Effects of Electrical Stimulation of the Dorsal Skin on Systemic and Mesenteric Microvascular Hemodynamics in Anesthetized Rats

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Abstract: The effects of electrical stimulation of the dorsal skin area on the mesenteric arterioles were investigated in anesthetized rats by the use of an intravital microscope-television system. Changes in the diameter of the mesenteric precapillary arterioles (10–40 μm in diameter) were measured with an image processor. Blood flow velocity in the mesenteric precapillary arterioles was monitored by the dual sensor method developed by the authors. Electrical stimulation was performed through two platinum electrodes placed at the right dorsal Th5–12 level skin area by the use of an electrical stimulator (0.2 ms, 20 Hz). Continuous stimulation lasting for 30 s (1–10 mA) and intermittent stimulation lasting for 10 min (3 mA) were applied. The pressor response following the depressor response was induced by a stimulus current above 8 mA. The decrease in mesenteric blood flow velocity was induced by stimulus current above 10 mA. These responses were abolished by lidocaine injection into the subcutaneous area where the electrodes were attached. No significant change in arteriolar diameter or heart rate were induced by the stimulation for 30 s. Electrical stimulation of the skin for 10 min evoked a decrease in the diameter of arterioles (−3.4 ± 2%, p < 0.01, n = 12). In the adrenalectomized group, electrical stimulation of the skin for 10 min elicited a slight increase in the diameter (1.1 ± 0.5%, n = 6). It is therefore suggested that electrical stimulation of the skin for 30 s reflexly evoked decreases in MAP and in blood flow velocity, and that the constriction of the mesenteric precapillary arterioles induced by the stimulation for 10 min was mediated by humoral adrenaline and noradrenaline released by somato-adrenal medullary reflex. [Japanese Journal of Physiology, 52, 257–265, 2002]

Key words: adrenal medulla, autonomic reflex, hemodynamics, microcirculation, somatosensory nerve.

Transcutaneous electrical nerve stimulation (TENS) has long been recognized to be an efficient treatment to alleviate pain [1]. Recently it has been reported that TENS also works to improve peripheral circulatory functions [2]. For example, the increased effect of TENS on changes in skin temperature was proven in humans by the use of thermography, suggesting that one TENS mechanism is mediated by an inhibition of sympathetic activity [3, 4].

Many investigators reported that electrical stimulation applied to somatosensory nerves modulates reflex hemodynamic functions [5]. Thus TENS is expected to elicit hemodynamic reflex responses through an activation of somatosensory nerves in the skin area contacting the surface electrodes and its vicinity. It is plausible that various neuronal mediators are released reflexly by the electrical stimulation targeting specific organs, including blood vessels, because in many clinical cases TENS is applied to a patient’s skin for more than 10 min [6–8].

It has been proved that neural control of the blood flow in the mesenteric microvasculature under normal conditions...
physiological conditions is mainly mediated by adrenergic sympathetic nerves [9, 10]. Furness [10] concluded that the neural control of the mesenteric vasculature is mediated by adrenergic nerves that act to constrict the principal arteries, small arteries, and terminal arterioles. Precapillary arterioles are thought not to be directly influenced by neural activities, but are probably under humoral control. The authors demonstrated that noxious mechanical stimulation lasting for 30 s applied to the rat hindpaw evoked reflex vasoconstriction in the mesenteric terminal arteriole, and that this stimulation did not affect the diameter of the precapillary arteriole [11]. Therefore it is thought that the precapillary arteriole is an appropriate site to observe the humoral response to electrical stimulation of the skin with respect to changes induced by circulating catecholamines.

In the present study, the neural responses in heart rate, blood pressure, blood flow velocity, and inner diameter in the mesenteric precapillary arterioles to electrical stimulation of the skin for 30 s were investigated in anesthetized rats by the use of an intravital microscope-television system. We noticed that the precapillary arterioles showed constriction during the repetitive stimulation. Therefore the humoral mechanism of change in the inner diameter of precapillary arterioles induced by electrical stimulation of the skin for 10 min was examined.

MATERIALS AND METHODS

Animal preparation. Experimental preparations and protocols were reviewed and approved by the Animal Care and Use Committee of the University of Tsukuba. Experiments were performed in adult male Wistar rats (250–360 g, n=32) anesthetized with urethane (1.1 g/kg I.M.). The trachea was cannulated, and respiration was maintained with a ventilator (Model 683, Harvard Apparatus, MA, USA). The systemic blood pressure was continuously recorded (Model 683, Harvard Apparatus, MA, USA). An intravital microscope-television system was used throughout the present experiments to observe the changes in blood flow velocity and arteriolar diameter. We identified microvessels by observing vascular architecture, flow direction, and inner diameter of the vessels. The terminal arterioles were 18–40 μm in inner diameter and did not form anastomoses with other arterioles; they became narrower and usually branched to form the precapillary arterioles. Most observable arterioles in rats with body weight of more than 250 g were identified as precapillary arterioles thought to be not innervated by sympathetic nerves [10]. Straight arterioles, at least 100 μm long, at the proximal sites were chosen for measurements of blood flow velocity and inner diameters. Before the start of each experiment, we confirmed that somato-sympathetic reflex vasoconstrictions were not produced in the arterioles chosen for observation accompanying pinch stimulation of the rat hindpaw [11]. During each experiment, an arteriole was continuously visualized by a CCD camera (CCD-IRIS, Sony, Tokyo, Japan) mounted on the top of an intravital microscope, and recorded by a videocassette recorder (BR-S800, Victor, Tokyo, Japan).

Blood flow velocity was measured by the dual-sensor method developed by the authors [12]. The inner diameter of the arteriole was measured on a replayed standstill TV frame by means of an image processor (ARGUS 20, Hamamatsu Photonics, Hamamatsu, Japan). A final magnification ×1,400 of an arteriole was obtained on a TV screen by the use of a recording lens (NFK, ×5, Olympus) and an objective lens (ULWD CDPlan, ×20, Olympus).

Bilateral adrenalectomy. To examine a possible involvement of catecholamine secreted by adrenal medulla, we surgically removed the bilateral adrenal glands in 6 rats before the mesentery was exteriorized into a chamber.

Electrical stimulation. Before the operation, the dorsal and abdominal hair of rats were depilated with depilatory cream (hair remover cream, Kanebo Co. Ltd., Tokyo, Japan), and two platinum electrodes (1×2 cm) were then attached to the depilated skin area, placed about 5 mm apart. The cathode was placed at the right dorsal area along Th5–12 level spine, and the anode was placed at the abdominal area in a direction parallel to the cathode. Electrical stimulation was performed with square wave pulses (0.2 ms, 20 Hz) by the use of an electrical stimulator (SEN-7203, Nihon Kohden). Two different types of stimulus parameters were applied. One was continuous stimulation lasting for 30 s (1–10 mA), and the other was intermittent stimulation lasting for 10 min, which consisted of 3 s of stimulus and 2 s of the rest period. In the experiments of electrical stimulation of
the skin for 10 min, a subthreshold stimulus intensity (3 mA), by which no response was confirmed to be elicited by electrical stimulation for 30 s, was adopted to avoid the reflex response induced by the stimulation. Furthermore, to stimulate repetitively the somatosensory nerve of the skin area, we applied intermittent stimulation of 20 Hz.

**Experimental protocols.** In the first series of experiments, we examined the effects of electrical stimulation of the skin for 30 s on HR, MAP, blood flow velocity, and the inner diameter of precapillary arterioles in the mesentery \( (n=6) \). After an injection of lidocaine (2% Xylocaine, Fujisawa, Osaka, Japan, 0.2 ml) into the subcutaneous area where the electrodes were attached, the effects of electrical stimulation of the skin for 30 s on the hemodynamic parameters were examined \( (n=2) \).

In the second series of experiments, a comparison of the effects of electrical stimulation of the skin for 10 min on MAP, blood flow velocity, and the inner diameter of the mesenteric precapillary arterioles was made between the stimulated group \( (n=12) \) and the nonstimulated control group \( (n=6) \), or between the prestimulus and stimulus values.

In the third series of experiments, the effects of the electrical stimulation of the skin on the diameter and MAP in the intact rats \( (n=12) \) were compared with those in the adrenalectomized group \( (n=6) \).

**Data analysis and statistics.** All the hemodynamic parameters of HR, MAP, and blood flow velocity were measured with a Chart software. The inner diameter was measured from a playback video image. These parameters were measured every second and expressed in terms of percentages of the mean values during stimulation to the prestimulus values. Data are expressed as mean±SEM. The statistical significance was determined by an analysis of variance (ANOVA) followed by Fisher’s least significant difference (LSD) test.

**RESULTS**

**Effects of electrical stimulation of the dorsal skin for 30 s on hemodynamic parameters**

In all the present experiments \( (n=32) \), blood flow velocity in the mesenteric precapillary arterioles and MAP measured under the resting conditions were in a range of 2–6 mm/s and 70–110 mmHg, respectively.

Figure 1 shows representative change (a) and summarized data \( (n=6) \) (b) in MAP accompanying electrical stimulation. Averaged changes in MAP \( (n=6, \text{mean}±\text{SEM}: \text{ANOVA}: * p<0.05, ** p<0.01, \text{statistical significance is expressed between prestimulus and during-stimulus values}).

![Fig. 1. Effects of electrical stimulation (20 Hz, 1–10 mA, 30 s) of the dorsal skin area (Th5–12) on MAP in anesthetized rats. a: Typical recordings of time-course changes in MAP accompanying electrical stimulation. b: Averaged changes in MAP \( (n=6, \text{mean}±\text{SEM}: \text{ANOVA}: * p<0.05, ** p<0.01, \text{statistical significance is expressed between prestimulus and during-stimulus values}).
Figure 2 shows the response in blood flow velocity (a) as well as inner diameter (b) of the mesenteric pre-capillary arteriole induced by electrical stimulation of the skin. Both in Fig. 2a and b, the left-hand side panel represents absolute value, and the right-hand side represents an averaged percent of the changes in the parameters. As shown in Fig. 2a, when the stimulus intensity was less than 5 mA, no response accompanying electrical stimulation was observed. A decrease response in blood flow velocity, though statistically insignificant, was induced after the beginning of stimulation at 8 mA. A higher electrical stimulation of 10 mA evoked a significant decrease in blood flow velocity at 10 s (−8.6±2%, p<0.05) and 20 s (−12.1±4%, p<0.01) after stimulus onset. In these experiments, blood flow velocity in two out of six rats increased temporarily after the end of the stimulus, then recovered to the baseline at 1 min after stimulation. In these experiments, blood flow velocity in two out of six rats increased temporarily after the end of the stimulus, then recovered to the baseline at 1 min after stimulation.

Effect of electrical stimulation of the skin for 10 min on the changes in hemodynamic parameters

Figure 4a shows a montaged photomicrograph of microvascular architecture in the rat mesentery. The inner diameter of the precapillary arteriole was measured at the portion indicated as a square. As shown in Fig. 4b, a remarkable constriction of the mesenteric precapillary arteriole in response to electrical stimulation applied to the dorsal skin area in an anesthetized rat was clearly observable under the intravital microscope (magnified ×100). The inner diameter before stimulation was 28.2±2 μm, and this value showed a
decrease to 25.8 ± 2 μm after the onset of stimulation in all 12 rats.

In the 12 rats, changes in the inner diameter of precapillary arterioles of the mesentery induced by electrical stimulation of the skin for 10 min were recorded. In 5 rats, vasoconstriction by more than 5% in diameter of precapillary arterioles compared to the prestimulus value was observed throughout the stimulation period (−11.8 ± 3.5%). Five rats among the 12 showed vasoconstriction by 1–5% when compared to the prestimulus value (−2.5 ± 0.3%), and the precapillary arterioles in 2 rats did not respond to the stimulation (0.2 ± 1.1%). Figure 5 summarizes time-course changes in the same parameters (d-f) as shown in the left panel in an anesthetized rat administrated lidocaine (2% Xylocaine, 0.2 ml) into the subcutaneous area where the electrodes were attached.
ulus value of the two groups. The stimulus group demonstrated a statistically significant decrease in the diameter of precapillary arterioles compared with those of the prestimulus value, particularly over a period from 3–4 min (2.2 ± 6.2%, p < 0.05), 4–5 min (3.4 ± 6.2%, p < 0.01), 7–10 min (3.0 ± 6.2%, -2.2 ± 6.2%, and 2.1 ± 6.2%, p < 0.05) after the onset of stimulation. These significant vasoconstrictions continued until 1–2 min (4.6 ± 3%, p < 0.01, compared to both prestimulus value and control group) and 2–3 min (3.8 ± 6.2%, p < 0.01, compared to prestimulus value) after the end of the stimulus.

Effects of adrenalectomy on precapillary arteriolar constriction in the mesentery and pressor induced by electrical stimulation

In 6 rats, adrenalectomy was bilaterally performed to examine the effects of secreted humoral adrenaline and noradrenaline on changes in the diameters of precapillary arterioles accompanying electrical stimulation of the skin lasting for 10 min (intermittent pulse wave of 3 mA, 20 Hz). Figure 6 shows a percentage (Δ%) of the changes to the prestimulus value in diameter observed at 4–5 min and in MAP at 2–3 min after the onset of the stimulation in the intact group (n = 12) and the adrenalectomized group (n = 6). The mean value of the prestimulus diameter in precapillary arterioles was 23.3 ± 1 μm in the adrenalectomized group and 28.2 ± 2 μm in the stimulated group (not significant, p > 0.05). As shown in Fig. 6a, the intact group showed a significant vasoconstriction (−3.4 ± 2%, p < 0.01) at 4–5 min after the onset of the stimulation compared with the prestimulus value, but the adrenalectomized group demonstrated a slight vasodilation (1.1 ± 0.5%). Figure 6b shows that an electrical stimulation of the skin evoked a significant pressor response at 2–3 min after stimulus onset in the intact group (5.1 ± 2.1%, p < 0.05). In the adrenalectomized group, no significant change in MAP was demonstrated (3.7 ± 1%) at 2–3 min after the stimulus. Thus it was demonstrated that the adrenal medulla was ac-
tually involved in the humoral vasoregulatory mechanisms of the precapillary arterioles of the rat mesentery and pressor response when electrical stimulation lasting for 10 min was applied to the dorsal skin area.

**DISCUSSION**

In the present study, electrical stimulation of the skin (20 Hz, 30 s) evoked decreases in blood flow velocity in the mesenteric precapillary arterioles and depressor responses. It was also confirmed that these responses were reflexly induced by the stimulation, since they were abolished by a local administration of lidocaine into the subcutaneous area where the electrodes were attached. Following the earlier report of Hunt [13] suggesting that the peripheral nerves contained two types of afferents, i.e., “pressor and depressor fibers,” many investigators have reported regarding the mechanism of blood pressure responses to somatic afferent stimulation [14]. The previous studies have shown that these opposite responses in arterial pressure to somatic afferent stimulation seem to be produced by a different depth of anesthesia [15, 16] and a different type of afferent fiber group [14]. In our previous experiment we have reported that under the same experimental condition as the present study, a noxious mechanical stimulation of hindpaw produced a significant pressor response [11]. The effects of noxious mechanical stimulation of various segmental areas on HR and MAP as well as cardiac and renal sympathetic activities were reported by Kimura et al. [17]. They reported that pinch stimulation of some segmental skin area produced an increase in HR, MAP, and sympathetic activities in the CNS-intact rats. Furthermore, Uchida et al. [18] reported that acupuncture-like stimulation of the cheek, forepaw, and hindpaw produced an increase in MAP, but that the stimulation of the back produced a decrease in MAP in the CNS-intact rats. Our data demonstrated that the pressor response following depressor response was induced by electrical stimulation applied to the dorsal skin area. It is thought that electrical stimulation of the skin activates the afferent fibers in the dorsal skin. The pressor response following depressor responses obtained in the present study seem to be caused by actions of different types of afferent fiber in the skin and/or underlying muscle. It is plausible that the activity of sympathetic promoter neurons in the rostral ventrolateral medulla (RVLM) is inhibited or excited by the activation of somatic afferents [14, 19, 20]. Furthermore, vasodilation is reported to be caused by axon reflex through the dichotomized fiber activity [21]. It is thought that depressor response induced by electrical stimulation is a consequence of vasodilation induced by axon reflex [22]. Therefore a decrease in mesenteric blood low velocity seemed to be caused by the passive response by the depressor and by the local regulation of smooth muscle in the mesenteric microvasculature.

In our experiments, the inner diameter of precapillary arterioles was not affected by an electrical stimulation of the skin for 30 s under conditions of all stimulus intensities. Furness [10] described that precapillary arterioles were not directly influenced by the sympathetic nerves, but were subjected to humoral control. In preliminary experiments by the authors, the mesenteric precapillary arterioles in the anesthetized rats exhibited a marked constriction by the topical administration of noradrenaline. The effects of various types of somatosensory stimulation, such as noxious [23], electrical [24], acupuncture-like stimulation [25], and electro-acupuncture stimulation [26] on adrenal sympathetic nerve activity and on the secretion of the adrenal medullary hormones, have been studied. In the present experiments, 10 out of 12 rats undergoing electrical stimulation of the skin for 10 min showed a significant vasoconstriction in the mesenteric precapillary arterioles, and 11 out of 12 rats demonstrated a significant pressor response. Sato et al. [25] reported that acupuncture-like stimulation induced three types of responses with respect to both catecholamine secretion and adrenal sympathetic nerve activity, i.e., decrease, increase, and no change. They noted that these different responses corresponded with three similar types of response in MAP; for example, when MAP increased during stimulation, the secretion rate of adrenaline increased significantly. In the present study, the bilateral adrenalectomized group exhibited no significant vasoconstriction or pressor effect by electrical stimulation of the skin lasting for 10 min. Therefore it is considered that the precapillary arterioles constricted through the action of catecholamines (adrenaline and/or noradrenaline) released from the adrenal medulla throughout electrical stimulation of the dorsal skin area after the pressor response was caused by the stimulation.

In fact, in clinical practice TENS is frequently applied for a relatively long duration from 10 to 60 min [6–8]. It is therefore expected that various reflex responses are evoked simultaneously throughout the period of the long-term stimulation, during which the humoral response could be induced by the stimulation [27]. However, the stimulus intensity (3 mA) adopted in the present study caused no sizable response in systemic blood pressure and vasoconstriction in mesenteric precapillary arterioles. These results suggest that
the response in precapillary arteriole to electrical stimulation of the skin might be induced by somatoadrenal medullary reflex through the activity of different threshold afferent fibers from those inducing the reflex responses in systemic arterial pressure. Furthermore, it was thought that repetitive electrical stimulation lasting for 10 min caused a release of catecholamines to blood.

In summary: (1) The pressor response following depressor response and decreases in precapillary blood flow velocity were intensity-dependently induced by electrical stimulation of the skin (20 Hz, 30 s); (2) These responses to an electrical stimulation of the skin were abolished by a preinjection of lidocaine into the subcutaneous area where the electrodes were attached; (3) No significant change in precapillary arteriolar diameter or HR were induced by an electrical stimulation of the skin (20 Hz, 1–10 mA, 30 s); (4) In the intact group, electrical stimulation of the skin for 10 min evoked a pressor response and a decrease in the diameter of precapillary arterioles; (5) In the adrenalectomized group, pressor response and a decrease in the diameter induced by electrical stimulation of the skin for 10 min were abolished. It is therefore suggested that the effect of electrical stimulation of the skin for 10 min on the hemodynamic changes of mesenteric precapillary arterioles was caused by humoral catecholamines released by the somato-adrenal medullary reflex.

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