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REVIEW ARTICLE

The Neuroendocrinology of Stress: A Never Ending Story

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Evolutionary success depends on our ability to adapt to changing circumstances. The neuroendocrine response to stress is an excellent example of a plastic system that responds to threats to homeostasis and alters its output to meet current and expected future demands. At the level of the hypothalamus, the corticotroph secretagogues corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) respond rapidly to an acute stressor but, following chronic stress, they adapt with a reduction of CRH but a major increase in AVP. The release of CRH and AVP activates pro-opiomelanocortin in anterior pituitary corticotroph cells and the release of adrenocorticotrophic hormone into peripheral blood from where it targets receptors in the adrenal cortex to release glucocorticoid hormones. These hormones (i.e. corticosterone in the rat and cortisol in man) are released in a pulsatile ultradian pattern which defines the normal circadian rhythm. The frequency of the pulses is increased under states of chronic stress, and in rats with genetically determined hyper-responsiveness of the hypothalamic-pituitary-adrenal axis. Interestingly, neonatal influences can also programme alterations in ultradian rhythmicity, implicating epigenetic factors in its regulation. At the level of tissue receptors, the alteration in pattern of glucocorticoid ultradian rhythm has differential effects on mineralocorticoid receptor and glucocorticoid receptor (GR) binding to DNA and offers a mechanism for tissue specific responses to altered glucocorticoid dynamics. The effects of neonatal experience are not only seen at the level of CRH and GR regulation, but also are evident in behavioural responses to stress and in the responsiveness of brain stem serotonergic pathways, as measured by tryptophan hydroxylase mRNA in the brain stem.

Key words: neurotrophin, brain-derived neurotrophic factor, adrenalectomy, glucocorticoids, rat brain neurotrophin, brain-derived neurotrophic factor, adrenalectomy, glucocorticoids, rat brain.

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Why should anyone be interested in stress? Besides the purely personal fascination of trying to understand how a phenomenon as pervasive as stress can acutely alter cognition, memory, cardiovascular activity and glucose, protein and fat metabolism, it is clearly an important aspect of global health to understand how chronic stressful stimuli can lead to increased morbidity and mortality from depression, cardiovascular disease and metabolic disorders.

Since the neuroendocrine system is such a good candidate for mediator of many of the diseases linked to chronic stress, the first areas that need to be addressed are the central mechanisms underlying the neuroendocrine stress response and how they might change when acute stressors become repeated or chronic.

Acute and chronic stress

The parvocellular cells of the paraventricular nucleus of the hypothalamus are the major information junction for the neuroendocrine response to stressors. Inputs from both limbic circuits and brain stem centres ensure these cells can be activated by both psychological and physical stressors (1), with a rapid increase in c-fos (2) followed by increased activation of many genes (3, 4), of which corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) are the most important for neuroendocrine activation of the hypothalamic-pituitary-adrenal (HPA) axis. The peptide products, CRH and AVP, are secreted into the hypophyseal portal blood and act in a synergistic fashion on the corticotroph cells of the anterior pituitary to increase pro-opiomelanocortin (POMC) transcription,

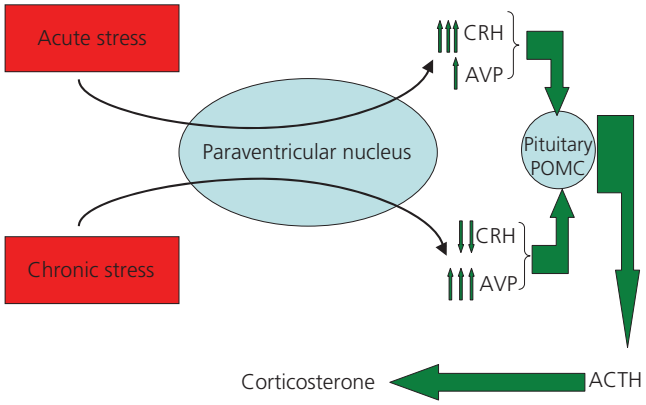

Fig. 1. The hypothalamic-pituitary-adrenal axis response to acute and chronic stressors. ACTH, adrenocortocotrophin; AVP, arginine vasopressin; CRH, corticotrophin-releasing hormone; POMC, pro-opiomelanocortin.

adrenocorticotrophin (ACTH) release into the circulation and consequent glucocorticoid hormone secretion from the adrenal cortex (Fig. 1).

In response to chronic stress, however, we find a markedly different hypothalamic response. In mycobacterial induced adjuvant arthritis (a very effective activator of HPA activity), although we find a chronic increase in POMC mRNA in the anterior pituitary, and both plasma corticosterone and adrenal weight (a well validated measure of chronic HPA activity), we actually find a paradoxical reduction both in paraventricular (PVN) CRH mRNA and in portal blood concentrations of CRH 41 (5). Interestingly, the decline in CRH mRNA and peptide were paralleled by a marked increase in AVP mRNA and portal blood AVP concentration (6), suggesting that, in this model of chronic stress, the central activation of HPA activity has been taken over by a predominant AVP rather than CRH drive.

We investigated this in more detail in a model of repeated daily acute stress (7). This confirmed that the ratio of AVP : CRH mRNA in the PVN increased with each episode of stress (8) and also that, eventually, there emerged an isolated AVP but not CRH response to subsequent restraints (7). This was in good keeping with the work from the laboratory of Aguilera and colleagues (9) on the HPA hormonal adaptation to repeated homotypic stressors (Fig. 1).

Stressors and hormone secretion

How do the changes in hypothalamic CRH and AVP transcripts manifest themselves in terms of physiological function? To answer this, we first need to know how plasma glucocorticoids are regulated under basal conditions and then what happens under conditions of acute and chronic stress. Although this sounds reasonably straightforward, taking multiple samples from conscious freely-moving unrestricted rodents is quite challenging, particularly since any contact with the animal will result in activation of the HPA axis.

Colin Ingram, Richard Windle and I therefore adapted the automated blood sampling system devised by Iain Robinson at Mill Hill (10) for use in our HPA studies. Using this equipment, we were able to demonstrate that there is not only a clear circadian rhythm of glucocorticoid secretion, but also that this circadian rhythm is actually made up from changes in the regulation of a much faster underlying ultradian rhythm consisting of pulses of corticosterone release that occur approximately one hourly (11).

This hourly pulsatile secretion is characterised by alternating episodes of HPA activation and inhibition. Interestingly, after each pulse of secretion, there is actually a period of inhibition (in effect a refractory period) during which the HPA is no longer sensitive to activation by a mild stressor (11). This provides an explanation for the well-known variability in the HPA responsiveness to stressors, as the magnitude of the response will depend on the stage of the endogenous secretory cycle at which the animal is exposed to the stressor. Furthermore, when the frequency of pulsatility increases, as it does in chronic stress (*vide infra*), there is an increased proportion of time when the animals are in a stress nonresponsive state, giving rise to apparent stress hyporesponsiveness (12).

Of course, we now need to know what happens to the pattern of HPA activity in animals under conditions of chronic stress with a raised hypothalamic AVP and reduced CRH drive to the pituitary. These animals do indeed have a markedly altered regulation both of their circadian and ultradian rhythms: the circadian rhythm is flattened or lost and the frequency of corticosterone pulses almost doubles (12).

Genetic and neonatal influences

There is great heterogeneity of stress responsiveness. The question we wanted to ask was whether this was related to the regulation of the underlying ultradian rhythm and, if so, how it might be related to genetic or epigenetic influences (Fig. 2).

First we looked at the HPA axis in two strains of histocompatible rat that Esther Sternberg and colleagues had shown to have markedly different HPA responses to stress (13). The Lewis rat had a completely normal circadian and ultradian rhythm, and also showed a normal post-pulse refractory period. The stress hyper-responsive Fisher rat, on the other hand, had a markedly abnormal rhythm with high amplitude corticosterone pulses occurring throughout the 24 h and, quite remarkably, showed no post-pulse inhibition (14). We have therefore been able to demonstrate that the genetic difference between these two very similar strains of rat resulted in marked abnormalities in the regulation of their pulsatile corticosterone secretion and, consistent with this, in their stress responsiveness.

The second major influence that we wanted to investigate was the epigenetic effect of neonatal programming on adult HPA ultradian rhythmicity. As long ago as 1967 in a seminal paper in *Science*, Levine had demonstrated that the HPA axis could be programmed by early life events (15). Using a model developed by Nola Shanks in Michael Meaney's laboratory (16), in which animals exposed to endotoxin during the first week of life become more stress responsive as adults, we investigated whether this change in HPA reactivity might be related to a change in the organisation of HPA pulsatility. We found that neonatal endotoxin resulted in both an increased frequency of pulses and increased corticosterone pulse

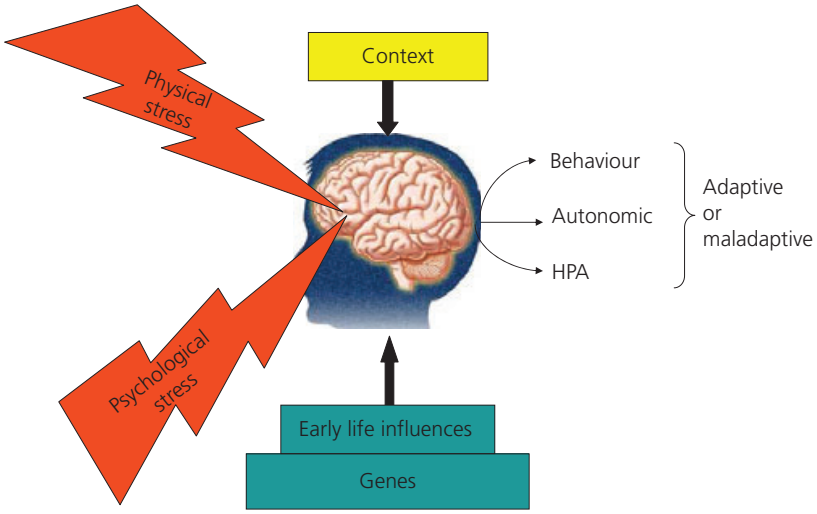

Fig. 2. Physiological and pathological responses to stress. The resilience or vulnerability of any one individual to stressful situations in adulthood will depend upon that person's genetic inherence and early life experiences.

amplitude (17), confirming that this neonatal stimulus had exerted long-term programming effects on the mechanisms generating basal pulsatile HPA activity in adult animals. We have now gone on to show that there are also organisational effects of the gonadal steroids that are normally secreted neonatally. Indeed, the presence or absence of circulating androgens perinatally can programme the activity of the HPA axis throughout the rest of the life of the animal (18, 19).

One of the things that fascinated us about the neonatal programming data was the fact that there were clear modifications of behaviour, as well as neuroendocrine hormone secretion. Thus, the animals that had been given neonatal lipopolysaccharide, showed much longer lasting activity responses to noise stress in adult life. We wanted to investigate this further and test the hypothesis that these altered responses might be related to a change in the regulation of serotonergic activity within the dorsal raphe nucleus. To test this, Chris Lowry and I collaborated with Paul Plotsky who used his maternal separation paradigm in which rats were exposed to either short (15 min) or long (180 min) periods of maternal deprivation during a critical period of development. Then, as adults, these neonatally-treated animals were exposed to the powerful psychosocial stressor of social defeat. Not only did we find that the different paradigms of maternal deprivation resulted in different behaviours, with the 180 min neonatal deprivation group showing more passive-submissive behaviour and less proactive coping behaviour (20), but also we were able to show that these behavioural effects were associated with marked differences in dorsal raphe tryptophan hydroxylase (TPH2) mRNA (21). Of particular interest was the fact that 15- and 180-min maternally-deprived rats that had undergone social defeat had much greater differences in TPH2 mRNA than unstressed maternally-deprived controls. This was a clear demonstration that the serotonergic differences following maternal separation were context specific and could be brought out by psychosocial stress in adult life. This, of course, has important implications for the effect of early life events on behavioural adaptation and on the susceptibility to affective and other stress-related disorders in adult life in man (Fig. 2).

Specificity of glucocorticoid signalling

The pulsatile nature of the glucocorticoid signal in the plasma provides scope for a digital, in addition to analogue, signal for tissue glucocorticoid receptors. To test whether tissues can detect different patterns of pulsatility, we need to demonstrate that rapid responses to individual glucocorticoid pulses can occur and define a mechanism through which different pulse frequencies could impart different information (Fig. 3).

We have approached this question by developing a model in which we can reproduce different pulse sizes and frequencies at will. This model, which was developed by a graduate student Crispin Wiles, consists of an adrenalectomised rat with an indwelling venous cannula connected to an automated infusion system. Using this system, Becky Conway-Campbell has been able to demonstrate an extremely rapid translocation of both the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) to the nucleus with subsequent DNA binding. A remarkable novel finding was that GR, unlike MR, then rapidly dissociates from the DNA (22). This may well be related to the lower affinity of corticosterone for GR than for MR (23). Using subcellular fractionation and western blotting, we found that GR was lost from the nucleus within the time course of a single 1-h interpulse interval, whereas MR remains. Changes in pulse frequency will therefore have differential effects on MR and GR binding and also probably on MR and GR homodimer and heterodimer formation. Similarly, the prolonged increase in plasma glucocorticoids in response to an acute stressor will result in a different pattern of GR and MR binding to DNA. Furthermore, the presence of different transcription factors and kinases, etc., in cells of different tissues will provide scope for multiple cell-specific responses to different digital signals.

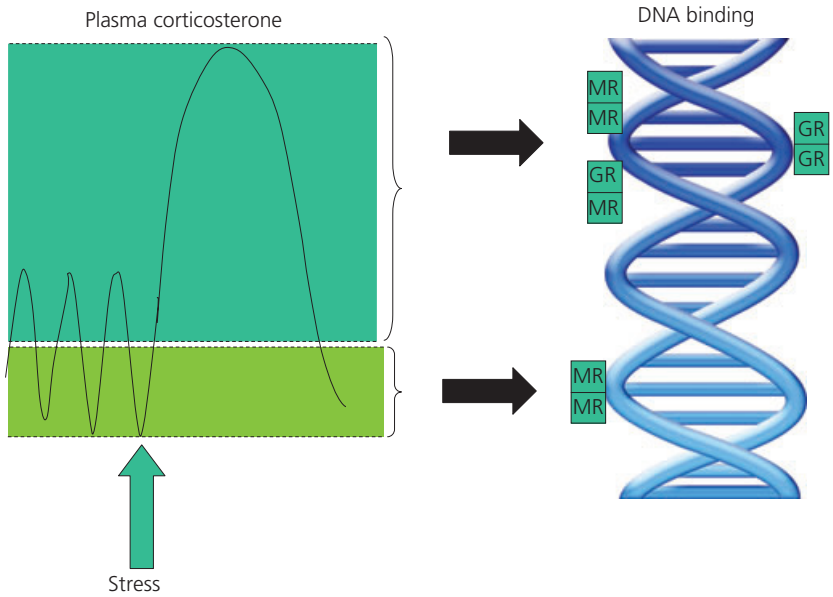

Fig. 3. The effect of glucocorticoid pulses and an acute stressor on glucocorticoid responsive genes. Note that, at nadir levels of corticosterone, there is only mineralocorticoid receptor (MR) binding to DNA but that, at peak and stress levels, there is both glucocorticoid receptor and MR binding.

Another area that we now need to approach to understand tissue selective transcription responses is the regulation of chromatin accessibility as a rate-limiting step, which will either make genes available or unavailable for GR binding and the subsequent transcriptional response. Work from the laboratory of Gordon Hager certainly suggests that preclusion of GR binding by a closed chromatin confirmation may provide a target for drugs that could change chromatin structure and GR responsivity. Their demonstration that, in different cell types, there are different chromatin profiles in the vicinity of GR binding sites (24) provides an exciting way ahead for our understanding of tissue specific GR responses.

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