Stress and the Gastrointestinal Tract
II. Stress and intestinal barrier function

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Sönderholm, Johan D., and Mary H. Perdue. Stress and the Gastrointestinal Tract. II. Stress and intestinal barrier function. Am J Physiol Gastrointest Liver Physiol 280: G7–G13, 2001.—The influence of stress on the clinical course of a number of intestinal diseases is increasingly being recognized, but the underlying mechanisms are largely unknown. This themes article focuses on recent findings related to the effects of stress on mucosal barrier function in the small intestine and colon. Experiments using animal models demonstrate that various types of psychological and physical stress induce dysfunction of the intestinal barrier, resulting in enhanced uptake of potentially noxious material (e.g., antigens, toxins, and other proinflammatory molecules) from the gut lumen. Evidence from several studies indicates that in this process, mucosal mast cells play an important role, possibly activated via neurons releasing corticotropin-releasing hormone and/or acetylcholine. Defining the role of specific cells and mediator molecules in stress-induced barrier dysfunction may provide clues to novel treatments for intestinal disorders.

THE INTESTINAL BARRIER

The intestinal mucosa is continuously exposed to an immense load of antigens from ingested food, resident bacteria, invading viruses, etc. The single-cell epithelial layer lining the gut lumen (surface area ~300 m²) has conflicting functions, playing a major role in the digestion and absorption of nutrients and at the same time constituting the organism’s most important barrier between the internal and external environments. Under normal circumstances, the epithelium allows only minute quantities of intact antigens to cross into the mucosa, where they interact with the mucosal immune system to downregulate inflammation (known as oral tolerance). On the other hand, it is necessary for enteric pathogens to activate immune cells and initiate the inflammatory response required to clear the infection. In some disease conditions, such as inflammatory bowel disease, excessive penetration of antigens through the epithelial layer may result in inappropriate immune stimulation, leading to chronic gastrointestinal inflammation. The ability of the epithelium to control uptake of molecules into the body is denoted as the intestinal barrier function.

The intestinal barrier includes physical diffusion barriers, regulated physiological and enzymatic barriers, and immunological barriers, all of which are under neurohormonal control and therefore possible targets for influence by stress. The continuous epithelial cell layer, interconnected by tight junctions, restricts both transcellular and paracellular permeation of molecules, thus constituting the principal component of the intestinal barrier. In addition, the epithelium exerts a important physiological defense by secretion of fluid and mucus, together with secretory IgA, into the lumen to dilute, wash away, and bind noxious substances.

A disturbance of intestinal barrier function has been suggested as an etiologic factor in Crohn’s disease (15, 24) and food allergy (6, 11). In several other disease states, an increased mucosal permeability is implicated in pathogenesis and development of complica-
tions, e.g., viral and bacterial gastroenteritis, ulcerative colitis, and multiple organ dysfunction syndrome in patients with sepsis and trauma. “Leaky gut syndrome” is a current topic of interest on the Internet. In this syndrome, uptake of various noxious substances from the gut lumen is considered to lead to a range of disorders; however, few of the claims are substantiated by conclusive scientific evidence.

The barrier properties of the intestinal epithelium are usually studied by assessing the permeability to various probe/marker molecules in vivo or in vitro with intestinal segments mounted in Ussing-type chambers. Although the in vivo studies are more physiological, the in vitro approach makes it possible to study epithelial permeability to a greater range of probes, including proteins, and to determine the mechanisms and routes of passage involved.

STRESS-INDUCED BARRIER DYSFUNCTION IN HUMANS

Studies using intestinal perfusion techniques in human jejunum have revealed effects of acute stress on intestinal secretion. Barclay and Turnberg (3) found that psychological stress induced by dichotomous listening reduced mean net water absorption and reversed net Na$^+$ and Cl$^-$ absorption to secretion. Because the stress effects were inhibited by atropine, the authors concluded that stress-induced ion secretion may be mediated by the parasympathetic nervous system. With the same technique, similar changes were found during cold-induced hand pain stress (4). By extending this model, Santos et al. (20) found that jejunal water secretion induced by cold pain stress was paralleled by luminal release of the mast cell mediators tryptase and histamine. These data suggested that there are signaling pathways between the central nervous system and intestinal mucosal mast cells during stress.

Although physical stressors such as surgery and trauma have been shown to increase intestinal permeability in humans, no studies to date have looked specifically at the influence of psychological stress on intestinal permeability in humans.

ANIMAL MODELS OF STRESS

There is a large body of evidence that severe physical stress (e.g., trauma, burns, major surgery) can cause gastrointestinal dysfunction and pathology, including stress ulcers, multiple organ dysfunction, bacterial translocation, increased intestinal permeability, etc. To study the possibility of a disease-promoting effect of life stressors, models of nontraumatic “physiological” stress have been developed. Animals may be exposed to stressors that include components of both psychological and physical stress. In choosing models, the trend has been to try to increase the psychological component and decrease the physical component, to better imitate the experience of ongoing environmental/life stress in humans.

The effects of stress resulting from the transport and handling of animals during shipping cannot be overlooked and have been shown to impair the intestinal barrier (16, 27). In fact, a rest period after arrival at the animal facility and daily handling of the animals for 1–2 wk before the study are commonly used to minimize the stress of unusual contact with humans. The most widely used experimental model for studies of intestinal function is acute (a single, relatively short exposure) restraint or immobilization stress in rodents. The animals are immobilized for times from 30 min to 4 h in an adjustable restraint device or by wrap restraint, i.e., gentle wrapping of the upper and lower limbs. These models are all referred to here as restraint stress (RS). A combination of RS with a cold environment [e.g., placing the restrained animal in a cold room (usually at 8°C)] is called cold restraint stress (CRS). Another variant of RS is water immersion restraint stress (WIRS), in which the restrained animals are placed vertically in 20°C water to the level of the xiphoid process for 2–5 h. These models involve elements of physical stress in addition to psychological stress. All have been shown to induce changes in gut barrier function.

Placing animals on a small platform surrounded by room temperature water, termed water avoidance stress (WAS), induces minimal physical stress and therefore may be a more appropriate model of psychological stress. Exposure of rodents to chronic (repeated exposure to a stressor) mild stress, is widely used by psychiatrists as a model of depression and has recently been introduced in studies of intestinal barrier function. Social defeat and deprivation have been used as stress models in primates in psychological research and also in some studies of intestinal barrier function.

STRESS AND INTESTINAL PERMEABILITY

Acute stress. In an early report of stress-induced permeability changes, Saunders et al. (23) found that rats subjected to 4 h of RS or CRS showed overt barrier dysfunction, as assessed by increased jejunal conductance and permeability to the inert marker molecules mannitol and $^{51}$Cr-EDTA, despite normal mucosal structure by light microscopy. A follow-up study showed an enhanced permeability response to CRS in Wistar-Kyoto rats (with low cholinesterase activity) compared with the parent Wistar strain (22), and the possibility that cholinergic nerves were involved in modulating barrier function was further emphasized by inhibition of the stress-induced gut pathophysiology by atropine.

To determine whether the stress-induced epithelial barrier defect extends to macromolecules with antigenic potential, Kiliaan et al. (13) studied transport of a model protein, hors eradish peroxidase (HRP; mol wt 45), across isolated jejunal segments. HRP can be used as a macromolecular probe for flux studies (intact molecule measured by assessing enzymatic activity) as well as to determine passage routes (HRP reaction product visualized in cells and tissues by electron mi-
microscopy). Compared with control rats, Wistar-Kyoto rats exposed to CRS for 2 h showed enhanced jejunal permeability to HRP and $^{51}\text{Cr}-\text{EDTA}$. Moreover, electron microscopy revealed an increased number and size of HRP-containing endosomes in enterocytes and increased HRP within the intraepithelial tight junctions of stressed rats (but not controls).

A stress-induced defect of the colonic barrier was recently reported by Santos et al. (21). Isolated colonic segments from Wistar-Kyoto rats showed elevated conductance, as well as greater permeability to HRP and the bacterial chemotactic peptide N-formylmethionyl-leucyl-phenylalanine (fMLP) after 2 h of CRS. In addition, electron microscopy showed an increased uptake of HRP via both the transcellular and paracellular pathways. The findings were mimicked by injecting rats peripherally with corticotropin-releasing hormone (CRH) and inhibited by CRH antagonists. These results implicate CRH in colonic epithelial pathophysiology after stress.

With in vivo techniques and sugar probes, Meddings and Swain (16) recently confirmed that acute stress increases intestinal permeability. With both RS and swimming stress, signs of abnormal paracellular permeability could be found throughout the gastrointestinal tract by assessing fractional absorption of sucrose, lactulose/mannitol, and sucralose. The barrier defect did not occur in adrenalectomized rats or those treated with a high dose of glucocorticoid receptor antagonist.

**Chronic stress.** A recent clinical study suggests that long-term perceived stress is more important than acute stressful life events for the risk of exacerbation of ulcerative colitis (14). To explore the effects of chronic psychological stress on gut mucosal function, a model of repeated 1-h sessions of WAS was developed (19). The impact of chronic stress on rat growth rate and jejunal epithelial physiology and the role of mast cells in these responses were studied using this model. Stressed rats reduced their food intake and lost weight over 5 days. After chronic stress, epithelial conductance and HRP fluxes were increased in wild-type rats, changes that took several days to repair. Mast cell-deficient rats exposed to the same protocol did lose weight but were normal with respect to epithelial barrier function, suggesting an important role for mast cells in the pathophysiology of stress-mediated barrier disturbances.

**STRESS AND BARRIER FUNCTION**

Secretion of ions and water in response to bacteria and noxious substances present in the epithelial microenvironment is another aspect of intestinal barrier function. In vivo techniques for assessing secretion involve measurements of water/ion fluxes in intestinal loops. As mentioned earlier, experimental stress in humans stimulates water secretion in the jejunum (3, 4, 20), and a similar response was found in ileum and colon of stressed rats (12).

In Ussing chambers, ion secretion can be studied by monitoring short-circuit current ($I_{sc}$) as an indicator of active luminaly directed anion secretion. This technique also allows for studies of bidirectional fluxes of radioactive isotopes, e.g., Cl$^-$ and Na$^+$. Studies with ion-free buffers also provide information on the specific ions involved in secretory responses.

After acute CRS in rats, an elevated baseline $I_{sc}$ was found in isolated jejunal segments (23). Substitution of another anion for Cl$^-$ in the buffers eliminated the abnormality, suggesting that stress stimulates Cl$^-$ secretion. In addition, the magnitude of the $I_{sc}$ response to electrical transmural stimulation of enteric nerves was significantly less in tissues from CRS rats than from control rats, suggesting an impaired neural responsiveness. However, the ability of the epithelium to secrete in response to exogenous stimulation with bezanexol (a cholinergic agonist) or vasoactive intestinal polyopeptide was unimpaired, implicating a neural change in acute stress. In a subsequent study, the finding of an elevated baseline $I_{sc}$ after stress was verified (22) and shown to involve cholinergic nerves. Similar findings regarding $I_{sc}$ were described in the rat colon after CRS (21). Chronic WAS also increased jejunal baseline $I_{sc}$ in wild-type but not mast cell-deficient rats (19).

To summarize, acute CRS in rats increases baseline Cl$^-$ secretion in the jejunum and colon by a mechanism involving neural stimulation, which would imply that stress induces a raised baseline activity of enteric neurons. The ability to respond to noxious substances may, however, be impaired. A similar pattern is seen in chronic WAS and involves mast cells.

**STRESS AND MUCUS SECRETION**

In a series of papers, Castagliuolo et al. (8–10) described the effects of RS on colonic mucin production. Thirty minutes of immobilization stress caused a significant increase in mucin release from colonic mucosal explants and goblet cell depletion by histological evaluation, paralleled by increased colonic mucosal levels of rat mast cell protease II (RMCP II, a product of rat mucosal mast cells), PGE$_2$, and cyclooxygenase-2 (COX-2) mRNA (8). The stress-associated changes were reproduced by injection of CRH in nonstressed rats, and pretreatment of rats with a CRH antagonist or the mast cell stabilizer lodoxamide inhibited the stress-induced effects. A follow-up study (9) confirmed the results and also showed that the peptide neurotensin was involved in the stress-induced release of mucin and activation of mast cells. To directly assess the contribution of mast cells, colonic responses to RS were compared in mast cell-deficient and normal mice (10). Stress stimulated colonic mucin release and goblet cell depletion in normal but not in mast cell-deficient mice, suggesting that mast cells regulate colonic mucin release in response to RS. The findings of stress-activated mucin release were recently corroborated by findings of goblet cell activation by environmental stress in rats (27). Rapid mucin release during acute stress would increase barrier properties and provide a degree of protection against invasion of a leaky epithe-
lium. However, over a longer time period, goblet cell depletion would be deleterious because of the reduced capacity to respond to ongoing or new threats.

STRESS AND OTHER ASPECTS OF BARRIER FUNCTION

Some additional studies deserve mention because they broaden the perspective of the effects of stress on intestinal mucosal function. Colitis models in specific gene knockout mice have in recent years highlighted the importance of the intestinal flora as an important part of mucosal defense. Mimicking stress with a high dose of dexamethasone (see also Ref. 16), Spitz et al. (25) found increased bacterial adherence to the mucosa in steroid-stressed rats associated with impaired colonic IgA secretion. Moreover, permeability to fMLP was increased. In a study of maternal separation at the time of weaning in Rhesus monkeys, Bailey and Coe (2) showed that separation of infants from the mother decreased the number of lactobacilli in their feces and increased their susceptibility to opportunistic bacterial infections. Thus it seems that stress can also alter the microenvironment for resident bacteria and change the conditions for enterocyte bacterial contacts. Finally, in an ultrastructural study of intestinal changes after environmental stress (27) (low vs. high individual activity in housing rooms), pathological changes were found both in the epithelium and in the capillary endothelium, which compromised the epithelial-endothelial exchange barrier.

MECHANISMS INVOLVED IN STRESS-INDUCED BARRIER DYSFUNCTION

Acetylcholine. The early study by Barclay and Turn-berg (3) showed that stress-induced Na\(^+\) and Cl\(^-\) secretion in the human jejunum could be inhibited by intravenous atropine infusion, suggesting a cholinergic parasympathetic nervous mechanism, and studies in rodents have confirmed and expanded these findings. Saunders et al. (22) showed that jejunal Cl\(^-\) secretion after CRS was inhibited by pretreating the rats peripherally (ip) with atropine or atropine methyl nitrate (does not cross the blood-brain barrier) but not with hexamethonium. The same pattern was found regarding changes in jejunal permeability to \(^{51}\)Cr-EDTA. Moreover, the magnitude of the stress response was inversely correlated with mucosal cholinesterase activity in the two rat strains (Wistar > Wistar-Kyoto). The increase in transcellular endosomal uptake and paracellular transport of HRP was also inhibited by atropine (13). Together, these results suggest that acetylcholine mediates stress-induced ion secretion in the jejunum and increased paracellular permeability and transcellular uptake of proteins via muscarinic receptors located in the gastrointestinal tract. The importance of cholinergic mechanisms for the regulation of the intestinal barrier to stress was also shown by Castagliuolo et al. (8), who demonstrated that atropine inhibited colonic mucin and RMCP II release after RS (8).

Mast cells. Studies from various groups have highlighted the importance of mast cells in stress-related changes in intestinal barrier function. Mast cell involvement in stress-induced mucosal changes was first observed as an increased release of RMCP II during RS in mice (8, 9). Moreover, stress-induced enhanced mucin or ion secretion was inhibited by pharmacological stabilization of mast cells with lodoxamide (8) or doxantrazole (21), respectively. Using intestinal perfusion in humans, Santos et al. (20) found that cold pain stress caused water secretion in the jejunum, paralleled by luminal release of the mast cell mediators tryptase and histamine. Studies of mucosal ultrastruc-
ture have found mucosal mast cell activation in combination with various signs of barrier disturbances during stress (13, 26, 27). These results suggest that the central nervous system has the ability to modulate intestinal mast cell activity and that mast cells play a role in stress-related gut mucosal dysfunction. More conclusive evidence for a role for mast cells comes from studies of mast cell-deficient mice and rats. As indicated above, Castagliuolo et al. (10) found that RS-induced mucin and prostaglandin release in the colon did not occur in mast cell-deficient mice, in contrast to normal animals. However, mast cell-deficient mice that had their mast cell population reconstituted by injection of mast cell precursors from normal animals had the same colonic response to stress as mast cell-replete mice. Similarly, Santos et al. (19) showed that the stress-induced rise in jejunal $I_{sc}$ and permeability to macromolecules were absent in mast cell-deficient rats. In wild-type rats subjected to 5-day chronic stress, activated mast cells were identified in the jejunal mucosa and the increase in macromolecule permeability lasted for 3 days after the experimental period. These studies provide direct evidence that intestinal barrier dysfunction after stress is dependent on mast cells and further underline the importance of mast cells in the regulation of intestinal physiology. In contrast to the mucosal changes involving mast cells, motility abnormalities and feeding behavior were not dependent on the presence of mast cells (Ref. 19; Söderholm and Perdue, unpublished observations).

**CRH.** Mast cells are often found close to neurons and are activated by certain neurotransmitter chemicals. Activation of mast cells has been associated with stress-related migraine. The pathways and chemical mediators leading to activation of mucosa mast cells during stress are still unsettled. CRH has been implicated in various stress-induced abnormalities, including those in the gastrointestinal tract. Peripheral (iv or ip) injection of CRH mimics stress-induced changes in colonic function regarding mucin release (8) and ion secretion and permeability (paracellular as well as transcellular) (21). These effects are associated with RMCP II secretion and activation of mast cells shown by ultrastructural examination and are inhibited by doxantrazole. Moreover, RS-induced functional changes of colonic epithelium can be inhibited by the CRH antagonist $\alpha$-helical CRH. On the other hand, the effects of CRH were not inhibited by blocking steroid synthesis, suggesting that adrenal function is not important in the CRH-mediated response (8, 17, 21). The involvement of neurons was suggested by modulation of the CRH-induced response by atropine, hexamethonium, and bretylium (8, 21). Together, the findings in these studies suggest that CRH is important for stress-induced changes in colonic epithelial function and that its effects are mediated by peripherally located receptors (possibly on nerves) and involve activation of mast cells. In another study, Castagliuolo et al. (9) suggested that neurotensin is a candidate for transmitting the CRH-induced effects within the colonic wall. In addition, other cells, including certain inflammatory cells present in the mucosa, have been shown to produce CRH and may be involved in the pathways leading to mucosal barrier dysfunction.

**Other chemical mediators.** In contrast to the lack of a role for glucocorticoids in CRH-induced permeability changes, Meddings and Swain (16) reported a critical role for glucocorticoids in the increase in vivo permeability in rats exposed to environmental stress of various degrees (16). Barrier defects have also been induced by high doses of dexamethasone (25). In addition, Alptekin et al. (1) reported an increased lipid peroxidation and reduced glutathione levels in intestinal tissue after WIRS in rats. Increased exposure to
reactive oxygen species during stress could be one mechanism for increased epithelial permeability.

SUMMARY AND CONCLUSIONS

The studies reviewed here indicate that various types of physical and psychological stress have an impact on several components of intestinal barrier function. Abnormalities have been described in enterocyte physiology as well as goblet cell function. Generally, stress stimulates secretion of ions, water, mucus, and even IgA. Stress-induced epithelial pathophysiology has been reported in several species including humans (although permeability changes per se have not been reported). The stress-induced barrier defect results in enhanced passage into the mucosa of both small molecules, including chemotactic peptides derived from bacteria, and macromolecules, such as intact proteins with antigenic potential. Although it has not been shown precisely, one would expect that ongoing exposure to such proinflammatory molecules would lead to gut inflammation. Certainly, animal models have demonstrated that luminal factors, particularly of bacterial origin, are necessary for the induction of chronic inflammation. Several types of murine genetic mutations that produce a barrier defect can lead to inflammatory bowel disease. In addition, where a specific immune sensitivity is involved (e.g., to a hapten), stress reactivates colitis in association with increased permeability. Finally, altered mucin secretion may affect the binding of microbes to the epithelial cell surface, a process also influenced by IgA. However, the initiation of a chronic intestinal inflammatory process by stress in the absence of an immunological defect has not yet been demonstrated in animals, let alone in humans. A diagram of how stress might impact health through effects on intestinal barrier function is presented in Fig. 1.

Although we are gaining insights into the mechanisms by which stress induces intestinal barrier dysfunction, it is clear that complex interactions are involved. Several studies have implicated mucosal mast cells and components of the hypothalamic-pituitary-adrenal axis.

Mast cells may be activated via neurons releasing CRH and/or acetylcholine, whereas the magnitude of the stress-induced response can be modulated by various other factors. Results are likely to vary in different species and regions of the gut. There are currently significant gaps in our understanding of mechanisms at the cellular and intracellular levels. A simplified schema derived from our own studies on the mechanisms involved in stress-related barrier dysfunction is shown in Fig. 2.

A final point concerns predisposition to stress-induced abnormalities. Certainly, human individuals differ considerably in their ability to cope with stress. Animal studies in rodents highlight strain-dependent genetic differences in gut pathophysiology induced by stress. Outbred rat strains such as Sprague-Dawley include rats that respond to stress with intestinal barrier dysfunction as well as those with no response; however, in specific inbred strains such as Wistar-Kyoto, the incidence of stress-induced intestinal pathophysiology is much higher, almost 100% (M. H. Perdue et al., unpublished observations). Rodent models developed for the study of stress-related reactive depression often also demonstrate stress-induced gastrointestinal abnormalities. In addition to genetic predisposing factors, prior experience is important, as shown by the effect of handling animals on the outcome of the experiment. The stress of being shipped causes increased intestinal permeability that lasts 1–2 wk. At this time, there are no reported studies on the effects and the duration of more severe types of psychological stress to induce intestinal mucosal pathophysiology.

In summary, there is a growing body of evidence that both psychological and physical stress can adversely increase epithelial permeability. What is not clear at the present time is the relevance of these changes for human disease. In the clinical situation, barrier dysfunction, which may initiate and/or promote pathogenic immune reactions, must be put in context with stress-induced immune dysfunction when dealing with symptoms and disease flare-ups related to stress, for example, in patients with inflammatory bowel disease. Much work is needed to define the role of each of the cellular elements and to understand the clinical relevance of these in the search for new therapies for stress-related gastrointestinal pathophysiology. However, it is likely that stress reduction will be shown to be positive for gastrointestinal health and normal function as has been shown for other organ systems.

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