Current Topics

Functional Changes Induced by Long-Term Stimulation of Nicotinic Acetylcholine Receptors

Cerebral Cortical Vasodilatation Mediated by Nicotinic Cholinergic Receptors: Effects of Old Age and of Chronic Nicotine Exposure

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Nicotinic acetylcholine receptors in the brain play a crucial role in the vasodilation in the cerebral cortex induced by basal forebrain cholinergic activation. Nicotinic receptors can be up-regulated or down-regulated in various conditions, *e.g.*, up-regulation (increase in number) is found in smokers and in rodents exposed to chronic nicotine, while down-regulation (decrease in number) is found in elderly humans and in aged rats. We found in anesthetized rats that the increase in cortical cerebral blood flow induced by bolus intravenous nicotine injection was due to a vasodilation mediated by nitric oxide following activation of nicotinic receptors, probably of $\alpha 4 \beta 2$ -like subtype, both in the basal forebrain nuclei (Meynert nucleus) and in the cortex. This vasodilative response was reduced by long-term nicotine treatment and also in aged rats. Our results show that functional changes in nicotinic receptors are not always in the same direction as the changes in number; they highlight the importance of investigating not only the changes in receptor numbers but also those in their functional activity.

Key words chronic nicotine; nicotinic acetylcholine receptor; cortical cerebral blood flow; vasodilation; aging

1. INTRODUCTION

The nucleus basalis of Meynert (Meynert nucleus) of the basal forebrain contains large cholinergic neurons whose projections reach all areas of the cortex. Degeneration of these neurons in Alzheimer's disease with cognitive deterioration¹⁾ points to the importance of this cholinergic system also for cognitive functions. The terminal axons of these neurons are distributed along cortical small blood vessels and on cortical neurons. Recently it has emerged that they dilate the cortical vessels, hence to increase cortical blood flow, by activating acetylcholine receptors, which are both of the nicotinic and the muscarinic type.^{2,3)} The exact location of these receptors is still under investigation; however, they are not present on the luminal side of the vessels, but within the nervous tissue itself, that is on the brain side of blood–brain barrier.

The muscarinic receptors in the brain are relatively stable, whereas nicotinic receptors can be up-regulated or down-regulated in various conditions. For example, in rodents, after chronic exposure to nicotine, there is up-regulation (increase in number) of the nicotinic acetylcholine receptors (nAChRs) but not of the muscarinic acetylcholine receptors (mAChRs) of the brain, especially in the cerebral cortex.^{4–6)} A similar increase in number of nicotinic receptors is found in the brains of human smokers.^{7,8)} In contrast, both in humans and in rodents there is a down-regulation (decrease in number) of nicotinic receptors in the cerebral cortex in old age.^{9,10)} In aged humans there is also a down-regulation of muscarinic receptors, but this is less marked than that of nicotinic receptors.¹⁰⁾

With our experiments we examined the effect that the up-regulation or down-regulation of nicotinic receptors may have on the cortical vasodilatation that is under the control of

the Meynert nucleus.^{11,12)} The functional activity of nicotinic receptors producing vasodilation was assessed in anesthetized rats by measuring the changes in cerebral cortical blood flow (CBF) after bolus intravenous injection of nicotine. Up-regulation of nicotinic receptors was obtained with chronic treatment with nicotine, while the effects of down-regulation were studied in aged rats. Here we review our results on (1) the mechanisms by which nicotine increases cortical cerebral blood flow; (2) their activity in rats chronically treated with nicotine; and (3) their activity in aged rats.

2. INCREASE IN CORTICAL CEREBRAL BLOOD FLOW BY NICOTINE

It is well established that an intravenous injection of nicotine produces a regional increase in cerebral blood flow, particularly in the cortex, independently of changes in systemic blood pressure.^{13,14)} We measured the blood flow in the frontal cortex by laser Doppler flowmetry in urethane-anesthetized artificially-ventilated rats, before and after intravenous bolus injection of nicotine. Nicotine at doses of 3–30 $\mu\text{g/kg}$ produced an increase in cortical blood flow, without significant changes in mean arterial pressure. The increase in cortical blood flow by 30 $\mu\text{g/kg}$ of nicotine usually started within 30–40 s after the injection, reached a maximum ($71 \pm 16\%$) at 10 min, and gradually returned to the pre-injection level after more than 30 min (Figs. 1A, B). Nicotine at a dose of 300 $\mu\text{g/kg}$ increased cortical blood flow in parallel with a marked increase in arterial pressure. The rapid increase in cortical blood flow after the injection of 300 $\mu\text{g/kg}$ of nicotine appears to be due to a passive increase in cortical blood flow in response to the increased arterial pressure. These results show that with nicotine doses of 30 $\mu\text{g/kg}$ or less the increased cortical blood flow is not accompanied by

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Fig. 1. Responses of Cortical Blood Flow to Bolus i.v. Injection of Nicotine

(A) Sample recordings of cortical blood flow (CBF) and mean arterial pressure (MAP). (B) Summary of cortical blood flow response (mean $\pm$ S.E.M.). Changes in cortical blood flow were calculated every 2–5 min. (C) Summary of cortical blood flow responses. (a) Control, (b) after C6 (20 mg/kg, i.v.), (c) after MEC (2 mg/kg, i.v.), (d) after bilateral destruction of the Meynert nucleus (NBM lesion), (e) after ATR (0.5 mg/kg, i.v.), (f) after L-NAME (30 mg/kg, i.v.). Peak responses of CBF were measured during the 5–10 min after the nicotine injection. * $p < 0.05$, ** $p < 0.01$; significantly different from preinjection basal values. ^a $p < 0.05$; ^b $p < 0.01$; significantly different from respective control responses. Modified from Uchida *et al.*^{11,39}

changes in systemic blood pressure, and we conclude, therefore, that it is due to active dilatation of the cortical vessels. Nicotine at the same dosages had a similar effect in the parietal cortex of our animals. In the hippocampus and the olfactory bulb there were similar responses, except at the low dosages of nicotine, which were ineffective, indicating that the threshold for the vascular response to nicotine is higher in these regions than in the neocortex.^{15,16}

The nicotine-induced increase in cortical blood flow is suppressed by the application of nicotinic receptor antagonists, when these are able to cross the blood–brain barrier. Intravenous administration of the nicotinic receptor antagonist hexamethonium (C6, 20 mg/kg), which does not cross the blood–brain barrier, has no effect, whereas mecamylamine (MEC, 2 mg/kg intravenously (i.v.)), which can cross the blood–brain barrier, inhibits the nicotine-induced increase in blood flow (Figs. 1Cb, c). The data indicate that nicotine acts on nicotinic receptors in the brain tissues to produce the cortical vascular response that is independent of the mean arterial pressure.

The nicotine-induced increase in cortical blood flow is due to increased excitation of cholinergic neurons in the Meynert nucleus, by activation of their nicotinic receptors, and it has been suggested that is not due to a possible activation of the nicotinic receptors located in the cortex itself.¹⁴ In rats subjected to bilateral destruction of the Meynert nucleus (by electro-coagulation), we too observed a marked increase in

cerebral blood flow; however, we also found a 50% reduction of the vascular response to nicotine administration (Fig. 1Cd). This observation indicates that nicotine-induced cortical vasodilation is due not only to activation of nicotinic receptors in the Meynert nucleus, as proposed by Linville *et al.*,¹⁴ but also to activation of nicotinic receptors in the cortex itself.

In our experiments, the extracellular concentration of acetylcholine (ACh) in the cerebral cortex of rats was increased following i.v. injection of nicotine at a dose of 30 μ g/kg. This result is in agreement both with a nicotine-induced activation of the neurons of the Meynert nucleus, and with the finding that nicotine induces ACh release from the cerebral cortex in anesthetized cats¹⁷ and in conscious rats,¹⁸ and also from synaptosomes obtained from the rat cerebral cortex.^{19,20}

The nicotine-induced ACh release in the cortex may produce cortical vasodilation, by activation not only of the nicotinic, but also of the muscarinic receptors. However, the nicotine-induced increase in cortical blood flow is not affected by i.v. injection of the muscarinic receptor antagonist (atropine: ATR, 0.5 mg/kg), in spite of the fact that this antagonist can cross the blood–brain barrier (Fig. 1Ce). This observation suggests that nicotinic receptors may play a greater role than the muscarinic receptors in the production of the nicotine-induced cortical vasodilation that we have observed.

Contribution of Nitric Oxide (NO) The nicotine-induced increase in cortical blood flow was partly but significantly inhibited by i.v. administration of a nitric oxide synthase (NOS) inhibitor, L-N^G-nitroarginine methylester (L-NAME) (Fig. 1Cf). The effect of the NOS inhibitor was counteracted with additional i.v. administration of 300 mg/kg of L-arginine, a physiological precursor of NO.

This evidence indicates that NO is a necessary element in the response of cortical blood flow to nicotine administration. Adachi *et al.*²¹ have already described that the increase in cortical blood flow induced by stimulation of the Meynert nucleus is inhibited by L-NAME, and they suggested that NO is a transmitter in the pathway between the cholinergic neurons of the Meynert nucleus and the cortical vessels. Cortical neurons that are NOS-immunoreactive (and presumably release NO) are detected in the proximity of blood vessels in the rat cerebral cortex,^{22,23} and some of these neurons receive cholinergic synaptic terminals that originate from the Meynert nucleus,²³ and have nicotinic receptors.^{24,25} On the basis of these data, we suggest that intravenously-injected nicotine activates nicotinic receptors on NOS-positive neurons in the cerebral cortex, and that NO released from these neurons produces vasodilation, resulting in an increase in local blood flow (Fig. 5A). Another possibility is that nicotine activates NOS-positive glial cells or endothelial cells, and that vasodilation is produced by NO released from these cells (Fig. 5A). In fact, some glial cells in the cortex are NOS positive,²⁶ and some astrocytes near cortical microvessels receive cholinergic terminals.^{27,28} Cerebral microvascular endothelial cells have nicotinic receptors.^{29,30} However, it is still uncertain whether the NOS positive glial cells have nicotinic receptors and whether the endothelial nicotinic receptors locate on the abluminal surface, *i.e.*, beyond the blood–brain barrier.

Subtypes of Nicotinic Receptors The $\alpha 4\beta 2$ and the

Fig. 2. Effect of DHβE and MLA on the Nicotine-Induced Response of Cortical Blood Flow

(A) DHβE: $\alpha 4\beta 2$ -like nicotinic receptor antagonist, 1.5–4 mg/kg, i.v. (B) MLA: $\alpha 7$ nicotinic receptor antagonist, 2 mg/kg, i.v. Responses of CBF were calculated 10 min after the nicotine injection (30 μ g/kg). ** $p < 0.01$; significantly different from preinjection basal values. # $p < 0.01$; significantly different from control response. From Uchida *et al.*¹²⁾

$\alpha 7$ subtypes are the most abundant and widespread in the mammalian brain, including cerebral cortex and Meynert nucleus.^{31–33)} The increase in cortical blood flow induced by nicotine was not influenced by methyllycaconitine (MLA), an $\alpha 7$ -selective nicotinic antagonist, while it was completely abolished by dihydro- β -erythroidine (DHβE), an $\alpha 4\beta 2$ -preferring nicotinic antagonist (Figs. 2A, B). These results suggest that activation of $\alpha 4\beta 2$ -like nicotinic receptors but not of $\alpha 7$ nicotinic receptors in the Meynert nucleus and the cerebral cortex is responsible for the nicotine-induced cortical vasodilation (Fig. 5A). In contrast, the nicotine-induced neurogenic vasodilation observed in the isolated basilar artery of the pig is mediated by nicotinic receptors of the $\alpha 7$ subtype.³⁴⁾ It appears that different subtypes of nicotinic receptors operate in the brain tissue and in the large cranial vessels.

3. CHRONIC ADMINISTRATION OF NICOTINE AND THE NICOTINE-INDUCED INCREASE IN CORTICAL BLOOD FLOW

We examined whether the nicotine-induced cerebral cortical vasodilation is affected by chronic subcutaneous nicotine treatment. The threshold dose of nicotine (3 μ g/kg, i.v.) producing vasodilation was not affected by chronic nicotine treatment (Fig. 3B). However, the vasodilation induced by nicotine at 30 μ g/kg was reduced after long-term (14 d) nicotine treatment (but not after short-term (1 h) exposure). The degree of reduction was marked and was statistically significant with high-dose (100 μ g/kg/h) nicotine; low-dose (33 μ g/kg/h) nicotine had a small effect that was not statistically significant (Fig. 3C). In contrast, the vasodilation in the cortical vessels examined during hypercapnia stimulation (inhalation of 10% CO₂) was not changed by chronic subcutaneous nicotine treatment.

These results indicate that long-term nicotine treatment reduces signal transmission through the nicotinic receptors that mediates cortical vasodilation, and the reduction depends on both the dose of chronic nicotine and the duration of treatment (Fig. 5B). Up-regulation (increase in number) of nicotinic receptors by chronic nicotine treatment depends on both the dose of nicotine⁵⁾ and the duration of treatment.³⁵⁾ Although the mechanism of the reduction of nicotinic recep-

Fig. 3. Effect of Chronic Nicotine Treatment on the Nicotine (i.v.)-Induced Response of Cortical Blood Flow

Peak responses of CBF were measured during the 2–10 min after the nicotine injection. * $p < 0.05$; ** $p < 0.01$; significantly different from pre-injection basal values. # $p < 0.05$; significantly different from the response in saline treated group. From Uchida *et al.*¹²⁾

tor-mediating cortical vasodilation by long-term nicotine treatment is not clear, nicotinic receptor desensitization and subsequent up-regulation might be involved in this process, as suggested previously.³⁶⁾ We need further studies to clarify whether the reduction of nicotine-induced cerebral cortical vasodilation after long-term nicotine treatment is due to desensitization of the nicotinic receptor in the Meynert nucleus or in the cerebral cortex.

The nicotine-induced increase in cortical blood flow is mediated by $\alpha 4\beta 2$ -like nicotinic receptors and not by $\alpha 7$ nicotinic receptors. Therefore, we can assume that the reduction of the acute effect of nicotine is due to the decreased functional activity of $\alpha 4\beta 2$ -like nicotinic receptors. Our observation that the long-term nicotine-treatment decreases the functional activity of $\beta 2$ -subunit-containing nicotinic receptors, agrees the previous studies by Grilli *et al.*³⁷⁾ and Besson *et al.*³⁸⁾ Grilli *et al.*³⁷⁾ reported that long-term nicotine treatment decreased nicotine-evoked release of ACh from rat hippocampal synaptosomes, and that the processes involve $\alpha 4\beta 2$ subtype nicotinic receptors. Besson *et al.*³⁸⁾ demonstrated that long-term nicotine treatment decreases the functional activity of $\beta 2$ -subunit-containing nicotinic receptors but increases the functional activity of $\alpha 7$ -containing nicotinic receptors, in the dopaminergic system of ventral tegmental area in mice.

4. EFFECT OF AGING ON THE NICOTINE-INDUCED INCREASE IN CORTICAL BLOOD FLOW

The data discussed above on the effect of nicotine on cortical blood flow were obtained from adult rats aged 3–10 months. The investigation was then extended to animals of older age. In rats of 23–26 months, a bolus injection of 30 μ g/kg of nicotine increased cortical blood flow by a very similar extent as in the younger rats. The lower dose of nicotine (3 μ g/kg), however, was ineffective. In other words, the intensity of the response to nicotine was unchanged in old rats, but the threshold was higher. In contrast, in 32–36 month old rats, nicotine had no significant effect on the cortical blood flow at the doses tested (Figs. 4, 5). This fall in the cortical blood flow response in the very old animals is probably due to a decline in the number of nicotinic receptors in

Fig. 4. Effect of Aging on the Nicotine (i.v.)-Induced Response of Cortical Blood Flow

Peak responses of CBF were measured during the 2–10 min after the nicotine injection. * $p < 0.05$; ** $p < 0.01$; significantly different from pre-injection basal values. From Uchida *et al.*¹¹⁾

Fig. 5. Diagram of the Mechanism of Nicotine-Induced Cerebral Cortical Vasodilation (A) and Its Changes by Chronic Nicotine Treatment (B) and by Aging (C)

the cortex, as has been observed in humans.^{9,10)}

Because the nicotinic receptors in the cerebral cortex decline with age, there may be a beneficial effect in activating the nicotinic receptors involved in cortical vasodilation by administering nicotinic receptor agonists in old subjects; this intervention would support or enhance to cholinergic vasodilative mechanisms. However, while it is known that chronic nicotine treatment up-regulates cortical nicotinic receptors,^{4–6)} we have shown that the nicotine treatment also reduces the functional activity of the nicotinic receptors that are involved in cortical vasodilation. These effects remain difficult to explain, but it is known that there can be a discrepancy between changes in number of nicotinic receptors and changes in nicotinic cholinergic activity. It will be important not only to extend the present data on up-regulation and the down-regulation of nicotinic receptors in the cortex, but also to investigate the changes in their functional activity.

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