Bacterial translocation can increase plasma corticosterone and brain catecholamine and indoleamine metabolism

TETSUYA ANDO,1 RHONDA F. BROWN,1 RODNEY D. BERG,2 AND ADRIAN J. DUNN1
Departments of 1Pharmacology and Therapeutics and 2Microbiology and Immunology, Louisiana State University Medical Center, Shreveport, Louisiana 71130

Received 18 January 2000; accepted in final form 10 August 2000

Ando, Tetsuya, Rhonda F. Brown, Rodney D. Berg, and Adrian J. Dunn. Bacterial translocation can increase plasma corticosterone and brain catecholamine and indoleamine metabolism. Am J Physiol Regulatory Integrative Comp Physiol 279: R2164–R2172, 2000.—The potential contribution of stress-induced bacterial translocation to the activation of the hypothalamo-pituitary-adrenocortical (HPA) axis and brain biogenic amines was assessed. Mice were restrained for various periods, and brain concentrations of tryptophan, catecholamines, serotonin, and their metabolites, plasma corticosterone, and the translocation of viable bacteria from the gastrointestinal tract to the mesenteric lymph nodes, spleen, and liver were measured. Restraint induced the translocation of indigenous gram-positive bacteria in only a small proportion of animals, but translocation of gram-negative bacteria did not occur. Restraint induced short-lived increases in plasma corticosterone and brain amine metabolism, whereas bacterial translocation was slower and persisted long after the HPA axis and neurochemical responses had dissipated. When mice were infected with Salmonella typhimurium, spontaneous translocation occurred and plasma corticosterone, interleukin-6 concentrations, and brain catecholamine and indoleamine metabolism were elevated. These findings indicate that the translocation of indigenous gastrointestinal bacteria did not contribute to the HPA axis and neurochemical changes induced by restraint. However, translocation of nonindigenous S. typhimurium with or without restraint did induce HPA and neurochemical responses.

Salmonella typhimurium; tryptophan; catecholamines; serotonin; corticosterone

VARIOUS STRESSORS (e.g., burn, surgery, and hemorrhagic shock) promote the translocation of indigenous bacteria from the gastrointestinal (GI) tract to extraintestinal sites, including the mesenteric lymph node (MLN) complex, liver, and spleen (4). The cell walls of gram-negative bacteria contain lipopolysaccharide (LPS), which is a strong immune stimulator. LPS stimulates not only the immune system but also has direct vascular effects and induces changes in the central nervous system (CNS). There is considerable similarity in the neurochemical and hypothalamo-pituitary-adrenocortical (HPA) axis responses to stressors such as electric shock and restraint and to LPS. They each activate the HPA axis, as well as brain catecholamine and indoleamine metabolism, and increase brain tryptophan concentrations (9, 10, 12, 13). Therefore, it is possible that LPS released from translocating gram-negative bacteria may contribute to these endocrine and neurochemical responses to stress. Thus we sought to determine whether 1) restraint induces the translocation of indigenous microflora bacteria in normal mice; 2) the translocation of indigenous microflora is associated with HPA axis activation and changes in brain catecholamine and indoleamine metabolism; and 3) bacterial translocation contributes to the restraint-induced changes in HPA axis activity and brain catecholamine and indoleamine metabolism.

MATERIALS AND METHODS

Animals. Male CD-1 virus antigen-free (VAFplus) mice (5–6 wk old) weighing 25–35 g were obtained from Charles River (Raleigh-Durham facility colony R16). Mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility under specific pathogen-free conditions on a 12:12-h dark-light cycle (lights on 7:00 AM) at 22–23°C. Food (Teklad food pellets) and acidified water (0.001 N HCl) were provided ad libitum. Three days before each experiment, mice were housed in individual cages to avoid problems associated with group housing.

Restraint. Mice were restrained by placing them in 45-ml centrifuge tubes for periods of 0.5–4 h as previously described (11).

Antibiotic decontamination and monoassociation with Salmonella typhimurium. Mice were given drinking water ad libitum containing streptomycin sulfate (2 mg/ml) and penicillin G (1,500 U/ml) for 3 days to reduce the indigenous GI microflora. S. enteritidis typhimurium was provided by Dr. Earl Steffen, University of Missouri-Columbia, and was cultured at 37°C overnight with shaking in brain-heart infusion. Mice were inoculated with S. typhimurium by placing the viable cultures on their food and in the drinking water. Antibiotic pretreatment followed by oral inoculation with S. typhimurium is a manipulation intended to reduce the indigenous GI microflora, allowing overgrowth by S. typhimurium and thereby promoting its translocation from the GI tract to extraintestinal sites (3, 16). Intestinal bacterial overgrowth

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is one of three primary mechanisms that promote bacterial translocation from the GI tract. The other two are impaired host-immune defenses and physical damage to the intestinal mucosa (3, 4). Each animal received approximately the same amount of viable *S. typhimurium* culture.

Mice were killed by decapitation, and trunk blood was collected in Eppendorf tubes containing 20 μl of 1.5 M EDTA-Na<sub>2</sub> for assay of corticosterone and interleukin-6 (IL-6). The brain was then rapidly excised and frontal cortex, hypothalamus, and brain stem were removed as previously described (13). Tissues were promptly weighed and frozen on dry ice.

**HPLC analysis of brain biogenic amines.** Brain tissue samples were processed for HPLC analysis as previously described (11). The following metabolites were separated and quantified: norepinephrine (NE), normetanephrine, 3-methoxy-4-hydroxyphenylethylenglycol (MHPG), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid, 3-methoxytyramine, serotonin (5-hydroxytryptamine; 5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and tryptophan. 

**Radioimmunoassay and ELISA.** Corticosterone was assayed by using [125I] radioimmunoassay kits supplied by ICN Biomedical (Costa Mesa, CA) as described by the manufacturer. Plasma IL-6 was determined by sandwich ELISA using anti-mouse IL-6 capture antibody and anti-mouse IL-6 detecting antibody purchased from PharMingen (San Diego, CA) according to the manufacturer’s protocol. The detection limits for the corticosterone and IL-6 assays were 12 ng/ml and 4 pg/ml, respectively.

**Assessment of bacterial translocation.** An incision was made with sterile instruments through the skin and peritoneum of the abdomen. The MLN complex, spleen, liver, and cecum were removed aseptically and placed in preweighed glass grinding tubes (Glas-Col Apparatus, Terra Haute, IN) containing sterile saline. The organs were weighed and then homogenized with glass-reinforced Teflon tissue grinders. Portions of the MLN, spleen, and liver were cultured aerobically on blood agar for gram-positive and gram-negative bacteria and on MacConkey agar select for gram-negative enteric bacilli. The plates were examined after 24-h incubation at 37°C, and the colony-forming units (CFUs) were counted. Bacteria from the colonies were gram stained.

**Statistical analysis.** Data are presented as means ± SE and were analyzed by one- or two-way ANOVA followed by Fisher's least-significant difference (LSD) test or Student’s *t*-test. Values of CFU and IL-6 were converted to their logarithms for comparisons. When zero values were recorded, one was added to every value before being converted to its logarithm. A nonparametric test (Kruskal-Wallis test) was used when appropriate. Comparisons of the incidence of translocation were made by using Fisher’s exact test. Significance was defined at the 0.05 level.

**RESULTS**

**Translocation of indigenous intestinal microflora.** In the first experiment, we measured bacterial translocation to the MLN, liver, and spleen as well as plasma corticosterone and brain catecholamines, indoleamines, and metabolites immediately after 30 min, 1, 2, or 4 h of restraint. No bacterial colonies were observed on the blood agar plates in samples from unrestrained mice, but when mice were restrained for 1 h or more, bacteria were detected on blood agar at a low incidence (Fig. 1). The percentages of mice showing translocation in any organs at 0.5, 1, 2, and 4 h were 0, 11, 50, and 30%, respectively. No colonies were detected on MacConkey agar plates, indicating the absence of gram-negative enteric bacteria.

All restraint treatments (0.5–4 h) resulted in significant elevations of plasma corticosterone concentrations (Fig. 2A). MHPG/NE ratios in frontal cortex were elevated after 1-h restraint, whereas MHPG/NE ratios in hypothalamus and brain stem were elevated at all times, with a tendency to decrease with increasing durations of restraint (Fig. 2B). 5-HIAA/5-HT ratios were elevated in frontal cortex after 1 h or more of restraint, and in hypothalamus and brain stem after 2 h or more of restraint (Fig. 2B). Tryptophan concentrations were increased significantly in frontal cortex after 1 h of restraint and in hypothalamus and brain stem at all measured times. Dopaminergic systems were also activated as indicated by increases in DOPAC/DA ratios in hypothalamus after 0.5, 2, and 4 h and in brain stem after 2 h (Fig. 2B). To compare plasma corticosterone concentrations and the various neurochemical measures between mice that exhibited translocation and those that did not at 2 and 4 h, Student’s *t*-test with Bonferroni correction was per-
formed. Although there was a tendency for the DOPAC/DA ratio in the brain stem of mice in which translocation occurred to be higher than that in mice in which it did not, the difference did not reach statistical significance. None of the other measures was significantly different.

A second experiment was performed in which mice were restrained for 4 h and then killed at various subsequent times (4, 8, 24, and 48 h) after restraint was initiated. Bacteria were detected in samples plated on blood agar with slightly higher magnitude and incidence (Fig. 3) than in the first experiment. The percentage of mice with translocation to any organs at 4, 8, 24, and 48 h were 60, 70, 40, and 50%, respectively. The peak magnitude and incidence of translocation in the MLN and liver were observed at 8 h (i.e., 4 h after the restraint was terminated), but translocation to the spleen occurred in only one animal at 48 h. No bacteria were detected on MacConkey agar plates, again indicating no translocation of gram-negative enteric bacilli.

Plasma corticosterone concentrations were elevated at the end of the 4-h restraint period but returned to (Fig. 3) than in the first experiment. The percentage of mice with translocation to any organs at 4, 8, 24, and 48 h were 60, 70, 40, and 50%, respectively. The peak magnitude and incidence of translocation in the MLN and liver were observed at 8 h (i.e., 4 h after the restraint was terminated), but translocation to the spleen occurred in only one animal at 48 h. No bacteria were detected on MacConkey agar plates, again indicating no translocation of gram-negative enteric bacilli.

Plasma corticosterone concentrations were elevated at the end of the 4-h restraint period but returned to
control levels by 8 h (Fig. 4A). MHPG/NE ratios in all three brain regions were elevated at the end of the restraint period but had returned to control levels by 8 h (Fig. 4B). 5-HIAA/5-HT ratios in the frontal cortex and hypothalamus, tryptophan concentrations in the frontal cortex and hypothalamus, and DOPAC/DA ratios in the hypothalamus and brain stem followed a similar pattern. However, 5-HIAA/5-HT ratios in the brain stem remained slightly elevated at 8 h, but not at 24 or 48 h (Fig. 4B). The average values of plasma corticosterone concentrations and the various neurochemical measures were compared between mice with and without translocation at 4, 8, 24, and 48 h by Student's t-test. The average 5-HIAA/5-HT ratios in the hypothalamus and brain stem at 4 h tended to be higher in mice with translocation (0.595 ± 0.036 and 0.880 ± 0.047, n = 6) than those without (0.548 ± 0.017 and 0.753 ± 0.019, n = 4), although the P values did not reach the significance level after adjustment by the Bonferroni procedure (P = 0.001 > 0.05/52 and P = 0.04 > 0.05/52, respectively). There were no statistically significant differences in plasma corticosterone concentrations and the neurochemical parameters in mice exhibiting translocation and those that did not (P > 0.1).

**Translocation of S. typhimurium.** Mice were “decontaminated” with oral penicillin plus streptomycin for 3 days and then “monoassociated” with *S. typhimurium* for 2 days. *S. typhimurium* is an invasive pathogen that translocates spontaneously across the intestinal mucosal barrier. Pretreatment of the mice with antibiotics reduces the indigenous GI microflora, allowing intestinal overgrowth by *S. typhimurium* and promoting its translocation from the GI tract (3, 16). Mice were subsequently restrained for 8 h and killed 48 h after initiation of the restraint (4 days after the initiation of *S. typhimurium* monoassociation).

![Fig. 4. The effects of 4-h restraint on plasma corticosterone (A) and brain NE, 5-HT, and DA metabolism 4, 8, 24, and 48 h after restraint was initiated (B). The same experiment was performed as in Fig. 3. One-way ANOVA indicated significant effects of restraint on corticosterone (F_{4,45} = 39, P = 0.0001); MHPG/NE ratios in the frontal cortex (F_{4,45} = 6, P = 0.0006), hypothalamus (F_{4,45} = 9.6, P = 0.0001), and brain stem (F_{4,45} = 24, P < 0.0001); 5-HIAA/5-HT ratios in the frontal cortex (F_{4,45} = 41.9, P = 0.0001), hypothalamus (F_{4,45} = 28, P = 0.0001), and brain stem (F_{4,45} = 74, P = 0.0001); tryptophan concentrations in the frontal cortex (F_{4,45} = 2.8, P = 0.039) and hypothalamus (F_{4,45} = 4.8, P = 0.003); and DOPAC/DA ratios in the hypothalamus (F_{4,45} = 6.8, P = 0.0002) and brain stem (F_{4,45} = 6.7, P = 0.0003). **Significantly different from the unrestrained control (P < 0.01; Fisher's LSD test).**

![Fig. 5. The effect of restraint on bacterial translocation of *Salmonella typhimurium*. Mice (n = 10) were decontaminated by oral pretreatment with antibiotics for 3 days and then inoculated orally with *S. typhimurium* for 2 days. *S. typhimurium* is an invasive pathogen that translocates spontaneously across the intestinal mucosal barrier. Pretreatment of the mice with antibiotics reduces the indigenous GI microflora, allowing intestinal overgrowth by *S. typhimurium* and promoting its translocation from the GI tract (3, 16). Mice were subsequently restrained for 8 h and killed 48 h after initiation of the restraint (4 days after the initiation of *S. typhimurium* monoassociation). Two-way ANOVA indicated a significant effect of monoassociation on *S. typhimurium* translocation to the MLN (F_{1,31} = 131, P = 0.0001), liver (F_{1,31} = 195, P = 0.0001), and spleen (F_{1,31} = 292, P = 0.0001). There was no effect of restraint on translocation of *S. typhimurium*. **Significantly different from the corresponding group not treated with *S. typhimurium* (P < 0.01, Student's t-test).**
Figure 5 shows the magnitude of bacterial translocation in mice with and without inoculation of *S. typhimurium*. There were no significant differences in mean cecal populations of *S. typhimurium* in restrained and unrestrained mice (\(1.1 \pm 0.2\) vs. \(1.8 \pm 0.5 \times 10^7/g\)). *S. typhimurium* was detected in the MLN, liver, spleen, and cecum of all infected mice 96 h after inoculation, indicating that translocation occurred in all animals (Fig. 5). Eight-hour restraint did not further increase the translocation of *S. typhimurium* to any organs. A small amount of translocation of indigenous gram-negative bacteria was seen in 6 of 10 vehicle-unrestrained mice (60%) and 5 of 10 vehicle, 8-h restrained mice (50%) not infected with *S. typhimurium*. Antibiotic treatment does not eliminate all 400–500 species of indigenous GI bacteria, and any species resistant to the antibiotic cocktail can grow to higher population levels and translocate from the GI tract (5). This limited translocation did not appear to be related to the effects on corticosterone concentrations or any of the neurochemical parameters (2-way ANOVA for effects of translocation, restraint, and their interaction, \(P > 0.1\)).

Plasma corticosterone and IL-6 concentrations (Fig. 6A) and brain MHPG/NE and 5-HIAA/5-HT ratios and tryptophan concentrations (Fig. 6B) were significantly elevated in *S. typhimurium*-infected mice compared with uninfected mice. The prior restraint treatment exerted no additional effects on these parameters in infected mice. Neither *S. typhimurium* infection nor 8-h restraint altered DA metabolism.

It is possible that the addition of 8-h restraint did not further increase the translocation of *S. typhimurium* because the translocation mechanism was saturated (i.e., a “ceiling” effect). Therefore, we performed another experiment in which one-half the animals were

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**Figure 6.** The effect of restraint on plasma corticosterone and interleukin-6 (IL-6; A) and brain NE, 5-HT, and DA metabolism (B) in *S. typhimurium*-monoassociated mice. The same experiment was performed as in Fig. 5. Two-way ANOVA indicated significant effects of *S. typhimurium* treatment on corticosterone (\(F_{1,36} = 36.2, P = 0.0001\)); IL-6 (\(F_{1,36} = 252.0, P = 0.001\)); MHPG/NE ratios in the hypothalamus (\(F_{1,36} = 42.6, P = 0.0001\)); 5-HIAA/5-HT ratios in the hypothalamus (\(F_{1,36} = 38.4, P = 0.0001\)) and brain stem (\(F_{1,36} = 52.3, P = 0.0001\)); and tryptophan concentrations in the hypothalamus (\(F_{1,36} = 13.4, P = 0.0003\)) and brain stem (\(F_{1,36} = 24.3, P = 0.0001\)) but not on DOPAC/DA ratios. There were no statistically significant effects of restraint on plasma corticosterone nor on brain NE, 5-HT, and DA metabolism. Significantly different from the corresponding group not treated with *S. typhimurium* (#\(P < 0.05\), ##\(P < 0.01\): Student’s t-test).
not pretreated with antibiotics before monoassociation with the pathogen. As expected, mice not given antibiotics showed lower cecal populations of \textit{S. typhimurium} and less translocation of \textit{S. typhimurium} than antibiotic-treated mice. However, restraint did not further increase \textit{S. typhimurium} translocation response in these animals; the only statistically significant effect was on the magnitude of translocation to the MLN in the mice not treated with antibiotics (Fig. 7).

Figure 8 shows the plasma corticosterone and IL-6 concentrations and neurochemical data in these same infected and uninfected mice (40 h after the restraint was terminated). Plasma corticosterone and IL-6 concentrations (Fig. 8A), cerebral NE and 5-HT metabolism, and tryptophan concentrations (Fig. 8B) were elevated in the antibiotic-treated groups compared with those not treated with antibiotics. The 8-h restraint did not significantly alter any of these parameters in either antibiotic-treated and untreated mice.

Figure 9 indicates the correlations between \textit{S. typhimurium} translocation and plasma corticosterone and IL-6 concentrations, and neurochemical parameters in the antibiotic-pretreated mice of this last experiment. The correlation coefficients are summarized in Table 1. 5-HIAA/5-HT ratios in brain stem correlated positively with the magnitude of \textit{S. typhimurium} translocation to the spleen \((P = 0.0005 < 0.05/30)\). Brain tryptophan concentrations in both hypothalamus and brain stem were also positively correlated with translocation to the MLN \((P = 0.0009 < 0.05/30; P = 0.0006 < 0.05/30)\). Plasma corticosterone and IL-6 concentrations and brain MHPG/NE ratios were not significantly correlated with the magnitude of the translocation, although they were elevated in \textit{S. typhimurium}-treated mice.

### DISCUSSION

Stress is associated with translocation of indigenous bacteria from the GI tract to the MLN, liver, spleen, and other organs (4, 8). For example, Anderlik et al. (1) documented the spontaneous translocation of gram-positive cocci in young mice, which was further increased after 24- to 48-h cold exposure, although this stressor induced the translocation of gram-negative bacteria in only 1 of 27 young mice. In contrast, Deitch and Bridges (7) reported that cold exposure (up to 16 h at 4°C) did not promote bacterial translocation in mice with normal GI microflora or those that were monoassociated with \textit{Escherichia coli} C25. However, significant trauma (e.g., femoral fracture/amputation) or thermal injury promoted translocation in both normal and \textit{E. coli}-monoassociated mice. These more invasive stressors (burn, surgery, hemorrhagic shock) are known to damage the GI tract mucosal barrier and thereby promote the translocation of indigenous bacteria from the GI tract to extraintestinal sites (4, 15), although the impact of less invasive stressors on the GI mucosa is not known.

Takaki et al. (22) found that immobilization of rats induced a rapid increase in portal LPS concentrations, which reached a peak 30 min after rats were immobilized. We did not measure portal LPS concentrations. Such an increase in LPS might have reflected translocation of gram-negative bacteria, but other sources are possible.

In the present series of experiments, 1–4 h of restraint induced the translocation of indigenous gram-positive bacteria, but in only a small proportion of animals. Translocation of indigenous gram-negative (i.e., LPS containing) bacteria was not observed after any duration of restraint tested. Consistent with this, Anderlik et al. (1) found that translocation of gram-negative bacteria was rarely seen even after cold stress. The greater translocation of gram-positive compared with gram-negative indigenous bacteria is likely related to their relative GI population levels in normal animals. Increased GI population levels (i.e., intestinal overgrowth) is a major mechanism promoting translocation of indigenous bacteria from the GI tract (3). In untreated rodents, obligate anaerobes, such as \textit{Bacteroides} and \textit{Clostridium}, comprising 99.9% of the indigenous microflora, do not translocate “spontaneously” from the GI tract even though they are normally present at the highest population levels \((10^{9–10}/g cecum)\) (19). It is not known why the obligate anaerobes do not translocate readily, but it may be due to their extreme sensitivity to oxygen. Gram-positive, facultatively anaerobic, indigenous bacteria, such as \textit{Lactobacillus} and \textit{Enterococcus}, translocate spontaneously in a proportion of untreated rodents, likely because they are present at intermediate population levels \((10^{8–9}/g cecum)\). The gram-negative, facultatively anaerobic, indigenous bacteria, such as \textit{E. coli} and other \textit{Enterobacteriaceae}, are almost never found to translocate spontaneously because they are present at the lowest population levels \((10^{4–5}/g cecum)\).
Little is known about the direct effects of constituents of gram-positive bacteria on CNS and HPA axis functions. Chen et al. (6) demonstrated recently that gram-positive cell walls derived from common intestinal indigenous bacteria can induce cytokine production from human peripheral blood mononuclear cells with potencies similar to LPS. This finding suggests that gram-positive bacteria may induce CNS and HPA axis responses through the actions of cytokines (14).

The HPA axis and neurochemical responses to restraint in these animals replicated findings from our own earlier studies (12, 13) and those of many other groups (20). The significant delay in the appearance of statistically significant increases in 5-HIAA (especially compared with tryptophan) is also consistent with changes observed after electric foot shock (9), although the delay was shorter in foot-shocked mice. The results of the first two experiments presented make it clear that the peak bacterial translocation response occurred long after the HPA axis and neurochemical responses. Conversely, at the time of maximal bacterial translocation, there were often no detectable changes in HPA axis or biogenic amine metabolism. Significantly different from the corresponding group not treated with antibiotics (#P < 0.05, ## P < 0.01: Student’s t-test).

Fig. 8. The effect of antibiotic pretreatment and restraint on plasma corticosterone and IL-6 (A) and brain NE, 5-HT, and DA metabolism (B) in S. typhimurium-monoassociated mice. The same experiment was performed as in Fig. 7. Two-way ANOVA indicated significant effects of antibiotic treatment on corticosterone ($F_{1,36} = 8.2, P = 0.01$); IL-6 ($F_{1,36} = 42.5, P = 0.0001$); MHPG/NE ratios in the hypothalamus ($F_{1,36} = 15, P = 0.001$) and brain stem ($F_{1,36} = 49, P = 0.0001$); 5-HIAA/5-HT ratios in the hypothalamus ($F_{1,36} = 14, P = 0.001$) and brain stem ($F_{1,36} = 29, P < 0.0001$); and tryptophan concentrations in the hypothalamus ($F_{1,36} = 19, P = 0.0001$) and brain stem ($F_{1,36} = 25, P < 0.0001$), but not on DOPAC/DA ratios. There were no statistically significant effects of restraint on plasma corticosterone nor on brain NE, 5-HT, and DA metabolism. Significantly different from the corresponding group not treated with antibiotics (#P < 0.05, ## P < 0.01: Student’s t-test).
It is notable that the responses to restraint included a prominent increase in DOPAC/DA ratios especially in the medial prefrontal cortex (9), whereas immune activation is not generally associated with significant changes in DA metabolism (14), although small changes are associated with LPS administration (10, 14). In the case of LPS, however, neurochemical changes in the medial prefrontal cortex are less prominent and are similar in magnitude to those seen in other brain regions (10, 14). Thus, at least in the particular mouse strain and mouse colony tested, there does not appear to have been any HPA axis or catecholamine or indoleamine activation associated with restraint-induced translocation. However, because we measured only live bacteria in this study, our findings do not negate a role for a stress-related increase of LPS in the circulation or any biosignals secreted by phagocytes in the process of translocating bacteria (18, 22). Future studies should employ quantification of dead bacteria in extraintestinal tissues or use small latex beads (3).

The question remained whether the translocation of gram-negative bacteria was capable of influencing CNS and HPA axis function. Therefore, we performed experiments using pathogenic *S. typhimurium*, a gram-nea-

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**Table 1. Correlations between translocation of *Salmonella typhimurium* to each organ and plasma corticosterone and IL-6, MHPG/NE, 5-HIAA/5-HT, tryptophan, or DOPAC in each brain region**

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<td>DOPAC/DA</td>
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<td>0.8743</td>
</tr>
<tr>
<td>Spleen</td>
<td>BS</td>
<td>MHPG/NE</td>
<td>0.4246</td>
<td>0.0620</td>
</tr>
<tr>
<td>Spleen</td>
<td>BS</td>
<td>5-HIAA/5-HT</td>
<td>0.7087</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Spleen</td>
<td>BS</td>
<td>Tryptophan</td>
<td>0.6497</td>
<td>0.0019</td>
</tr>
<tr>
<td>Spleen</td>
<td>BS</td>
<td>DOPAC/DA</td>
<td>−0.1611</td>
<td>0.4974</td>
</tr>
</tbody>
</table>

MLN, mesenteric lymph nodes; HT, hypothalamus; BS, brain stem; r, Pearson's correlation coefficient; IL-6, interleukin-6; MHPG, 3-methoxy-4-hydroxyphenylethylene glycol; NE, norepinephrine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; DOPAC, 3,4-dihydroxyphenylacetic acid; DA, dopamine. *P < 0.05/30.

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It is notable that the responses to restraint included a prominent increase in DOPAC/DA ratios especially in the medial prefrontal cortex (9), whereas immune activation is not generally associated with significant changes in DA metabolism (14), although small changes are associated with LPS administration (10, 14). In the case of LPS, however, neurochemical changes in the medial prefrontal cortex are less prominent and are similar in magnitude to those seen in other brain regions (10, 14). Thus, at least in the particular mouse strain and mouse colony tested, there does not appear to have been any HPA axis or catecholamine or indoleamine activation associated with restraint-induced translocation. However, because we measured only live bacteria in this study, our findings do not negate a role for a stress-related increase of LPS in the circulation or any biosignals secreted by phagocytes in the process of translocating bacteria (18, 22). Future studies should employ quantification of dead bacteria in extraintestinal tissues or use small latex beads (3).

The question remained whether the translocation of gram-negative bacteria was capable of influencing CNS and HPA axis function. Therefore, we performed experiments using pathogenic *S. typhimurium*, a gram-nega-
tive bacterium containing LPS and which translocates spontaneously through the intestinal mucosa. When mice were depleted of their indigenous GI microflora (i.e., “decontaminated”) and infected (i.e., monoassociated) with \textit{S. typhimurium} (16), plasma corticosterone concentrations increased, as did brain tryptophan and catecholamine and indoleamine metabolism. Plasma concentrations of IL-6 were also increased. The cause of this increase is not known, although LPS administration has long been known to stimulate IL-6 secretion (17). Thus the increase observed here could be due to LPS derived from translocating \textit{Salmonella}. Increases in circulating LPS have been observed after immobilization (22), and increases in plasma IL-6 have also been reported in response to restraint or immobilization (21, 25). The mechanism of these effects is not known, although peripheral and central NE (21) and corticotropin-releasing factor (2) have all been implicated. Systemic administration of IL-6 increases plasma corticosterone concentrations and affects brain tryptophan and 5-HT metabolism (23). Therefore, the elevation of plasma IL-6 suggests that IL-6 may mediate the neurochemical and corticosterone responses to the translocation (24). This is supported by the significant correlations between the extent of translocation and the increases in plasma IL-6 and corticosterone and brain tryptophan and 5-HIAA/5-HT ratios.

In summary, these findings indicate that 4-h restraint did not induce the translocation of indigenous gram-negative (i.e., LPS containing) bacteria in normal mice but did induce the translocation of indigenous gram-positive bacteria in a few animals. Nevertheless, this translocation of indigenous gram-positive bacteria was not associated with additional HPA axis activation nor with changes in cerebral biogenic amine metabolism. This translocation of indigenous GI bacteria did not appear to contribute significantly to the stress-induced HPA axis and CNS neurochemical changes. However, the translocation of a pathogenic bacterial strain (\textit{S. typhimurium}) did induce changes in HPA axis function and brain tryptophan and catecholamine and serotonin metabolism.

We thank Lynn Pittman-Cooley and Glenn Farrar for skilled technical assistance.

This research was supported by National Institute of Neurological Disorders and Stroke Grant RO1 NS-25370.


Present address of R. F. Brown: Medical Psychology Unit, Univ. of Sydney, 2050 Sydney, Australia.

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