Simulated microgravity enhances vasoconstrictor responsiveness of rat basilar artery

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Zhang, Le-Ning, Li-Fan Zhang, and Jin Ma. Simulated microgravity enhances vasoconstrictor responsiveness of rat basilar artery. J Appl Physiol 90: 2296–2305, 2001.—Recently, hypertrophy and increased myogenic tone of brain vessels have been observed in rats after simulated microgravity. It is expected that simulated microgravity may also induce hyperreactivity of brain vessels. To test this hypothesis, Sprague-Dawley rats were subjected to a 4-wk tail-suspended hindlimb unloading (TS) to simulate the cardiovascular deconditioning effect of microgravity. After 4 wk, the vasoreactivity of isolated basilar arterial rings from TS rats to both receptor- and non-receptor-mediated vasoconstrictors, such as KCl, arginine vasopressin, or 5-hydroxytryptamine (5-HT), and vasodilators such as ACh, thrombin, adenosine, or sodium nitroprusside were examined and compared with those from simultaneous control (Cn) rats. In the first part of this study, it was found that the maximal isometric contractile responsiveness evoked by vasoconstrictors such as KCl, arginine vasopressin, or 5-HT was enhanced in basilar arterial rings from TS rats, whereas vasodilatory responsiveness to vasodilators showed no significant difference between TS and Cn rats. In the second part of this study, it was found that removal of the endothelium had no effects on the contractile responsiveness to 5-HT in basilar arterial rings from TS rats but enhanced markedly the responsiveness in basilar arterial rings from Cn rats to an extent comparable with that of TS rats. Application of tetraethylammonium also had no effects on the contractile response to 5-HT in basilar arterial rings from TS but significantly increased the responsiveness of basilar arterial rings from Cn rats with endothelium intact. These results showed that 4-wk simulated microgravity enhanced the vascular contractile responsiveness of basilar arterial rings to both receptor- and non-receptor-mediated vasoconstrictors, and the enhancement of 5-HT-induced contraction in TS rat basilar arteries was due to an impairment of endothelium-dependent mechanism. These results suggest that endothelium-derived hyperpolarizing factors are responsible for this endothelium-dependent attenuating modulatory mechanism in contractile responsiveness of rat basilar arteries to 5-HT.

tail-suspended hindlimb unloading; potassium chloride; arginine vasopressin; 5-hydroxytryptamine; acetylcholine; thrombin; adenosine; sodium nitroprusside; endothelium-derived hyperpolarizing factors

WHEN ASTRONAUTS RETURN FROM the microgravity environment into the 1-G environment of Earth, obvious cardiovascular dysfunction is generally present among them during the early postflight stage, which may lead to significant orthostatic intolerance and lowered upright exercise capacity (3, 20, 52, 56). Now it is well recognized that multiple mechanisms might account for this and that hypovolemia is not the unique cause and sometimes not a necessary one (3, 4, 56).

Evidence from recent bed rest and spaceflight/postflight human studies has indicated that inadequate vasoconstrictor responsiveness is an important factor in postflight orthostatic intolerance (1, 3–5, 31, 56), which should be the new focal point in further studies. Although this compromised ability to raise peripheral vascular resistance may result from alterations in neurally mediated vascular tone due to defective central integration (3, 4) or functional changes at receptor level (3, 4, 10, 11, 56), recent studies strongly support the notion that the adaptive changes in the vessels are of prime importance (14, 50, 64–66).

Furthermore, evidence is also accumulating that adaptational changes in cerebral arterial vasculature in microgravity may also contribute to the genesis of postflight orthostatic intolerance (3, 4, 23, 56, 57, 67), and this could be reasonably explained by the differential adaptation of vessels in different body regions during simulated microgravity (56, 57, 64, 66). However, it still needs further studies to clarify.

Exposure to microgravity undoubtedly results in redistribution of transmural pressures and blood flows within the arterial vasculature due to the removal of hydrostatic pressure gradients. Therefore, blood vessels in dependent body regions are chronically exposed to lower than normal upright 1-G blood pressure, whereas vessels in upper body regions are exposed to higher than normal 1-G blood pressure (27, 56). In the late 1980s, Hargens and colleagues (26, 27, 56) proposed that these chronic changes may well differentially alter the structure and function of the arterial vessels in different body regions. In recent years, there has been an increased interest in studies on vascular adaptation to microgravity in ground-based studies using the tail-suspended hindlimb-unloaded rat model to mimic the vascular effect of microgravity. For example, Delp et al. have indicated that prolonged hindlimb
unweighting induces a diminished vasoconstrictive responsiveness of aortic smooth muscle (13, 15) and structural and functional adaptations of arterial microvasculature of hindlimb skeletal muscles (14). Zhang et al. (34, 37, 64, 65) have demonstrated that differential structural remodeling and perivascular innervation state changes in opposite directions occur in large- and medium-sized arteries and arterioles of the fore and hind body regions of rats during simulated microgravity. They have also demonstrated the downregulation of the structure and function of the rat hindquarter medium-sized conduit arteries and arterial vascular beds after simulated microgravity (33, 36, 64, 65). Collectively, these studies support the hypothesis that at least part of the inability to elevate peripheral vascular resistance of the astronauts postflight results from an adaptive downregulation in structure and function of the arterial vasculature, especially the altered intrinsic vasoconstrictor properties of the resistance vasculature (14, 64). With respect to the vessels in fore body regions, our laboratory’s previous work has demonstrated that simulated microgravity induces hypertrophic remodeling changes in the common carotid and basilar arteries (36, 38) and plastic changes of the perivascular adrenergic and peptidergic nerve fibers in a hyperinnervation state (37). Recently, the smooth muscle hypertrophy in the basilar artery has been reported by Wilkerson et al. (58), which is consistent with our findings. It has been further reported by Geary et al. (24) that simulated microgravity increases myogenic tone of cerebral arteries through both NOS-dependent and -independent mechanisms. Despite the aforementioned studies, the simulated microgravity-induced changes in cerebral vasoreactivity to both vasoconstrictors and vasodilators remain unknown.

Interestingly, the reported cerebral vascular changes by simulated microgravity are quite similar in certain respects to the structural and functional adaptations induced by hypertension. It has been established that hypertension produces vascular hypertrophy (28, 42, 45), enhances myogenic tone (43), and alters endothelial morphology and function (17). Further studies have shown that endothelial release of both NO and endothelium-derived hyperpolarizing factors (EDHF) is reduced in isolated mesenteric arterial preparations from stroke-prone spontaneously hypertensive rats (SHRSP) (54), that the 5-hydroxytryptamine (5-HT)-induced contraction in basilar arteries is attenuated to a much lesser extent in spontaneously hypertensive rats (SHR) than in Wistar-Kyoto (WKY) rats (62), and that endothelial dysfunction may also contribute to enhanced 5-HT-induced constriction in large cerebral arteries of SHRSP (39). Thus it is important to examine whether similar impairment in endothelial function on 5-HT may be detected in cerebral arteries from rats after simulated microgravity.

Therefore, the purposes of the present study were 1) to examine whether vasoreactivity of cerebral basilar artery is altered by adaptation to medium-term simulated microgravity and 2) to determine whether endothelial modulation to 5-HT response is impaired in rats after simulated microgravity.

**MATERIALS AND METHODS**

**Animals**

The protocol and procedures described below were approved by the Animal Care and Use Committee of the Fourth Military Medical University and were in accordance with the guidelines on the care and use of animals required by the American Physiological Society.

Sixty male Sprague-Dawley rats weighing between 180 and 200 g were randomly assigned to either tail-suspended (TS, n = 30) or control groups (Cn, n = 30). The tail-suspension hindlimb-unloading technique (60) with modification from our laboratory (7, 8, 63) was used to induce antishortstatic hypokinesia in the rat. The modified method for tail suspension has been described in detail previously (7, 8). Briefly, the tail was cleaned and dried. Tincture of benzoin and resin were successively sprayed on the tail to protect the skin from irritation and form a sticky surface. A normal-width strip of adhesive tape looped over a plastic bar in the middle of the tape was then attached laterally along the proximal portion of the tail. The tape was then secured to the tail by wrapping the taped portion of the tail in three tail-width tape strips that twined separately around the tail and then a layer of mesh netting. The tail was divided into four quadrants, and only an opposite pair of quadrants (lateral or dorsalventral) was used for applying the tape. Tape was replaced weekly, using the alternate pair of quadrants. The rats were then housed in a room maintained at a 12:12-h light-dark cycle. After the 28-day head-down tilt and hindlimb-unloading period, animals from the Cn and TS groups were anesthetized with pentobarbital sodium (40 mg/kg ip) and killed by exsanguination via the abdominal aorta. Brains were rapidly removed from the cranial cavity and placed in a dissecting dish with cold oxygenated Krebs solution.

**Vessel Preparation**

The basilar artery was carefully isolated from the brain with the aid of a dissecting microscope. The vessel was freed of fat and connective tissues and cut into rings (2 mm in length) with scissors. In protocols examining responsiveness in the absence of vascular endothelium, the endothelial cells were destroyed at this stage by rubbing the intimal surface gently with a stainless steel wire (200 μm in diameter) inserted through the lumen.

For tension measurement, two stainless steel wires (each 40 μm in diameter) were carefully passed through the lumen of an arterial ring under a dissecting microscope. The two ends of each wire were fixed to a specially made plastic holder designed according to Mulvaney and Halperrn (41) with modifications. Then each wire was stretched tight by adjusting two fine screws on each side of the holder. One holder was fixed to a micrometer-controlled device to allow the vessel to be stretched by known increments. The other was tightly attached to a force-displacement transducer (TB-651T, Nikon Kohden, Tokyo, Japan), which was connected via an...
amplifier to a polygraph recorder (RM-6000, Nihon Kohden) for isometric tension recording. Each vessel apparatus was mounted in a 20-ml organ bath of 20-ml volume filled with Krebs solution. The Krebs solution was maintained at 37°C and was continuously bubbled with a gas mixture of 95% O₂-5% CO₂. In a preliminary study, the optimal initial resting force of rings was determined by repeated exposures to 100 mM KCl at increasing vessel diameters. After 60-min equilibration at zero resting force, the vessel rings were individually stretched by 40-mg increments. As each increment of passive resting force, the contractile response to 100 mM KCl was determined until optimal resting force was identified as ~200 mg. Thus all subsequent pharmacological experiments were started after the rings were allowed to equilibrate at this initial resting force for 60 min.

Experimental Design
Two separate protocols were performed in this study.

Protocol 1. In this first series of experiments, the changes in vascular responsiveness of basilar arterial rings from Cn (n = 10) and TS (n = 10) rats to several vasoconstrictors and dilators were examined. KCl, arginine vasopressin (AVP), and 5-HT were chosen as vasoconstrictors to allow comparison of contractile responses resulting from activation of voltage-gated Ca²⁺ channels (KCl) and receptor-mediated (AVP and 5-HT) mechanisms. Concentration-response relationships were determined by cumulative addition of KCl (10–100 mM), AVP (10⁻¹³–10⁻⁶ M), or 5-HT (10⁻⁹–10⁻⁵ M) to rings with endothelium intact.

After the vasoconstrictor experiments, vasodilator responses were determined using the same arterial rings. To examine whether there was any change in vasodilatory responsiveness, concentration-response relationships to the cumulative addition of ACh (10⁻⁵–10⁻⁵ M), thrombin (0.01–1 unit/ml), adenosine (Ado; 10⁻⁹–10⁻⁴ M), and sodium nitroprusside (SNP; 10⁻⁹–10⁻⁴ M) were determined, respectively, in the rings precontracted by 40 mM KCl. These vasodilators were chosen because they induce vasodilator responses through different mechanisms. ACh and thrombin induce relaxation indirectly through the release of the endothelium-derived relaxing factor (nitric oxide), which induces dilation through the guanylate cyclase-cGMP pathway (55). Ado induces dilation through an A₁-receptor-adenylate cyclase mechanism (6). SNP has a direct dilatory effect on the vascular smooth muscle by activation of guanylate cyclase (2).

Protocol 2. To assess the effects of endothelium modulatory role on change of contractile responsiveness, the contractile responsiveness of basilar arterial rings with endothelium-denuded from Cn (n = 10) and TS (n = 10) rats was measured. The results indicated that endothelium had an inhibitory effect on vascular contractile responses to 5-HT in Cn rat basilar arterial rings rather than in TS rat arteries. To determine which endothelium-derived vasodilators were related to this inhibitory effect of endothelium, 5-HT-induced concentration-response relationships of arterial rings from Cn (n = 10) and TS (n = 10) rats were also performed in the presence of 10⁻⁵ M indomethacin, 10⁻⁵ M N⁶-nitro-L-arginine (L-NNA), or 3.2 mM tetraethylammonium (TEA). Indomethacin prevented the production of vasoactive prostanoi ds, L-NNA inhibited nitric oxide synthesis, and TEA blocked K⁺ channels activated by EDHF.

Solutions and Drugs
The Krebs solution contained (in mM) 118.3 NaCl, 4.7 KCl, 25 NaHCO₃, 1.2 MgSO₄, 1.2 KH₂PO₄, 2.5 CaCl₂, 11.1 glucose, and 0.026 EDTA; pH 7.4. All drugs and chemicals were obtained from Sigma Chemical (St. Louis, MO). Indomethacin was dissolved in ethanol for stock solutions, and other drugs were dissolved in distilled water.

Statistical Analysis
Contractile responses to drugs are presented as absolute values in milligrams of force. The agonist concentration that produced 50% of the maximal vasoconstrictor response was designated as EC₅₀. The relaxation of arterial rings to drugs was expressed as a percentage, taking the amplitude of precontraction induced by 40 mM KCl as 100%. The vasodilator concentration that produced 50% of the maximal relaxation of KCl precontraction was designated as IC₅₀. Statistical significance was determined using unpaired t-test, or one-way ANOVA for multiple comparisons. Results are given as means ± SE. Means were considered significantly different when the P value was <0.05.

RESULTS
Vasoconstrictor Responses
As shown in Fig. 1, cumulative application of KCl, AVP, or 5-HT elicited concentration-dependent contractile responses in ring preparations of basilar arteries from Cn and TS rats. TS treatment significantly increased (P < 0.05) the maximal responses to KCl (714.56 ± 53.37 mg), AVP (895.26 ± 41.06 mg), and 5-HT (665.72 ± 57.56 mg) relative to that of basilar arteries from Cn rats (KCl, 537.57 ± 26.60; AVP, 689.73 ± 24.77; 5-HT, 114.84 ± 22.88 mg). However, sensitivity (EC₅₀) of basilar arterial rings from TS rats to KCl (34.82 ± 1.31 mM), AVP (6.46 ± 1.69 × 10⁻⁶ M), and 5-HT (7.08 ± 2.51 × 10⁻⁶ M) was not significantly different from that of Cn rats (KCl, 33.81 ± 0.92 mM; AVP, 4.68 ± 1.82 × 10⁻⁴ M; 5-HT, 5.13 ± 3.15 × 10⁻⁶ M).

Vasodilator Responses
The vasorelaxants ACh, thrombin, Ado, and SNP all induced concentration-dependent decreases in force in basilar arterial rings precontracted by 40 mM KCl for both groups. The data summarized in Table 1 show that the maximal vasorelaxant effects of the four kinds of vasodilators on basilar arteries from TS rats were comparable with from Cn rats. Furthermore, the sensitivity (IC₅₀) of basilar arterial rings to these vasodilators from both groups were also not significantly different.

Effects of Endothelium Removal on Vascular Contractile Responses to 5-HT
Deendothelialization eliminated the difference in maximal response to 5-HT between the TS and Cn groups. Fig. 2A shows typical tracings of contractile responses to 5-HT in TS and Cn basilar arterial rings with endothelium. Cumulative application of 5-HT elicited a much higher concentration-dependent contractile response in TS than that in Cn. Fig. 2B shows that removal of the endothelium dramatically enhanced the contractile response to 5-HT in Cn basilar arterial ring, resulting in responses comparable with
that in TS. In contrast, the maximal contractile responses to 5-HT were basically not affected by endothelium removal in TS basilar arterial rings. Mean concentration-response curves for 5-HT are showed in Fig. 3A. Removal of endothelium significantly increased the maximal responses of Cn rat basilar arteries to 5-HT from 114.84 ± 22.88 to 504.70 ± 30.74 mg (P < 0.05), which was not significantly different from that of TS rat basilar arteries without endothelium (561.59 ± 36.57 mg) but still significantly lower than that of endothelium-intact arteries from TS rats (665.72 ± 57.56 mg). These observations strongly suggest that the enhancement of contractile responses to 5-HT in TS rats is due, at least partially, to an impairment in endothelial modulatory function on smooth muscle contraction that is normally present in Cn rat basilar arteries.

Effects of l-NNA, Indomethacin, and TEA on Vascular Contractile Responses to 5-HT

To determine whether endothelium-derived vasodilators such as NO, PGI2, and EDHF that activated K+ channels on smooth muscle cells to cause relaxation of vessels may contribute to this endothelium-dependent mechanism, effects of l-NNA, indomethacin, and TEA on vascular contractile responses to 5-HT in Cn and TS basilar arteries with endothelium were examined. Fig. 3B shows that treatment with 10⁻⁵ M indomethacin or l-NNA did not significantly affect the concentration-dependent responses to 5-HT in either Cn or TS, indicating that the cyclooxygenase product and NO are not involved. Furthermore, the resting force was not affected by 10⁻⁵ M indomethacin but was altered by 10⁻⁵ M l-NNA with an increment of 99.31 ± 3.20 mg in Cn and 98.68 ± 5.58 mg in TS, indicating that NO is released by basilar arteries in basal condition for both groups.

These results have suggested that the endothelium-dependent attenuation of 5-HT-induced contraction may be mediated by the opening of K⁺ channels activated by EDHF. Therefore, the effect of TEA (a K⁺-channel blocker) on Cn basilar arterial rings to 5-HT was examined. The typical tracings shown in Fig. 4 demonstrated that the application of TEA dramatically augmented the 5-HT-induced contractions and spontaneous motions of Cn basilar arterial ring with endothelium intact. Mean concentration-response curves shown in Fig. 3A further demonstrated that applica-

Table 1. Maximal relaxation and IC₅₀ values of ACh, thrombin, adenosine, and SNP in basilar arterial rings from Cn and TS rats precontracted by 40 mM KCl

<table>
<thead>
<tr>
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<th>Maximal Relaxation, %</th>
<th>IC₅₀ Value</th>
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<tr>
<td></td>
<td>Cn (n = 10)</td>
<td>TS (n = 10)</td>
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<tr>
<td>ACh (10⁻⁶ M)</td>
<td>47 ± 5</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>Thrombin (10⁻² U/ml)</td>
<td>59 ± 1</td>
<td>57 ± 4</td>
</tr>
<tr>
<td>Adenosine (10⁻⁶ M)</td>
<td>51 ± 5</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>SNP (10⁻⁶ M)</td>
<td>92 ± 6</td>
<td>96 ± 4</td>
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Values are means ± SE; n, number of rats per group. SNP, sodium nitroprusside; IC₅₀, 50% inhibitory concentration. There were no significant differences between control (Cn) and 4-wk tail-suspended (TS) rats for any of these parameters.
tion of TEA significantly enhanced the contractile responses of Cn basilar arteries with endothelium intact to 5-HT but increased only to a much lesser extent the contractile responses in TS arteries. Additionally, the resting forces of the basilar arterial rings were significantly increased after application of 3.2 mM TEA by nearly the same amplitude in Cn (135.68 ± 5.61 mg) and TS (137.86 ± 7.52 mg) arteries, suggesting that the basal tone of rat basilar arteries for both groups is mediated by the activation of K\textsuperscript{+} channels. These results suggest that the endothelium-dependent mechanism attenuating 5-HT responsiveness in Cn rats is related to K\textsuperscript{+} channels.

DISCUSSION

One of the purposes of the present study was to test the hypothesis that the tail-suspended hindlimb-unloading treatment induces a generalized enhancement of vascular maximal force development in rat basilar arterial rings to both receptor- and non-receptor-mediated agonists. The results support the hypothesis in that the maximal contractile force elicited by the vasoconstrictors KCl, AVP, or 5-HT was much greater in basilar arterial rings from TS compared with that from Cn rats. These data have demonstrated that the simulated microgravity may induce in brain vessels not only hypertrophic change (36, 38, 58), hyperinnervation state of perivascular nerves (37), and increase in the myogenic tone (24), but also the vasoreactivity enhancement to both receptor- and non-receptor-mediated agonists. However, alterations in vasodilator reactivity were not found in the present study. With 40 mM KCl-induced submaximal preconstriction, vasodilatory responses induced by ACh, thrombin, Ado, and SNP were not significantly different between groups. The second purpose of this study was to determine whether this hyperreactivity of the brain vessels is associated with an impairment in the modulatory function of the endothelium. The results that endothelium removal or TEA application can dramatically increase the vasoconstrictor responses to 5-HT only in basilar arteries from Cn but not TS rats have provided evidence that the enhanced contractility to 5-HT in basilar arteries resulted from simulated microgravity may be explained by the inability of endothelium to release an EDHF-like substance but not by augmented release of endothelium-derived contracting factor.

Although the prediction that microgravity-induced chronic changes in regional vascular transmural pressures may well alter differentially the structure of vessels in different body regions to accommodate the local changes in stress was first made by Hargens and coworker (26, 27) in the late 1980s, findings providing direct evidence of differential structural and functional
adaptations of vessels to simulated microgravity were reported (64, 65) in the late 1990s. Using the tail-suspension hindlimb-unloaded rat model to mimic the redistribution of transmural pressures and flows in the vasculature during microgravity, investigators observed structural remodeling changes in opposite directions in large and medium-sized vessels in fore and hindquarters, respectively, of rats in simulated microgravity (37, 64, 65). For example, in the arteries of the fore body regions, e.g., the common carotid and basilar arteries, hypertrophic changes, such as significant increases in both the lumen diameter and total medial area, increases in the number of layers of smooth muscle cells, hyperplasia and conversion of smooth muscle cells of contractile phenotype into that of synthetic phenotype and migration into the subendothelial layer were observed. On the contrary, in the hindquarter arteries, e.g., the femoral and anterior tibial arteries, atrophic changes, such as significant decreases in both lumen diameter and medial area of the vascular wall, reduction in the number of layers of smooth muscle cells, and fewer myofilaments in smooth muscle

Fig. 3. Effects of endothelium removal and tetroethylammonium (TEA) (A), or N\textsuperscript{G}-nitro-L-arginine (L-NNA) and indomethacin (Ind) (B) on 5-HT-induced contractions in rings of basilar arteries from Cn and TS rats. Values are means ± SE; n = 10 per group. A: *response significantly different from other groups’ responses (P < 0.05); †response of rings with endothelium intact from TS significantly different from Cn rings with endothelium removed (P < 0.05); †response of TS rings in the presence of 3.2 mM TEA significantly different from response of Cn rings in the presence of 3.2 mM TEA (P < 0.05). B: *response of Cn groups significantly different from TS groups’ responses (P < 0.05).

Fig. 4. Tracings showing the effects of TEA on 5-HT-induced contractions in a basilar arterial ring with endothelium intact from a control rat. 5-HT was added cumulatively at concentrations shown above the tracings as −log M. W, washout of drugs. Note that application of 3.2 mM TEA increased the resting force from 204 to 327 mg and significantly enhanced the spontaneous vasomotion in TS ring (lower tracing).
cells, were observed. More recently, the hypertrophy of basilar artery after 2 wk of simulated microgravity was reported by Wilkerson et al. (58), which is consistent with our findings (36, 38). It is well known in vascular biomedicine that structural and functional changes in vessels should consistently be interdependent in their adaptations to changes in local stresses (18). Although it has been reported that simulated microgravity resulted in diminished vasoconstrictor responsiveness in hindquarter arteries, such as the abdominal aorta and mesenteric and femoral arteries (12–15, 33, 48), the changes in vasoreactivity of brain vessels have not yet been reported. However, with perfused isolated middle cerebral arterial segments, Geary et al. (24) have shown that simulated microgravity results in an increase in myogenic tone in resistance-sized cerebral arteries. Because the pressure-mediated myogenic tone and agonist-mediated vasoconstrictor responsiveness may share a common intrinsic smooth muscle control mechanism, therefore, the findings of the present study are expected. Collectively, the present study is an important supplement to the previous work concerning differential structural and functional adaptations of vessels to microgravity (24, 33, 36, 58, 64), demonstrating that the enhanced vasoreactivity and myogenic tone of brain vessels are the functional consequences of the cerebral vascular structural adaptation to simulated microgravity. It is intriguing to indicate that the aforementioned cerebral arterial remodeling and hyperreactivity in rats by simulated microgravity are quite similar to the vascular adaptations induced by hypertension (16, 19, 43, 44), suggesting that these changes are induced largely by sustained elevations in transmural pressures in cerebral vessels.

Although the precise mechanisms for this generalized enhancement of vasoconstrictor responsiveness remain yet to be elucidated, the findings of the present study suggest that there is not an obligatory modification of receptor-second messenger signal transduction pathways (13, 15), because the increase in maximal isometric contractile force was evident for both receptor-mediated agonist (AVP, 5-HT) and non-receptor-mediated agonist (KCl). Because the hypertrophy of the basilar artery is evident in rats after simulated microgravity, then the increased amount of contractile apparatus involving cellular calmodulin protein expression, and myosin light chain kinase activation or activity (12, 13, 15), and this may also be due to changes in mechanisms that regulate membrane excitability (24). However, it needs further clarification.

In addition to mechanisms intrinsic in the vascular smooth muscle of the basilar artery, the present study also provides evidence indicating that defects in modulatory function of endothelium induced by simulated microgravity are involved. The endothelium modulates underlying vascular smooth-muscle tone by releasing relaxing factors such as nitric oxide (NO) (21, 30, 46), prostacyclin (PGL2) (47), and EDHF (9), as well as endothelium-derived contracting factors, such as the endothelins (61) and products of cyclooxygenase (prostaglandin H2 and thromboxane A2) (25). In the present study, it was found that the contractile responses to 5-HT in TS basilar arteries were not markedly changed by treatment of endothelium removal; however, endothelium denuding significantly augmented the contractile responses in Cn rats (Figs. 2 and 3A). Two important implications can be derived from our results. First, the attenuating mechanism to 5-HT-induced contraction in rat basilar arteries is due to the release of relaxing factors from the endothelium rather than contracting factors. Second, the augmented 5-HT-induced contraction in TS basilar arteries is apparently due to an impairment in the attenuating effect. To determine what vasodilator factors mediated the attenuating effect, 5-HT-induced contractile responses in basilar arteries were examined in the presence of L-NNA, indomethacin, or TEA (Figs. 3 and 4). The results showed that application of L-NNA or indomethacin did not change the contractile responses to 5-HT in either Cn or TS basilar arteries, indicating that neither NO nor PGL2 is responsible for the endothelium-dependent attenuating effect on 5-HT-induced contraction. Furthermore, treatment with TEA also markedly enhanced the contractile responses to 5-HT in Cn rat basilar arteries, but not in TS, having an effect similar to the treatment of endothelium removal. These data strongly suggest that EDHF may be the relaxing factor responsible for the endothelium-dependent attenuating effect on 5-HT-induced contractile responses, because it has been established that the action of EDHF is mediated through activating K+ channels on membrane of vascular smooth muscle cells, and TEA is a nonselective K+ channel blocker (9, 22, 40). It is intriguing that similar changes have been reported for SHR. Yokota et al. (62) have shown that the endothelium of basilar artery exerts an attenuating influence on the responsiveness of smooth muscle to 5-HT in WKY rats but to a much lesser extent in SHR because the inability of the endothelium to release an EDHF-like substance. The possible explanation to this similarity is that the transmural pressures in the brain vessels are consistently elevated in both SHR and TS rats and the resultant changes in local tissue stress trigger the events of vascular adaptation (24, 36, 56, 58).

Furthermore, perivascular adrenergic and peptidergic nerve fibers may contribute to the local vascular adaptations, because vascular smooth muscle, endothelium, and perivascular nerves are integrated as a functional entity to accomplish the complex and unified structural and functional autoregulation (64, 66). This has been supported by the findings from previous work. On the one hand, simulated microgravity may induce differentiated adaptational changes in the innervation state of vessels in different body regions (34, 37, 64). Hyperinnervation of both the adrenergic and peptidergic (NPY-, CGRP-, and SP-containing) perivascular nerve fibers around the cerebral arteries has been
shown in rats during simulated microgravity (37). On the other hand, increased sympathetic innervation of NA- and NPY-containing nerve fibers supplying the basilar artery was seen in spontaneously hypertensive rats (49).

In the present study, we measured the vasodilator reactivity of rat basilar arteries to a variety of vasodilators, such as ACh, thrombin, Ado, and SNP. However, the present study showed that there were no significant differences in the vasodilatory reactivity of basilar arteries to these vasodilators between TS and Cn rats (Table 1). Unlike the mentioned similarity in the impairment of endothelial attenuating influence on the 5-HT-induced vasoconstriction between TS and SHR, the present findings are inconsistent with those in hypertensive rats regarding changes of vasodilatory reactivity (62). The maximal relaxation induced by ACh in 5-HT-precontracted basilar arteries is smaller in SHR than in WKY rats (62). The reason for this discrepancy is not yet clear. One possible explanation is that the order of severity in endothelial dysfunction is different between pathological adaptation and pathological changes. Even in pathological conditions, the severity of impairment may not be of the same order. For example, endothelium-dependent relaxation is more prominently impaired in SHRSP than in conventional SHR (53, 54). In addition, the degree of dilation may also be related to the magnitude of preconstriction tension, kind of preconstrictors used (15), and strain of rats (13, 48). Therefore, further studies are needed to examine whether there is any impairment in the vasodilatory reactivity of basilar vessels after simulated microgravity.

Although it is now well recognized that the stimulus initiating the cerebral vascular adaptations is due to the local tissue-stress changes causally related to the sustained elevation of transmural pressure and/or flows within the brain vessels, direct measurements of cerebral vascular pressures are still lacking. Direct measurement indicated that mean aortic pressure increased from 124 ± 7 mmHg during standing to 145 ± 2 mmHg after 14 days of tail-suspended hindlimb unloading (58). During standing, the basilar artery pressure has been estimated to be 80% of aortic pressure (58). If the percentage remains unchanged during head-down tilt, it is estimated that the basilar artery mean blood pressure would be increased by ~17 mmHg during tail suspension compared with during standing. Data indicating changes in cerebral blood flow in rat during simulated microgravity are also rare. Existing data concerning hemodynamic changes in human cerebral circulation during both bed rest and real microgravity are fewer and inconclusive (56, 67). However, the changes revealed in the en face preparations (that the length and width of the endothelial cells of the common carotid arteries of the suspended rats were lengthened and shortened, respectively) have suggested an enhanced blood flow to the head region during simulated microgravity (35). Whether this is due to an increase in extracranial blood flow with cerebral blood flow unchanged remains to be clarified. Measurements using radiolabeled microspheres have indicated that acute hindlimb unloading induces reductions in blood flow to most regions of the brain, but these lower flows tend to return to control levels with prolonged hindlimb unloading (59).

It is possible that the above-stated structural and functional adaptations in cerebral vessels may also occur in humans during simulated and/or real microgravity. Folkow (18) has postulated that, in rats, locally induced vascular structural adaptation can be largely completed within 2 wk and, in humans, within a few months, presumably reflecting the five- to sixfold lower metabolic rate in humans. There is evidence that suggests that vascular adaptations may occur in humans. For example, a greater cerebral vasoconstriction response to 30° head-down tilt, suggesting an enhanced cerebral vasoconstriction, perhaps resulting from acclimation to chronic cerebral hypertension in microgravity, was reported by Gazenko et al. (23). Furthermore, the effect was substantially more apparent and prolonged after longer flights (23, 56). In a ground-based human study, an impairment in autoregulation of cerebral circulation and an attenuated increase in muscle sympathetic nerve activity during LBNP testing were observed in subjects after a 2-wk bed rest (67). Furthermore, it has been suggested that microgravity-induced remodeling and hyperreactivity in cerebral arterial vasculature may also play an important role in the genesis of postflight orthostatic intolerance by the so-called “cerebrovascular syncpe-initiating mechanism” (56, 57) or by exacerbating the decrease in cerebral blood flow associated with hypotension (32). Another important physiological implication of the adaptation of cerebral vessels to microgravity would be the prevention of cerebral edema and possibly stroke (27, 58, 64, 65). In this respect, studies on hypertensive rats have provided evidence illustrating the role of structural and functional adaptation of cerebral vessels in edema and stroke prevention and blood-brain barrier maintenance in stroke-prone rats (29, 51).

In conclusion, the findings of the present study have shown that tail-suspended hindlimb unloading induces a generalized enhancement in basilar arterial responses to agonist-mediated vasoconstriction. On the basis of the responses of the arteries to various vasconstrictor agonists, it appears that this hyperreactivity may be causally related to the hypertrophic remodeling changes of the brain vessels. Furthermore, the hyperreactivity to 5-HT may also be due to an impairment in endothelial modulatory function. This is the first study to examine the alterations in arterial contractile properties of cerebral vessels caused by simulated microgravity and is an important supplement to the previous relevant studies (24, 36, 37, 58, 64) on cerebral vascular adaptations to microgravity.

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