Presynaptic α7-Nicotinic Acetylcholine Receptors Mediate Nicotine-Induced Nitric Oxidergic Neurogenic Vasodilation in Porcine Basilar Arteries

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ABSTRACT
We previously reported that nicotine-induced nitric oxide (NO)-mediated neurogenic vasodilation in the porcine basilar artery was dependent on intact sympathetic innervation. We further demonstrated in this artery that nicotine acted on nicotinic acetylcholine receptors (nAChRs) on presynaptic sympathetic nerve terminals to release norepinephrine (NE), which then acted on β2-adrenoceptors located on the neighboring NOergic nerve terminals to release NO, resulting in vasodilation. The nature of the nAChRs has not been determined. The nAChR subtype mediating nicotine-induced dilation in isolated porcine basilar arterial rings denuded of endothelium was therefore examined pharmacologically and immunohistochemically. Results from using an in vitro tissue bath technique indicated that relaxation induced by nicotine (100 μM) was blocked by preferential α7-nAChR antagonists (methyllycaconitine and α-bungarotoxin) and nonspecific nAChR antagonist (meacamylamine) in a concentration-dependent manner, but was not affected by dihydro-β-erythroidine (a preferential α2-nAChR antagonist). These nAChR antagonists did not affect relaxation elicited by transmural nerve stimulation (8 Hz) or that by sodium nitroprusside and NE. Results from double-labeling immunohistochemical studies in whole-mount porcine basilar and middle cerebral arteries and in cultured porcine superior cervical ganglia (SCG) indicated that α7-nAChR and tyrosine hydroxylase immunoreactivities were colocalized in same nerve fibers. These results suggest the presence of functional α7-nAChRs on postganglionic sympathetic adrenergic nerve terminals of SCG origin, which mediate nicotine-induced neurogenic NOergic vasodilation. These findings are consistent with our hypothesis that nicotine acts on nAChRs on presynaptic sympathetic nerve terminals to release NE, which then acts on presynaptic β2-adrenoceptors located on the neighboring NOergic nerve terminals, resulting in release of NO and dilation of porcine basilar arteries.

Molecular biological studies have demonstrated that nAChRs in the central nervous system are composed of a diverse array of subunits (α2-α4, β2-β4) and have a range of pharmacological properties. This has led to increased interest in presynaptic nAChRs that act to modulate the release of transmitter from presynaptic terminals (McGehee and Role, 1995; Lindstrom et al., 1995; Wonnacott, 1997; Kaiser and Wonnacott, 2000). It has long been known that sympathetic neurons have presynaptic nAChRs on their nerve terminals in target tissues, where nicotinic agonists cause the release of NE (Su and Bevan, 1970; Starke, 1977; Haass et al., 1991). In contrast to the central nervous system, the biological significance and subtype of these presynaptic nAChRs in the peripheral nervous system are less defined (Kristufek et al., 1999).

We have demonstrated that nicotine-induced nitric oxide (NO)-mediated neurogenic vasodilation is dependent on intact sympathetic, adrenergic innervation in porcine basilar arteries and cat middle cerebral arteries (Zhang et al., 1998; Lee et al., 2000). This is based on the observations that nicotine-induced NO-mediated cerebral neurogenic vasodilation is abolished by guanethidine, a specific sympathetic neuronal blocker, and by chemical denervation of sympathetic nerves with 6-hydroxydopamine. These treatments, however, do not affect transmural nerve stimulation (TNS)-elicited NO-mediated neurogenic vasodilation in the same preparations. Furthermore, relaxation induced by exogenous NE in porcine basilar arterial rings was blocked by nitro-L-arginine (L-NNA) (Zhang et al., 1998). Accordingly, it was hypothesized that nicotine acted on nicotinic receptors located on sympathetic nerve terminals, resulting in release of NE, which then diffused to act on β2-adrenoceptors located on the neighboring NOergic nerve terminals to release NO and therefore vasodilation (Zhang et al., 1998; Lee et al., 2000).

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; NE, norepinephrine; NO, nitric oxide; TNS, transmural nerve stimulation; L-NNA, N-nitro-L-arginine; SCG, superior cervical ganglion; TTX, tetrodotoxin; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate; MLA, methyllycaconitine; α-BGTX, α-bungarotoxin; DHβE, dihydro-β-erythroidine.
The exact nature of the nAChR receptors located on pre-synaptic sympathetic nerves mediating release of NE, which then elicits NO release, in cerebral arteries has not been clarified. The present study, therefore, was designed to pharmacologically and immunohistochemically characterize in isolated porcine basilar arteries and cultured superior cervical ganglia (SCG), the origin of cerebrovascular sympathetic nerves (Lee, 1981), the pre-synaptic nAChR subtype located on postganglionic sympathetic nerves, which mediate nicotine-induced NOergic vasodilation.

Materials and Methods

General Procedure. Fresh heads of adult pigs (60–100 kg) of either sex were collected at local packing companies (Excel, Beardstown, IL, and Y.T., Springfield, IL). The entire brain, with dura matter attached, was removed and placed in Krebs' bicarbonate solution equilibrated with 95% O₂ and 5% CO₂ at room temperature. The composition of the Krebs' solution was as follows: 122.0 mM NaCl, 5.16 mM KCl, 1.2 mM CaCl₂, 1.22 mM MgSO₄, 25.6 mM NaHCO₃, 0.03 mM ethylenediamine-tetraacetic acid, 0.1 mM L-ascorbic acid, and 11.0 mM glucose, pH 7.4. Basilar and middle cerebral arteries were dissected and cleaned off surrounding tissue under a dissecting microscope.

In Vitro Tissue Bath Studies. The ring segment (4 mm in length) was cannulated with a stainless steel rod (30-gauge hemispherical section) and a short piece of platinum wire and mounted horizontally in a plastic tissue bath containing 6 ml of Krebs' bicarbonate solution. The platinum wire was bent into a U shape and anchored to a gate. The stainless steel rod was connected to a strain gauge (UC2; Gould, Cleveland, OH) for isometric recording of changes in force, as described in our previous report (Lee et al., 1976). The temperature of the Krebs' solution equilibrated with 95% O₂ and 5% CO₂ was maintained at 37°C. Tissues were equilibrated in the Krebs' solution for an initial 30 min and then mechanically stretched to a resting tension of 750 mg (Zhang et al., 1998).

The basal arterial ring segments were then precontracted with U-46619 (0.3–3 μM) to induce an active muscle tone of 0.5 to 0.75 g. TNS at 2, 4, and 8 Hz and concentrations of nicotine (1–100 μM) were applied to induce a relaxation. Since the maximum relaxation induced by TNS was at 8 Hz, and that by nicotine at 100 μM, only TNS at 8 Hz and 100 μM nicotine were examined in some experiments. After relaxation induced by TNS at various frequencies and 100 μM nicotine, the arteries were washed with prewarmed Krebs' solution. A similar magnitude of active muscle tone was induced with U-46619 again, and TNS at various frequencies was repeated (to serve as a control compared with the relaxation elicited by TNS prior to the wash). Effects of experimental drugs were then administered, and TNS and nicotine at the same concentration prior to the wash were repeated. To avoid possible development of tachyphylaxis upon repeated applications of nicotine (Zhang et al., 1998), at least 90 min with six washes (every 15 min) was allowed before the next application of nicotine (Zhang et al., 1998; Lee et al., 2000). Experimental drugs were added at least 30 min before TNS application of nicotine. After this, the arteries were washed with prewarmed Krebs' solution again. A similar magnitude of active muscle tone was induced with U-46619 again, and TNS at various frequencies and nicotine at 100 μM were repeated (to serve as a second control compared with the relaxation elicited by TNS and nicotine prior to the drug application).

For TNS, tissues were electrically, transmurally stimulated with a pair of electrodes through which 100 biphasic square-wave pulses of 0.6-ms duration and 200 mA in intensity were applied at various frequencies (Zhang et al., 1998). Stimulation parameters were continuously monitored on a Tektronix oscilloscope. The neurogenic origin of this TNS-induced relaxation response was verified by its complete blockade by tetrodotoxin (TTX) (0.2 μM). At the end of each experiment, papaverine (100 μM) was added to induce a maximum relaxation. The magnitude of a vasodilator response was expressed as a percentage of the maximum response induced by papaverine (Zhang et al., 1998).

For examining effects of experimental drugs on relaxation induced by sodium nitroprusside and NE, concentration-response relationships for sodium nitroprusside and NE were obtained by a cumulative technique in arteries without endothelial cells in the presence of active muscle tone induced by U-46619. After the arterial rings were washed with prewarmed Krebs' solution, a similar magnitude of active muscle tone was again induced by U-46619. The experimental drugs were then added, and 15 min later, concentration-response relationships for sodium nitroprusside and NE were repeated. EC₅₀ values (the concentration that produces 50% of the maximum relaxation) were determined for each arterial ring. From these values, the geometric means EC₅₀, with 95% confidence intervals (Fleming et al., 1972) were calculated.

The endothelial cells of all arterial ring segments were mechanically removed by a standard brief gentle rubbing of the intimal surface with a stainless steel rod having a diameter (25–30-gauge) equivalent to the lumen of the arteries (Zhang et al., 1998; Lee et al., 2000). A complete removal of endothelial cells was verified by lack of effect of l-NAME in increasing basal tone (Zhang et al., 1998; Lee et al., 2000).

SCG Cell Culture. Freshly dissected SCG from animals were placed in cold Hibernate A (Life Technologies, Gaithersburg, MD) solution (Liu et al., 2000). After being cut into smaller pieces, the ganglia were transferred to Mg²⁺/Ca²⁺ free Hank's balanced salt solution containing papain (2 U/ml; Sigma, St. Louis, MO), collagenase D (1.2 mg/ml; Roche Molecular Biochemicals, Indianapolis, IN), and Dispase (4.5 mg/ml; Life Technologies), and were incubated for 50 min at 37°C. Cells were released by gentle tritutating at the end of the incubation. The cell suspension was centrifuged at 300g for 5 min. The pellet was gently resuspended in Neurobasal culture medium (Life Technologies), containing B27 (1:50 dilution; Life Technologies), 0.5 mM L-glutamine, 25 μM L-glutamate, and nerve growth factor (50 ng/ml; Alomone Lab Ltd, Jerusalem, Israel) (Brewer, 1997). All medium and Hank's balanced salt solution contained 100 U/ml penicillin and 100 U/ml streptomycin. The cell suspension was plated into a four-well culture plate with a poly(D-lysine)-coated (50 μg/ml; Sigma) glass coverslip (12 mm in diameter; Fisher Scientific, St. Louis, MO) in each well and incubated with air containing 5% CO₂ at 37°C. The growth medium was changed every 6 days.

Double-Labeling Immunohistochemistry. Fresh porcine brain arteries obtained from local slaughterhouses were dissected and placed into picric acid-periodate-paraformaldehyde-lysine (PPPL) fixative (Yu et al., 1988) overnight at 4°C. After five washes in PBS (pH 7.4), the arteries were permeabilized and nonspecific sites were blocked with 2.5% normal donkey serum in 0.25% Triton X-100 PBS for 30 min at room temperature. The arteries were incubated with primary antibodies (anti-rabbit α₁ nAChR antibody 1:40, Santa Cruz Biotechnology Inc. Santa Cruz, CA, and anti-mouse TH antibody 1:40, Chemicon International Inc., Temecula, CA) at 4°C for 24 to 48 h. After being rinsed with PBS, pH 8.2, three times, the arteries were incubated with the secondary antibodies for 1 h at room temperature. The secondary antibodies were fluorescein isothiocyanate (FITC)-conjugated donkey anti-rabbit IgG, and tetramethylrhodamine isothiocyanate (TRITC)-conjugated donkey anti-mouse IgG (1:40, Jackson Immunoresearch, West Grove, PA) (Liu et al., 2000). After being rinsed with PBS, pH 8.2, each artery was whole-mounted with Vectashield mounting medium on Vectabond coated slides (Vector Laboratories, Burlingame, CA). The labeled specimens were observed and photographed under a fluorescence microscope fitted with proper filters (Olympus BX50 microscope). Negative controls were obtained following the same procedure without the primary antibody (Liu et al., 2000).

For double-immunostaining of the SCG cells, the cells in culture for 7 to 10 days were removed and washed with PBS, pH 7.4, and...
fixed with PPPL fixative overnight at 4°C. The remaining procedures were the same as those described above for artery immunostaining. The SCG cells were also stained with anti-rabbit neurofilament 200 (Sigma) as a marker of neuronal cells (Liu et al., 2000).

**Drugs Used and Statistical Analysis.** The following drugs were used: (−)-nicotine, methyllycaconitine (MLA), α-bungarotoxin (α-BGTX), mecamylamine, dihydro-β-erythroidine (DHβE), L-NNA, NE, paraformaldehyde, sodium nitroprusside, TTX, papaverine (all from Sigma), Triton X-100 (Amersharm Pharmacia Biotech, Arlington Heights, IL) and U46619 (Upjohn, Kalamazoo, MI). All drugs, otherwise stated, were dissolved in deionized water, and added directly to the tissue baths. The drug concentrations reported were the final concentration in the bath.

Results were expressed as means ± S.E.M. Statistical analysis was evaluated by one-way analysis of variance, and Student's *t* test for paired or unpaired samples as appropriate. The *p* < 0.05 level of probability was accepted as significant.

**Results**

**Nicotine- and TNS-Induced Neurogenic Vasodilation in Porcine Basilar Arteries.** Consistent with our previous reports (Zhang et al., 1998; Lee et al., 2000), the porcine basilar arteries without endothelial cells, in the presence of active muscle tone induced by U-46619 (0.3 μM), relaxed exclusively upon TNS at various frequencies (2, 4, and 8 Hz), and applications of nicotine (1–100 μM) in a concentration-dependent manner (Fig. 1). The relaxation induced by nicotine was significantly blocked by TTX (0.3 μM, *n* = 7) and was abolished by L-NNA (30 μM, *n* = 6) and cold-storage denervation (*n* = 6, data not shown). These results suggest that the relaxation induced by TNS and nicotine was due to release of neurogenic NO.

**α7-nAChR Antagonists Blocked Nicotine-Induced Neurogenic Vasodilation.** Since TNS at 8 Hz and nicotine at 100 μM induced maximum relaxation, these parameters, which have previously been used by us and many others (Toda and Okamura, 1998; Zhang et al., 1998; Lee et al., 2000), were used in the subsequent studies. As reported previously by many investigators, neurogenic vasodilation induced by nicotine diminished upon repeated applications of this agonist with short time intervals (Zhang et al., 1998). Accordingly, in the present study, a 90-min interval with six washes was allowed before repeating each application of nicotine. Three consecutive, reproducible relaxations induced by nicotine diminished upon repeated applications of this agonist with short time intervals (Zhang et al., 1998). The blockade of MLA on nicotine-induced relaxation was concentration-dependent. The IC50 values were 8.63 (2.34–31.84) μM, and 9.48 (3.21–27.99) × 10−8 M, respectively. The rank order of potency was α-BGTX > mecamylamine = MLA. The nicotine-induced relaxation, however, was not appreciably affected by α7-nAChR antagonists DHβE (10 μM, *n* = 6, Fig. 4). MLA, α-BGTX, mecamylamine, and DHβE at concentrations used did not affect the TNS-elicted relaxation (Figs. 1–4), nor did these antagonists affect sodium nitroprusside- and NE-induced relaxation in porcine basilar arteries (data not shown).

**Fig. 1.** Effects of MLA on relaxation of porcine basilar arteries induced by nicotine and TNS. A, representative tracing showing effect of the preferential α7-nAChR antagonist MLA on relaxation elicited by nicotine (100 μM) and TNS at 2, 4, and 8 Hz in a basilar artery in the presence of active muscle tone induced by U-46619 (0.3 μM). MLA at 10 μM almost abolished the relaxation induced by nicotine, without affecting the relaxation elicited by TNS at various frequencies. The results are summarized in B. MLA blockade of nicotine-induced relaxation was fully recovered after the arteries were washed with fresh prewarmed Krebs’ solution (B, *n* = 6). The blockade of MLA on nicotine-induced relaxation was concentration-dependent. The IC50 values were 8.63 (2.34–31.84) × 10−8 M, *n* = 6). Arrowheads in A indicate repeated washings. Values are means ± S.E.M. *p* is number of experiments, *p* < 0.05 indicates significant difference from respective controls.
In Vitro Growth of Porcine SCG Neurons. Isolated SCG cells started to adhere to the poly(D-lysine)-coated surface of glass coverslips 2 to 3 h after incubation. At this stage, they were spherical with various sizes. Some of the cells started to extend processes within 24 to 48 h of incubation. After a week, the processes of the cells were well developed and formed networks at places where the cell density was high. Growing cells always stayed close to each other to form several high-density cell “islands” and left other areas nearly blank. Generally, there were two types of cells that could be visually distinguished in the culture: cells with small spindle-liked soma (the majority) and those with large round soma. Most large soma cells were monopolar or bipolar cells, while most small soma cells were bipolar or tripolar cells. Cells survived in culture at least for 4 weeks. When cultured
for a longer time (>4 weeks), the individual cell became ambiguous with a membrane-like substance that appeared around cell soma, and the cells began to detach from the coverslips (data not shown). Therefore, cells between 7 and 10 days in culture were used for immunocytochemical study.

**Immunohistochemistry.** Results from double-labeling immunostaining studies, i.e., FITC-conjugated second antibody to detect the α7-nAChR and TRITC-conjugated second antibody to detect tyrosine hydroxylase, indicated the presence of α7-nAChR (Figs. 5A and 6A) and tyrosine hydroxylase-immunoreactive fibers (Figs. 5B and 6B) in whole-mount basilar and middle cerebral arteries, and of cultured SCG cells (7–10 days). The neuronal nature of cultured SCG was verified by positive immunoreactivities of both soma and dendrites of SCG for neurofilament 200, a neuron marker (data not shown). Almost all tyrosine hydroxylase-immunoreactive fibers were coincident with α7-nAChR-immunoreactive fibers in the whole-mount preparations, and α7-nAChR-immunoreactivities and tyrosine hydroxylase-immunoreactivities were coexpressed in both dendrites and soma of the same SCG cells. α7-nAChR immunoreactive bundle fibers were frequently found to be slightly thicker than the corresponding tyrosine hydroxylase immunoreactive fibers in whole-mount preparations (Fig. 5), while these two immunoreactive fibers of cultured SCG cells were almost identical (Fig. 6).

For negative controls, no immunoreactivities of α7-nAChR, tyrosine hydroxylase, or neurofilament 200 were observed in whole-mount or cultured preparations by omitting the respective primary antibodies (data not shown).

**Discussion**

Nicotine has been shown to elicit neurogenic NOergic vasodilation in peripheral and cerebral arteries in many species (Jiang et al., 1997; Toda et al., 1997; Uchiyama et al., 1997; Okamura et al., 1999). It was assumed that nicotine acted directly on NOergic nerve terminals to release NO, resulting in NO-mediated neurogenic vasodilation. This assumption however was questioned. Our recent studies demonstrated for the first time that nicotine-induced NO-mediated relaxation in porcine basilar arteries was dependent exclusively on intact sympathetic innervation (Zhang et al., 1998). Following a complete blockade of sympathetic transmission with guanethidine, or chemical denervation of sympathetic nerves with 6-hydroxydopamine, nicotine never induced a relaxation, although TNS-elicited NO-mediated relaxation in the same preparations remained unchanged. This latter finding was consistent with morphological observations that NOergic innervation remained intact, while adrenergic nerves were completely denervated following treatment with 6-hydroxy-

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**Fig. 4.** Failure of DHβE in affecting relaxation in porcine basilar arteries induced by nicotine and TNS. A, representative tracing showing effect of the preferential α4-nAChR antagonist DHβE on relaxation elicited by nicotine (100 μM) and TNS at 8 Hz in a basilar artery in the presence of active muscle tone induced by U-46619 (0.3 μM). DHβE at 10 μM had no effect on the relaxation induced by nicotine or TNS. The results are summarized in B. Arrowheads in A indicate repeated washings. Values are means ± S.E.M. n is number of experiments.

**Fig. 5.** α7-nAChR- and tyrosine hydroxylase immunoreactive fibers in a whole-mount porcine basilar artery. Fluorescence photomicrographs showing almost all α7-nAChR immunoreactive fibers (FITC-labeling, arrowheads in A) were coincident with tyrosine hydroxylase immunoreactive fibers (TRITC-labeling, arrowheads in B). Scale bar, 50 μm.
dopamine (Zhang et al., 1998). These results indicate that in porcine basilar arteries nicotine does not act directly on NOergic nerves to release transmitter NO. Rather, nicotine acts on the nicotinic receptors located on sympathetic nerves to release NE, which then diffuses to act on adrenoceptors located on the neighboring NOergic nerves, causing release of NO from these nerves (Zhang et al., 1998; Lee et al., 2000). The role of NE as the mediator released from the sympathetic nerves was further supported by the findings that β2-adrenoceptors located on the NOergic nerves mediated nicotine-induced relaxation (Lee et al., 2000). This functional axonal interaction is also supported by morphological evidence 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 (Iwayama et al., 1970; Edvinsson et al., 1977; Lee, 1981; Barroso et al., 1996). This nicotine-induced, sympathetic-dependent neurogenic vasodilation also has been demonstrated in the mesenteric vascular beds (Shiraki et al., 2000).

Release of NE by nicotine acting on nicotinic receptors located on sympathetic adrenergic nerve terminals is well established (Su and Bevan, 1970; Haass et al., 1991). The exact nature of the nAChR on adrenergic sympathetic nerves mediating transmitter release in regulating vascular function, including cerebral circulation, however, has not been determined. Results of the present study indicated for the first time that α7-nAChR located on the sympathetic nerve terminals mediated nicotine-induced neurogenic NOergic vasodilation in porcine basilar arteries. This conclusion was based on the findings that nicotine-induced relaxation was blocked by preferential α7-nAChR antagonists but not by preferential α4-nAChR antagonists. The findings that preferential α7-nAChR antagonists (α-BGTX and MLA) and nonspecific nAChR antagonist (mecamylamine) almost completely block nicotine-induced relaxation, with the former more potent (α-BGTX) than or equally potent (MLA) to the latter, further suggest that α7-nAChR on sympathetic nerves play a major role in modulating transmitter NE release in porcine basilar arteries.

The presence of postganglionic sympathetic innervation of superior cervical ganglionic origin in cerebral circulation in many species is well established (Lee et al., 1976; Lee, 1981). Nicotine-induced relaxation in isolated porcine basilar arteries, therefore, suggests that functional α7-nAChRs are located on the postganglionic sympathetic neurons and terminals. This conclusion is supported by the immunohistochemical findings that α7-nAChR- and tyrosine hydroxylase-immunoreactive fibers were almost completely coincident in whole-mount basilar arterial preparations, and that α7-nAChR- and tyrosine hydroxylase-immunoreactive activities were completely coexpressed in both dendrites and soma of cultured SCG. These results provide strong evidence for the presence of α7-nAChRs on tyrosine hydroxylase-containing adrenergic, sympathetic nerves. These findings also suggest the presence of α7-nAChRs on porcine SCG neurons, a result consistent to that found by electrophysiological study in the rat SCG (Cuevas et al., 2000).

In whole-mount arterial preparations in the present study, most α7-nAChR-immunoreactive fibers were found thicker than tyrosine hydroxylase immunoreactive fibers, while the coexpressed α7-nAChR- and tyrosine hydroxylase immunoreactive fibers in cultured SCG are identical. This is consistent with the suggestion that sympathetic and nonsympathetic (parasympathetic) nerves run closely together, suggesting that α7-nAChRs also are present on nonsympathetic or parasympathetic nerves. The reason for the lack of effect of α7-nAChRs located on nonsympathetic NOergic nerves in mediating nicotine-induced relaxation (Zhang et al., 1998; Lee et al., 2000) remains to be determined. The present results from immunohistochemical studies, however, support our hypothesis that nicotine acts on presynaptic nicotinic receptors (α7-nAChR) on the adrenergic nerve terminals to release NE, which then acts on the presynaptic β2-adrenoceptors located on the neighboring NOergic nerves to cause release of NO and therefore vasodilation (Lee et al., 2000).

The superior cervical ganglionic neurons of the rat have been shown to express a variety of nAChR genes, including α7, and display nicotine-induced responses (Brown and Fumagalli, 1977; Kristufek et al., 1999; Skok et al., 1999;
Erkman et al., 2000). The α7-nAChRs have a high relative permeability to calcium-dependent events in neurons, including release of neurotransmitter from presynaptic sites in the brain and periphery (McGehee et al., 1995; Gray et al., 1996; Brodie and Leslie, 1999; Fu et al., 1999). Furthermore, α7-nAChRs in most instances have been found to develop nicotine responses that rapidly desensitize and are blocked by α-BGTX (Zorumski et al., 1992; Alkondon and Albuquerque, 1993; Zhang et al., 1994, Blumenthal et al., 1997; Cuevas et al., 2000). These findings on characteristics of α7-nAChRs are consistent with our present and previous observations that nicotine-induced relaxation in isolated porcine bararteries develops tachyphylaxis upon repeated applications of nicotine in short time intervals (Zhang et al., 1998; Lee et al., 2000).

The suggestion that α7-nAChRs on sympathetic nerves mediate NE release in porcine bararteries in the present study is consistent with findings in several reports, for examples, in rat dorsal raphe neurons (Li et al., 1998) and rat hippocampus (Fu et al., 1999). Other investigators, however, have demonstrated that non-α7-nAChRs such as α9β2 subunits on sympathetic nerves in the rat stomach (Yokotani et al., 2000) and rat hippocampal synaptosomes (Luo et al., 1998), and α7β2 in rat hippocampal slices (Sershen et al., 1997) are involved in regulating nicotine-induced NE release. It appears that regional variations exist in functional subunits of nAChRs on adrenergic neurons in regulating NE release.

In summary, the present study demonstrates for the first time that α7-nAChRs are present on perivascular postganglionic, sympathetic nerves of SCG origin in porcine bararteries. This α7-nAChR appears to be the main type of nAChR that is functional in mediating nicotine-induced release of NE. This result further supports our hypothesis that nicotine acts on nAChR on sympathetic nerve terminals to release NE, which then diffuses to act on presynaptic β2-adrenoceptors located on the neighboring NOergic nerves, causing release of NO and therefore vasodilatation.

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References


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