Attenuation of Clonidine-Induced Vascular $\alpha_1$-Antagonistic Action in Hypercholesterolemic Rabbit Common Carotid Arteries

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ABSTRACT—We examined the effect of hypercholesterolemia on vasodilatory responses to clonidine in isolated and perfused rabbit common carotid arteries that had been preconstricted by phenylephrine. The responses decreased in rabbits fed an atherogenic diet for 4 or 8 weeks, whereas the responses to acetylcholine, nitroglycerin and substance P were not changed after the cholesterol feeding. Attenuated responses to clonidine were maintained for 24 weeks after cessation of the atherogenic diet, suggesting that this response might be an early marker of atherosclerosis.

Keywords: Common carotid artery (rabbit), Clonidine, Hypercholesterolemia

Although clonidine is a potent $\alpha_2$-adrenoceptor agonist, this drug acts as a weak $\alpha_1$-agonist in non-treated, isolated peripheral vasculatures and exceptionally as an $\alpha_1$-antagonist in phenylephrine-treated isolated rabbit peripheral vasculatures (1). We previously reported that in hypercholesterolemic common carotid arteries, vasoconstrictory responses to clonidine were attenuated in rabbits fed a high cholesterol supplement diet for 4 or 8 weeks (2). Since the responses to various vasoactive substances are generally considered to be augmented in hypercholesterolemic rabbit aorta (3-5) and the hyper-reactivities might contribute to atherogenesis, the decreased response in $\alpha$-adrenergic stimuli to clonidine (2, 5) appears paradoxical. In the present study, we investigated the effects of hypercholesterolemia and of cessation of an atherogenic diet on clonidine-induced vasodilatory responses in rabbit common carotid arteries by the cannula insertion method developed and modified by Hongo and Chiba (6) and Tsuji and Chiba (7) to clarify the mechanism for the attenuated response to clonidine in atherosclerotic vessels.

Five groups of male albino rabbits, aged 10-12 months and weighing 2.0-2.5 kg, were used: rabbits fed a normal diet (control); rabbits fed daily a diet supplemented with 120 g of 1% cholesterol and 4% lard for 4 weeks (group 2) or 8 weeks (group 3); and rabbits fed a high cholesterol diet for 8 weeks, following by a normal diet for 12 weeks (group 4) or 24 weeks (group 5). Rabbits used in all groups were age-matched ones. Both control and atherogenic diets were obtained from the Oriental Food Company, Tokyo. Rabbits were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), and the animals were killed by rapid exsanguination from the abdominal aorta after treatment with sodium heparin (200 units/kg, i.v.). Common carotid arteries were carefully isolated, and each vessel (2.0-3.0 cm) was cannulated with a 16 gauge, 4-cm-long cannula with small side holes 5 mm distant from the blind end. The cannulated vascular segment preparation was placed in a cup-shaped glass container maintained at 37°C and perfused at a constant flow rate (4 ml/min) with perfusate containing 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM KHPO$_4$, 1.2 mM MgSO$_4$, 25 mM NaHCO$_3$ and 10 mM glucose, which was bubbled with 95% O$_2$ and 5% CO$_2$. The perfusion pressure was continuously measured with an electric manometer, and vasodilation was calculated as the % decrease in perfusion pressure. To induce preconstrictions, 10$^{-5}$ M phenylephrine (PE) was continuously infused to obtain a stable long-lasting vasoconstriction. The method of cannula insertion has been described previously (5, 6). The drugs used were clonidine hydrochloride (CL; Boehringer Ingelheim, Germany), nitroglycerin (NTG; Nihon Kayaku, Tokyo), acetylcholine chloride (ACh; Daiichi, Tokyo), substance P (Sigma, St. Louis, MO, USA) and phenylephrine hydrochloride (PE; Kowa, Tokyo). The drug solution was administered into the rubber tubing close to the cannula in a volume of 0.01-0.03 ml for a period of 4 sec. The data are presented as mean values ± S.E.M. Statisti-
cal differences between two means and between curves were determined by the Student’s *t*-test, and significance was accepted when *P* < 0.05.

When clonidine was injected into the cannulated carotid artery preconstricted by 10^{-5} M PE, a vasodilator response was readily induced in a dose-related manner (Fig. 1). We previously reported that the responses were not modified by treatment with an α2-adrenoceptor antagonist or by endothelial denudation (1). We also found that in PGF2α- or 5-hydroxytryptamine (5-HT)-preconstricted vessels, clonidine caused a vasoconstriction that was suppressed only by α1-adrenoceptor blockade (1), suggesting that the vasodilatory response was due to antagonistic action against α1-adrenoceptors. In the experiments described here, plasma cholesterol (PC) levels were increased from 64±5 to 2312±72 mg/dl and triglyceride (TG) levels from 56±5 to 122±15 mg/dl in rabbits fed a high cholesterol diet for 8 weeks. Nevertheless, there were no microscopic atherosclerotic lesions in the vessel tested. Vasodilatory responses to clonidine were attenuated significantly in the high cholesterol fed groups at both 4 and 8 weeks. On the contrary, ACh-, NTG- and substance P-induced responses were not modified in any group (Fig. 1). After 8 weeks of high cholesterol feeding, normal diets were resumed to examine whether cessation of the atherogenic diet resulted in recover-

ey of vasodilatory properties. Even 24 weeks after cessation of the atherogenic diet, the responses were not changed and the attenuation was maintained (Fig. 2), even
when PC and TG levels had returned almost to normal (data not shown).

It has been reported that vasoconstrictions caused by phenylephrine, 5-HT or ergonovine, especially in the common carotid arteries of hypercholesterolemic rabbits, were not different from those in the controls (4) and that the responses to norepinephrine or clonidine were slightly decreased (5). We also reported that vasoconstrictions in response to norepinephrine, methoxamine and clonidine might be attenuated due to a decrease in \( \alpha \)-adrenoceptors, without accompanying atherosclerotic changes and endothelial dysfunction (2). On the other hand, Tesfamariam et al. (3) reported that responses to adrenergic nerve stimulation significantly became greater in carotid arteries from cholesterol-fed rabbits and that cholesterol feeding impaired the inhibitory influence of the endothelium-derived relaxing factor.

Previously, Fujiwara and Chiba (1) reported that clonidine could act as both an \( \alpha \)-adrenoceptor agonist and antagonist in the same vessel at the smooth muscle level. Thus, we examined the vasodilatory changes in hypercholesterolemic rabbits exposed to clonidine. The vasodilatory responses to clonidine were significantly decreased in the 4- and 8-week-cholesterol-fed groups, while the responses to other vasodilators were not significantly changed (Fig. 1). Verbeuren et al. (8) reported that endothelium-dependent relaxations induced by \( \alpha \)-adrenoceptor stimuli were not significantly changed in the aortas of atherosclerotic rabbits. Jayakody et al. (9) also suggested that the impairment of \( \alpha \)-adrenoceptor stimuli could be an early functional marker in the development of atherosclerosis. They used rabbit aortic strips that revealed atherosclerotic changes after cholesterol-feeding. On the other hand, our preparations showed no pathological change and \( \alpha \)-adrenoceptor stimuli were well-maintained (2). This discrepancy might be due to species and regional differences. Aortic atherosclerosis produced by continuous feeding of a high cholesterol diet to rabbits has been shown to regress on removal of the cholesterol fraction from the diet (10). Although we examined the response to clonidine for up to 24 weeks after cessation of the atherogenic diet, the attenuated responses were still detected (Fig. 2). NTG-, \( \alpha \)-adrenoceptor stimuli were also maintained under these conditions (data not shown).

It has been reported that clonidine exerts its anti-hypertensive action by stimulating histamine \( H_2 \)-receptors in peripheral tissues as well as in the central nervous system (11). However, in our preparations, the clonidine-induced responses were not influenced by cimetidine, a histamine \( H_2 \) antagonist (data not shown). From these results, we suggest that the attenuation of clonidine-induced vasodilatory responses by hypercholesterolemia may be due to decreased sensitivity to \( \alpha \)-adrenoceptor stimuli in this vessel and is one of early markers of atherosclerosis.

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