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EXPERT REVIEWS

Variability in stress system regulatory control of inflammation: a critical factor mediating health effects of stress

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Chronic stress has been associated with disease but the biological pathways are not completely understood. Stress systems such as the hypothalamus–pituitary–adrenal axis and the autonomic nervous system are prime candidates but alterations in their baseline activity are not consistently found in chronic stress. Evidence suggests that stress-related changes in the sensitivity of inflammatory pathways towards glucocorticoid regulation, that is, the development of glucocorticoid resistance, might help explain inflammatory disinhibition and the subsequent development of disease. Recent data show a similarly important role for sympathetic and parasympathetic modulation of the inflammatory cascade for the maintenance of health. This article argues that variation of target tissue sensitivity towards anti-inflammatory effects of the hypothalamus–pituitary–adrenal axis, as well as sympathetic and parasympathetic signaling, might be involved in the development of low-grade inflammation under chronic psychosocial stress.

Keywords: acetylcholine • acute stress • catecholamine • chronic low-grade inflammation • chronic stress • cytokines • glucocorticoid • HPA axis • parasympathetic nervous system • sympathetic nervous system

Cardiovascular disease, Type 2 diabetes, insulin resistance, the metabolic syndrome and cancer are the most relevant diseases of all time and are responsible for the majority of deaths in the developed world. However, the relative percentage of mental health problems is on the rise. Although frequently regarded as distinct diseases, physical and mental health problems bear some significant similarities. Inflammatory processes, for example, are a central component in atherosclerotic plaque development and thus important in cardiovascular disease [1]. They are involved in pathways leading to insulin resistance and are thus linked to Type 2 diabetes and the metabolic syndrome [2], and they have been implied in age-related morbidity and mortality [3].

Inflammatory processes are also found to be upregulated in psychiatric disorders (e.g., depression [4]) and in individuals exposed to life stressors or traumatic events (e.g., in posttraumatic stress disorder [PTSD] and in familial caregivers [5–7]), and have been suggested to be involved in the pathophysiology of depressive symptoms [8,9].

It has therefore been suggested that inflammatory processes, or more specifically failure of the body to efficiently control inflammation, provides a link between adverse states of the CNS (such as depression, PTSD and chronic stress) and pathophysiology of medical diseases. This helps to explain why depression, trauma and chronic stress are statistically associated with cardiovascular disease, Type 2 diabetes or general decreases in healthy lifespan [10,11]. One important theory in this context is the allostatic-load model, which proposes that long-term or repeated stress exposure is associated with accumulation of damage in peripheral systems and CNS structures, ultimately leading to tissue damage and age-related diseases [12,13].

One important component of this theory is that the body's stress systems, that is, the hypothalamus–pituitary–adrenal (HPA) axis

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and the autonomic nervous system (ANS), exert a modulatory influence on the immune system and particularly on inflammatory processes. In situations of acute stress, these are thought to upregulate and thus prepare the immune system for potential pathogen infections, but also to downregulate and thus shut off potentially harmful biological mechanisms when the danger has passed [14]. Independent of psychosocial stress, the HPA axis is part of a feedback loop in which inflammatory stimuli from the periphery activate the axis and increase glucocorticoid (GC) concentrations to exert suppressive control on peripheral inflammation [15]. In situations of chronic stress, overall daily stress system activity and intact diurnal rhythms are thought to be important for successful control of target organ systems, including the inflammatory response [12]. However, current data are not always compatible with the notion that changes in stress systems mediate dysregulation of dependent systems, suggesting that we are missing important pieces of the puzzle. For example, depression has traditionally been seen as a disorder with increased HPA axis activity and thus increased circulating GCs [16]. The fact that depression is also found to be associated with a disinhibition of peripheral inflammation is theoretically incompatible with anti-inflammatory GC effects. Similarly, it was recently reported that the chronic stress of caring for a family member dying from brain cancer is associated with the development of inflammatory disinhibition, although changes in HPA axis activity were absent [17].

A potential solution for these apparent inconsistencies is to assume that stress hormone effects on target tissues are not a constant force but, instead, have to be viewed as a dynamic phenomenon. There is sufficient evidence now for HPA axis signaling to the periphery, and in particular, inflammatory processes, demonstrating that acute, as well as chronic stress, induces marked changes in the ability of the target tissue to receive and respond to suppressive GC signals [17–19] and insufficient GC signaling has been identified as a major component of stress-related diseases [20].

While the literature reviewed so far mainly focuses on the HPA axis, it has to be pointed out that the HPA axis is only one of at least three relevant stress-responsive systems. For example, anti-inflammatory properties have also been described for both arms of the ANS, for the sympathetic nervous systems (SNS) [21] and parasympathetic nervous system (PNS) [22], and since both ANS arms are activated by peripheral inflammation through similar pathways as the HPA axis, similar feedback loops have been proposed for the SNS and PNS [22–24].

Based on the complexity of HPA, SNS and PNS regulation of inflammatory pathways, a considerable variability of target tissue sensitivity can be assumed. This has previously been demonstrated for HPA axis (GC) sensitivity inflammatory pathways, and will have to be shown for SNS (catecholamine [CAT]) and PNS (acetylcholine [ACh]) control of inflammation. The aim of this article is to review current literature regarding GC, CAT and ACh sensitivity of peripheral inflammation, and to propose that modulation of stress system sensitivity by acute and chronic stress will allow a better understanding of stress–disease pathways.

Sensitivity of the inflammatory system to stress system regulation

The number of published articles describing stress system sensitivity of inflammatory target tissues and how it is modulated by acute and chronic stress, or stress hormones, differs markedly between stress systems. The vast majority of literature describes HPA axis (GC) sensitivity of inflammatory and other target systems, while significantly fewer studies have been published investigating CAT sensitivity under stress, and even fewer have investigated cholinergic (ACh) sensitivity.

HPA axis regulation of inflammation (GC sensitivity)

Glucocorticoids exert their effects through binding to cytosolic GC receptors (GRs) and mineralocorticoid receptors and through subsequent translocation of the hormone–receptor complex to the nucleus, where they interact with the DNA to stimulate or inhibit gene expression. Of particular relevance for GC effects on inflammatory activity of target cells is the ability of the GC–GR complex to negatively interfere with DNA-binding activity of the major inflammatory transcription factor nuclear factor-κB (NF-κB). This multistep process is subject to modification at virtually all levels of the cascade, from the modulation of extracellular GC availability by corticosteroid-binding globulin, over modifications of the GR chaperone and co-chaperone complex, to activity of NF-κB (for a detailed description see [19,25–27]), allowing for short- and long-term changes in tissue sensitivity to GC modulation.

Glucocorticoid sensitivity has been investigated in animals and humans using whole blood *in vitro* methods in which blood is incubated with inflammatory stimuli (e.g., lipopolysaccharide [LPS]) and different GC concentrations to stimulate and dose-dependently suppress inflammatory cytokine production. Earlier human studies have established that short-term modulation of GC sensitivity can be induced by acute exercise, leading to relative GC resistance [28,29] and by acute psychosocial stress, leading to increases or decreases in GC sensitivity, depending on age and sex-steroid concentrations [19]. Furthermore, chronic stressors and psychiatric disorders were shown to be associated with long-term changes in GC sensitivity. Miller *et al.* proposed a GC resistance model based on the finding that relatives of cancer patients showed changes in baseline HPA activity in conjunction with lower GC sensitivity [18]. Lower GC sensitivity was also found in individuals suffering from fatigue [30,31] and hypertension [32]. Increased GC sensitivity of the inflammatory system was found in patients with PTSD [33]. Most studies of GC sensitivity using laboratory animals have employed a social disruption stress (SDR) paradigm, in which group-housed mice with an established social hierarchy are subjected to an aggressive intruder. Measuring GC sensitivity of mononuclear cells harvested from the spleen, all published studies consistently show development of GC resistance as a consequence of chronic social disruption stress [34]. Taken together, these early studies demonstrated that GC sensitivity can be modulated in a rapid fashion in response to acute stress and exercise but also in a more chronic, long-term manner, suggesting that the organism can fine-tune responsivity to hormonal signals at the target tissue level.

Recently, our knowledge of how GC sensitivity of inflammatory target tissues is modulated by acute and chronic stress has significantly improved. Traditional whole blood-based *in vitro* assays have been complemented by methods that employ gene-expression microarrays or make use of immune cell redistribution patterns. These mechanisms are beginning to be understood and longitudinal studies have shed some light on the long-term development of altered GC sensitivity.

Traditional whole blood techniques

Using traditional whole blood *in vitro* techniques, Miller *et al.* found that although individuals with clinical depression did show higher GC sensitivity at baseline compared with controls, they developed a relative GC resistance of LPS-stimulated production of IL-6 and TNF-α in response to acute stress [35]. Using a similar experimental design, Wirtz *et al.* found that a higher BMI was associated with relative GC resistance after acute psychosocial stress in a group of young-to-middle-aged men [36]. Acute stress exposure of fatigued breast cancer survivors, by contrast, revealed lower HPA axis activation, but no acute responses in GC sensitivity of LPS-stimulated inflammatory cytokine production and no baseline or post-stress differences [37]. This finding represents an interesting contrast to our earlier study in which we found that healthy, nonfatigued women with lower HPA axis activation by acute stress did, in fact, appear to compensate their blunted cortisol responses with increases in post-stress GC sensitivity [38]. This compensatory potential might thus be lost in fatigued breast cancer survivors.

A few more recent cross-sectional studies have further addressed GC sensitivity in chronic stress and related conditions. No differences in GC sensitivity of LPS-stimulated TNF-α production were found in 56 individuals with burnout compared with 38 healthy controls [39]. In contrast to this, a lower GC sensitivity of IL-5 and IFN-γ, but not IL-13, production by peripheral blood mononuclear cells stimulated with phorbol 12-myristate 13-acetate and ionomycin was found in children with asthma who reported low social support by their families [40]. In 2007, De Kloet *et al.* investigated GC signaling in traumatized veterans with (n = 29) or without (n = 29) PTSD and healthy controls [41]. GR number was found to be significantly reduced in both groups of traumatized veterans, while binding affinity did not differ. Functional assays did not show many differences between the groups but veterans with PTSD displayed relative GC resistance of phytohemagglutinin (PHA)-stimulated T-cell proliferation compared with traumatized and healthy controls. GC stimulation of IL-10 production was significantly greater in healthy controls compared with the two traumatized groups. While these results are in contrast to our earlier findings of increased GC sensitivity in PTSD [33], these inconsistencies might be explained by differences in the group of traumatized individuals (i.e., veteran soldiers vs civilian war victims) or *in vitro* methods, and the overall tendency towards GC resistance in PTSD is in line with the literature on GC sensitivity under chronic stress.

In one of the few published longitudinal studies, we followed up on a group of relatives of brain cancer patients and matched controls over the course of the patients' treatment, thereby assessing whole blood-based *in vitro* GC sensitivity at four time points relative to the patients' treatment progression. While we did not find any significant changes in HPA axis activity and diurnal rhythm over time, GC sensitivity of LPS-stimulated IL-6 production revealed the development of relative GC resistance over time, with the lowest sensitivity at approximately 18 weeks after diagnosis. This change occurred in conjunction with a decrease in expression of anti-inflammatory genes (inhibitory-κB and GR-α relative to GR-β) and an increase in plasma concentrations of C-reactive protein [17]. Another longitudinal study comparing girls with chronic fatigue syndrome with nonfatigued controls did not find any significant differences in GC sensitivity of LPS-stimulated inflammatory cytokine production; however, a subgroup of 26 consistently fatigued girls displayed a relative GC resistance of mitogen-stimulated production of IFN-γ by T cells [42].

GC regulation of leukocyte distribution patterns

It has recently been suggested that GC sensitivity can be assessed through interpretation of leukocyte distribution relative to GC concentrations. This method is based on the effect of GCs to induce an increase in the number and percentage of circulating lymphocytes and monocytes and to induce a decrease in neutrophils [43]. Consequently, it has been suggested that the relationship between circulating GC concentrations and specific leukocyte subpopulations at any given time can be interpreted as an index of leukocyte GC sensitivity. Cole *et al.* used this method in a study of approximately 1000 Taiwanese older adults (mean age 68 years), of which overnight urinary cortisol excretion (as well as leukocyte numbers) from a blood sample taken the following morning was available [44]. In the entire group of participants, a neutrophil-to-lymphocyte and a neutrophil-to-monocyte ratio was calculated and found to be significantly correlated with overnight cortisol. These correlations were absent in a subgroup of participants with high loneliness self-ratings and this was interpreted as a sign for the resistance of leukocytes to GC signaling [44]. The same approach was used in a longitudinal study of rhesus macaques, which were exposed to repeated changes in housing conditions by being transferred to cages with other macaques. Cortisol concentrations and leukocyte numbers were analyzed in blood samples taken on nonstress days and inverse correlations between lymphocyte numbers and cortisol were found. In animals randomized to the unstable social relationship condition, this correlation deviated repeatedly from this inverse baseline state more frequently during the 18 study months than in animals in the stable condition, indicating a disruption of leukocyte GC sensitivity [45]. Currently, no further data using these methods have been reported, but this approach might be very useful in larger populations in which functional assays are not possible but cortisol and differential blood count data can be obtained.

Gene-expression microarrays & expression of anti-inflammatory genes

Another recent addition to the canon of methodologies for assessing GC sensitivity employs gene-expression patterns. In simple terms, researchers have started to interpret gene expression at

a given time point, either obtained through real-time reverse-transcriptase PCR or microarrays in light of upstream regulators of the expressed genes. To assess the efficiency of the GC signaling pathway, the relative number of genes regulated by GC response elements can be quantified and compared with genes regulated by inflammatory or other signaling pathways [46]. If more genes regulated by the GC response elements are expressed relative to, for example, inflammatory genes, the respective system would be regarded as more GC sensitive, while a relative overexpression of inflammatory genes would be interpreted as relative GC resistance.

Using this approach, Miller *et al.* found diminished GR-mediated relative to inflammatory gene expression in relatives of brain cancer patients, that is, in individuals suffering from extreme stress, in comparison with unstressed matched controls, who indicated GC resistance [47]. Importantly, stressed individuals also showed increased plasma concentrations of the inflammatory biomarkers C-reactive protein and IL-1 receptor antagonist, despite unchanged HPA axis activity. In a related study, the same group of authors found similar differences in GC sensitivity between a group of approximately 100 healthy adults between 25 and 40 years of age based on their childhood socioeconomic status. Participants with lower parental socioeconomic status during the first 5 years of their lives were characterized by underactivity of GR-activated genes relative to inflammatory genes, again in the absence of any HPA axis differences [48]. This study is in line with data presented so far pointing to GC resistance in stressful life conditions, because it further supports the idea that specific, potentially stressful, conditions during critical phases can exert a lifelong impact on the ability of target tissues to receive stress system signals.

No studies have yet applied this methodology to patients with PTSD or depression but, recently, fatigue in breast cancer survivors was found to be associated with a similar pattern of GC resistance assessed using the same methodology [49]. It is likely that future studies using this approach will greatly advance our understanding of GC signaling pathways in stress-related disorders.

Mechanism

As previously summarized, there is good evidence demonstrating that acute, as well as chronic, stress changes GC sensitivity of inflammatory or immune target tissues. Acute stress effects were more dependent on individual characteristics such as age or sex hormone concentrations, while chronic stress almost uniformly induced GC resistance. An important question in this regard is: how are these changes mediated?

Studies on laboratory animals have shed some light on the mechanisms underlying chronic stress-induced development of GC resistance. In the SDR model, GC resistance was found to be strongest in subordinate mice and in those that were wounded and physically defeated [50,51]. Several follow-up studies demonstrated that SDR mainly affects $CD11b^+$ cells, which had been released in higher quantity under chronic stress from the bone marrow and had subsequently accumulated in the spleen [52,53]. A recent study further revealed that dendritic cells (DCs; $CD11c^+$ cells) are affected by SDR in a similar fashion as macrophages: they appear to accumulate in the spleen, display more activity markers and become GC resistant [54]. Quan *et al.* found that SDR did not induce GR mutations, but that in macrophages ($CD11b^+$), LPS-induced GR synthesis was reduced and nuclear translocation of GR after GC stimulation was impaired [55]. The search for mediators of this response revealed that proinflammatory cytokines appear to play an important role in the development of GC resistance. SDR has been shown to increase the expression of proinflammatory cytokines in splenocytes and, as Engler *et al.* report, suppression of IL-1 signaling through IL-1 receptor knockout abolishes the development of SDR-induced GC resistance [56].

Reber *et al.* extended the literature on chronic stress in mice with a chronic subordinate colony housing (CSC) paradigm, in which subordinate mice were housed with different aggressive intruders for a period of 19 days, thus realizing a long-term chronic stress exposure [57]. In accordance with earlier rodent data [58], CSC induced GC resistance of splenocytes [57]. In a more recent study, however, the same authors showed that leukocytes harvested from lymph nodes remained GC responsive during chronic stress, with the exception of IL-4 production, which developed a relative GC resistance after 19 days of CSC [59].

Further data support the notion that inflammatory cytokines have the potential to modulate their own sensitivity towards GC regulation. As reviewed by Pace *et al.*, cytokine effects on GC signaling have been shown to be mediated by mitogen-activated protein kinases that interfere with GR function [27]. IL-1 and other cytokines, for example, activate p38 mitogen-activated protein kinase by inducing GR phosphorylation and thus blocking GR translocation to the nucleus [27]. Similar effects were also found for c-Jun N-terminal kinases [60]. A further mechanism of cytokine-induced GC resistance might be mediated through NF-κB itself: as described previously, one important pathway of anti-inflammatory GC effects is direct protein–protein interaction of the GR–GC complex with NF-κB [25]. This interaction works in both directions, that is, activation of NF-κB might negatively interfere with the efficiency of GR-mediated gene expression (see [27] for a discussion of further inflammatory pathways affecting GC signaling).

Although the role of genetic polymorphisms in modulating GC sensitivity has been discussed [61] and potentially meaningful polymorphisms have been identified in genes coding for the GR chaperone complex (e.g., co-chaperone FK506-binding protein [62]), it is currently unclear if and how these genetic factors are relevant in acute and chronic stress responses (for a discussion of these mechanisms in relation to GC sensitivity in depression and PTSD, see [63]).

Taken together, it appears that we are beginning to understand some potential pathways leading to changes in GC resistance, particularly in response to chronic stress. In animal models of social stress, GC resistance might be interpreted as a beneficial response, because physical defeat and wounding frequently occur during social defeat, and not suppressing immune mechanisms would permit faster and more efficient clearing of infections associated with wounding [58]. Compared with humans, where wounding is not a component of most social stress situations, a response like this would most likely have adverse health consequences through damaging effects of inflammatory processes. Even less is known

about GC sensitivity modulation in acute stress. Given the potential pathways already reviewed, one might hypothesize that acute inflammatory responses might induce acute changes in GC sensitivity but, in previous studies, changes in GC sensitivity have been found to occur much faster than increases in peripheral inflammatory cytokine concentrations [19,64]. Since the sympathetic neurotransmitter norepinephrine has been shown to rapidly upregulate NF-κB DNA-binding activity in response to stress [65], and NF-κB negatively interferes with GC signaling, SNS responses may be a potential pathway to GC resistance. Future studies will have to further explore this and other possibilities.

SNS regulation of inflammation (CAT sensitivity)

Similar to the HPA axis, the SNS is an important signaling pathway from the CNS to the periphery. In contrast to the HPA axis, the SNS consists of a large array of neural projections that reach target organs through pre- and post-ganglionic pathways. These pathways are characterized by distinct, target organ-specific activity patterns, making it necessary to view SNS activity in light of the target tissue of interest [66,67]. One of the target organs of the SNS is the immune system and immune cells express adrenergic receptors [68–70]. Early findings showed a differential modulation of adaptive immunity by β-adrenergic signaling through differential expression of adrenergic receptors on lymphocyte subtypes [71]. However, relatively soon after these findings, it was demonstrated by several studies that LPS-stimulated production of inflammatory cytokines by whole blood *in vitro* was suppressed by norepinephrine via β-adrenergic receptors [72]. Similar results were found *in vivo* after LPS injection into mice [73]. Based on these findings, a neuroendocrine immune feedback loop was suggested similar to and in addition to the loop involving the HPA axis, in which peripheral immune activation would signal to the CNS and activate the SNS (in addition to the HPA axis), thus allowing the CNS to sense and subsequently control peripheral inflammation [21,23,74,75]. It should be noted that while *in vitro*-stimulated inflammatory activity appears to be suppressed by adrenergic signaling, the opposite seems to be true *in vivo*. Epinephrine injection or acute psychosocial stress with increased secretion of endogenous norepinephrine has been shown to activate intracellular inflammatory pathways [65,76].

Based on the complexity of the cascade mediating adrenergic immune modulation and the fact that adrenergic receptor balance differs between leukocyte subtypes [71] and within subtypes depending on the activation state of the cell [77], it was assumed that alterations in the efficiency of SNS signaling (to the immune system) or CAT sensitivity might be a relevant pathophysiological mechanism in human disease (for a summary and proposed mechanism, see [78]). More specifically, the same group of authors demonstrated that children suffering from arthritis displayed a significantly altered profile of adrenergic receptors on peripheral immune cells. The expression of α-1 adrenergic receptors, which are not present in cells of healthy children, allows increased inflammatory responses to stress in arthritic children [79]. Furthermore, it was found that, *in vitro*, GCs and CATs are capable of inducing α-1 receptor expression of lymphocytes, thus changing CAT sensitivity of these cells [80]. In two studies investigating CAT sensitivity in inflammatory bowel disease and ulcerative colitis, no differences in CAT suppression of proinflammatory cytokines were found between groups, but both bowel disease groups failed to show an increase of anti-inflammatory IL-10 response to β-adrenergic stimulation [81,82].

Given the findings already mentioned, it might be expected that acute as well as chronic stress would be associated with adaptive or maladaptive alterations in CAT sensitivity. In fact, Kavelaars *et al.* studied CAT sensitivity in 15 girls with chronic fatigue syndrome and healthy controls, and found reduced CAT sensitivity of LPS-stimulated TNF-α and IL-10 production in whole blood [30]. In a longitudinal study of approximately 100 caregivers to Alzheimer's disease patients, Mausbach *et al.* assessed β_2-adrenergic receptor sensitivity by *in vitro* isoproterenol stimulation of peripheral blood mononuclear cells and found that CAT sensitivity decreased over the 5-year study period and that stronger decreases were associated with higher perceived stress and lower self-reported mastery [83]. Similarly, in animals, the development of diminished CAT sensitivity of LPS-stimulated TNF-α production by splenocytes was found using the SDR model of chronic stress [84]. Only one study has measured CAT sensitivity in response to acute psychosocial stress. Pawlak *et al.* exposed patients with systemic lupus erythematosus (SLE) and healthy controls to acute psychosocial stress and found no difference in neuroendocrine stress responses. However, numbers of β_2-adrenoceptors increased significantly in response to acute stress in healthy controls but not in SLE patients [85]. This indicates that, in healthy individuals, CAT sensitivity might be upregulated in response to acute stress and that this mechanism is missing in SLE patients. Further studies are needed to ensure that increased numbers of β_2-receptors are in fact associated with increased CAT sensitivity.

Taken together, the number of studies investigating the impact of psychosocial stress on CAT sensitivity is still low. However, the few available studies allow the cautious conclusion that chronic stress is associated with the development of relative CAT resistance in inflammatory cells, while the one current acute stress study does not allow any conclusions to be made yet.

PNS regulation of inflammation (ACh sensitivity)

The HPA axis and SNS are generally regarded as the classical human stress systems because their activation and subsequent release of stress hormones and neurotransmitters induces many of the significant changes in response to stress. The PNS, as a third system, is often overlooked because it is not activated in response to stress but, by contrast, is reduced in its neural activity. Nevertheless, owing to the importance of its tonic activity, stress-related decreases of PNS activity play an important role in homeostasis during acute and chronic stress. Vagal innervation of the heart is probably the most salient function of the PNS but, as recently described, the PNS also exerts important actions on the immune system.

Borovikova *et al.* found that the vagal neurotransmitter ACh suppressed LPS-stimulated production of proinflammatory (TNF-α, IL-1β, IL-6 and IL-18) but not anti-inflammatory (IL-10)

cytokines in differentiated human macrophage cultures [86]. This mechanism was mediated by α7-nicotinic receptors and seemed to be post-transcriptional as TNF gene expression was not altered. Using an animal model of sepsis, they further underscored the role of the vagus in inflammatory control, because vagotomy significantly increased mortality, while vagal stimulation restored inflammatory suppression and survival [86]. These results allowed Tracey *et al.* to propose a third feedback loop between the immune system and the CNS, which was termed the cholinergic anti-inflammatory pathway, while the anti-inflammatory vagal response to peripheral inflammation was termed 'inflammatory reflex' [22].

However, one major difference between PNS/vagal modulation of inflammation versus regulation of inflammation by the HPA axis or SNS is that the vagus does not use endocrine signaling mechanisms. While both the HPA axis and SNS do secrete hormones into the blood, where they can directly affect leukocytes, PNS effects are restricted to neural or indirect mechanisms. It has been suggested that cholinergic anti-inflammatory communication involves signaling through the spleen, where direct neural-to-immune cell contact has previously been described for SNS neurotransmitters [87]. Consistent with this assumption, removal of the spleen was associated with increased mortality in *in vivo* models of sepsis [88]. However, as Nance and Sanders point out, the spleen does not receive cholinergic neural input [89]. Rosas-Ballina and Tracey have proposed alternative pathways explaining the involvement of the spleen. One suggestion involves ACh neurotransmission in the celiac ganglion, which would activate the splenic nerve to stimulate norepinephrine signaling to immune cells circulating through the spleen. Another suggestion involves β_2 adrenergic activation of intermediate immune cells, such as lymphocytes, which would then secrete ACh, which binds to α7-nicotinic receptors on macrophages [90].

Regardless of the details of this mechanism, the same group of authors has since repeatedly demonstrated that incubation of LPS-stimulated whole blood with α7-nicotinic agonists induces dose-dependent suppression of inflammatory cytokine production [91,92]. Since leukocytes pass through the spleen and receive neural signals, whole blood or separated leukocyte subsets might be an excellent target to study stress-induced changes in the efficiency of the cholinergic anti-inflammatory pathway, or ACh sensitivity of inflammatory cells. Recent data from Hunter *et al.,* which demonstrate that hippocampal expression of α7-nicotinic receptors is modulated by chronic stress and GCs, lends further support to the assumption that chronic (and probably acute) psychosocial stress might modulate peripheral ACh sensitivity in a similar fashion to their regulation of GC and CAT sensitivity.

Given the potential importance of alterations in the efficiency of the cholinergic anti-inflammatory pathway, or specifically ACh sensitivity, a logical question is whether changes have been reported, either in medical disease or in response to acute and chronic psychosocial stress. Tracey *et al.* mainly discuss the inflammatory reflex in the most relevant context, for example, autoimmune/inflammatory diseases and sepsis [93]. Bruchfeld *et al.* have tested ACh sensitivity in rheumatoid arthritis (RA) patients and controls [92]. Heart rate variability measures revealed lower vagal activity and higher stimulated TNF-α production in RA patients. ACh sensitivity, however, was not different and not related to vagal activity [92]. Nevertheless, given that psychosocial stress has been found to be associated with changes in vagal activity [94], chronically altered vagal innervation of immune cells in chronic stress, depression or PTSD might induce alterations in ACh sensitivity, which would add the potential for compensation but also for further dysregulation of inflammatory control. Future studies will have to include measures of ACh sensitivity to better understand its contribution.

Expert commentary

Taken together, the literature reviewed in this article reveals marked differences in the amount of data available regarding modulation of peripheral inflammation by different stress systems. While there is convincing evidence that the HPA axis, SNS and PNS are important in modulating peripheral inflammation, only HPA axis regulation of inflammation has consistently been shown to respond to acute as well as chronic stress. The most consistent finding across methodologies and species is that chronic psychosocial stress is associated with the development of relative GC resistance. This, in many cases, is associated with increases in circulating inflammatory mediators, although changes in HPA axis activity are often absent. Acute stress-induced modulation of GC sensitivity appears to be dependent on individual modulating factors such as age and sex hormone status. Fewer data are available on CAT sensitivity in relation to stress, but available studies allow the cautious conclusion that chronic stress induces CAT resistance in humans and animals, while acute stress studies are too few to draw any conclusions. Virtually no studies have investigated whether acute or chronic psychosocial stress affects ACh sensitivity or the efficiency of the cholinergic anti-inflammatory pathway.

Earlier conceptual review articles have convincingly argued for the importance of GC regulation of peripheral and central dependent systems, including inflammatory pathways for health, or the failure of GC regulation in stress-related diseases (e.g., see [20,95]). Furthermore, dysregulation of sympathetic/CAT modulation of inflammation, in conjunction with alterations of HPA axis activity, have been shown to play a major role in the pathophysiology of RA [96]. Thus, the most significant recent advancement in our understanding of stress system regulation of peripheral inflammation is the addition of the SNS as a second, and the PNS as a third neuroendocrine immune feedback loop, as summarized by Sternberg [23]. We need to be aware of the fact that it is currently not clear how well changes in sensitivity to stress hormone signals are related across target tissues. While we have, for example, shown that acute GC-induced changes in inflammatory sensitivity are related to changes in CNS sensitivity [97], other studies have found no association of different target tissues' GC sensitivity [98] and no data are available on the sensitivity to SNS or PNS signals.

Nevertheless, now that the importance of each of the three stress systems in inflammatory control and, thus, maintenance of homeostasis and health are generally accepted, our next task will be to further our knowledge of how acute and chronic psychosocial stressors might disrupt any or all of these loops and thus endanger our health.

Five-year view

Based on the fact that the literature on GC sensitivity modulation by psychosocial stress has evolved significantly over the past 5 years, driven by longitudinal studies and the development of novel methodological tools, it can be assumed that similar advances will be made with regard to CAT and ACh sensitivity within the next 5 years. While we know a good deal about how GC sensitivity changes under stress, we still do not know enough about how these changes are mediated. Current data point to the fact that it is more complicated than simple ligand-dependent downregulation. Rather, the complex assembly of stress hormone/neurotransmitter receptor pathways, including chaperones and co-chaperones, as well as for example nonclassical modes of action (e.g., nongenomic GC effects) will be targeted and better understood. Related to that, we will be able to better understand which changes in sensitivity are related across target tissues. Future studies will explore how acute and chronic stress affects inflammatory and cognitive GC sensitivity. It is further expected that we will learn more about the role of stress system sensitivity of different target tissues within the aging process. A recent study has shown that chronic stress for approximately 1 year induces profound increases in peripheral inflammation and related changes in target system sensitivity to GC signals [17]. This bears signs of an accelerated aging process and aging studies will have to test the hypothesis that the same changes occur while we age, only on a slower scale. It can also be assumed that, in the near future, we will develop a better understanding of how alterations in sensitivity to stress system regulation, in acute and chronic stress, are related or similar to changes seen in psychiatric disorders, predominantly depression. Finally, we will begin to understand the interactions between the three feedback loops. We know, for example, that GCs can affect adrenergic signaling [78] and (at least in the CNS) α7-nicotinic signaling too [99]. It will be exciting to investigate whether dysregulations in one system can be compensated by other systems or if they negatively affect each other.

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Key issues

- Hypothalamus–pituitary–adrenal axis/glucocorticoid (GC) control of peripheral inflammatory regulation is part of a neuroendocrine immune feedback loop that is relevant for the maintenance of health.
- Chronic psychosocial stress induces GC resistance and acute psychosocial stress induces age and sex steroid-dependent modulation of GC sensitivity.
- The sympathetic nervous system is part of a second neuroendocrine immune feedback loop that is also relevant for the maintenance of health.
- Emerging data suggest the potential of acute and chronic stress-induced modulation of the sympathetic nervous system feedback loop through changes in catecholamine sensitivity.
- The parasympathetic nervous system is part of a third neuroendocrine immune feedback loop that is similarly relevant for the maintenance of health.
- No data are currently available regarding potential modulation of the parasympathetic nervous system feedback loop (cholinergic anti-inflammatory pathway) by stress through changes in acetylcholine sensitivity.

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