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'Keep on taking the tablets': pharmacological adaptation during long-term drug therapy

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Many drug treatments of disease, whether curative or prophylactic, require prolonged therapy (Table 1). Examination of the long-term pharmacology of such treatments often brings to light pharmacological effects which are the consequence of drug exposure over a long period of time. Such effects may be due to a homeostatic response and reduce the efficacy of the treatment. On other occasions a complex set of responses occurs, seemingly unrelated to the immediate therapeutically relevant drug effect.

The outcome of such processes can be several (Table 2). The chronic pharmacological adaptive response can be the instrument of therapeutic efficacy, as is the case with ACE inhibitors in heart failure and probably so with antidepressant drug therapy. It can be a nuisance, as with resistance to diuretic therapy in severe heart failure or tolerance to nitrates in the treatment of angina. It may result in serious adverse effects, as with tardive dyskinesia during the treatment of schizophrenia with neuroleptic drugs. Chronic drug therapy may induce a sleeping tiger, which awakens when the drug therapy is stopped and results in rebound withdrawal effects with serious consequences, as with many drug addictions.

The problems this presents to the Clinical Pharmacologist are several. First, there is a need to understand the mechanisms by which chronic effects are exerted, for if they are good they may be enhanced, but if adverse, means may be sought to avoid them. Secondly, if the chronic effects are therapeutic and the mechanisms are understood, different pharmacological approaches may be developed which are more direct or effective, and which cause fewer adverse effects.

There is a further challenge for Clinical Pharmacologists, because during long-term drug therapy the correlation between plasma concentrations and the pharmacodynamic effects, based on acute pharmacology, may be erroneous, and new objective measures of drug effect have to be developed to take into account the changed relationship. In addition, the inability to mount appropriate endogenous pharmacological adaptive responses maybe at the heart of the causation of certain conditions (e.g. depression).

Table 1 Situations requiring chronic drug therapy.

Asthma	Epilepsy
Heart failure	Gout
Ischaemic heart disease	Depression
Hypertension	Anxiety
Hyperlipidaemia	Schizophrenia
Diabetes	Parkinson's disease
Oral contraception	Immunosuppression
HRT	HIV/AIDS
Osteoporosis	Tuberculosis
Arthritis	

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One might pause to consider in evolutionary terms where these adapative responses come from. They have not evolved as a result of exposure to drugs, they have evolved as homeostatic responses to endogenous pharmacological disturbances, occurring either physiologically or as part of disease. I believe that biology gets confused by unknown or excessive insults and sometimes responds predictably, but in a therapeutically undesirable way. For instance, suppression of pituitary ACTH secretion by exogenous glucocorticoid therapy is part of a physiological feed-back mechanism which results in potentially harmful adrenocortical atrophy in patients taking therapeutic glucocorticoids. Although the precise details of the causation of tardive dyskinesia associated with neuroleptic therapy are still unknown, one can think of them as nature's attempt to try and overcome pharmacological blockade of dopaminergic function. The pharmacology is plausible, but evolutionary confusion occurs in the biology.

Four examples will be dealt with here. Two of them, nitrate tolerance and diuretic resistance, are examples of the increasing ineffectiveness of therapy, and commonly cause clinical difficulty. Vascular and cardiac re-modelling during ACE inhibitor therapy and the treatment of depression with anti-depressant drugs and ECT are examples where pharmacological adaptive responses are an important part of the mechanism by which the therapeutic effect is mediated.

Nitrate tolerance

The prolonged and constant administration of organic nitrates for the treatment of ischaemic heart disease, or in the vasodilator therapy of heart failure, leads to nitrate tolerance. This means that treatment with nitrates no longer produces a pharmacodynamic effect nor does it have its required efficacy.

Before this became common knowledge in therapeutics, organic nitrate withdrawal was mentioned in every large textbook of pharmacology as part of the adaptive response to nitrate exposure. Munitions workers using nitroglycerine (glyceryl trinitrate) suffered 'Monday morning headaches'. The explanation for this was that during the week, exposure to nitroglycerine led to tolerance of its vasodilatory effects. At the weekend the tolerance wore off, and on Monday morning when they started work again, they were sensitive once more and suffered the well-known nitrate headache.

Table 2 Pharmacological adaptive responses occurring during drug therapy.

1.	*Increasing ineffectiveness of therapy*
	Nitrate tolerance, diuretic resistance
2.	*Adaptive responses producing a therapeutic effect*
	ACE inhibitor induced vascular and cardiac remodelling
	Antidepressants (and ECT)
3.	*Adaptive responses producing adverse effects*
	Neuroleptics and tardive dyskinesia
4.	*Adaptive responses causing drug addiction*
	Morphine, cocaine, benzodiazepines, nicotine, alcohol
5.	*Withdrawal syndromes* (other than drugs of dependency)
	β-adrenoceptor blockers, glucocorticoids

Lange *et al.* [1] reported patients in a munitions factory who developed coronary artery spasm on cessation of exposure to nitroglycerin, i.e. rebound vasoconstriction.

Tolerance to the general haemodynamic effects of nitrates [2] and loss of the antianginal effect on prolonged exposure to nitrates [3, 4] are well established. In nitrate-naive patients with coronary artery disease, intra-coronary infusion of glyceryl trinitrate increases coronary arterial diameter. Patients who had been pre-treated with glyceryl trinitrate by long-term infusion for 24 h lost the vasodilating effect of intra-coronary GTN and had evidence of activation of the renin-angiotensin system. After 72 h of GTN infusion, nitrate tolerance was still present, but evidence for increased renin-angiotensin activity had disappeared [5].

In the past it was thought that nitrate tolerance was due to depletion of sulphydryl groups, resulting in reduced transformation of organic nitrates to an active vasodilating moiety [6]. Other mechanisms suggested have been desensitisation of guanyl cyclase, increased vascular superoxide anion production and neurohumoral adaptation through the renin-angiotensin system, changes in plasma volume and increased vasopressin activity. However, the discovery of nitric oxide (NO: endothelial derived relaxing factor) and the conversion of organic nitrates to nitric oxide, together with the realisation that the endothelium has complex pharmacological functions, have given further insight into possible explanations of nitrate tolerance.

Recent work by Münzel *et al.* [7] has revealed an intriguing new mechanism. Rabbits were made nitrate tolerant by the application of glyceryl trinitrate patches. Their aortas were taken and segments were prepared and studied in organ chambers for contraction and relaxation. Nitrates were less potent in causing relaxation in nitrate tolerant aortic segments, and tolerant segments contracted more in response to vasoconstrictors, e.g. angiotensin (Figure 1), 5-HT, phenylephrine, KCl, and the direct activator of protein kinase C, phorbol 12,13-dibutyrate. Protein kinase C antagonists annulled the hypersensitivity to vasoconstrictors in tolerant slices. However, vasoconstrictor responses to endothelin were decreased in nitrate tolerant slices. When the aortas of control rabbits were examined for endothelin content by immunocytochemical analysis, none was found in the media. In contrast, endothelin was plentiful in the aortas of nitrate tolerant rabbits (see Figure 2). When normal rabbit aortic segments were pre-incubated with endothelin the vasoconstrictor responses to angiotensin, 5-HT, phenylephrine, and KCl were markedly enhanced,

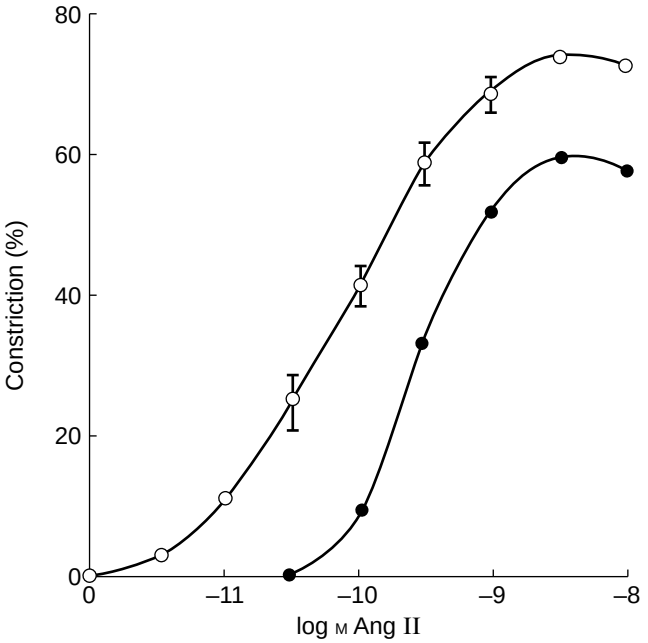

Figure 1 The effect of nitrate tolerance on the vasoconstrictor action of angiotensin II.

In segments of thoracic aorta removed from rabbits treated for 3 days, the responses of aortic segments to angiotensin II (○) showed increased sensitivity as compared with controls (●). This increased sensitivity was inhibited by the protein kinase C inhibitor calphostin. (From Münzel *et al.* [7]).

and this enhancement was blocked by an inhibitor of protein kinase C, calphostin C.

Münzel *et al.* [7] interpreted these results as follows: nitrate-tolerant vessels produce more endothelin in their smooth muscle cells to maintain, by vasoconstriction, homeostasis in vessel luminal diameter, and that endothelin sensitizes the vasculature to vasoconstrictors by a protein kinase C dependent mechanism. They speculated that during nitrate therapy the renin-angiotensin system is activated (as they have shown), that angiotensin II induces expression of pre-pro-endothelin mRNA, and that this accounts for the increased amounts of medial endothelin in nitrate tolerant aorta (see Figure 3). In summary, at a physiological level, at least part of nitrate tolerance is due to vasoconstrictor influences cancelling out vasodilatation. Münzel & Bassenge [8] have gone on to show in dogs that long-term ACE inhibition with enalapril retards nitrate tolerance in large epicardial arteries and prevents rebound coronary vasoconstriction *in vivo*.

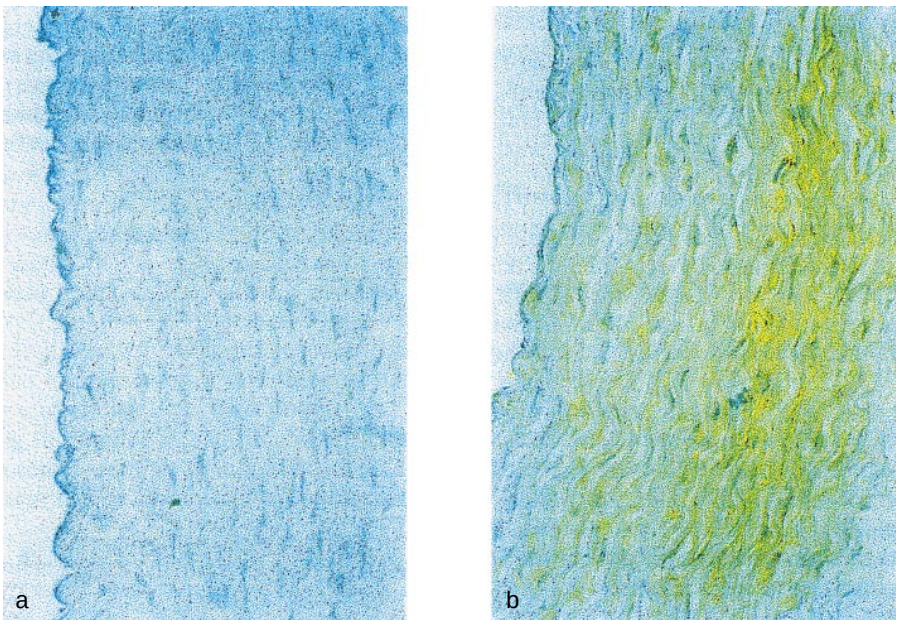

Figure 2 Nitrate induced expression of endothelin in rabbit aorta.

A section of rabbit aorta from (**a**) control rabbit and (**b**) a nitrate-tolerant rabbit. Endothelin has been demonstrated by an immunocytochemical reaction. The yellowish-brown staining in the right hand section indicates increased endothelin content. (From Münzel *et al.* [7]).

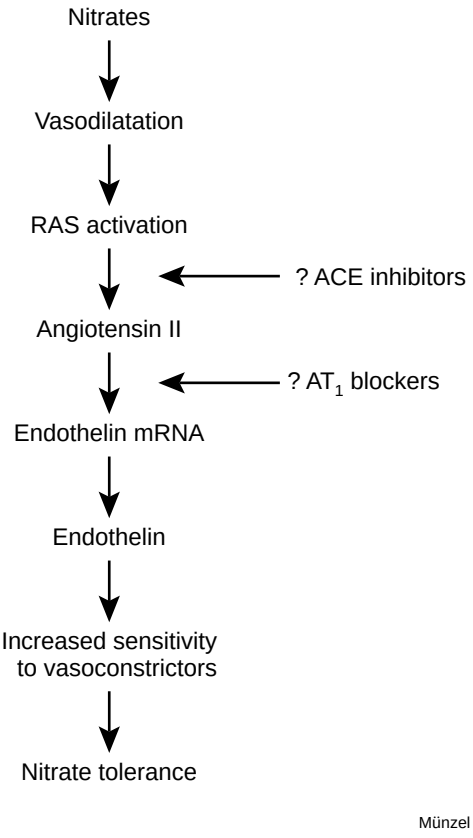

Figure 3 The suggested biological cascade by which endothelin levels in nitrate-tolerant aorta are increased.

Currently the therapeutic approach to nitrate tolerance is empirically based. The principle is one of intermittent therapy [9]. The administration of intravenous nitrates continuously for 24–72 h often produces apparent tolerance. The following options are available for management:

(i) increase the dose and observe effect
(ii) rest from nitrates for 12 h then resume and observe
(iii) find alternative treatment

To avoid the occurence of nitrate tolerance:
(i) in the case of patches, advise a 10–12 h patch off period, usually at night
(ii) with isosorbide dinitrate SR and mononitrate, administer dosage at 08.00 and 14.00 h to allow daily washout of greater than 12 h

The use of ACE inhibitors or AT_1 blocking agents to prevent tolerance has yet to be clinically tested.

Diuretic resistance

Diuretic resistance in severe cardiac failure is an all too common occurrence. It may be due to the severity of the heart failure, but very often diuretic resistance occurs during chronic diuretic therapy, and can often be overcome with appropriate intervention. In practical terms the causes are several. They include lack of compliance, inadvertent prescription of an NSAID, worsening of the heart failure, volume depletion and poor renal function, humoral adaptation, and the newly discovered phenomenon of hypertrophy of the tubular cells of the distal nephron. Brater [10] has dealt very clearly with some of the determinants of the response to a loop diuretic, such as the bioavailability of the diuretic in its pharmaceutical form, the pharmacokinetic factors determining the delivery of the diuretic to the glomerulus, the renal clearance of the diuretic, and the concentration of the diuretic in the tubular fluid (see Figure 4).

I shall concentrate on the pharmacodynamics. It is known that the loop diuretics act from within the renal tubule, so the operative concentration of the drug is that in the tubular fluid. The site of action is the thick ascending limb of the loop of Henle and the molecular locus the $Na^+/K^+/Cl^-$ co-transporter on the luminal side of the tubular cell. Inhibition of this transporter increases the volume of, and the sodium concentration in, the luminal fluid reaching the distal convoluted tubule (DCT). Diuretic-induced reduction in the extracellular fluid volume turns on a number of homeostatic mechanisms, which promote greater Na^+

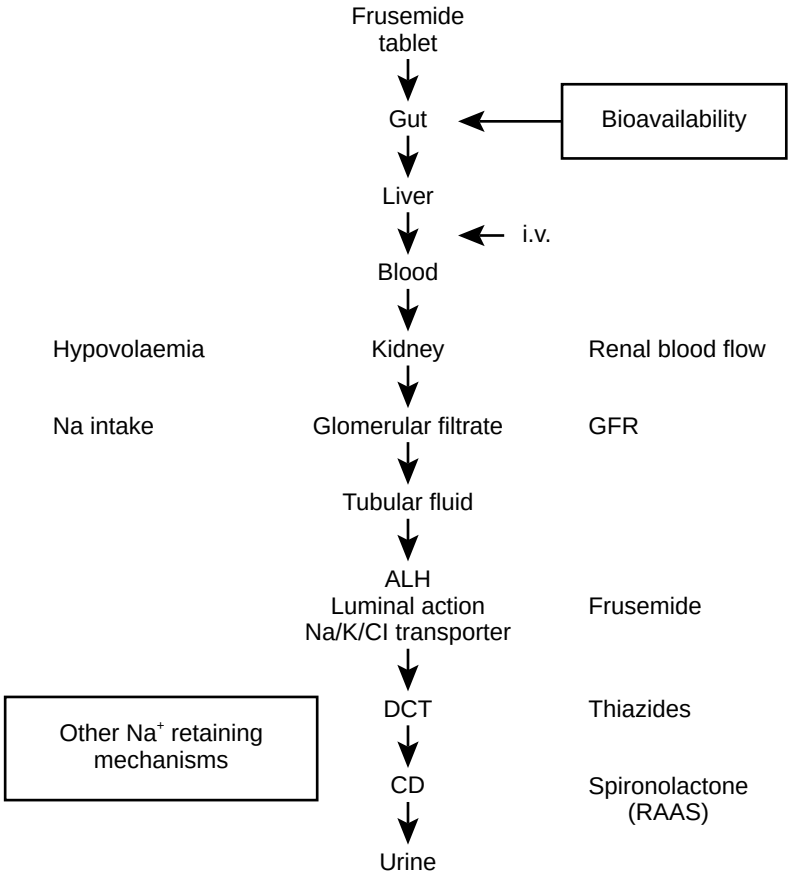

Figure 4 Pharmaceutical, pharmacokinetic and pharmacodynamic links in the diuresis induced by frusemide.

Frusemide if in tablet form, dissolves in intestinal fluid and is absorbed across the gut wall, passes up the portal vein into the liver, passes through the hepatic veins and then through the pulmonary circulation and into the systemic circulation. The drug in blood in the glomerular tuft is filtered in glomerular filtrate and passes in the tubular fluid to the ascending Loop of Henle where it exerts its pharmacological action which is dependent upon its concentration in the tubular fluid. It inhibits the Na/K/Cl transporter in the cells lining the ascending Loop of Henle. Sodium reabsorption in the ascending Loop of Henle is blocked and therefore the fluid entering the distal convoluted tubules and collecting duct has an increased sodium concentration. The sites of action of frusemide, thiazides and spironolactone are shown, as are the sites of some sodium retaining mechanisms. The actions of hypovolaemia to affect renal blood flow, the importance of GFR and the importance of sodium intake in determining renal tubular sodium load are indicated.

absorption in the distal tubule. A final factor is the hypertrophy of the distal renal tubules, which occurs as a response to the increased Na^+ concentrations to which they are exposed (see Figure 5).

Loon *et al.* [11] have shown in man that chronic frusemide treatment is associated with a reducing sodium loss, compared with losses in the drug naive state. This is accompanied by decreased Na^+ and diuretic responses to intravenous infusion of frusemide, despite an adequate concentration of frusemide in the urine. The glomerular filtration rate remained normal in these studies. There was no evidence that increased activity of the renin-angiotensin-aldosterone system was responsible for the diminished frusemide effect. They did find evidence for increased Na^+ reabsorption in a thiazide-sensitive nephron segment probably including the DCT. Kaissling *et al.* [12] and Ellison *et al.* [13] found impressive structural hypertrophy of tubular cells in the DCT, connecting tubule, and cortical collecting ducts in rats treated with frusemide by infusion for 6 to 7 days (Figures 6 and 7). These changes did not depend on mineralocorticoids, as they occurred in adrenalectomized rats, and they did not relate to arginine vasopressin concentrations. The conclusion is that in some way, the increased Na^+ load in the distal nephron promotes hypertrophy of distal tubular cells and increases their reabsorption of sodium.

Current methods for dealing with diuretic resistance are shown in Table 3 [10, 14]. However,these findings form a firm clinical basis for combining thiazide diuretics with loop diuretics in the treatment of resistant oedema in congestive cardiac failure. Ellison [14] has also suggested that thiazides may reduce the DCT cell hypertrophy and thereby prevent the cellular adaptive response to loop diuretics. This might be achieved with low doses of metolazone 2.5 mg or hydrochlorothiazide 25 mg administered two or three times a week. A controlled prospective study of this strategy on the prevention of diuretic resistance in severe heart failure, accompanied by a benefit *vs* risk analysis, would be very valuable indeed.

Vascular and cardiac re-modelling in response to ACE inhibition

Clinical studies have shown that treatment of heart failure, post-myocardial infarction, and hypertension with ACE inhibitors results in cardiac 're-modelling', i.e. a diminution in LV hypertrophy and improved LV function [15, 16]. Experimental studies have shown that ACE inhibitors prevent vascular hypertrophy, attenuate experimental atherosclerosis, and inhibit the neointimal hyperplasia of re-stenosis [17].

Initially it was supposed that many of these effects were mediated through the pressure off-loading associated with the vasodilating effects of ACE inhibitors, and indeed these effects may play a part. Recent advances, however, have implicated a trophic effect of local angiotensin, producing hypertrophic change in both heart and arteries and consequently an antitrophic effect of ACE inhibitors in the heart and arteries because of blockade of angiotensin II production.

The heart and arteries have been shown to possess local renin-angiotensin systems (RAS). Baker *et al.* [15] and Raman *et al.* [18] have reviewed the role of the intracardiac

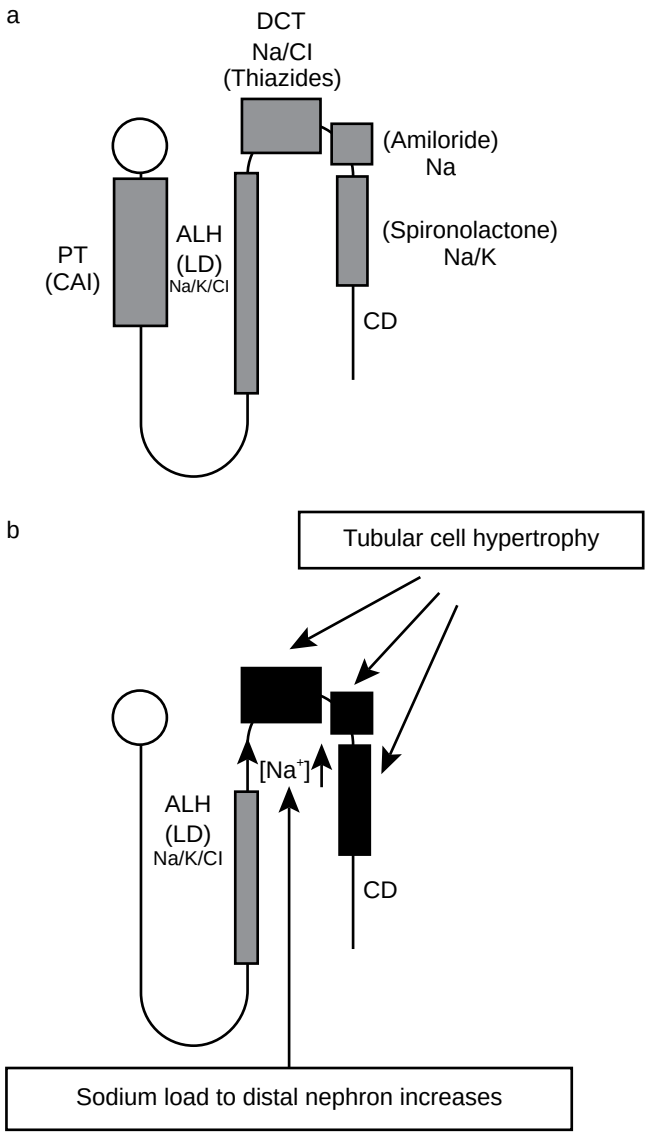

Figure 5 (a) This is a stylised diagram of the sites of action of diuretics on the nephron; carbonic and hydrase inhibitors (CAI) act in the proximal tubule to inhibit carbonic anhydrase and its dependent reabsorption of sodium. The loop diuretics (LD) act in the ascending Loop of Henle (ALH) to inhibit the Na/K/Cl transporter. In the distal convoluted tubule (DCT) thiazides act to inhibit the Na/Cl transporter, and amiloride to inhibit sodium reabsorption. Spironolactone acts more distal to thiazides to inhibit the action of aldosterone on the Na/K transporter.

(b) In this figure is depicted the sites of increased sodium load in the distal nephron and of the tubular cell hypertrophy resulting from sustained inhibition of the Na/K/Cl transporter in the ascending Loop of Henle by a loop diuretic.

renin-angiotensin system and the action of angiotensin II on the heart. Angiotensin II binding sites are situated on myocardial sarcolemmal membrane, and it is suggested that the AT_1 sub-type of angiotensin II receptor mediates the cardiac hypertrophic effects of angiotensin. There is clear evidence that in the spontaneously hypertensive rat, ACE inhibitors prevent cardiac hypertrophy, an effect not wholly due to lowering the blood pressure, as some other antihypertensive drugs that lower the blood pressure equally do not have this effect.

Kang *et al.* [19], using the rat isolated heart perfusion system (thereby eliminating the systemic RAS), have

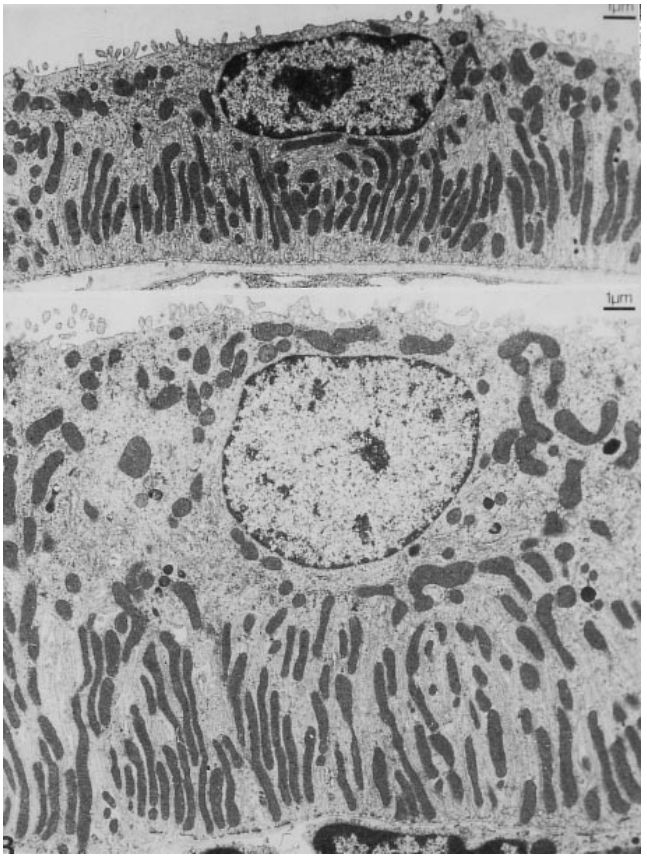

Figure 6 In the upper part of the figure is shown an electromicrograph of a normal distal convoluted tubular cell and below a markedly hypertrophied distal convoluted tubular cell from an animal treated by a constant frusemide infusion delivered by an osmotic minipump. Both groups of animals were adrenalectomised. (From Kaissling & Stanton, [12])

demonstrated that increased dynamic left ventricular stretch increases the steady state expression of c-fos mRNA, and this was inhibited by an AT_1 receptor blocker. There was also increased protein kinase C activation. Renin activity is rather low in the heart, whereas ACE activity is higher, and both angiotensinogen mRNA and ACE mRNA are plentifully expressed and subject to physiological control [20]. Several studies in spontaneously hypertensive rats have shown that treatment with ACE inhibitors reverses left ventricular hypertrophy and the myocardial fibrosis that accompanies it [21]. Ishiye *et al.* [22] have shown that losartan, an angiotensin II receptor antagonist, reduces the cardiac hypertrophy in rats produced by surgically-induced aortic insufficiency. Locally produced angiotensin II in the heart is made both by the actions of angiotensin converting enzyme and another enzyme, the cardiac angiotensin II forming serum proteinase (human heart chymase). The respective roles of these two different routes for angiotensin II formation in the human heart has yet to be worked out [23]. Because of the possibility of two enzymatic sources for angiotensin II, the possibility that angiotensin II receptor blockers might be more efficacious in preventing or reversing left ventricular hypertrophy in hypertension and ischaemic heart disease is under consideration, and a clinical study of this is being carried out [24].

The functional molecular biology of the control of the synthesis and secretion of angiotensin II in the heart is still

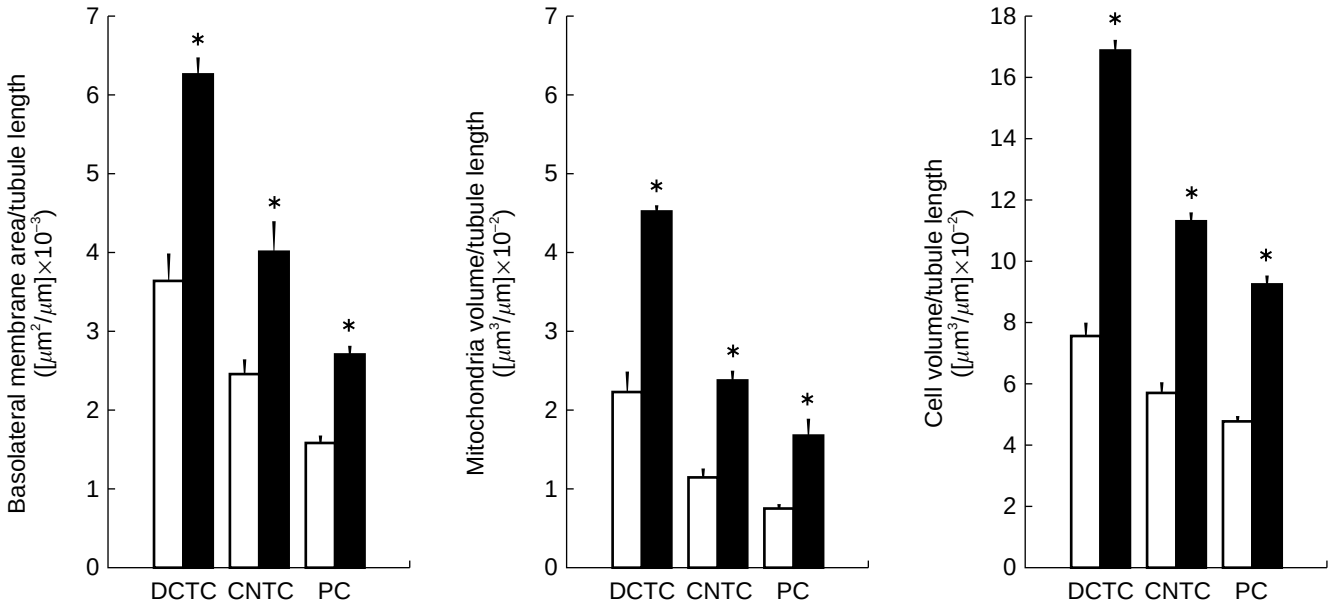

Figure 7 The effect of frusemide on tubular cells in the distal nephron.

This figure summarises the ultra-structural changes in the ultra-structure of distal convoluted (DCTC), connecting tubule (CNTC), and principle cells (PC) produced by frusemide. Controls of experimental groups were both adrenalectomised. Frusemide treated groups are depicted by the filled-in bars, controls by the open bars.

Table 3 What to do about diuretic resistance in congestive cardiac failure?

- Check compliance
- Check not taking NSAID
- Check sodium intake (?restrict)
- Should patient be on ACE inhibitor?
- Is inotropic support indicated?

Having taken the above into consideration:

1. Increase dose of loop diuretic and observe response.
2. Give loop diuretic i.v. and observe response.
3. Give i.v. infusion (avoid between dose Na^+ retention).
4. Add thiazide
   (beware excess diuresis and electrolyte loss).
5. Prevention?
   2.5 mg metolazone or 25 mg hydrochlorothiazide
   2–3 times per week

Ellison [14].

being worked out. Haemodynamic load causes the expression of intermediate-early genes (IEGs) and cytoskeletal mRNAs [25]. Kim *et al.* [26] have shown that angiotensin II infused into rats increases LV mRNAs for skeletal alpha-actin, beta-myosin heavy chain, atrial naturetic peptide, and fibronectin. These effects were blocked by an angiotensin II receptor blocking agent. Lindpaintner *et al.* [27] have shown that in rats 5 days after coronary ligation, there was an increase in angiotensinogen mRNA in non-infarcted ventricles, falling to normal after 25 days. This is relevant to the role of ACE inhibitors in aiding cardiac re-modelling after myocardial infarction. Kent & McDermott [28] have shown that passive loads or angiotensin II produced different patterns of gene expression and protein synthesis in isolated cardiac myocytes. Angiotensin did cause the expression of c-fos mRNA in this system. However, it does look from these experiments as if not all long-term responses to passive load are mediated by angiotensin.

The situation, in principle, is not too different in the vascular system. The demonstration that arteries have their own renin-angiotensin system (RAS) has altered one conception of the pharmacology of vascular hypertrophy and hyperplasia, including the pathogenesis of atherosclerosis. Dzau [29] has written an excellent review of the subject.

In essence, the proposal is that angiotensin II, whether manufactured through the agency of the systemic RAS or via a localized system in the vessel wall, is a factor that promotes the hypertrophy of vascular smooth muscle cells, besides being a vasoconstrictor. It may also be related to the expression of autocrine growth factors, such as platelet derived growth factor (PDGF) and fibroblast growth factor (β-FGF). Angiotensin II may also promote the expression of TGF-β, which may counteract the actions of the trophic factors. Therefore, a balance between the proliferative and the anti-proliferative actions of angiotensin II may be important in the development of vessel hypertrophy. This is the basis for the explanation of the effects of ACE inhibitors in preventing and reversing vascular hypertrophy

in hypertension, i.e. vascular re-modelling. Dzau [17] has also drawn attention to the effects of angiotensin II on the genesis of atheroma by its actions on the vessel wall. There is evidence that high doses of ACE inhibitors in experimental animals can prevent intimal hypertrophy after intimal injury, and ACE inhibitors or angiotensin II receptor blocking agents may be effective in delaying the genesis of atheromatous lesions.

What is the mechanism by which angiotensin II brings about these changes in the heart and in the blood vessel wall? Are there any commonalities?

In terms of the initial signal transducing mechanisms, there are similarities. Angiotensin interacts with its receptor to stimulate the breakdown of phosphatidyl inositol, with the production of inositol trisphosphate and diacylglycerol, which increase intracelluar calcium concentrations and activate protein kinase C. This sets in train a number of processes which result in the triggering of a set of instructions for gene expression, intermediate early gene expression, such as c-fos, being an early step in this cascade in both the heart and the blood vessel. The phenotype of the myocardial cell and the smooth muscle cell in the vessel wall will determine which genes are expressed down the line (see Figure 8).

One could argue that the effects of ACE inhibitors on cardiac hypertrophy and vessel wall hypertrophy are not so much examples of adaptive pharmacology, as simply indicating a longer term anti-trophic pharmacology underlying a gradual and long-lasting therapeutic response. What is seen though is an immediate response produced by ACE inhibitors in both hypertension and heart failure mediated by vasodilatation, and then the later effect of a more chronic pharmacology involving gene expression and changing cellular growth and responses in the long term.

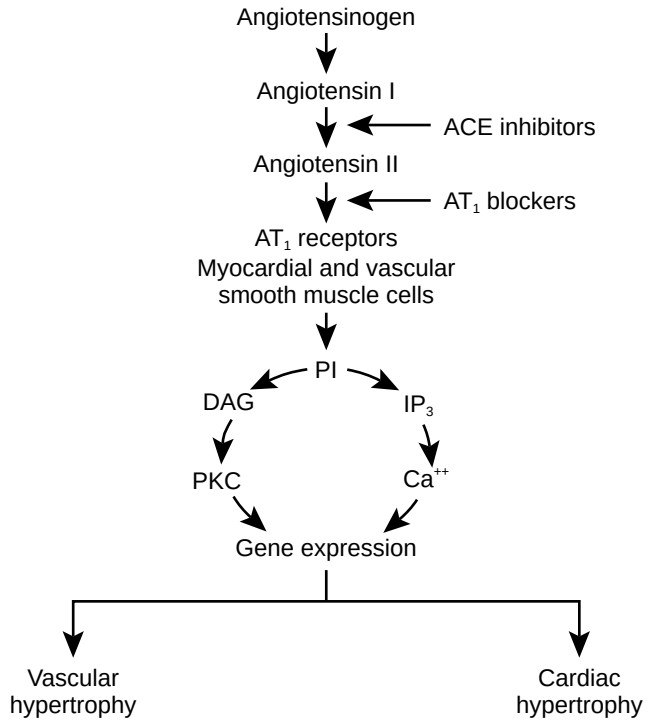

Figure 8 The biological cascade of angiotensin-mediated effects on the heart to produce cardiac hypertrophy and in arteries to produce vascular hypertrophy.

Note: In the heart the human chymase can bypass angiotensin converting enzyme and that AT_1 blockers may theoretically therefore be more effective in reducing the cardiac hypertrophy produced by angiotensin II.

These are new and rather unexpected phenomena in therapeutic pharmacology, brought to light by careful clinical observations made over a long period of time, in large scale clinical trials (meta-analyses), put on to a firm basis by systematic review, and providing an empirical basis for the clinical scientist to question whether the acute actions of ACE inhibitors really account for their spectacular long-term effects in reducing death rates in cardiac failure and re-modelling the heart and arteries in hypertension.

Antidepressant drug therapy: an example of an adaptive response mediating a therapeutic effect

Most drugs are developed on the basis of an acute pharmacology. All the antidepressants, the tricyclics, selective serotonin re-uptake inhibitors (SSRIs), MAO inhibitors, and the newer drugs such as nefazadone and trazodone, have been developed on the basis of their acute actions on noradrenaline and/or 5-HT function.

Antidepressant treatments take 2–4 weeks to produce a clear therapeutic effect. If they are discontinued quickly after recovery, relapse is more likely than if they are continued for several months. ECT twice weekly requires 3–4 treatments before a therapeutic response is seen. Improvement then tends to occur quickly with subsequent treatments. Antidepressant drugs must be continued for a period of months after a course of ECT to decrease the likelihood of relapse, suggesting some kind of synergy between ECT and antidepressants [30].

These phenomena suggest something more than an acute simple and transient point-to-point pharmacological effect of the treatments. Why does it take so long for the therapeutic effect to appear, when, as can be safely predicted from what is known of the pharmacokinetics and pharmacodynamics of many of these drugs, monoamine function in the brain is affected within hours or less of the first dose? My colleagues and I, over many years, have been studying the chronic adaptive pharmacology of antidepressant drugs, ECT (using repeated electroconvulsive shock in rats as an analogy) and also lithium, as it too may owe its therapeutic effects to an adaptive and chronic pharmacology. A summary of our accumulated findings on the effects of antidepressant drugs, electroconvulsive shock, and lithium on 5-HT function is given in Table 4.

Because of methodological problems it has been very difficult to mirror these animal experiments in man and study the chronic adaptive pharmacology of tricyclic antidepressants in patients with depression. However, a number of clinical studies have been done in which 5-HT agonist strategies have been used to stimulate neuroendocrine function in normal people and in patients with depression taking antidepressant drugs, and these do suggest neuroadaptive changes [31–40].

Taking the animal studies and clinical studies together, a few clear general conclusions can be drawn:

1. Antidepressant drugs and ECS, when administered to

Table 4 Effects of repeated long-term administration of antidepressant drugs, monoamine oxidase inhibitors, electroconvulsive shock, and lithium upon 5-HT-mediated behavioural functions in the rat and mouse. (Grahame-Smith [59]).

	8-OH-DPAT rat 5-HT behavioural syndrome (5-HT$_{1A}$ receptor)	*8-OH-DPAT hypothermia in the rat (5-HT$_{1A}$ receptor)*	*8-OH-DPAT hypothermia in the mouse (5-HT$_{1A}$ receptor)*	*Mouse head-twitch (5-HT$_2$ receptor)*
Anti-depressant drugs	All aspects decreased	Attenuated	Attenuated	Decreased
Monoamine oxidase inhibitors	All aspects decreased	Attenuated	Attenuated	Decreased
Electro-convulsive shock	Stereotypes decreased Locomotor activity ?increased	Attenuated	Attenuated	Increased
Lithium	All aspects increased	No change	Attenuated	Decreased

rats and mice, cause delayed effects on various aspects of 5-HT function. Human studies also point to delayed effects of these treatments on monoamine function.

2. Changes occur in the number of many neurotransmitter receptor binding sites and their signalling cascades during chronic antidepressant drug treatment, ECS, and lithium treatment, but so far these changes do not form a coherent final explanation for the functional behavioural changes observed.

3. Some of these effects in animals depend on the function of multiple neurotransmitters, e.g. interactions between 5-HT, noradrenaline, and GABA.

Recent advances in molecular neurobiology have thrown much light on the sort of adaptive responses in the brain which might be expected during antidepressant treatment and which may underlie the therapeutic effects of these drugs.

Take for instance the work of Kandel [41] on memory in *Aplysia*. In the marine mollusc *Aplysia* there is a form of avoidance behaviour to noxious stimuli which involves a withdrawal reaction of its gill, and this behaviour is sensitised by electric shock, a form of learning, i.e. avoidance behaviour. The *Aplysia* has a simple nervous system, and one essential feature of it is a sensory neurone which synapses with a motor neurone that controls the withdrawal reflex of the gill. There are facilitatory neurones modulating synpatic traffic between the sensory neurone and the motor neurone, and 5-HT is a facilitatory neurotransmitter at these synapses.

There are two phases of memory formation in *Aplysia*, short-term and long-term. Short-term memory depends on small molecular modifications of existing synaptic functional elements, whereas long-term memory depends on the production of new macromolecular components and synaptic re-modelling. In the production of long-term memory in *Aplysia*, 5-HT release from facilitatory neurones on to axo-axonal synapses of the sensory neurones acts to stimulate the production of cyclic AMP; this then stimulates a protein kinase A, which in turn phosphorylates a cyclic AMP response binding protein, which leads to a sequence of gene expression, resulting in the production of a group of macromolecules known to be involved in synaptic re-modelling. This re-modelling leads to alterations in the circuitry linking the sensory and motor neurone, such that during the process of sensitization, there is sprouting of the sensory neurones and new synapse formation linking sensory neurones to motor neurones. Here in microcosm is a system illustrating the neurobiological phenomena involved in synaptic and neural plasticity [41]. Some will object to drawing analogies between the mollusc nervous system and the human, but nature is a tinkerer and during evolution does not design systems from scratch if she does not need to.

Long-term potentiation (LTP) is another phenomenon illustrating the mechanisms of neuronal adaptation. A repetitive train of stimuli delivered to one of the afferent pathways of the hippocampus produces a subsequent increase in the excitatory synaptic potential in the post-synaptic hippocampal neurones, which *in vitro* lasts for hours and *in vivo* in the rat for days or weeks. LTP requires coincident presynaptic and postsynaptic activity, [42, 43]. In general terms this corresponds to Hebb's Rule: 'When an axon of cell A excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells so that A's efficiency as one of the cells firing B is increased' [44].

LTP may be related mechanistically to memory and learning. There are at least two overlapping phases in the development of LTP, in principle not unlike the short-term and long-term memory processes in *Aplysia*. First the stimulation causes activation of postsynaptic NMDA receptors. This produces a rise in intracellular Ca^{++} concentrations in the postsynaptic neurone. This results in activation of kinases and a range of post-translational biochemical changes, which alter pre-synaptic and post-synaptic function in the short term. However, these initial changes produce a biochemical cascade, which

eventually results in transcriptional changes that confer the much longer maintenance of LTP. Many recent studies have shown that during LTP, and generally dependent on NMDA receptor stimulation, there is increased expression in the hippocampus of BDNF mRNA, the neurotrophic factor receptor Trk-B mRNA, suggesting that neurotrophic factors are acting to produce changes in synaptic structure, and producing neuronal modelling, leading to a longer term LTP. Edwards [45], Lipton & Kater [46], and Geinisman *et al.* [47] have all shown convincing structural changes in neuronal and synaptic architecture in the hippocampus during LTP, which suggests that neuronal and synaptic re-modelling are part of the biological process leading to the long-term change in the physiological function of the hippocampus during LTP. Although the phenomenon of memory may seem quite unlike mood and its disorders, perhaps there are reasons for considering that each possesses an analogous neurobiology. If this is the case, one might expect to find similar biological changes occurring as a result of antidepressant drug treatment to those one finds during the establishment of memory.

There are four other models illustrating neural plasticity in the adult brain. These are:

a) Kindling [48]
b) Seizures (re ECT) [49]
c) Vision [50]
d) Regeneration after lesions [51].

This body of work shows that neuronal and synaptic plasticity are true neurobiological phenomena in adult nervous systems and allows them to be considered as potential targets for the pharmacological changes in monoamine function brought about by antidepressant drugs.

It is always dangerous to equate what a drug does pharmacologically to the primary causation of a disease. Diuretics are helpful in the treatment of heart failure, but act on the kidneys and not the heart! Nevertheless, in the state of understanding of the causation of mental illness at the moment, and our ignorance of neurobiological causes, there is a temptation to do that. Duman *et al.* [52] have stated, 'The genetic abnormality leading to psychiatric illness may not be the production of abnormal gene products, but instead an abnormality in the regulation of normal gene products that produces abnormal neural plasticity and adaptability.' This brings us to an understanding of the biological cascades which might lead from drug effects on neurotransmitter function, e.g. antidepressant effects on monoamine uptake, to the regulation of gene expression which itself might be involved in synaptic and neural plasticity. The fundamental cascade is shown in Figure 9. This scheme provides the background upon which one can build an understanding of the processes by which the brain adapts to experience, by which short-term and long-term homeostasis of neural programmes is controlled, and by which the long-term effects of psychotrophic drugs, (such as antidepressants) and of ECT can be explained. For instance Duman *et al.* [52] proposed that stress normally results in activation of noradrenergic systems that lead to adaptive responses, which prevent a disturbance of the homeostasis in monoamine neurotransmitter function and which might otherwise result in depression. One proposal is that glucocorticoids produced in response to stress are potentially neurotoxic, and that the disturbance of neuronal function produced thereby, may result in depression and anxiety.

There is mounting evidence that antidepressant drugs or ECT affect gene expression through changes in neurotransmitter action. Chronic, but not acute, treatment with fluoxetine or desmethylimipramine results in an increased expression of the intermediate-early gene, c-fos, in the frontal cortex and hippocampus in response to the 5-HT_2 receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) [53]. Lipton & Kater [46] have argued that neurotransmitters, in addition to immediate trans-synaptic signal transfer, can also induce a spectrum of effects on neuronal cytoarchitecture, ranging from neurite sprouting to dendritic pruning and even cell death. They have proposed that these actions may constitute part of the normal functioning and structuring of the nervous system as well as contributing to pathology. What is the evidence that 5-HT might be involved in these neurotrophic effects? Emerit *et al.* [54] and Whitaker-Azmitia *et al.* [55] have provided evidence that 5-HT function is of importance in brain development, playing an important role in the organisation of neuronal connectivity. Of course, this role during development may be different from that in the adult brain, but Nishi *et al.* [56] have shown that synaptophysin and Map 2 in the cortex and hippocampus of the adult rat are reduced by 5-HT depletion with parachloroamphetamine treatment, and that this loss of synaptophysin is reversed by *in vivo* treatment with ipsapirone, a 5-HT_{1A} receptor agonist. When one considers the incredibly intricate, widespread, and heavy innervation of many areas of the brain with a web of fine 5-HT projection fibres and considers also the fact that many of these fibres do not form close synapses with target cells, implying that they release 5-HT in a cloud [57], it does seem that 5-HT may act as a neuromodulatory system, one action of which might be to maintain synaptic homeostasis by trophic effects. It is of interest that several drugs of addiction produce long-term neuronal adaptive responses of a not dissimilar molecular neurobiological nature, which are probably responsible for the addictive state, with its manifestations of craving and associated drug-seeking behaviour, withdrawal syndromes, and tolerance [58].

Clinical relevance

What is there about depression that might respond to such a neurotrophic effect of antidepressant treatments? Is there anything about the actions of lithium that might be invoked to maintain synaptic homeostasis?

The genetics of depressive illness are not incompatible. One would propose that the genetic defect produces a biology in an individual which is defective and leads to a disorder of neuronal adaptation when the individual is faced with psychological stress. In a clinical context this would produce vulnerability to depression associated with adverse life events, which is a feature of recurrent depressive illness. Because of this predisposition, depression would be recurrent.

Lithium remains an enigma. We know about a number of its pharmacological actions, in particular its very clear effect during chronic treatment on 5-HT behavioural functions [59]. Its clearest biochemical effect is on the

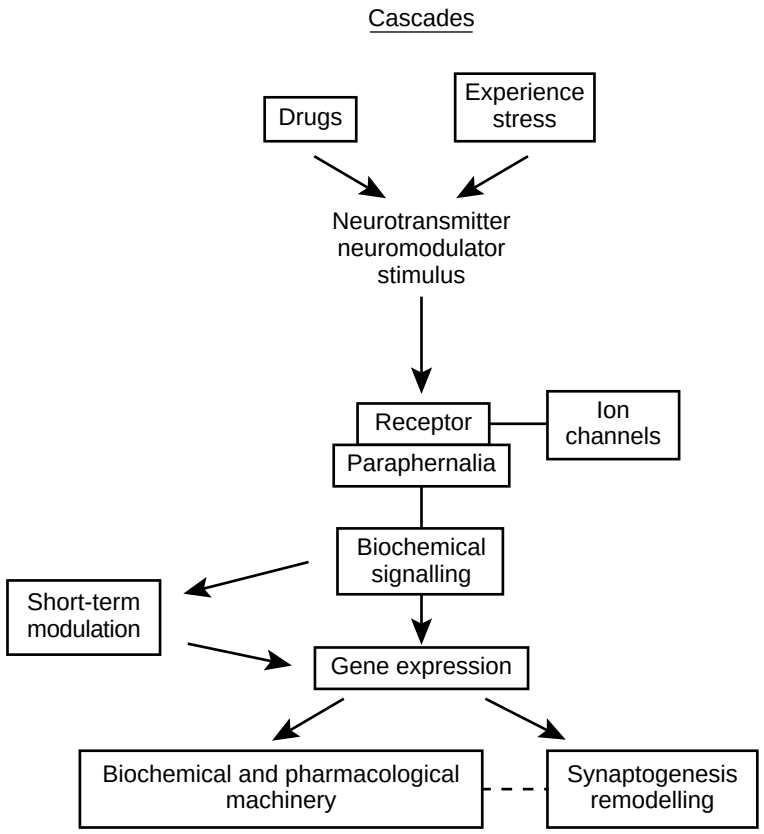

Figure 9 Mechanisms of neuronal and synaptic remodelling and plasticity

In this biological cascade are shown the processes initiated via neurotransmitter and neuromodulator actions to produce short- and long-term changes in neuronal function, and via gene expression, synaptogenesis and neuronal remodelling.

inositol phosphate-diacylglycerol signalling cascade [60], which superficially looks simple but which so far has not provided an explanation for the effects of lithium on 5-HT function [61]. The clear and complex effects on 5-HT function might fit the proposed neurotrophic role of 5-HT. Lithium, through its actions on 5-HT function, could maintain synaptic homeostasis in a brain vulnerable to its breakdown, hence its prophylactic effect in manic-depressive disease.

The deep biological nature of depression is also reflected in the demonstration of circadian phase shifts, sleep disturbance, hormonal changes, and physical symptoms. These clinical features all suggest a biology of the brain which is slow changing, unapparent to the observer, below the surface of consciousness, and which although autonomic is also responsive to experience. The proposition, therefore, is that the neurobiological problem in depression lies in an abnormality which does not permit the usual responses of neuronal plasticity to cope with stress. Synaptic homeostasis in certain neuronal networks of the brain is then easily disturbed, and because of this the vulnerable individual is deprived of the neurobiological (and therefore the psychological) substrates to withstand emotional stress.

Conclusion

If one considers the biological processes mediating the pharmacological adaptive responses to nitrates, ACE inhibi-

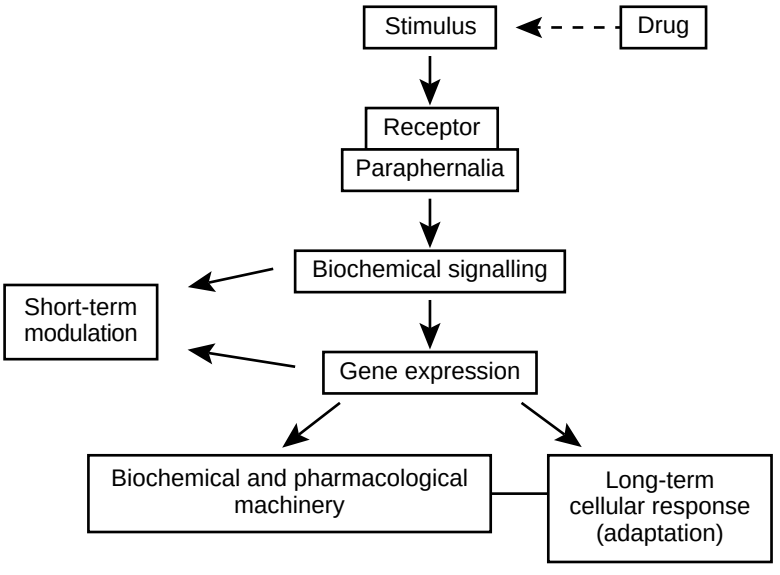

Figure 10 Commonality of mechanisms producing pharmacological adaptation to chronic drug therapy.

The purpose of this figure is to stress the commonality of mechanisms by which long-term pharmacological treatment may lead to chronic adaptive changes in tissues and physiological systems. It seems likely that nitrate tolerance, ACE inhibitors, angiotensin II blocking agents, antidepressants and electroconvulsive therapy, all produce their chronic adaptive effects via this type of scheme. Whether or not diuretics produce tubular cell hypertrophy via this mechanism, secondary to cellular signals produced by increased sodium load in the tubular fluid, has yet to be investigated.

tors, antidepressants (and future investigation might also apply them to the tubular cell hypertrophy produced by diuretics) a common theme emerges (Figure 10).

An initial change in a stimulus produced by a small molecule brought about by an acute drug action, leads on long-term dosing to changes in intracellular messenger biochemical cascades. These result in enduring changes in gene expression, leading to the expression of functional and/or structural proteins, so that cellular function is altered for an extended time. An understanding of these mechanisms may allow strategies for enhancing these effects when they are beneficial (e.g. ACE inhibitors and antidepressants), and counteracting them when they harmful (e.g. drug addiction, diuretic resistance, and nitrate tolerance).

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References

1 Lange RL, Reid MS, Tresch DD, Keelan MH, Bernhard VM, Coolidge G. Nonatheromatous ischemic heart disease following withdrawal from chronic industrial nitroglycerin exposure. *Circulation* 1972; **46**: 666–678.
2 Zelis R, Mason DT. Isosorbide dinitrate. Effect on the vasodilator response to nitroglycerin. *JAMA* 1975; **234**: 166–170.
3 Munzel T, Kurz S, Heitzer T, Harrison DG. New insights into mechanisms underlying nitrate tolerance. *Am J Cardiol* 1996; **77**: 24C–30C.
4 Klemsdal TO, Gjesdal K. Intermittent or continuous transdermal nitroglycerin: still an issue, or is the case closed? *Cardiovasc Drugs Ther* 1996; **10**: 5–10.
5 Münzel T, Heitzer T, Kurz S, *et al*. Dissociation of coronary vascular tolerance and neurohormonal adjustments during long-term nitroglycerin therapy in patients with stable coronary artery disease. *J Am Coll Cardiol* 1996; **27**: 297–303.
6 Needleman P, Johnson EM Jr. Mechanism of tolerance development to organic nitrates. *J Pharmacol Exp Ther* 1973; **184**: 709–715.
7 Münzel T, Giaid A, Kurz S, Stewart DJ, Harrison DG. Evidence for a role of endothelin 1 and protein kinase C in nitroglycerin tolerance. *Proc Natl Acad Sci USA* 1995; **92**: 5244–5248.
8 Münzel T, Bassenge E. Long-term angiotensin-converting enzyme inhibition with high-dose enalapril retards nitrate tolerance in large epicardial arteries and prevents rebound coronary vasoconstriction *in vivo*. *Circulation* 1996; **93**: 2052–2058.
9 Thadani U. Nitrate tolerance. How to prevent it or minimize its effect. *Postgrad Med* 1992; **91**: 307–312, 315–318.
10 Brater DC. Diuretic resistance: mechanisms and therapeutic strategies. *Cardiology* 1994; 84 Suppl 2: 57–67.
11 Loon NR, Wilcox CS, Unwin RJ. Mechanism of impaired natriuretic response to furosemide during prolonged therapy. *Kidney Int* 1989; **36**: 682–689.
12 Kaissling B, Stanton BA. Adaptation of distal tubule and collecting duct to increased sodium delivery. I. Ultrastructure. *Am J Physiol* 1988; **255**: F1256–F1268.
13 Ellison DH, Velazquez H, Wright FS. Adaptation of the distal convoluted tubule of the rat. Structural and functional effects of dietary salt intake and chronic diuretic infusion. *J Clin Invest* 1989; **83**: 113–126.
14 Ellison DH. The physiologic basis of diuretic synergism: its role in treating diuretic resistance [see comments]. *Ann Intern Med* 1991; **114**: 886–894.
15 Baker KM, Booz GW, Dostal DE. Cardiac actions of angiotensin II: Role of an intracardiac renin-angiotensin system. *Ann Rev Physiol* 1992; **54**: 227–241.
16 Blaufarb IS, Sonnenblick EH. The renin-angiotensin system in left ventricular remodeling. *Am J Cardiol* 1996; **77**: 8C–16C.
17 Dzau VJ. Vascular renin-angiotensin system and vascular protection. *J Cardiovasc Pharmacol* 1993; **22** Suppl 5: S1–S9.
18 Raman VK, Lee YA, Lindpaintner K. The cardiac renin-angiotensin-aldosterone system and hypertensive cardiac hypertrophy. *Am J Cardiol* 1995; **76**: 18D–23D.
19 Kang PM, Nakouzi A, Simpson T, Scheuer J, Buttrick PM. Role of endogenous renin-angiotensin system in c-fos activation and PKC-epsilon translocation in adult rat hearts. *Am J Physiol* 1996; **270**: H2177–H2183.
20 Iwai N, Shimoike H, Kinoshita M. Cardiac renin-angiotensin system in the hypertrophied heart. *Circulation* 1995; **92**: 2690–2696.
21 Brilla CG, Matsubara L, Weber KT. Advanced hypertensive heart disease in spontaneously hypertensive rats. Lisinopril-mediated regression of myocardial fibrosis. *Hypertension* 1996; **28**: 269–275.
22 Ishiye M, Umemura K, Uematsu T, Nakashima M. Effects of losartan, an angiotensin II antagonist, on the development of cardiac hypertrophy due to volume overload. *Biol Pharm Bull* 1995; **18**: 700–704.
23 Urata H, Nishimura H, Ganten D. Mechanisms of angiotensin II formation in humans. *Eur Heart J* 1995; **16** Suppl N: 79–85.
24 Dahlof B. Effect of angiotensin II blockade on cardiac hypertrophy and remodelling: a review. *J Hum Hypertens* 1995; **9** Suppl 5: S37–S44.
25 Rozich JD, Barnes MA, Schmid PG, Zile MR, McDermott PJ, Cooper G 4th. Load effects on gene expression during cardiac hypertrophy. *J Mol Cell Cardiol* 1995; **27**: 485–499.
26 Kim S, Ohta K, Hamaguchi A, Yukimura T, Miura K, Iwao H. Angiotensin II induces cardiac phenotypic modulation and remodeling in vivo in rats. *Hypertension* 1995; **25**: 1252–1259.
27 Lindpaintner K, Lu W, Neidermajer N, *et al*. Selective activation of cardiac angiotensinogen gene expression in post-infarction ventricular remodeling in the rat. *J Mol Cell Cardiol* 1993; **25**: 133–143.
28 Kent RL, McDermott PJ. Passive load and angiotensin II evoke differential responses of gene expression and protein synthesis in cardiac myocytes. *Circ Res* 1996; **78**: 829–838.
29 Dzau VJ. Molecular biology of angiotensin II biosynthesis and receptors. *Can J Cardiol* 1995; 11 Suppl F: 21F–26F.
30 Gelder M, Gath D, Mayou R, Cowen P (eds) *Oxford Textbook of Psychiatry*, Oxford University Press, 1996.
31 Meltzer HY, Lowy M, Robertson A, Goodnick P, Perline R. Effect of 5-hydroxytryptophan on serum cortisol levels in major affective disorders. III. Effect of antidepressants and lithium carbonate. *Arch Gen Psychiatry* 1984; **41**: 391–397.
32 Meltzer HY. Serotonergic function in the affective disorders: the effect of antidepressants and lithium on the 5-hydroxytryptophan-induced increase in serum cortisol. *Ann NY Acad Sci* 1984; **430**: 115–137.

33 Siever LJ, Uhde TW, Jimerson DC, *et al*. Clinical studies of monoamine receptors in the affective disorders and receptor changes with antidepressant treatment. *Prog Neuropsychopharmacol Biol Psychiatry* 1983; **7**: 249–261.
34 Glue PW, Cowen PJ, Nutt DJ, Kolakowska T, Grahame Smith DG. The effect of lithium on 5-HT-mediated neuroendocrine responses and platelet 5-HT receptors. *Psychopharmacology Berl* 1986; **90**: 398–402.
35 Price LH, Charney DS, Heninger GR. Effects of trazodone treatment on serotonergic function in depressed patients. *Psychiatry Res* 1988; **24**: 165–175.
36 Price LH, Charney DS, Delgado PL, Anderson GM, Heninger GR. Effects of desipramine and fluvoxamine treatment on the prolactin response to tryptophan. Serotonergic function and the mechanism of antidepressant action. *Arch Gen Psychiatry* 1989; **46**: 625–631.
37 Price LH, Charney DS, Delgado PL, Heninger GR. Lithium and serotonin function: implications for the serotonin hypothesis of depression. *Psychopharmacology* 1990; **100**: 3–12.
38 Cowen PJ, McCance SL, Cohen PR, Julier DL. Lithium increases 5-HT-mediated neuroendocrine responses in tricyclic resistant depression. *Psychopharmacology Berl* 1989; **99**: 230–232.
39 Sargent P, Williamson DJ, Cowen PJ. Brain 5-HT neurotransmission during paroxetine treatment. *Br J Psychol* 1997; (in press).
40 Sargent P, Williamson DJ, Pearson G, Odontiadis J, Cowen PJ. Effect of paroxetine and nefazodone on 5-HT_{1A} receptor sensitivity. *Psychopharmacology* 1997; (in press).
41 Bailey CH, Alberini C, Ghirardi M, Kandel ER. Molecular and structural changes underlying long-term memory storage in Aplysia. In *Molecular and Cellular Mechanisms of Neurotransmitter Release*, eds Stjarne L, Greengard P, Griller SE, Hokfelt TGM, Ottoson DR, New York: Raven Press, 1994; 529–544.
42 Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol Lond* 1973; **232**: 331–356.
43 Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 1993; **361**: 31–39.
44 Hebb DO. *The organisation of behaviour: a neuropsychological study* Wiley, 1949.
45 Edwards FA. Anatomy and electrophysiology of fast central synapses lead to a structural model for long-term potentiation. *Physiol Rev* 1995; **75**: 759–787.
46 Lipton SA, Kater SB. Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. *Trends Neurosci* 1989; **12**: 265–270.
47 Geinisman Y, Morrell F, deToledo Morrell L. Remodeling of synaptic architecture during hippocampal 'kindling'. *Proc Natl Acad Sci USA* 1988; **85**: 3260–3264.
48 Lindvall O, Kokaia Z, Elmer E, Bengzon J, Kokaia M. Neurotrophins in kindling epilepsy: neuronal protection or induction of sprouting and epileptogenesis? In *Life and death in the nervous system*, eds Ibanez CF, Hokfelt T, Olson I, Fuxe K, Jornvall H, Ottoson D, Wenner-Gren International Series, 1995; **67**: 417–438.
49 Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 1995; **15**: 7539–7547.
50 Rosenzweig MR. Aspects of the search for neural mechanisms of memory. *Ann Rev Psychol* 1996; **47**: 1–32.
51 Raisman G, Field PM. Synapse formation in the adult brain after lesions and after transplantation of embryonic tissue. *J Exp Biol* 1990; **153**: 277–287.
52 Duman RS, Heninger GR, Nestler EJ. Molecular psychiatry. Adaptations of receptor-coupled signal transduction pathways underlying stress- and drug-induced neural plasticity. *J Nerv Ment Dis* 1994; **182**: 692–700.
53 Tilakaratne N, Yang ZL, Friedman E. Chronic fluoxetine or desmethylimipramine treatment alters 5-HT2 receptor mediated c-fos gene expression. *Eur J Pharmacol—Molecular Pharmacology Section* 1995; **290**: 263–266.
54 Emerit MB, Riad M, Hamon M. Trophic Effects of Neurotransmitters During Brain Maturation. *Biol Neonate* 1992; **62**: 193–201.
55 Whitaker-Azmitia PM, Druse M, Walker P, Lauder JM. Serotonin as a developmental signal. *Behav Brain Res* 1996; **73**: 19–29.
56 Nishi M, Whitaker-Azmitia PM, Azmitia EC. Enhanced synaptophysin immunoreactivity in rat hippocampal culture by 5-HT1A agonist, S100b, and corticosteroid receptor agonists. *Synapse* 1996; **23**: 1–9.
57 Seguela P, Watkins KC, Descarries L. Ultrastructural relationships of serotonin axon terminals in the cerebral cortex of the adult rat. *J Comp Neurol* 1989; **289**: 129–142.
58 Hyman SE, Nestler EJ. Initiation and adaptation: a paradigm for understanding psychotropic drug action. *Am J Psychiatry* 1996; **153**: 151–162.
59 Grahame-Smith DG. Serotonin in affective disorders. *Int Clin Psychopharmacol* 1992; **6** Suppl 4: 5–13.
60 Berridge MJ, Downes CP, Hanley MR. Neural and developmental actions of lithium: a unifying hypothesis. *Cell* 1989; **59**: 411–419.
61 Wang H, Grahame-Smith DG. The effects of rubidium, caesium and quinine on 5-HT-mediated behaviour in rat and mouse—3. Quinine. *Neuropharmacology* 1992; **31**: 425–431.

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