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 (5±6% versus 54±4% [mean±SE] in response to 3×10⁻⁴ 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±5% 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.

Key Words • acetylcholine • atherosclerosis • carotid arteries • monkeys • potassium channels
Fig 1. Relaxation in response to aprikalim under control (Con) conditions in the presence of vehicle and in the presence of glibenclamide (Glib) in carotid arteries from normal (left panel), atherosclerotic (middle panel), and regression (right panel) monkeys. To examine responses to aprikalim, arteries were precontracted submaximally with prostaglandin F\(_2\alpha\). Values are mean±SE (n=9 normal, 6 atherosclerotic, and 7 regression animals). Relaxation in response to aprikalim in the presence of vehicle was significantly reduced in the atherosclerotic (P<.05 vs normal) and regression groups. Relaxation in response to aprikalim in arteries from atherosclerotic (middle panel), and regression (right panel) monkeys. To examine responses to acetylcholine, arteries were precontracted submaximally with prostaglandin F\(_2\alpha\). Values are mean±SE (n=9 normal, 6 atherosclerotic, and 7 regression animals). Relaxation in response to acetylcholine in the presence of vehicle was significantly reduced in the atherosclerotic (P<.05 vs normal at 3x10^{-8}, 10^{-7}, 10^{-6}, and 3x10^{-5} mol/L acetylcholine) and regression (P<.05 vs normal at 3x10^{-8}, 10^{-7}, 3x10^{-6}, and 10^{-5} mol/L acetylcholine) groups.

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, CaCl\(_2\) 2.5, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 25, ethylenediaminetetraacetic acid calcium 0.026, glucose 11.1, pH 7.4. The buffer was maintained at 37°C and aerated with 95% O\(_2\)/5% CO\(_2\). Vessels were gradually stretched to a resting tension of approximately 2 g, which produced optimal contraction in response to KCl and prostaglandin F\(_2\alpha\) in vessels from all three groups of animals. To examine relaxation responses, rings were precontracted submaximally (approximately 50% to 60% of maximum) with prostaglandin F\(_2\alpha\).

After reaching a stable contraction plateau, vascular rings were exposed to acetylcholine, nitroprusside, and aprikalim. Aprikalim [(trans-(-)-N-methyl-2-(3-pyridyl)-2-tetrahydrothio-pyran carbothiamide-1-oxide)], formerly RP52891, is a direct activator of ATP-sensitive potassium channels.\(^5\) In all three groups of animals, parallel experiments were performed in the presence of glibenclamide (4 μmol/L), a specific inhibitor of ATP-sensitive potassium channels.\(^1\) Stock solutions of aprikalim and glibenclamide (both 1 mmol/L) were prepared in dimethyl sulfoxide (DMSO). Control experiments were performed in the presence of the DMSO vehicle. All experiments were performed in the presence of indomethacin (10 μmol/L).

Statistical analysis was performed using unpaired \(t\) tests to compare different groups of animals with a Bonferroni correction for multiple comparisons. All values are 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.\(^6\)

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 (3x10^{-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 (Fig 1). As in normal arteries, 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, an endothelium-independent vasodilator, produced relaxation of normal arteries that was not affected by glibenclamide (Fig 3). Relaxation in response to nitroprusside tended to be less in atherosclerotic than in normal arteries (Fig 3). Responses to low concentrations of nitroprusside were inhibited significantly, but relaxation to higher concentrations of nitroprusside was not significantly different in normal and atherosclerotic blood vessels (Fig 3). Relaxation in response to nitroprusside was similar in carotid arteries
Responses to Acetylcholine

There is substantial evidence for endothelial dysfunction in atherosclerotic blood vessels. Atherosclerosis impairs basal and agonist-induced production or activity of EDRF and endothelium-dependent relaxation in humans and animal models of atherosclerosis.12-15 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 EDRF.19 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.15

In some blood vessels, endothelium-dependent relaxation in response to acetylcholine appears to be mediated, in part, by release of an EDRF that activates ATP-sensitive potassium channels.4,20 Activation of these potassium channels produces hyperpolarization of vascular muscle and relaxation that is inhibited selectively by glibenclamide.3,4,20 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 arteries 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.13,21 Sodium nitroprusside relaxes vascular muscle by activating guanylate cyclase, which produces an accumulation of cyclic guanosine monophosphate.22 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 vasodilation, 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 Aprikalim

Relaxation of the carotid artery in response to aprikalim was significantly impaired in atherosclerotic arteries. Relaxation of carotid arteries in response to aprikalim was inhibited almost completely by glibenclamide, which suggests that aprikalim activates ATP-sensitive potassium channels.2,5,7 Effects of glibenclamide were selective because the antagonist did not alter relaxation in response to nitroprusside or acetylcholine. These findings suggest that ATP-sensitive potassium channels are present and functional in the carotid artery. The concentration of glibenclamide used was efficacious because it produced marked inhibition of relaxation in response to aprikalim.

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 was also impaired during atherosclerosis. Thus, impairment of relaxation in response to aprikalim is due, at least in part, to nonspecific reduction in dilator responses of the carotid artery during atherosclerosis. In contrast to responses to acetylcholine, this nonspecific impairment of relaxation was not restored toward normal by regression of atherosclerosis.

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Potassium channels exist as a heterogeneous group including calcium-dependent and ATP-sensitive potassium channels.2,4 Two lines of evidence suggest strongly that aprikalim produces relaxation by activation of ATP-sensitive potassium channels.5,7 First, responses to...
aprikalim are inhibited by glibenclamide.\textsuperscript{5,7,23,24} Glibenclamide is considered to be a selective inhibitor of ATP-sensitive potassium channels, and it has been used widely for this purpose.\textsuperscript{2,4} Second, vasodilation in response to aprikalim is not attenuated by an inhibitor of nitric oxide synthase\textsuperscript{24} or inhibitors of other potassium channels (apamin and charybdotoxin).\textsuperscript{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.\textsuperscript{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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