Stauss, Harald M., Jens-Ulrich Stegmann, Pontus B. Persson and Heinz-Joachim Häbler. Frequency response characteristics of sympathetic transmission to skin vascular smooth muscles in rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R591–R600, 1999.—Sympathetic modulation of cutaneous vasomotor waves in humans is most effective at frequencies up to 0.1 Hz. In contrast, sympathetic modulation of mesenteric vasomotor waves in rats is strongest in the frequency band between 0.2 and 0.75 Hz. Therefore, we addressed the question as to whether these different frequency response characteristics are due to species- or organ-specific disparities. Eleven Sprague-Dawley rats were instrumented with catheters in the carotid artery and in the jugular vein, together with electrodes on the centrally sectioned left lumbar sympathetic trunk (LST) and laser Doppler flow probes directed to the plantar surface of the skin of the left and right hind paws. In anesthetized rats, the LST was electrically stimulated at eight different stimulation frequencies, and the responses in laser Doppler blood flow were recorded in the skin of the ipsilateral and contralateral paw. At stimulation frequencies <0.2 Hz, LST stimulation induced corresponding oscillations in skin blood flow in the ipsilateral, but not in the contralateral, paw. These dynamic responses to LST stimulation in the ipsilateral paw were strongest at 0.05 and 0.075 Hz. At higher stimulation frequencies a tonic vasoconstriction was observed. It is concluded that organ-specific disparities exist in sympathetic transmission to vascular smooth muscles, whereas no species-specific differences are apparent in sympathetic transmission to cutaneous blood vessels of humans and rats.

Methods

Animal maintenance during the experimental protocol. The experiments were performed in 11 3-mo-old male Sprague-Dawley rats.
Dawley rats (Tierzucht Schönwalde) weighing 310 ± 3 g (mean ± SE). Anesthesia was initiated by an intraperitoneal bolus injection of 60 mg/kg pentobarbital sodium (Nembutal, Sanofi Santé Animale) and maintained during the experiments by intravenous injections of 10 mg/kg pentobarbital sodium (in saline 1:2 vol/vol) every hour through a catheter placed in the left jugular vein. A second catheter was inserted into the right carotid artery for continuous blood pressure recording. The pentobarbital sodium injections were applied before baseline registrations to avoid differences in the depth of anesthesia between a stimulation period and the preceding baseline recording that served as a direct control. In addition, after an intravenous injection of pentobarbital sodium, the experimental protocol was interrupted until stationary blood pressure and heart rate recordings were obtained. A sufficient depth of anesthesia was judged from the absence of gross fluctuations in blood pressure and heart rate. During the experiments, rats were paralyzed with pancuronium bromide (Curamed, CuraMED Pharma, 76202 Karlsruhe, Germany, 1 mg/kg iv initially and further doses of 0.4 mg/kg iv when necessary) and artificially ventilated with room air through an orally inserted tracheal cannula at a respiration rate of 75 respirations/min (1.25 Hz). End-expiratory CO2 was continuously monitored, and the tidal volume was adjusted to maintain an end-expiratory CO2 concentration of 4% (34). Arterial blood gases and arterial acid-base status were assessed (AVL 990, AVL Scientific, Roswell, GA) at the end of the experimental protocol. O2 tension was 88.3 ± 4.5 mmHg, CO2 tension was 36.9 ± 1.5 mmHg, and pH was 7.45 ± 0.01 (means ± SE). To control body core temperature, the experiments were performed on a servocontrolled heating table maintained at 38°C. The study was conducted in accordance with the National Institutes of Health guidelines for health and care of experimental animals.

LST preparation. Preparation of the left LST and placement of the stimulation electrode was performed as described previously (14). Briefly, the LST was identified bilaterally between paravertebral ganglia L2 and L4, using a retroperitoneal approach. Then, both LST were cut caudally to ganglion L2, and a bipolar platinum hook electrode was placed on the distal portion of the left LST for electrical stimulation. At this location, the LST almost exclusively supplies the ipsilateral hindlimb (1). Consistent with a former study (13), sectioning of the left LST caused a marked increase in skin blood flow of the ipsilateral hind paw, indicating removal of tonic postganglionic sympathetic vasoconstrictor activity to this vascular bed (Fig. 1). Finally, a pool was formed from skin flaps, and the exposed tissue was covered with warm paraffin oil.

Hemodynamic recordings. The arterial blood pressure signal was obtained from the carotid artery catheter connected to a pressure transducer (DTX Plus, Ohmeda) and a pressure processor amplifier (Gould 4600 Series, Gould Instrument Systems). Heart rate was calculated offline from the arterial blood pressure signal using a freely available analyzing software (XmANA, ftp://sunsite.unc.edu/pub/Linux/science/lab). Superficial skin blood flow was recorded in arbitrary “flux” units within the L5 innervation territory on the central plantar skin, proximal to the foot pads of the left and right hind paws using a dual-channel laser Doppler flowmeter device (MBF3D, Moor Instruments). The single fiber laser Doppler flow probes (P10b, 0.45 mm diameter, Moor Instruments) were positioned at a distance of 2 mm above the surface of the skin and did not touch the paws. Both laser Doppler flux signals were low-pass filtered by an analog filter provided by the MBF3D with a time constant (τ) of 0.1 s (corner frequency 10.0 Hz) to avoid aliasing effects. This technique is well established and has been used by one of the authors (H.-J. Häbler) in a large number of studies (11–14). Arterial blood pressure, together with the laser Doppler flux signals from the ipsilateral and contralateral paw, was recorded on a computer-based monitoring system (XmA) using a sampling rate of 400 Hz for each channel.

LST stimulation and experimental protocol. LST stimulation was performed using a commercially available stimulator unit (Isolated Pulse Stimulator model 2100, A-M Systems) externally triggered by a frequency generator. Electrical

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**Fig. 1.** Original recording during section of left lumbar sympathetic trunk. From top to bottom: arterial blood pressure (BP), heart rate (HR), and laser Doppler blood flow to the skin of contralateral hind paw (SBF(contr)) and to ipsilateral hind paw (SBF(ipsi)). Vertical line indicates time point of section. After a time delay of 5 s, blood flow to skin of ipsilateral paw markedly increased. bpm, Beats/min.

**BP (mmHg)**

150

100

50

0

50

100

150

**HR (bpm)**

350

400

450

500

**SBF(contr) (arb. units)**

1000

600

400

200

0

10

20

30

40

50

60

70

80

90

100

**SBF(ipsi) (arb. units)**

100

50

0

0

10

20

30

40

50

60

70

80

90

100

**time (sec)**
stimulations were applied via bipolar platinum hook electrodes as short trains of four rectangular impulses of 5 V, 0.5-ms duration for each impulse, and an intratrain frequency of 20 Hz (time constant $\tau = 50$ ms). Therefore, the duration of each train was 150 ms. During the experimental protocol, such trains were applied to the caudal end of the sectioned LST with eight different stimulation frequencies ranging from 0.02 to 1.0 Hz. Each recording period involved a 3-min baseline recording followed by another 3-min recording during LST stimulation. Thus for each stimulation period an individual control recording was obtained. The stimulation frequencies were 0.02, 0.05, 0.075, 0.1, 0.2, 0.5, 0.75, and 1.0 Hz. The responses in skin blood flow to these stimulations were highly reproducible in all 11 rats. As an example, Fig. 2 shows the cutaneous vascular responses in the ipsilateral paw of each individual rat to LST stimulation at a stimulation frequency of 0.05 Hz ($\tau = 20$ s). In each rat, periodic stimulation at this frequency caused synchronous fluctuations of skin blood flow.

Signal analysis. Each experimental protocol consisted of eight stimulation periods and eight preceding baseline recordings. Thus each experiment involved 16 recording segments, each of 3 min duration. Eleven rats were included in the study; therefore, the database consisted of 176 3-min recordings, each sampled at 400 Hz. All 176 recordings were visually inspected on the computer screen, and artifact-free segments of 164 s (65,536 values) were selected for further analysis. Then, heart rate together with systolic blood pressure, mean blood pressure, and diastolic blood pressure was derived from the pulsatile arterial blood pressure signal. For each rat and for each of the 16 recording segments, mean values were calculated for heart rate, systolic blood pressure, mean blood pressure, diastolic blood pressure, and for the laser Doppler skin blood flows in the ipsilateral and contralateral paws. Finally, hemodynamic effects of LST stimulation were determined by computing the differences between the mean values during stimulation and the mean values during the respective baseline recordings for all parameters and for each stimulation frequency.

Power spectral analysis was performed from the laser Doppler signals obtained from the ipsilateral (stimulated) and contralateral (nonstimulated) paw. The spectra were calculated from the 164-s-long time series sampled at 400 Hz using the fast Fourier transform based on 65,536 values. Relative spectra were obtained by dividing the power spectra by the variance of the time domain signals (i.e., the total power of the spectra). The relative spectra of all 11 rats were averaged and are presented in Fig. 6. For each stimulation frequency, the area under the curve of the absolute power spectra computed from the baseline recording and from the recording during stimulation was calculated in a frequency band centered around the respective stimulation frequency. The frequency bands for calculation of the areas under the curve were 0.02 ± 0.005, 0.05 ± 0.01, 0.075 ± 0.025, 0.1 ± 0.025, 0.2 ± 0.05, 0.5 ± 0.1, 0.75 ± 0.15, and 1.0 ± 0.2 Hz. The changes in spectral power due to LST stimulation were assessed by the delta values obtained for each stimulation frequency by subtracting the area under the curve during stimulation from the area under the curve during the respective baseline recording. In each rat, these changes in spectral power were fitted to a damped oscillator model as described by Cooke et al. (7) (see Fig. 7A). The model used was

$$X(f) = \frac{a}{2\pi \sqrt{(2\pi f)^2 + b^2 + 4c^2f^2}}$$

In this model, $X(f)$ is the system output, $a$ is the amplitude of the driving force of the oscillator, $b$ is the resonance frequency, and $c$ is a damping parameter. Therefore, calculation of the parameter $b$ allows estimation of the resonance frequency at which periodic oscillations in sympathetic tone are most effectively translated into corresponding oscillations of skin blood flow.

It is well known that sympathetic transmission to vascular smooth muscles behaves like a low-pass filter (38, 39, 41). To further characterize the low-pass filter of sympathetic transmission to cutaneous blood vessels, the corner frequency of...
the attenuation curve of this low-pass filter was calculated for all rats. The corner frequency is the frequency at which the response in skin blood flow is attenuated by 3 dB. For each rat, Bode plots were constructed by plotting the attenuation of the cutaneous vascular response against the stimulation frequency on a logarithmic scale (see Fig. 7B). Attenuation at each stimulation frequency was calculated as 20 log(A/B), where A is the amplitude of skin blood flow oscillations and B is the maximum tonic decrease in cutaneous blood flow at the highest stimulation frequency of 1.0 Hz. The amplitude (A) was calculated from the spectral modulus (square root of spectral power) of skin blood flow at each stimulation frequency by the formula

\[
\text{amplitude} = \frac{\text{spectral modulus}}{0.205 - 0.002}
\]

This relation between spectral modulus and the amplitude of an oscillation has been validated by Bertram et al. (3). Finally, the Bode plots were used to calculate the corner frequency of the underlying low-pass filter by the intersection of the linear portion of the attenuation curve with the frequency axis plotted on a logarithmic scale (3).

Statistical analysis. All data are presented as means ± SE. Statistical comparisons between values obtained during stimulation and during the baseline period before stimulation were performed using the paired Student's t-test. Comparisons between the baseline recordings within individual rats were performed by the one-way analysis of variance for repeated measures. Post hoc Newman-Keuls tests were used to identify differences between individual baseline recordings if the analysis of variance revealed statistical significance.

RESULTS

Hemodynamic baseline characteristics. The experimental protocol consisted of eight recordings during which the LST was electrically stimulated with increasing stimulation frequencies. Each stimulation period was preceded by an individual control recording without stimulation. Systolic, diastolic, and mean blood pressure and heart rate, together with blood flow to the skin of the ipsilateral (stimulated) and contralateral (nonstimulated) paw during each of the eight baseline recordings, are presented in Fig. 3. During the total timeframe of the experimental protocol, baseline hemodynamic parameters did not change significantly, indicating that constant hemodynamic conditions were preserved throughout the duration of the experiments.

Effects of LST stimulation. An original recording during baseline conditions and during LST stimulation at 0.075 Hz is shown in Fig. 4. LST stimulation at this frequency caused strong periodic oscillations of skin blood flow in the stimulated but not in the contralateral paw. This dynamic response in skin blood flow in the ipsilateral paw was accompanied by a mild tonic vasoconstriction as indicated by the reduction in mean blood flow during LST stimulation. As the stimulation frequency was increased >0.2 Hz, the dynamic oscillatory response to LST stimulation subsided, and a strong tonic, nonoscillatory vasoconstriction occurred.

Tonic effects. The hemodynamic effects of LST stimulation on arterial blood pressure, heart rate, and skin blood flow in the ipsilateral (stimulated) and contralateral (nonstimulated) paw are illustrated in Fig. 5. Increasing stimulation frequencies caused continuous reductions in skin blood flow in the ipsilateral paw, indicating sympathetically mediated vasoconstriction. In addition, arterial blood pressure increased steadily as the stimulation frequency was accelerated. As a result, blood flow to the skin in the nonstimulated paw was augmented with higher stimulation frequencies,
most likely due to the increased perfusion pressure at the higher stimulation frequencies. Heart rate did not change with LST stimulation.

Dynamic effects. LST stimulation at lower stimulation frequencies caused a dynamic response of skin blood flow in the ipsilateral paw as shown in Figs. 2 and 4. These dynamic responses were analyzed by power spectral analysis. The power spectra of the blood flow to the skin in the stimulated and nonstimulated paw are shown in Fig. 6. In this graph, the relative spectra of all 11 rats are averaged and the stimulation frequencies are given on the y-axes, whereas the spectral frequencies are plotted on a logarithmic scale on the x-axes. In the skin blood flow signal of the stimulated paw, stimulation frequencies ranging from 0.02 to 0.2 Hz evoked clearly distinguishable peaks in the power spectrum.
spectra. Even at a stimulation frequency of 0.5 Hz, a small peak could be detected. The quantitative analysis of the absolute power spectra is presented in Fig. 7A. Significant increases in spectral power due to LST stimulation were found in the skin blood flow signal of the stimulated paw but not in the contralateral control side. Cutaneous blood vessels in the rat responded to periodic LST stimulation with rhythmic vasoconstrictions and vasodilations at stimulation frequencies between 0.02 and 0.1 Hz. However, the largest and probably most relevant dynamic responses were found at stimulation frequencies of 0.05 and 0.075 Hz. Accordingly, the resonance frequency was found at 0.065 ± 0.003 Hz (Fig. 7A).

Sympathetic transmission to the skin exhibited frequency characteristics similar to a first-order low-pass filter. This property of the system was evidenced by a linear decrease of the normalized oscillatory responses in the frequency range from 0.05 to 1.0 Hz, with an average slope of −17.1 ± 0.8 dB per decade (Fig. 7B). This value was very close to the theoretically expected value of −20 dB per decade that characterizes a first-order low-pass filter. The corner frequency is the frequency at which the response to sympathetic trunk stimulation is attenuated by 3.0 dB. This frequency was determined as 0.085 ± 0.018 Hz (Fig. 7B).

**DISCUSSION**

Using electrical stimulations of the LST and simultaneously recording blood flow to the plantar skin of the hind paws in intact rats, we were able to demonstrate that periodic vasoconstrictions of cutaneous blood vessels can be triggered by sympathetic excitations at stimulation frequencies ranging from 0.02 to 0.1 Hz. At higher stimulation frequencies oscillatory fluctuations in skin blood flow ceased and tonic vasoconstriction occurred. Cutaneous blood flow was recorded in the plantar hairless skin of the hind paw that may be comparable to the vascular bed of the palm of the hand in humans, as investigated in a former study (38). In humans, the same frequency response characteristics of sympathetic transmission to skin vascular smooth muscles were observed as in the present study in rats. Consistent with these results, spontaneous oscillations in blood flow to the skin of the volar surface of the forearm were found in humans at frequencies ~0.1 Hz (2). These spontaneous oscillations were markedly reduced by sympathetic blockade. Therefore, no species differences between rats and humans can be postulated in sympathetic transmission to cutaneous blood vessels. Furthermore, on the basis of two of our former studies, in which we showed that sympathetic transmis-
The findings of our present study raise a number of questions. First, do organ-specific differences in sympathetic transmission to vascular smooth muscles exist within one species? If so, can one conclude that sympathetic transmission to vascular smooth muscles within one species can differ from one vascular bed to the other?

The question as to why mesenteric vascular responses to sympathetic stimuli are faster than those of the skin may rely on potential time-limiting steps in sympathetically mediated vascular smooth muscle contraction. The first step is the release of the neurotransmitters and their passage from the sympathetic varicosities to the vascular smooth muscle receptors. It is known that mesenteric arteries of rats have a very dense sympathetic innervation (10, 15, 16) to other vascular regions such as the skin. Therefore, one cannot completely rule out the possibility that variation in adrenergic synaptic density and distribution (4) play a role in the faster response to sympathetic stimulation in the mesenteric vasculature than in the skin. Therefore, one may argue that sympathetic transmission to the blood vessels may be altered, because the sympathetic stimulations were applied to a vascular bed that was lacking the basal vascular tone. However, in a dilated blood vessel one would expect that the transition from the dynamic to the tonic vascular response to periodic sympathetic stimulations is, if at all, shifted toward higher and not to lower frequencies. Furthermore, in both of our former studies (39, 41), the frequency response characteristics of sympathetic transmission to the mesenteric vasculature were similar, although, in one study the splanchnic nerve was sectioned (41). In the second study (39) the nerve was intact. Therefore, the reduced basal vascular tone in the skin area due to sectioning the LST cannot explain the slower sympathetic responses in the skin than in the mesenteric vascular bed.

Vascular autonomic modulation can be differentially regulated in different districts. For example, in some species (cats and dogs) the arterioles of skeletal muscle are innervated by noradrenergic and cholinergic sympathetic nerve fibers. As a result, different emotional stimuli can result in either muscular vasconstriction or vasodilation (24). Similarly, the sweat glands of the skin are innervated by sympathetic cholinergic nerves. Stimulation of these nerves elicits not only sweating but also marked cutaneous vasodilation. This vasodilation seems to be mediated by acetylcholine and vasoactive intestinal polypeptide that acts as a neurotransmitter in human skin as demonstrated by immunocytochemistry (21). The complex interplay of sympathetic vasodilator and vasoconstrictor fibers in the skin may contribute to the different frequency response characteristics of sympathetic transmission to vascular smooth muscles in the skin versus the mesenteric vascular bed that lacks a sympathetic vasodilator system.
nels via action potentials elicited by α1-adrenergic receptors and by P2X-purinergic receptors that are stimulated by ATP that is coreleased together with norepinephrine from the sympathetic varicosities (21). Differences in the kinetics may account for the faster mesenteric than cutaneous sympathetic responses. As an example, the P2X-purinergic receptors are faster than the α1-adrenergic receptors in eliciting action potentials and subsequently causing vascular smooth muscle contractions. Indeed, stimulation of perivascular sympathetic nerves in the rat tail artery causes two distinct mechanical responses (5). An early contraction is sensitive to (α1, β-methylene)-ATP, a P2X-purinergic antagonist, whereas a second, delayed contraction is sensitive to α1-adrenergic antagonists (37). P2X-purinergic receptors are present in a number of large arteries such as the renal (6, 35) and the mesenteric arteries (8, 36), but are rare in other vascular regions such as the cerebral or hindquarter circulation (8). Therefore, the faster frequency response characteristics of sympathetic transmission to vascular smooth muscles in the mesenteric vascular region compared to the skin may be due to a different distribution of fast P2X-purinergic receptors in these two vascular beds. Other potential time-limiting steps in sympathetically mediated vascular smooth muscle contraction are all aspects of the electromechanical coupling inside the cell or the reuptake of norepinephrine into the sympathetic varicosities and, therefore, may also be responsible for the organ-specific differences in sympathetic transmission to vascular smooth muscles.

Our results demonstrate that there are no species differences in the frequency response characteristics of sympathetic transmission to the cutaneous vasculature in humans and rats. However, sympathetic modulation of arterial blood pressure fluctuations differs markedly in humans and rats. In humans, sympathetic modulation of arterial blood pressure fluctuations is most effective in a frequency band centered at 0.1 Hz (27, 28), whereas in rats this frequency range is located between 0.2 and 0.8 Hz (3, 19, 20, 31). If sympathetic transmission to the vasculature is comparable in humans and rats, it remains unclear why sympathetic modulation of arterial blood pressure differs in humans and rats. This question is particularly interesting, because it has been shown that sympathetic modulation of arterial blood pressure is mostly secondary to rhythmic fluctuations in sympathetic vasomotor tone to several regional circulations (18, 20). At least two explanations are conceivable. First, sympathetic transmission to other vascular regions than the skin may differ in humans and rats. Second, supposed sympathetic transmission to the vascular smooth muscles would be similar in all vascular regions in humans and rats; different distributions of cardiac output to the various organs could explain the differences in the frequency characteristics of sympathetic modulation of arterial blood pressure. If a large share of cardiac output is distributed to vascular regions that respond very fast to sympathetic stimuli, one would expect that sympathetic modulation of arterial blood pressure can operate at higher frequencies than in the case when a large amount of cardiac output flows into vascular regions that respond slowly to sympathetic excitations. In contrast to humans, blood flow to the mesenteric vascular bed in resting rats is extremely high (17% of cardiac output) and equals the blood flow to the kidneys (9). Therefore, differences in the distribution of cardiac output may indeed be involved in the faster sympathetic modulation of arterial blood pressure in rats than in humans.

It has been demonstrated in a very impressive set of experiments by Bertram and colleagues (3) that LF blood pressure oscillations (also called Mayer waves) in rats can be explained by positive feedback properties of the arterial baroreceptor reflex. In this study, the aortic depressor nerve was electrically stimulated at various stimulation frequencies in anesthetized rats, and the responses of arterial blood pressure were investigated. The authors found a resonance frequency of the feedback system at 0.42 Hz that is within the LF range in rats (0.2–0.8 Hz). On the basis of our results, the skin cannot respond to periodic sympathetic stimuli with oscillations in vascular tone at a frequency of 0.42 Hz. Therefore, it is reasonable to assume that sympathetic transmission to the skin is not involved in the generation of the LF Mayer waves found in arterial blood pressure of rats. It is more likely that sympathetic transmission to other vascular beds such as the mesenteric circulation that can respond much faster to sympathetic stimuli than the skin is responsible for the resonance phenomenon that might be the origin of the LF Mayer waves in arterial blood pressure.

In summary, we have demonstrated that sympathetic transmission to the cutaneous circulation in rats operates most effectively in a frequency band between 0.02 and 0.1 Hz. This frequency response characteristic differs markedly from sympathetic transmission to other circulatory regions in the rat, such as the mesenteric vascular bed. In addition, the frequency response characteristics of sympathetic vascular control of the skin is similar in humans and rats. It is concluded that organ-specific disparities exist in sympathetic transmission to vascular smooth muscles, whereas there are no species-specific differences in sympathetic transmission to cutaneous blood vessels in humans and rats. The mechanisms underlying this apparent organ specificity remain to be elucidated.

Perspectives

In this study, we were able to demonstrate that the dynamics of sympathetic transmission to cutaneous vascular smooth muscles are similar in humans and rats. However, the frequencies of spontaneously occurring LF blood pressure oscillations differ between humans and rats. Therefore, it is an intriguing question, whether sympathetic transmission to vascular beds other than the skin differs among species. This question could be addressed by future experiments, which may use ultrasound Doppler techniques to record blood flow to internal organs such as the kidney or the mesentery in humans.
In a former study (40) we found that heat stress is associated with a strong increase in spectral power in a frequency range between 0.01 and 0.20 Hz. It is interesting that the largest dynamic response of cutaneous blood flow to sympathetic stimulation in our present study was found at a resonance frequency of 0.065 Hz that is located within the frequency band in which spectral power is increased by heat stress. Because the skin is the main target organ for thermoregulatory adjustments, one may speculate whether thermoregulation exhibits positive feedback properties at a frequency close to 0.065 Hz in rats. Further experiments could be designed to investigate whether a positive feedback exists in the system regulating core temperature and whether the resonance frequency of this system is really located at 0.065 Hz, the frequency at which the skin most effectively responds to periodic sympathetic stimulation.

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