Several long-term antigravity defense mechanisms are activated in the lower extremity veins and microvessels when an organism is exposed to chronic orthostatic load. These mechanisms involve acute pressure-induced myogenic response, counterregulatory K+ channels, functional and structural remodeling of sympathetic innervation, and vascular network properties.

Antigravity defense reactions probably represent one of the phylogenetically oldest physiological adaptation mechanisms of the terrestrial animals. Gravitational load for an animal on land is larger by a factor of ~10^6 than for an animal floating in the sea. Consequently, physiological adaptation to this huge gravitational stress represented one of the greatest evolutionary challenges for adapting to terrestrial life.

The upright body posture of humans introduced additional orthostatic complications. The long hydrostatic columns that exist in the large blood vessels in the upright posture impose gravitational forces that substantially raise the transmural pressure and vascular wall stress in the lower part of the body, especially in the highly compliant veins. In the absence of effective compensatory mechanisms, the consequent orthostatic venous pooling of the blood and suppressed cardiac output result in an immediate and profound fall of the arterial pressure, accompanied by syncpe. The consequences of the failure of these mechanisms are frequently seen after long-lasting space flight or chronic bed rest.

Much is known about the short-term mechanisms responsible for the cardiovascular antigravity defense responses, including carotid and aortic baroreceptor mechanisms, vestibulosympathetic reflexes, and hormonal components such as catecholamines, angiotensin II, aldosterone, vasopressin, etc. (1, 7, 17, 20). However, long-term vascular adaptation processes supporting the orthostatic tolerance of the organism are not well understood, especially those of the veins (12). We have therefore developed a new model to investigate the physiological responses to a sustained orthostatic load of veins in the lower extremity of rats, as well as a precise computerized videomicroangiometry and techniques for quantitative micromorphological investigations. Comparative studies were carried out by using human lower and upper limb veins.

A novel rat model of long-term orthostatic gravitational loading

An original experimental method was developed to keep animals in long-term quasi-orthostatic body position without substantial locomotor restrictions and nonspecific stress (13, 14). In brief, specially designed, long, tube-like, tiltable cages were constructed from transparent acrylic plastic for adult rats. The animals were housed individually for 2 wk in these cages, which were set in an oblique position (45°) to chronically maintain an orthostatic (head-up tilt) posture. They could walk up and down on the steep metal grid of the cage but could not turn around. Free access to food and tap water was provided at the upper end of the cage. Control rats were kept in the same type of tubular cage but in a horizontal body position. Animals were removed for 1-h periods each day and were allowed to groom freely in a traditional rat cage. This time was also used for measurement of body weight and daily food and water intake and for cleaning the cages. The experimental procedures conformed to The Principles of Laboratory Animal Care (US National Institutes of Health Publication no. 86-23, revised 1985), as well as to the related Hungarian Laws on Protection of Animals (no. 243/1998).

Studies were initially performed to characterize the rats living under these conditions for at least 2 wk. It was shown that the head-up tilt position resulted in an immediate and permanent doubling of saphenofemoral venous blood pressure, whereas mean pressure in the femoral artery did not change substantially (13). Water content of the lower extremity muscles was not influenced in this orthostatic model, and edema did not develop (5). Feeding and drinking activity of the animals was not inhibited by the long-term tilted body position (14). The locomotor activity of the rats was characterized by using a computer-based infrared video tracking method that continuously recorded and quantitated the movement of these rats throughout the day (6). It was demonstrated that the circadian pattern of locomotor activity of chronically tilted rats did not differ substantially from that of control rats kept in horizontal cages.

Plasma levels of two “stress hormones,” ACTH and corticosterone, were found to rise transiently above normal resting values only during the first day of tilting (13). In these studies, rats appeared calm and comfortable in the tube cages following a short orientation reaction observed during the first few hours of the first day. After daily grooming sessions, they walked spontaneously back to their individual cages from the palm of the assistant. Together, these studies indicate that the long-term changes of vascular properties observed in the head-up tilted animal groups are not likely to be due to nonspecific stress effects.
Pressure-induced myogenic response of rat saphenous vein and its augmentation due to long-term orthostatic body position

Myogenic responsiveness of saphenous veins from both control and tilted rats was studied by using isolated, perfused, and superfused cylindrical vessel segments subjected to pressures covering the full physiological range. Recently, a new computerized videomicroangiometry was developed to determine precisely the pressure-induced changes in vascular stress-strain characteristics (Experimetria, Budapest, Hungary). Acute pressurization in the physiological range and in normal medium resulted in a significant myogenic tone generation. This myogenic tone controlled about one-third of the total venous lumen capacity. Both in vitro and in vivo electrophysiological measurements proved that the above active biomechanical responses were coupled with proportional and reversible vascular smooth muscle membrane depolarization (11).

After 2 wk of maintaining animals in the experimental orthostatic body position, the active myogenic response of saphenous vein segments to the acute pressurization was twice as large as that of veins isolated from control rats. The anatomic “passive” diameter of the veins and the smooth muscle count in the vessel wall increased, but overall wall thickness did not change significantly (11, 13). Thus intrinsic myogenic tone generated in the lower extremity veins was significantly augmented by the long-term orthostatic-type gravitational loading. This augmentation contributed substantially to the ability of these veins to increase their vascular wall tone appropriately and resist the distending pressure when it increased acutely.

High myogenic responsiveness of human saphenous vein and its counterregulation via K⁺ channels

Studies in our laboratory have shown that the myogenic tone in the isolated side branches of human saphenous vein is steeply enhanced with acute increases of intraluminal pressure. This response is endothelium independent. The contribution of this myogenic tone to maintaining vascular caliber against pressure averages a quarter of the total vascular diameter at a physiological pressure of 20 mmHg and thus can control more than half of the total lumen capacity of the vein. This represents a substantial range for resisting the distending pressure and reflects a powerful intrinsic venous capacity autoregulation (11). This is especially remarkable when we consider that human saphenous vein is regularly exposed to high (up to 60–80 mmHg) pressure in the standing position over many decades. In contrast, human cephalic veins, being normally subjected to a low (<10 mmHg) pressure load, do not exhibit significant myogenic tone. Furthermore, it was demonstrated by using specific ion channel blockers (iberiotoxin and 4-aminopyridine) that Ca²⁺-activated and voltage-gated K⁺ channels counterregulate the myogenic tone of human saphenous vein, supposedly by restoring the membrane depolarization in the smooth muscle cell membrane (18). This effect was found to be dependent on intraluminal pressure (Fig. 1). The role of the large-conductance Ca²⁺-activated K⁺ channels was confirmed by Milesi et al. (10) in this human vein by using patch-clamp techniques. Indeed, these results suggest that this ion channel may represent a target site for alleviation of conditions of increased venous tone. It should be noted, however, that not all veins have been found to respond in the same manner. No evidence was found for the participation of these K⁺ channels in the regulation of vascular tone in either the human cephalic (Fig. 1) or in the rat saphenous veins (18). Together, these observations suggest that a higher level of venous myogenic tone correlates well with higher chronic pressure loads and that the development of its adaptive ionic tuning may be a long-term process, being perhaps an evolutionary development.

Pathological aspects of venous adaptation to chronic pressure

There is firm evidence in the literature that the range of normal physiological adaptation of extremity veins to chronically elevated transmural pressure is limited. Extreme pressure loads induce pathological changes.

Human saphenous vein segments are frequently used as arterial grafts in aortocoronary and femoropopliteal bypass surgery. These grafted veins respond to the high arterial pres-

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**FIGURE 1.** Effect of tetraethylammonium chloride (TEA, 10 mM), a general inhibitor of cell membrane K⁺ channels, on human saphenous (A) and cephalic (B) vein segments as a function of pressure. Data are %values of control (physiological salt solution (PSS)) diameter. It was shown that TEA administration induced a dose- and pressure-dependent vasoconstriction in the human saphenous vein (*P < 0.05). In human cephalic vein, however, there was no change in the diameter after TEA administration at any pressure level. Modified from Ref. 18.
sure with abnormal fibrosclerotic increases in wall thickness, reducing the extremely high average mechanical wall stress back to normal, but the active tone is lost (11, 12). In rabbit, less pronounced but sustained elevation of extremity venous pressure above normal by increasing the outflow resistance also results in a significant increase of wall thickness, with high vascular tone maintained (4). Acutely, normal human saphenous vein segments were found to fully distend at intraluminal pressures of 50 mmHg or greater, and serotonin still can constrict these veins substantially (16). Several data in the literature suggest that sustained high venous pressure due to valvular insufficiency contributes to the development of varicosity, the most frequent disease of lower extremity veins.

Saphenous vein rings from hypertensive patients are less distensible, slower to relax, and more reactive to vasoactive agonists than those of normotensive patients (10). Diabetes mellitus causes an impaired dilatation of human saphenous vein, an impairment mediated through a reduction of ATP-sensitive K⁺ channels (19). Similar alterations in ion channel activity may occur with diabetes-induced vascular complications such as vasospasm and even hypertension. It has been suggested by Yontem et al. (19) that ATP-sensitive K⁺ channels may also play a role in the altered hemodynamic performance of human grafted veins.

**Striking increases in sympathetic innervation of lower extremity vessels due to chronic experimental orthostasis**

Using quantitative electron microscopy, immunohistochemistry, and the orthostasis model described above, recent studies proved that 2 wk of experimental head-up tilted body position causes significant increases (52–95%) in the sympathetic innervation density of saphenous vein (14). These increases involve both the density of adventitial nerve terminals and their norepinephrine-containing synaptic microvesicle population (Fig. 2). Similar changes were identified in the adventitia of saphenous artery, although the increase in synaptic vesicle count was substantially larger in the vein. In contrast, innervation density of the arteries and veins of the upper extremities did not respond significantly to chronic tilt. These results are consistent with earlier findings, which show that the sympathetic component of smooth muscle cell membrane potential, measured in vivo by using intracellular microelectrodes, is significantly augmented in the veins of the lower extremities but not in the vessels of the upper extremities after 2 wk of head-up tilting (13). Interestingly, Zhang (20), who studied perivascular innervation density of the hindquarter arterioles in a tail-suspension rat model of microgravity, found hypoinnervation of the hindquarter muscle arterioles. Both this and our study demonstrate a high degree of plasticity of the vascular sympathetic innervation. In another study (2), it was found that sympathoadrenergic responses may significantly amplify the pressure-induced myogenic response in extremity small veins via norepinephrine release. Thus we have good reasons to assume that both functional and structural remodeling of sympathetic innervation in the extremity blood vessels contribute substantially to the adaptive augmentation of venous myogenic response observed after chronic orthostatic-type gravitational load. However, recent studies (3, 9) indicate that local transmural pressure changes of long duration may also participate in the induction of adaptive alterations of the venous myogenic response. It has been demonstrated that long-term experimental inverse orthostatic body position does not result in a diminution of innervation density in the adventitia of saphenous vein (9), whereas it decreases significantly the acute pressure-induced myogenic response of this vein (3, 9).

**FIGURE 2.** Effect of 2 wk head-up tilt on innervation density of saphenous vein and artery (left), as well as of brachial vein and artery (right), in the rat. Nerve terminal density (NTD, top) is the average number of sympathetic nerve terminals per cross-section of 100-μm² adventitia. Synaptic vesicle density (SyVD, bottom) is the average number of synaptic microvesicles per cross-section of 10 nerve terminals. The long-term upright body position resulted in a significant increase in the sympathetic innervation density of saphenous vessels, involving both density of adventitial nerve terminals and their synaptic microvesicle population (*P < 0.05; **P < 0.01). Modified from Ref. 13.
Microvascular network remodeling induced by chronic experimental orthostatic body position

Chronic head-up tilt also influences the microcirculatory network properties. A significant rarefaction of microvessels was found in the hindlimb oxidative skeletal muscles of the rat after 2 wk of experimental orthostasis (Fig. 3). Capillary/muscle fiber ratio in the oxidative part of musculus tibialis anterior and in the musculus soleus were decreased significantly (4). Other network characteristics, including decreased branching angle and increased vessel diameter in the superficial venous system of the saphenous region in rats, also exhibited changes of an adaptive nature in response to long-term orthostatic gravitational load (8). These structural changes in the microcirculation could be determined by local hemodynamics and by humoral mediators. Regardless of the mechanism, the reduced capillary density in the muscle and the optimization of venous network properties could serve to minimize edema formation in the dependent limbs.

Endothelial vesicular system in the saphenous vein responding to long-term gravitational load

Recently, with the use of electron microscopy, large amounts of membrane-bound, electron-dense microvesicles of ~0.1- to 0.2-μm diameter were observed in the endothelial cells of saphenous vein segments from both tilted and control rats (15). Neither acidic phosphatase activity nor lipid content could be identified in these vesicles, indicating that they are neither secondary lysosomes nor lipid inclusions characteristic of apoptosis. A computer-based image analyzer was used to measure the size of vesicles on digitalized electron micrographs. It was found that during 2 wk of experimental orthostasis the total amount of these vesicles decreased relative to the total cell cross-sectional area from 5 to 2.5% (15). It is hypothesized that these structures are secretory vesicles containing a vasoactive agent that influences vascular tone and/or permeability, and thus the endothelium may also participate in vascular adaptation to sustained gravitational changes. Further quantitative electron microscopic histochemical studies are needed to identify the content of the vesicles and to elucidate questions related to their physiological function.

Summary and conclusions

The novel experimental technique developed for modeling chronic orthostatic gravitational load has contributed substantially to identification of unknown local vascular control mechanisms supporting long-term orthostatic tolerance (Fig. 4). These mechanisms include among others augmentation of the acute pressure-induced myogenic response of the saphenous vein and an elevated smooth muscle count in the absence of a significantly increased wall thickness. In healthy human saphenous vein, the myogenic reactivity was found to be higher than that of the experimental animals. This myogenic response of human vein is counterregulated via Ca$^{2+}$-
activated and voltage-gated K⁺ channels. These active ion channels are absent in the human cephalic vein where myogenic reactivity is negligible, and they are also absent in the rat saphenous vein.

In parallel with the augmentation of myogenic response, the sympathetic innervation density of the rat saphenous vein adventitia also increases significantly during 2 wk of experimental orthostatic body position. This morphological remodeling of innervation is also reflected by a significant increase in the sympathetic component of smooth muscle cell membrane potential of the vein. The neural components may play a role in the augmentation of the vascular myogenic response.

In addition to the long-term myogenic and local neural responses of the saphenous vein, microvascular density of oxidative muscles and network characteristics of superficial veins of the lower limb also exhibit changes of an adaptive nature after 2 wk of experimental orthostasis. An electron-dense vesicular system can be seen electron microscopically in the endothelium cells of rat extremity veins. This structure may belong to a secretory transport system that is influenced by chronic orthostatic gravitational stress in the lower extremity.

In conclusion, long-term orthostatic gravitational effects may activate and augment several mechanisms in the extremity vessels. These mechanisms seem to operate in harmony to support orthostatic tolerance of the whole organism effectively.

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