Mechanisms That Produce Nitric Oxide–Mediated Relaxation of Cerebral Arteries During Atherosclerosis

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Background and Purpose—The first goal of the present study was to examine the hypothesis that relaxation of cerebral arteries to nitric oxide in primates is dependent on activation of soluble guanylate cyclase (sGC). The second goal was to determine whether the role of sGC in mediating responses to nitric oxide is altered in atherosclerosis.

Methods—Basilar arteries from normal and atherosclerotic monkeys were studied in vitro. After precontraction with prostaglandin F$_{2a}$ (0.1 to 1 μmol/L), concentration-response curves to authentic nitric oxide (1 nmol/L to 1 μmol/L), sodium nitroprusside (10 nmol/L to 10 μmol/L; a nitric oxide donor), and papaverine (10 nmol/L to 10 μmol/L; a non–nitric oxide, non–sGC-dependent stimulus) were generated in the presence and absence of 1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 1 and 10 μmol/L; an inhibitor of sGC). The effect of ODQ on basal tone of basilar arteries from normal and atherosclerotic monkeys was also examined.

Results—Nitric oxide, sodium nitroprusside, and papaverine produced relaxation that was similar (P>0.05) in normal and atherosclerotic monkeys. ODQ produced marked inhibition (P<0.05) of vasorelaxation in response to nitric oxide and nitroprusside but not papaverine. For example, relaxation of the basilar artery in response to nitric oxide (0.1 μmol/L) was inhibited by approximately 85% and 73% by ODQ (1 μmol/L) in normal and atherosclerotic monkeys, respectively. ODQ produced contraction of the basilar arteries, and the increase in tension to ODQ was greater in normal (2.7±0.3 g; mean±SE) than in atherosclerotic monkeys (1.4±0.4 g; P<0.05). In contrast, contraction to prostaglandin F$_{2a}$ was similar in the basilar artery from normal and atherosclerotic monkeys.

Conclusions—These findings suggest that (1) relaxation of cerebral arteries in primates in response to nitric oxide is normally dependent, in large part, on activation of sGC and (2) the influence of sGC (via reduced production and/or activity of basal nitric oxide) on cerebral vascular tone is reduced in atherosclerosis. (Stroke. 2001;32:761-766.)

Key Words: atherosclerosis ■ basilar artery ■ guanylate cyclase ■ nitric oxide ■ vasodilation ■ monkeys

Nitric oxide is a potent dilator of cerebral arteries and microvessels.1–3 Nitric oxide may relax blood vessels by pathways that are dependent and/or independent of soluble guanylate cyclase (sGC).4,5 sGC-dependent relaxation involves nitric oxide binding to the heme portion of sGC with resultant cGMP formation. A rise in cellular cGMP results in smooth muscle relaxation via reduction of intracellular calcium and/or potassium channel activation. Nitric oxide has also been shown to directly activate potassium channels independent of cGMP formation; however, in cerebral vessels, a role for this pathway remains uncertain.

Previous studies from our laboratory and others have provided evidence that sGC plays a major role in mediating relaxation of cerebral blood vessels to nitric oxide.6–11 For example, we found previously that 1H-[1,2,4]-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; an inhibitor of sGC) almost completely abolished relaxation to acetylcholine (which causes production of endogenous nitric oxide) and nitroprusside (a nitric oxide donor) in both mouse cerebral arterioles and rat basilar artery.9,10 There are species differences in regulatory mechanisms in the cerebral circulation; however, the role of sGC in cerebral vessels of primates is not known. Thus, the first goal of the present study was to test the hypothesis that nitric oxide–induced relaxation of primate cerebral arteries is dependent on activation of sGC.

Previous studies have described either preserved or impaired formation of cGMP as well as impaired relaxation of extracranial vessels to nitric oxide during atherosclerosis, suggesting diminished sGC activity.12–16 In cerebral vessels, the effect of atherosclerosis on responses to nitric oxide is not known. Thus, the second goal of the present study was to determine whether the role of nitric oxide–induced activation of sGC in primate cerebral vessels is altered in atherosclerosis.

Materials and Methods

Experimental Groups
Adult male cynomolgus monkeys (Macaca fascicularis) were randomly assigned to receive either a normal or an atherogenic diet, as described previously.17 Seven monkeys (normal group) were fed commercial laboratory chow (Tekland Monkey Chow), and 11 monkeys (atherosclerotic group) were fed an atherogenic diet for 3.6 months.
Vascular Ring Preparation

Animals were euthanatized with pentobarbital sodium (200 mg/kg IV) and exsanguinated. Basilar arteries were dissected from the brain stem and placed in Krebs’ buffer (pH 7.4) with the following ionic composition (mmol/L): NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, glucose 11. Loose connective tissue was removed from the adventitial surface, and each artery was then cut into 4 ring segments (each 4 mm in length). Vascular rings were mounted on a pair of triangular hooks and suspended in individual organ chambers containing 20 mL Krebs’ solution maintained at 37°C and bubbled continuously with 95% O2 and 5% CO2. The rings were connected to a force transducer to measure isometric tension (contraction and relaxation). Resting tension was increased stepwise to reach final resting tension of 1 g. This amount of tension was found to be optimal for this artery in our own preliminary experiments and in previous studies.

We have used these methods previously.

Response of Cerebral Arteries to Vasodilator Agonists

Cerebral arteries were allowed to equilibrate for 60 minutes before addition of vasodilator agonists. Krebs’ buffer was replaced with fresh buffer every 15 minutes throughout the study, except during generation of concentration-response curves. To determine the influence of cGMP on cerebral vascular tone under basal conditions, rings were randomly chosen to be incubated with either vehicle (dimethyl sulfoxide; DMSO) or ODQ (1 or 10 μmol/L) for 30 minutes. Vascular rings were then contracted submaximally (70% to 80% of baseline) with prostaglandin F2α (PGF2α) on the response to PGF2α.

Atherosclerotic Monkeys

Effect of ODQ on Baseline Tone

ODQ produced a significant increase in baseline tone of basilar arteries from normal and atherosclerotic monkeys (Figure 1). In contrast, incubation of vessels with vehicle had no effect on baseline tone in either normal (change in tension of 0.1 g) or atherosclerotic (change in tension of 0.05) on baseline tone in either normal (change in tension of 0.05) on baseline tone in either normal (change in tension of 4.3 versus 2.2 g [n = 7]).

Plasma Lipids and Vascular Morphology

Total plasma cholesterol was significantly higher in atherosclerotic monkeys than in normal monkeys (356 ± 3 versus 93 ± 2 mg/dL, respectively; P < 0.001). No gross or microscopic evidence of atherosclerotic lesions was detectable in basilar arteries of normal monkeys. By gross visual examination, atherosclerotic lesions were noted in basilar arteries of atherosclerotic monkeys (5 of 11 animals). This observation was confirmed on microscopic examination of sections stained with Oil Red O (data not shown). Vascular reactivity was examined in vascular segments adjacent to lesions (either proximal or distal). However, vessel segments that were studied did not contain significant lesions themselves.

Vascular Responses in Normal and Atherosclerotic Monkeys

Authentic nitric oxide produced concentration-dependent relaxation of basilar arteries from normal and atherosclerotic monkeys (Figures 3 and 4). Relaxation to nitric oxide was similar in normal and atherosclerotic monkeys (Figure 4; P > 0.05), which indicates that responses to nitric oxide are preserved in basilar arteries during atherosclerosis. Nitroprusside also produced concentration-dependent relaxation of basilar arteries that was similar in normal and atherosclerotic monkeys (P > 0.05; data not shown).

ODQ (1 μmol/L) inhibited (P < 0.05) the majority of the relaxation to nitric oxide (Figures 3 and 4) and nitroprusside (data not shown) in basilar arteries from normal and atherosclerotic monkeys, suggesting that activation of sGC is the major mechanism of relaxation of primate cerebral arteries in...
response to nitric oxide. At submaximal concentrations of nitric oxide, virtually all of the vasorelaxation was inhibited by ODQ. For example, 0.1 μmol/L nitric oxide relaxed the basilar artery from normal monkeys 61 ± 6% and 10 ± 3% in the absence and presence of ODQ (1 μmol/L), respectively. A higher concentration of ODQ (10 μmol/L) had a similar inhibitory effect (P < 0.05) but did not produce any greater inhibition than that obtained with 1 μmol/L ODQ in response to nitric oxide or nitroprusside in either group of monkeys.

Papaverine appears to produce relaxation of smooth muscle through inhibition of L-type calcium channels and possibly through phosphodiesterase inhibition. Neither mechanism is dependent on nitric oxide or sGC.21,22 In the present study papaverine produced concentration-dependent relaxation of basilar arteries from normal and atherosclerotic monkeys, which was not affected by ODQ (Figure 5; P > 0.05). Thus, these findings suggest that the inhibitory effects of ODQ on responses to nitric oxide and nitroprusside were due to selective inhibition of sGC.

Discussion
There are several major findings in the present study. First, the findings with ODQ suggest that activation of sGC is the major mechanism of relaxation to nitric oxide in primate cerebral arteries. Second, ODQ produced less contraction of the basilar artery in atherosclerotic monkeys, suggesting that the influence of sGC on baseline vascular tone is reduced during atherosclerosis. This reduced response to ODQ may reflect reduced basal production and/or activity of nitric oxide in cerebral vessels during atherosclerosis. Third, cerebral vascular reactivity to nitric oxide was generally similar in atherosclerotic and normal monkeys, suggesting that functional activation of sGC by nitric oxide is preserved in atherosclerosis. Thus, although basal nitric oxide release may be reduced, responsiveness to nitric oxide in primate cerebral arteries is generally preserved in atherosclerosis.

Role of sGC in the Cerebral Circulation Under Normal Conditions
The results of the present study are consistent with previous findings that have suggested that endothelium-dependent
responses and vasodilation to nitric oxide donors are dependent on activation of sGC in the carotid artery and in cerebral blood vessels. Previous studies using ODQ have suggested that most or all of the response to nitric oxide (both endogenously produced nitric oxide in response to endothelium-dependent stimuli and exogenously administered nitric oxide donors) is mediated by sGC. These previous findings were obtained in large cerebral arteries and cerebral microvessels (including parenchymal vessels) from nonprimate species, including mouse and rat. The present findings with ODQ suggest that a similar mechanism is present in cerebral arteries from primates.

ODQ appears to be a very effective and selective inhibitor of sGC. In purified preparations, ODQ is a potent inhibitor of sGC. At the same concentrations used in these experiments, ODQ does not inhibit endothelial nitric oxide synthase or vascular responses to cGMP, adenosine, or papaverine. Consistent with these studies, we found that ODQ did not alter relaxation of primate cerebral arteries to papaverine. In contrast to other inhibitors of sGC, namely LY-83583 and methylene blue, ODQ does not produce superoxide, which rapidly inactivates nitric oxide. It appears that the effect of ODQ in the present study was maximal, because effects of 10 μmol/L ODQ were not greater than that of 1 μmol/L ODQ. The present and previous findings in the cerebral circulation related to the role of sGC in mediating vascular responses to nitric oxide are also consistent with studies in extracranial vessels from humans as well as vessels from normal and genetically altered mice.

As in previous studies, ODQ was particularly effective in inhibiting responses to submaximal concentrations of nitric oxide. However, at high concentrations of nitric oxide, not all of the vasorelaxation was inhibited by ODQ. Residual vasorelaxation to higher concentrations of nitric oxide in the presence of ODQ may be accounted for by additional mechanisms, such as direct activation of potassium channels by nitric oxide or inhibition of 20-hydroxyeicosatetraenoic acids (HETE) formation.

### Effects of Atherosclerosis on Cerebral Vessels

Previous studies have demonstrated impaired endothelium-dependent responses in various experimental models of atherosclerosis and in vessels from atherosclerotic patients. Mechanisms underlying this dysfunction are not completely defined but may include a reduction in agonist-induced nitric oxide production, enhanced inactivation of nitric oxide by reactive oxygen species, and/or increased levels of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase. Additionally, vascular dysfunction in atherosclerosis may also involve an alteration in basal nitric oxide production, which normally plays an important role in modulation of vascular tone. For example, functional studies have demonstrated that pharmacological inhibition of endothelial nitric oxide synthase results in significantly less contraction in atherosclerotic versus normal vessels, suggesting that basal nitric oxide production/release is reduced in atherosclerosis.

In the present study ODQ produced marked increases in basal tone, which suggests that activation of sGC, presumably via tonic release of nitric oxide, influences vascular tone. This finding is consistent with in vivo observations that ODQ produces constriction of the rat basilar artery. The amount of contraction produced by ODQ in the present study was significantly greater in basilar artery from normal monkeys than in atherosclerotic monkeys, suggesting a reduction of basal production/release of nitric oxide in atherosclerotic vessels. Nevertheless, basilar arteries from atherosclerotic monkeys in the present study maintained their ability to relax to nitric oxide, suggesting that mechanisms that mediate responses to nitric oxide are intact.

We have previously demonstrated that endothelium-dependent responses of extracranial vessels (ie, carotid artery) from atherosclerotic monkeys are impaired compared with those obtained from normal monkeys. Consistent with these previous studies, preliminary findings from the same atherosclerotic monkeys used in the present study also demonstrate impaired endothelium-dependent relaxation to acetylcholine in extracranial vessels. Taken together, our previous studies and those employing carotid artery from the same monkeys used in the present study suggest that long-term treatment with an atherogenic diet selectively impairs endothelium-dependent responses of extracranial vessels. In the present study we did not examine responses to endothelium-dependent stimuli. We chose not to pursue this direction because of the limited number of animals available and the fact that cerebral vessels in primates do not relax to acetylcholine. Thus, we are unable to compare responses of extracranial vessels with those of intracranial vessels in terms of endothelium-dependent relaxation.

Previous studies have demonstrated that atherosclerosis impairs responsiveness to acetylcholine in extracranial arteries (ie, aorta and carotid artery) but not in intracranial arteries (ie, basilar and branches of the middle cerebral artery) in a rabbit model of atherosclerosis. These differences have been attributed to the presence of lesions in extracranial arteries and relative absence of lesions in intracranial arteries. Thus, previous studies have suggested that intracranial arteries are relatively spared from lesions and hence maintain normal vascular responsiveness. In the present study atherosclerotic lesions were present in approximately half of basilar arteries from monkeys that were fed an atherogenic diet. The absence of lesions in intracranial vessels in previous studies and the presence of lesions in the present study are most likely related to the experimental model and length of time on the atherogenic diet. Monkeys in the present study were fed an atherogenic diet for 3 years, whereas in previous studies, using a rabbit model, the length of the atherogenic diet was 10 to 12 weeks. Both the response to nitric oxide and the amount of inhibition of nitric oxide-induced relaxation by ODQ was similar in normal and atherosclerotic monkeys, which suggests preservation of sGC activation in response to nitric oxide during atherosclerosis. In this respect, the present results are consistent with a previous study in carotid artery in which normal production of...
cGMP and preserved relaxation to authentic nitric oxide was observed in rabbits fed an atherogenic diet for 12 weeks. A major strength of the present study relates to the use of a primate model of atherosclerosis, which is thought to more closely resemble the development of atherosclerosis in humans.

In summary, results of the present study suggest that activation of sGC is the major mechanism that produces relaxation of cerebral arteries in primates to nitric oxide under normal conditions. This response is generally preserved during atherosclerosis. A reduction in the influence of sGC under basal conditions appears to be present in atherosclerotic monkeys. This effect is probably related to a reduction in production and/or activity of basal nitric oxide during atherosclerosis.

References


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