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Effect of Noise on Microvascular Integrity in Laboratory Rats

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Housing rats in an environment with high personnel activity increases microvascular leakiness to albumin in the mesenteric microcirculation and causes mast cell degranulation. In this study, rats were exposed to daily 15-min episodes of 90-dB SPL noise to determine whether similar effects occurred and whether vitamin E with α-lipoic acid or Traumeel (a homeopathic anti-inflammatory–analgesic) reduced these effects. Groups of rats fed a control diet (1000 IU/kg vitamin E) only, the control diet with Traumeel, or a diet with 10,000 IU/kg vitamin E and 1.65 g/kg lipoic acid were exposed to daily noise for 3 to 5 wk; a fourth group of rats, fed control diet, was housed with no excess noise. The rats were anesthetized, the superior mesenteric artery cannulated, and a portion of the microvasculature perfused for 1 min with fluoroscein isothiocyanate–albumin before fixing for microscopy. All groups exposed to excess noise had significantly more leaks per venule length and greater leak area per venule length than did the quiet group. However, the number and area of leaks in the rats that received Traumeel or vitamin E were significantly smaller than those in rats exposed to noise only. In addition, mast cell degranulation was significantly lower in rats given Traumeel. Thus exposure of rats to excessive noise produces structural damage in the mesenteric microvasculature that is significantly reduced by dietary supplements.

Abbreviations: FITC, fluoroscein isothiocyanate; HBS, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffered saline

Stress can alter the physiologic responses of research animals.[9,17,20,28-30] In a previous study we showed that high personnel activity within animal housing facilities produced mesenteric microvascular leakage and intestinal disorders in rats.[29,30] The mesentery often is used in studies of microvascular function because it is a thin tissue that can be transilluminated easily. When determining microvascular permeability, control preparations should show low basal permeability to macromolecules so that the experimental findings are not confounded by extraneous parameters. The basic cellular mechanisms responsible for the structural changes that occurred in response to high personnel activity in the animal facility remain obscure but, as was shown in a recent study,[3] excess noise may have been a key contributing factor.

Most previous studies concerning the effects of noise on microvascular integrity have involved the cochlea.[10,19,21] These studies showed increased microvascular permeability and localized periods of blood flow stasis that were irreversible after exposure of the animals to 110 dB noise for 23 min. Excess reactive oxygen species and increased expression of induced nitric oxide synthase were found in the walls of blood vessels of cochlear stria vascularis, and it was hypothesized that the stasis could have produced ischemia leading to the formation of excess reactive oxygen species, thus exacerbating microvascular damage.[21]

In another study, rats were exposed to chronic intermittent noise to determine whether it would reduce exogenous bradykinin-induced extravasation from the synovium.[23] The rationale was that stress causes rodents to release corticosterone, and corticosterone inhibits increases in microvascular permeability. Plasma corticosterone concentrations did not habituate to noise in the rats, and noise-related bradykinin-induced extravasation developed 24 h or more after the end of the noise exposure period. The authors hypothesized that exposure to noise led to release of a factor that promoted endogenous bradykinin-induced extravasation and that this release masked a delayed inhibitory effect of noise on extravasation. Histamine and testosterone were mentioned as possible candidates for this factor. This study supports our hypothesis that the increased mesenteric microvascular leakage observed in rats exposed to an animal facility with high personnel activity was due to noise. Previous experiments[29] lacked a 24-h period between exposure to personnel activity and the beginning of the experiment, so an insufficient time was available for development of a possible inhibitory effect.

In the current study, we tested the hypothesis that exposure to noise causes microvascular extravasation in the mesentery and that this leakage could be ameliorated by treatment with anti-inflammatory agents. Although the mechanism for nonauditory, noise-induced tissue damage is not known, the effects of noise on the intestinal mucosa (such as mast cell degranulation and recruitment of eosinophils[3]) indicate an inflammatory response. The present study also evaluated the agents Traumeel (a combination homeopathic remedy containing derivatives of 12 different herbs and 1 mineral in isotonic saline) and α-tocopherol acetate (vitamin E) with α-lipoic acid. Vitamin E is the most potent antioxidant in the lipid phase,[8] and it protects the rat intestine from oxidative stress associated with inflammation.[11,22] This vitamin is nontoxic, nonimmunogenic, inexpensive, able to penetrate cells, and approved for use in animals and humans. Because vitamin E has a prolonged metabolic half-life, it can be included in food as a dietary supplement. When vitamin E quenches reactive oxidant species, the resulting vitamin E radicals must be recycled back to the reduced form. Alpha lipoic acid can perform this task.

Alternatively, Traumeel is one of the most popular alternative medicines in Germany, but compared with vitamin E, less experimental evidence is available regarding its efficacy. However, Traumeel has been shown to attenuate the course of experimentally induced local injury and edema,[16] and it can inhibit the secretion of proinflammatory mediators from a range of immune cells in vitro.[18] Its mechanism of action appears to

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be due to modulation of the release of reactive oxygen species from activated neutrophils and to inhibition of the release of inflammatory mediators from activated macrophages.[13]

Materials and Methods

Experimental protocol. Male Sprague-Dawley rats (300 to 350 g) were obtained from Harlan Labs (Indianapolis, IN); monthly serology, bacteriology, and parasitology evaluations are performed on animals from each virus-free barrier at Harlan. The rats were transported to the animal facility at the US Veterans Hospital, Tucson, AZ, by truck; this facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, and all experimental protocols have been approved by the institutional animal care and use committee. On arrival, the animals were housed under the same conditions but in 2 different rooms. The animal facility is small with low personnel activity, and the 2 rooms (each 3 × 4 m) were remote from noisy air vents, cage washers, and so forth. Only rats used in this study were housed in the rooms. A technician entered both rooms once a day to feed and tend to the rats. Apart from the investigator, the technician was the only person to enter the rooms, and he was instructed to perform his duties at the same time each day. Both rooms had identical lighting arrangements of the same intensity, and each room was thoroughly disinfected before use to remove extraneous odors. The temperature of the rooms varied between 22 and 23 °C, and humidity was kept between 55% and 60%.

The rats in each room were housed 2 per cage and were on similar diets (AIN-93M purified diet (Harlan), which contains 1000 IU vitamin E per kg) and light cycles (lights on 0600 to 1800). The cages were 39 × 29 × 29 cm (that is, larger than standard rat cages) and contained standard bedding (Sanichip, Harlan). Each cage contained a shelf (29 × 10 cm wide) that had a ladder to the floor of the cage, a 10-cm diameter black plastic tube, and a small food-bowl. The water bottle was attached to the outside of the cage.

The one intentional difference between the environments of the 2 rooms was that for 3 wk the rats in 1 room (the 'noise' rats, n = 6 to 10) received an additional stimulus each day at 1800 (that is, just before the lights were switched off). The stimulus was activation of an electronic noise generator (90 dB overall noise level) for 15 min. The noise spectrum was of highest intensity (77 to 85 dB SPL) over the frequency range of 800 to 8000 Hz. Rat hearing is most sensitive within the range of 800 to 32,000 Hz,[24] so the noise spectrum was well matched to this range. Frequencies below 250 Hz are below the hearing range of rats,[14] but are easily audible by humans, who can hear sound frequencies down to 20 Hz[25]. The noise level of 90 dB SPL (reference pressure level, 0.0002 dyn/cm^2), averaged over frequencies from 10 to 10,000 Hz, is similar to that produced by poorly maintained cage washers and air conditioners and is relatively low compared with the 110-dB tolerance level of rats.[1] The rats in the other room ('quiet' rats; n = 6 to 10) did not receive the noise stimulus. Ambient and experimental noise sound pressure levels were measured as described in a previous study.[7]

Experiment 1. After 3 wk, all of the quiet rats and half of the noise rats were anesthetized, and experiments were performed in an order randomized with respect to pretreatment to compare the extent of microvascular damage, as judged by increased leakiness to fluorescent albumin. Surgeries to harvest the mesentery for analysis were completed throughout the next 2 wk; during this time the remaining noise rats continued to receive daily noise. A previous study[29] found that the effects of noise on microvascular integrity did not change between 3 and 7 wk of noise exposure. Surgeries were always performed at the same time of day (starting at 0800) in order to avoid chronobiologic variation. After these surgeries were completed, the noise generator was switched off, and the remaining rats were left in quiet for another 3 wk ('recovery' rats).

Experiment 2. For this experiment, the conditions of Experiment 1 were repeated, but the noise generator was moved to the other room so that possible room-specific effects could be eliminated.

Experiment 3. For Experiment 3, the conditions for Experiment 1 were repeated, but the noise generator was operated in both rooms. The rats in 1 room were exposed to daily noise for 1 wk and then were treated with either vitamin E (tocopheryl acetate, DSM Nutritional Products, Heerlen, The Netherlands) together with α-lipoic acid (Sigma-Aldrich, St Louis, MO) or with Traumeel (HEEL-BHI, Albuquerque, NM) while receiving the daily noise for another 2 to 4 wk. Concurrently a third group of rats was housed in a third room that was identical to the other 2 with regard to background noise, dimensions, furniture, and fixtures. This third room had been used for accommodating control rats in previous studies, and few microvascular leaks had been noted in those animals (data not shown). For Experiment 3, each group consisted of 6 rats.

Methodologies. The key experimental methodologies were: generation of white noise, cannulation and perfusion of rat mesentery, assessment of venular leakage (epifluorescence microscopy), evaluation of mesenteric mast cell degranulation, and measurement of superoxide (O_2^-) in rat plasma.

Generation of noise. The background noise in all 3 animal rooms was measured as described previously[6] by use of a calibrated microphone (type 4133, Bruel and Kjaer, Norcross, GA) sound-level calibrator (type 4230, Bruel and Kjaer), and variable filter (model 3202, Krohn-Hite,Brockton, MA) and was 50 dB SPL when averaged over frequencies from 50 to 10,000 Hz (ranging from 25 to 42 dB at frequencies above 250 kHz). The noise was generated from an audio recording (90 dB), which was played in a looped mode by use of a compact-disc player and transmitted to 1 or 2 rooms, depending on the experiment. To ensure uniformity of sound delivery between the rooms, the frequency response of each speaker was equalized by use of 'pink noise' in conjunction with a one-third octave equalizer. Pink noise is low-pass-filtered white noise and provides equal energy with the one-third octave bands. Loudspeakers were selected that reproduce frequencies in the range of 50 to 10,000 Hz, and the noise level was adjusted to 90 dB averaged over those frequencies.

Cannulation and perfusion of rat mesentery. We have developed a method of preparing the rat mesentery in which we perfuse the microvasculature of 3 to 4 contiguous mesenteric windows,[4] with a window defined as the mesenteric tissue between 2 pairs of feeding arterioles and collecting venules. Perfusion of a small network ensures that the pressure is maintained throughout the venous circulation. Another advantage of this preparation is that it allows concentrated tracer to be applied intravascularly and precisely to a small region of mesentery and intestine. Each rat was anesthetized with a ketamine–acepromazine cocktail followed by intraperitoneal injection of sodium pentobarbital (6 mg/100 g body weight). After tracheotomy, the abdomen was slit along the linea alba, and a well-vascularized mesenteric window was selected and spread out flat over a small gauze platform. Next, the superior mesenteric artery was cannulated close to the selected mesenteric window, and the appropriate arterioles and venules bordering the window were clamped to allow perfusion of the chosen windows only. The mesenteric window was then flushed clear of blood with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-buffered saline (HBS, pH 7.4) containing 0.5% bovine serum albumin.

All surgery was performed under a cool fiberoptic light, and exposure of the mesenteric window to light was minimized because exposure to light can cause leaky sites in venules.[26] In all cases the mesenteric windows to be perfused were close to the cecum, to reduce experimental variation.

Assessment of venular leakage (epifluorescence microscopy). After the microvascular network was flushed free of blood, the animal was killed by intravenous injection of Beuthanasia (Schering-Plough Animal Health Corp, Union, NJ). Immediately after euthanasia, the mesenteric vasculature was perfused with 0.05% fluoroscein isothiocyanate (FITC)-labeled albumin (Sigma) dissolved in HBS to test for venular leaks. As soon as the vasculature of the window was filled with tracer, the pressure was adjusted to 30 mm Hg, and the portal vein, which acts as the flow outlet, was clamped. After treatment for 1 min, the clamp was removed, and 1.5 ml of fixative (3% formaldehyde in HBS) was perfused via the cannula at a pressure of 60 mm Hg. Next, the pressure was reduced to 30 mm Hg, the portal vein was clamped, and fixation was continued for 60 min. We chose the pressure of 30 mm Hg because Gore[12] found the pressures of cat mesenteric capillaries to be on the order of 34 mm Hg; the pressure in venules is lower than that in capillaries[15] and so an inlet pressure of 30 mm Hg seems appropriate. In some cases, we followed this procedure with perfusion of 3% formaldehyde, 0.1% Triton X100, and rhodamine phalloidin (10 U/ml; Molecular Probes, Eugene, OR) in HBS for 30 min at 4 °C to promote staining of endothelial actin fibers. After staining, the vasculature was perfusion-fixed for 30 min with 3% formaldehyde and then flushed with HBS. The mesenteric tissue was excised carefully, and half of the vascularized windows were mounted individually between 2 thin glass coverslips by use of aqueous mounting medium (Vectashield, Burlington, CA) for evaluation by use of epifluorescence microscopy. The extravasated albumin is fixed in place and continues to mark leaky sites after the tissue has been excised and mounted.

Overall vascular leakage was assessed by measuring the number and area of regions with extravascular FITC-albumin. Slides were examined by use of a microscope (Axioplan with 10× objective and numerical aperture of 0.6, Carl Zeiss, Oberkochen, Germany) fitted for epifluorescence. The light source was a 100-W Hg lamp for epifluorescence and a halogen lamp for transmitted illumination. A video camera (VI470, Optronix, Goleta, CA) was mounted at the camera port of the microscope. Images of each microvascular network (usually 1 or 2 per slide), produced by epifluorescence with the appropriate FITC excitation and barrier filters (488 nm and 515 nm, respectively) inserted into the light path, were viewed on a monitor and recorded on a video recorder. Each network was scanned systematically (from left to right and top to bottom) to minimize overlap of adjacent fields of view. Usually each network could be thoroughly scanned in 10 to 12 fields of view. Networks smaller than 10 fields of view were not used because they rarely respond to stress or to inflammatory mediators by leaking.[29] The length of time for which each slide was exposed to fluorescence was standardized.

In addition, recordings of the networks were made when they were transilluminated, to provide records of all networks and not just the leaky areas. Videotaped images were analyzed by use of an analog-to-digital converter and appropriate free use software (NIH-Image). The images were coded and were analyzed by a technician who did not know the code, to eliminate bias. The following measurements were made on the images: the length and diameter of each venule (both leaky and intact), the number of leaks per venule, and the area of each leak. The length of a venule was defined as the visible length between branching points. If more than 1 venule contributed to a leak, the leak area was divided by the number of venules. In all cases, the area of each leak was calculated automatically by NIH-Image software. Obviously the area of the leak will depend on the settings used on the microscope and videorecorder when obtaining the image and on the settings used with NIH-Image when analyzing the image. To ensure reproducibility, the settings on the microscope and videorecorder were adjusted so that the image on the screen exactly reflected that viewed through the microscope. These settings rarely had to be altered between experiments. The settings on NIH-Image were chosen so that every visible leak was counted and so that the leak intensity did not saturate the system. These settings were not changed throughout the experiment. For each animal, we calculated the average number of leaks per micron of venule length (venules without leaks were included in this average) and average leak area per micron of venule.

Evaluation of mesenteric mast cell degranulation. The mesenteric windows that were not used to assess microvascular leakage were suffused with 1% toluidine blue for 15 min, washed in saline, and mounted on microscope slides for viewing by light microscopy. Toluidine blue stains mast cells so that it is easy to distinguish between those that are intact and those that have degranulated. The number of mast cells within an area of 1.13 mm^2 (the field of view of the microscope when a 20 × objective and 10× eyepiece are used) was counted. For each mesenteric window, 30 randomly placed fields were counted to determine the percentage of cells that had degranulated, and 5 mesenteric windows were examined per rat.

Measurement of O_2^- in rat plasma. From 6 noise rats and 6 quiet rats from Experiment 1, 6 ml of blood was collected after cannulation of the superior mesenteric artery and just prior to euthanasia. The blood was centrifuged for 15 min at 1200 rpm (40 g) and the plasma harvested and frozen. After all the samples had been collected, they were thawed and the concentrations of O_2^- were measured by use of a lucigenin-enhanced chemiluminescence technique.[7] The light reaction between O_2^- and lucigenin was detected in a scintillation counter with 6 photomultiplier tubes. For each measurement, 1.5 ml plasma was added to 0.5 ml 0.001M lucigenin in a scintillation vial, and the photon emission at a wavelength of 510 nm (excitation wavelength, 495 nm) was recorded every 10 s for 60 s. The readings were averaged over the 60-s period. The system was calibrated by substituting HBS for the plasma and by adding 50 µl of the superoxide generator potassium superoxide (KO_2) to each sample at concentrations ranging from 0 to 0.4 M, giving final concentrations in the range of 0 to 0.01 M.

Modifications of diet. The vitamin E- and α-lipoic acid-modified diet was professionally prepared (Harlan Teklad) and consisted of the same AIN-93M purified diet that was fed to all the other animals but to which was added 10,000 IU DL-α-tocopherol acetate and 1.65 g α-lipoic acid per kilogram of diet. The unsupplemented AIN-93M diet contained 75 IU DL-α-tocopherol acetate/kg diet. The Traumeel group received the unsupplemented AIN-93M diet but also received Traumeel in their drinking water. We used the contents of injectable vials of Traumeel to avoid exposure of the rats to ethanol, which is a component of the oral liquid form of the agent. Each day, 1 vial of Traumeel (2.2 ml) was stirred into 60 ml drinking water and shaken for 10 s; this bottle was shared between the 2 rats per cage. The volume of 60 ml was chosen because this was the average volume of water consumed per pair of rats daily based on records collected during the past 3 years. If the rats consumed this volume before the end of the day, unsupplemented drinking water was provided.

Statistical analysis. Statistical analysis was performed using

Sigma Stat 3.1 (Systat Software, Richmond, CA). Data groups were tested for normality and equal variance. Data that passed both tests were compared between groups by use of analysis of variance. If a significant difference was found between groups, pairwise multiple comparisons were performed by use of the Tukey test. All values are presented as mean ± standard error of the mean. A stronger statistical test, 1-way analysis of variance with blocking, was used on the leakage data from Experiment 1 together with those from Experiment 2 (rooms reversed) to determine whether there was a greater difference between 'noise' versus 'quiet' in one room versus the other room (regardless of which room contained the noise generator). Data groups that failed the normality test (mast cell degranulation data) were compared by Kruskal-Wallis 1-way analysis of variance on ranks. To isolate the group(s) that differed from the others, a pairwise multiple comparison procedure (Dunn method) was used. In all cases, n was the number of rats in a group and a P value of <0.05 indicated significance. The minimum number of animals per group to ensure statistical validity was determined as described.[31]

Results

Effects of noise exposure on microvascular leakage and mast cell degranulation (Experiment 1). Rats from the noise group (n = 9) demonstrated significantly ($P < 0.05$) more leakage sites (mean ± standard error of the mean, $3.84 \pm 0.46 \times 10^{-3}/\mu m$; n = 95 venules) and a significantly ($P < 0.05$) greater leakage area per length of venule ($3.20 \pm 0.49\ \mu m^2/\mu m$) than did rats from the quiet group (n = 10 rats; $1.38 \pm 0.26 \times 10^{-3}/\mu m$ and $0.30 \pm 0.06\ \mu m^2/\mu m$, respectively; n = 123 venules) or the recovery group (n = 6 rats; $1.40 \pm 0.24 \times 10^{-3}/\mu m$ and $0.63 \pm 0.16\ \mu m^2/\mu m$, respectively; n = 108 venules). Rats from the recovery and quiet groups showed similar numbers of leaks per length of venule. However the recovery group demonstrated significantly ($P < 0.05$) greater leak area per venule length than did the quiet group, although still significantly ($P < 0.05$) less than that for the noise group (Figure 1). The percentages of venules that contained leaks in the noise, quiet, and recovery groups were 73%, 37%, and 39%, respectively. Light micrographs of typical FITC-albumin-perfused microvascular networks from rats from the noise and quiet groups showed extensive leakage of fluorescent material in the network from the noise-group rat, but few leaks in the network from the quiet-group rat (Figure 2). Leaks were quantified by use of NIH-Image software to obtain an objective evaluation (Figure 3). In non-leaky regions of venules (Figure 4 A), endothelial peripheral actin rims were more continuous than in leaky regions (Figure 4 C). The leaks were coincident with endothelial cell junctions, as outlined by rhodamine phalloidin staining of peripheral actin rims (Figure 4 B, C). The precise positions of the leaks coincided with gaps in the peripheral actin rims. We have observed this phenomenon previously in preparations treated with histamine.[3] This type of disruption rarely occurred in control preparations. In control preparations, the peripheral actin rims formed fairly continuous boundaries around each cell (Figure 4 A).

In Experiment 1, the mean number of degranulated mast cells per microscopic field of view (1.13 mm^2) was significantly greater for the noise group ($P < 0.05$) and recovery group ($P < 0.05$) than for the quiet group (Table 1). Light micrographs showing mast cells in the mesenteric tissue of 1 rat from each of the 3 groups are shown in Figure 5. All mast cells in micrograph from the quiet group are intact (Figure 5 A), most of the mast cells are degranulated in that from the noise group (Figure 5 B), and some are degranulated in the recovery-group image (Figure 5 C). However, it should be noted that mast cell degranulation occurred in localized areas and was not distributed homogeneously. Therefore, to accurately assess the degree of degranulation, we examined 150 fields per rat. These results indicate that daily noise markedly increases microvascular permeability in rats and that this change may be stimulated by mast cell degranulation.

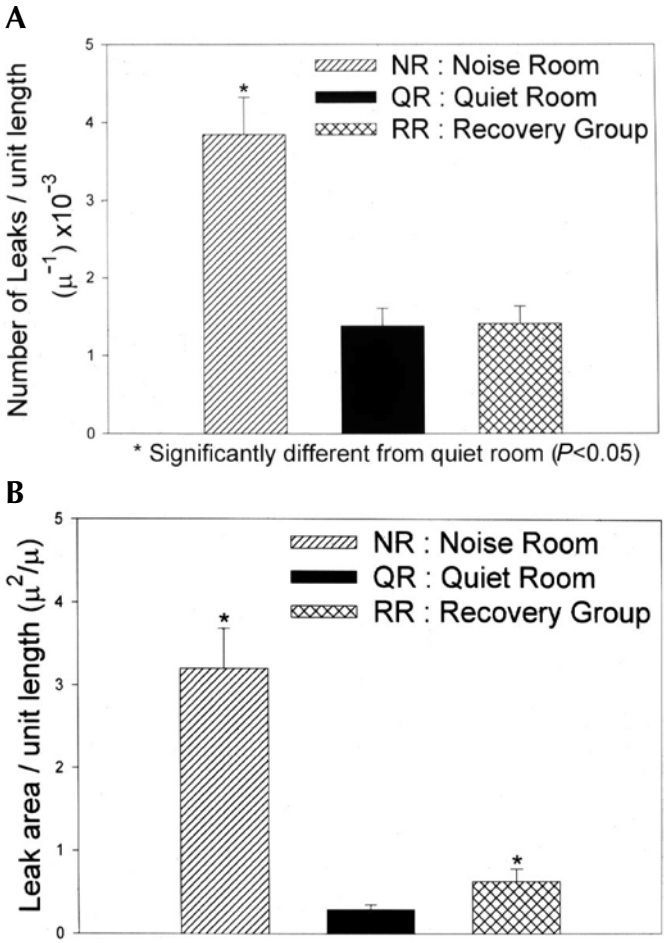

Figure 1. Histograms demonstrating effect of noise on microvascular leakage (Experiment 1). (A) Number of leaks per unit length of venule. (B) Area of leakage per unit length of venule.

Microvascular leakage and mast cell degranulation in Experiment 2. This experiment was performed to confirm that the differences in microvascular leakage and mast cell degranulation seen in rats in Experiment 1 were due to the presence or absence of noise rather than to other differences between the rooms. The noise generator was switched to the room previously used as the quiet room so that it became the new noise room.

We obtained similar results regarding noise-induced microvascular leakage when the noise and quiet rooms were reversed. Rats from the noise group (n = 6) demonstrated significantly ($P < 0.05$) more leakage sites ($1.40 \pm 0.14 \times 10^{-3}/\mu m$, n = 151 venules) and a significantly ($P < 0.05$) greater leakage area per length of venule ($1.92 \pm 0.32\ \mu m^2/\mu m$) than did rats from the quiet group (n = 6 rats; $0.31 \pm 0.04 \times 10^{-3}/\mu m$ and $0.16 \pm 0.03\ \mu m^2/\mu m$, respectively; n = 247 venules). One-way analysis of variance with blocking demonstrated a significant ($P < 0.02$) difference between noise and quiet groups for leak numbers and leak areas ($P < 0.02$ and $P = 0.001$, respectively) but not between rooms ($P = 0.15$ and $P = 0.22$, respectively), indicating that the data were not confounded by intrinsic differences between the rooms themselves.

Effects of antioxidants or antioxidant-like materials on noise-

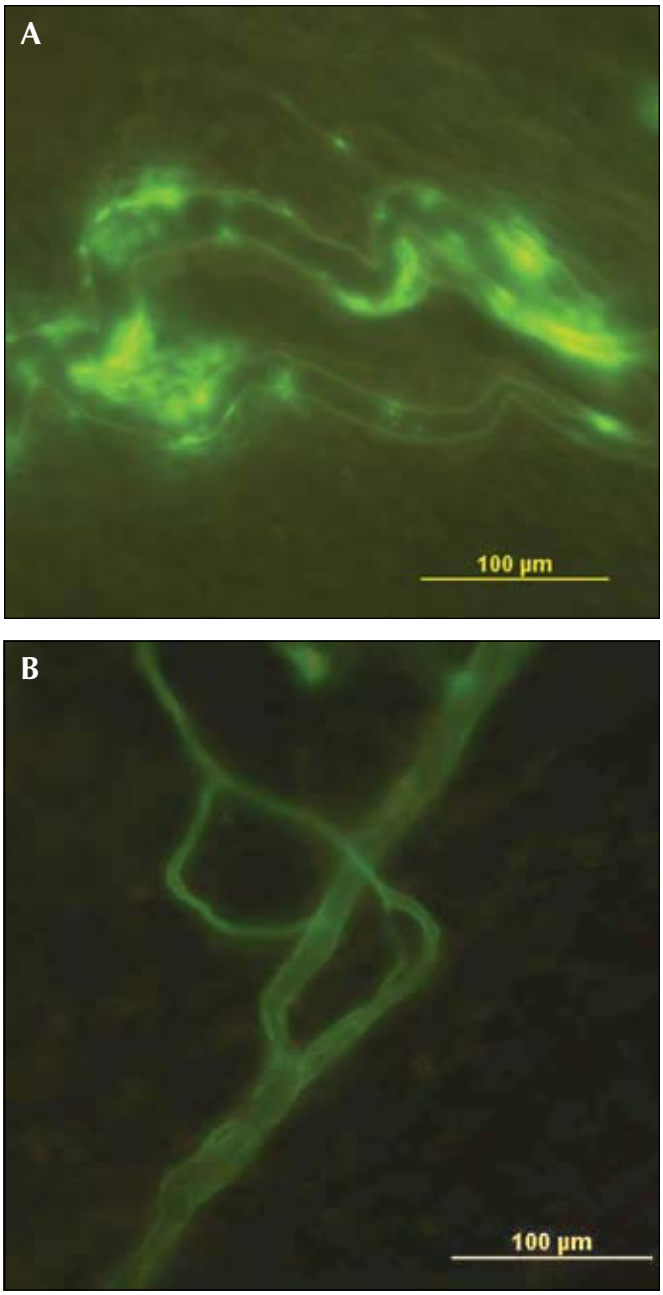

Figure 2. Light micrographs of mesenteric microvascular networks after perfusion with fluorescent-labeled albumin. (A) Network from an animal that had been exposed to daily noise. Many leaks of labeled albumin from the venules are visible. (B) Network from an animal that had not been exposed to daily noise. No leaks can be seen.

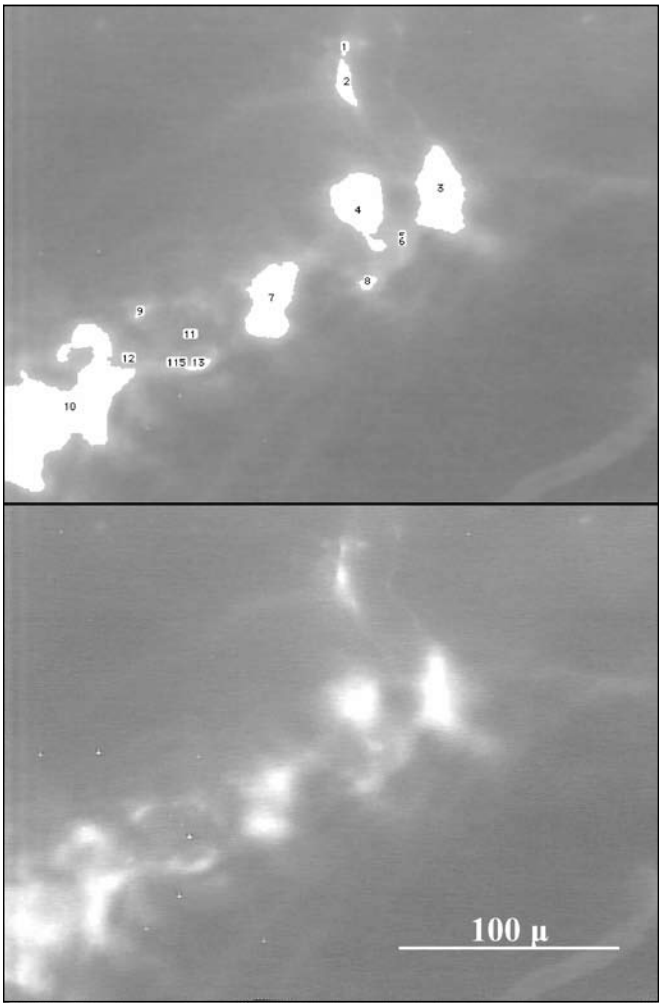

Figure 3. Light micrograph of mesenteric microvascular network from animal exposed to daily noise after perfusion with fluorescent-labeled albumin. The upper panel shows the same image after image processing and analysis to reveal the correspondence between the observed leaks and those that were counted and measured.

induced microvascular leakage (Experiment 3). Both vitamin E with α-lipoic acid and Traumeel, significantly ($P < 0.05$) reduced noise-induced venular leakage of fluorescent albumin to similar degrees, although not to control levels (Figure 6). Comparing Figures 1 and 6 reveals that the numbers of leaks were several-fold higher for Experiment 3 than for Experiment 1 for both noise and quiet groups, although the leak areas were more similar. Experiment 3 was performed later than the other 2 experiments, and by this time the analysis system had been improved to detect smaller leaks. Thus comparisons are only made between groups in Experiment 3 and not between experiments. The quiet control animals (n = 6) had a mean number of leaks per length of venule of $0.44 \pm 0.06 \times 10^{-2}/\mu m$ (n = 341 venules) compared with $3.05 \pm 0.32 \times 10^{-2}/\mu m$ (n = 294 venules, n = 6 rats) for noise alone, $1.04 \pm 0.19 \times 10^{-2}/\mu m$ (n = 304 venules, n = 6 rats) for noise and vitamin E with α-lipoic acid, and $1.50 \pm 0.11 \times 10^{-2}/\mu m$ (n = 300 venules, n = 6 rats) for noise and Traumeel; the results for leak area per length of venule were 0.44 ± 0.10, 6.60 ± 0.88, 1.90 ± 0.51, and $2.33 \pm 0.29\ \mu^2/\mu m$, respectively. Thus leak number was reduced by about 66% with vitamin E and α-lipoic acid ($P < 0.05$) and by about 50% ($P < 0.05$) with Traumeel. Leak area was reduced even more: by 70% with vitamin E and α-lipoic acid ($P < 0.05$) and by about 65% with Traumeel ($P < 0.05$).

In Experiment 3, the degree of mast cell degranulation in control rats was only a fraction of that observed in Experiment 1. In Experiment 3, vitamin E with α-lipoic acid did not significantly reduce mast cell degranulation, which was not surprising because it was already very low. However, treatment with Traumeel significantly decreased mast cell degranulation to below control (quiet room) levels (Table 2).

Effects of noise exposure on plasma superoxide concentration. Superoxide production in plasma taken from noise rats (5102 ± 56 counts/10 s in 1.5 ml) did not differ significantly from that from quiet rats (5162 ± 42 counts/10 s in 1.5 ml). These counts approximated the value obtained for an equivalent volume of 0.01 M KO_2 (of 5140 ± 11 counts/10 s in 1.5 ml); the background count was 269 ± 4 counts/10 s in 1.5 ml.

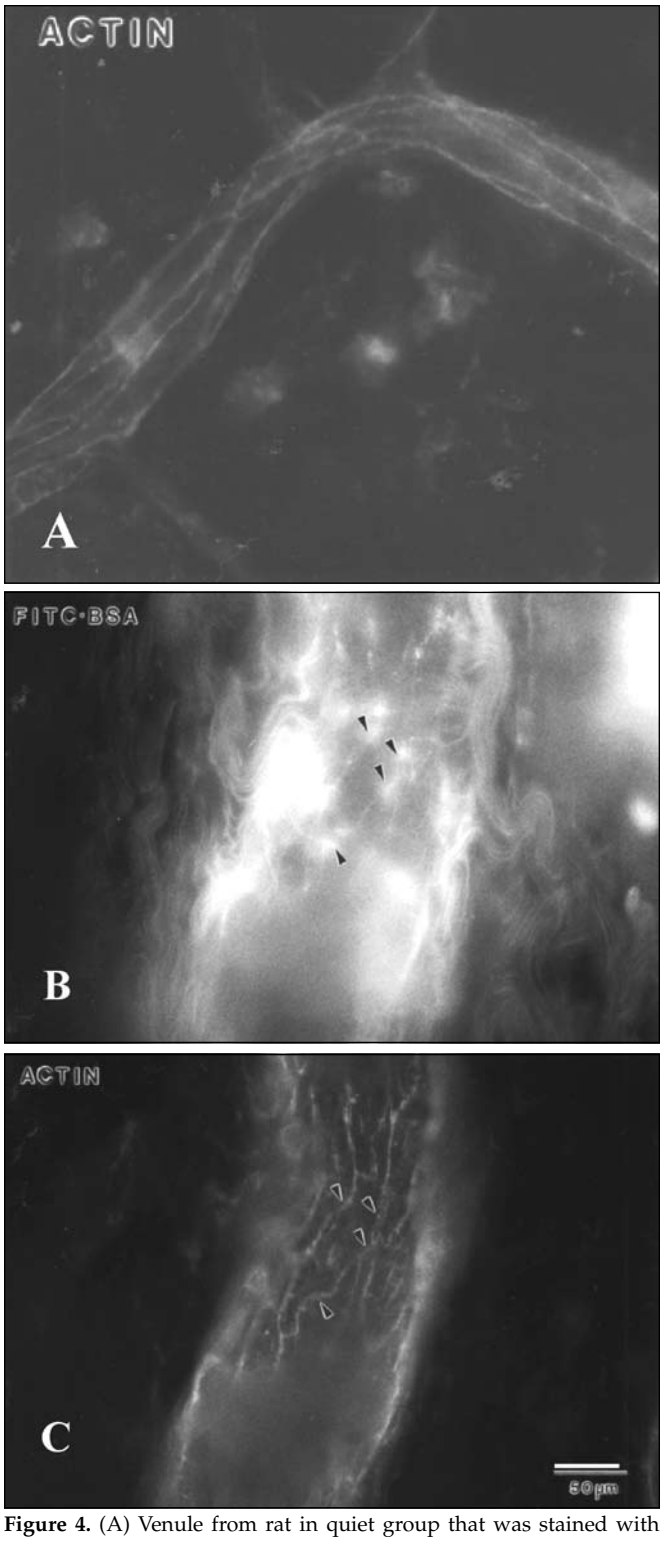

Figure 4. (A) Venule from rat in quiet group that was stained with rhodamine phalloidin for F-actin to demonstrate endothelial peripheral actin rims. (B) Venule from rat in noise group shows leakage of fluroescent-labeled albumin. (C) Same image as in panel B but taken through a rhodamine filter to reveal staining for F-actin. Arrowheads indicate positions of leaks coincident with discontinuous regions of peripheral actin rims. Bar, 50 µm.

Discussion

This study demonstrates for the first time that daily brief exposure to noise at an intensity that is encountered in many research animal facilities in the United States increases leakage of albumin from the rat mesenteric microvasculature. These results are similar to those obtained when comparing rats housed in an environment with high personnel activity with those housed in a low-activity environment.[29] In the current study, the noise occurred at the same time every day, whereas in the high personnel-activity environment the loud-noise periods were less predictable. However, the effects of noise on microvascular integrity were similar in the 2 studies. The abrupt onset of noise may cause a startle response that leads to stress. If so, perhaps masking the sudden onset of noise by increasing the background noise level would reduce the deleterious effects of noise on rodent physiology.

Table 1. Experiment 1—Effect of noise on mast cell degranulation

	No. (mean ± standard error) of degranulated mesenteric mast cells per microscopic field of view (1.13 mm^2)
Noise room rats (n = 9)	13.75 ± 0.77
Quiet room rats (n = 10)	7.43 ± 0.36
Recovery group rats (n = 6)	12.09 ± 0.90

Although noise intensity cannot always be reduced to ideal levels in animal facilities, our results clearly show that providing rats with dietary supplements significantly reduces the deleterious effects of noise on microvascular integrity. Previous preliminary results[2] also show that supplementation with vitamin E alone significantly reduces noise-induced damage of the intestinal mucosa. Therefore, dietary supplementation with antioxidants or (in the case of Traumeel) antioxidant-like compounds is an effective regimen for minimizing this response in experimental animals. However, because it did not reduce noise-induced microvascular leakage to control levels, supplementation should be used in conjunction with efforts to reduce environmental noise, such as using acoustical panels and electronic noise-canceling equipment and requiring animal caretakers to perform their duties as quietly as possible.[5] Minimization of uncontrolled noise exposure in experimental animals is essential because the effects may confound the data obtained from the animals and thus raise questions concerning data validity. The use of dietary supplements also will confound experimental outcomes, requiring determination of whether their use would significantly affect the experimental outcome being measured.

In this study, we tested 2 different supplements—a combination of 2 antioxidants, vitamin E and α-lipoic acid, and a homeopathic remedy, Traumeel—to determine whether they would reduce microvascular leakage. Both treatments were comparable at inhibition of microvascular leakage, but Traumeel was more effective at preventing mast cell degranulation. This result is consistent with an in vitro study showing that Traumeel inhibited secretion of proinflammatory mediators from immune cells such as monocytes and T cells.[18] Therefore Traumeel may act in ways other than just as an antioxidant, possibly by stabilizing immune cells. In this experiment (Experiment 3), the degree of mast cell degranulation in control rats was only a fraction of that observed in Experiment 1. There are 2 possible reasons for this observation. First, the animals were housed in a different facility, which may have had less background noise than that in Experiment 1; and second, the technicians were more experienced with the surgeries and thus were less likely to produce mast cell degranulation inadvertently by their handling of the tissue. In addition, unlike Experiment 1, exposure of the rats to noise in Experiment 3 did not significantly increase mast cell degranulation (Table 2). It is not obvious why the results differ between the 2 experiments. One possibility is a threshold of background mast cell degranulation below which it is difficult

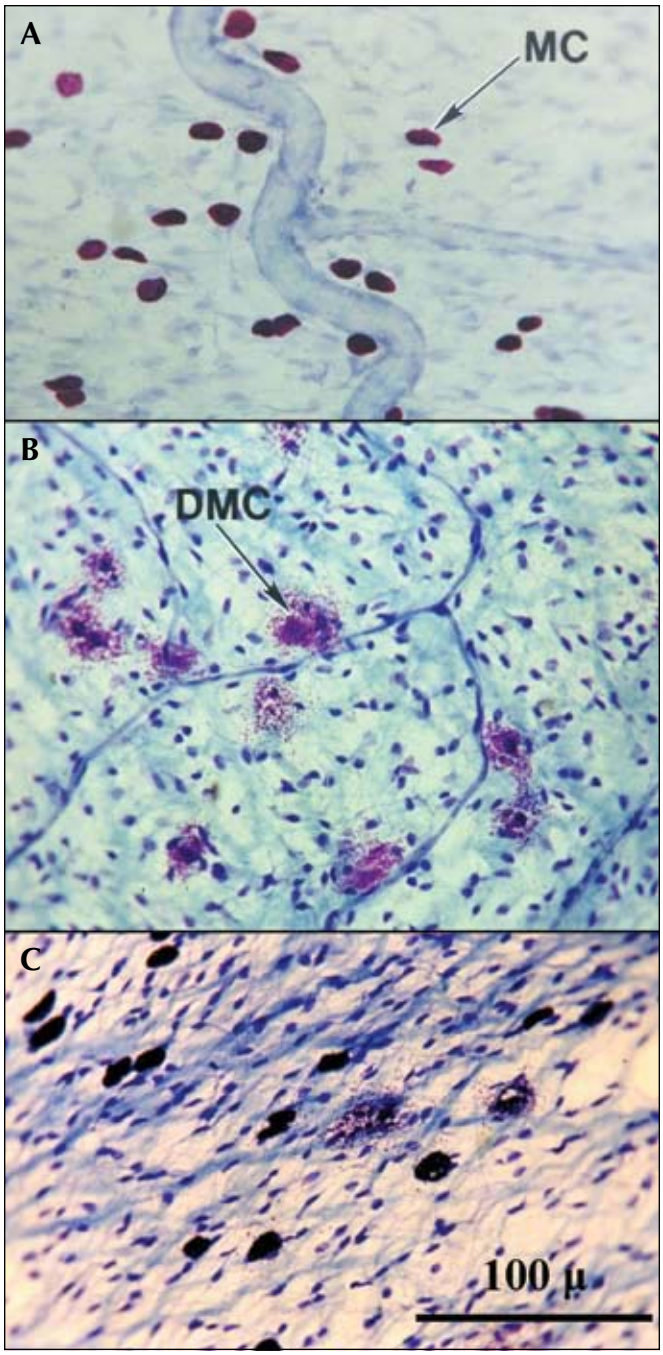

Figure 5. Light micrographs of mesenteric tissue after staining with toluidine blue to demonstrate mast cells. (A) Tissue from an animal that had not been exposed to noise. Mast cells (arrows) are intact. (B) Tissue from an animal that had been exposed to daily noise. These mast cells (arrows) are degranulated. (C) Tissue from an animal that had been exposed to daily noise for 3 wk and then to quiet for 3 wk. In this image, some of the mast cells are intact, whereas others are degranulated.

to activate the cells; this threshold is regulated by polymerization of filamentous F-actin.[27]

When designing housing for research rodents, an awareness of the effects of the environment on their microvascular structural integrity is essential, because such effects can confound experimental results. Although little scientific investigation of this problem has been conducted, the evidence we present here points to a complex situation regarding the mechanisms by which outside influences impinge on an animal's physiology. In some cases, such as in the present experiments, noise can cause tissue changes, whereas in other situations, increased release of

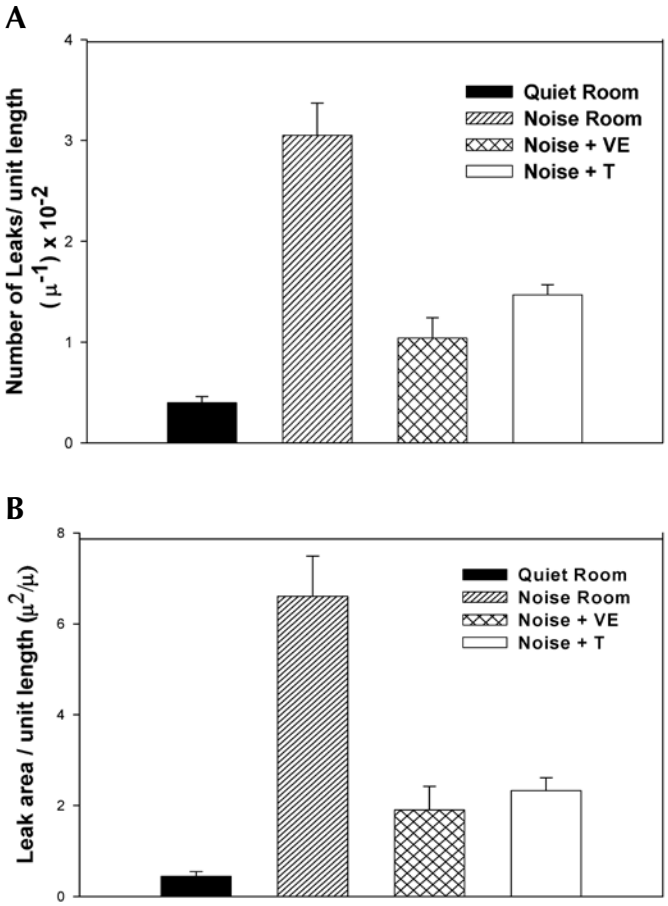

Figure 6. Histograms demonstrating effect of noise on microvascular leakage (Experiment 3). (A) Number of leaks per unit length of venule. (B) Area of leakage per unit length of venule.

Table 2. Experiment 3—Effect of noise on mast cell degranulation

	No. (mean ± standard error) of degranulated mesenteric mast cells per microscopic field of view (1.13 mm^2)
Noise room rats	1.62 ± 0.12
Noise + vitamin E + α lipoic acid	1.11 ± 0.10
Noise + Traumeel	0.39 ± 0.05
Quiet room rats	1.37 ± 0.20

Each group included 6 rats.

corticosterone may conceal that response.[6] An important result of the present study is the finding that the effects of noise on the structural integrity of the microvasculature can be controlled with readily available dietary supplements. Supplementation could be used as an additional prophylactic to prevent the effects of environmental noise. However, such supplementation only inhibits a symptom, and so care should also be taken to control environmental noise.

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