α7-Nicotinic Acetylcholine Receptors on Cerebral Perivascular Sympathetic Nerves Mediate Choline-Induced Nitrergic Neurogenic Vasodilation

Min-Liang Si, Tony J.F. Lee

Abstract—It has been suggested in isolated porcine cerebral arteries that stimulation by nicotine of α7-nicotinic acetylcholine receptors (α7-nAChRs) on sympathetic nerves, but not direct stimulation of parasympathetic nitrergic nerves, caused nitrergic neurogenic dilation. Direct evidence supporting this hypothesis has not been presented. The present study, which used in vitro tissue bath and confocal microscopy techniques, was designed to determine whether choline, a selective agonist for α7-nAChRs, induced sympathetic-dependent nitrergic dilation of porcine basilar arterial rings. Choline and several nAChR agonists induced exclusive relaxation of basilar arterial rings without endothelium. The relaxation was blocked by tetrodotoxin, nitro-L-arginine, guanethidine, and β2-adrenoceptor antagonists. Furthermore, the relaxation was blocked by methyllycaconitine and α-bungarotoxin (preferential α7-nAChR antagonists) and mecamylamine but was not affected by dihydro-β-erythroidine (a preferential α5-nAChR antagonist). Confocal microscopic study demonstrated that choline and nicotine induced significant calcium influx in cultured porcine superior cervical ganglionic cells but failed to affect calcium influx in cultured sphenopalatine ganglionic cells, providing direct evidence that choline and nicotine did not act directly on the parasympathetic nitrergic neurons. The increased calcium influx in superior cervical ganglionic cells was attenuated by α-bungarotoxin and methyllycaconitine but not by dihydro-β-erythroidine. These results support our hypothesis that activation of α7-nAChRs on cerebral perivascular sympathetic nerves causes calcium influx and the release of norepinephrine, which then act on presynaptic β2-adrenoceptors located on the neighboring nitrergic nerve terminals, resulting in NO release and vasodilation. Endogenous choline may play an important role in regulating cerebral sympathetic activity and vascular tone. (Circ Res. 2002;91:62-69.)

Key Words: choline ▪ α7-nicotinic acetylcholine receptors ▪ cerebral neurogenic vasodilation ▪ nitric oxide ▪ sympathetic-parasympathetic interaction

The α-bungarotoxin (α-BGTX)-sensitive nicotinic acetylcholine receptor (nAChR), which is one of the predominant nAChR subtypes in the brain, consists of the α7-subunit, possesses calcium permeability, and exhibits rapid desensitization.1 Choline, which is a precursor of acetylcholine (ACh) synthesis and a product of ACh hydrolysis by acetylcholinesterase, has been shown to act as a relatively selective agonist for α7-nAChRs in the central nervous system.2,3 By activating presynaptic α7-nAChRs, choline has been shown to elicit the release of neurotransmitters, including norepinephrine (NE).2,4 The presence of nAChRs on sympathetic adrenergic nerve terminals is also well established.5,6 However, the functional significance of choline and α7-nAChRs in the perivascular neurons has not been clarified.

We reported recently that nicotine-induced NO-mediated neurogenic vasodilation in porcine basilar arteries and feline middle cerebral arteries was dependent on intact perivascular sympathetic adrenergic innervation.7–9 Results from pharmacological studies using nicotinic receptor antagonists further suggest that nicotine acts on α7-nAChRs located on presynaptic sympathetic nerve terminals to release NE, which then acts on presynaptic β2-adrenoceptors located on the neighboring nitrergic nerve terminals, resulting in the release of NO and dilation of porcine basilar arteries. These results do not support the idea that nicotine acts directly on nitrergic nerves to induce nitrergic vasodilation.7–9 However, direct evidence supporting this “indirect” nitrergic neurogenic vasodilation mediated by α7-nAChRs located on presynaptic sympathetic neurons has not been presented.

Nicotine is a nonspecific nAChR agonist.1,2 To further confirm that nicotine-induced cerebral neurogenic vasodilation is mainly mediated initially through sympathetic presynaptic α7-nAChRs, we used choline (a selective α7-nAChR agonist)6 and, for comparison, several nonspecific nAChR agonists, namely, epibatidine, 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP), and cytisine,10,11 to determine whether these nicotinic agonists induced an NO-
mediated relaxation in porcine basilar arteries and whether the relaxation was blocked by guanethidine (a sympathetic neuronal blocker) and antagonists of β2-adrenoceptors and α1-nAChRs. In addition, cultured porcine superior cervical ganglionic (SCG) cells (the origin of cerebral perivascular sympathetic nerves) and sphenopalatine ganglionic (SPG) cells (one of the major origins of cerebral perivascular parasympathetic nitrergic nerves) were used to determine whether nicotine and choline induced α1-nAChR-mediated calcium influx in the former but not the latter cells.

Materials and Methods

General Procedure

Fresh heads of adult pigs (60 to 100 kg) of either sex were collected at local packing companies (Excel and Y-T). The entire brain, with dura matter attached, was removed and placed in Krebs’ bicarbonate solution equilibrated with 95% O2/5% CO2 at room temperature. The composition of the Krebs’ solution was as follows (mmol/L): NaCl 122.0, KCl 5.16, CaCl2 1.2, MgSO4 1.22, NaHCO3 25.6, EDTA 0.03, l-ascorbic acid 0.1, and glucose 11.0 (pH 7.4). Basilar arteries were dissected under a dissecting microscope.7

In Vitro Tissue Bath Studies

An arterial ring segment (4 mm long) was cannulated with a stainless-steel rod and a short piece of platinum wire in a plastic tissue bath containing 6 mL Krebs’ bicarbonate solution. The stainless-steel rod was connected to a strain-gauge transducer for isometric recording of changes in force, as described in our previous report.7 The temperature of the Krebs’ solution was maintained at 37°C. Tissues were equilibrated in the Krebs’ solution for an initial 30 minutes and then mechanically stretched to a resting tension of 750 mg.7-9

The basilar arterial ring segments were then contracted with U-46619 (0.3 to 3 μmol/L) to induce an active muscle tone of 0.5 to 0.75-g transmural nerve stimulation (TNS) at 8 Hz, and concentrations of nicotinic agonists (0.1 to 1000 μmol/L) were applied to induce a relaxation. The concentrations that induce the maximum relaxation by most nicotinic agonists were used in subsequent experiments. After relaxation induced by TNS at 8 Hz and nicotinic agonists, the arteries were washed with prewarmed Krebs’ solution. A similar magnitude of active muscle tone was induced with U-46619 again, and TNS was repeated (to serve as a control for comparison with the relaxation elicited by TNS before the wash). Experimental drugs were then administered 30 minutes before repeating TNS and application of nicotinic agonists at the same concentration before the wash. To avoid the possible development of tachyphylaxis on repeated applications of nAChR agonists, at least 90 minutes with 6 washes (every 15 minutes) was allowed before the next application of nicotinic agonists.7-9

For TNS, tissues were electrically and transmurally stimulated with a pair of electrodes through which 100 biphasic square-wave pulses of 0.6 ms in duration and 200 mA in intensity (continuously monitored on a Tektronix oscilloscope) were applied at various frequencies.7-9 The neurogenic origin of this TNS-induced response was verified by its complete blockade by tetradotoxin (TTX, 0.3 μmol/L). The magnitude of a vasodilator response was expressed as a percentage of the maximum relaxation induced by 100 μmol/L papaverine, which was added at the end of each experiment.7-9 EC50 values (the concentration that produces 50% of the maximum relaxation) and IC50 values (the concentration that produces 50% inhibition of agonist-induced relaxation) were determined for each arterial ring. From these values, the geometric means E-50 and IC-50, with 95% CIs,13 were calculated.

Choline and Cerebral Neurogenic Vasodilation

The following drugs were used: (-)-nicotine, ACh, choline chloride, cytisine, DMPP, hemicholinium, atropine, methyllycaconitine (MLA), α-BGTX, mecamylamine, dihydro-β-erythroidine (DHβE), atenolol, guanethidine, propranolol, L-NNA, tetrodotoxin, and papaverine (all from Sigma). ICI 118,551 hydrochloride (Imperial Chemical), U46619 (Upjohn), and fluo 4-AM (Molecular Probes Inc). All drugs, otherwise stated, were dissolved in deionized water and added directly to the tissue baths. The drug concentrations reported were the final concentrations in the bath.

Results

The porcine basilar arteries without endothelial cells in the presence of active muscle tone induced by U-46619 (0.3 μmol/L) relaxed exclusively with TNS at 8 Hz and applications of choline (10 to 1000 μmol/L) in a concentration-dependent manner (Figures 1 and 2). Similar to choline and nicotine, several nAChR agonists, such as epibatidine, DMPP, and cytisine, induced dilation of porcine basilar arteries without endothelial cells (Figure 2 and online Table, which appears in the online data supplement available at http://
The rank order of potency of these agonists is epibatidine > DMPP > cytisine > nicotine > choline.

The relaxation of porcine basilar arteries induced by TNS, choline, and nAChR agonists was almost completely blocked by TTX (0.3 μmol/L, Figures 1A and 1C, n=4) and L-NNA (30 μmol/L, Figure 1D, n=4), was abolished by cold-storage denervation (n=6, data not shown). These results suggest that the relaxation induced by TNS, choline, and other nAChR agonists was due to the release of neurogenic NO. In parallel studies, ACh (up to 1 mmol/L) usually induced a constriction of arteries without endothelial cells (Figures 1A and 1B). Only in the presence of atropine (10 μmol/L) did ACh induce a small relaxation, which was blocked by MLA. On the other hand, choline-induced relaxation was not affected by atropine, which potentiated TNS-induced relaxation (Figure 1B).

Guanethidine, ICI 118,551, and Propranolol Blockade of Relaxation Induced by Choline and Other Nicotinic Agonists

Choline-induced relaxation in isolated porcine basilar arteries without endothelial cells was blocked by guanethidine, propranolol, and ICI 118,551 (a preferential β2-adrenoceptor antagonist) in a concentration-dependent manner. The IC50 values for guanethidine and ICI 118,551 were 6.98 (4.22 to 11.54) μmol/L and 10.9 (4.9 to 24.2) μmol/L, respectively (Figures 3A and 3B, n=5). However, the choline-induced relaxation was not affected by atenolol (a preferential β1-adrenoceptor antagonist) even at concentrations as high as 10 μmol/L (Figure 3A, n=6). Similarly, relaxation induced by cytisine, DMPP, and epibatidine was blocked by guanethidine, ICI 118,551, and propranolol but not atenolol (data not shown). The blockade was readily recovered after washing off these antagonists in the bath (Figure 3A, n=5 for each antagonist). However, these antagonists had no effect on TNS-induced vasodilation (Figure 3A).

Effects of Choline Uptake Inhibition on Choline-Induced Relaxation

Choline is known to be taken up via choline transporters on the synaptic membrane into nerve terminals for the synthesis of ACh. Preincubation with hemicholinium (10 μmol/L), a neuronal choline uptake inhibitor, potentiated relaxation induced by choline (1 mmol/L, n=8). However, hemicholinium...
had no significant effect on relaxation induced by TNS at 8 Hz or other nAChR agonists, such as nicotine and cytisine at the maximum concentrations examined (n=5; see online Figure, available at http://www.circresaha.org).

Blockade by α7-nAChR Antagonists of Neurogenic Vasodilation Induced by Choline and Other nAChR Agonists

Because TNS at 8 Hz and most nicotinic agonists at 100 μmol/L (except choline at 1 mmol/L and epibatidine at 10 μmol/L) induced maximum relaxation, these parameters were used in the subsequent studies. In the presence of active muscle tone induced by U-46619 in basilar arteries without endothelial cells, relaxation induced by choline was concentration-dependently blocked by selective α7-nAChR antagonists MLA and α-BGTX17 (Figure 4A), with the IC50 values of 5.16 (1.39 to 19.21)×10⁻⁷ mol/L and 5.15 (1.10 to 24.23)×10⁻⁸ mol/L, respectively (Figure 4B). Choline-induced relaxation was also blocked by mecamylamine (10 μmol/L, a nonspecific nAChR antagonist; Figure 4A). Blockade of choline-induced relaxation by these specific and nonspecific α7-nAChR antagonists was fully recovered after washing off these antagonists (Figure 4A). However, choline-induced relaxation was not appreciably affected by α4-nAChR antagonist DHβE (10 μmol/L, Figure 4A; n=6). Similar results were found; ie, these nAChR antagonists (MLA, α-BGTX, and mecamylamine but not DHβE) blocked relaxation induced by epibatidine, DMPP, and cytisine (Figure 5). MLA, α-BGTX, mecamylamine, and DHβE at the...
Choline- and Nicotine-Induced Calcium Influx in Cultured SCG and SPG Cells

We previously reported that cultured SCG cells, like cerebral perivascular sympathetic neurons, contain dense \( \alpha_7 \)-nAChRs.\(^9\) Because \( \alpha_7 \)-nAChRs form membrane cation channels that possess high calcium permeability,\(^1\) we used the intracellular calcium imaging indicator fluo 4-AM to determine whether the activation of \( \alpha_7 \)-nAChRs by choline and nicotine would induce calcium influx. As shown in Figure 6, both nicotine (100 \( \mu \text{mol/L} \)) and choline (1 mmol/L) induced a significant increase in calcium image in the SCG cells (1818 of 2108 cells in 10 plates from at least 8 animals, respectively). Quantitative analysis on single cells (Figure 7A) indicated that both nicotine (100 \( \mu \text{mol/L} \)) and choline (1 mmol/L) significantly increased calcium image in the SCG cells by 2.94±0.18-fold (n=10 cells from 10 different animals) and 3.32±0.45-fold (n=8 cells from 8 different animals), respectively. The addition of KCl (50 mmol/L) did not further increase intracellular calcium. The nicotine- and choline-induced calcium influx was drastically attenuated in cells pretreated with \( \alpha \)-BGTX (1 \( \mu \text{mol/L} \), Figure 6 [panel C3] and Figure 7B) and MLA (10 \( \mu \text{mol/L} \), Figure 7C), both of which alone did not affect calcium influx (Figure 6 [panel C2] and Figure 7B and 7C). In the presence of blockade of calcium influx by \( \alpha \)-BGTX and MLA, KCl (50 mmol/L) still induced significant calcium influx (Figure 6 [panel C4] and Figures 7B and 7C), which was comparable to that seen in preparations in the absence of antagonists (Figure 7A), suggesting the specificity of blockade by \( \alpha \)-BGTX and MLA. In parallel studies, \( \alpha_2 \)-nAChR antagonist DH\( \beta \)E (10 \( \mu \text{mol/L} \)) had no significant effect on nicotine-, choline-, or KCl-induced calcium influx in cultured SCG cells (Figure 7D).

In contrast to the calcium influx found in SCG cells, choline and nicotine in the same concentrations did not increase calcium influx in cultured SPG cells (1343 cells in 8 plates from at least 8 animals, Figure 7E). However, significant calcium influx was induced by the addition of KCl (50 mmol/L) in the same cells, which was comparable to that seen in the SCG cells.

Discussion

Our previous studies demonstrated that nicotine-induced NO-mediated neurogenic relaxation in porcine basilar arteries was dependent exclusively on intact sympathetic innervation.\(^7\) This conclusion was based on the findings indicating that after a complete blockade of sympathetic transmission with guanethidine or chemical denervation of sympathetic nerves with 6-hydroxydopamine, nicotine never induced a relaxation, whereas TNS-elicited NO-mediated relaxation in the same preparations remained unchanged.\(^7\) These results suggest that in porcine basilar arteries, nicotine does not act directly on nitrergic nerves to release transmitter NO. Rather, nicotine acts on the nicotinic receptors located on sympathetic nerves to release transmitter NE, which then diffuses to act on adrenoceptors located on the neighboring nitrergic nerves, causing release of NO from these nerves and relaxation of the smooth muscle.\(^7\) The role of NE as the mediator was supported by the findings that \( \beta_2 \)-adrenoceptors on the nitrergic nerves but not adrenoceptors on the smooth muscle mediated nicotine-induced relaxation.\(^8\) This functional axon-axonal or sympathetic-parasympathetic interaction is also supported by morphological evidence indicating that close apposition (25 nm) between the adrenergic nerve terminals and the nonadrenergic nerve terminals is a characteristic of innervation of cerebral arteries at the base of the brain in several species.\(^18\)–\(^21\)

Our findings, based on studies using nicotinic receptor antagonists, further suggested that \( \alpha_7 \)-nAChRs on sympathetic nerves were mediating nicotine-induced nitricergic neurogenic vasodilation in porcine basilar arteries.\(^9\) This was supported by the presence of \( \alpha_7 \)-nAChR immunoreactivities on both cerebral perivascular sympathetic nerves and SCG cells.\(^9\) This is further supported by results of the present study, which indicated that choline acts as a selective agonist for \( \alpha_7 \)-nAChR on sympathetic nerves in porcine basilar
arteries to induce NO-mediated neurogenic vasodilatation with a mechanism of action similar to that of nicotine. This conclusion was based on the findings that choline-induced relaxation was neurogenic, nitrergic, and sensitive to guanethidine, ICI 118,551, and propranolol (10 μmol/L) but not atenolol. Furthermore, the relaxation induced by choline was blocked specifically by preferential α7-nAChR antagonists. Similar results of the blockade of neurogenic vasodilation induced by various nAChR agonists in the presence of guanethidine, preferential β2-adrenoceptor antagonists, and preferential α7-AChR antagonists were obtained. Finally, both nicotine and choline induced significant calcium influx into sympathetic ganglionic neurons, and the influx was specifically blocked by preferential α7-nAChR antagonists.

The sympathetic-dependent nitrergic neurogenic vasodilation induced by choline and other nicotinic agonists was further supported by the fact that choline and nicotine did not elicit any calcium influx, whereas KCl did, in the parasympathetic SPG cells. This result provides convincing evidence supporting our hypothesis that choline and other nicotinic agonists do not act directly on the SPG cells to release NO. Rather, these agonists act initially on α7-nAChRs on sympathetic nerves, resulting in NE release and the aforementioned nitrergic vasodilation (Figure 8).

α7-nAChRs have been shown to belong to the evolutionarily oldest group of nAChRs.22 It is possible that choline rather than ACh was originally the natural transmitter for cholinergic receptors. It appears feasible that in the developed cerebral vascular system, choline rather than ACh remains to be the endogenous ligand for α7-nAChRs. In the present study in isolated porcine basilar arteries without endothelial cells, choline and other nAChR agonists consistently induced NO-mediated neurogenic vasodilation. In contrast, ACh

Figure 6. Effect of nicotine and choline on calcium influx in cultured porcine SCG cells. Cultured SCG cells were loaded with fluo 4-AM (3 μmol/L) in physiological buffer and incubated at room temperature for 30 minutes (panels A1, B1, and C1). Nicotine (100 μmol/L) or choline (1 mmol/L) followed by KCl (50 mmol/L) was applied to the medium, and the calcium image in the neuronal cells was examined. Both choline (panel A2) and nicotine (panel B2) induced a significant calcium influx. α-BGTX (1 μmol/L, panel C2), which did not change the basal intracellular calcium concentrations, blocked choline-elicited calcium influx (panel C3). In the presence of α-BGTX blockade of the choline effect, KCl (50 mmol/L) induced significant calcium influx in these cells (panel C4).

Figure 7. Summary of the effects of choline (1 mmol/L), nicotine (100 μmol/L), and KCl (50 mmol/L) on intracellular calcium concentration ([Ca2+]i) in cultured porcine SCG cells (A through D), as described in Figure 6, and SPG cells (E). A, Changes in intracellular calcium were compared with the basal control calcium concentration, which served as control (100%). *P<0.05 vs respective control. B and C, Effects of 1 μmol/L α-BGTX (B) and 10 μmol/L MLA (C) on choline-, nicotine-, and KCl-induced calcium influx. ⋅P<0.05 vs respective agonist. D, Failure of DHβE in blocking calcium influx induced by choline, nicotine, or KCl. ⋅P<0.05 vs blocker. DHβE alone did not increase calcium influx. E, Summary showing that neither choline nor nicotine significantly increased [Ca2+]i in the cultured porcine SPG cells. However, the addition of KCl significantly increased calcium influx in these cells. Values are mean±SEM; n indicates number of experiments.
**Figure 8.** Summary diagram showing close apposition of an adrenergic and a cholinergic-nitric nerve terminal35 in large cerebral arteries at the base of the porcine brain. The axonal distance between these 2 different nerve terminals is closer than that between the nerves and the smooth muscle. Choline, nicotinic agonists, and ACh act on presynaptic α7-nicotinic receptors located on the adrenergic nerve terminal, causing release of NE (+), which then acts on presynaptic β-adrenoceptors located on the adjacent cholinergic-nitric nerve terminal. This effect of NE results in stimulating NO release (+), which activates guanylate cyclase (GC), increases cGMP synthesis from GTP, and relaxes the smooth muscle. However, NE released from sympathetic adrenergic nerves is a weak postsynaptic transmitter (as indicated by a question mark). NO is not stored in vesicles and is synthesized from L-arginine (L-Arg) in the presence of NO synthase (NOS). L-Citrulline (L-Cit), the byproduct of NO synthesis, is actively converted to L-Arg.34 This L-Citrulline-L-Arg cycle provides evidence for the neuronal source of NO. Furthermore, NO is colocalized and coreleased with ACh, which is stored in neuronal vesicles. ACh released from cholinergic-neuronal vesicles acts on presynaptic muscarinic M2 receptors (M2), resulting in G-protein–mediated negative coupling (−) to the N-type calcium channels, which leads to suppression of calcium influx through this type of calcium channel,34 a decreased NOS activity accompanied by a decrease in NO formation and release, and diminished relaxation of the smooth muscle cell. ACh, like NE, is a very weak or negligible postsynaptic transmitter, inasmuch as these 2 classic transmitter substances released on TNS do not directly affect the smooth muscle cell tone on the basis of animal studies.6,8

never induced a relaxation but did induce a small contraction in these arterial preparations. This lack of effect of ACh in inducing NO-mediated neurotransmitter release is consistent with previous findings that ACh attenuates TNS-induced nitricergic relaxation of porcine cerebral arteries by acting on presynaptic muscarinic M2 receptors on nitricergic nerves, which are negatively coupled to the neuronal calcium influx via N-type calcium channels, resulting in decreased release of its cotransmitter NO12,23 (Figure 8).

The contraction induced by ACh is most likely because of its direct effect on muscarinic receptors on the smooth muscle cells, resulting in calcium influx via L-type calcium channels.24 However, in the presence of atropine, ACh-induced contraction was converted to a relatively small relaxation compared with that induced by choline in the same preparations (Figure 1B). The residual relaxation was blocked by MLA, suggesting that ACh also acted on α7-nAChRs. In addition, desensitization to choline-induced relaxation occurred after pretreatment of ACh, suggesting that ACh acts on nicotinic receptors with significantly less efficacy than does choline. In contrast, choline does not seem to bind the muscarinic receptors, inasmuch as choline-induced nitricergic vasodilation was not significantly affected by atropine, which nevertheless potentiated TNS-induced relaxation (a result similar to our previous findings25). These results suggest that choline is more effective than ACh as an endogenous ligand for α7-nAChRs on the sympathetic nerves of SCG origin.

The present study demonstrates that α7-nAChR selective and nonselective agonists induce relaxation in porcine basilar arteries with different potency and efficacy. Epibatidine, a well-described nAChR agonist,25 is the most potent agonist, with the highest efficacy among the agonists examined. Although choline is a full α7-nAChR agonist, a relatively high concentration (high EC50) is needed to induce a relaxation. However, this concentration is comparable to the concentration of choline needed to induce neurotransmitter release in the central nervous system.26

The possible physiological significance of choline is further supported by the finding of the present study, ie, that NO-mediated neurogenic vasodilation induced by choline but not nicotine or cytisine is potentiated by hemicholinium (a choline uptake blocker). Furthermore, choline is present in significant concentration in the cerebral spinal fluid27 and blood serum.28 Accordingly, activation of α7-nAChRs by endogenous choline may play an important role in regulating cerebral sympathetic activity and vascular tone.

The sympathetic-dependent neurogenic vasodilation induced by nAChR agonists has also been demonstrated in the mesenteric vascular beds,29 although the specific subtype of nAChRs in this vascular bed is not determined. Furthermore, acute cigarette smoking has been shown to cause pulmonary vasodilation in pigs30 and to cause increased cerebral blood flow in humans.31,32 Because nicotine is a major constituent in the cigarette and because NE is a vasoconstrictor, it is very likely that these vasodilatory effects of cigarette smoking are due to the initial release of NE, which then diffuses to the neighboring nerves to release potent dilator transmitters from these nerves. Together, it is very likely that activation of sympathetic nerves will lead to activation of parasympathetic nerves. Surprisingly, the subtype(s) of nicotinic receptors found on the sympathetic nerves in peripheral vasculatures is practically unavailable; however, α7-nAChRs on sympathetic nerves in the rat stomach22 have been shown to be involved in regulating nicotine-induced NE release. It is possible that variations in the functional subunits of nAChRs on adrenergic neurons in regulating NE release in different vascular beds may exist. Because choline is an endogenous ligand for α7-nAChRs, identification of the nAChR subtype becomes critical for a complete understanding of the sympathetic control of systemic circulation.

In conclusion, the present study demonstrates for the first time that choline and all nAChR agonists examined do not act directly on the nitricergic nerves but bind α7-nAChRs on sympathetic nerve terminals, causing subsequent nitricergic vasodilation in porcine basilar arteries. This is consistent with our hypothesis that activation of α7-nAChRs on cerebral...
perivascular sympathetic nerves results in calcium influx and release of NE. The released NE then diffuses to act on \(\beta\)-adrenoceptors located on the neighboring nitricergic nerves, causing release of NO and, therefore, vasodilation (Figure 8). The endogenous choline may play an important role in regulating cerebral perivascular sympathetic activity and, therefore, the cerebral circulation.

**Acknowledgments**

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**References**

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online fig 1

The diagram shows the percentage of ppv-induced relaxation in response to various treatments. The treatments include TNS, 8H2, Choline, 1mM, Cysteine, 100μM, and Nicotine, 100μM. The data is presented for Control and Hemicholinium, 10μM conditions. The y-axis represents the percentage of ppv-induced relaxation, while the x-axis lists the different treatments. The diagram indicates a significant difference between the control and the treatment with Hemicholinium, 10μM.
Online supplement data

Table 1. The EC\textsubscript{50} and E\textsubscript{max} of relaxation\textsuperscript{*} of porcine basilar arteries\textsuperscript{\gamma} induced by different nAChR agonists

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<th>Epibatidine</th>
<th>Nicotine</th>
<th>Cytisine</th>
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<th>Choline</th>
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<td>EC\textsubscript{50} (\mu M)</td>
<td>0.405 (0.208-0.788)</td>
<td>7.80 (5.51-11.04)</td>
<td>5.94 (2.97-11.89)</td>
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<td>E\textsubscript{max}</td>
<td>56.10±5.85</td>
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<td>26.90±1.50</td>
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\* determined as percent of papaverine-induced maximum relaxation; n = number of experiments.

\gamma denuded of endothelium

Figure 1. Effect of choline uptake inhibitor hemicholinium on choline-induced relaxation, estimated as percent of papaverine (PPV)-induced maximum relaxation, in porcine basilar arteries denuded of endothelium. Number in parenthesis indicates number of experiments. Values are means ± SEM. *p<0.05 indicates significant difference from the respective controls