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Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress

JOHAN D. SÖDERHOLM,[1] DERRICK A. YATES,[1] MÉLANIE G. GAREAU,[1]
PING-CHANG YANG,[1] GLENDA MACQUEEN,[2] AND MARY H. PERDUE[1]
[1]*Intestinal Disease Research Program, Department of Pathology and Molecular Medicine, and* [2]*Department of Psychiatry, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5*

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Söderholm, Johan D., Derrick A. Yates, Mélanie G. Gareau, Ping-Chang Yang, Glenda MacQueen, and Mary H. Perdue. Neonatal maternal separation predisposes adult rats to colonic barrier dysfunction in response to mild stress. *Am J Physiol Gastrointest Liver Physiol* 283: G1257–G1263, 2002. First published August 28, 2002; 10.1152/ajpgi.00314.2002.—Intestinal dysfunction is related to stress and early life events, but the mechanisms are largely unknown. Our aim was to determine whether early trauma predisposes adult rats to intestinal mucosal dysfunction in response to stress. Neonatal Sprague-Dawley rats were individually separated from their mothers for 3 h/day at 4–21 days of age. Between *days 80* and *90*, separated and control rats were subjected to mild acute stress (30-min water avoidance) or sham stress. Mucosal barrier function and ion transport were assessed in colonic tissues mounted in Ussing chambers. Mild stress increased short-circuit current, conductance, and transepithelial transport of macromolecules in separated rats, while having minimal effects in controls. Pretreatment of the separated rats with a corticotropin-releasing hormone (CRH) antagonist, the peptide α-helical CRH(9–41) injected intraperitoneally 20 min before stress, abolished the stress-induced mucosal changes. Our results indicate that neonatal trauma can induce phenotypic changes in adulthood, including enhanced vulnerability of the gut mucosa to stress via mechanisms involving peripherally located CRH receptors.

behavior; corticotropin-releasing hormone; electron microscopy; ion transport; intestinal permeability

HUMANS EXPERIENCE STRESS OF various types during daily life, and adequate responses to these stressors are necessary for survival. If the severity or the chronicity of the stressful experience exceeds the adaptive capacity, the individual will be predisposed to illness and disease in multiple organ systems (21). A large number of studies have shown that early-life experience plays an important role in stress responsiveness throughout life (8). In animal models, neonatal adversity can result in permanent functional changes in the stress-mediating systems of the central nervous system (15). For example, newborn rats subjected to maternal separation demonstrate increased release of corticotropin-releasing hormone (CRH), altered expression of glucocorticoid receptors, as well as changes in the norepinephrine and GABA systems (3, 4). Recently, it was also shown in humans that adverse early-life events are associated with hyperresponsiveness to stress and alterations in the hypothalamic-pituitary-adrenal (HPA) axis (10).

There is evidence that early-life trauma and ongoing psychological stress can affect the clinical course of intestinal disorders (16, 17, 20, 26) and also reactivate inflammation in experimental colitis (6, 9, 22, 25). The mechanisms underlying stress-induced exacerbations of intestinal diseases are, however, largely unknown. One possible link between intestinal disease and stress is a change in mucosal function. Our previous studies have shown that acute stress in rats induces enhanced intestinal epithelial permeability to macromolecules and increased chloride secretion, by mechanisms involving CRH and mast cells (32). We also found that genetic factors modulate the mucosal stress response. For example, inbred Wistar-Kyoto rats have an augmented stress-induced mucosal dysfunction compared with their background Wistar strain (30). On the other hand, outbred Sprague-Dawley rats are relatively stress resistant (P. R. Saunders and M. H. Perdue, unpublished data). Recently, Coutinho et al. (7) reported that rats exposed to neonatal trauma in the form of maternal separation showed visceral hyperalgesia and increased colonic motility in response to stress at 2 mo of age. Also, Al Chaer et al. (1) demonstrated that local manipulation of the colon in neonatal rats induces chronic visceral hypersensitivity to painful stimuli. Thus neonatal trauma can change colonic neuromuscular function in adult rats. However, effects of early-life experience on the function of the intestinal mucosa have not been reported previously.

Our aim in this study was to determine, in outbred Sprague-Dawley rats, if early psychological trauma predisposes adult rats to develop intestinal mucosal dysfunction in response to mild stress. In other words, we tested the hypothesis that early-life experience can

Address for reprint requests and other correspondence: M. H. Perdue, Intestinal Disease Research Program, HSC-3N5C, McMaster Univ., 1200 Main St. West, Hamilton, Ontario, Canada L8N 3Z5.

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result in long-lasting phenotypic changes in mucosal function. Rats subjected to maternal separation in the neonatal period were exposed to mild acute stress as adults, and colonic mucosal function was studied. We found that, indeed, early psychological trauma was a predisposing factor for stress-induced hyperresponsiveness in the colonic mucosa, resulting in a secretory state and defective barrier function. Such changes may be important in the etiology and/or pathophysiology of intestinal disorders, such as irritable bowel syndrome or inflammatory bowel disease.

METHODS

Animals

Primiparous timed-pregnant Sprague-Dawley female rats were obtained from Charles River Laboratories (St. Constant, Quebec) on gestational *days 15–16*. Dams were individually housed in cages containing bedding material on a 12:12-h light-dark cycle (lights on at 8:00 AM) and provided with food and water ad libitum. The Animal Care Committee at McMaster University approved all procedures.

Study Design

Rat pups were separated from the dams from *days 4* to *21*, whereas control pups remained with their mothers. Behavioral tests were performed at *days 50–70*. At *days 80–90*, separated and control rats were subjected to mild acute stress, and intestinal function was studied. In a second set of experiments, to study involvement of CRH, the rats were injected intraperitoneally with a CRH antagonist 30 min before the stress exposure.

Maternal Separation Protocol

Dams and their litters were assigned to the control (nonseparated) protocol or to the maternal separation protocol. Separated pups were removed from their cages and dams at postnatal *days 4–21* for 180 min daily by placing them in individual compartments within plastic cages, in an adjacent room from their home cages. The new cages were lined with chip bedding and kept at 37 ± 0.5°C by using a heating pad placed underneath the cages. Separation occurred at 9:00 AM ± 30 min each day to minimize the effect of circadian rhythm. Control pups were not handled and were maintained in their home cages with the dams until they were weaned. Litters were culled to eight pups per dam at postnatal *day 7* to ensure even litter sizes and maternal care. At *day 23*, the pups' sex was determined, and they were weaned and housed in individual cages. After weaning, pups were weighed weekly. Only male animals were utilized in this study to avoid variations due to hormonal cycling.

Behavioral Tests

At postnatal *days 50–70*, the rats underwent behavioral tests. These tests have previously been shown to be affected by maternal separation in rodents (2, 12, 27) and were mainly performed to evaluate the separation model. The three procedures were conducted in the following order.

Sucrose test. Rats were habituated to sucrose for 3 days by having free access to 50 ml/day of 5% sucrose solution. On *days 4–6*, the amount of sucrose ingested over 30 min was recorded and averaged for each rat.

Novel object test. Rats were habituated to a new cage for 1 h before a novel object was placed inside the cage. Exploratory behavior was videotaped for 10 min and analyzed for latency to smell, frequency of smell, latency to touch, frequency of touch, and duration of touch.

Activity test. Locomotor activity was monitored and quantified with rats placed individually in an open-field activity system consisting of a 40 × 40 × 40-cm clear Plexiglas box within a 4 × 4 matrix of photo beams, which electronically monitored horizontal and vertical locomotive movement for 90 min. The software counted and stored total horizontal as well as vertical beam interruptions in 5-min bins.

Mild Acute Stress

At postnatal *days 80–90*, separated and control rats were subjected to either water avoidance stress (WAS) or sham stress (SS). All experimental stress procedures were performed between 9:30 and 11:30 AM, to minimize the effect of circadian rhythm. For WAS, rats were placed on a platform in a container filled with warm water (25°C, to 2 cm below the platform) for 30 min. Rats avoided the aversive stimulus by remaining on the platform. For SS, rats were placed in an empty container (no water) on a platform for 30 min. After the stress or sham period, rats were immediately killed by decapitation. Colonic segments were removed and placed in 37°C oxygenated Krebs buffer.

Role of CRH

In a second set of experiments, to study mechanisms involved, separated and control rats were injected ip with the nonselective CRH_1/CRH_2-receptor antagonist α-helical CRH(9–41) (Peninsula Laboratories, Belmont, CA), or vehicle, 30 min before being subjected to WAS or SS. The α-helical CRH was dissolved according to the manufacturer's instructions, aliquoted, and kept frozen until used. Immediately before the experiments, the peptide was diluted in saline for intraperitoneal injections of 250 μg/kg body wt. This dose has previously been shown to inhibit CRH-induced barrier dysfunction (28).

Ussing Chamber Studies

The distal colon was removed, placed in 37°C oxygenated Krebs buffer, stripped of longitudinal muscle and myenteric plexus, and opened along the mesenteric border. Four adjacent pieces from each rat were mounted in Ussing chambers (W-P Instruments, Narco Scientific, Mississauga, Ontario). The chamber opening exposed 0.6 cm^2 of tissue surface area to 8 ml of circulating oxygenated Krebs buffer at 37°C. The buffer contained (in mM) 115 NaCl, 1.25 $CaCl_2$, 1.2 $MgCl_2$, 2.0 KH_2PO_4, and 25 $NaHCO_3$, pH 7.35 ± 0.02. In addition, the serosal buffer also contained 10 mM glucose as an energy source that was osmotically balanced by 10 mM mannitol in the mucosal buffer. The chambers contained agar-salt bridges to monitor the potential difference across the tissue and to inject the required short-circuit current (I_{sc}) to maintain a zero potential difference as registered via an automated voltage clamp (W-P Instruments). I_{sc} (μA/cm^2) was recorded continuously by a computer connected to the voltage clamp system. Tissue conductance was calculated according to Ohm's law and expressed as millisiemens per centimeter squared. Baseline values for I_{sc}, as an indicator of ion secretion, and conductance, an indicator of ion permeability, were calculated at equilibrium, 15 min after the tissues were mounted.

Macromolecular Permeability

Horseradish peroxidase (HRP) (Sigma) was used as a model protein probe to examine macromolecular permeability. Fifteen minutes after the tissues were mounted, type VI

Table 1. *Behavioral studies in separated and nonseparated rats*

	Nonseparated Controls	Maternal Separation
n	6–9	7–8
Sucrose test, ml/30-min period	19.7 ± 2.9	10.9 ± 2.9*
Novel object test		
Latency to smell, s	9.0 ± 3.2	12.8 ± 3.8
Frequency of smell, no./10 min	66.2 ± 5.4	42.8 ± 5.9*
Latency to touch, s	15.0 ± 4.6	224 ± 81.8*
Frequency of touch, no./10 min	9.3 ± 1.7	3.1 ± 0.8*
Duration of touch, s/10 min	27.4 ± 7.2	9.5 ± 2.7*

Values are means ± SE; *n,* no. of rats. Sucrose test, after habituation to sucrose for 3 days, the amount of sucrose ingested over 3 separate 30-min periods was recorded. Novel object test, a novel object was placed inside the cage, and exploratory behavior was videotaped and analyzed. *$P < 0.05$ compared with nonseparated rats.

HRP (10^{-5} M) was added to the luminal buffer and allowed to equilibrate for 30 min. Serosal samples (0.5 ml) were obtained at 30-min intervals for 2 h and replaced with buffer to maintain a constant volume in the chambers. HRP activity was determined by a modified Worthington method, as previously described (14). In brief, 150 μl of serosal sample were added to 800 μl of phosphate buffer containing 0.003% H_2O_2 and 80 μg/ml *o*-dianisidine (Sigma). Enzyme activity was determined from the rate of increase in optical density at 460 nm. The mucosal-to-serosal flux of HRP was the average value of two consecutive stable flux periods and expressed as picomoles per centimeter squared per hour.

Epithelial Uptake of HRP

In separate chambers, colonic tissues were removed 90 min after the addition of HRP into the luminal compartment. The tissues were immediately fixed in 2.5% gluteraldehyde in 0.1 M sodium-cacodylate buffer (pH 7.4) for 2 h at 22°C, rinsed for 18 h (4°C) with 0.05 Tris buffer (pH 7.6), and washed three times, for 5 min each time. Methods for HRP product identification have been previously described (14). Quantitative analysis of HRP uptake in intracellular endosomes and paracellular HRP transport were performed on coded high-magnification photomicrographs, 12 per rat (3 rats/group). The total area of HRP-containing endosomes within colonocytes was determined in an area of 300 μm^2 in the apical region of the cells, by using a computerized image analysis system (Kontron Mop Videoplan, Kontron, Eching, Germany) (28, 29).

Statistics

Results are expressed as means ± SE unless otherwise stated. Groups were compared with ANOVA or Student's *t*-test as applicable. Differences with $P < 0.05$ were considered significant.

RESULTS

Maternal Separation Reduced Weight Gain and Altered Behavior

At weaning, immediately after the separation period, body weight was similar in separated (47 ± 3.5 g) and control (49 ± 2.8 g) rats. However, subsequently, the separated rats gained less weight, so that at *day 90* they weighed less than the control rats (269 ± 23 and 340 ± 4 g, respectively, $P < 0.01$).

The ingestion of sucrose solution (mean volume/30 min on 3 consecutive days) was significantly decreased in rats subjected to maternal separation (Table 1). Also, the response to a novel object differed between the separated and control rats. The separated rats showed less exploratory behavior when a novel object was put into the cage compared with the control rats (Table 1).

Moreover, locomotor activity, assessed in an activity box, demonstrated an altered movement pattern in the separated group. Compared with controls, separated rats showed reduced movement in the horizontal plane and less time in motion (Table 2).

Maternal Separation Altered Stress-induced Colonic Mucosal Physiology in Adult Rats

In control rats, exposure to a 30-min period of WAS at *day 90* did not alter mucosal conductance and macromolecular permeability compared with SS (Fig. 1). Conversely, in separated rats, the mild stress induced a significant increase in colonic mucosal conductance, from 28 ± 4.2 to 42 ± 6.3 mS/cm^2 ($P < 0.05$; Fig. 1*A*) and in macromolecular permeability assessed as flux of HRP (10 ± 2.8 vs. 35 ± 8.1 pmol·h^{-1}·cm^{-2}, $P < 0.05$; Fig. 1*B*). In addition, increased endosomal uptake of HRP into epithelial cells was demonstrated by endosomes in colonic tissues from separated rats subjected to WAS (see Fig. 4) (Table 3; $P < 0.01$, SS vs. WAS).

Ion transport, assessed as baseline I_{sc}, was low in tissues from sham-stressed control rats (Fig. 2). Thirty-minute WAS induced a rise in I_{sc}, from 30 ± 6.6 to 50 ± 7.1 μA/cm^2 ($P < 0.05$) in these rats. Sham-stressed separated rats showed a higher baseline secretory state (47 ± 4.5 μA/cm^2) compared with controls ($P < 0.05$), which was further increased in rats exposed to WAS (69 ± 8.0 μA/cm^2; $P < 0.01$ vs. controls) (Fig. 2). Our laboratory (31) previously showed that the acute stress-induced rise in I_{sc} was due to luminally directed Cl^- secretion.

Table 2. *Locomotor activity in an open-field monitoring system in separated and nonseparated rats*

	Nonseparated Controls	Maternal Separation
n	6	9
Movements, no./5 min	31 ± 2.7	78 ± 9.0*
Movement time, s/5 min	33 ± 4.0	12 ± 2.3*
Horizontal activity, beam interruptions/5 min	760 ± 71	398 ± 80*
Vertical activity, beam interruptions/5 min	24 ± 4.9	102 ± 11*

Values are means ± SE; *n,* no. of rats. Locomotor activity was monitored with rats placed individually in an open-field activity system in a Plexiglas box within a 4 × 4 matrix of photo beams, which electronically monitored movement in 5-min bins for 90 min. *$P < 0.05$ compared with nonseparated rats.

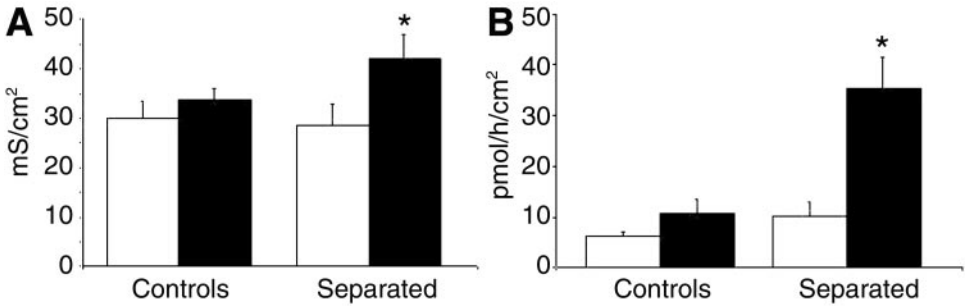

Fig. 1. Effects of maternal separation on mucosal barrier function assessed as electrical conductance (*A*) and transmucosal flux (*B*) of the macromolecule horseradish peroxidase (HRP). Nonseparated controls (*left*) and rats subjected to maternal separation (*right*) were exposed to sham stress (open bars) or 30-min water avoidance stress (WAS; solid bars). Values are means ± SE; n = 6–7 rats/group. *$P <$ 0.05 vs. sham-stressed nonseparated and separated rats.

CRH Antagonism Abolished the Stress-induced Colonic Mucosal Responses in Separated Rats

In a second set of experiments, to elucidate the involvement of CRH in the altered mucosal response to mild acute stress in separated rats, we studied the effects of intraperitoneal injection of the specific, nonselective CRH antagonist α-helical CRH(9–41) before exposure of rats to WAS or SS. In the separated rats, α-helical CRH inhibited the stress-induced increases in macromolecular permeability (HRP flux, from 20 ± 6.2 to 8.5 ± 3.6 pmol·h^{-1}·cm^{-2}; $P < 0.05$) and ion secretion (I_{sc}, from 58 ± 7.6 to 28 ± 5.1 μA/cm^2; $P < 0.05$) (Fig. 3). The enlarged area of HRP containing endosomes was also diminished by intraperitoneal α-helical CRH (Fig. 4*C*, Table 3; $P < 0.05$, WAS + α-helical CRH vs. WAS + saline). No significant effects of α-helical CRH were seen in sham-stressed separated animals (SS + α-helical CRH: conductance, 26 ± 2.1 mS/cm^2; HRP flux, 7.4 ± 0.6 pmol·h^{-1}·cm^{-2}; I_{sc}, 34 ± 1.5 μA/cm^2; $P > 0.05$ vs. SS + saline; n = 5–6 rats/group). In this second set of experiments, WAS induced no significant changes in control rats regarding mucosal physiology (data not shown). Neither did α-helical CRH(9–41) affect permeability or ion transport in stressed control rats (WAS + α-helical CRH: conductance, 22 ± 3.6 mS/cm^2; HRP flux, 6.2 ± 1.2 pmol·h^{-1}·cm^{-2}; I_{sc}, 29 ± 1.5 μA/cm^2; n = 5 rats/group, $P > 0.05$ vs. WAS).

Table 3 *Effects of acute stress and CRH antagonism on endosomal uptake of HRP in colonocytes of separated and control rats*

	Endosomal Area, μm²/300 μm²		
	Sham stress	WAS + Saline	WAS + α-CRH
Controls	1.9 ± 0.2	3.8 ± 0.9	2.2 ± 0.4
Separated	2.0 ± 0.2	7.5 ± 0.8*†	2.9 ± 0.3‡

Values are means ± SE. Twenty minutes before being subjected to sham stress or 30-min water avoidance stress (WAS) the rats were injected intraperitoneally with saline or α-helical corticotropin-releasing hormone (CRH) (9–41). Data show total area of horseradish peroxidase (HRP)-containing endosomes within colonocytes determined in an area of 300 μm^2 in the apical region of the cells (n = 3 rats per group; 12 randomly chosen cells assessed per rat). *Increased compared with separated sham, $P < 0.01$. †Increased compared with WAS + saline in controls, $P < 0.05$. ‡Decreased compared with WAS + saline, $P < 0.01$. Stress effects on endosomal uptake of HRP in the control group were not statistically significant (ANOVA).

DISCUSSION

In the present study, we show that rats exposed to postnatal maternal separation develop a vulnerability to acute stress later in life that is manifest by colonic mucosal dysfunction. Sprague-Dawley rats, which are normally relatively stress resistant, demonstrated increased permeability to antigens (mainly via endosomal uptake) and augmented ion secretion in response to a mild stressor. In addition, we found that the stress-induced mucosal changes in separated rats were abolished by intraperitoneal injection of a nonselective CRH receptor antagonist. Our results suggest that neonatal psychological trauma can alter the adult phenotype of the intestinal mucosa via mechanisms involving peripherally located CRH receptors.

A more tentative behavior and decreased gain of body weight after weaning were features of the separated rats. These findings agree with previous studies from behavioral research (15). Maternal separation initiates a complex bio-behavioral response, including decreased secretion and suppression of cell responses to trophic hormones, e.g., growth hormone and insulin, leading to growth retardation. It is also well established that neonatal psychological trauma in rodents is associated with increased CRH content in hypothalamic neurons and increased release in response to stress (2, 3, 11, 24), which leads to an enhanced HPA

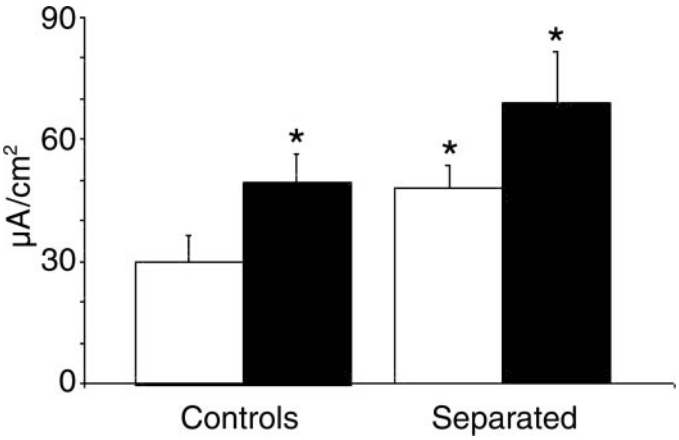

Fig. 2. Effects of maternal separation on mucosal ion transport assessed as short-circuit current. Nonseparated controls (*left*) and separated rats (*right*) were subjected to sham stress (open bars) or 30-min WAS (solid bars). Values are means ± SE; n = 6–8 rats/group. *$P < 0.05$ vs. sham-stressed controls.

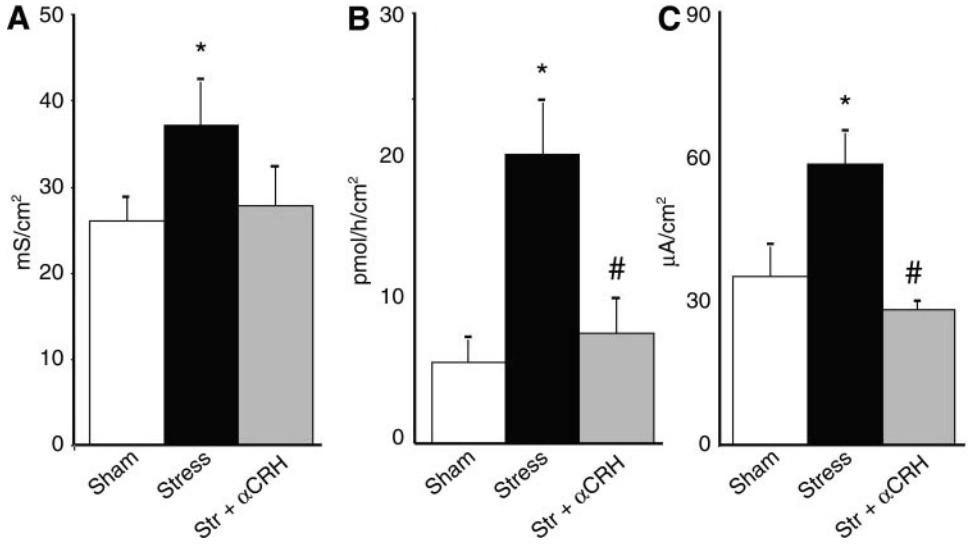

Fig. 3. Effects of corticotropin-releasing hormone (CRH) antagonism on separation-induced alterations in mucosal response to acute stress. Data are given for electrical conductance (*A*), transmucosal flux of HRP (*B*), and short-circuit current (*C*). Twenty minutes before being subjected to sham stress (open bars) or 30-min WAS (solid bars), separated rats were injected intraperitoneally with saline (solid bars) or α-helical CRH(9–41) (shaded bars). Values are means ± SE; n = 5–8 rats/group. Str, stress; α, α-helical. *Increased compared with sham, $P < 0.05$; #decreased compared with stress + saline, $P < 0.05$. α-Helical CRH(9−41) did not affect mucosal variables in sham-stressed animals.

response to stress in adulthood (15). Moreover, separation leads to an increased CRH release and an enhanced postsynaptic response in the limbic system. This CRH neuronal hyperactivity is associated with anxiety disorders and is believed to mediate certain behavioral symptoms in depression (3).

CRH has also been implicated in stress-induced intestinal abnormalities (5, 28, 34). CRH injected into the central nervous system increased motility, mucin release, and mucosal mast cell degranulation in the rat colon (34). This central action of CRH to stimulate colonic motor function was shown to be unrelated to activation of the HPA axis but rather to parasympathetic modulation. In addition, peripheral (iv or ip) injection of CRH mimicked stress-induced changes in colonic function regarding mucin release (5), ion secretion, and permeability (28). Moreover, acute stress-induced functional changes of colonic epithelium were inhibited by intraperitoneal injection of the CRH antagonist α-helical CRH (28). On the other hand, CRH effects could not be inhibited by blocking steroid synthesis, suggesting that glucocorticosteroids are not important in the CRH-mediated mucosal stress response (5, 22, 28). The involvement of neurons was suggested by modulation of the CRH response by atropine, hexamethonim, and bretylium (5, 28). Peripheral effects of CRH have been shown to regulate colonic motor activity (18), and expression of CRH and CRH receptors is also found in the colonic mucosa of rodents (19) and humans (13, 23). Coutinho et al. (7) recently reported that maternal separation induced visceral hyperalgesia and increased colonic motility in adult rats. The

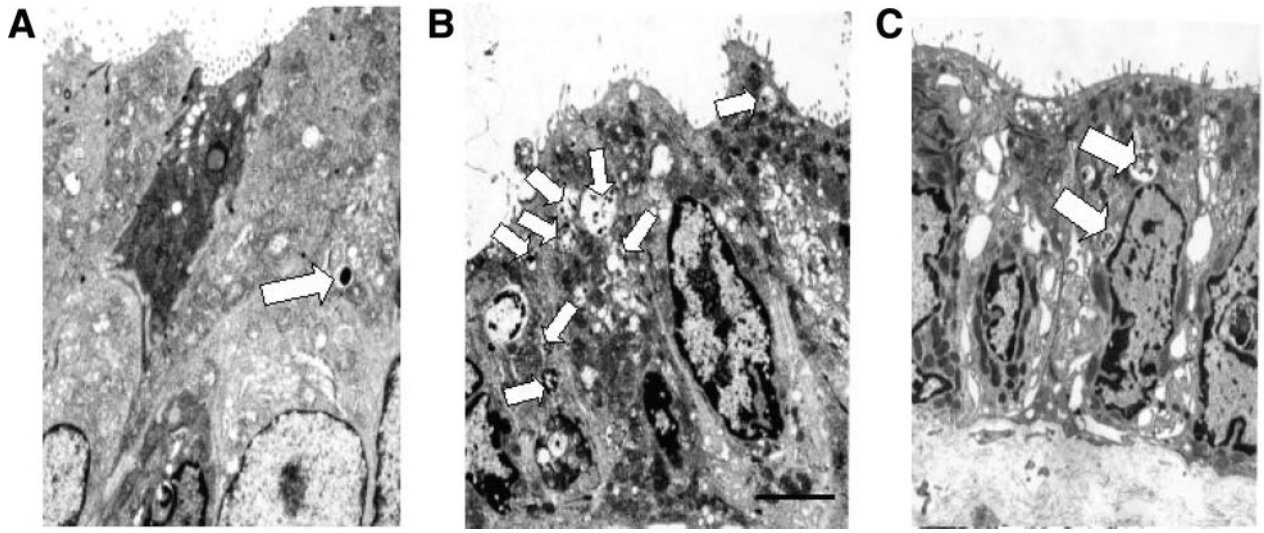

Fig. 4. Effects of stress and CRH antagonism on endosomal uptake of HRP in colonocytes of separated rats. *A*: transmission electron photomicrograph showing few HRP-filled endosomes (arrow) in colonocytes after exposure to sham stress. *B*: larger and more numerous HRP-filled endosomes (arrows) in the colonic epithelium of a separated rat subjected to WAS after intraperitoneal injection of saline. *C*: colonocytes with less endosomal uptake of HRP (arrows) from a rat injected with the CRH antagonist α-helical CRH(9–41), 20 min before being subjected to WAS. Original magnification: ×5,000.

mechanisms mediating that abnormality have not been fully elucidated.

In the present study, we found that a specific, nonselective CRH_1/CRH_2-receptor antagonist injected peripherally had no effects on mucosal physiology by itself, but abolished the mucosal hyperresponsiveness to stress in the separated rats. Although passage of small amounts of CRH through the blood-brain barrier in stressed animals cannot be ruled out, our data suggest that peripheral CRH receptors, possibly located within the colonic mucosa, are involved in the mucosal changes induced by early-life stress. These findings indicate that CRH is important for the early-life stress-induced changes in colonic epithelial function, and that its effects could be mediated by peripheral release and/or peripherally located receptors. Further studies are needed to characterize the CRH receptor subclasses and location of these receptors.

In previous studies, our laboratory (30) found an enhanced mucosal response to acute stress in genetically susceptible Wistar-Kyoto rats (deficiency in cholinesterase activity) compared with the parent Wistar strain. A genetic variation in intestinal stress response has also been documented in other rat strains, e.g., Fischer and Lewis rats (18). In this study, we sought to determine whether neonatal stress, which causes phenotypic changes in behavior and systemic parameters, also affects the sensitivity to stress-induced functional changes of the colonic mucosa in genetically stress-resistant Sprague-Dawley rats. The separated Sprague-Dawley rats developed increased macromolecular permeability in response to a relatively mild acute stressor (30-min WAS) in adult life. Whereas barrier function was unaffected by WAS in the control group, the separated rats demonstrated barrier dysfunction to a similar extent as the stress-susceptible Wistar-Kyoto rats in previous studies (35). In other words, our data support the hypothesis that environmental as well as genetic factors are important in determining stress responsiveness of the intestinal mucosa.

The stress-induced mucosal barrier defect in separated rats was a combination of increased paracellular leakage via tight junctions, as shown by increased conductance, and increased transcytosis of macromolecules, as shown by endosomal uptake and HRP flux. In two recent studies (29, 33), our laboratory found that rats exposed to chronic stress develop a prolonged barrier defect to macromolecules and epithelial mitochondrial damage (29). Longer exposure to stress induces bacterial internalization into the epithelium and inflammatory cell infiltration in the lamina propria, suggesting that stress can be important in the initiation of intestinal inflammation (33). Thus neonatal stress-induced mucosal abnormalities may have implications for pathogenesis as well as symptoms in intestinal diseases. An increased tendency for intestinal ion and fluid secretion would make stress-susceptible individuals more prone to diarrhea, and a more vulnerable intestinal barrier would predispose stress-susceptible individuals to inflammation because of uptake of proinflammatory luminal antigens. Our studies predict that peripheral CRH receptors may be a useful target for treatment of stress-related symptoms in patients with irritable bowel syndrome and/or inflammatory bowel disease.

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