The Influence of Chronic Sympathectomy on Cutaneous Blood Flow in the Rat Tail

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ABSTRACT. Tail blood flow (TBF) in the rat markedly increases during sympathetic withdrawal such as hyperthermia or lumbar sympathetic blockade. However, a long-term alteration of TBF after chronic sympathetic denervation is not well understood. In the present study, TBF following lumbar sympathectomy (LSX) was observed to ascertain whether subsequent changes in TBF occur in the absence of the sympathetic nervous activity in the rat tail. Assessed by recording tail and rectal temperature, the LSX immediately caused an increase in TBF. TBF was gradually decreased along with time and returned to the sham operated (SO) control level within 4 days. About a week after the surgery, a rapid increase in TBF in response to whole body heating was almost abolished in denervated animals. Neither hexamethonium (20 mg/kg, i.v.) for ganglion blockade nor intra-arterial infusion of α-receptor antagonist, phentolamine (10, 100 μg) produced vasodilation in LSX animals. Nitroprusside, a donor of nitric oxide, produced an increase in TBF in both LSX and SO animals. These results indicate that the tail vasculature after LSX constricts with capability to be vasodilated independent of sympathetic reinnervation. Quantification of the tail vascular mRNA expression by reverse transcriptase-polymerase chain reaction showed less endothelial nitric oxide synthetase in LSX group than that in SO group whereas endothelin-1 was not significantly different in both groups. It is suggested that functional changes in tail vascular endothelium takes at least a part in the reduction in TBF after LSX.

KEY WORDS: body temperature regulation, hyperthermia, sympathetic denervation, tail skin circulation.

The tail of the rat is frequently used for a cutaneous circulatory model because of its wide range for changes in blood flow [22, 24, 25, 29]. The tail of the rat is capable of conducting 10% of the cardiac output or of dissipating 25% of resting heat production during periods of heat stress whereas its blood flow is maintained nearly zero in thermoneutral conditions [11, 23]. O’Leary et al. [20] observed that preventing lumbar sympathetic activity immediately increased tail blood flow (TBF) to the equivalent levels as observed during hyperthermia suggesting that TBF rise to its maximal volume with sympathetic withdrawal. However, to our knowledge, TBF after chronic sympathetic denervation is not well understood. A large amount of TBF caused by lumbar sympathectomy (LSX) increases heat dissipation from the body core region and that would not be favorable for homeothermic animals. We thought that TBF must be diminished even in LSX conditions to prevent heat loss from the tail.

In several arteries, long-term influences of sympathectomy on the vascular characteristics have been shown as well as its immediate, short-term changes in vascular tone [4, 8]. Long-term sympathectomy produces a variety of changes such as in the mass, composition and elasticity of the blood vessel wall, lumen size, agonist sensitivity, smooth muscle proliferation, endothelial function, and small vessel number [6, 16, 19]. These changes in blood vessels are thought to affect blood flow as well.

We hypothesised that quantities of TBF after LSX are not long lasting and produce adaptive changes in tail vasculature independent of sympathetic reinnervation. To test this hypothesis, we observed TBF following LSX and compared vasodilatory responses with those in sympathetic intact rats. We also quantified mRNA expression levels of endothelial nitric oxide synthetase (eNOS) and endothelin-1 (ET-1) in the tail artery to examine functional changes in arterial endothelial cells.

MATERIALS AND METHODS

Methods for Blood Flow Measurement

TBF measurements for short periods: TBF for short periods (<2 to 3 hr) of experiments was assessed by tail blood volume pulsations (TBP) monitored with a tail-cuff which was a part of apparatus for indirect blood pressure monitoring (BP98, Softron, Tokyo). A light-emitting diode and a phototransistor mounted in the cuff were served as the light source and photodetector, respectively. These cells have a maximum light transmission at a wavelength of near 900 nm so that the photocell can detect blood volume between the cells. These signals were AC amplified to obtain pulsatile blood volume changes, i.e. heart beat synchronized TBP which were shown in Fig. 1 with ECG. The amplitudes of TBP, whose baseline were 0.3–0.8 mV, indicate blood flow rate and are referred as TBF in this paper. Changes in TBF were expressed as the ratio to control values. The rubber in the tail-cuff was not inflated throughout any experiments.

TBF measurements for long periods: Because TBF measurements with tail-cuff method were difficult for long
periods of experiments (see below in detail), TBF was assessed semi-continuously for more than 7 days by tail skin (Tts), rectal (Tr) and ambient temperature (Ta). The principal for TBF assessment by these temperatures is as follows; Tts is a function of blood flow within the extremes of Ta and deep body temperature (Tb). Consider the tail as a homogeneous compartment with respect to Tts. Heat loss to the environment through the tail skin is proportional to temperature gradient to air (Tts - Ta) and a heat transfer constant (K). Furthermore, assume that blood enters and leaves the tail at a flow rate of F and with temperatures of Tb and Tts, respectively. If the heat capacity of blood equals that of tail tissues, heat conservation requires that

\[(T_{ts} - T_{a}) \cdot K = (T_{b} - T_{ts}) \cdot F\]

or

\[\frac{F}{K} = \frac{T_{ts} - T_{a}}{T_{b} - T_{ts}}\]

(1)

showing that \(F/K\), calculated from \(T_{ts}, T_{b}\) and \(T_{ts}\), is proportional to TBF provided if \(K\) is not influenced by TBF. Respecting Eq. 1 tail skin circulatory index (TCI) was derived as an indication of TBF with following equation

\[TCI = \frac{T_{ts} - T_{a}}{T_{b} - T_{ts}}\]

(2)

where \(T_{b}\) is representing \(T_{ts}\) [3, 10, 26].

We measured TBF using these two methods because of the following reasons. Photoelectric TBP could detect very small changes in blood volume if the sensor cuff was appropriately placed on the base of the tail. Its amplitudes represent relative values of TBF in each heart beat. But it has no meaning to compare TBP amplitudes unless the tail and the cuff positions are fixed to each other. It seemed difficult to fix the cuff around the tail for a week or to set exactly at the same position of the tail for measurements in every 6 hr. TCI expresses relative value as well and is rather an indirect index of TBF. However, it is based on absolute values of rectal, tail and ambient temperature. It was suitable for non-continuous long-term measurement. In addition, TCI is independent of systemic blood pressure or body temperature and thus could detect immediate increase in TBF after the LSX (Fig. 2) in spite of hypotension with anesthesia and associated low body temperature. So, we assessed TBF with TCI for long-term and with TBP for short period of experiments.

Experimental Preparations

Male Wistar rats aged from 8 to 10 weeks were used in all experiments. The animals were kept at room temperature of 23 to 25°C and fed commercial pellets and water ad libitum.

LSX or sham operation procedures: The animals were randomly separated into LSX or sham operation (SO) groups. LSX was performed following the method of O’Leary et al. [20]. Each animal was anesthetized with pentobarbital sodium (50 mg/kg i.p.). The abdomen was opened with midline incision. The sympathetic chains locating at dorsal to the aorta were isolated from surrounding tissues and cut and removed at levels of L3 to L5. After the sympathectomy the abdominal cavity was closed. The effect of sympathetic denervation was confirmed by the rapid increase in TBF immediately after LSX and by the absence of response in TBF to cold-stimulation test with an ice pack placed on the abdomen for 30 sec. For the SO animal, sympathetic chains were only exposed and abdominal cavity was sutured. All these procedures in both groups were performed under sterile conditions.

Postoperatively, the animals received penicillin (100,000 U i.m.) and topical antibiotics at the abdominal wound, after then they were kept in their individual home cages.
Changes in TBF for 7 days after LSX: Before the surgery, each animal was restrained within a temperature controlled \((T_a = 25\, ^{\circ}\mathrm{C})\) cylinder made of 5 mm mesh wire for measurement of \(T_r\) and \(T_{ts}\). \(T_r\) was measured with a copper-constantan thermocouple inserted 3 cm into the rectum and \(T_{ts}\) was measured with another thermocouple placed on the ventral surface of the base of the tail. The measurements of \(T_r\) and \(T_{ts}\) were repeated in the same manner after the LSX or SO and every 6 hr for the following 7 days. TCI were calculated with these parameters to estimate TBF.

The effects of whole body heating on TBF in sympathectomized rats: After seven to nine days from the LSX or SO, the animals were anesthetized with a mixture of urethane \((750\, \text{mg/kg i.p.})\) and \(\alpha\)-chloralose \((60\, \text{mg/kg i.p.})\). Each animal was placed into a heat exposure chamber which was initially set at 30°C and the cuff was set at the base of the tail to measure TBF. A thermoelectrode was inserted 3 cm into the rectum to measure \(T_r\). Care was taken so that the placement of the thermoelectrode did not affect the position of the tail-cuff. Whole body heating was performed by raising the chamber temperature to 40°C after 10 min of pre-heating period. Changes in TBF, heart rate and \(T_r\) were recorded every 3 min on a floppy disk with an analytical program (SBP-4, Softron, Tokyo). When \(T_r\) exceeded 40°C during whole body heating, experiment was finished.

The effects of vasodilatory agents or ganglion blockade on TBF in sympathectomized rats: After seven to nine days after the LSX or SO, the animals were anesthetized with a mixture of urethane \((750\, \text{mg/kg i.p.})\) and \(\alpha\)-chloralose \((60\, \text{mg/kg i.p.})\). The left carotid artery was cannulated to measure BP. The iliac artery was cannulated until tip of the aorta for bolus injections of phentolamine mesylate \((10\, \text{and } 100\, \mu\text{g})\) or sodium nitroprusside dihydrate \((10\, \text{and } 20\, \mu\text{g})\). The femoral vein was cannulated for i.v. injection of sympathetic ganglion blockade, hexamethonium chloride \((20\, \text{mg/kg})\). TBF and BP were recorded before and after the drug injections.

Quantification of eNOS, ET-1 mRNAs by reverse transcriptase-polymerase chain reaction: Seven to nine days after the LSX or SO, animals were killed by decapitation. The tail artery was dissected from surrounding connective tissue and immediately frozen in liquid nitrogen and stored at -80°C. Total RNA from each tissue sample was extracted by the method of Chomczynski and Sacchi [9] using Trizol reagent (Gibco BRL Life Technologies Inc.). Quantitative polymerase chain reaction (PCR) was performed. Isolated total RNA \((1\, \mu\text{g})\) was reverse transcribed with Ready-To-Go T-Primed First-Strand Kit (Pharmacia Biotec Inc.). To confirm the absence of genomic contamination in the cDNA, PCR was done at the same condition as below with no-reverse transcribed total RNA instead of single-strand solution.

For PCR amplification, \(1\, \mu\text{l}\) of resultant cDNA solution was used. The primers utilized are listed in Table 1. PCR was performed with pellic thermal cycler (PTC-200, MJ Research Inc.) in 20 \(\mu\text{l}\) reaction volume with 0.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems Inc.), 50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl\(_2\), 0.2 mM dNTP and 0.2 \(\mu\text{M}\) oligonucleotide primer pair. Reaction profile after 9 min pre-heating at 94°C was as follows: 30 sec at 94°C, 30 sec at 60°C and 1 min at 72°C. Amplification cycles were 38 times for eNOS, 25 for ET-1, respectively. Characterization of this assay indicated that there was linearity of responses with these cycles. For standardization, gyleraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified from the same samples for 25 cycles. Four-\(\mu\text{l}\) aliquots of the PCR products were separated on a 2% agarose gel and stained with 0.1% ethidium bromide. Under UV illumination, the gel image was captured by a computer with CCD camera, and densitometrically analyzed by an image software (Scion Image, Scion). The amount of amplified mRNA was measured and standardized to the amount of amplified GAPDH mRNA.

Data analysis
The results were expressed as the means ± SEM. Statistical comparisons were made with Student’s \(t\)-test. Differences were considered significant at \(P<0.05\).

RESULTS
Changes in TBF for 7 days after the LSX: TCI changes through 168 hr after the LSX or the SO are shown in Fig. 1. The LSX caused an increase in TCI indicating an increase in TBF. The amplitude of TBP also increased as high as 10.2 ± 0.8-fold \((\text{mean ± SEM, } n=6)\) of the values before sympathectomy. Such changes were the similar extent to that in the sympathetic intact SO rats exposed to body heating (see below). TCI in the LSX group decreased progressively indicating the occurrence of tail vasoconstriction. After 84 hr, TCI was not significantly different from that in the SO group (Fig. 2).

The effects of whole body heating on TBF in sympathectomized rats: Changes in rectal temperature before and during whole body heating in each animal are shown in Fig. 3. In both groups, rectal temperature continued to fall during pre-heating and first 10 minutes of heating period, and thereafter it began to increase.

Figure 4 shows the relationship between TBF and rectal temperature during whole body heating in each animal of the SO and the LSX group. At 38 to 40°C of rectal temperature a rapid increase (more than 2-fold increase within 3 min) in TBF was observed in each animal in the SO group, while only a slight increase was observed in the LSX group. The maximal TBF response to the whole body heating in the LSX group was 1.6 ± 0.6-fold \((\text{mean ± SEM, } P<0.01\, \text{vs. pre-heating control period})\) and significantly lower than that in the SO group \((10.9 ± 1.8\,-\text{fold, mean ± SEM, } P<0.01\, \text{vs. LSX group, } P<0.01\, \text{vs. pre-heating control period})\).

The effects of vasodilatory agents or ganglion blockade on TBF in sympathectomized rats: The baseline values of...
heart rate and blood pressure are shown in Table 2. The heart rate and blood pressure in both groups are not different from each other.

Phentolamine induced a dose-dependent increase in TBF and decrease in blood pressure in the SO group but it failed to increase TBF in the LSX group (Fig. 5). Nitroprusside induced moderate increases in TBF and considerable decreases in blood pressure and the differences are not significant between the groups (Fig. 6).

Hexamethonium induced a large increase in TBF and decrease in blood pressure in the SO group, whereas TBF in the LSX group was not changed ($P>0.1$ compared with control values, Fig. 7).

Quantification of eNOS, ET-1 mRNAs by RT-PCR: Figure 8 shows the effect of LSX on expression of eNOS and ET-1 mRNA in the tail artery. Expression of eNOS mRNA ratio to GAPDH mRNA was significantly lower in LSX group ($P<0.05$) whereas expression of ET-1 mRNA ratio to GAPDH mRNA was not significantly different in both group ($P>0.1$).

DISCUSSION

Preventing lumbar sympathetic activity increases TBF to the equivalent degree with whole body heating in sympathetic intact rats, which was first shown by O'Leary et al. [20]. The results of the present study indicate that the increased TBF by LSX returns to non-denervated control level without sympathetic adrenergic vasoconstrictor tone and that the thermoregulatory reflex vasodilation was markedly diminished by LSX. Quantitative mRNA analysis showed significantly decreased expression of eNOS mRNA in denervated tail artery suggesting that NO secretion from endothelial cells would be decreased in a week after LSX.

If a large amount of TBF is maintained it causes dissipation of body core temperature from the tail skin [11, 23]. To prevent this, it seems reasonable to reduce TBF to the lower levels after LSX as shown in this study.

Several possible mechanisms were thought to be responsible for TBF reduction after LSX, i.e. sympathetic reinnervation or functional changes in the tail vascular wall. The results from this study indicate sympathetic reinnervation is not occurred. Neither hexamethonium nor phentolamine did elicit TBF responses in LSX rats (Figs. 5, 7), indicating that the tail vascular tone was not being influenced by ganglion-mediated or $\alpha$-adrenergic sympathetic activity. Further, in LSX rats whole body heating failed to produce rapid increase in TBF that was characteristics of TBF in sympathetic intact rats (Fig. 4). These results suggest that TBF reduction following the LSX was not attributed to centrally mediated thermoregulatory sympathetic nervous activity.

In regard to vascular function, we thought that secretions of locally mediated substances, i.e. NO or ET-1, are altered with LSX. Long-term sympathectomy causes a decrease in NO synthase and increase in ET-1 immunoreactivity in endothelial cells [2]. The results from mRNA quantification suggested decreased endothelial NO secretion by LSX, whereas ET-1 levels were not as much as to be influenced. It could be due to organ difference with the previous study. Because thermoregulatory vasodilation in rat tail is derived
Table 1. Oligonucleotides used for PCR

<table>
<thead>
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<th>Structure</th>
<th>Sequence</th>
<th>Position</th>
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<tbody>
<tr>
<td>eNOS mouse eNOS cDNA</td>
<td>5'GAG TGG TTT GCC CTG CGT CC 3'</td>
<td>5 position 965</td>
</tr>
<tr>
<td>ET-1 rat preproET-1 cDNA</td>
<td>5'TTC CCA AGA CAA AGC CAC CC 3'</td>
<td>5 position 480</td>
</tr>
<tr>
<td>GAPDH rat GAPDH cDNA</td>
<td>5'GGA TCC TGG GCT CAC CTG GA 3'</td>
<td>5 position 1188</td>
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Fig. 5. Effects of intra-caudal arterial infusion of phenolamine on TBF and blood pressure (BP) in sham operated (open columns, n=5) and LSX (closed columns, n=6) rats. Each column and vertical bar indicates mean ± SEM. *; P<0.05 vs control values; †; P<0.05 vs each other.

Fig. 6. The effects of intra-caudal arterial infusion of nitroprusside on TBF and blood pressure (BP) in sham operated (open columns, n=5) and LSX (closed columns, n=6) rats. Each column and vertical bar indicates mean ± SEM. *; P<0.05 vs control values.

Table 2. Baseline values of heart rate (HR) and blood pressure (BP) in sham operated (SO) and LSX rats

<table>
<thead>
<tr>
<th>Group</th>
<th>HR (bpm) ± SEM</th>
<th>BP (mmHg) ± SEM</th>
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<tr>
<td>SO (n=5)</td>
<td>392.0 ± 21.5</td>
<td>99.4 ± 4.7</td>
</tr>
<tr>
<td>LSV (n=6)</td>
<td>351.3 ± 23.5</td>
<td>110.5 ± 6.0</td>
</tr>
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Values are means ± SEM. The differences between each group are not significant in both HR and BP.
these peptidergic nerve is possibly involved in eNOS expression [28]. It is also possible that perivascular nervous activity influences second messenger cascades such as activation of protein kinase C pathways in endothelial cells which is responsible for the production of vasoactive molecules [15]. However it is unlikely that changes in blood flow itself are responsible for the eNOS reduction. Increase in TBF imparts mechanical shear stress to vascular endothelial cells and shear stress has been demonstrated to induce dilation of small arteries through eNOS activation [13, 18]. Whether this pathway is modulated by sympathectomy or not is of interest.

In addition to endothelial function, reactivity in smooth muscle cells are known to be affected by chronic denervation. Chronic sympathetic denervation tends to increase constriction and to decrease dilation in resistance arteries [5]. But we could not recognize such changes in the preparations employed in this study. At least, LSX does not seem to alter reactivity to NO donor nitroprusside.

The observed phenomena might be thought as an autoregulation of TBF. Autoregulation is the tendency for blood flow to remain constant despite changes in perfusion pressure [12]. Indeed, TBF remained almost constant, probably suitable levels for the tail vasculature, in the last half of the observed period (Fig. 2). However, it took as long as 3 days to return control levels. This seems much more slower response compared with autoregulation in other organs, such as myocardium, brain, kidney and intestine which show autoregulation in rapid vascular responses in demand of continuous oxygen within a second or no longer than a few minutes [1, 7, 14, 21]. Thus it is implied that the mechanisms responsible for constant TBF in LSX conditions are induced by different chemical actions from autoregulation in other organs. Myogenic or metabolic autoregulation may be involved in initial stage of TBF.

Fig. 7. The effects of hexamethonium (20 mg/kg, i.v.) on TBF and blood pressure (BP) in sham operated (open column, n=5) and LSX (closed column, n=6) rats. Each column and vertical bar indicates mean ± SEM. *: P<0.05 vs control values; #: P<0.05 vs each other.

Fig. 8. A: representative RT-PCR amplification of eNOS, ET-1 and GAPDH mRNA in the tail artery from 7 to 9 days of sham operation (SO) or LSX. Each lane represents RNA from one animal. B: summary of eNOS and ET-1 mRNA expression in tail artery from 7 to 9 days of SO (n=4) or LSX (n=4). Data are expressed as mean ratio of eNOS or ET-1 mRNA to GAPDH mRNA. Bars indicate SEM. *: P<0.05 vs each other.
reduction but not acting mainly.

In summary, the results of the present study demonstrate that the tail vasodilation after the LSX is reduced with the independency on reinnervation of the sympathetic nervous system within a week. We suggest that decrease in endothelium derived NO takes at least a part in TBF reduction.

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REFERENCES


