Impaired Relaxation of the Carotid Artery During Activation of ATP-Sensitive Potassium Channels in Atherosclerotic Monkeys

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Background and Purpose
This study examined the hypotheses that (1) atherosclerosis impairs relaxation of the carotid artery in response to activation of adenosine triphosphate (ATP)-sensitive potassium channels and (2) regression of atherosclerosis restores the response toward normal.

Methods
Isometric tension was measured in rings of carotid artery taken from normal, atherosclerotic, and regression monkeys and precontracted submaximally with prostaglandin F2α.

Results
Relaxation in response to acetylcholine was less in atherosclerotic compared with normal arteries (54±4% [mean±SE] in response to 3×10⁻⁶ mol/L acetylcholine, P<.01). Relaxation in response to aprikalim, a direct activator of ATP-sensitive potassium channels, was also less in atherosclerotic than in normal arteries (32±7% versus 69±2% during 10⁻⁴ mol/L aprikalim, P<.01). Relaxation in response to aprikalim but not to acetylcholine or nitroprusside was inhibited almost completely by glibenclamide (4 μmol/L), a selective inhibitor of ATP-sensitive potassium channels. Relaxation in response to low but not high (10⁻⁶ to 10⁻⁵ mol/L) concentrations of sodium nitroprusside was less in atherosclerotic than in normal arteries. Regression of atherosclerosis tended to restore responses to acetylcholine, but not responses to nitroprusside or aprikalim, toward normal.

Conclusions
These findings suggest that atherosclerosis impairs relaxation of the carotid artery in response to activation of ATP-sensitive channels. Impaired responses may be due, in part, to nonspecific impairment of relaxation. Regression of atherosclerosis did not restore responses of the carotid artery toward normal. (Stroke. 1994;25:178-182.)

Key Words
- acetylcholine
- atherosclerosis
- carotid arteries
- monkeys
- potassium channels

See Editorial Comment, page 182

Impaired relaxation of the carotid artery in response to activation of ATP-sensitive potassium channels. We also determined whether dietary treatment of atherosclerosis (regression) restores responses to activation of ATP-sensitive potassium channels toward normal.

Materials and Methods
We studied three groups of adult cynomolgus monkeys. Nine normal monkeys were fed commercial chow (Purina Monkey Chow, Ralston Purina, Richmond, Ind), which produced plasma cholesterol concentrations of 119±6 (mean±SE) mg/dL. In a second group of six monkeys, atherosclerosis was induced by feeding the monkeys an atherogenic diet that contained 41% of total calories from fat and 0.8% cholesterol for 27±2 months. Plasma cholesterol concentrations in this group were 611±43 mg/dL. A third group of seven monkeys was fed an atherogenic diet for 21±1 months and was subsequently fed standard monkey chow for 16±4 months. The plasma cholesterol in this group was 694±57 mg/dL while they received the atherogenic diet and 117±8 mg/dL when they received the normal diet. We have described this primate model of regression of atherosclerosis and the morphological changes that occur in the carotid circulation in detail previously.16

Animals were sedated with 12 mg/kg ketamine and then anesthetized with 75 to 100 mg/kg α-chloralose intravenously. Supplemental anesthesia was administered as needed. The trachea was cannulated and the monkey ventilated mechanically with room air and supplemental oxygen. One common carotid artery was exposed, ligated proximally and distally with suture, and the isolated segment of artery was removed and placed into Krebs solution.
Segments of carotid artery were cut into 5-mm rings and suspended in organ baths using two stainless-steel stirrups. A force transducer was used to record isometric tension in vessels continuously bathed with a modified Krebs solution with the following composition (in mmol/L): NaCl 118.3, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, ethylenediaminetetraacetic acid calcium 0.026, glucose 11.1, pH 7.4. The buffer was maintained at 37°C and aerated with 95% O₂/5% CO₂. Vessels were gradually stretched to a resting tension of approximately 2 g, which produced optimal contraction in response to KCl and prostaglandin F₂α in vessels from all three groups of animals. To examine relaxation responses, rings were precontracted submaximally (approximately 50% to 60% of maximum) with prostaglandin F₂α.

After reaching a stable contraction plateau, vascular rings were exposed to acetylcholine, nitroprusside, and aprikalim. Aprikalim [(trans-)-N-methyl-2-(3-pyridyl)-2-tetrahydro-10-1-carboxamidio-1-oxide], formerly RP52891, is a direct activator of ATP-sensitive potassium channels, and the DMSO vehicle had no significant effect on vascular tone in precontracted arteries. Glibenclamide produced almost complete inhibition of relaxation of the carotid artery in response to aprikalim (Fig 1). Aprikalim also produced relaxation in atherosclerotic arteries, but responses were significantly impaired compared with responses in normal arteries (Fig 1). For example, 10⁻⁶ mol/L aprikalim produced relaxation in normal and atherosclerotic arteries by 69±5% and 32±7% (P<.01 versus normal), respectively. Regression of atherosclerosis was not associated with improvement of relaxation in response to aprikalim (P>.05)(Fig 1). As in normal animals, relaxation in response to aprikalim in arteries from atherosclerotic and regression animals was inhibited by glibenclamide (Fig 1).

Acetylcholine, an endothelium-dependent vasodilator, produced relaxation of carotid arteries from normal animals that was not altered by glibenclamide (Fig 2). Relaxation in response to acetylcholine was significantly impaired in atherosclerotic arteries and was restored partially toward normal in arteries from regression animals (Fig 2).

Sodium nitroprusside was used as a control to compare different groups of animals with a Bonferroni correction for multiple comparisons. All values were expressed as mean±SE. A value of P<.05 was considered significant.

**Results**

The carotid artery from normal monkeys had no gross or microscopic evidence of atherosclerotic lesions. In contrast, the carotid artery from atherosclerotic monkeys was observed to have lesions that ranged from fatty streaks to fibrofatty plaques. We have previously examined this artery morphometrically and observed marked intimal proliferation in this model. Aprikalim, a direct activator of ATP-sensitive potassium channels, produced marked relaxation of the carotid artery from normal monkeys (Fig 1). The highest concentration of aprikalim (3×10⁻⁶ mol/L) relaxed normal carotid arteries by approximately 80%. We did not examine the effects of higher concentrations of aprikalim because such experiments would have required relatively large quantities of aprikalim.

Glibenclamide, an inhibitor of ATP-sensitive potassium channels, and the DMSO vehicle had no significant effect on vascular tone in precontracted arteries. Glibenclamide produced almost complete inhibition of relaxation of the carotid artery in response to aprikalim (Fig 1). Aprikalim also produced relaxation in atherosclerotic arteries, but responses were significantly impaired compared with responses in normal arteries (Fig 1). For example, 10⁻⁶ mol/L aprikalim produced relaxation in normal and atherosclerotic arteries by 69±5% and 32±7% (P<.01 versus normal), respectively. Regression of atherosclerosis was not associated with improvement of relaxation in response to aprikalim (P>.05)(Fig 1). As in normal animals, relaxation in response to aprikalim in arteries from atherosclerotic and regression animals was inhibited by glibenclamide (Fig 1).

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from atherosclerotic and regression animals ($P>0.05$) (Fig 3).

**Discussion**

There are two major findings in the present study. First, relaxation of the carotid artery in response to the endothelium-dependent agonist acetylcholine is impaired during atherosclerosis and tended to improve with dietary regression of atherosclerosis. These findings confirm previous studies describing impaired endothelium-dependent relaxation in other blood vessels during atherosclerosis. Relaxation of the carotid artery in response to acetylcholine was not altered by glibenclamide, suggesting that responses of this blood vessel to acetylcholine are not dependent on activation of ATP-sensitive potassium channels.

Second, activation of ATP-sensitive potassium channels with aprikalim produces glibenclamide-sensitive relaxation of the carotid artery. Thus, ATP-sensitive potassium channels appear to be present in the carotid artery in primates. Relaxation of the carotid artery in response to aprikalim was impaired significantly by atherosclerosis. Relaxation in response to low concentrations of nitroprusside tended to be less than relaxation in normal arteries. This difference was statistically significant for low concentrations of nitroprusside. In contrast, maximum relaxation in response to higher concentrations of nitroprusside was not impaired significantly by atherosclerosis.

These findings in carotid arteries of monkeys, that atherosclerosis impairs relaxation in response to low but not high concentrations of a nitrovasodilator, are similar to findings in human coronary arteries. Sodium nitroprusside relaxes vascular muscle by activating guanylate cyclase, which produces an accumulation of cyclic guanosine monophosphate. The tendency for reduction of relaxation of atherosclerotic arteries in response to nitrovasodilators may be due to impairment in the guanylate cyclase system, or it may reflect structural alterations in the vessel wall. Nonspecific impairment of vasodilatation, if present, would presumably contribute to reduced relaxation in response to all agonists, but impairment of responses to aprikalim and acetylcholine in atherosclerotic vessels tended to be greater than impairment of responses to nitroprusside, especially at higher concentrations.

**Responses to Acetylcholine**

There is substantial evidence for endothelial dysfunction in atherosclerotic blood vessels. Atherosclerosis impairs basal and agonist-induced production or activity of EDHF and endothelium-dependent relaxation in humans and animal models of atherosclerosis. In agreement with this concept, we observed marked impairment of relaxation of the carotid artery in monkeys in response to acetylcholine, the “classic” agonist in studies of endothelium-dependent relaxation and EDHF. Relaxation of the carotid artery in response to acetylcholine was restored partially toward normal by dietary treatment of atherosclerosis. This observation supports previous studies suggesting that regression of atherosclerosis improves endothelium-dependent responses.

In some blood vessels, endothelium-dependent relaxation in response to acetylcholine appears to be mediated, in part, by release of an EDHF that activates ATP-sensitive potassium channels. Activation of these potassium channels produces hyperpolarization of vascular muscle and relaxation that is inhibited selectively by glibenclamide. In the present study, relaxation of the carotid artery in monkeys in response to acetylcholine was not altered by glibenclamide, which suggests that activation of ATP-sensitive potassium channels does not contribute to endothelium-dependent relaxation in response to acetylcholine in this artery. The concentration of glibenclamide used was efficacious because it produced marked inhibition of relaxation in response to aprikalim.

**Responses to Sodium Nitroprusside**

Relaxation of atherosclerotic carotid artery responses to nitroprusside tended to be less than relaxation in normal arteries. This difference was statistically significant for low concentrations of nitroprusside. In contrast, maximum relaxation in response to higher concentrations of nitroprusside was not impaired significantly by atherosclerosis.

First, relaxation of the carotid artery in response to nitroprusside in monkeys in response to nitroprusside was significantly impaired by atherosclerosis. Atherosclerosis impaired relaxation in response to nitroprusside, especially at lower concentrations. Thus, relaxation in response to nitroprusside tended to be less than relaxation in normal arteries. This difference was statistically significant for low concentrations of nitroprusside. In contrast, maximum relaxation in response to higher concentrations of nitroprusside was not impaired significantly by atherosclerosis.

These findings in carotid arteries of monkeys, that atherosclerosis impairs relaxation in response to low but not high concentrations of a nitrovasodilator, are similar to findings in human coronary arteries. Sodium nitroprusside relaxes vascular muscle by activating guanylate cyclase, which produces an accumulation of cyclic guanosine monophosphate. The tendency for reduction of relaxation of atherosclerotic arteries in response to nitrovasodilators may be due to impairment in the guanylate cyclase system, or it may reflect structural alterations in the vessel wall. Nonspecific impairment of vasodilatation, if present, would presumably contribute to reduced relaxation in response to all agonists, but impairment of responses to aprikalim and acetylcholine in atherosclerotic vessels tended to be greater than impairment of responses to nitroprusside, especially at higher concentrations.
Impaired Relaxation of Carotid Artery

Faraci et al

aprikalim are inhibited by glibenclamide.5,7,22,24 Glibenclamide is considered to be a selective inhibitor of ATP-sensitive potassium channels, and it has been used widely for this purpose.2,4 Second, vasodilation in response to aprikalim is not attenuated by an inhibitor of nitric oxide synthase24 or inhibitors of other potassium channels (apamin and charybdotoxin).24

At least two mechanisms may account for impaired relaxation of the carotid artery in response to activation of ATP-sensitive potassium channels during atherosclerosis. First, a recent study suggests that atherosclerosis alters activity of calcium-dependent potassium channels in vascular muscle of the human aorta.25 It is possible that atherosclerosis also produces functional changes in ATP-sensitive potassium channels. Second, the finding that relaxation in response to low concentrations of nitroprusside was impaired during atherosclerosis suggests that nonspecific impairment of dilator responses may be present. Thus, nonspecific impairment may contribute to reduced relaxation in response to aprikalim in atherosclerotic carotid arteries.

In summary, the present study suggests that in addition to impairment of endothelium-dependent responses, atherosclerosis impairs relaxation of the carotid artery in response to activation of ATP-sensitive potassium channels. This impairment may be due to specific effects on ATP-sensitive potassium channels and nonspecific impairment of relaxation during atherosclerosis. Impairment of normal mechanisms that mediate vasodilatation may contribute to vasospasm during atherosclerosis.

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