OXYGEN FREE RADICALS ENHANCE ERGONOVINE-INDUCED CANINE CORONARY VASOCONSTRICTION

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Summary. In order to examine the effects of oxygen free radicals on the ergonovine (EM)-induced coronary vasoconstriction in vivo, we administered EM (50μg) into the ostium of the left coronary artery (LCA) and angiographically evaluated the change of diameter of the left anterior descending (LAD) and the left circumflex (LCX) coronary artery in eight dogs before and after selective administration of oxygen free radicals, generated by xanthine (X)-xanthine oxidase (XO) reaction, into the LCX. To investigate the participation of serotonin in EM-induced vasoconstriction, the concentrations of serotonin in the LCA and the coronary sinus (CS) were measured before and after administration of X + XO. The diameter of the LCX remained essentially unchanged after administration of X + XO. However, EM-induced constriction was greater in the LCX than in the LAD. The difference of serotonin (S) concentrations in the CS and in the ostium of the LCA [(S in CS) − (S in LCA)] gradually increased after administration of X + XO. Electron microscopy of endothelial surface revealed marked changes in the LCX, but such changes were not observed in the LAD. These results suggest that the enhancement of the EM-induced vasoconstriction of coronary artery by oxygen free radicals may probably be due to the morphological change and the rise in the S produced by oxidative injury.

INTRODUCTION
Coronary artery spasm plays an important role in the pathogenesis of ischemic heart disease, not only in variant angina but also in unstable angina, myocardial infarction, and sudden cardiac death [1–4]. However, the pathogenesis of coronary spasm is still unknown, and the elucidation of its mechanism remains an important clinical issue. Enhanced vasoconstriction to vasoactive stimuli has been noted in experimental

models with atherosclerotic blood vessels brought about by mechanical arterial injury [5–8]. In patients with variant angina, coronary vasoconstriction in response to provocative agents is also demonstrated at a site of organic stenosis or minimal atherosclerosis [9–11]. Thus, atherosclerotic change of coronary blood vessels may play an important role in coronary hyperconstriction.

Ergonovine maleate is the agent that has been used frequently to provoke coronary vasospasm in human [12] and experimental models [5,6,8]. Ergonovine-induced coronary hyperconstriction is thought to be mediated by activation of serotonergic receptors and a subsequent increase in calcium influx into vascular smooth muscle cells [13,14]. Serotonin is a spasm-provocating agent. It is released from platelets and plays a major part in the platelet-dependent coronary vasoconstriction after arterial injury [15]. Recently, we have reported an augmented response to ergonovine noted in canine coronary artery immediately after oxidative injury [16,17]. However, in that experimental condition, the ergonovine-induced coronary vasocostriction was observed as diffuse coronary constriction because the solution containing oxygen radicals was infused into the ostium of the left coronary artery (LCA). The purpose of this study is to confirm that the increasing vasoconstrictor response to ergonovine with oxidative injury is a result of enhanced sensitivity to ergonovine brought about by oxygen radicals produced in locus quo and to examine the possible participation of serotonin in in vivo conditions. Thus, oxygen radicals are injected not into the ostium but into the branch of the LCA.

METHODS

Instrumentation

Eight mongrel dogs weighing 9 to 22 kg (15.4 ± 3.5 kg) were anesthetized with an intravenous administration of sodium pentobarbital (30 mg/kg). After intubation, the dogs were mechanically ventilated with room air. A 7.2-F catheter sheath was introduced into the abdominal aorta via the right femoral artery to measure the aortic pressure by means of a strain-gauge transducer (DT-4817, Spectramed Inc., Oxnard, California, USA). For the selective coronary angiography, a 5-F preshaped catheter (Judkins or Amplatz, Cordis Japan Inc., Japan) was advanced into the orifice of the LCA through the sheath under the guidance of fluoroscopy in the x-ray system. A 6-F preshaped catheter (Fansac, Clinical Supply Co. Ltd., Japan) was also placed at the coronary sinus (CS) via the left internal jugular vein for blood sampling. The electrocardiogram (limb leads I, II, and III) was monitored throughout the experiment. Heparin was administered intravenously at 200 IU/kg and then 1000 IU was added every hour by bolus.

Experimental protocol

To generate oxygen radicals, xanthine (X; 2 mM, Sigma, St. Louis, Missouri, USA) and xanthine oxidase (XO; 10 U/L, Sigma) were dissolved in the Krebs–Henseleit solution containing (in mM) NaCl 120.0, NaHCO₃ 25.5, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 1.25, and glucose 11.0. This X + XO solution was oxygenated