic infusions of BDNF or its saline vehicle were made near the dorsal raphe nucleus, a cluster of 5-HT cell bodies that innervate the forebrain[9]. When tested one week later, the vehicle-infused rats that had been pre-exposed to inescapable shock showed the typical 'learned helplessness' profile. They demonstrated fewer and greatly delayed escape behaviours when exposed to shocks that could now be avoided by moving to another area. Animals that had received BDNF infusions showed behaviours that are normally associated with antidepressant drugs. This was evidenced by escape latencies and frequencies that equalled those of animals that had not been pre-exposed to inescapable shocks. BDNF-infused animals also showed an inhibition of the learned helplessness that vehicle-infused animals displayed after their repeated experiences with forced swimming[9]. Both tests are routinely used to discover the antidepressant potential of drugs.

Small molecules boost neurotrophin message

Although infusions of exogenous BDNF could produce antidepressant-like effects, it remained unknown whether there was a link between endogenous neurotrophins and treatments for depression. Duman and colleagues have shown that treatment with antidepressants, including specific inhibitors of 5-HT or noradrenaline reuptake, or inhibitors of monoamine oxidase, elevate BDNF messenger RNA levels in the rat hippocampus[10,11]. Reminiscent of the delay in treating depression in humans, these compounds augmented BDNF mRNA after three weeks of chronic treatment, but not after one day of treatment. It was particularly intriguing that these commonly prescribed antidepressants also elevated the mRNA for the high-affinity BDNF receptor, tropomyosin receptor-related kinase B (TrkB). Stimulants such as cocaine and other psychoactive drugs that do not treat depression failed to augment BDNF or TrkB levels, even when administered chronically[10].

Understanding how monoamine reuptake blockers or monoamine oxidase inhibitors elevate BDNF message could lead to novel ways to upregulate endogenous protein levels of the growth factor. This is particularly important because BDNF is a large, lipophobic protein and, when given peripherally, does not cross the blood–brain barrier. 5-HT receptors (including the 5-HT_{2A} subtype), phosphodiesterase inhibition and β-adrenoceptors appear to be positively coupled to the production of BDNF mRNA in some brain areas[10–12]. However, it is necessary to test a more diverse number of clinically effective antidepressants to determine how consistent their effects are on the mRNA for BDNF, or for NT-3, which has yet to be tested. Small-molecule agonists that selectively target one or more of these functional sites could represent a new generation of antidepressants with greater specificity than currently used 5-HT- or noradrenaline-reuptake-blocking drugs.

Stress, neurotrophins and antidepressants

Further evidence linking BDNF and depression comes from studies in which stress, often a precipitating factor in depression, has been shown to decrease BDNF mRNA. The stress of being immobilized[10,11,13–15] (which induces learned helplessness) or systemic injections of glucocorticoids[16,17] lower BDNF but not NT-3 mRNA levels in the hippocampus and other brain areas. Glucocorticoids can also depress the activity-dependent

expression of BDNF mRNA within cultured hippocampal neurones[18]. Chronic treatment of animals with antidepressants can prevent the stress-induced lowering of BDNF mRNA (Refs 10, 11). Interestingly, seizures in rats induced by pilocarpine[19] or by electroshock[10,20], which mimics the ECT used to treat severe depression, also elevate BDNF mRNA in hippocampal, cortical and other brain areas. As with antidepressant drugs, ECT prevents the decrease of hippocampal BDNF mRNA due to immobilization stress[10]. These and other *in vivo* studies are summarized in Fig. 1.

Areas for future research

An important issue in this area is whether antidepressants or chronic ECT can actually increase brain levels of BDNF or TrkB protein, and where such increases might occur. Although other examples with more sensitive methods will undoubtedly follow, to date only the study by Smith and colleagues[20] has shown that BDNF protein was elevated in the ECT-treated brain. Such increases occurred in the mossy-fibre terminals of hippocampal dentate granule neurones. It will also be important to determine if BDNF levels or BDNF receptor number are decreased in the brains of depressed patients who were not receiving drugs before death. If lower than normal, will BDNF or its receptor be normalized with chronic antidepressant treatment? The role in depression of other neuronal growth factors that augment 5-HT and noradrenergic neurone functions, such as NT-3 and NT-4 but not NGF (Refs 1, 2, 4, 5), remains to be clarified.

Such important questions can be answered from post-mortem studies of human brain tissue using radiolabelled neurotrophins and ELISA (enzyme-linked immunosorbant assay)-based neurotrophin protein assays, or possibly from evaluations of the BDNF content of platelets. Human platelets contain BDNF mRNA (Ref. 21) and protein[22]. Platelets, which have been linked to depression by way of 5-HT and the 5-HT_2 receptor[8,23], could be a peripheral marker for alterations in BDNF levels in depression, as well as the BDNF response of the brain to antidepressant therapy.

Another pressing question is whether drugs that selectively boost neurotrophin levels could treat depression faster than the currently popular 5-HT or noradrenaline reuptake blockers. Such drugs would be of obvious benefit to suicide-prone depressed patients and to those with treatment-resistant depression. However, recalling the neurotrophic effects of NT-3 and BDNF and the neurotoxic effects of glucocorticoids and learned helplessness, the possibility that depression itself results from a subtle atrophy or diminished function of BDNF-responsive noradrenergic[24,25] or serotonergic neurones must be considered. Indeed, atrophies of the hippocampus[26], frontal cortex, cerebellum and striatum[27] are fairly consistent findings in major depression. Because antidepressants can promote the axonal regeneration of noradrenergic neurones that have atrophied as a result of exposure to neurotoxins[24,25] or of chronic stress[28], it is worth considering whether neurotrophins, including BDNF, mediate such trophic effects. If depression is a subtle form of neurodegenerative disease, the commonly observed delay of several weeks between the start of ECT or antidepressant medication and therapeutic response could reflect the requirement for serotonergic or noradrenergic nerve-terminal regrowth.

Small molecules that boost the endogenous levels of BDNF or NT-3 might also be useful for treating temporally protracted and severe forms of neurodegenerative disease, such as Alzheimer's or Parkinson's disease. Both of these diseases reveal a compromise of a broad number of neurotrophin-responsive neuronal populations[4]. For example, it is interesting to note that ECT elevates BDNF mRNA (Ref. 10) and protein[20] in animal models, and also improves both mood and motor function in depressed Parkinson's disease patients[29,30]. Could these clinical improvements be mediated by the beneficial effects of neurotrophins on 5-HT- and dopamine-containing neurones[1–5], respectively? A small molecule that passes through the blood–brain barrier and subsequently boosts endogenous neurotrophin levels could be used to test the long-held, but as yet unproven, strategy of treating CNS neurodegenerative disease with neurotrophic factors.

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Are ion-exchange processes central to understanding drug-resistance phenomena?

Paul D. Roepe and James A. Martiney

Drug resistance in malarial parasites is arguably the greatest challenge currently facing infectious disease research. In addressing this problem, researchers have been intrigued by similarities between drug-resistant malarial parasites and tumour cells. For example, it was originally thought that the role of pfMDR (*Plasmodium falciparum* multidrug resistance) proteins was central in conferring antimalarial multidrug resistance. However, recent work has questioned the precise role of MDR proteins in multidrug resistance. In addition, recent ground-breaking work in identifying mutations associated with antimalarial drug resistance might have led to identification of yet another parallel between drug-resistant tumour cells and malarial parasites, namely, intriguing alterations in transmembrane ion transport, discussed here by **Paul Roepe and James Martiney.** This further underscores an emerging paradigm in drug-resistance research.

The similarities and differences between multidrug resistance (MDR) phenotypes exhibited by tumour cells, certain bacteria and malarial parasites (*Plasmodium falciparum*) have intrigued many researchers for a long time[1–3]. A key role for ATP-binding cassette (ABC) proteins has dominated the drug-resistance literature in recent years; however, it appears that these proteins are unable to explain all phenomena, including levels of resistance and the biophysics of altered drug traffic in many examples of resistant cells[2–7]. In fact, it can be argued convincingly that the role of ABC proteins in conferring MDR phenomena has been overemphasized[8,9]. Recently, several reports that initially appear to be unrelated underscore an unanticipated and interesting role for ion exchange processes in drug-resistance phenomena[10–12]. Dysregulation of ion exchange might be central to the evolution of drug resistance in tumour cells, malarial parasites and some bacteria. The implications of this observation are important because the findings confirm a central prediction of the 'indirect' drug-partitioning model[3,5,13]. This indirect model suggests that altered drug accumulation in resistant cells and microorganisms is fundamentally a result of alterations in pH gradients, electrical membrane potential and perhaps other biophysical parameters, and is not necessarily a result of direct drug trafficking by putative nonspecific hydrophobic drug pumps. If confirmed, the observations predict an important new set of 'targets' for improved therapy of drug-resistant cells and microorganisms, and propose a new way to envisage the emergence of drug-resistance phenomena.

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Background

It is estimated that drug-resistant tumour cells are responsible for 500 000 deaths annually in the USA alone, and that deaths from malaria worldwide could approach ten times that number shortly after the millennium. The rapid geographical spread of drug-resistant malaria, as well as the continued rapid evolution of drug-resistant parasites and bacteria is arguably the most pressing concern in pharmacology today.

Thus, there is great excitement about the realization that some common features appear to be shared among drug-resistant tumour cells, malarial parasites and certain gram-negative bacteria. The most important of these features are that drug resistance is typically accompanied by altered intracellular accumulation of the drugs to which cells or microorganisms are resistant, and that certain 'chemomodulators' (such as the Ca^{2+} channel blocker, verapamil) appear to reverse more than one type of drug-resistance phenomenon (for example, both tumour and antimalarial MDR). Thus, although it is still not understood precisely how verapamil reverses MDR in these systems, it is reasonable to expect that molecular concepts from the study of drug-resistant tumour cells might also apply to drug-resistant malarial parasites, and vice versa. Such was the hope when *pfMDR* (*P. falciparum* multidrug resistance) genes were first cloned[14,15] and shown to be similar to the *hu MDR 1* gene, which is overexpressed in a variety of model drug-resistant tumour cell lines[6], and which has been proposed to encode a nonspecific drug pump. Initially, it was thought that drug pumps for chloroquine and other antimalarials must therefore exist in drug-resistant *P. falciparum*, and that these would be similar to the drug pump proposed for tumour cells (thought to be encoded by *hu MDR 1*) that is believed by some investigators to translocate vinblastine, doxorubicin and other anti-tumour drugs. Moreover, this logic predicts that similar compounds might interact with the homologous pumps (for example, verapamil), providing a pharmacological paradigm for reversal of drug resistance in both systems.

However, progress in capitalizing on this idea has been painfully slow. Along with poor progress in understanding the mechanism of verapamil 'chemomodulation', subsequent work on antimalarial drug resistance showed that many drug-resistant *P. falciparum* are not genetically linked to mutation or increased expression of *pfMDR* genes[16,17] and, consequently, other genetic events must be linked to the evolution of drug resistance. At around the same time, an increasing importance for overexpression of other genes was beginning to be

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