The factors regulating susceptibility and severity of autoimmune diseases are poorly understood. That neuroendocrine factors are critical modulators in this regard is self-evident. For example, there are major gender differences in susceptibility with women at greater risk than men of, for example, rheumatoid arthritis (RA) and multiple sclerosis (MS). The hypothalamo-pituitary-adrenal (HPA) axis has rightly attracted a considerable amount of attention. Of particular interest has been the hypothesis that susceptibility to autoimmune disease may be related to an impaired responsiveness of the HPA axis; that is, an inability to mount an appropriate cortisol response with which to down-regulate the immune system might allow the immune system to rampage unchecked and attack self. This hypothesis links regulation of the release from the adrenal gland of the potent anti-inflammatory glucocorticoids to the disease process. Endogenous glucocorticoids are crucial for the regulation of the severity of the disease process. The hypothesis proposing a link between a hypo-responsive HPA axis and susceptibility to disease is compelling. However, evidence from a number of sources has suggested that this may not be the entire story and alterations in the activity of the HPA axis have not been consistently observed in patients with RA. This review will concentrate on recent findings concerning the HPA axis in determining susceptibility to, and in regulating the severity of, inflammatory processes in autoimmune disease. These studies have revealed that a single exposure to endotoxin can confer protection to subsequent development of inflammation in an arthritis model in both neonatal and adult rats. Behavioural differences within a single population of rats are associated with differences in the plasma corticosterone responses to stress. However, relative hyporesponsiveness is not reflected by an increase in the severity of inflammation. In humans with RA the dexamethasone–corticotrophin-releasing factor (CRF) test has revealed two distinct sub-populations of patients. Studies in patients with MS have shown that this is not related to depression but rather to the severity of the disease. A better understanding of these complex neuroendocrine interactions may lead to novel clinical interventions. Experimental Physiology (2002) 87.5, 519–525.
hypophysial portal blood, increased release of ACTH and cortisol into the blood, increased POMC mRNA in the anterior pituitary and increased CRF and AVP mRNAs in the PVN (Fig. 1; Buckingham et al. 1997).

It has long been assumed that the HPA axis represents a homeostatic mechanism: following termination of a stress, homeostatic mechanisms would return hormonal levels back to baseline within an hour or so. It is generally accepted that the HPA axis would not be permanently altered by a single acute stress. However, recent studies by a number of groups have questioned this assumption. Instead, it has been suggested that exposure to stress results in long-term alterations to the HPA axis that are manifest days or weeks after the acute stress episode. For example, it has been shown that exposure to the same stressor days or even weeks after initial exposure can alter responsiveness to a variety of stressors including interleukin-1, footshock, social defeat, immobilisation, and others (Schmidt et al. 1995; Tilders et al. 1999; Marti et al. 2001). The stress history of individuals is clearly important and confirms the need for contemporary controls in both clinical studies and pre-clinical studies using animal models.

The HPA axis and autoimmune disease and animal models

The HPA axis is of fundamental importance in regulating autoimmune disease. Treatment of RA patients with metyrapone (which blocks the synthesis of endogenous glucocorticoids in the adrenal cortex), results in a flare of disease activity (Panayi, 1992). In patients with Cushing’s syndrome, adrenalectomy has resulted in the development of RA and autoimmune thyroid disease (Takasu et al. 1990; Yakushiji et al. 1995). In pre-clinical studies in rodents, adrenalectomy results in an earlier onset of disease and an increase in severity of adjuvant-induced arthritis (AA) and experimental allergic encephalomyelitis (EAE) (Mason et al. 1990; Harbuz et al. 1993b). If left untreated, the animals will die, whilst steroid replacement can prevent this outcome. The normally resistant Fischer strain of rat can develop arthritis if treated with a corticosteroid receptor antagonist and the severity of inflammation can be reduced in the susceptible Lewis strain by corticosterone treatment (Sternberg et al. 1989b). Together these data confirm the crucial importance of a functioning HPA axis in autoimmune disease in humans and in disease models in animals.

HPA axis regulation and inflammation: increased role for AVP

AA is a T cell-mediated disease model involving chronic activation of the immune system. This chronic inflammatory stress is associated with an increase in plasma ACTH and corticosterone and a loss in the normal diurnal rhythm. POMC mRNA levels in the anterior pituitary are also elevated. Despite the increased activity of the pituitary-adrenal axis there is a paradoxical decrease in CRF mRNA and CRF peptide release into the hypophysial portal blood. This decrease in CRF activity is first apparent at the time of onset of inflammation and reaches a nadir at the time of maximum severity (Harbuz et al. 1992). This paradoxical decrease in CRF activity has been reported in the Piebald-Viral-Glaxo (PVG) (Harbuz et al. 1992), the Lewis (Brady et al. 1994) and the Wistar (Chover-Gonzalez et al. 2000) strains of rat, suggesting that it is a common mechanism in response to inflammation in AA.

The drive to the axis may come from AVP as AVP concentrations in the portal blood and AVP mRNA in the parvocellular cells of the PVN are increased in AA. These data suggest that in the presence of permissive levels of CRF, AVP is able to take over as the major stimulator of the HPA axis in rats with AA (Harbuz et al. 1992; Chowdrey et al. 1995). These data support findings from repeated stress studies that have proposed an increased role for AVP in chronic stress situations. The role of AVP in RA, MS and other autoimmune diseases has recently been reviewed (Chikanza et al. 2000).

This alteration in hypothalamic regulation with a decrease in CRF activity appears to be a common feature in a wide variety of immune-mediated disease models. A decrease in CRF mRNA is associated with peak clinical symptoms in EAE. Following recovery, CRF mRNA levels return to normal (Harbuz et al. 1993a). Increased HPA axis activity has been reported in patients with MS under basal conditions and in response to challenge tests (Michelson et al. 1994; Reder et al. 1994; Grasser et al. 1996; Wei & Lightman, 1997; Fassbender et al. 1998; Then Bergh et al. 2001). Post-mortem analysis of brains from MS patients has revealed an increase in the number of CRF-positive, AVP-positive neurons in the PVN with no change in the CRF-positive, AVP-negative population (Erkut et al. 1995; Purba et al. 1995). These data suggest an increased role for AVP in MS. Further support for an increased role for AVP is provided by the observation that MS patients have an increased HPA axis response to AVP compared with controls (Michelson et al. 1994).

Eosinophilia-myalgia syndrome (EMS) is an immune-mediated disease that reached epidemic proportions on the West Coast of the USA in the late 1980s. Investigation identified the source of the epidemic to a batch of impure l-tryptophan (L-Trp) derived from genetically modified bacteria. The L-Trp found its way into a number of health food preparations as a diet supplement. In order to confirm the responsible agent, Lewis rats were treated with comparable doses (w/w) of the impure L-Trp to those ingested by humans. These animals developed similar pathological features of human EMS. The appearance of symptoms of EMS in the rat model was found to be associated with a decrease in CRF mRNA in the PVN (Brady et al. 1994).

Altered hypothalamic regulation is not solely a feature of rat disease models. The MRL strain of mouse has been used as a model of the autoimmune disease systemic lupus erythematosus. With increased age these mice show increased symptoms of lupus, which in turn are associated
with a corresponding decrease in CRF mRNA (Shanks et al. 1999). The protozoan *Leishmania donovani* causes an immune-mediated parasitic liver infection prevalent in the tropics and some Mediterranean areas. Following inoculation with the parasite into a susceptible mouse strain there is a decrease in CRF mRNA associated with infection (Harbuz et al. 1995).

These data suggest that an alteration in the hypothalamic control of the HPA axis may be part of the adaptive response to chronic immune-mediated disease/inflammatory stressors. This change can be seen in a number of different disease models in different rodent species and may also occur in humans. From the available evidence it would appear that AVP takes over as the major stimulator of the HPA axis while CRF provides a secondary permissive role in chronic inflammatory stress.

**Implications for the stress response**

The implications of these changes in hypothalamic regulation on the ability to respond to stress are profound. This is, perhaps, not surprising as CRF is considered to be the major stimulator of the acute stress response. AA rats are unable to respond to the predominantly psychological stressors of restraint and noise or to the physical stress of i.p. injection of hypertonic saline (Harbuz et al. 1993b; Aguilera et al. 1997; Windle et al. 2001). This inability to respond to acute stress appears to be related to the alteration in pulsatile corticosterone release patterns seen in AA rats (Windle et al. 2001). Patients with RA have also been reported to be unable to respond to stress. Chikanza and colleagues (1992) reported that there was no increase in plasma cortisol in RA patients following joint replacement surgery whereas in patients with osteoarthritis an increase

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**Figure 1**

The hypothalamo-pituitary-adrenal axis. CRF is synthesised in the parvocellular cells of the paraventricular nucleus (PVN) which is located within the hypothalamus. Activation of the axis results in the synthesis and release of corticosteroids from the adrenal cortex (cortisol in man, corticosterone in rodents). See text for further details.
was observed. They also noted a normal response to the CRF stimulation test in RA patients indicating the presence of intact pituitary and adrenal responses and further supporting the case for a hypothalamic defect associated with inflammatory disease. However, in a more recent study, no such hyporesponsiveness to stress was found (Eijsbouts et al. 1998), suggesting that further investigation is required in humans with RA.

An inability to mount an HPA axis response to either acute or chronic immune stimulation is potentially life threatening. However, alterations in susceptibility to infection are not a feature in animal disease models or in patients with autoimmune disease. Studies have revealed an intact HPA response at all levels of the axis to acute injection with lipopolysaccharide (LPS) (Harbuz et al. 1999). These data show that although the ability to respond to acute stress is impaired in rats with AA, the ability to respond to acute immune stimulation is intact, presumably reflecting the potentially life-threatening nature of the latter challenge. These data suggest activation of alternative pathway(s) to these stimuli and provide a further example of differential control of the response to acute stress and acute immune stimulation.

**Does a poor stress response increase susceptibility to immune-mediated disease?**

**a. Animal studies.** In the late 1980s it was proposed that susceptibility to autoimmune disease resides in an inability to mount an appropriate response to stress. This hypothesis evolved from a comparison of the susceptible Lewis rat strain with the Fischer strain (resistant to streptococcal cell wall-induced arthritis) and the PVG strain of rat (resistant to EAE) (Sternberg et al. 1989a,b; Mason, 1991). The Lewis strain is hyporesponsive to a variety of acute stressors. In contrast, the Fischer and PVG strains are relatively hyperresponsive. Although compelling, this hypothesis has recently been questioned for a number of reasons. First, despite a robust response to stress the PVG strain is susceptible to AA suggesting that the hypothesis does not hold true for all disease models (Harbuz et al. 1993b). Second, a number of laboratories have failed to replicate the difference in stress response between the Lewis and the Fischer strains (Dhabhar et al. 1993; Rivest & Rivier, 1994; Grotta et al. 1997). Third, comparison of HPA axis responsiveness in different strains of rat did not predict disease susceptibility (Steffe et al. 1999). Fourth, the use of different strains has an inherent disadvantage in that by definition they are genetically different. Although their HPA axis responses to stress may differ, it is likely that there are other major differences in neuroendocrine and immune factors that contribute to susceptibility/resistance. To rigorously test the hypothesis that a difference in HPA axis responsivity can alter disease susceptibility or severity it is pertinent to do so in a single population of the same strain. The use of an outbred strain guarantees genotypic heterogeneity and is likely to be closer to the human condition than comparison of inbred strains.

**b. Behavioural differences in animals.** Behavioural tests can be used to divide a single population into subpopulations. The open field test is an established measure of anxiety and can easily be used to separate animals with high and low anxiety from within a population. Significant differences in stress responsiveness were revealed between the low and high anxiety groups. However, despite these differences no difference in susceptibility to, or severity of, AA were revealed (Chover-Gonzalez et al. 1998).

The ‘learned helplessness’ (LH) paradigm has been used as an animal model of depression. Initially, all the animals are exposed to an uncontrollable footshock. When exposed to a subsequent escapable footshock in a shuttle box one group of rats make no attempt to escape the shock. These are termed learned helpless (LH+), that is, they exhibit depression-like symptoms. In contrast, another subpopulation very rapidly learn to escape and these animals receive few footshocks (LH−). There is a striking difference in corticosterone responses to acute stress between the LH+ (i.e. depression-like) group and the LH− group with a further significant increase in plasma corticosterone levels in the LH− rats compared to the increase in the LH+ rats. The hypothesis would predict that the LH− rats should be relatively protected by the larger increase in plasma corticosterone. However, and contrary to the hypothesis, the LH− rats develop inflammation sooner and with increased severity when compared to the LH+ animals, suggesting that HPA axis responsiveness is not a good predictor of disease activity (Chover-Gonzalez et al. 2000). In another behavioural study it was demonstrated that the latency of an animal to attack an intruder was inversely correlated with the disease score in EAE. Animals that did not attack an intruder were more resistant to the disease (Kavelaars et al. 1999). Together, these data suggest a link between behavioural characteristics of an individual and subsequent disease severity. The precise nature of these interactions remains to be determined.

**c. Human studies.** There is evidence to support a hyporesponsive HPA axis both in animal models and in autoimmune disease in man once the disease is established. However, in man it is rather more difficult to determine if this hyporesponsiveness was present prior to, and is therefore contributory to, the onset of disease. A pertinent study was carried out in patients with African sleeping sickness. This is a potentially lethal parasitic disease caused by the protozoan Trypanosoma brucei that if left untreated is eventually fatal. It has been shown that individuals with sleeping sickness are unable to mount a cortisol response to challenge with either ACTH or CRF suggesting that the HPA axis is altered (Reincke et al. 1994). However, following anti-parasitic treatment and recovery ACTH and cortisol responses to CRF are normal, which suggests that the defective HPA axis responsiveness was as a consequence of the disease and not inherent to the individual prior to infection. It is therefore unlikely that HPA axis responsiveness is a good predictor of susceptibility to autoimmune disease.
Acute immune challenge and alterations in susceptibility

The question of whether acute immune activation might influence the susceptibility to disease is of interest. LPS injected in neonatal rats confers resistance to AA when these animals are injected with adjuvant in adulthood (Shanks et al. 2000). These observations are in keeping with the well-established effects of neonatal treatments in rodents affecting HPA axis and behavioural activities in adulthood (Francis et al. 1999). Recently, it has been shown that exposure of adult rats to LPS can confer resistance to AA when the adjuvant is injected a number of weeks later (Harbuz et al. 2002). This does not appear to be an adaptive stress-related effect as exposure of rats to footshock has no effect on the development of AA under these conditions.

Is the HPA axis altered in patients with autoimmune disease?

The precise role of the HPA axis in autoimmune disease remains obscure. Concerning RA, the evidence in the literature suggests that HPA axis activity is not significantly different from normal individuals (Harbuz & Jessop, 1999). However, the inability of the axis to respond to the development of inflammation and suppress immune activation may be evidence for an abnormality. There are differences in diurnal profiles of cortisol in autoimmune disease and of corticosterone in disease models. One way to assess the HPA axis is through a challenge to the system. In patients with MS, the results of injecting the hypothalamic releasing factors CRF and AVP have indicated that AVP may be more important than CRF in maintaining HPA axis activity (Michelson et al. 1994). In patients with MS, the cortisol response to CRF differs depending on the disease status of the individual (Wei & Lightman, 1997). Similarly, in patients with RA, the cortisol response to challenge with releasing hormones is impaired in individuals with established disease (Gudbjornsson et al. 1996), but not in newly diagnosed untreated RA patients (Templ et al. 1996).

The dexamethasone–CRF test has been used extensively to assess HPA axis dysfunction in psychiatric disorders (Holsboer et al. 1985; Zobel et al. 2001). Individuals with depression have an altered feedback regulatory system such that dexamethasone suppression of endogenous HPA axis activity is incomplete and cortisol levels remain elevated. In response to infusion of CRF, these patients are able to escape dexamethasone suppression and elevated cortisol is observed. This may be the result of lower glucocorticoid receptor sensitivity in depressed patients reflecting feedback resistance (Modell et al. 1997). The involvement of corticosteroid receptors is supported by evidence of decreased type I and type II receptor mRNAs in suicide victims with a history of depression (Lopez et al. 1998). Following successful treatment, dexamethasone suppression prevents the response to CRF. Continued non-suppression is associated with early relapse (Zobel et al. 2001). These data provide evidence that alterations in HPA axis activity are not necessarily inherent to an individual but may be a consequence of the disorder itself.

Studies in patients with autoimmune disease demonstrated abnormalities in dexamethasone suppression. For example, approximately 50% of MS patients did not suppress cortisol after dexamethasone administration (Reder et al. 1994). This is similar to the finding in patients with major depression and greater than that found in the normal control population. Depression scores between the suppressors and the non-suppressors were not different suggesting this was not a major contributory factor. Combining the dexamethasone suppression with a CRF challenge revealed a heterogeneous response with 15 out of 19 patients responding to CRF with increased cortisol, and six of these mounting an exaggerated response. The remaining four subjects did not respond (Grasser et al. 1996). Subsequent studies have confirmed that MS patients are able to escape from dexamethasone suppression following challenge with CRF, suggesting a hyperactivity of the HPA axis that is significantly correlated to disease activity (Fassbender et al. 1998; Kumpfel et al. 1999; Then Bergh et al. 1999). However, although one study related this escape to higher depression and anxiety scores in MS patients compared to controls (Fassbender et al. 1998), others have not found any evidence to relate the cortisol response to CRF with increased incidence of depression (Kumpfel et al. 1999; Then Bergh et al. 1999). The cortisol response to the dexamethasone–CRF test has recently been investigated in patients with RA (M. S. Harbuz, E. Korendowych, D. S. Jessop, A. Crown, S. L. Lightman & J. Kirwa, manuscript in preparation). Three out of seven patients escaped the dexamethasone-induced suppression of HPA axis activity while four remained suppressed. No correlation between disease activity or severity and non-suppression was noted in this study suggesting the existence of sub-populations of patients with RA. The impact of these differences in HPA axis responsivity on disease progression in RA remains to be elucidated.

Summary

The cause of autoimmune disease remains obscure. The HPA axis is crucial in regulating the severity of disease. In the absence of corticosteroids the immune system is unrestricted and its activation by either acute or chronic immune challenge is likely to be fatal. The question of susceptibility and/or resistance may be relative and could be influenced by a variety of factors including behavioural responses, exposure to pathogens in early or later life and the stress history of the individual. The use of challenge tests such as the dexamethasone suppression test and the dexamethasone–CRF test suggest that there are sub-populations of patients with RA and MS. These data suggest that some patients have an altered glucocorticoid feedback whereas in others feedback remains normal. The relative proportion of these sub-populations in earlier studies may underlie the conflicting reports of HPA axis involvement of autoimmune disease. It has been suggested that responsiveness is related to severity of disease. Whether
these differences in response to the tests reflect the subsequent progression of disease in individuals remains to be determined. Accumulating evidence suggests that questions of susceptibility and resistance are not simply due to a hyporesponsive HPA axis. Rather, there is a more complex interaction between the HPA axis, the immune system and disease progression. Understanding these interactions may lead to novel treatment regimes targeting specific components of these systems.


