Mechanism of Nicotine-Induced Relaxation in the Porcine Basilar Artery

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ABSTRACT

The present experiment was designed to examine possible influence of adrenergic nerves on nicotine-induced neurogenic vasodilation in porcine basilar arteries denuded of endothelium. Nicotine and transmural nerve stimulation (TNS) induced relaxation of basilar arteries. Tetrodotoxin (TTX) abolished the relaxation elicited by TNS, but only partially blocked that induced by nicotine. Relaxation induced by both nicotine and TNS was abolished by N-nitro-L-argine. The N-nitro-L-arginine inhibition of both TNS- and nicotine-induced relaxation was reversed by L-argine but not by D-argine. Hexamethonium abolished the relaxation induced by nicotine, but did not affect that elicited by TNS. Relaxation induced by nicotine was diminished by guanethidine, which did not affect the relaxation induced by TNS, suggesting that guanethidine blockade of nicotine-induced relaxation is not due to its local anesthetic effect. Results from histochemical studies indicated that catecholamine fluorescence and NADPH-diaphorase fibers were not appreciably affected by guanethidine. Following incubation with 6-hydroxydopamine for 1 hr, the catecholamine fluorescence fibers in the basilar arteries completely disappeared, although the NADPH-diaphorase fibers were not affected. In these adrenenergically denervated arteries, nicotine-induced relaxation was abolished, while the TNS-elicited relaxation was not affected. Furthermore, norepinephrine-induced relaxation in basilar arteries was blocked by N-nitro-L-arginine, but was not affected by N-nitro-D-arginine or hexamethonium. These results suggest that in porcine cerebral arteries nicotine-induced nitric oxide-mediated relaxation is dependent on an intact adrenergic innervation. Nicotine appears to act on nicotinic receptors on the presynaptic adrenergic nerve terminals to release norepinephrine or a related substance, which then stimulates release of nitric oxide from the neighboring nitric oxidergic nerves. The TNS-elicited nitric oxide-mediated relaxation, however, is results from direct depolarization of nitric oxidergic nerves.

Results from pharmacological studies in isolated vascular preparations have indicated that cerebral arteries from several species receive vasodilator and constrictor nerves (Lee et al., 1975, 1976, 1982; Duckles et al., 1977; Toda 1981). Using in vitro tissue bath technique, TNS or field electrical stimulation of the intramural nerves has been shown to elicit vasoconstriction, dilation, or bi-model responses in isolated cerebral arteries, depending on the region of the arteries and species of the experimental animals examined (Lee et al., 1975, 1976, 1982; Duckles et al., 1977; Toda 1981). The neurogenic vasodilator response on TNS, however, is predominant in cerebral arterial segments from most species examined (Lee et al., 1975, 1982; Winquist et al., 1982; Toda 1981). Few cerebral arteries such as the basilar arteries from the rabbits (Lee et al., 1976) and sheep (Duckles et al., 1977) constricted predominantly upon TNS. The constriction resulted from excitation of the adrenergic sympathetic nerves, and the dilation from that of the non-adrenergic nerves (Lee, 1994). Because TNS depolarizes all intramural nerves, these TNS-elicited responses, therefore, are the net results of constriction and relaxation. For this reason, experiments that were designed to characterize the nature of the neurogenic vasodilation, an adrenergic neuronal blocker such as GUA was administered to eliminate any potential influence from the adrenergic components (Lee et al., 1975, 1982).

In the rabbit basilar arteries, TNS-elicited sympathetic vasoconstriction was reversed to a vasodilation in the presence of GUA (Lee et al., 1976), suggesting that sympathetic nerves are functional in regulating the rabbit basilar arterial tone. In parallel studies in cerebral arteries (basilar and middle cerebral arteries, and the circle of Willis) from several other species including the pig and cat, TNS elicited exclusive relaxation. GUA, however, did not significantly affect this TNS-elicited vasodilation (Lee et al., 1975, 1982). In these vascular beds sympathetic nerves may play a minimum

ABBREVIATIONS: ACh, acetylcholine; GUA, guanethidine; 6-OHDA, 6-hydroxydopamine; L-NNA, N-nitro-L-arginine; D-NNA, N-nitro-D-arginine; NE, norepinephrine; NADPH, nicotinamide adenine dinucleotide phosphate; NADPHd, NADPH-diaphorase; NO, nitric oxide; SNP, sodium nitroprusside; TNS, transmural nerve stimulation; TTX, tetrodotoxin; NOS, nitric oxide synthase; NBT, nitro blue tetrazolium; GSH, glutathione; PB, phosphate buffer; PPV, papaverine; PBS, phosphate-buffered saline.
role in regulating vascular tone, even though these arteries receive dense catecholamine fluorescence fibers (Lee et al., 1982; Asada and Lee, 1992) with high content of endogenous NE (Lee et al., 1980) which is readily to be released upon TNS (Duckles and Rapoport, 1979; T.J.-F. Lee, unpublished data). It is possible that release of transmitters such as NE from the sympathetic nerves into the synapses upon TNS is too low to elicit a response in the smooth muscle cells (Lee et al., 1982). However, it has been shown that the postsynaptic adrenoceptors mediating cerebral arterial constriction are rather insensitive to catecholamines (Lee et al., 1976, 1980; Duckles and Bevan, 1976). The functional significance of transmitter NE in these cerebral arteries remains unclear.

The nature of the transmitter substance for cerebral vasodilation was first suggested based on in vivo physiological studies to be ACh (Chorobski and Penfield, 1932). In isolated cerebral arteries of the cat, Lee (1982) demonstrated that ACh constricted the vascular smooth muscle, although TNS elicited a relaxation in the same arterial ring segment without endothelial cells. This finding provides strong evidence against ACh as a transmitter for cerebral neurogenic vasodilation at least in the major arteries of the brain. Since then, several potential substances such as vasoactive intestinal polypeptide and calcitonin gene-related peptide have been suggested to be transmitters for cerebral vasodilation (Lee et al., 1988). NO, however, has recently been shown to mediate a major component of the TNS-elicited vasodilation in cerebral arteries of the pig, cat and other species (Lee, 1994). Similar result also has been shown that nicotine-induced vasodilation in isolated cerebral arteries from several species including human is mainly due to NO (Toda, 1993).

The mechanism of action of nicotine in inducing cerebral vasodilation is different from that of TNS in that its effects are receptor-mediated (Toda and Okamura, 1990; Boeckxstaens et al., 1993; Toda et al., 1995). It has been demonstrated that nicotine acts on nicotinic receptors on the adrenergic nerves to release NE (Su and Beven, 1970), and on the nitric oxide-producing nerves to release NO (Toda and Okamura, 1990; Toda et al., 1995). It is logical to assume that nicotine-induced NO-mediated relaxation is not affected by GUA in a similar way to that elicited by TNS (Lee et al., 1982, 1996; Lee and Sarwinski, 1991). Surprisingly, effect of GUA on nicotine-induced, NO-mediated relaxation in cerebral arteries has not been clarified. Our preliminary results have indicated that unlike TNS-elicited relaxation, the nicotine-induced relaxation in the porcine basilar arteries was abolished by GUA at the concentrations that did not affect the TNS-elicited relaxation. Our experiment was, therefore, designed to examine the mechanism of action of nicotine in inducing NO-mediated neurogenic vasodilation in isolated porcine basilar arteries. Our results indicate that the nicotine-induced NO-mediated cerebral vasodilation is dependent on intact adrenergic sympathetic nerves.

Methods

General procedure. Fresh heads of adult pigs of either sex were collected from 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 (mM): NaCl, 122.0; KCl, 5.16; CaCl₂, 1.2; MgSO₄, 1.22; NaHCO₃, 25.6; ethylenediamine-tetraacetic acid, 0.03; l-ascorbic acid, 0.1; and glucose, 11.0 (pH 7.4). Basilar arteries were dissected and cleaned off surrounding tissue under a dissected microscope.

In vitro tissue bath studies. The ring segment (4 mm long) of the basilar artery was dissected and cannulated with a stainless-steel rod (30–28 gauge) of 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 (UC3, Gould) for isometric recording of changes in force, as described in our previous report (Lee, 1982). The temperature of the Krebs' solution in the tissue baths, 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 were mechanically stretched to a resting tension of 0.75 g for another 30 min.

The basilar arterial ring segments were then precontracted with U-46619 (0.3–3 μM) to induce an active muscle tone of 0.5–0.75 g. TNS at 8 Hz and a single concentration of nicotine (100 μM) were then added to induce a relaxation. The arteries were then washed with prewarmed Krebs' solution. A similar magnitude of active muscle tone was induced with U46619, and TNS at 8 Hz was repeated (to serve as a control comparing to the relaxation elicited by TNS before the wash). Effects of experimental drugs were then administered and TNS at 8 Hz and nicotine at the same concentration before the wash was repeated. To avoid possible development of tachyphylaxis, the washes were repeated (5–6 washes every 15 min) with six washes every 15 min before the next application of nicotine. GUA was added at least 30 min before TNS and application of nicotine.

For TNS, tissues were electrically, transmurally stimulated with a pair of electrodes through which 100 biphasic square-wave pulses of 0.6 msec in duration and 200 mA in intensity were applied at various frequencies (Lee, 1982). Stimulation parameters were continuously monitored on a Tektronix oscilloscope. The neurogenic origin of this TNS-induced response was verified by its complete blockade by TTX (0.3 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 relaxation response induced by papaverine (Lee, 1982).

For examining effects of experimental drugs on relaxation induced by NE and SNP, concentration-response relationships for NE or SNP were obtained by a cumulative technique in arteries (without endothelial cells) in the presence of active muscle tone induced by U46619. After the arterial rings were washed with prewarmed Krebs' solution, a similar magnitude of active muscle tone was again induced by U46619. The experimental drugs were then added, and 15 min later, concentration-response relationships for NE or SNP 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 (Lee, 1982). A complete removal of endothelial cells was verified by lack of effect of L-NNa in increasing basal tone (Lee and Sarwinski, 1991; Lee et al., 1996).

Catecholamine fluorescence. At the end of in vitro tissue bath studies, basilar arteries were immersed in ice-cold (4°C) 2% glyoxylic acid (pH 7.4) for 30 min (Lee et al., 1982). They were then dried in a stream of warm air with a hair drier for 15 min, and heated in a oven at 110°C for 6 min. The specimens were then covered with xylene and examined under a Zeiss Universal fluorescence microscope and photographed.
NADPHd histochemical staining. NADPHd activity in perivascular nerves of the porcine basilar arteries was examined histochemically according to our previous report (Chen and Lee, 1995). Briefly, at the end of in vitro tissue bath studies, basilar arteries were fixed in 4% paraformaldehyde and 0.05% glutaraldehyde in 0.1 M PB (pH 7.4) overnight (4°C), and incubated in 0.1 M PB (pH 8.0) containing 0.5 mg/ml of NADPH, 0.1 mg/ml of NBT and 0.3% Triton X-100 at 37°C for 1 hr. The tissues were then rinsed with 0.01 M PBS (pH 7.4) and examined under a Zeiss light microscope and photographed.

Chemical denervation with 6-OHDA. In vitro incubation with 6-OHDA were carried out according to the method of Aprigliano and Herrmsmeyer (1976). 6-OHDA is extremely susceptible to oxidation at neutral and alkaline pH. GSH (20 μM), therefore, was added to the unbuffered Krebs’ solution (NaHCO3 was omitted from the solution) to reduce the rate of oxidation of 6-OHDA. Basilar arterial segments were incubated in Krebs’ solution (37°C) containing 6-OHDA (2 mM) for 30 min twice with an interval of 20 min in normal Krebs’ solution.

Cold-storage denervation. Isolated porcine cerebral arteries were stored in cold Krebs’ solution at 4°C for 7 days to achieve cold-storage denervation (Lee et al., 1975). Although there is a possibility that some biological properties of the tissue may have changed after cold storage, changes in biochemistry of cerebral blood vessels, particularly related to the amino acid conversion, should be minimal (Muller-Schweinitzer, 1994; Chen and Lee, 1995). In addition, our previous reports have indicated that cold-storage denervation did not affect endothelium-independent and endothelium-dependent relaxation in cerebral arteries (Lee, 1982; Lee et al., 1982).

Drug used. D-Arginine, L-arginine, glutaraldehyde, glyoxylic acid, 6-OHDA, NADPH, L-NNA, NBT, NE, paraformaldehyde, SNP, TTX (all from Sigma Chemical Co., St. Louis, MO), atropine sulfate (Calbiochem., San Diego, CA), guanethidine sulfate (Ciba Geigy, Summit, NJ), hexamethonium (RBI, Natick, MA), D-NNA (RBI, Natick, MA), phenolamine (Ciba Geigy, Summit, NJ), triton X-100 (Amersham Corp., IL) and U46619 (Upjohn, Kalamazoo, MI) were used. All drugs, otherwise stated, were dissolved in deionized water, and examined under a Zeiss light microscope and photographed. Statistical analysis was evaluated by Student’s t test for paired or unpaired samples as appropriate. The P < .05 level of probability was accepted as significant.

Results
Nicotine- and TNS-induced neurogenic vasodilation in the basilar arteries. In the presence of active muscle tone induced by U46619 (0.3 μM) elicited by nicotine (100 μM) and transmural nerve stimulation (TNS) at 8 Hz. Tetrodotoxin (TTX) (0.3 μM) abolished the relaxation elicited by TNS although it partially but significantly blocked the relaxation induced by nicotine. This was summarized in B. Similarly, cold-storage denervation at 4°C for 7 days abolished the relaxation elicited by TNS, but partially blocked the relaxation induced by nicotine (C). Relaxation was estimated as a percentage of maximum relaxation induced by papaverine (PPV) (300 μM). Values are means ± S.E.M.; n, number of experiments. *P < .05 and **P < .01 indicate significant difference from the respective control.

Fig. 1. A representative tracing (A) showing relaxation of basilar arteries, in the presence of active muscle tone induced by U46619 (0.3 μM) and nicotine (100 μM) in same arteries with 90-min interval and six washes between two applications. There was no significant difference among the three responses. Relaxation was estimated as a percentage of maximum relaxation induced by papaverine (PPV) (300 μM). Values are means ± S.E.M.; n, number of experiments. obtained and they were not significantly different (fig. 2). The relaxations elicited by repeated TNS at 8 Hz, as with previous reports in the porcine cerebral arteries (Lee et al., 1982, 1983, 1984) were reproducible relaxations induced by nicotine (100 μM) were reproducible relaxations induced by nicotine (100 μM).
abolished by L-NNA (30 \text{ M}) (63.6 ± 6\% after L-arginine, \text{P} > .05, n = 5) and nicotine (63.6 ± 6\% in control and 58.8 ± 2.2\% after L-arginine, \text{P} > .05, n = 5) was complete.

Hexamethonium (10 \text{ M}) abolished the relaxation induced by nicotine (100 \text{ M}), but did not affect that elicited by TNS in the same arteries without endothelial cells (fig. 4). The residual relaxation in the presence of hexamethonium was abolished by TTX (data not shown). The nicotine-induced relaxation was not affected by atropine (1 \text{ M}, n = 8, data not shown).

Hexamethonium (10 \text{ M}) was diminished by GUA in a concentration-dependent manner (fig. 5A and B). Thirty min after its application, GUA at 10 \text{ M} abolished the nicotine-induced relaxation. Blockade of nicotine-induced relaxation by GUA (10 \text{ M}) was fully recovered after the arteries were washed with fresh pre-warmed Krebs’ solution (fig. 5B). However, GUA (10 \text{ M}) never affected the TNS-elicited relaxation (fig. 5B) or relaxation induced by SNP (0.01–100 \text{ M}) and TNS at 8 Hz. GUA blockade of nicotine-induced but not TNS-elicited neurogenic vasodilation. Relaxation of the isolated basilar arteries without endothelial cells induced by nicotine (100 \text{ M}) was diminished by GUA in a concentration-dependent manner (fig. 5A and B). Thirty min after its application, GUA at 10 \text{ M} abolished the nicotine-induced relaxation. Blockade of nicotine-induced relaxation by GUA (10 \text{ M}) was fully recovered after the arteries were washed with fresh pre-warmed Krebs’ solution (fig. 5B).

Results from histochemical studies indicated that dense catecholamine fluorescence fibers in the arterial wall decreased drastically. But a very small residual amount of both fibers was still present in most of the arteries, although the TNS-elicited relaxation was not affected (fig. 6A).

**Effect of chemical denervation of sympathetic nerves.** After incubation with 6-OHDA (2.0 \text{ mM}) for 1 hr, the adrenergic innervation as indicated by catecholamine fluorescence fibers in the basilar arteries was completely disappeared (n = 6, fig. 6C). This treatment did not seem to appreciably affect the density of NADPHd fibers in the same arteries (fig. 6C).

**Fig. 3. A representative tracing (A) showing effect of N-nitro-l-arginine (L-NNA) on relaxation induced by nicotine (100 \text{ M}) and transmural electrical stimulation (TNS) at 8 Hz in a basilar artery in the presence of active muscle tone induced by U46619 (0.3 \text{ M}). L-NNA (30 \text{ M}) abolished the relaxation induced by nicotine (100 \text{ M}), and almost abolished that induced by TNS (B). Relaxation was estimated as a percentage of maximum relaxation induced by papaverine (PPV) (300 \text{ M}). Values are means ± S.E.M.; n, number of experiments.**

**Fig. 4. Effects of hexamethonium on relaxation of the basilar arteries induced by nicotine (100 \text{ M}) and transmural electrical stimulation (TNS) at 8 Hz. Hexamethonium (10 \text{ M}) abolished the relaxations induced by nicotine while it does not affect the relaxation induced by TNS. Relaxation was estimated as a percentage of maximum relaxation induced by papaverine (PPV) (300 \text{ M}). Values are means ± S.E.M.; n, number of experiments.**
and B). The nicotine-induced relaxation was not affected by GSH-containing Krebs’ solution used to dissolve 6-OHDA for same time (fig. 7B).

**L-NNA blockade of NE-induced relaxation.** NE induced a concentration-dependent relaxation in the basilar arteries without endothelial cells (fig. 8); a result similar to those reported previously (Lee et al., 1982). The relaxation induced by lower concentrations of NE in the presence of phentolamine (1 μM), was significantly blocked by L-NNA (30 μM, n = 8) (fig. 8A), but was not affected by D-NNA (30 μM, n = 5, data not shown) or hexamethonium (10 μM, n = 8) (fig. 8B).

**Discussion**

Our study demonstrated that both nicotine and TNS induced exclusive relaxation in the porcine basilar arteries without endothelial cells. Both relaxations were neurogenic in nature and were mediated primarily by NO. The nicotine-induced relaxation, however, was different from that induced by TNS. The former was dependent on intact adrenergic innervation.

Cerebral blood vessels from several species including pigs have been shown to receive dense nitric oxideergic innervation (Chen and Lee, 1995; Bredt et al., 1990; Yu et al., 1997; Lee, 1994). Neurogenic vasodilation in isolated cerebral arteries induced by TNS and nicotine has been shown to be primarily due to release of neuronal NO (Lee and Sarwinski, 1991; Chen and Lee, 1995; Toda and Okamura, 1990; Toda et al., 1995; Lee et al., 1996). Relaxation elicited by TNS is believed due to depolarization of all different types of intramural nerve terminals resulting in a dilator or constrictor response depending on the predominance of dilator or constrictor nerves, respectively (Lee, 1994). Because NO is the most potent vasodilator, TNS accordingly induces a predominant vasodilation in cerebral blood vessels in most species including the pigs (Lee and Sarwinski, 1991; Chen and Lee, 1993; Lee et al., 1996).

The mechanism of action of nicotine in inducing neurogenic vasodilation is less understood. Nicotine has been frequently used to release transmitters from autonomic nerves including NE and NO (Su and Bevan, 1970; Toda and Okamura, 1990). It has been shown that nicotine acts on the prejunctional nicotinic receptors and results in NE release by triggering action potentials and by nonpropagated local depolarization (Haefely, 1974; Su and Beven, 1970). In our study, nicotine-induced relaxation was blocked by hexamethonium. Because hexamethonium did not affect the relaxation induced by SNP and NE, hexamethonium appeared to be specific for nicotinic receptors as already been reported by others (Furchgott et al., 1975; Toda, 1981).

Furthermore, nicotine, as with TNS, induced predominant vasodilation, which was blocked by inhibitors of NO synthesis. This result supports the role of NO as the primary mediator for cerebral neurogenic vasodilation in cerebral arteries of the pigs (Lee et al., 1996) and other species (Toda and Okamura, 1990; Toda et al., 1995). The neuronal origin of NO in mediating nicotine-induced relaxation is less convincing than that elicited by TNS. The relaxation induced by nicotine after cold-storage denervation, but that induced by nicotine was only partially blocked by TTX and cold-storage denervation. Although the residual relaxations induced by nicotine after cold-storage denervation and TTX treatment were comparable, the exact reason for the residual relaxation is not clear. Whether it is related to the small residual catecholamine fluorescence and NADPHd fibers seen in arteries after cold-storage denervation remains to be determined. The failure of TTX to abolish the nicotine-induced relaxation, however, is in agreement with several reports by others (Furchgott, 1975; Toda, 1981). Porcine cerebral smooth muscles do not synthesize or release NO (Lee et al., 1996), unless it is challenged by lipopolysaccharides (Ueno and Lee, 1993). In addition, only basilar arteries without endothelial cells were used in our study. It is most likely that nicotine and TNS released NO from the perivascular NOergic nerves in the basilar arteries.

The presence of dense adrenergic innervation in cerebral arteries of several species including pigs has been well established (Lee et al., 1982; Winquist et al., 1982). Exogenously applied NE relaxes the porcine basilar arteries exclusively, and the relaxation was blocked by propranolol (Lee et al., 1982; Winquist et al., 1982), suggesting that NE released from the sympathetic nerves in the porcine cerebral arteries is a potential transmitter for vasodilation. TNS-elicited relaxation in this artery, however, was not affected by agents that blocked NE release or postsynaptic β-adrenergic receptors (Lee et al., 1982). NE can be released from the sympathetic nerves upon TNS in cerebral arteries of the rabbit (Duckles and Rapoport, 1979) and the pig (our preliminary results). These results suggest that concentration of NE re-
leased from the sympathetic nerves upon TNS is probably too low to induce a relaxation, a similar result in the cerebral arteries from other species has been reported (Lee et al., 1975). The findings in vitro are consistent with the in vivo findings that electrical stimulation of sympathetic nerves did not significantly alter the cerebral vascular tone or cerebral circulation in normotensive animals (Edvinsson and McKenzie, 1976; Heistad et al., 1980). Thus, the exact functional role of NE released from cerebral perivascular sympathetic nerves in regulating cerebral blood flow remains unclarified, although it has been demonstrated that sympathetic innervation plays a protective role in cerebral circulation when the animals become hypertensive (Heistad et al., 1980).

The major finding of our study is that nicotine-induced relaxation was blocked by GUA in a concentration-dependent manner. GUA, however, did not affect the TNS-induced relaxation, a result similar to those reported previously (Lee et al., 1996). This latter finding suggests that GUA did not directly affect synthesis or release of NO from the NOergic nerves, and also rules out the possibility that the blockade by GUA is due to its possible local anesthetic effect. The GUA blockade was reversible, because nicotine-induced relaxation was fully recovered after GUA was washed away. It is well established that GUA is an adrenergic neuronal blocker, in part, by blocking NE release from the sympathetic nerves. A complete blockade of NE release from sympathetic nerves in the presence of GUA at 10 µM in mesenteric vascular beds (Yamamoto et al., 1997) and porcine basilar arteries (Sato, E., Sarwinski, S., and Lee, T.J-F., unpublished data) occurs about 20 min after its application. Together with the presence of catecholamine after GUA treatment (fig. 6), these results indicate that GUA completely block release of NE before depleting it completely. This is the reason that we examined effect of GUA on nicotine-induced relaxation 30 min after application of GUA when blockade of NE release is complete. These results also explain that nicotine-induced relaxation recovers after GUA is washed away, and further indicate that nicotine-induced relaxation is dependent on adrenergic nerves. An alternative explanation is that nicotine-induced relaxation is mediated by NE released from sympathetic nerves, which acts directly on the postsynaptic beta adrenoceptors (Lee et al., 1982; Winquist et al., 1982, Wang and Lee, 1986). This possibility is questionable, because the relaxation induced by nicotine was abolished by...
This is consistent with findings reported by others that nicotine-induced relaxation in cerebral blood vessels is mediated by NO (Toda and Okamura, 1990; Toda, 1993).

It is possible that nicotine acts preferentially on the adrenergic nerves to release NE or a related substance that then acts on the neighboring NOergic nerve terminals to release NO and vasodilation. This is supported by the findings that a complete chemical denervation with 6-OHDA, as indicated by the absence of catecholamine fluorescence fibers, abolished the nicotine-induced relaxation without affecting the relaxation induced by transmural electrical stimulation (TNS). The physiological solution containing glutathione (GSH) (or vehicle), used to dissolve 6-OHDA did not affect the relaxation induced by nicotine or TNS (B). Relaxation was estimated as a percentage of maximum relaxation induced by papaverine (PPV) (300 μM). Values are means ± S.E.M.; n, number of experiments.

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The possibility that GUA blocked the nicotine-induced neurogenic vasodilation by acting on the effector smooth muscle is unlikely. This is based on the finding that SNP-induced relaxation was not affected by GUA, suggesting that GUA did not directly affect the cyclic GMP mechanism.

Fig. 7. A representative tracing (A) showing effect of 6-hydroxydopamine (6-OHDA) (2 mM) incubation for 1 hr on the relaxation of basilar arteries, precontracted with U46619 (0.3 μM), elicited by nicotine (100 μM) and transmural nerve stimulation (TNS) at 8 Hz. 6-OHDA incubation for 1 hr resulted in complete blockade of relaxation induced by nicotine without affecting the relaxation induced by transmural electrical stimulation (TNS). The physiological solution containing glutathione (GSH) (or vehicle), used to dissolve 6-OHDA did not affect the relaxation induced by nicotine or TNS (B). Relaxation was estimated as a percentage of maximum relaxation induced by papaverine (PPV) (300 μM). Values are means ± S.E.M.; n, number of experiments.

Fig. 8. Concentration-effect curves showing effects of N-nitro-l-arginine (L-NNA) and hexamethonium on norepinephrine-(NE) induced relaxation in the basilar arteries without endothelial cells. NE-induced concentration-dependent relaxation was significantly inhibited by L-NNA (30 μM) (A), but was not affected by hexamethonium (10 μM) (B). Relaxation was estimated as a percentage of maximum relaxation induced by papaverine (PPV) (300 μM). Values are means ± S.E.M.; n, number of experiments. *P < .05 and **P < .01 indicate significant difference from the respective control.
In summary, our study demonstrated for the first time that nicotine-induced NO-mediated relaxation in porcine cerebral arteries was dependent on the intact adrenergic innervation. It appears that nicotine acts on presynaptic nicotinic receptors on adrenergic nerves to release NE or a related substance, which then diffuses to the neighboring NOergic nerve terminals to release NO, and therefore, vasodilation.

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References


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