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Modulation of Stress Responses: How we cope with excess glucocorticoids*

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We are admirably equipped to respond to survive the acute slings and arrows of outrageous fortune. With acute challenges of sufficient magnitude, activity of the hypothalamo-pituitary-adrenal (HPA) axis is stimulated and the protean adrenocortical hormones, glucocorticoids, are secreted to do their job throughout all cell types in the body, most of which contain the appropriate receptors. The consequent actions of the glucocorticoids, together with the rapidly acting adrenomedullary catecholamines, alter metabolic, cardiovascular, immune and central neural responses to the ongoing condition so that we can effectively sustain life at the moment. Circulating glucose is increased with an abundant supply for the potential need for fighting or fleeing, as is arterial blood pressure; the immune system is prevented from over-responding to acute bacterial threats; contextual brain-generated behaviors are enhanced by both increased attention and drive (13). Thus, the glucocorticoids engage many of the body systems for optimal responses to acute challenge. These responses enable and sustain life.

However, many of life's challenges cannot be escaped, or otherwise be behaviorally modified, this then entails recruitment of the brain chronic stress response network, with potential persistent elevations in glucocorticoid secretion. It seems abundantly clear that mechanisms have evolved to reduce activity in the brain chronic response network so that individuals undergoing inescapable challenge do not become debilitated, as occurs by definition with individuals with Cushing's syndrome, who have unregulated glucocorticoid exposure. High glucocorticoid concentrations that persist, may rapidly curtail life through marked shifts in energy stores from the periphery to the center of the body, hypertension, muzzling the immune system and carving the brain into an alert and anxious, but insensible mess of responsiveness that no longer can use learning and memories appropriately. Thus, persistently elevated glucocorticoid concentrations are far too much of a once good thing (12).

Given this down-side of chronic exposure to persistently high glucocorticoids, it is not surprising that mechanisms have been selected for that damp their secretion in chronically stressed individuals. These mechanisms have been explored in many organisms, primarily rodents, exposed repeatedly or chronically to challenge. It turns out, again unsurprisingly, that there appears to be a host of mechanisms that allow strong damping of the HPA responses. However, with persistent life-threatening stimuli that may be unanticipated, it is also of key importance to maintain full responsiveness of the system, even under conditions of chronic challenge. How does this happen?

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The model that has been most examined is that of the damping of subsequent HPA responses to repeated stimuli; this is termed “habituation” to (homotypic) stressors. Although there is marked diminution of the HPA response to repeated restraint, low intensity foot-shock and other stimuli, there is always, at least a slight response that can be detected. Nonetheless, with repetition the HPA response diminishes markedly, thus limiting the overall exposure to glucocorticoids experienced by the animal during repeated bouts of the same stimulus. Despite the habituation that frequently occurs to a stimulus that may be distressing, or boring but not life-threatening, the HPA system maintains, or even augments its responsivity to new (heterotypic) stimuli.

The most frequently studied homotypic stimulus, repeated restraint, has begun to yield its secrets about how habituation of the HPA response occurs. Comparison of the responses of repeatedly restrained rats to those experiencing a first exposure to the stimulus shows that in the site of the motor output of the HPA axis, the hypothalamic paraventricular nuclei (PVN), all indices of activity are diminished after repetition. Corticotropin-releasing factor (CRF) and vasopressin (AVP) synthesis is decreased as is consequent ACTH and corticosterone secretion (6). This is reflected by the finding that a measure of activation of the CRF neurons, c-Fos staining, shows a reduced number of excited cells, and that the responsive CRF neurons are more ventrally located in the nuclei (20). Thus, the final hypothalamic motor output is both damped and altered with repeated restraint, suggesting that other sites in brain, with input to the PVN, modulate its activity.

When activity in the whole brain is compared in singly vs repeatedly restrained rats with Fos staining, responses are shown to be different throughout the CNS, with decreased sensory input, thalamic amygdala and cortical activation, suggesting markedly diminished input to the PVN (1,6). Real insight into the mechanism of decreased HPA activity induced by repeated homotypic stressors came with the finding that neurotoxin lesions of the posterior midline paraventricular thalamus, known as the neuroendocrine thalamus, abrogated habituation to repeated stressors (2). Moreover, habituation appears to be mediated by the corticosteroids, since injection of a combination of mineralocorticoid- and glucocorticoid receptor antagonists into the paraventricular thalamus before each of the repeated restraint bouts also blocked the acquisition of habituation (8). The paraventricular thalamus is highly, and directly connected to brain sites of emotional learning and memory, such as the prefrontal cortex, accumbens and the amygdala (18), and also is innervated by both primary and visceral sensory as well as cortical afferents (11). Thus, this midline thalamic cell group is ideally positioned to serve as a learning and memory hub that plays a large part in habituation to repeated stimuli. It is curiously appropriate that the glucocorticoids secreted after stimulation of the HPA axis also importantly control their own decreased secretion under the threat of over exposure to them.

The glucocorticoids also appear to be key to both the maintenance of HPA activity and responses to heterotypic stressors in chronically stressed animals. In this case, it is an action of the glucocorticoids on increased secretion of CRF from the amygdala that occurs with heterotypic stressors. Amygdalar CRF synthesis and secretion is stimulated by glucocorticoid activity in amygdalar cells (3,15–17) and CRF activity appears to be essential for the engagement of the chronic stress response network (4,14). Thus, with repeated stressors that are not life-threatening, glucocorticoid-mediated mechanisms in brain that both inhibit homotypic and also allow novel, heterotypic, responses have been shown, although there is much further work to be done.

But, what about repeated stressors that are life-threatening, not just simply nasty, distressful and boring? The situation is different when there must be glucocorticoid responses each time the stimulus occurs. Because of the requisite immediate actions of the glucocorticoids, the response cannot be allowed to be damped, but excessive glucocorticoid secretion must be

curtailed for the well being of the animal. Are there also mechanisms that modulate the HPA responses to such repeated stimuli?

The paper by Amhold et al. in this issue provides the beginning of a resoundingly positive response to the question. The paradigm, of intermittent and limited water availability used by these authors, was first reported to affect the HPA axis by the Levine lab in 1973, and its characteristics were actively explored thereafter (e.g., (5,⁷,¹⁰)). The paradigm used by Amhold et al. employed 23.5 hours of water restriction, followed by 0.5h of ad lib drinking. Unlike other, less-threatening stressors, with this potentially lethal stimulus, peak corticosterone is maintained and even augmented in magnitude throughout numerous repetitions. Plasma corticosterone concentrations begin to increase above controls with ad lib water approximately six hours before, and reach a plateau two hours prior to water provision (22). Thus the restriction provides a daily excess of corticosterone to the body.

However, perhaps in compensation, the response decays much faster than with other stimuli, and faster than the usual 25 min half-disappearance time for corticosterone (Figure, compare left and right panels). This very rapid decay is not a result of inhibition of ACTH secretion, plasma expansion occurring through drinking, or increased clearance of the steroid. Plasma ACTH occasionally does decrease slightly during this time, but the adrenal corticosterone response is strikingly inhibited by 15 min (Amhold et al, *this issue*). Moreover, when corticosterone is replaced in adrenalectomized rats and the water restriction paradigm applied, plasma corticosterone concentrations decrease little, if at all (23). The inhibition of adrenal and plasma corticosterone has been observed as early as 3 and 6 minutes after presentation of water, well before changes in ACTH occur (21).

To determine the changes that occur in the hypothalamic PVN during the water replenishment that is accompanied by such marked inhibition of circulating corticosterone, the authors measured Fos immunoreactivity in cells identified phenotypically as CRF or AVP secreting. Although the numbers of CRF and AVP expressing cells did not change, either up or down with respect to unstressed controls after either acute or repeated water restriction, the numbers of these neurons that were co-stained with Fos, indicating acute activation, decreased markedly in the parvocellular PVN after the 6th exposure to water, but not after the first exposure. By contrast, AVP magnocellular neurons, that send axons to the posterior pituitary and secrete AVP into the general circulation, showed reductions in Fos co-staining after both the 1st and 6th period of access to water and this was reflected by the decrease in osmolality and in circulating AVP in both conditions. As with non-threatening but distressing repeated stressors, the Fos staining of CRF cells just before water was reduced on the 6th compared to the 1st bout of dehydration, despite the increased circulating corticosterone.

Previous studies by Engeland's group have elegantly tested the role of the sharp decrease in circulating AVP on circulating corticosterone under conditions of water deprivation (24). These showed unequivocally that the reduction in plasma AVP is highly correlated with the reduction in corticosterone, and that injection of an AVP-neutralizing antiserum reduced corticosterone in water-deprived but not ad lib drinking rats. However those studies also failed to find a direct effect of AVP on dispersed adrenocortical cells in culture, and suggested that the drinking-induced drop in AVP may explain some, but not all of the decrease in corticosterone observed (24). The present paper shows that the number of magnocellular Fos-positive AVP cells reflects precisely that drop in plasma AVP that occurs, and suggests that the decreased magnocellular AVP drive, coupled with the results from the previous paper, may contribute to the drop in corticosterone observed.

It is quite possible, as Arnhold et al. suggest, that the remaining portion of the inhibition of corticosterone after repeated exposure to the paradigm may be reflected by the decrease in Fos

staining in parvocellular AVP and CRF cells in the PVN, and that this may reflect a conditioned response, indicating memory of the fact that water will become available. However, the timing of the response seems too slow to further explain the dramatic decrease in circulating corticosterone that occurs so fast after receiving water.

Engeland's lab has provided strong evidence in previous studies that the splanchnic neural innervation of adrenocortical cells is of key importance in upregulation of adrenal sensitivity to ACTH diurnally (9) and to the response to water deprivation (19), however, this innervation appears to be entirely stimulatory to corticosterone production and secretion, not inhibitory. It may well turn out that there is dual innervation of adrenocortical cells, and that a second type of innervation is responsible for the very rapid decrease in adrenal and plasma corticosterone concentrations that follow drinking in dehydrated rats. New results from this lab are to be anticipated with relish.

Although the studies so far do not entirely mechanistically account for the phenomenon of very rapid inhibition of circulating corticosterone after drinking in dehydrated rats, the phenomenon itself is absolutely clear. It makes great sense teleologically, in that whatever the mechanisms are, they reduce the overall daily exposure to this potent hormone under conditions in which daily secretion is greater than normal. It seems quite possible that the mechanism being explored so well by the Engeland lab may also be used under conditions of other chronic life-threatening stressors that are relieved periodically, such as exposure to very intense shock, predator odor or adverse social conditions. The HPA axis does not adapt to these stimuli with repetition, and the corticosterone response is either of consistent amplitude, or increases. It seems likely that there is a similar mechanism in place under those conditions to rapidly relieve the organism of excessive corticosterone secretion once the stimulus is relieved. Limiting the increased corticosterone burden through a variety of mechanisms is physiologically important, and should serve to prolong life and more normal function under conditions of chronic challenge.

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Figure. Comparison of the modification in corticosterone responses after repetition of stimuli that are either distressful, but not life-threatening (restraint) or potentially life-threatening (water restriction)

On the 5th repetition, the peak corticosterone response to restraint is markedly damped, and decays normally once the stimulus is removed (left panel; from (6)). On the 6th repetition, the peak corticosterone response to water deprivation is significantly increased. However the response decays remarkably fast, and faster on the 6th than the 1st exposure. Under both conditions, there are physiological mechanisms in place that insure limiting overall exposure to the potent glucocorticoid.