Stress and the Gastrointestinal Tract
I. Stress and hepatic inflammation

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Swain, Mark G. Stress and the Gastrointestinal Tract. I. Stress and hepatic inflammation. Am J Physiol Gastrointest Liver Physiol 279: G1135–G1138, 2000.— Stress is an ever-present part of modern life. The “stress response” constitutes an organism’s mechanism for coping with a given stress and is mediated via the release of glucocorticoids and catecholamines. Patients often complain of stress-related worsening of their liver disease; however, the interrelationship between stress and hepatic inflammation is incompletely understood and has received little scientific attention. Considering the broad impact glucocorticoids and catecholamines have on immune cell function, it is very likely that stress has a significant impact on the hepatic inflammatory response. This themes article discusses studies of the stress response and its peripheral effectors (glucocorticoids and catecholamines) in liver disease and their impact on hepatic inflammation and outlines potential areas for future scientific investigation.

glucocorticoids; catecholamines; hypothalamic-pituitary-adrenal axis; inflammation

THE GENERAL CONCEPT of “stress” is broadly accepted; however, a specific definition of stress that is relative to a given individual is more difficult to achieve. A reasonable working definition of “stress” has been suggested (16). Survival requires the maintenance of a stable internal milieu or steady state, which has been termed homeostasis. Stressors therefore can be defined as external or internal forces that attempt to disrupt this internal homeostasis, and stress can be defined as “threatened homeostasis” (16). Stressors can include physical or mental forces or combinations of both. The reaction of an individual to a given stressor involves the stimulation of pathways within the brain leading to activation of the hypothalamic-pituitary-adrenal (HPA) axis and the central sympathetic outflow. Activation of these pathways results in the “stress response” and ultimately to the release of the key peripheral mediators of the stress response, namely, glucocorticoids and catecholamines.

Although stress has been implicated as a cofactor in the severity and progression of a number of diseases, the potential role of stress in aggravating liver disease in general, and hepatic inflammation specifically, has received very little scientific attention. Anecdotally, in the clinical setting patients often complain of a worsening of their liver disease, at least symptomatically, in times of increased stress. Moreover, in patients with alcohol-induced liver disease the degree of psychosocial stress appears to correlate with the extent of hepatic fibrosis and inflammation (7). In addition, chronic liver disease in itself constitutes a psychological stressor.

Traditionally, it has been suggested that the principal effect of stress on the liver is related solely to changes in hepatic blood flow (12). Specifically, this hypothesis suggested that emotional stress leads to vasospasm and centrilobular hypoxia and ultimately to liver damage. However, as the broader physiological effects of the mediators of the stress response have become better understood, it is clear that this earlier explanation of the effects of stress on the liver appears to be overly simplistic.

HPA AXIS IN LIVER DISEASE

Activation of the HPA axis is a central component of the stress response, resulting ultimately in the release of glucocorticoids (cortisol in man, corticosterone in rodents) from the adrenal cortex. This release of glucocorticoids during stress is known to be critical for survival. However, in recent years glucocorticoids released during the activation of the HPA axis have been shown to be intimately involved in the control of the inflammatory response in that removal of endogenous glucocorticoid release (by adrenalectomy), inhibition of the biological effect of endogenous glucocorticoids, or intrinsic biologically defective HPA axis activity results in a significant augmentation of the inflammatory response (16, 17). Moreover, tissue inflammation itself, through the release of cytokines into the circulation and by stimulation of vagal afferents, can activate the HPA axis. This interaction between the neural and
immune systems has been termed the neuroimmune axis.

Abnormalities in the HPA axis have not been adequately studied in patients with hepatic inflammatory disease. Certainly, patients with hepatic failure caused by acute hepatic inflammation exhibit HPA axis activation. In addition, patients with chronic liver disease have delayed cortisol clearance and altered cortisol binding in plasma. However, dynamic evaluations of the HPA axis in patients with ongoing hepatic inflammation have not been performed. Interestingly, elevated plasma cytokine levels have been documented in patients with chronic hepatic inflammation, and chronic exposure to these elevated circulating levels of cytokines may lead to HPA axis dysregulation (23). Indeed, elevated plasma cytokine levels and alterations in HPA axis responsiveness appear to accompany experimental chronic liver disease in rodents. Specifically, experimental cholestatic liver injury results in elevated circulating tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels. Moreover, chronic cholestatic liver injury in rats results in decreased hypothalamic mRNA and protein expression of corticotropin-releasing hormone (the main central regulator of the HPA axis) (19). When exposed to psychological stress, cholestatic animals display defective activation of the HPA axis and a significant attenuation in the resultant release of glucocorticoids into the circulation compared with control animals (19). This finding suggests that chronic inflammatory liver disease itself may be associated with defective stress-induced glucocorticoid release. Low levels of circulating glucocorticoid after stress may theoretically exacerbate the underlying hepatic inflammatory disease.

The end result of HPA axis activation is the release of glucocorticoids from the adrenal cortex. Glucocorticoids have powerful inhibitory effects on the inflammatory response by direct or indirect effects on immune cell function, adhesion molecule expression, immune cell recruitment, and inflammatory mediator and cytokine generation (4). Given their broad range of immunosuppressive effects, it is clear that endogenously released glucocorticoids should directly impact hepatic inflammatory disease. However, this area of study has received limited scientific attention and has focused generally on the effects of pharmacological doses of exogenously administered synthetic glucocorticoids (e.g., dexamethasone), the biological effects of which may be quite different from those that would be observed with physiological concentrations of endogenous glucocorticoids.

Acute toxin-induced hepatic inflammation in the rat is associated with activation of the HPA axis and an elevation of circulating glucocorticoid levels (18). Inhibition of the physiological effects of glucocorticoids released in this model of acute hepatic inflammation results in increased mortality and an enhanced hepatic inflammatory response (18). These deleterious effects of glucocorticoid deficiency can be mitigated by exogenous glucocorticoid administration. The hepatoprotective effects of endogenously released glucocorticoids appear to be caused, at least in part, by enhanced glucocorticoid-mediated synthesis and release of IL-10 within the liver (18). IL-10 is mainly produced by Kupffer cells within the liver, and it has hepatoprotective effects in a number of animal models of acute hepatic inflammation. Kupffer cells (liver-fixed macrophages) are the main hepatic source of a number of inflammation-modulating mediators including IL-1, IL-6, TNF-α, leukotrienes, PGE2, and nitric oxide. The production of these inflammatory mediators is in general upregulated during hepatic inflammation. Moreover, glucocorticoids inhibit the production of these inflammatory mediators from Kupffer cells either directly (at the level of mRNA stability and gene transcription) or indirectly by inhibiting the production of proinflammatory transcription factors such as nuclear factor (NF)-κB and activator protein (AP)-1. However, there appears to be a differential sensitivity to glucocorticoid-mediated suppression of proinflammatory cytokine synthesis in vivo (TNF-α > IL-1 > IL-6), which may have direct clinical relevance. The concept that glucocorticoids inhibit Kupffer cell cytokine production has been challenged by the observations of Liao et al. (13), where corticosterone infusion (at stress and nonstress concentrations) into an isolated, perfused rat liver increased the release of TNF-α and IL-6 into the hepatic effluent. Interestingly, psychological stress itself can induce both IL-6 and TNF-α within the liver, suggesting a potential direct link between psychological stress and hepatic inflammation (21). These results are consistent with the existence of a complex interaction between endogenous glucocorticoids and classic mediators of the inflammatory response during liver inflammation.

Chemokines are increasingly recognized as important mediators of hepatic inflammation. Chemokine expression is increased within the liver in patients with various forms of hepatic inflammatory disease, including those induced by alcohol, toxins (acetaminophen), and viruses (hepatitis C), and has been directly implicated in inflammatory cell recruitment in these liver diseases. In general, inflammatory mediator (i.e., endotoxin, cytokine)-induced expression of chemokines in immune cells is attenuated by pretreatment with a synthetic glucocorticoid (i.e., dexamethasone; Ref. 6). However, the effects of physiologically relevant concentrations of naturally occurring glucocorticoids in vitro and stress-induced glucocorticoid release in vivo on basal or induced chemokine levels within the liver or liver-derived cells have not been studied.

T cells are critically involved in the propagation and maintenance of chronic hepatic inflammatory disease, and glucocorticoids have profound effects on T cell function. Recently, adrenal steroids have received scientific attention as regulators of T helper (Th) lymphocyte cytokine secretion patterns. Glucocorticoids inhibit the production of Th1 cytokines [e.g., interferon (IFN)-γ, IL-2]. Dehydroepiandrosterone is another adrenal steroid hormone that circulates in plasma in an inactive form (DHEAS) and is activated in tissues (especially the liver) to its active form (DHEA). DHEA
inhibits glucocorticoid-induced suppression of Th1 cytokine release and also directly enhances Th1 cell activity (3). Therefore, activation of the HPA axis by stress, with the associated release of adrenal glucocorticoid, would be expected to drive the cytokine profile to be more Th2 dominant. Interestingly, restraint stress in mice results in a Th2-dominant response in the spleen (10); however, similar data for the liver are not available. Most hepatic inflammatory diseases are associated with a predominant Th1 cytokine profile, and similar observations have been made in animal models of hepatic inflammation (21). Glucocorticoids also influence T cell dynamics by inducing apoptosis. Therefore, the increased circulating glucocorticoid levels observed in hepatic inflammatory disease or after HPA axis activation should enhance T cell apoptosis within the liver, although this has not been directly examined. Interestingly, Tamada et al. (20) demonstrated in mice that IL-4-producing hepatic NK1.1+ T cells are resistant to glucocorticoid-induced apoptosis and suggested that this may play a role in determining the hepatic Th1/Th2 balance in times of stress. Whether other hepatic cell types also exhibit differential glucocorticoid sensitivity to apoptosis is unknown.

Recruitment of neutrophils to the liver is important in hepatic inflammatory disease, especially in the earlier phases. Glucocorticoids have profound inhibitory effects on neutrophil recruitment by downregulating endothelial cell adhesion molecule expression. Therefore, increased circulating glucocorticoid levels would be expected to decrease hepatic neutrophil recruitment. We demonstrated previously (22) that endogenous glucocorticoids released during experimental liver disease inhibit leukocyte recruitment to inflammatory sites outside the liver. The role of endogenous glucocorticoid release in the control of hepatic neutrophil recruitment warrants further study.

In many animal models of hepatic inflammation, and very likely in their human correlates, endotoxin has been directly implicated in the induction of hepatic inflammation. The level of endotoxin found in the portal circulation is a function of the integrity of the gut barrier. Therefore, increased gut permeability would be expected to lead to enhanced endotoxin absorption and thus portal endotoxemia. Recently, psychological stress in the rat has been shown to be associated with a significant increase in gut permeability mediated by the release of endogenous glucocorticoids (14). This stress-related increase in gut permeability likely is associated with increased portal endotoxin levels and may therefore provide a further possible link between stress and hepatic inflammation.

SYMPATHETIC OUTFLOW AND HEPATIC INFLAMMATION

The second major arm of the stress response involves stimulation of the locus coeruleus-norepinephrine system within the central nervous system and activation of central sympathetic nerve pathways and peripheral sympathetic outflow. This results in the release of catecholamines from autonomic nerve endings and from the adrenal medulla. The catecholamines thus released constitute the peripheral effector of this arm of the stress response (16). Catecholamines have well-established cardiovascular effects; however, their effects on the immune response and inflammation are less clear. In patients with either acute liver failure or hepatic cirrhosis, plasma catecholamine levels are elevated. This observation suggests that activation of the sympathetic outflow arm of the stress response occurs in these patients. Moreover, plasma catecholamine (especially norepinephrine) levels are elevated in the setting of acute toxin-induced hepatic inflammation in the rat (1). Catecholamines released in the setting of acute experimental liver inflammation appear to augment the hepatic inflammatory response and accentuate hepatocellular necrosis. Hepatic inflammation induced by carbon tetrachloride is attenuated by spinal transection or by administration of adrenergic blocking drugs and augmented by the coadministration of catecholamines with carbon tetrachloride (1, 15). Moreover, footshock stress enhances carbon tetrachloride-induced liver damage in rats, an effect mediated in significant part via the release of catecholamines (9).

Although catecholamines appear to augment hepatic inflammation, the mechanism underlying this catecholamine-induced proinflammatory effect is unclear. Earlier studies suggested that catecholamine effects were related to altered hemodynamics; however, this explanation appears to be overly simplistic. It is becoming increasingly apparent that catecholamines can directly influence the immune response, because catecholamine receptors have been identified on immunocompetent cells and activation of these receptors has biological effects (2).

Cytokines play a critical role in the initiation and propagation of the hepatic inflammatory response. The cytokines that have received the most scientific scrutiny in this regard have been IL-1, IL-6, and TNF-α. Immobilization stress in rodents increases hepatic IL-6 levels, mainly within hepatocytes, and this effect can be mimicked in vitro by incubating hepatocytes with norepinephrine (11). Moreover, IL-1β production is enhanced in hepatic nonparenchymal cells after incubation with norepinephrine in vitro (11). Furthermore, Kupffer cells demonstrate increased TNF-α release on stimulation with norepinephrine. The enhanced release of these cytokines, induced by catecholamines, would be expected to augment the hepatic inflammatory response. Interestingly, catecholamines appear to inhibit the release of the chemokine macrophage inhibitory protein (MIP)-1α from endotoxin-treated macrophages in vitro (8). This interaction of catecholamines and chemokines has obvious inflammation-regulating potential and is an area of significant potential interest with respect to hepatic inflammation.

Activation of the sympathetic nervous system also appears to be able to modulate Th1 and Th2 cytokine profiles. Specifically, sympathetic activation drives the Th1/Th2 balance toward more Th2. This effect has been shown in vitro to be caused by β-adrenergoreceptor-
induced inhibition of IL-12 production and augmentation of IL-10, IL-4, and IL-5 production. A catecholamine-induced increase in IL-10 release from monocytes also appears to occur in vivo (24), although whether this effect also occurs within the liver is unknown.

**SUMMARY**

Advances in our understanding of the stress response and its peripheral mediators have enhanced our appreciation of the impact that stress may have on the inflammatory response. Specifically, the diverse physiological effects of glucocorticoids and catecholamines released into the circulation during stress are receiving increasing scientific attention. Moreover, recent work has shed some light in helping us to understand the complex interactions that occur between stress system effectors and inflammation. However, the role of stress in the setting of hepatic inflammatory disease is still a relatively unexplored area. The future study of stress in the setting of hepatic inflammatory disease is still a complex interaction that occurs between stress system effectors and inflammation must utilize physiologically relevant classes and concentrations of glucocorticoids and catecholamines in experimental paradigms that examine both in vivo and in vitro effects. A better understanding of the impact of stress on the hepatic inflammatory response should provide us with better tools to deal with these issues in the clinical setting.

**REFERENCES**

2. Chambers DA, Cohen RL, and Perlman RL. Alterations of glucocorticoid and catecholamine-induced increase in IL-10 release from monocytes also appears to occur in vivo (24), although whether this effect also occurs within the liver is unknown.