many of the effects of NO and cGMP in target tissues, such as vascular smooth muscle cell dilatation[6,7]. Of the two mammalian PKG genes, only the one that encodes PKG-1 is expressed in heart. Studies of PKG-1 knockout mice support the notion that NO and cGMP decrease the force of cardiac contraction through PKG-1, and PKG-1 expression in heart cells inhibits hypertrophic signaling[7–9]. Hypertrophy can be exacerbated by the loss of cGMP-generating enzymes in mice, like the atrial natriuretic peptide receptor[10]—and natriuretic peptides have promise as therapeutic agents in heart failure. Sildenafil increases cGMP in target tissues, activating cGMP-binding proteins, especially PKG-1.

This new work strongly supports the notion that activation of PKG-1 mediates the inhibitory effect of sildenafil on the heart's response to hypertrophic stimulation. Takimoto and Champion *et al.*[1] showed that PDE5A inhibition by sildenafil (as well as by a second selective PDE5A inhibitor) suppressed heart chamber enlargement, cellular hypertrophy and myocardial fibrosis after pressure overload induced by transaortic constriction (TAC, or aortic banding). Furthermore, sildenafil reversed the fetal gene expression program stimulated by TAC, as well as the TAC-mediated decrease in expression of several myocardial calcium-regulatory proteins. In TAC hearts, PDE5A activity was substantially increased, and PDE5A inhibition with sildenafil activated PKG-1. The observed improvement in cardiac function with sildenafil is further evidence that hypertrophy is not a necessary compensatory event and that inhibiting hypertrophy is a promising therapeutic approach[2,3].

The molecular events responsible for cardiac hypertrophy are complex and involve both mechanical stress- and neurohumoral-mediated stimulation of gene transcription and cardiomyocyte enlargement[2,3,11]. Pathological hypertrophy causes reemergence of fetal cardiac metabolism and gene expression, with altered expression of proteins that regulate calcium responsiveness and contractility[2,3,11]. Which hypertrophic signaling pathways were interrupted by sildenafil treatment?

The data suggest that the increase in cGMP and PKG-1 by sildenafil affected levels and activity of the phosphatase calcineurin, and activation of the protein kinase Akt, both of which mediate major hypertrophic pathways in the heart. Other affected molecules included MAP kinase (Erk 1/2) and GSK-3, a downstream target of Akt, but how inhibition of hypertrophy by sildenafil actually occurs remains obscure.

Many of the signals that stimulate cardiac hypertrophy act through the heterotrimeric G-protein subunit $G_{\alpha q}$. Activation of $G_{\alpha q}$ is both necessary and sufficient to alter expression and activity of transcription factors and fetal genes that mediate pressure overload hypertrophy[12–14]. $G_{\beta\gamma}$ subunits released by $G_{\alpha q}$ activation can activate PI3Kγ, another hypertrophic stimulus. Though the authors show that sildenafil decreased TAC-induced PI3Kα activation, PI3Kγ activity was not examined, and may be more relevant for cardiac hypertrophy[15] (**Fig. 1**).

Myocardial cGMP and PKG-1 should now be viewed as potential antihypertrophic molecules. To explore their potential, the relevant substrates of PKG-1 that cause regression of the hypertrophic program in cardiomyocytes need to be uncovered. There are many possibilities. For instance, several calcium regulatory proteins in or near the sarcoplasmic reticulum have been identified as PKG substrates, such as the L-type calcium channel, the ryanodine receptor calcium release channel, and troponin I (**Fig. 1**).

One especially intriguing possibility, the regulator of G-protein signaling RGS2, has emerged from studies of vascular smooth muscle cells. In these cells, PKG-1 mediates NO-induced relaxation. PKG-1α binds to, phosphorylates and activates RGS2, which disrupts activation of intracellular calcium signaling by turning off G_q-coupled signaling[16].

A similar role for RGS2 in cardiomyocytes would provide an explanation for the observed effects of sildenafil. RGS2 knockout mice are hypertensive[16], and testing whether these mice are also insensitive to protection against cardiac hypertrophy by sildenafil might prove revealing. Other molecules downstream of PKG-1 merit serious consideration for a role in cardiac cells, including PP1M and the IRAG protein, which mediates PKG-1β inhibitory actions on smooth muscle sarcoplasmic reticulum[17,18] (**Fig. 1**) . So do antihypertrophic genes known to be induced by PKG activation, like MAP kinase phosphatase-1 (ref. 19; **Fig. 1**)

It is not clear whether the effect of sildenafil on pressure overload hypertrophy in mice will extend to humans. Can sildenafil reduce the rate of cardiovascular events resulting from hypertrophy in hypertensive men taking the compound for erectile dysfunction? Will PDE5A inhibitors reduce hypertrophy in women? Will there be a role for PDE5A inhibitors in the treatment of the familial hypertrophic cardiomyopathies? These questions should be straightforward to test and deserve to be explored soon.

1. Takimoto, E. *et al.* (*Nat. Med.* **11**, 214–222 (2005).
2. Frey, N., Katus, H.A., Olson, E.N. & Hill, J.A. *Circulation* **109**, 1580–1589 (2004).
3. Olson, E.N. & Schneider, M.D. *Genes Dev.* **17**, 1937–1956 (2003).
4. Shah, A.M. & MacCarthy, P.A. *Pharmacol. Ther.* **86**, 49–86 (2000).
5. Massion, P.B., Feron, O., Dessy, C. & Balligand, J.L. *Circ. Res.* **93**, 388–398 (2003).
6. Lincoln, T.M. *Cyclic GMP: Biochemistry, Physiology and Pathophysioogy.* (R.G. Landes Company, Austin, Texas, 1994).
7. Feil, R., Lohmann, S.M., de Jonge, H., Walter, U. & Hofmann, F. *Circ. Res.* **93**, 907–916 (2003).
8. Wegener, J.W. *et al. Circ. Res.* **90**,18–20 (2002).
9. Fiedler, B. *et al. Proc. Natl. Acad. Sci. USA* **99**,11363–11368 (2002).
10. Kishimoto, I., Rossi, K. & Garbers, D.L. *Proc. Natl. Acad. Sci. USA* **98**, 2703–2706 (2001).
12. Force, T., Michael, A., Kilter, H. & Haq, S. *J. Card. Fail.* **8** Suppl 6, S351–S358 (2002).
12. Akhter, S.A. *et al. Science* **280**, 574–577 (1998).
13. Dorn, G.W. II & Brown, J.H. *Trends Cardiovasc. Med.* **9**, 26–34 (1999).
14. Wettschureck, N. *et al. Nat. Med.* **7**, 1236–1240 (2001).
15. Patrucco, E. *et al. Cell* **118**, 375–387 (2004).
16. Tang, K.M. *et al. Nat. Med.* **9**, 1506–1512 (2003).
17. Surks, H.K. *et al. Science* **286**,1583–1587 (1999).
18. Schlossmann, J. *et al. Nature* **404**,197–201 (2000).
19. Pilz, R.B. & Casteel, D.E. *Circ. Res.* **93**,1034–1046 (2003).

Stressed and depressed

Huda Akil

Knocking out a stress hormone receptor in the forebrain of mice generates symptoms of depression, which are alleviated by the antidepressant imipramine. The findings begin to unravel the intricate relationship between stress and depression.

Major depression is a severely debilitating illness that is a prototypical 'complex genetic disorder.' It arises from the equal interplay of vulnerability genes on the one hand and developmental and environmental factors on the other. The role of psychosocial stress (so-called 'life events') in triggering the early episodes of depression has long been appreciated, and abnormalities in the 'stress system' of at least a subset of depressed individuals have been documented[1–3].

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Over the last decade, our view of the role of stress in the etiology of depression has evolved. We have learned that stress is not simply a trigger that can be neatly separated from the disease process. Rather the neural network that encodes and evaluates a given event as stressful is the very brain circuit that underlies negative emotions and moods, and is dysregulated in severe depression.

More recently, key molecules expressed in specific anatomical components of this emotional circuitry have become the focus of attention, both as possible causative factors in depression and as potential targets for novel treatments of the illness[3,4].

A recent study by Boyle *et al.*[5] is the latest advance in this evolution. In the 11 January issue of the *Proceedings of the National Academy of Sciences*, the authors show in mice that a targeted disruption of the glucocorticoid receptor in the forebrain leads to behavioral and neuroendocrine changes that are parallel to human depression. Interestingly, these changes develop over the course of the animal's life and are reversible by the classical antidepressant imipramine, even though this is a monoaminergic drug not primarily targeted to the stress system. The direct use of drugs that block the stress system represents a new avenue for the treatment of types of depression that are resistant to classic antidepressants.

A major component of stress responsiveness in mammals is the limbic-hypothalamo-pituitary-adrenal (LHPA) axis which, when activated, leads to the synthesis and release of the corticosteroid hormones—cortisol in humans and corticosterone in rodents. A stressful event, be it physical or psychological, activates neural circuits involved in emotional responses, cognition and endocrine control (**Fig. 1a**,**b**). Relevant brain regions include the brainstem (the first relay station for many physiological stressors), the amygdala (which processes fear and anxiety responses), the hippocampus (which mediates learning and memory and encodes the salience, or importance of a stimulus, to the organism) and the frontal cortex (which has important cognitive and executive functions).

The stress-induced neural activation converges on the hypothalamus, triggering a cascade of signaling events (**Fig. 1a**,**b**). The adrenal corticosteroids are the final products of this cascade and mediate their actions through activation of two receptors—the mineralocorticoid receptor, which senses basal levels of corticosteroids, and the glucocorticoid receptor, which is sensitive to higher stress levels of steroids. These ligand-gated transacting factors have both immediate actions and long-term impact through altered expression of target genes. Stress hormones have broad biological effects throughout the body that are adaptive but can become damaging when chronically elevated. In the short term they change energy metabolism to provide the resources needed for coping. But in the long term, stress hormones can inhibit immune function, cause broad endocrine dysregulation, lead to apoptosis and accelerate the aging process[6].

Indeed, the high potency of stress hormones requires that the organism keep their levels under tight control, which is why the termination of the stress response is as important as its initiation. Nested negative feedback loops limit the stress response and the hippocampal glucocorticoid receptors are an important link in this process (**Fig. 1a**,**b**).

The specific role of brain glucocorticoid receptors in stress and mood disorders has been investigated in a number of animal models, yielding what might appear to be contradictory findings. A set of studies have suggested that high levels of the receptors in the brain can make the animal behave in a more anxious or depressive manner and that, conversely, blocking the receptors with an antagonist or knocking it down in brain can make the animal appear less anxious[7–11]. Yet, these studies seem to fly in the face of the notion put forward by Boyle *et al.*[5] that a low level of glucocorticoid receptors can produce a depression-like syndrome.

Both lines of evidence, however, make sense if one considers forebrain glucocorticoid receptors both as a sensor of stress and as a braking mechanism that limits the stress response once it has taken place (**Fig. 1a**,**b**).

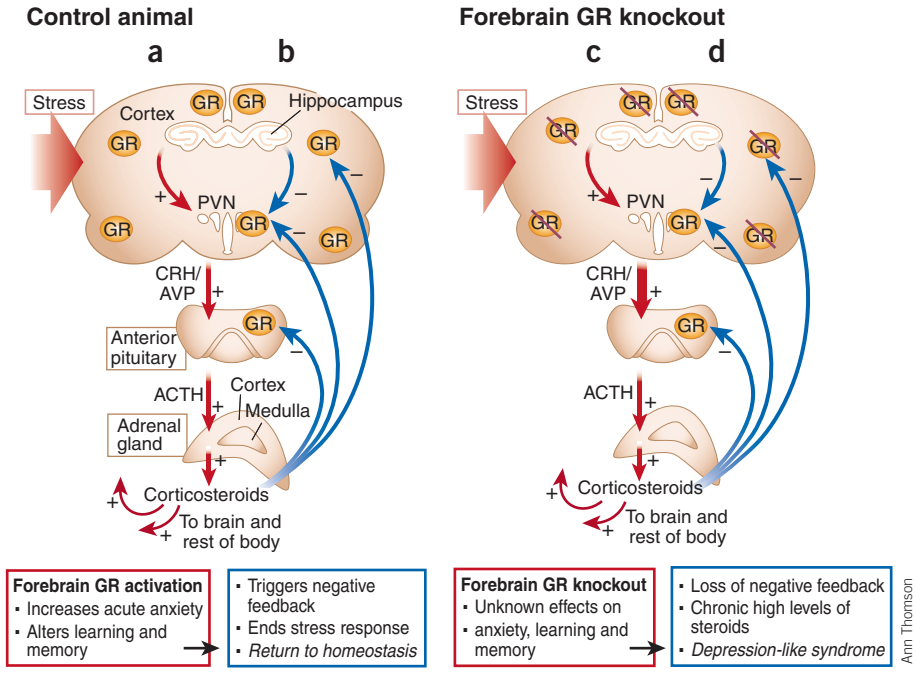

Figure 1 Stress and depression. (**a**) Activation of the limbic-hypothalamo-pituitary-adrenal (LHPA) axis by stress (in red). A stressful stimulus activates neural circuits that converge on a region of the hypothalamus, the paraventricular nucleus (PVN). Two neuropeptides, corticotropin releasing hormone (CRH), and arginine vasopression (AVP) are then released into the portal blood, targeting the anterior lobe of the pituitary gland. This leads to the increased release of adrenocorticotropic hormone (ACTH) into the general circulation—in turn triggering the synthesis and release of steroids from the cortex of the adrenal gland. These corticosteroids then diffuse freely throughout the body, including the brain, and are detected by the glucocorticoid receptor (GR). GR is in part a sensor of stress and its activation results in increased anxiety behavior and alterations in learning and memory. (**b**) Negative feedback inhibition (in blue). The increase in glucocorticoids also triggers a set of nested negative feedback mechanisms that limit further activation of the stress system. Thus, GR activation in the anterior pituitary, hypothalamus, hippocampus and frontal cortex is thought to limit the stress response. (**c**,**d**) Targeted disruption of GR. Boyle *et al* show that the specific loss of GR in the forebrain (but not in the hypothalamus or the pituitary) interferes with the negative feedback exerted by the hippocampus and the frontal cortex. As a result, there is increased activity of the PVN, increased circulating levels of ACTH and corticosteroids, with increasing impact on the brain. This results in a 'depression-like' syndrome and can be reversed by a tricyclic antidepressant.

If an animal is exposed to a stressful condition, it will adapt to become more cautious and take fewer risks, which would be measured as an increase in anxiety-like responses and can alter learning and memory strategies. High levels of glucocorticoid receptors in the forebrain would detect the elevation in glucocorticoids and promote this anxious-like behavior[8,11] and low levels of receptors would encourage increases in basal exploration[7,10]. Moreover, elevated levels of forebrain glucocorticoid receptors lead to increased environmental reactivity and emotional lability, mimicking some aspects of bipolar illness[11].

By contrast, selective removal of forebrain glucocorticoid receptors—especially if it occurs after the early period of development and so avoids generating dramatic differences in the wiring of the affective circuitry[5]—uncovers the substantial loss in negative feedback, leading to a sustained increase in circulating stress hormones. This increase can then cause sequelae that resemble the effects of depression (**Fig. 1c**,**d**).

The exact mechanisms whereby increased stress hormones lead to this pattern remain to be discovered, although Boyle *et al.*[5] suggest that part of the effect is mediated through changes in mineralocorticoid receptors. The forebrain-specific glucocorticoid receptor knockout mice resemble in many ways mice exposed to chronic unpredictable stress; such stress also raises resting levels of glucocorticoids, alters the ratio of glucocorticoid to mineralocorticoid receptors in the hippocampus, alters the expression of serotonin receptors and responds to chronic treatment by imipramine[12].

Discovering a direct link between specific molecules of the stress system and mood disorders has important implications for devising new approaches for treatment-resistant depression, or for accelerating the impact of classical antidepressants. Thus, a number of pharmaceutical firms are attempting to produce effective antagonists to corticotropin releasing hormone (CRH) to block stress both centrally and peripherally[4]. A recent study used blockade of glucocorticoids synthesis in the adrenal gland in humans as an adjunct to classical antidepressants to accelerate their actions[13]. Whereas these studies block the activation cascade, there are also some clinical efforts to block the glucocorticoid receptor itself, not because of its role as a mediator of negative feedback, but rather as a sensor of environmental stress that produces emotional instability and cognitive dysfunction. Thus, Belanoff *et al.*[14], in preliminary findings, showed that blockade of glucocorticoid receptors could ameliorate symptoms in patients with psychotic depression. More recently, Young *et al.*[15] showed that blockade of glucocorticoid receptors leads to improvements in cognition and mood in patients with bipolar disorder.

Regardless of the specific genes that lead to vulnerability to depression, it is likely that the disease process sooner or later engages the stress system. Stress in turn can contribute to the deteriorating course of the illness. Both overactivation and underactivation of the stress response are damaging to the organism, and effective treatment of depression requires the 'resetting' of the stress system. This can often be achieved through classical antidepressants, but sometimes may require direct intervention by altering specific molecular components of the stress system to restore its balance.

1. Holsboer, F. J. *Affect. Disord.* **62**,77–91 (2001).
2. Young, E.A., Haskett, R.F., Murphy-Weinberg, V., Watson, S.J. & Akil, H. *Arch. Gen. Psychiatry* **48**, 693–699 (1991).
3. Young, E.A., Lopez, J.F., Murphy-Weinberg, V., Watson, S.J. & Akil, H. *Arch. Gen. Psychiatry* **60**, 24–28 (2003).
4. Keck, M.E. & Holsboer, *F. Peptides* **22**, 835–844 (2001).
5. Boyle, M.P.,*et al. Proc. Natl. Acad. Sci.* USA **102**, 473–478 (2005).
6. McEwen, B.S. & Seeman T. Ann. NY Acad. Sci. **896**, 30–47 (1999).
7. Barden, N., Stec, I.S., Montkowski, A., Holsboer, F. & Reul, J.M. *Neuroendocrinology* **66**, 212–220 (1997).
8. Kabbaj, M., Devine, D.P., Savage, V.R. & Akil, H. *J. Neurosci.* **20**, 6983–6988 (2000).
9. Karanth, S., Linthorst, A.C., Stalla, G.K., Barden, N., Holsboer, F. & Reul, J.M. *Endocrinology* **138**, 3476–3485 (1997).
10. Tronche, F. *et al. Nat. Genet.* **23**, 99–103 (1999).
11. Wei, Q. *et al. Proc. Natl. Acad. Sci.* USA **101**, 11851–11856 (2004).
12. Lopez, J.F., Chalmers, D.T., Little, K.Y. & Watson, S.J. *Biol. Psychiatry* **43**, 547–573 (1998)
13. Jahn, H., Schick, M., Kiefer, F., Kellner, M., Yassouridis, A. & Wiedemann, K. *Arch. Gen. Psychiatry* **61**, 1235–1244 (2004).
14. Belanoff, J.K. *et al. Biol. Psychiatry* **52**, 386–392 (2002).
15. Young, A.H., Gallagher, P., Watson, S., Del-Estal, D., Owen, B.M. & Nicol Ferrier, I. *Neuropsychopharmacology* **29**, 1538–1545 (2004).

ACE sets up fertilization

Pierre Corvol

The ACE enzyme, a target of blood pressure medications, now gains a new function in mice. The enzyme cleaves proteins linked to the cell membrane by GPI linkages, an activity required for fertilization (pages 160–166).

Angiotensin I converting enzyme (ACE, encoded by *Ace*) acts as a peptidase that regulates enzymes responsible for maintaining blood pressure. But studies in mice have shown that ACE also has effects on fertility, as *Ace* knockout mice are infertile. In this issue, Kondoh *et al.*[1] discover why. They find that ACE cleaves GPI-linked proteins, some of which mediate the fusion of sperm with egg.

The renin-angiotensin system has a major role in blood pressure regulation and cardiovascular function. ACE, a key element of this system, converts the inactive decapeptide angiotensin I into the vasoactive peptide angiotensin II, and also inactivates the vasodilator peptide bradykinin. These actions of ACE are mediated by its zinc metallopeptidase activity. ACE inhibitors designed according to the knowledge of the ACE catalytic site are widely used in the treatment of hypertension, heart failure and renal insufficiency.

ACE is an ectoenzyme anchored to the plasma membrane with the bulk of its mass exposed at the cell surface. There are two ACE isoforms: a somatic form of around 150–180 kDa, which bears two catalytically active sites, and a smaller isoform (90–110 kDa) found in the testes, which contains a single active site[2]. Testicular ACE is found exclusively in male germinal cells after meiosis and is transcribed from the same gene as the somatic ACE by a potent testis-specific promoter[2].

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