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ORIGINAL PAPER

Electrical stimulation of the mesencephalic ventral tegmental area evokes skeletal muscle vasodilatation in the cat and rat

Kanji Matsukawa · Tomoko Nakamoto · Nan Liang

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Abstract To test the hypothesis that the mesencephalic ventral tegmental area (VTA) plays a role in autonomic control of the cardiovascular system, we examined the cardiovascular effects of electrical stimulation of the mesencephalic ventral areas in anesthetized, paralyzed cats and rats. Electrical stimulation of the VTA for 30 s (100-μA current intensity; 40–50-Hz pulse frequency; 0.5–1-ms pulse duration) increased femoral blood flow by 130–162% in anesthetized cats and rats, whereas the identical stimulation of the substantial nigra (SN) failed to increase femoral blood flow. Electrical stimulation of the VTA also increased the arterial blood pressure and heart rate in anesthetized rats, but did not alter them in anesthetized cats. Accordingly, femoral vascular conductance was increased by 102–134% in both cats and rats. Atropine methyl nitrate (0.1 mg/kg) injected intravenously in the cats markedly attenuated the increases in femoral blood flow and vascular conductance. VTA stimulation was able to produce substantial increases in femoral blood flow and vascular conductance following a decerebration procedure performed at the premammillary and precollicular level in the cats, although their responses tended to attenuate to 55–69% of the control before the decerebration. Thus, it is likely that electrical stimulation of the VTA, but not the SN, is capable of evoking skeletal muscle vasodilatation, particularly via a sympathetically mediated cholinergic mechanism in the cat, and that the ascending projection from the VTA to the forebrain may not be responsible for the muscle vasodilatation.

Keywords Ventral tegmental area · Substantia nigra · Muscle vasodilatation · Central command · Sympathetic cholinergic nerve

K. Matsukawa (✉) · T. Nakamoto · N. Liang
Department of Physiology, Graduate School of Health Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
e-mail: matsuk@hiroshima-u.ac.jp

Introduction

The mesencephalic ventral tegmental area (VTA), one of the dopaminergic origins in the central nervous system, has been emphasized so far in behavioral motivation and reward function. Indeed, ascending dopaminergic efferent fibers from the VTA to the forebrain are widely distributed throughout the limbic, motor, and association areas of the cerebral cortex and the striatum [1–3]. Recent immunohistochemical studies, however, revealed that axons of dopaminergic neurons in the VTA terminate within the diencephalic and brain stem areas known as the autonomic and cardiovascular centers, in particular the lateral hypothalamus, the periaqueductal gray region, the dorsal raphe nucleus, and the parabrachial nucleus [2, 4–9]. Furthermore, VTA neurons receive synaptic input from the limbic, motor, and association areas of the cerebral cortex and the striatum [2, 10]. Therefore, it is speculated that the higher cortical structures may excite neurons in the VTA, whose descending pathways to the diencephalon and mesencephalon may convey a central command signal for regulating the autonomic and cardiovascular systems.

To test this hypothesis, we examined the effects of electrical stimulation of the VTA on femoral blood flow and vascular conductance as well as arterial blood pressure (AP) and heart rate (HR) compared with the cardiovascular effects of the identical stimulation of the substantia nigra

(SN). Since autonomic adjustment of the cardiovascular system happens to differ in a species-dependent manner, it is important to determine whether the cardiovascular effects of electrical stimulation of the VTA and the SN are a common phenomenon irrespective of animal species. For this reason, we examined the cardiovascular effects of electrical stimulation of the ventral mesencephalic areas using both anesthetized cats and rats. Since electrical stimulation of the VTA actually increased femoral blood flow and vascular conductance, we examined whether the evoked muscle vasodilatation was mediated with the same sympathetically mediated cholinergic mechanism as muscle vasodilatation produced by stimulation of the hypothalamic defense area [11–13]. Finally, to examine whether the ascending efferent projection from the VTA to the forebrain is relevant to the muscle vasodilatation, the increases in femoral blood flow and vascular conductance by electrical stimulation of the VTA were examined following a decerebration procedure performed at the precollicular-premammillary level in cats.

Methods

The experiments were performed using eight cats (body weight 3.8 ± 0.3 kg) and four male Wistar rats (body weight 428 ± 28 g) in accordance with the "Guiding Principles for the Care and Use of Animals in the Fields of Physiology Sciences" approved by the Physiological Society of Japan. The experimental protocols and procedures were also approved by the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development, Hiroshima University.

Anesthetized preparations

To examine the cardiovascular effects of electrical stimulation of the ventral mesencephalic areas, the cats and rats were anesthetized with pentobarbital (30–40 mg/kg ip for cats and 50–60 mg/kg ip for rats). Electrocardiogram (ECG), HR, and rectal temperature were continuously monitored, and respiratory thoracic movement was visually observed. Rectal temperature was maintained at 37–38°C with a heating pad and an external lamp. The trachea was exposed and an endotracheal tube was inserted into the airway. The animals spontaneously breathed oxygen-enriched room air through the endotracheal tube. Polyurethane catheters were inserted into the left external jugular vein for administering drugs and the left carotid artery for measuring AP with a pressure transducer (DPT-6100, Kawasumi Laboratories, Tokyo, Japan). The animal head was mounted on a stereotaxic frame (SN-2N or SR-6R, Narishige, Tokyo, Japan), and a temporal part of the skull was removed for electrical stimulation of the ventral mesencephalic areas. HR was derived from the R wave of ECG by a tachometer (model 1321, GE Marquette Medical Systems, Tokyo, Japan).

Recording of femoral blood flow

To identify the response in skeletal muscle blood flow during electrical stimulation of the ventral mesencephalic areas, femoral blood flow was recorded using a flow probe implanted on the left femoral artery (Transonic 1.5R or 2R probe for cats; Transonic 1RB probe for rats), which was connected to a Doppler flowmeter (T206, Transonic Systems, Ithaca, NY). Femoral vascular conductance was estimated from a ratio of femoral blood flow and mean arterial blood pressure (MAP).

Experimental protocols

After all surgical and preparatory procedures were finished, a muscle relaxant [pancuronium bromide (2 mg) for cats and D-tubocurarine chloride (0.6 mg) for rats] was intravenously administered, and the lungs were artificially ventilated with a respirator. If an increase in HR and/or AP in response to a noxious pinch of the paw and/or a surgical procedure was observed under muscle paralysis in the pentobarbital-anesthetized condition, supplemental pentobarbital (3–5 mg/kg iv) was administered to maintain an appropriate level of surgical anesthesia. The effects of electrical stimulations of the ventral mesencephalic areas (the VTA and the SN) on femoral muscle blood flow and vascular conductance were examined using anesthetized cats ($n = 8$) and rats ($n = 4$). To do this, a monopolar tungsten microelectrode (tip diameter 5 μm; shaft diameter 0.2 mm; UJ-3002, Unique Medical, Tokyo, Japan) was stereotaxically inserted into the mesencephalic ventral areas, according to the atlases of the cat and rat brain [14, 15], and an indifferent ground electrode was inserted in the occipital muscle. The current intensity of electrical stimulation was 100 μA for 30 s at a frequency of 40–50 Hz and 0.5–1.0 ms in pulse duration. At the end of the experiments, a small lesion was made by passing a negative DC current of 100 μA for 30–40 s to identify the stimulated sites.

To examine a possible role of acetylcholine in the femoral blood flow response during electrical stimulation of the VTA, the changes in femoral blood flow and vascular conductance were measured after intravenous administration of atropine methyl nitrate (0.1 mg/kg, Sigma-Aldrich, St. Louis, MO). Furthermore, to examine a possible role of the ascending projection from the VTA in the femoral vasodilatation induced by the VTA stimulation, a decerebration procedure at the premammillary and precollicular level was performed with an electrocoagulation

method in three cats, according to previous studies [16, 17]. A stainless steel electrode (bare diameter 0.25 mm), whose insulation was removed along a length of 4 mm from the tip, was inserted into the hypothalamus rostral to the mammillary body. A negative direct current (1 mA) was passed for 30–40 s through the electrode. The electrode was then withdrawn by 4 mm, and the same current was passed again. This procedure was repeated over a total of 42 tracks at 0.5-mm intervals on the frontal plane. Dexamethasone (0.2 mg, Sigma-Aldrich, St. Louis, MO) was intravenously administered to minimize cerebral edema.

Histology

The animals were killed with an overdose of pentobarbital. The brains were perfused with saline followed with a 10% formalin solution, and were removed and immersed in the formalin solution. Subsequently, the midbrain was sliced in 40–50-μm-thick coronal sections with a freezing microtome and stained using the Nissl method. The mesencephalic stimulating sites marked with the electrolytic lesions were examined histologically.

Data and statistical analyses

Timing at the start of electrical stimulation was manually marked with an electric switch. HR, AP, femoral blood flow, and the marking signal were simultaneously recorded on a pen-writing recorder (8M14, GE Marquette Medical Systems, Tokyo, Japan) and were stored in a computer via an analog to digital converter (BIOPACK MP100 system, Santa Barbara, CA) at a sampling frequency of 1 kHz. The mean values of HR, AP, and femoral blood flow and vascular conductance over 1 s were sequentially calculated. The baseline value of each variable was defined as the value prior to electrical stimulation of the ventral mesencephalic areas. Using a paired or unpaired *t* test, the peak cardiovascular changes in response to electrical stimulations of the mesencephalic areas were statistically assessed from the baseline levels and were compared between the VTA and the SN. Furthermore, the cardiovascular changes during the VTA stimulation were compared by a paired *t* test before and after administration of atropine and before and after the decerebration. A level of statistical significance was defined at $P < 0.05$ in all cases. The data in the text and figures are expressed as mean ± SE.

Results

The baseline MAP, HR, femoral blood flow, and femoral vascular conductance in anesthetized cats were 103 ± 4 mmHg, 203 ± 11 beats/min, 6.4 ± 1.0 ml/min, and 65 ± 12 μl/min/mmHg, respectively; their values in anesthetized rats were 107 ± 4 mmHg, 425 ± 12 beats/min, 0.78 ± 0.18 ml/min, and 7 ± 1 μl/min/mmHg, respectively.

Responses in femoral blood flow and vascular conductance to stimulation of the VTA and SN

Typical examples of the cardiovascular changes in response to electrical stimulations of the ventral mesencephalic areas are demonstrated in Figs. 1 and 2. In an anesthetized cat, electrical stimulation of the VTA obviously increased femoral blood flow with a slight change in AP (e and f in Fig. 1B), whereas stimulation of the SN did not alter femoral blood flow at all (b, c, and d in Fig. 1B). Similarly, in an anesthetized rat, electrical stimulation of the VTA produced an increase in femoral blood flow (d in Fig. 2B), whereas electrical stimulation of the SN did not substantially increase femoral blood flow (a in Fig. 2B).

The average cardiovascular responses to electrical stimulations of the VTA and the SN are summarized in Fig. 3. In anesthetized cats, electrical stimulation of the VTA significantly increased femoral blood flow by 6.8 ± 1.6 ml/min (130 ± 41% of the baseline level, $P = 0.004$) and femoral vascular conductance by 64 ± 13 μl/min/mmHg (134 ± 41%, $P = 0.002$). However, the VTA stimulation altered neither HR nor MAP significantly ($P > 0.05$) (Fig. 3). In anesthetized rats, electrical stimulation of the VTA significantly increased femoral blood flow by 0.94 ± 0.09 ml/min (162 ± 47% of the baseline level, $P = 0.002$) and femoral vascular conductance by 4 ± 1 μl/min/mmHg (102 ± 55%, $P = 0.049$), respectively. Electrical stimulation of the VTA also increased MAP by 55 ± 11 mmHg ($P = 0.015$). When the electrode tip was displaced upward by 0.5 mm from the target locus of the VTA, the increases in femoral blood flow and vascular conductance disappeared or greatly diminished.

In contrast to the VTA, electrical stimulation of the SN evoked no significant changes in femoral blood flow and vascular conductance, HR, and MAP in the cats (Fig. 3); the increase in femoral blood flow during the SN stimulation was 1.4 ± 0.8 ml/min ($P = 0.134$), and the increase in femoral vascular conductance was 13 ± 6 μl/min/mmHg ($P = 0.009$). On the other hand, the SN stimulation in the rats significantly increased femoral blood flow by 0.16 ± 0.03 ml/min ($P = 0.013$) and femoral vascular conductance by 1 ± 0.1 μl/min/mmHg ($P = 0.001$), respectively (Fig. 3); the increase in femoral blood flow during the SN stimulation was much smaller ($P = 0.003$) than that during the VTA stimulation. Accordingly, the responses of femoral blood flow and vascular conductance were significantly different ($P = 0.003$–0.019) between the VTA and

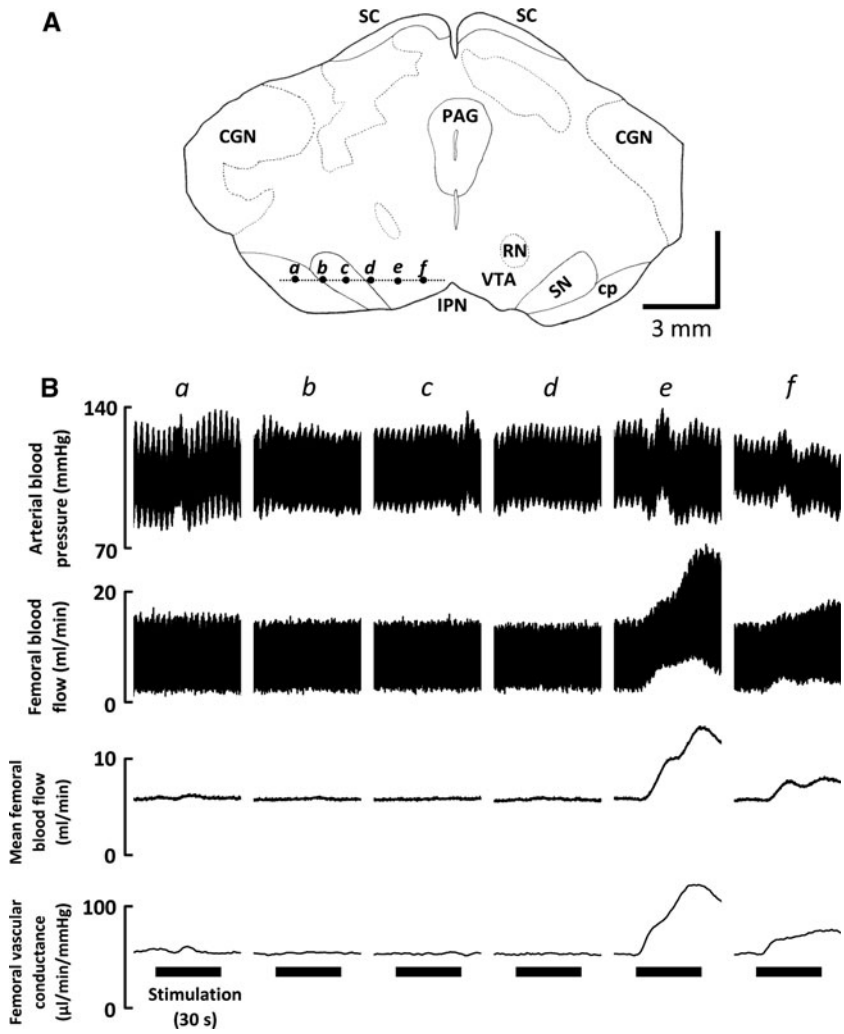

Fig. 1 Typical recordings showing the effects of electrical stimulations of the mesencephalic ventral areas on arterial blood pressure, original and mean femoral blood flow, and femoral vascular conductance in an anesthetized, paralyzed cat. **A** The six sites of the stimulating electrode are shown in the frontal plane 3.0–3.5 mm anterior to the interaural line [14]. **B** *Horizontal bars* at the *bottom* indicate the 30-s period of electrical stimulation of each mesencephalic site. *SC* superior colliculus, *CGN* nucleus corporis geniculati medialis, *PAG* periaqueductal gray, *RN* red nucleus, *SN* substantia nigra, *VTA* ventral tegmental area, *IPN* nucleus interpeduncularis, *cp* cerebral peduncle

SN stimulation in both species, except the response in femoral vascular conductance in the rats (Fig. 3).

Effect of atropine on the femoral blood flow response to the VTA stimulation

The effects of intravenous administration of atropine on the changes in femoral blood flow and vascular conductance during electrical stimulation of the VTA in anesthetized cats are summarized in Fig. 4. Although atropine did not significantly affect the baseline values of HR, MAP, and femoral blood flow and vascular conductance, atropine almost abolished the increases in femoral blood flow and vascular conductance during the VTA stimulation to 0.4 ± 1.6 ml/min (6% of the control response of 6.2 ± 1.7 ml/min, $P = 0.036$) and to 2 ± 17 μl/min/mmHg (3% of the control response of 63 ± 16 μl/min/mmHg, $P = 0.027$), respectively (Fig. 4). Atropine also blunted the increase in HR from 12 ± 2 to 2 ± 1 beats/min ($P = 0.007$) during the VTA stimulation.

Effect of decerebration on the femoral blood flow response to the VTA stimulation

To examine whether the ascending efferent projection from the VTA to the forebrain was relevant to the muscle vasodilatation evoked by electrical stimulation of the VTA, the increases in femoral blood flow and vascular conductance during the VTA stimulation were compared before and after the decerebration procedure performed at the precollicular-premammillary level in three cats. As exemplified in Fig. 5, the increase in femoral blood flow in response to the VTA stimulation tended to attenuate from 12.2 ± 2.8 to 6.7 ± 2.4 ml/min ($P = 0.155$) after the decerebration; the increase in femoral vascular conductance in response to the VTA stimulation also tended to attenuate from 118 ± 29 to 81 ± 27 μl/min/mmHg ($P = 0.234$). However, it was noted that the remaining substantial increases in femoral blood flow and vascular conductance were produced by the VTA stimulation even following the decerebration.

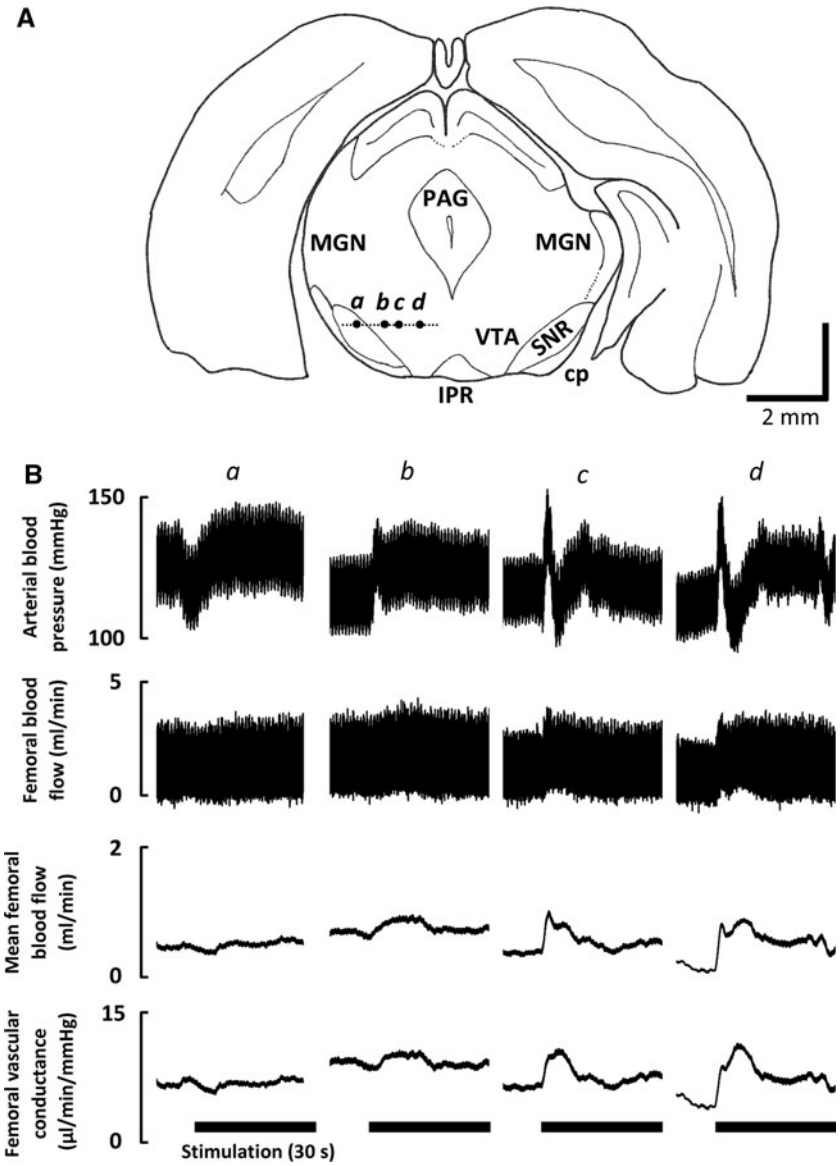

Fig. 2 Typical recordings showing the effects of electrical stimulations of the mesencephalic ventral areas on arterial blood pressure (AP), original and mean femoral blood flow, and femoral vascular conductance in an anesthetized, paralyzed rat. **A** The four sites of the stimulating electrode are shown in the frontal plane at the Bregma −5.30 to 5.60 [15]. **B** *Horizontal bars* at the *bottom* indicate the 30-s period of electrical stimulation of each mesencephalic site. A decrease in AP in **B**-*a* spontaneously appeared prior to electrical stimulation of the SN and was not related to the electrical stimulation. *MGN* medial genic nucleus, *PAG* periaqueductal gray, *SNR* substantia nigra pars reticulata, *VTA* ventral tegmental area, *IPR* interpeduncular nucleus, *cp* cerebral peduncle

Discussion

To test the hypothesis that the mesencephalic VTA plays a role in autonomic control of the cardiovascular system, we examined the cardiovascular effects of electrical stimulation of the mesencephalic ventral areas. The major findings of the present study are (1) that electrical stimulation of the VTA caused prompt increases in muscle blood flow and vascular conductance in both anesthetized, paralyzed cats and rats, whereas the identical electrical stimulation of the SN did not alter them at all; (2) that intravenous administration of atropine abolished the increases in femoral blood flow and vascular conductance by the VTA stimulation in the cats; and (3) that, even following the decerebration procedure performed at the premammillary and precollicular level, the VTA stimulation was able to induce substantial femoral vasodilatation. Thus, it is likely that electrical stimulation of the VTA is capable of evoking skeletal muscle vasodilatation, particularly via a sympathetically mediated cholinergic mechanism in the cat, and that the ascending projection from the VTA to the forebrain may not be responsible for the muscle vasodilatation.

The effect of stimulation of the VTA on skeletal muscle circulation

The previous cardiovascular effects of electrical and chemical stimulations of the VTA have been controversial [18–22]. Electrical stimulation of the VTA evoked a pressor response, bradycardia, and intense vasoconstriction of the renal vascular bed in pentobarbital-anesthetized rabbits [18]. Chemical stimulation of the VTA by injecting a tachykinin agonist induced a long-lasting pressor response and tachycardia in conscious rats [19–21],

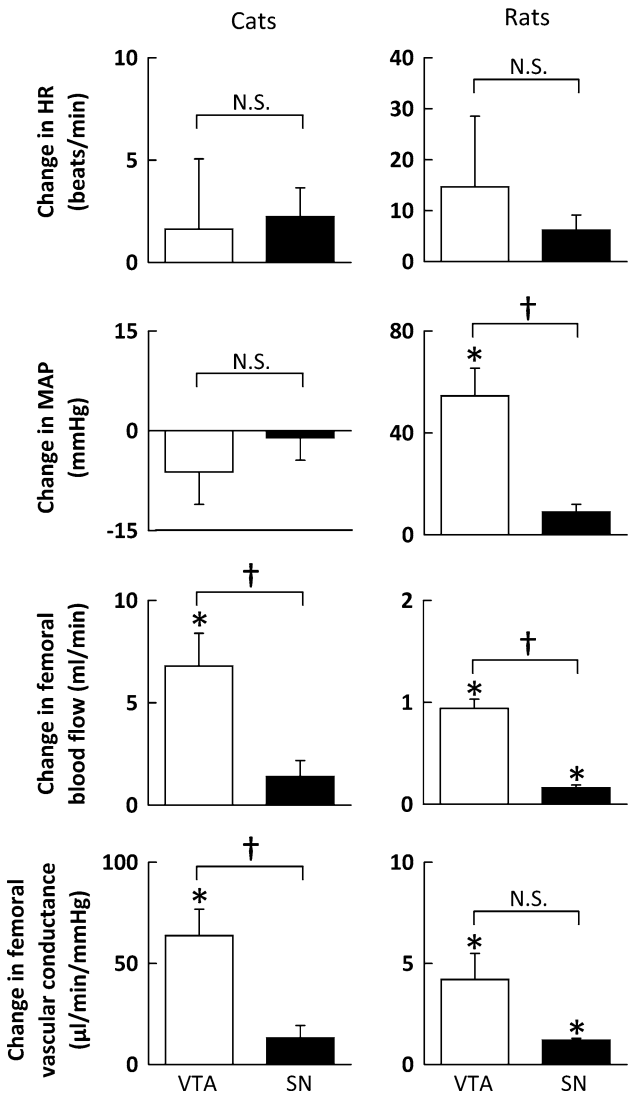

Fig. 3 The changes in heart rate (HR), mean arterial blood pressure (MAP), and femoral blood flow and vascular conductance in response to electrical stimulations of the VTA and the SN are summarized in anesthetized cats and rats. Electrical stimulation of the VTA in cats ($n = 8$ cats) increased femoral blood flow and vascular conductance significantly ($P < 0.05$), while electrical stimulation of the VTA in rats ($n = 4$ rats) increased all of HR, MAP, and femoral blood flow and vascular conductance significantly ($P < 0.05$). In contrast to the VTA, electrical stimulation of the SN in the same cats and rats did not significantly ($P > 0.05$) increase any variables, except the HR in the rats. *Asterisk* indicates significant change from the baseline ($P < 0.05$). *Dagger symbol* indicates significant difference between the VTA and the SN ($P < 0.05$). *NS* not significantly different between the VTA and the SN ($P > 0.05$)

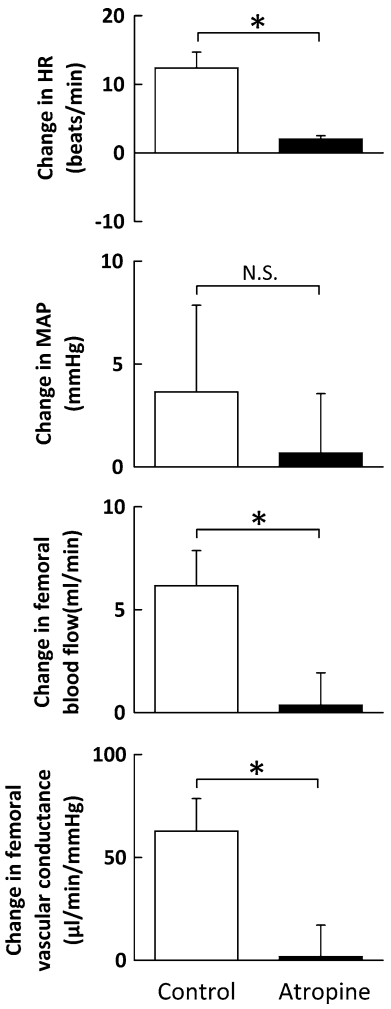

Fig. 4 The effects of atropine methyl nitrate (0.1 mg/kg) on the changes in HR, MAP, and femoral blood flow and vascular conductance evoked by electrical stimulation of the VTA ($n = 6$ cats). Atropine markedly blunted the increases in femoral blood flow and vascular conductance evoked by the VTA stimulation. *Asterisk* indicates significant difference ($P < 0.05$) before and after atropine. *NS* not significantly different ($P > 0.05$) before and after atropine

whereas chemical stimulation of the VTA by injecting L-glutamate induced a short-lasting depressor response and bradycardia in chloralose-anesthetized rats [22]. The cardiovascular effects produced by the tachykinin agonist and L-glutamate were blunted by intravenous pretreatment with antagonists for dopamine D_1 and/or D_2 receptors [19–22]. Because the pressor or depressor response depends on a balance between vasodilatation and vasoconstriction in peripheral vascular beds, the discrepancy can be explained at least partly by a differential influence on the vascular responses among the organs. Indeed, electrical stimulation of the VTA increased femoral vascular conductance in this study, but it decreased renal vascular conductance in another study [18]. If intense muscular vasodilatation exceeds both vasoconstriction in other vascular beds and an increase in cardiac output, the VTA stimulation may cause a depressor response rather than a pressor response. It was important for better understanding of autonomic function of the VTA to measure the responses in regional blood flows as well as systemic AP.

The characteristics of increased femoral blood flow evoked by electrical stimulation of the VTA in the cat are

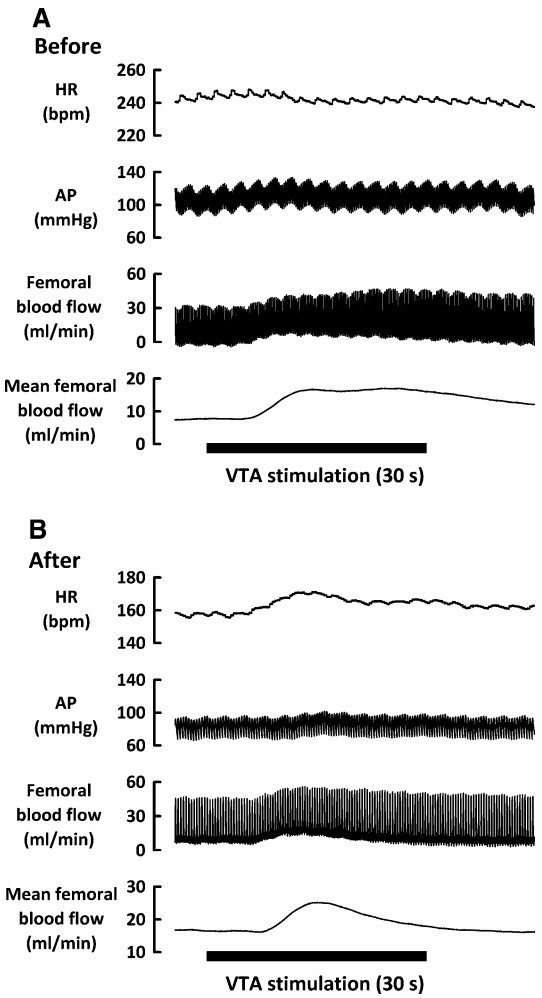

Fig. 5 The responses in HR, AP, and original and mean femoral blood flow evoked by electrical stimulation of the VTA before and after a decerebration procedure performed at the premammillary and precollicular level in an anesthetized, paralyzed cat. The substantial increase in femoral blood flow appeared during the VTA stimulation even following the decerebration procedure

in good agreement with those of the increases in brachial and femoral blood flows evoked by stimulation of the hypothalamic defense area [11–13]. When the actual changes in the internal diameter of small arteries in the cat triceps surae muscle during the hypothalamic stimulation were visualized with an X-ray angiography, a profound increase in vessel diameter of small arteries in the skeletal muscle was recognized during the hypothalamic stimulation [12]. The increases in vessel diameter of the intramuscular small arteries and limb blood flow were markedly blunted either by intra-arterial injection of atropine or by cutting the sciatic nerve, but not by a combined injection of phentolamine and propranolol [11–13]. Taken together, sympathetic cholinergic fibers stimulated by the hypothalamic stimulation is able to cause vasodilatation in small arteries of feline skeletal muscle. This concept is also likely to fit for the muscle vasodilatation evoked by electrical stimulation of the VTA.

The insignificant effect of stimulation of the SN on skeletal muscle circulation

Unlike the VTA, femoral blood flow and vascular conductance failed to increase during electrical stimulation of the SN, as well as systemic hemodynamics. In agreement with this finding, chemical stimulation of the SN with L-glutamate or a substance P analogue elicited no cardiovascular responses in anesthetized rats [21] and produced a significant but smaller pressor response than in the VTA with little effect on HR in conscious rats [18]. The SN is subdivided into the substantia nigra pars compacta (SNC) and the substantia nigra pars reticulata (SNR). The SNC primarily contains dopaminergic cells, whereas the majority of neurons in the SNR are devoid of dopamine and display intense GABA and glutamic acid decarboxylase immunoreactivity [1–3]. Although the GABAergic neurons in the SNR may play a role in regulating posture and locomotion [23, 24], the SN appears to have no significant roles in regulation of the autonomic and cardiovascular systems.

Efferent projection from the VTA

It is known that ascending dopaminergic efferent fibers from the VTA to the forebrain are widely distributed throughout the limbic, motor, and association areas of the cerebral cortex and the striatum [1–3]. The ascending efferent pathways are classified into the three systems: the mesocortical pathway (to the prefrontal, insular, motor, sensory, and association cortices), the mesolimbic pathway (to the limbic cortices, septo-hippocampal complex, accumbens, and amygdala), and the mesostriatal pathway (to the striatum). These ascending efferent pathways of the VTA have been emphasized so far in behavioral motivation and reward function [1–3], and they were also expected to be responsible for the skeletal muscle vasodilatation induced by electrical stimulation of the VTA. Contrary to this idea, substantial increases in femoral blood flow and vascular conductance were evoked by VTA stimulation even after decerebration was performed at the precollicular-premammillary level (Fig. 5). Thus, it is obvious that the descending pathways from the VTA to the diencephalon and the mesencephalon probably play a role in regulating the autonomic and cardiovascular systems. Indeed, recent immunohistochemical studies revealed that axons of dopaminergic neurons in the VTA terminate within the diencephalic and brain stem areas known as the autonomic and cardiovascular centers. Particularly

dopaminergic neurons in the VTA project to the lateral hypothalamus, periaqueductal gray region, dorsal raphe nucleus, and parabrachial nucleus [2, 4–9].

Limitations

There are some potential problems involved in this study. First, electrical stimulation of the central nervous system is known to excite not only cell bodies but also axonal fibers of passage in the vicinity of the electrode [25, 26]. Electrical stimulation at 100 μA may excite cells or axons located within 500 μm from the tip of the stimulating electrode [25]. In agreement with the previous finding, when the electrode tip was displaced upward by 0.5 mm from the target locus of the VTA in this study, the increases in femoral blood flow and vascular conductance disappeared or greatly diminished. To clarify whether neurons in the VTA play a role in evoking vasodilatation in skeletal muscle, chemical stimulation of the ventral mesencephalic areas, exciting cell bodies alone, will be needed. Second, a small sample size in the experimental interventions of atropine administration and decerebration might lose statistical power for detecting significance. However, as far as the effects of atropine on the muscle vasodilatation during VTA stimulation, the effects were obvious and statistically significant. Third, since the increases in femoral blood flow and vascular conductance during VTA stimulation tended to be blunted to 55–69% of the control by decerebration at the precollicular-premammillary level, it is possible that the reductions may be evoked via the ascending efferent projection from the VTA or may be related to deterioration of animal preparations, brain edema, and/or hemorrhage due to the intervention. Nevertheless, the remaining increases in femoral blood flow and vascular conductance cannot be explained by the ascending projection from the VTA.

Functional significance

Central command from higher brain centers plays an important role in feedforward control of the cardiovascular system during voluntary exercise [27–30]. However, little is known about the origin within the central nervous system and the descending pathways responsible for central command. Based on the present evidence, we can speculate that the mesencephalic VTA may contain neural circuits crucial for generating central command, which may be triggered by descending output from the cerebral cortex [30]. The concept is supported by the anatomical evidence demonstrating reciprocal connections between the VTA and the medial prefrontal, cingulate, and insular cortex [2, 10].

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