Presynaptic β₂-adrenoceptors mediate nicotine-induced NOergic neurogenic dilation in porcine basilar arteries

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Lee, Tony J. F., W. Zhang, and S. Sarwinski. Presynaptic β₂-adrenoceptors mediate nicotine-induced NOergic neurogenic dilation in porcine basilar arteries. Am J Physiol Heart Circ Physiol 279: H808–H816, 2000.—We previously reported that nicotine-induced nitric oxide (NO)-mediated cerebral neurogenic vasodilation was dependent on intact sympathetic innervation. We hypothesized that nicotine acted on sympathetic nerve terminals to release norepinephrine (NE), which then acted on adrenoceptors located on the neighboring nitric oxidergic (NOergic) nerve terminals to release NO, resulting in vasodilation. The adrenoceptor subtype in mediating nicotine-induced vasodilation 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 propranolol and preferential β₁-adrenoceptor antagonists (ICI-118,551 and butoxamine), in a concentration-dependent manner, blocked the relaxation induced by nicotine (100 μM) without affecting the relaxation elicited by transmural nerve stimulation (TNS, 8 Hz). In contrast, preferential β₂-adrenoceptor antagonists (atenolol and CGP-20712A) did not affect either nicotine- or TNS-induced relaxation. Results of double-labeling studies indicated that β₂-adrenoceptor immunoreactivities and NADPH diaphorase reactivities were colocalized in the same nerve fibers in basilar and middle cerebral arteries. These findings suggest that NE, which is released from sympathetic nerves upon application of nicotine, acts on presynaptic β₂-adrenoceptors located on the NOergic nerve terminals to release NO, resulting in vasodilation. In addition, nicotine-induced relaxation was enhanced by yohimbine, an α₂-adrenoceptor antagonist, which, however, did not affect the relaxation elicited by TNS. Prazosin, an α₁-adrenoceptor antagonist, on the other hand, did not have any effect on relaxation induced by either nicotine or TNS. The predominant facilitatory effect of β₂-adrenoceptors in releasing NO may be compromised by presynaptic α₂-adrenoceptors.

nitric oxide; norepinephrine; porcine cerebral arteries

MORPHOLOGICAL STUDIES have demonstrated that the close apposition between the adrenergic nerve terminal and the nonadrenergic or nitric oxide synthase (NOS) immunoreactive nerve terminal has frequently been found in large cerebral arteries at the base of the brain in several species (1, 11, 13). The axo-axonal distance between these different types of nerve terminals is substantially closer than the synaptic distance between the adventitial nerve terminals and the outermost layer of smooth muscle in the media (11, 13, 18). This morphological feature suggests that a functional axo-axonal interaction between nerve terminals is more likely to occur than that between the nerve and muscle. Thus transmitters released from one nerve terminal may modulate the release of transmitters from the neighboring nerve terminals and therefore the neurogenic vasomotor responses (5, 13).

We have recently demonstrated that nicotine-induced nitric oxide (NO)-mediated neurogenic vasodilation is dependent on intact sympathetic, adrenergic innervation in porcine basilar arteries. 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 (6-OHDA), although these treatments do not affect transmural nerve stimulation (TNS)-elicited NO-mediated neurogenic vasodilation in the same preparations. Furthermore, relaxation induced by exogenous norepinephrine (NE) in porcine basilar arterial rings was blocked by Nω-nitro-L-arginine (L-NNA; see Ref. 34). Accordingly, it was hypothesized that nicotine acts on nicotinic receptors located on sympathetic nerve terminals, resulting in release of NE or a related substance that then diffused to act on its receptors located on the neighboring nitric oxidergic (NOergic) nerve terminals to release NO and therefore induce vasodilation (34). The exact nature of the receptors located on NOergic nerves mediating nicotine-elicted NO release in cerebral arteries has not been clarified. Our preliminary results indicated that nicotine-induced neurogenic vasodilation in porcine basilar arteries was blocked by propranolol, suggesting the possible involvement of β-adrenoceptors in nicotine-induced neurogenic vasodilation. This latter finding further supported the notion that NE or a related substance was the mediator released from sympathetic nerve terminals to cause NO release from NOergic nerves. The present study, therefore, was designed to
pharmacologically and immunohistochemically characterize, in large cerebral arteries of the pig, the pre-

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% O2-5% CO2 at room temperature. The composition of the Krebs solution was as follows (in mM): 122.0 NaCl, 5.16 KCl, 1.2 CaCl2, 1.22 MgSO4, 25.6 NaHCO3, 0.03 EDTA, 0.1 L-ascorbic acid, and 11.0 glucose (pH 7.4). Basilar and middle cerebral arteries were dissected and cleaned of surrounding tissue after a dissecting microscope.

In vitro tissue bath studies. The ring segment (4 mm long) was cannulated with a stainless-steel rod (30-gauge hemispherical section) and a short piece of platinum wire and was 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) for isometric recording of changes in force, as described in our previous report (20). The temperature of the Krebs solution equilibrated with 95% O2-5% CO2 was maintained at 37°C. Tissues were equilibrated in the Krebs solution for an initial 30 min and then were mechanically stretched to a resting tension of 750 mg (34).

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 applied to induce a relaxation. The arteries were then washed with warmed Krebs solution. A similar magnitude of active muscle tone was induced with U-46619 again, and TNS at 8 Hz was repeated (to serve as a control compared with the relaxation elicited by TNS before the wash). Experimental drugs were then administered, and TNS at 8 Hz and nicotine at the same concentration before the wash were repeated. To avoid possible development of tachyphylaxis upon repeated applications of nicotine (34), at least 90 min with six washes (every 15 min) were allowed before the next application of nicotine (34). Experimental drugs were 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 ms in duration and 200 mA in intensity were applied at various frequencies (17). 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 response induced by papaverine (17).

For examining effects of experimental drugs on relaxation induced by NE, concentration-response relations for 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 relations for NE were repeated. EC50 values were determined for each arterial ring. From these values, the geometric means EC50 with 95% confidence intervals (8) 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 (17). A complete removal of endothelial cells was verified by the lack of effect of L-NNa in increasing basal tone (19).

Double-labeling immunohistochemistry. Fresh porcine basilar and middle cerebral arteries obtained from local slaughterhouses were dissected and placed into periodate-

Results were expressed as means ± SE. Statistical analysis was evaluated by 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. In the presence of active muscle tone induced by U-46619 (0.3 µM), the basilar arteries without endothelial cells relaxed exclusively upon TNS at various frequencies (2, 4, and 8 Hz) and application of nicotine (1–100 µM). A complete removal of endothelial cells was verified by the lack of effect of L-NNA in increasing basal tone (19). Because TNS at 8 Hz and nicotine at 100 µM induced maximum relaxation, these parameters, which have previously been used by us and many others (17, 29, 34), 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 (34). 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 (100 µM) were obtained, which were not significantly different (34). Furthermore, the relaxation elicited by repeated TNS at 8 Hz, like other reports in the porcine cerebral arteries (17, 19), was reproducible and not different (34).

The relaxation induced by both nicotine (100 µM) and TNS (8 Hz) was significantly blocked by L-NNA (30 µM, $n = 6$), TTX (0.3 µM), and cold storage denervation for 7 days (data not shown). Furthermore, the relaxation induced by nicotine (100 µM) was diminished by guanethidine (1–10 µM) in a concentration-dependent manner. Blockade of nicotine-induced relaxation by guanethidine (1–10 µM) was fully recovered after the arteries were washed with fresh prewarmed Krebs solution ($n = 5$). On the other hand, guanethidine at

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**Fig. 1.** A: representative tracing showing relaxation of a basilar arterial ring without endothelial cells induced by transmural nerve stimulation (TNS) at 8 Hz and 100 µM nicotine (Nic). B: propranolol blockade of relaxation induced by nicotine but not by TNS. Relaxation was estimated as a percentage of the respective control (C). Values are means ± SE; $n$, no. of experiments. *$P < 0.05$, significant difference from controls. #Complete blockade of nicotine-induced relaxation by propranolol. Brackets denote concentration. PPV, papaverine.
similar concentrations never affected the TNS-elicited relaxation. These results were similar to those reported previously (34).

**Blockade of nicotine-induced neurogenic vasodilation by β-adrenoceptor antagonists but not by α-adrenoceptor antagonists.** In the presence of active muscle tone induced by U-46619 (0.3 μM) in basilar arteries without endothelial cells, nicotine (100 μM)-induced relaxation was significantly inhibited by propranolol (0.1–10 μM) in a concentration-dependent manner (Fig. 1, A and B). At 10 μM, propranolol abolished the nicotine-induced relaxation without any effect on the TNS-elicited relaxation in the same preparations (Fig. 1, A and B).

The relaxation induced by nicotine in basilar arteries was not significantly affected by prazosin (1–10 μM, n = 8; Fig. 2A) but was significantly enhanced by yohimbine (0.1–1 μM, n = 4; Fig. 2B). Neither prazosin nor yohimbine at the concentrations tested affected the TNS-elicited relaxation (Fig. 2, A and B).

**β2-Adrenoceptor but not β1-adrenoceptor antagonists blocked nicotine-induced neurogenic vasodilation.** In the presence of active muscle tone induced by U-46619 (0.3 μM) in basilar arteries without endothelial cells, relaxation induced by nicotine (100 μM) was diminished by ICI-118,551 and butoxamine (selective β2-adrenoceptor antagonists) in a concentration-dependent manner (0.01–10 μM; Fig. 2, A and B). The nicotine-induced relaxation, however, was not appreciably affected by β1-adrenoceptor antagonists such as atenolol (0.01–10 μM, data not shown) and CGP-20712A (0.01–10 μM, data not shown). At similar concentrations, ICI-118,551, atenolol, butoxamine, and CGP-20712A did not affect the TNS-elicited relaxation in the same preparations (data not shown).

**Effect of ICI-118,551, atenolol, and CGP-20712A on NE-induced relaxation in basilar arteries.** In the presence of phentolamine (1 μM) and active muscle tone induced by U-46619 (0.3 μM), porcine basilar arteries without endothelial cells relaxed upon application of NE in a concentration-dependent manner, a result similar to that reported previously (17). The relaxation was blocked by ICI-118,551 (Fig. 3A), atenolol (Fig. 3B), and CGP-20712A (Fig. 3C), as indicated by parallel shift of the concentration-response curves to the right. CGP-20712A and atenolol appeared to be more potent than ICI-118,551. Both CGP-20712A and atenolol at 0.1 μM already significantly shifted the concentration-response curve to the right, whereas ICI-118,551 at same concentration was without any significant effect on NE-induced relaxation (Fig. 3).

Fig. 2. Bar graphs showing effects of prazosin (A), yohimbine (B), ICI-118,551 (C), and butoxamine (D) on relaxation of isolated basilar arterial rings without endothelial cells induced by nicotine (100 μM) and TNS at 8 Hz. Relaxation was estimated as a percentage of the respective control. Values are means ± SE; n, no. of experiments. *P < 0.05, significant difference from the respective controls. #At 10 μM, butoxamine almost completely blocked nicotine-induced relaxation.
Immunocytochemistry. Results from double-staining studies, i.e., single immunolabeling followed by histochemical staining for NADPHd, which is a reliable marker for neuronal NOS (33), indicated the presence of \( \beta_2 \)-adrenoceptor immunoreactive (Fig. 4A) and NADPHd reactive fine fibers and bundles (Fig. 4B) in the same whole-mount basilar and middle cerebral arteries. In both arteries, almost all NADPHd reactive fibers were coincident with \( \beta_2 \)-adrenoceptor immunoreactive fibers, but not all \( \beta_2 \)-adrenoceptor immunoreactive fibers were NADPHd reactive. These NADPHd reactive and NADPHd negative fibers were frequently found to run close to each other. It appeared that \( \beta_2 \)-adrenoceptor immunoreactive bundles were composed of NADPHd reactive and NADPHd negative fibers. Therefore, \( \beta_2 \)-adrenoceptor immunoreactive bundles were frequently found to be thicker than the corresponding NADPHd reactive bundles (Fig. 4A and B). For negative controls by omitting primary antibodies, no immunoreactivities of \( \beta_2 \)-adrenoceptors were observed (data not shown).

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**DISCUSSION**

The major finding of the present study was that nicotine-induced neurogenic vasodilation in porcine basilar arteries was blocked by preferential \( \beta_2 \)-adrenoceptor antagonists but not by preferential \( \beta_1 \)-adrenoceptor antagonists. The preferential \( \beta_1 \)-adrenoceptor antagonists, however, are more potent than preferential \( \beta_2 \)-adrenoceptor antagonists in blocking the postsynaptic \( \beta \)-adrenoceptor-mediated relaxation induced by exogenously applied NE. \( \beta_2 \)-Adrenoceptor immunoreactivities and NADPHd reactivities were found to colocalize in the same nerve fibers. Because NADPHd is a reliable marker for neuronal NOS in porcine cerebral arteries (33), these results support our hypothesis that nicotine acts on presynaptic nicotinic receptors located on the adrenergic nerve terminals to release NE, which then acts on the presynaptic \( \beta_2 \)-adrenoceptors located on the neighboring NOergic nerves to cause release of NO and therefore induces vasodilation (Fig. 5).
It is well established that nicotine releases NE by acting on nicotinic receptors located on sympathetic adrenergic nerve terminals (10, 27). Accordingly, nicotine was assumed to act directly on NOergic nerve terminals to release NO, resulting in NO-mediated cerebral neurogenic vasodilatation in many species (28). This assumption, however, was questioned, since our recent studies demonstrated for the first time that nicotine-induced NO-mediated relaxation in porcine cerebral arteries was dependent exclusively on intact sympathetic innervation (34). After a complete blockade of sympathetic transmission with guanethidine, or chemical denervation of sympathetic nerves with 6-OHDA, 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 after treatment with 6-OHDA (34). These results indicate that in cerebral 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 the neighboring NOergic nerves and causes the release of NO from these nerves (34). The morphological evidence of close apposition (25 nm) between the adrenergic nerve terminals and the nonadrenergic nerve terminals for such axo-axonal interaction has been demonstrated to be a characteristic of cerebral vessel innervation in several species (1, 5, 11, 13).

Evidence that NE or a related catecholamine mediated nicotine-induced NOergic vasodilation was supported by results of the present study that nicotine-induced relaxation was blocked by propranolol (a nonspecific β-adrenoceptor antagonist). Further studies demonstrated that this effect of nicotine was mediated specifically by presynaptic β₂-adrenoceptors, since nicotine-induced relaxation was blocked by preferential β₂-adrenoceptor antagonists in a concentration-dependent manner, but was not affected by preferential β₁-adrenoceptor antagonists. Both preferential β₁- and preferential β₂-adrenoceptor antagonists, like propranolol, however, significantly blocked postsynaptic β-adrenoceptor-mediated relaxation induced by exogenous NE, a result consistent to those reported previously (17, 23, 31, 32). Furthermore, propranolol and all preferential β₁- and preferential β₂-adrenoceptor antagonists at concentrations examined in the present studies did not affect TNS-elicited relaxation. This result is also consistent to those reported previously (17, 31, 32), suggesting that blockade of nicotine-induced relaxation by propranolol and β₂-adrenoceptor antagonists was not due to any possible local anesthetic effects of these agents at the concentrations used. Together, these results suggest that presynaptic β₂-adrenoceptors on the NOergic nerves mediate NE-induced NO release. Evidence has been presented that NE but not dopamine or epinephrine is found in cerebral arteries, including basilar and middle cerebral arteries from different species (14, 17), further indicating that NE is the most likely transmitter released by nicotine from sympathetic nerves to cause release of NO from the neighboring NOergic nerves.

It should be noted that the porcine cerebral vascular smooth muscle, unlike that of other species, contains mainly β-adrenoceptors, with β₁-adrenoceptors as the predominant subtype in mediating NE-induced relaxation (23, 31, 32). This is further supported by results of the present study that β₁-adrenoceptor antagonists were more potent than β₂-adrenoceptor antagonists in
blocking exogenous NE-induced relaxation. In particular, CGP-20712A (a preferential β₁-adrenoceptor antagonist) appears to be the most potent β-adrenoceptor antagonist in blocking relaxation induced by exogenous NE. At 3 μM, CGP-20712A almost abolished the maximum relaxation induced by NE but did not appreciably affect nicotine-induced neurogenic vasodilation. A similar result was found with a different β₁-adrenoceptor antagonist (atenolol). On the other hand, ICI-118,551 at 0.1 μM, which did not significantly affect the NE concentration-relaxation response relationship (Fig. 3C), significantly inhibited the nicotine-induced relaxation (Fig. 2C). These results indicate that neurally released NE induced by nicotine does not act on the postsynaptic β₁-adrenoceptors located on the smooth muscle cells to induce a relaxation. Rather, NE acts as a presynaptic transmitter on presynaptic β₂-adrenoceptors on the NOergic nerves to cause release of NO and therefore vasodilation. The presence of presynaptic β₂-adrenoceptors on the NOergic nerves is supported by results from double-labeling studies that NADPHd reactive fibers (markers for NOS-I fibers) are almost coincident with β₂-adrenoceptor immunoreactive fibers in both basilar and middle cerebral arteries. However, not all β₂-adrenoceptor immunoactive fibers were NADPHd reactive. This is an expected result, since β₂-adrenoceptors are also found in association with other types of nerves such as the noradrenergic neurons (24).

Similar results were found in isolated large cerebral arteries at the base of the cat brain in which nicotine-induced vasodilation, which was sensitive to L-NNA, was blocked by β₂- but not β₁-adrenoceptor antagonists (our preliminary data). In these cerebral arteries of the cat, postsynaptic α-adrenoceptors are predominant. Accordingly, exogenous NE has been shown to induce a constriction exclusively (16, 22). These findings clearly indicate that nicotine-induced vasodilation in the cat cerebral arteries cannot be due to a direct effect of NE on the postsynaptic smooth muscle cells. Rather, NE as a presynaptic transmitter acts on presynaptic β₂-adrenoceptors located on NOergic nerves to cause release of NO, which then induces vasodilation. This conclusion is consistent with the reported biochemical findings that neurogenic vasodilation in cerebral arteries from different species induced by either TNS or nicotine is accompanied by an increase in cGMP but not cAMP (9, 15, 29), suggesting that the terminal transmitter act-
ing on the smooth muscle to induce a relaxation is NO (known to increase cGMP synthesis) or a related substance but not NE (known to increase cAMP synthesis by its β-adrenoceptor activity).

In addition to that found in the cerebral arteries in the present studies, the presence of β2-adrenoceptors on NOergic nerves in the pulmonary and mesenteric arteries of the rat has been speculated (21, 25). Adrenergic nerve terminals in several preparations have been shown to contain β2-adrenoceptors, and these receptors mediate positive feedback of NE release (24, 30). Blockade of these presynaptic receptors by propranolol and other β-adrenoceptor antagonists only partially decreases the release of NE or NE-mediated vascular responses induced by nicotine or field electrical nerve stimulation (24, 30). This is different from the present findings that nicotine-induced NE-mediated NOergic vasodilation was abolished by propranolol and preferential β2-adrenoceptor antagonists. It is possible that the presynaptic β2-adrenoceptors on NOergic nerve terminals are more sensitive than those located on the adrenergic nerve terminals to β-adrenoceptor antagonists.

It should be pointed out that NE is generally considered to be a weak agonist for β2-adrenoceptors in the cardiovascular system (24). The possibility that other receptor subtypes such as the β3-adrenoceptors and β1-adrenoceptors (12) are involved in NE-mediated NO release remains to be clarified. However, the complete blockade of nicotine-induced relaxation by propranolol, which is not a ligand for β3-adrenoceptors (12), and the failure of CGP-20712A, which is a β1- and β4-adrenoceptor antagonist (12), in blocking nicotine-induced relaxation render this possibility tenuous.

The involvement of the presynaptic α2-adrenoceptors in mediating inhibition of NO release from NOergic nerves and NE release from adrenergic nerves in peripheral vascular preparations has been reported (2). This appears to be true also in the porcine cerebral arteries, since nicotine-induced relaxation was potentiated by yohimbine but not by prazosin. This is consistent with the present hypothesis that increased NE release after blocking presynaptic α2-adrenoceptors on the sympathetic nerves by yohimbine can result in increased NO release from the NOergic nerves and enhanced vasodilation. The relative significance of α2-adrenoceptors located on adrenergic sympathetic nerve terminals and NOergic nerve terminals in mediating nicotine-induced NO-mediated relaxation remains to be determined. The presynaptic β2-adrenoceptors on NOergic nerve terminals, however, appear to be predominant in cerebral perivascular nerves, since NE-mediated nicotine-induced NOergic vasodilation in the absence of yohimbine was demonstrated.

Results of our previous (34) and present studies indicate that nicotine-induced NO-mediated neurogenic vasodilation in porcine basilar arteries is indirectly mediated by release of NE from sympathetic nerves. Nicotine does not act directly on NOergic nerves to elicit an NO-mediated vasodilation (34). This effect of nicotine in inducing NO-mediated neurogenic vasodilation is different from that by TNS. The latter depolarizes the NOergic and sympathetic nerve terminals simultaneously, resulting in NO-mediated relaxation, although NE also is released upon TNS (our preliminary results). It is possible that direct depolarization of the NOergic nerves by TNS at various frequencies, resulting in NO release, is already at the maximum enzyme stimulating capacity of each frequency. An additional modulatory effect elicited by simultaneous release of NE from the sympathetic nerves may be relatively small and therefore is not detected. This may explain the well-established findings of the failure of guanethidine (a blocker of sympathetic adrenergic transmission), propranolol, preferential β2-adrenoceptor antagonists, yohimbine, and other α-adrenoceptor antagonists in affecting TNS-elicited NO-mediated neurogenic vasodilation in cerebral arteries (Refs. 16, 17, 32, and 34 and the present results).

In summary, the present study demonstrated that nicotine-induced, NO-mediated relaxation in porcine basilar arteries was inhibited by blocking presynaptic β2-adrenoceptors and was enhanced by blocking presynaptic α2-adrenoceptors. Although the distribution of both presynaptic β2- and α2-adrenoceptors in adrenergic and NOergic nerves remains to be determined, results from the present studies suggest that these receptors are present on NOergic nerve terminals. Similar results were found in the cat middle cerebral arteries and porcine internal carotid arteries (our preliminary results). These findings together with those from previous studies provide strong evidence that NE is the most likely mediator, released from the adrenergic nerve terminals upon application of nicotine, and acting predominantly on β2-adrenoceptors located on NOergic nerve terminals to cause release of NO and the resulting cerebral vasodilation (Fig. 5). NE therefore acts predominantly as a presynaptic transmitter. Accordingly, it is possible that regional vasoconstriction induced by electrical stimulation of the sympathetic nerves in vivo may be offset by immediate vasodilation in the same regions due to NO release from NOergic nerves. This finding may provide an explanation for the reported observations that electrical stimulation of the sympathetic nerves to cerebral circulation in normal experimental animals in general results in a very weak effect or no response in cerebral vascular tone and cerebral blood flow (3, 6, 7). This concept of presynaptic modulation of NOergic nerves by sympathetic adrenergic nerves appears to be supported by reports from some in vivo experimentation that the functional consequence of neuronal NO and NE interaction may play a role in blood pressure regulation (26).

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REFERENCES

1. Barroso CP, Edvinsson L, Zhang W, Cunha e Sa M, Springall DR, Polark JM, and Gulbenkian S. Nitroxidergic inner-


