Effect of Acupuncture-Like Stimulation on Cortical Cerebral Blood Flow in Anesthetized Rats

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Abstract: The effect of acupuncture-like stimulation of various areas (cheek, forepaw, upper arm, chest, back, lower leg, hindpaw, perineum) on cortical cerebral blood flow (CBF) was examined in anesthetized rats. An acupuncture needle (diameter, 340 μm) was inserted into the skin and underlying muscles at a depth of about 5 mm and twisted to the right and left once a second for 1 min. CBF of the cortex was measured using a laser Doppler flowmeter. Stimulation of the cheek, forepaw, upper arm and hindpaw produced significant increases in CBF, but stimulation of the chest, back, lower leg and perineum did not produce significant responses. Stimulation of the cheek, forepaw, and hindpaw produced an increase in mean arterial pressure (MAP), while stimulation of the back produced a decrease in MAP. Stimulation of the upper arm, chest, lower leg and perineum did not produce a significant MAP response. After spinal transection at the 1st to 2nd thoracic level, the blood pressure response to stimulation of the cheek and forepaw was suppressed, whereas an increase in CBF still took place. The increase in CBF induced by forepaw stimulation was abolished by severance of the somatic nerves at the brachial plexus. Forepaw stimulation enhanced the activity of the radial, ulnar and median nerves. Furthermore, in the present study, passing of an electric current through acupuncture needles showed that excitation of group III (Aδ) and group IV (C) afferent fibers in the somatic nerve was capable of producing an increase in CBF, whereas excitation of group I (Aα) and group II (Aβ) fibers was ineffective. The increase in CBF induced by forepaw stimulation was almost abolished by intravenous administration of muscarinic and nicotinic cholinergic blocking agents (atropine 5 mg/kg and mecamylamine 20 mg/kg), and by bilateral lesions in the nucleus basalis of Meynert. Acupuncture-like stimulation of a forepaw increased acetylcholine release in the cerebral cortex. We concluded that the increase in CBF, independent of systemic blood pressure, elicited by acupuncture stimulation is a reflex response in which the afferent nerve pathway is composed of somatic group III and IV afferent nerves, and efferent nerve pathway includes intrinsic cholinergic vasodilators originating in the nucleus basalis of Meynert. [Japanese Journal of Physiology, 50, 495–507, 2000]

Key words: acupuncture, neural regulation of cerebral blood flow, intracranial cholinergic vasodilative system, nucleus basalis of Meynert, extracellular acetylcholine.

Acupuncture has been used to improve disturbances of visceral autonomic functions [1–3]. Recent acupuncture studies in animals demonstrated that acupuncture-like stimulation delivered to anesthetized animals produced reflex responses of various visceral functions, for example, gastric motility [4, 5], bladder contraction [5, 6], cardiovascular responses [7], adrenal medullary hormonal function [8, 9], muscle blood flow [10], etc. These acupuncture-like stimulation-induced responses have been proven to be re-
flexes in which afferents are cutaneous, and muscle somatic afferent nerves and efferents are autonomic efferent nerves.

Disturbances in cerebral blood flow (CBF) may affect consciousness, motor and visceral functions, etc. Acupuncture has been used to improve these dysfunctions produced by disturbances in CBF [11–13], but the mechanisms of the improvement have not yet been clarified.

Recent studies in our laboratory have shown that cortical CBF is regulated by intracranial nerves (see review by Sato and Sato [14]). Particularly, excitation of cholinergic nerve fibers originating in the basal forebrain nucleus (the nucleus basalis of Meynert) releases extracellularly acetylcholine (ACh) in the cortex [15], resulting in an increase in cortical CBF [16], independent of metabolic vasodilation [17]. These intracranial cholinergic neurons have been shown to be excited by cutaneous mechanical sensory stimulation, for example, by pinching of the skin [18]. ACh is released in the cortex at this time [19], and consequently cortical CBF is increased [20]. These results indicate that insufficient cerebral circulation is improved by stimulation of the skin and underlying muscles through acupuncture.

In the present study, we examined whether acupuncture-like stimulation to the skin and underlying muscles increased CBF, and if so, attempted to further examine the neural mechanisms of these acupuncture-induced responses in cerebral blood flow.

**MATERIALS AND METHODS**

Fifty-two male Wistar rats (body weight, 300–440 g, 4–9 months old) were anesthetized with urethane (1.1 g/kg, I.P.). Respiration was maintained using an artificial respirator (SN-480-7, Shinano, Tokyo) through a tracheal cannula. End-tidal CO2 concentration was measured continuously using a thermistor and kept at around 37.5°C using an infrared lamp and heater system. Collection of the perfusate began 1 h after the initiation of perfusion, and the perfused fluid was collected every 3 min in a sample cup kept on ice. The perfusate in each sample (6 μl) was mixed with 6 μl (120 fmol) of ethylhomocholine, an internal standard, dissolved in aCSF.

**Measurement of extracellular ACh in the cerebral cortex.** Extracellular ACh in the cerebral cortex was measured by microdialysis in 12 rats. A microdialysis probe (outer diameter, 0.5 mm; length of perfusion, 3 mm: CMA/12, Carnegie Medicine AB, Sweden) was inserted in the cortex at an angle of 30° to the vertical line to a depth of 3.5 mm from the cortical surface at AP=+3, L=+3 mm for frontal, AP=+0.2, L=+4 for parietal, and AP=−6.3, L=+5 mm for occipital lobes as described originally by Kurosawa et al. [15].

The microdialysis probe was perfused with artificial cerebral spinal fluid (aCSF) composed of 128 mM NaCl, 2.6 mM KCl, 1.3 mM CaCl2, 0.9 mM MgCl2, 20 mM NaHCO3, and 1.3 mM Na2HPO4 containing 5 μM physostigmine to inhibit acetylcholinesterase, at a speed of 2 μl/min. The recovery rate of ACh of the microdialysis probe in vitro was about 13% at room temperature. Collection of the perfusate began 1 h after the initiation of perfusion, and the perfused fluid was collected every 3 min in a sample cup kept on ice. The perfusate in each sample (6 μl) was mixed with 6 μl (120 fmol) of ethylhomocholine, an internal standard, dissolved in aCSF.

ACh was measured by high-performance liquid chromatography (HPLC) using an electrochemical detector as described by Kurosawa et al. [23]. The mobile phase (pH 8.4), consisting of 50 mM disodium phosphate and 0.5 mM EDTA·2Na, was pumped at a rate of 60 μl/min through a microbore separation column (1×530 mm, BAS, Tokyo), and then ACh was converted to hydrogen peroxide and betaine by immobilized acetylcholinesterase and choline oxidase packed into a column (2×7 mm, BAS). Both the sepa-
Acupuncture-Induced Cerebral Vasodilation

Nerve activity was continuously recorded on a thermistor (Nihon Kohden). The parameters of electric stimulation were 0.5 ms duration, 20 Hz frequency and 0.1–10 mA intensity for 30 s.

**Recording of action potentials from the somatic afferent nerves.** In 4 rats, action potentials were recorded from radial, ulnar and median nerves during manual acupuncture-like stimulation delivered to a forepaw. Radial, ulnar and median nerves were separated from the surrounding tissue near the joint of the elbow and cut there. These separated and cut nerves were kept in a pool of warm paraffin oil. The peripheral cut end of each nerve was placed in contact with bipolar platinum-iridium wire electrodes, and the nerve activity was amplified with a preamplifier at a time constant of 0.01 s (S-0476, Nihon Kohden). Nerve activity was continuously recorded on a thermal recorder (WS-682G, Nihon Kohden).

In 4 rats, the evoked action potentials were recorded from a radial nerve following a single electrical stimulus (pulse duration, 0.1 ms for myelinated fibers and 0.5 ms for unmyelinated fibers) to a forepaw via a pair of acupuncture needles at various intensities. Evoked action potentials were amplified (time constant, 0.3 s), and observed after averaging using an averaging instrument (ATAC3700, Nihon Kohden). The maximum conduction velocities of myelinated and unmyelinated fibers were calculated by the length of the nerve between the proximal stimulating and recording electrodes (3.0–4.0 cm) and the latencies of both evoked action potentials.

**Severance of autonomic nerves innervating the cerebral blood vessels.** In 4 rats, bilateral cervical sympathetic nerves innervating the cerebral blood vessels were cut at the cervical levels. Also, bilateral parasympathetic nerves innervating the cerebral blood vessels were cut at the level of the palatine ganglia of the facial nerves.

**Intravenous administration of blockers of nerve receptors.** The following drugs were used intravenously as adrenergic or cholinergic receptor blockades: phenolamine (phenolamine mesylate, Ciba-Gaigy, Switzerland) at 10 mg/kg as an α-adrenergic receptor blockade, and propranolol (Indelal, Sumitomo Pharmaceutical Co. Ltd., Osaka) at 2 mg/kg as a β-adrenergic receptor blockade. Atropine (atropine sulfate, Sigma, USA) at 5 mg/kg, blood brain barrier (BBB) permeable or methylatropine (mecamylamine hydrochloride, Sigma, USA) at 20 mg/kg, BBB impermeable or hexamethonium (hexamethonium bromide, Sigma, USA) at 20 mg/kg, BBB permeable or hexamethonium (hexamethonium bromide, Sigma, USA) at 20 mg/kg, BBB impermeable, were used as muscarinic acetylcholine receptor blockades. Mecamylamine (mecamylamine hydrochloride, Sigma, USA) at 20 mg/kg, BBB permeable or hexamethonium (hexamethonium bromide, Sigma, USA) at 20 mg/kg, BBB impermeable, were used as nicotinic acetylcholine receptor blockades.

**Naloxone hydrochloride (Sigma) at 4 mg/kg was used intravenously as an opioid receptor antagonist.**

**DeSTRUCTION OF THE NUCLEUS BASALIS OF MEYNER (NBM) AND SUBSTANTIA INNOMINATA (SI).** The NBM and SI with a spherical volume of 2–3 mm in diameter were electrically lesioned bilaterally by passing a direct current of 3 mA intensity for 15 s via a coaxial metallic electrode (o.d. 0.3 mm) positioned 1.4 mm posterior to the bregma, 2.5 mm lateral to the midline and 7.6 mm vertically beneath the bregma according to Paxinos and Watson’s atlas [21]. After lesioning and before starting to test the cortical CBF response by acupuncture-like stimulation, we waited for 1 h, or more if necessary, to eliminate the effect of in-
jury current of this lesion on the neurons. The areas of the lesions were histologically confirmed postmortem.

Intravenous administration of nicotinic cholinergic agonist. After lesioning of the bilateral NBM and SI, (−)-nicotine (30 μg/kg, Tokyo Kasei Kogyo, Japan) was intravenously used as a nicotinic cholinergic agonist.

Spinal transection. The spinal cord was transected at the 1st to 2nd thoracic (T1–2) level in 40 rats. After transection of the spinal cord, 4% Ficoll (Ficoll 70, Pharmacia, Uppsala, Sweden) was intravenously injected to maintain systolic blood pressure above 60 mmHg.

Statistical analysis. Data were expressed as means±SEM. Statistical testing was performed using a paired t-test, analysis of variance (ANOVA) followed by Dunnet’s Multiple Comparison test, or Student’s t-test.

RESULTS

CBF responses in the parietal cortex to acupuncture-like stimulation to a forepaw

CBF in the parietal cortex and mean arterial blood pressure (MAP) measured under resting conditions were 280±16 mV and 91±3 mmHg, respectively (n=6, mean±SEM). As shown in Fig. 1A, acupuncture-like manual stimulation delivered to a forepaw for 1 min produced increases in CBF in the parietal cortex ipsilateral to the stimulating site and MAP. Figure 1B summarizes the time courses of the responses of CBF (upper trace) in the parietal cortex ipsilateral to the stimulating site and MAP (lower trace) measured every 10 s in 6 rats. Both CBF and MAP started to increase 10 s after the onset of stimulation and significantly increased 20 s after the onset of stimulation. The responses of CBF and MAP reached nearly their maximum 40–50 s after the onset of stimulation. The maximum CBF and MAP responses were 108±1 and 109±2%, respectively. CBF and MAP remained at the increased levels for about 1 min after the end of stimulation and gradually returned to the control level afterward.

Acupuncture-like stimulation to various areas

Acupuncture-like manual stimulation delivered to various segmental areas produced various responses for CBF in both the parietal cortex and MAP. As shown in the sample recordings in Fig. 2A and B, stimulation of the cheek, forepaw, upper arm and hindpaw produced relatively obvious increases in CBF and MAP compared to stimulation to the chest, back, lower leg and perineum. Figure 2C and D summarizes the maximum magnitudes of CBF responses (in C) and MAP responses (in D) to stimulation to the various areas tested. The CBF response was expressed both ways, ipsilateral and contralateral to the stimulating site. Both ipsilateral and contralateral CBF responses were almost identical, and no significant differences were found as shown in Fig. 2C. The summarized CBF responses show that stimulation of the cheek, forepaw, upper arm and hindpaw produced significant increases in CBF, but stimulation of the chest, back, lower leg and perineum did not produce significant responses. Stimulation of the cheek, forepaw and hindpaw produced an increase in MAP, while stimulation of the back produced a decrease in MAP. Stimulation of the upper arm, chest, lower leg and perineum did not produce a significant MAP response. Upper arm stimulation produced an increase in CBF, but there was no MAP response. Although there were slight discrepancies between CBF and MAP responses, as stated above, there was a general tendency to produce both CBF and MAP responses in parallel.
Responses of CBF and MAP after spinal transection at the T1–2 level

The spinal cord was transected at the T1–2 level to eliminate any influence of pressor MAP responses following stimulation on CBF. In 6 spinalized rats, CBF and MAP measured under resting conditions were 268 ± 6 mV and 50 ± 6 mmHg, respectively. In this spinal preparation, acupuncture-like manual stimulation to 2 different areas (i.e., the cheek and forepaw) produced an increase in CBF without any MAP responses, as shown in sample recordings in Fig. 3A and B. The CBF response to stimulation of these 2 areas in the spinal rats was almost identical to the CBF response in the spinal cord intact preparation. Figure 3C and D summarizes the maximum magnitudes of both CBF and MAP responses to stimulation in spinal rats. The summarized results demonstrate that following the stimulation of these 2 areas, there were no responses of MAP, but CBF responses were around 112 to 116%. Stimulation to the chest, back, lower leg, hindpaw or perineum produced no CBF responses, but MAP was increased following stimulation of the chest and back as shown in Fig. 3B and D. Stimulation of the upper arm produced increases in both CBF and MAP (Fig. 3C and D). Figure 4 shows that after spinal transection, manual acupuncture-like stimulation to the forepaw no longer produced MAP responses, but still produced CBF responses. Both ipsilateral and contralateral CBF responses were almost identical, and no significant differences were found.

Effect of cutting the somatic afferent nerve on CBF response

The increased CBF following acupuncture-like manual stimulation to a forepaw for 1 min in the spinalized rats noted above without any MAP response was totally abolished after severance of the brachial nerves, as shown in the sample recording of Fig. 5. This finding was confirmed in 3 other cases.

Recordings of nerve discharges following acupuncture-like manual stimulation to a forepaw

Acupuncture-like manual stimulation was delivered to a forepaw between the 2nd and 3rd digits, and discharge activity was recorded from the distal cut end of the radial, ulnar and median nerves during manual stimulation, as shown in the sample recordings in Fig. 6A–C. The discharges in the radial, ulnar and median nerves were observed during stimulation for 1 min. This finding was confirmed in 3 other cases.
Electric stimulation via 2 acupuncture needles in a forepaw

Single-shock electrical stimulation to 2 acupuncture needles in a forepaw produced an evoked action potential in the distal cut end of an ipsilateral radial nerve. With increased stimulus intensity, 3 different action potential waves corresponding to group II, III and IV nerve fibers were gradually elicited in that order, as shown in Fig. 7A and B. The thresholds of the group II, III and IV nerve fibers were different, low to high in that order, as shown in the plotted graph in Fig. 7C.

In the present experiments, the thresholds of group II, III and IV nerve fibers were 0.28±0.06, 0.55±0.10, 3.25±0.63 mA, respectively, as shown in Table 1. Evoked action potentials were identified by the maximum conduction velocities as listed in the Table 1; 44.7±1.19 m/s for group II, 17.3±1.38 m/s for group III, and 0.98±0.10 m/s for group IV.

Figure 8A and B shows sample records of CBF and
MAP responses by electrical stimulation of a forepaw via 2 acupuncture needles at different intensities in a spinalized rat. Only CBF response, not MAP response, was significantly elicited by stimulation at intensities of 1.5, 5.0 and 10.0 mA, but not at 0.5 mA. Figure 8C and D summarizes the maximum CBF and MAP responses at different stimulus intensities. CBF was increased only when stimulus intensity was above the threshold of group III nerve fibers (Fig. 8C and D).

Severance of autonomic nerves innervating the cerebral blood vessels

In 4 spinal rats transected at the T1–2 level, the sympathetic nerves and parasympathetic nerves innervating the cerebral blood vessels were cut at the cervical level for sympathetic nerves and at the palatine ganglia of the facial nerves for parasympathetic nerves, bilaterally as shown in Fig. 9A. The basal CBF level was not significantly changed either before or after severance of these nerves. The CBF response elicited by electrical stimulation of a forepaw before severance of these autonomic nerves did not significantly change after the severance of these sympathetic and parasympathetic nerves as shown in the sample record in Fig. 9C and in the results summarized in Table 1. Thresholds (means±SEM) and conduction velocities of group II, III and IV fibers of the radial nerve (n=4).

<table>
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<th>Group</th>
<th>Threshold (mA)</th>
<th>Conduction velocity (m/s)</th>
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<td>II</td>
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was increased only when stimulus intensity was above the threshold of group III nerve fibers (Fig. 8C and D).


Fig. 6. Samples of activities of radial (A), ulnar (B) and median (C) nerves responding to acupuncture-like stimulation of a forepaw.

Fig. 7. A, B: Sample recording of the evoked potentials of myelinated (A) and unmyelinated (B) fibers from the radial nerve by electro-acupuncture of a forepaw (pulse duration, 0.1 ms for myelinated fibers and 0.5 ms for unmyelinated fibers). C: Strength-response curves of evoked group II, III and IV fiber potentials. Abscissa: stimulus strength in mA. Ordinate: magnitude of evoked potentials expressed as a percent of maximum potential.

Fig. 8. Relationships between intensities of electrical stimulation of a forepaw and magnitude of increase in CBF and MAP. A, B: Sample recordings of CBF (A) and MAP (B). C, D: Summarized graphs of relationships between stimulus intensity and magnitude of response of increase in CBF (C) and MAP (D) (n=5). * p<0.05, ** p<0.01; significantly different from prestimulus control values using a paired t-test. TII, TIII and TIV show the mean threshold intensity of II, III and IV fibers of radial nerves, respectively.

Table 1. Thresholds (means±SEM) and conduction velocities of group II, III and IV fibers of the radial nerve (n=4).

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Fig. 9D. MAP was not significantly changed either before or after the severance of these nerves, as summarized in Fig. 9E.

**Effect of adrenergic receptor blockades on CBF response**

The basal CBF level was not significantly changed after the administration of adrenergic receptor blockers. The CBF response elicited by the electrical stimulation of a forepaw in the spinalized rats did not significantly change following intravenous injection of either propranolol, a β-adrenergic receptor blockade, or phentolamine, an α-adrenergic receptor blockade, as shown in the sample records in Fig. 10A–C, and in the results of 4 rats summarized in Fig. 10D.

**Effect of cholinergic receptor blockade on CBF response**

The basal CBF level was not changed following the administration of cholinergic blockers. The CBF response elicited by electrical stimulation of a forepaw in the spinalized rats was not significantly affected after the intravenous injection of methylatropine (5 mg/kg) or an additional injection of hexamethonium (20 mg/kg), blood brain barrier impermeable muscarinic and nicotinic receptor antagonists, respectively, as shown in Fig. 11C and D. However, these responses were attenuated after the intravenous injection of atropine, a blood brain barrier permeable muscarinic receptor antagonist, and were further attenuated by an additional injection of mecamylamine, a blood brain barrier permeable nicotinic receptor blockade, as shown in Fig. 11A and B. The increase in CBF during stimulation in the control before the injection of drugs was 123±3% and was attenuated to

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**Fig. 9. Effects of bilateral severance of cervical sympathetic nerves and parasympathetic nerves (facial nerves) on the response of CBF and MAP to electrical stimulation of a forepaw in spinalized rats. A. Schematic diagram of the innervation of cerebral blood vessel by sympathetic and parasympathetic nerves. B, C. Sample recordings of CBF and MAP. D, E. Summary of CBF and MAP responses (n=4). *p<0.05, **p<0.01; significantly different from prestimulus control values using a paired t-test. n.s.: non-significant (p>0.05) difference between the responses by Student’s t-test.**

**Fig. 10. Effects of adrenergic receptor blockers on CBF and MAP responses to electrical stimulation of a forepaw in spinalized rats. Sample recordings (A–C) and summary of CBF responses (D) (n=4). Data for adrenergic receptor blockers were taken 10–15 min after administration of the drugs. Other details are the same as in Fig. 9.**

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**Fig. 11. Effects of cholinergic receptor antagonists on CBF responses induced by electrical stimulation in spinalized rats. A. B. Cholinergic receptor antagonists permeable to BBB. C, D. Cholinergic receptor antagonists impermeable to BBB. A, C. Sample recordings of CBF (n=5); B, D. Summary of CBF responses. Data for cholinergic receptor antagonists were taken 10–15 min after administration of the drugs. *p<0.05, **p<0.01; significant difference between the responses by Student’s t-test. Other details are the same as in Fig. 9.**
112±2% following atropine injection, and further attenuated to 104±2% following an additional injection of mecamylamine.

**Extracellular ACh response in the cortex**

Extracellular ACh release in the parietal cortex measured every 3 min under resting conditions in 6 central nervous system (CNS)–intact rats was in the range of 35–93 fmol/3 min at the beginning of the experiment. Each cortical ACh release value in the same animal was stable in the resting condition.

Extracellular ACh in the parietal cortex was increased following acupuncture-like manual stimulation delivered to the forepaw, back or hindpaw, either ipsilateral or contralateral to the stimulation site when tested in 6 CNS-intact rats, as shown in Fig. 12A–C. For example, stimulation of a forepaw increased ACh release from the basal level of 58±14 to 128±16 fmol/3 min, stimulation of the back increased ACh release from the basal level of 69±25 to 123±30 fmol/3 min, and stimulation of a hindpaw increased ACh from the basal level of 80±9 to 172±23 fmol/3 min in the parietal cortex ipsilateral to the stimulating site. The increased cortical ACh release returned to the control level during the 3 min after the end of stimulation. There were no significant differences between ACh responses due to stimulation of the ipsi- and contralateral sites.

Extracellular ACh release in the frontal, parietal and occipital cortices measured every 3 min under resting conditions in 6 spinalized rats was 42±2, 40±4 and 41±2 fmol/3 min, respectively.

Acupuncture-like manual stimulation of a forepaw in the spinalized rats produced an increase in ACh in the frontal, parietal and occipital cortices, as shown in Fig. 13B and D, in addition to an increase in CBF in the frontal, parietal and occipital cortices whether ipsilateral or contralateral to the stimulation site, as previously mentioned and as shown in Fig. 13A and C. The response of ACh to unilateral forepaw stimulation was elicited bilaterally as seen in comparison with the responses ipsilateral and contralateral to the stimulation site in Fig. 13B and D. The increases in ACh in the ipsilateral frontal, parietal and occipital cortices were 33±5, 40±12 and 20±2 fmol/3 min, respectively. The increases in ACh in the contralateral frontal, parietal and occipital cortices were 43±8, 37±7 and 30±9 fmol/3 min, respectively.

**Effect of bilateral lesion of the NBM**

The bilateral NBM were electro-coagulated, and we waited for approximately 60 min. We found that the CBF response elicited by electrical stimulation of a forepaw in spinalized rats was totally abolished after lesioning of the bilateral NBM (Fig. 14A and B). The basal level of CBF was not significantly changed following destruction of the NBM.

After noting that electrical stimulation of a forepaw produced no more CBF response following bilateral lesioning of the NBM, we tested the effect of nicotine, which is known to produce increased CBF [24] on CBF, and found that the intravenous injection of nicotine (30 μg/kg), lasting about 1 min, produced a long-lasting increase in CBF without affecting MAP, as shown in Fig. 14 C and D.

**Effect of naloxone**

Naloxone (4 mg/kg), an opioid receptor antagonist, was intravenously injected into 4 rats, and we waited for 10–15 min. We found that the CBF response
elicited by electrical stimulation of a forepaw in the spinalized rats was not influenced by the intravenous injection of naloxone, as shown in Fig. 15. The basal CBF level was not significantly changed following the administration of naloxone.

**DISCUSSION**

The present study is the first report proving that acupuncture-like stimulation of the skin and underlying muscles at various segmental areas produces an increase in CBF in the cortex as a reflex response, independent of emotional responses and blood pressure responses, in anesthetized rats. This response was proven to be a reflex in which the afferent arc consisted of somatic group III and IV afferents and the
efferent arc involved intracranial cholinergic nerve fibers originating in the NBM (Fig. 16).

It has been very difficult to prove true reflex CBF responses following acupuncture in conscious humans or animals because somatic sensory stimulation by acupuncture produces various emotional responses that are difficult to distinguish from true reflex responses. Since the present study was performed on anesthetized unconscious rats, emotional responses were eliminated, and the present rat preparation made it possible to objectively evaluate true reflex CBF responses independently of the conscious state.

Acupuncture-like stimulation has been demonstrated to produce cardiovascular responses in anesthetized animals an increase or a decrease in arterial blood pressure [7, 8]. Since the autoregulation of CBF is insufficient to work against the MAP response during a short time period of a matter of seconds [25], we must keep in mind that CBF can be influenced passively in a time period of seconds by MAP responses. In the present study, we employed a special rat preparation in which the spinal cord was transected at T1–2, as developed by Adachi et al. [20]. This preparation has no descending neural connections from the brainstem to the spinal thoracic and lumbar sympathetic preganglionic neurons. Therefore, no afferent inputs entering in brainstem or cervical spinal cord above the T1 level can affect the activity of spinal preganglionic sympathetic neurons. This spinal preparation proved that acupuncture-like stimulation to a forepaw produced an increase in CBF of the same magnitude as that shown before the spinal transection, but no pressor MAP response. Thus, the increase in CBF could not be a passive vasodilative CBF response to pressor MAP response. The present finding of CBF response during acupuncture-like stimulation is partially a confirmation of the finding of CBF response to cutaneous pinching in anesthetized rats reported by Adachi et al. [20].

The present study showed that acupuncture-like stimulation in a forepaw excited somatic afferent nerve fibers, and CBF responses were lost after severing these somatic afferents in spinalized rats whose spinal cords were cut at the T1–2 level, indicating an inevitable role of somatic afferents in the acupuncture-like simulation induced CBF response. Stimulation of group III and IV somatic afferent fibers, but not of group II fibers, was necessary to produce CBF responses. Therefore, it is concluded that the response of CBF following acupuncture-like stimulation was elicited by the excitation of group III and IV somatic afferent fibers due to acupuncture-like stimulation. Adachi et al. [20] demonstrated that CBF response was elicited by cutaneous pinching exciting group III and IV afferent fibers, but not by cutaneous brushing exciting group II afferent fibers in anesthetized rats, indicating the importance of group III and IV somatic afferent fibers as afferent arcs for the cutaneous-CBF reflex response. However, Kurosawa et al. [26] noted the contribution of group II somatic afferent fibers excited by brushing to the increase in extracellular ACh in the cortex of conscious rats. It is likely that neural pathways from peripheral group II somatic afferent fibers to the central NBM neurons working in the conscious state are depressed in the anesthetized state. At this moment, we hesitate to give acupuncture-like stimulation to conscious rats to examine the effect on CBF because of the possible unpleasant stimulation. Therefore, it is not yet clear whether the excitation of group II afferent fibers during acupuncture-like stimulation affects CBF in conscious rats.

In traditional acupuncture, spots or points of acupuncture have been emphasized as important for effectiveness or response. However, the present experiments indicate that the effective areas of stimulation are rather diffuse and not strictly localized in small areas.
spots. The accuracy of spots appears to depend on the responses observed. For example, cardiovascular responses have the most effective areas of stimulation in the legs, while inhibitory responses of micturition contraction of the urinary bladder due to parasympathetic inhibition [6] and inhibitory gastric motility responses due to sympathetic activation [4] have the most effective areas in the perineum and abdomen, respectively, and not in the legs (see the review by Sato et al. [27]). CBF responses originating in efferent paths of the NBM are similar to cardiovascular responses. Both responses appear to be controlled strongly by the supraspinal structure, but other responses of the urinary bladder and gastric motility have strong segmental reflex control. It is likely that stimulation of the legs is important for supraspinal regulation, while stimulation of the spinal segments is important for segmental regulation.

The intracranial cholinergic vasodilative system, discovered by Sato and his colleagues in 1989 [15, 16] and reviewed by Sato and Sato in 1992 [14], have the following characteristic properties: (1) There are diffuse projections from the NBM to the frontal, parietal and occipital cortices. (2) Extracellular ACh is released in the cortex and activates the muscarinic and nicotinic ACh receptors. (3) This cholinergic vasodilative system causes neurogenic, but not metabolic vasodilation. The present study examining CBF response following acupuncture-like stimulation proved that CBF response was diffuse in the parietal, frontal and occipital cortices, and also bilateral to acupuncture-like stimulation at a unilateral site. Both muscarinic and nicotinic ACh receptor blockers (only blood brain barrier permeable blockers) attenuated the responses. Other muscarinic and nicotinic ACh receptor blockers that are blood brain barrier impermeable were not effective, indicating that the receptors in the parenchyma, but not inside the blood vessels in the cortex are important for producing responses. Lesioning of the bilateral NBM abolished CBF responses following acupuncture-like stimulation. Therefore, the NBM originating cholinergic system seems to be involved in the present responses. In fact, Akaishi et al. [18] demonstrated the excitation of NBM neurons by cutaneous pinching in anesthetized rats and demonstrated an increase in CBF [20] induced by exciting the NBM cholinergic vasodilative system. Thus cutaneous pinching and acupuncture-like stimulation to the skin and underlying muscles have similar effects on CBF responses, probably via excitation of the somatic group III and IV afferent fibers. Therefore, cutaneous and muscle efferent fibers have similar connections to the NBM neurons.

The present findings in healthy normal and anesthetized rats may lend support to the application of acupuncture for patients with disturbances in cerebral blood flow by activating NBM neurons and eventually increasing CBF in the cortex.

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