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# Sustained subcutaneous infusion of nicotine enhances cholinergic vasodilation in the cerebral cortex induced by stimulation of the nucleus basalis of Meynert in rats

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ABSTRACT

The present study examined the effects of sustained nicotine exposure on the cholinergic vasodilative system originating in the nucleus basalis of Meynert (NBM) and projecting to the cerebral cortex in rats. Rats received sustained subcutaneous nicotine (100 μg/kg/h) for 14 days. Under urethane anesthesia, the vasodilation response and acetylcholine release in the parietal cortex induced by electrical stimulation of the NBM (10–200 μA) were measured. The basal level of acetylcholine release was significantly higher in nicotine-treated rats than in saline-treated control rats. In the control rats, both the acetylcholine release and blood flow were increased by NBM stimulation in a stimulus intensity-dependent manner, and a threshold of 50 μA. In nicotine-treated rats, the threshold intensity of NBM stimulation producing increases in acetylcholine release and blood flow was reduced to 20 μA. The stimulus intensity-dependent acetylcholine release and vasodilation by NBM stimulation were significantly larger in nicotine-treated rats than in control rats. We conclude that sustained subcutaneous infusion of nicotine enhances cholinergic vasodilative system in the cerebral cortex originating in the NBM.



## 1. Introduction

A cholinergic neural vasodilative response in the cortex, independent of systemic blood pressure and metabolic vasodilation, has been reported to occur when cholinergic neurons originating in the magnocellular nucleus of the basal forebrain (the nucleus basalis of Meynert; NBM) and projecting to the cortex were activated (Biesold et al., 1989; Sato and Sato, 1992). For example, focal electrical stimulation of the unilateral NBM produces an increase in cerebral blood flow in the parietal cortex ipsilateral to the site of stimulation without an effect on systemic blood pressure in anesthetized rats (Biesold et al., 1989). This cholinergic vasodilative system, which operates by increasing acetylcholine release, relies upon the activation of both muscarinic and nicotinic receptors in the parenchyma of the cortex (Biesold et al., 1989; Kurosawa et al., 1989). This neural vasodilation system has been suggested to have a physiologically preparatory role in starting cerebral neuronal activity before the metabolic vasodilation mechanism begins in these neurons (Sato and Sato, 1992).

Recently, prolonged exposure to nicotine for about 1–2 weeks was reported to enhance trophic support to basal forebrain cholinergic neurons by increasing nerve growth factor (NGF) content (Brown et al., 2006) and the number of NGF-like immunolabelled neurons (Martínez-Rodríguez et al., 2003) in the cortex, or by increasing the levels of NGF receptors tyrosine kinase receptor A (TrkA) in basal forebrain cholinergic neurons (Formaggio et al., 2010). NGF is essential for the survival and function of cholinergic basal forebrain neurons (Cuello et al., 2007). These results indicate that prolonged nicotine exposure may enhance basal forebrain cholinergic function. The basal level of acetylcholine release in the cerebral cortex has been reported to increase after nicotine treatment for about 2 weeks in rats (Nordberg et al., 1989). Therefore, prolonged nicotine exposure may enhance the cholinergic neural vasodilative response in the cortex by stimulation of the NBM. The present study aimed to clarify whether the NBM stimulation-induced cortical vasodilation as well as acetylcholine release is affected by sustained subcutaneous infusion of nicotine for 2 weeks.

## 2. Materials and methods

The experiments were performed on 23 male adult Wistar rats (body weight, 320–430 g, 4–9 months old). All animal experiments were conducted according to the Guidelines for Animal Experimentation prepared by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

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### 2.1. Sustained subcutaneous infusion of nicotine

The rats were divided into two groups; (1) a control group (n = 11), and (2) a nicotine-treated group (n = 12). The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and a minipump (Alzet, model #2002, 0.5 μl/h, 14 days duration) containing either (−) nicotine (Tokyo Kasei Kogyo, Japan) (13–15 mg /200 μl, calculated as the free base) or saline was inserted into a subcutaneous pocket via a small incision over the shoulders (Fig. 1). In the control group, 7 of 11 rats were treated with saline and the other 4 rats were left untreated. The dose of nicotine used in the present experiment was in the same range as the doses previously reported to affect the expression of neurotrophin receptors in the basal forebrain cholinergic neurons (Formaggio et al., 2010), or cortical blood flow response (Uchida et al., 2009). The wound was sutured with a cotton thread and the rats were returned to their cage. After awakening, the animals were housed at an ambient temperature of 22 ± 2 °C and fed laboratory food with water ad libitum.

### 2.2. General surgery and anesthesia

Fourteen days after minipump implantation, the responses of acetylcholine release in the cerebral cortex and cerebral cortical blood flow to focal electrical stimulation of the NBM were examined under general anesthesia (Fig. 1), as described previously (Uchida et al., 2000). Briefly, the rats were anesthetized with urethane (1.1 g/kg, i.p.). Respiration was maintained by means of an artificial respirator (model 683, Harvard, USA) through a tracheal cannula. End-tidal $CO_2$ concentration was maintained at 3.0–4.0% by monitoring using a respiratory gas monitor (Microcap, Oridion Medical, Jerusalem, Israel). Arterial blood pressure was measured through a catheter inserted into a femoral artery with a pressure transducer (TP-400 T, Nihon Kohden, Tokyo). Body temperature was measured rectally and continuously using a thermistor, and maintained at approximately 37.5 °C by means of an infrared lamp and a heater system (ATB-1100, Nihon Kohden). The depth of anesthesia was adjusted by additional urethane doses (100 mg/kg, i.v. via a catheter inserted into a femoral vein) when necessary and by monitoring body movement, stability of blood pressure and respiratory movement.

### 2.3. Measurement of acetylcholine release in the cerebral cortex

The animals were mounted on a stereotaxic instrument (SR-5, Narisige) in a prone position, and the parietal cortex was exposed by removing a portion of the skull and dura. Extracellular acetylcholine in the parietal cortex was collected by a microdialysis technique. A microdialysis probe (outer diameter, 0.5 mm; length of perfusion, 3 mm; CMA/12, CMA/Microdialysis AB, Sweden) was inserted in the right parietal cortex at an angle of 30° to the vertical line to a depth of 3.5 mm from the cortical surface at AP = +0.2, L = +4, as described originally by Kurosawa et al. (1989). The microdialysis probe was perfused at a speed of 2 μl/min with an artificial cerebral spinal fluid (aCSF) containing (in mM) NaCl (122.7), KCl (2.4), $CaCl_2$ (1.5), $MgCl_2$ (1.1), $NaHCO_3$ (27.5), $KH_2PO_4$ (0.6), $Na_2SO_4$ (0.5), glucose (6), and the acetylcholinesterase inhibitor physostigmine (5 μM). The aCSF was bubbled with 95% $O_2$/5% $CO_2$ to adjust the pH to 7.4. The recovery rate of acetylcholine of the microdialysis probe in vitro at room temperature was usually 23–24%. The recovery rates of acetylcholine of two microdialysis probes used for one saline-treated rat and one nicotine-treated rat were 14–15%. In these two probes, the recovery rates were adjusted to 24% by calculation. 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 isopropylhomocholine, an internal standard, dissolved in aCSF. Acetylcholine was measured by high-performance liquid chromatography (HPLC) using an electrochemical detector (HTEC-500, Eicom, Kyoto) (Fig. 2A). The mobile phase, consisting of 50 mM $KHCO_3$, 400 mg/l Sodium 1-Decanesulfonate (Tokyo Kasei Kogyo, Japan), and 50 mg/l EDTA 2Na, was pumped at a rate of 150 μl/min through a microbore separation column (AC-GEL, 2 X 150 mm). The acetylcholine was converted to hydrogen peroxide and betaine by immobilized acetylcholinesterase and choline oxidase packed into a column (AC-ENZYMEPAKII, 1.0 X 4 mm). Both the separation column and enzyme column were maintained at 33 °C. Hydrogen peroxide was measured using an electrochemical detector, and acetylcholine was calculated by hydrogen peroxide measurement. The platinum working electrode was held at 0.45 V vs Ag/AgCl.

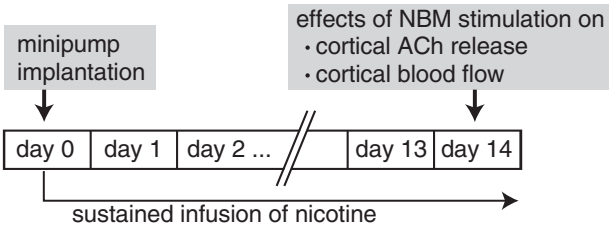


**Fig. 1.** Diagram showing the time course of the present experiments.

### 2.4. Measurement of cortical cerebral blood flow

The probe (diameter 0.8 mm) of a laser Doppler flowmeter (ALF21D, Advance, Tokyo) was placed on the surfaces of the right or left parietal lobe (AP = +1 to −2 mm, L = +4 to +6 mm), and was fixed with a balancing holder (ALF-B, Advance). The output of the laser Doppler flowmeter was expressed in mV and recorded on a polygraph. In 9 rats, cortical blood flow and acetylcholine release in the parietal cortex were measured simultaneously. In other rats, however, either cortical blood flow or acetylcholine release was measured.

### 2.5. Stimulation of the NBM

A coaxial metal electrode of 0.3 mm outer diameter was stereotactically inserted into the NBM ipsilateral to the parietal cortex where acetylcholine and/or blood flow was measured. Focal electrical stimulation of the NBM was performed by means of a stimulator (SEN-7203, Nihon Kohden, Tokyo) and stimulus isolation unit (SS-202 J, Nihon Kohden). The parameters of electric stimulation were 0.5 ms duration, 17 or 50 Hz frequency for 90 s or 3 min. Either parameter is reported to produce supramaximal responses in acetylcholine release in the parietal cortex (Kurosawa et al., 1989). Stimulus intensity was varied from 10 to 200 μA. Histological verification of the tip position of the stimulating electrode was performed after the end of experiments on frozen transverse brain sections. The stimulated area was located 1.8–2.4 mm posterior to the bregma, 3.6–4.0 mm lateral from the midline, and 6.2–7.6 vertical under the bregma height in both the control rats and nicotine-treated rats. All of the tip positions in 23 rats were confirmed within the regions of the NBM according to Paxinos and Watson's atlas (2009).

### 2.6. Statistical analysis

All values are presented as means ± S.E.M. Statistical comparisons were carried out by means of one-way repeated-measures ANOVA followed by a Dunnett's multiple comparison test, two-way factorial ANOVA and paired *t*-test or unpaired *t*-test. A *P*-value of $<0.05$ was considered to be statistically significant.

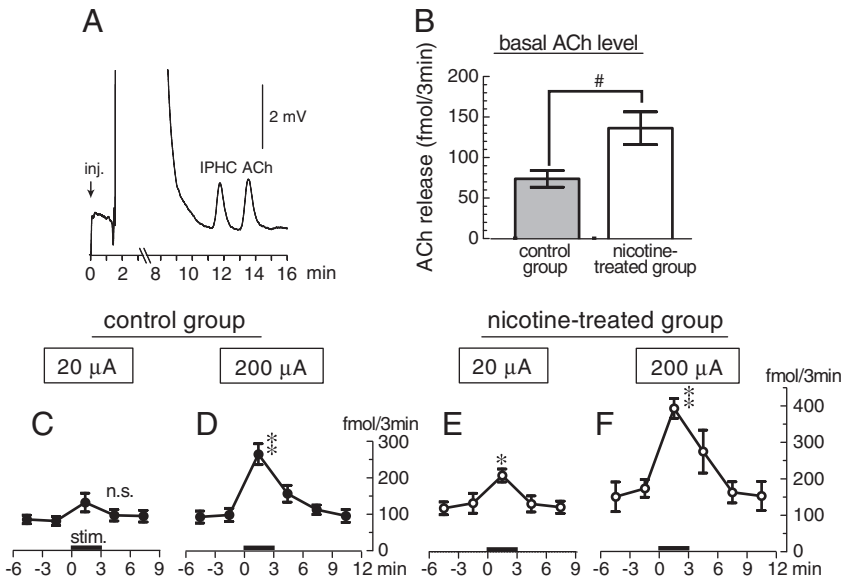


**Fig. 2.** Responses of acetylcholine (ACh) release in the parietal cortex to focal electrical stimulation (50 Hz, 3 min) of the unilateral NBM ipsilateral to the parietal cortex in saline-treated control group and nicotine-treated group at 20 and 200 μA. A: Chromatograms of 10 μl of mixed solution containing sample (taken from a saline-treated control rat in a resting condition) and internal standard (isopropylhomocholine: IPHC). B: Summary of basal level of ACh releases (mean ± S.E.M.). Values of two samples at the beginning of experiments were averaged in each rat. #P<0.05; significant difference between the saline-treated control group and nicotine-treated group, tested by unpaired *t*-test. C–F: The level of extracellular acetylcholine measured every 3 min are plotted as the absolute values in saline-treated control group (C, D, closed circles, n = 6) and nicotine-treated group (E, F, open circles, n = 6). Each point and vertical bar represents a mean ± S.E.M. *P<0.05; **P<0.01; significantly different from prestimulus control values (−3 to 0 min) using one-way repeated-measures ANOVA followed by Dunnett's multiple comparison test.

## 3. Results

### *3.1. Effect of sustained subcutaneous infusion of nicotine on the NBM stimulation-induced increase in acetylcholine release in the cerebral cortex*

The basal acetylcholine release at the beginning of the experiments in the parietal cortex was significantly higher in nicotine-treated rats than saline-treated control rats (Fig. 2B). The basal level of acetylcholine release was stable throughout the experiment.

Fig. 2C–F demonstrates the time course of acetylcholine release in the parietal cortex in response to the NBM stimulation at 20 μA and 200 μA in saline-treated control rats and nicotine-treated rats. In the control rats, 3 of 6 rats produced an increase in acetylcholine release during the NBM stimulation at 20 μA, but the total responses in all 6 rats were insignificant (Fig. 2C). During the stimulation at 200 μA, acetylcholine release was significantly increased to 266 ± 29 fmol/3 min in the control rats, and returned to the prestimulus level after the stimulation ended (Fig. 2D). In nicotine-treated rats, acetylcholine release was increased during the NBM stimulation at 20 μA in all 6 rats tested, and the response (reaching 209 ± 18 fmol/3 min) was statistically significant (Fig. 2E). During the stimulation at 200 μA, acetylcholine release was significantly increased to 393 ± 27 fmol/3 min in nicotine-treated rats (Fig. 2F). The increased acetylcholine release returned to prestimulus level after the end of stimulation. The time courses of the responses of acetylcholine release to NBM stimulation were similar in both the control rats and nicotine-treated rats.

The changes in acetylcholine release during the NBM stimulation at different intensities of stimulation (10–200 μA) are summarized in Fig. 3. Cortical acetylcholine release was increased by NBM stimulation in a stimulus intensity dependent manner in both saline-treated control rats and nicotine-treated rats. In the control rats, acetylcholine release was significantly increased during the NBM stimulation when the intensity of stimulation was more than 50 μA. In nicotine-treated rats, the threshold intensity of the NBM stimulation for producing a significant increase in acetylcholine release was decreased to 20 μA. The stimulus intensity-dependent acetylcholine release by NBM stimulation was higher in the nicotine-treated group than in the control group, and the difference was statistically significant.

### *3.2. Effect of sustained subcutaneous infusion of nicotine on the NBM stimulation-induced increase in cortical blood flow*

The basal cortical blood flow in the 6 saline-treated control rats at the beginning of the experiments was 277 ± 50 mV. This value was similar to that in 6 nicotine-treated rats (252 ± 45 mV).

Fig. 4 demonstrates the time course of cortical cerebral blood flow in the parietal cortex in response to NBM stimulation at 20 μA and 200 μA in saline-treated control rats and nicotine-treated rats. As

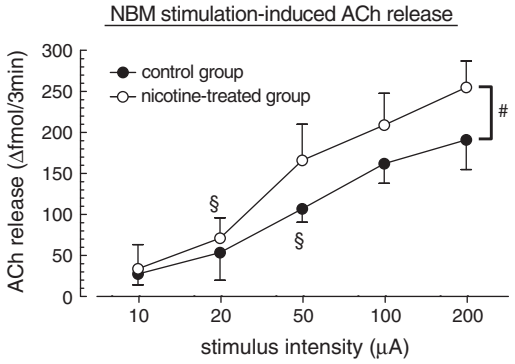


**Fig. 3.** Changes in ACh release in the parietal cortex in response to electrical stimulation of the unilateral NBM (50 Hz, 3 min) at different intensities in 6 saline-treated control rats and 6 nicotine-treated rats. Relationships between stimulus intensity (abscissa) and magnitude of increase in ACh release from the basal level of each rat (ordinate) are summarized as the mean ± S.E.M. §: threshold intensity for producing significant increase in acetylcholine release from the basal level, tested by paired *t*-test. #P<0.05; all the stimulus intensity-dependent responses in the nicotine-treated group were significantly different from those in control group, as tested by two-way factorial ANOVA.

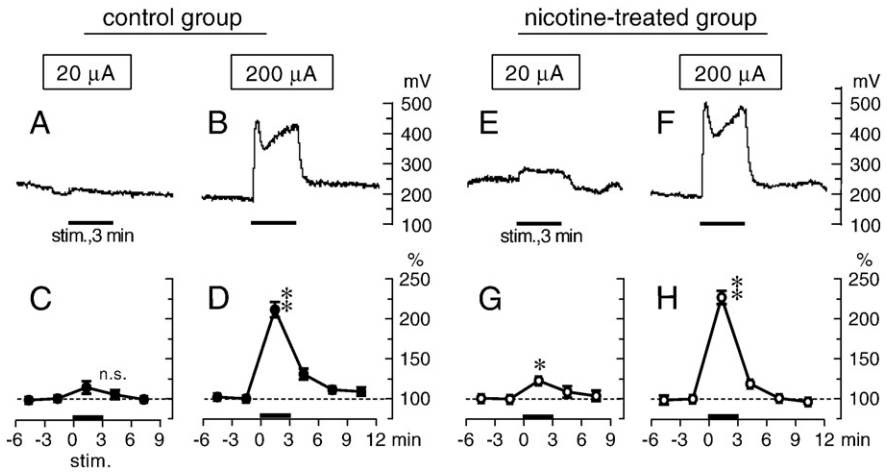


**Fig. 4.** Responses of cerebral blood flow in the parietal cortex to focal electrical stimulation (50 Hz, 3 min) of the unilateral NBM ipsilateral to the parietal cortex in the saline-treated control group (A–D) and the nicotine-treated group (E–H) at 20 and 200 μA. A, B, E, and F: Sample recordings of cerebral blood flow in response to electrical stimulation of the NBM with different intensities for 3 min indicated by the black bars. C, D, G, and H: Reponses of cerebral blood flow during a 3-min period were plotted every 3 min as a percentage of the prestimulus values in the saline-treated control group (C, and D, closed circles, n = 6) and the nicotine-treated group (G, and H, open circles, n = 6). Each point and vertical bar represents a mean ± S.E.M. *P<0.05; **P<0.01; significantly different from prestimulus control values (−3 to 0 min) using one-way repeated-measures ANOVA followed by Dunnett's multiple comparison test.

shown in the sample recordings (Fig. 4A, B, E, and F), cortical cerebral blood flow was increased during the NBM stimulation at the intensity of 200 μA, but not 20 μA in a control rat (Fig. 4A, and B), while cortical cerebral blood flow was increased at 20 and 200 μA in a nicotine-treated rat (Fig. 4E, and F). In control rats, 3 of 6 rats produced an increase in cortical cerebral blood flow during the NBM stimulation at 20 μA, but the total responses in all 6 rats were insignificant (Fig. 4C). At 200 μA, cortical cerebral blood flow was increased significantly during NBM stimulation, reaching 212 ± 10% of the prestimulus basal value in control rats (Fig. 4D). In nicotine-treated rats, cortical cerebral blood flow was increased during stimulation in all 6 nicotine-treated rats at 20 μA, and the response reached 123 ± 5% of the prestimulus basal value, which was statistically significant (Fig. 4G). At 200 μA, cortical cerebral blood flow was increased significantly during NBM stimulation reaching 227 ± 8% of the prestimulus basal value in nicotine-treated rats (Fig. 4H). The increased blood flow returned to the prestimulus level after the end of stimulation. The time courses of the responses of cortical cerebral blood flow to NBM stimulation were similar between the control rats and nicotine-treated rats.

The cortical cerebral blood flow responses during NBM stimulation at different intensities of stimulation (10–200 μA) are summarized in Fig. 5. Cortical cerebral blood flow was increased by NBM stimulation in a stimulus intensity dependent manner in both the control rats and nicotine-treated rats. The threshold intensity of the NBM stimulation producing significant vasodilation was 50 μA in control rats, but was reduced to 20 μA in nicotine-treated rats. The stimulus intensity-dependent increase in cortical cerebral blood flow by NBM stimulation was higher in the nicotine-treated group than in the control group, and the difference was statistically significant.

## 4. Discussion

The present study demonstrates that cholinergic vasodilative system in the cerebral cortex originating in the NBM is enhanced by sustained subcutaneous infusion of nicotine (Fig. 6). The threshold intensity of NBM stimulation for producing increases in both acetylcholine release and blood flow in the parietal cortex was lower in nicotine-treated rats (20 μA) than in saline-treated control rats (50 μA). Enhanced reactivity of cerebral cortical vasodilation to NBM stimulation in nicotine-treated rats may be due to the enhanced responsiveness of cortical acetylcholine release to NBM stimulation by sustained nicotine treatments.

Our results showed that basal level of acetylcholine release in the parietal cortex was significantly higher in nicotine-treated rats than in saline-treated control rats. This observation is in accord with the study of Nordberg et al. (1989), who reported that the repeated subcutaneous administration of nicotine (0.45 mg/kg twice daily for 16 days) increases basal cortical acetylcholine release in rats. Our results showed that sustained subcutaneous administration of nicotine using osmotic infusion pump (100 μg/kg/h for 14 days) increases basal cortical acetylcholine release in rats. Further, we demonstrated that sustained nicotine treatment decreases the threshold intensity of NBM stimulation for producing increases in cortical acetylcholine release, and enhances the stimulus intensity-dependent cortical acetylcholine release. The majority of cholinergic fibers in the cortex originate in the NBM (Herrera-Marschitz et al., 1990; Johnston et al., 1981). We observed that similar levels of choline uptake in cortical synaptosomes prepared from both nicotine-treated

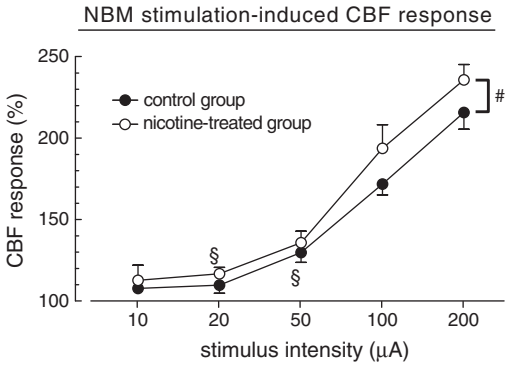


**Fig. 5.** Relationships between stimulus intensity (abscissa) and magnitude of cerebral blood flow (CBF) responses (ordinate) in 10 control rats (6 saline-treated rats and 4 untreated rats) and 10 nicotine-treated rats. The magnitude of the responses during the stimulus period is expressed as percentages of the prestimulus control value for 90 s or 3 min just before the stimulation and are summarized as the mean ± S.E.M. Stimulus parameters were 17 Hz for 90 s (n = 4), and 50 Hz for 3 min (n = 6) in each group. n = 6 for 10 μA, n = 10 for 20–200 μA in each group. §: threshold intensity for producing significant increase in cerebral blood flow from the prestimulus values, tested by paired *t*-test. #P<0.05; all the stimulus intensity-dependent responses in the nicotine-treated group were significantly different from those in control group, as tested by two-way factorial ANOVA.

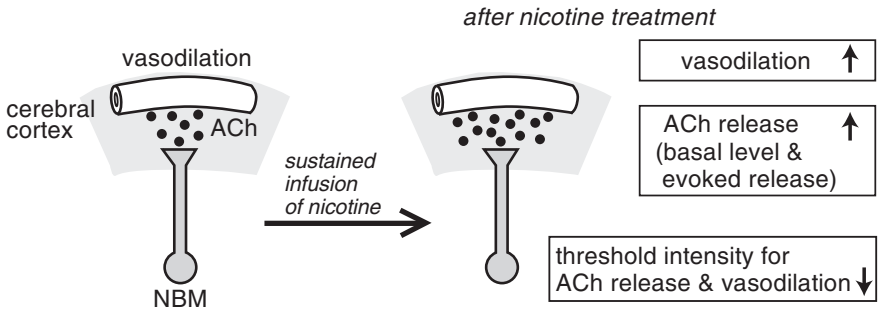


**Fig. 6.** Diagram of the summary of the present results.

rats and saline-treated control rats (unpublished observations) demonstrate that sustained infusion of nicotine does not change the choline transporter (CHT)-mediated high-affinity choline uptake, which is the rate-limiting step of acetylcholine synthesis. The present observations of enhancements of cortical acetylcholine release by sustained infusion of nicotine are therefore likely due to the enhanced excitability of NBM cholinergic neurons projecting to the cerebral cortex, which may enhance the firing rate of NBM cholinergic neurons at the resting condition and may also increase the numbers of NBM cholinergic neurons activated by NBM stimulation. Enhanced positive feedback of acetylcholine release by increased presynaptic nicotinic receptors in response to sustained nicotine treatment may also be involved (Rowell and Winkler, 1984). However, we cannot exclude the possible involvement of intrinsic cortical cholinergic neurons as another acetylcholine origin of the increased basal acetylcholine release in the cerebral cortex in nicotine-treated rats (Herrera-Marschitz et al., 1990; Johnston et al., 1981).

Recently, prolonged exposure to nicotine was reported to enhance the trophic support of basal forebrain cholinergic neurons by increasing NGF content (Brown et al., 2006) and the number of NGF-like immunolabelled neurons (Martínez-Rodríguez et al., 2003) in the cortex, or increasing the levels of NGF receptors (TrkA) in basal forebrain cholinergic neurons (Formaggio et al., 2010). Continuous intracerebral administration of NGF increases potassium-evoked acetylcholine release from cerebral cortical synaptosomes of adult rats (Rylett et al., 1993). Therefore, the enhancement of trophic support to basal forebrain cholinergic neurons by sustained nicotine treatment may contribute to the enhanced responsiveness of acetylcholine release in the cerebral cortex by NBM stimulation demonstrated in the present results.

In the present study, increases in both cortical acetylcholine release and cortical blood flow induced by NBM stimulation were enhanced by sustained infusion of nicotine. The NBM stimulation-induced cortical vasodilation occurs by increasing acetylcholine release during the stimulation (Biesold et al., 1989). Enhanced reactivity of cerebral cortical vasodilation to NBM stimulation in nicotine-treated rats may be due to the enhanced responsiveness of cortical acetylcholine release to NBM stimulation by sustained nicotine treatments. In contrast, sustained infusion of nicotine increased the basal level of cortical acetylcholine release in the resting condition, but did not change the basal level of cortical blood flow as measured by laser Doppler flowmetry. Laser Doppler flowmetry is valid for recording the time course of instantaneous changes in local cerebral blood flow and evaluating relative changes in perfusion rather than absolute values (Stern, 1975). Further study is needed to compare the basal level of cortical blood flow between saline and nicotine treated rats, using other techniques, such as microspheres or autoradiographic techniques, that measure the absolute values of cerebral cortical blood flow.

An earlier report by Linville et al. (1993) showed that bolus intravenous administration of nicotine enhanced both the NBM stimulation-induced blood flow response and resting blood flow in the cerebral cortex of anesthetized rats, only for a short period of time (less than 15 min). Using a low dosage of nicotine (100 μg/kg/h) that is subthreshold for acute effect (Linville et al., 1993), the present study demonstrated that sustained (14 days) subcutaneous infusion of nicotine can produce the enhancement of the NBM stimulation-induced cortical vasodilation.

The cerebral cortical vasodilative response induced by NBM stimulation has been shown to involve activation of both nicotinic receptors and muscarinic receptors within the parenchyma in the cortex (Biesold et al., 1989). Bolus intravenous administration of a small dose of nicotine (3–30 μg/kg) increases cortical cerebral blood flow without affecting systemic blood pressure, via activation of nicotinic receptors in the brain (Uchida et al., 1997). Previously, we showed that sustained subcutaneous infusion of nicotine, administered using the same procedure employed here, reduced the bolus i.v. nicotine-induced increase in cortical blood flow (Uchida et al., 2009; Uchida and Hotta, 2009). Sustained subcutaneous infusion of nicotine did not affect the threshold dose of nicotine (3 μg/kg, i.v.) producing vasodilation, but reduced (about 40% decrease) the vasodilation induced by nicotine at 30 μg/kg (Uchida et al., 2009). In contrast, our present study showed that sustained subcutaneous infusion of nicotine decreases the threshold intensity of NBM stimulation for producing cerebral cortical vasodilation and increases the magnitudes of vasodilative response. Although both cortical vasodilative responses induced by the bolus i.v. nicotine and by NBM stimulation involve nicotinic receptor activation, these two vasodilative responses were modulated differently by sustained infusion of nicotine. The reason of this opposite modulatory effect of sustained nicotine exposure may be due to enhancement of NBM stimulation-induced cortical acetylcholine release, which operates cortical vasodilation. In addition, different pharmacological properties of nicotine and acetylcholine to neuronal nicotinic receptors may be involved (Eaton et al., 2004). Facilitation of brain muscarinic receptor function after repeated nicotine treatment (Wang and Sun, 2005), or an increase of nicotine-metabolizing enzyme in the brain after repeated nicotine treatment (Miksys et al., 2000) may also be involved.

Our present study suggests that sustained low-dose nicotine administration enhances physiological coupling between NBM cholinergic tone and cerebral blood flow responses in rats. The enhanced cholinergic coupling of cerebral blood flow could result in providing sufficient oxygen and glucose to the cerebral cortex in concert with neuronal activity, which appear to be beneficial for protection of the cortical neurons (Hotta et al., 2002) and may lead to an enhancement of cognitive function (Rezvani and Levin, 2001). Furthermore, sustained nicotine treatment may enhance the levels of cortical arousal, attentiveness, sensory processing and consciousness, etc., those are reported as the roles of NBM cholinergic neurons projecting to the cortex (Jones, 2004; Pepeu and Giovannini, 2006; Sarter et al., 2005) besides the cerebral cortical vasodilation system (Biesold et al., 1989; Sato and Sato, 1992). To add to the NBM cholinergic system, effects of sustained nicotine infusion on the activity of other central cholinergic neurons are also important future research subjects.

In conclusion, the results of the present study demonstrated for the first time that sustained subcutaneous nicotine treatment increased sensitivity in cortical acetylcholine release and cortical blood flow responses to electrical stimulation of NBM.

## Acknowledgements

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