Diverse Relaxation Responses of Canine Large and Small Conductive Coronary Arteries to Glyceryl Trinitrate and Nitric Oxide on the One Hand and to 8-Bromoguanosine 3':5' Cyclic Monophosphate on the Other

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ABSTRACT—Glyceryl trinitrate (GTN) and nitric oxide (NO) preferentially relaxed the large (2 mm in diameter) conductive coronary artery (CCA) of the dog, while 8-bromoguanosine 3':5' cyclic monophosphate preferentially relaxed the small one (0.6 mm in diameter). Neither L-cysteine nor L-acetylcysteine affected GTN-induced relaxation in small- and large-CCA. These results indicate that not different biotransformation of GTN to NO, but a process or processes operative between activation of guanylate cyclase and that of cyclic GMP-dependent protein kinase seems to be responsible for the preferential dilatation of large-CCA.

Keywords: Glyceryl trinitrate, 8-Br-cGMP, Coronary artery

The weak response of coronary microvessels with a diameter less than 100 μm to vasodilatory action of glyceryl trinitrate (GTN) is recently explained by a less abundant presence of sulfhydryl groups necessary for conversion of GTN to its vasoactive metabolite, nitric oxide (NO) (1, 2). However, there exists differences in the relaxant effects of GTN even in larger conductive arteries with a diameter above 200 μm (3); the vasorelaxant effects are more potent in larger epicardial coronary arteries (large conductive coronary artery: large-CCA) than in small epicardial ones (small conductive coronary artery: small-CCA).

Although several possible mechanisms have been proposed, the subject is still under debate (4). More than a sufficient amount of sulfhydryl groups would present in the conductive coronary arteries (5).

It is now well-accepted that in association with the relaxation of the vascular smooth muscle by NO-forming or NO-liberating vasodilators including GTN, there occurs activation of guanylate cyclase and resultant accumulation of cyclic GMP within the cells. Cyclic GMP activates cyclic GMP-dependent protein kinase (cGMP-kinase) and produces relaxation of the smooth muscles (6). However, what step(s) in this signal transduction pathway is responsible for the diversity observed with respect to the relaxation of large- and small-CCA has not been elucidated. The purpose of the present study was to delineate the key step or steps in the signal transduction pathway that is responsible for heterogeneity of relaxant responses within conductive coronary arteries to GTN.

Mongrel dogs of either sex weighing 7–17 kg were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and bled. The heart was rapidly excised and placed in a cold bathing solution of the following composition: 125.0 mM NaCl, 2.7 mM KCl, 2.0 mM CaCl₂, 1.2 mM MgCl₂, 11.0 mM D-glucose, 23.8 mM Tris-base. The pH of the solution was adjusted to 7.4 with HCl. Epicardial coronary artery of the left circumflex branch (large-CCA, 2 mm in diameter) and its distal branch (small-CCA, 0.6 mm in diameter) were carefully dissected out from the surrounding tissue. After removal of endothelium with a stainless rod of the respective size, helical strips (1–2 mm in width and 10–15 mm in length) were made and placed in an organ bath filled with the above-described bathing solution (37°C, gassed with O₂). The isometric tension of the strip was recorded as described elsewhere (7).

After 2 hr of equilibrium under a resting tension of 2 g, the bathing solution was changed to a high K⁺ solution (127.7 mM, NaCl of the bathing solution was replaced with equimolar KCl) to induce contraction. Administration of relaxing agents began after attainment of a steady tension. The concentration-response curve for GTN

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(Nihon Kayaku Co., Ltd., Tokyo) was constructed by administering the drug cumulatively. When effects of l-cysteine (Sigma, St. Louis, MO, USA) or N-acetylcysteine (N-acetyl-l-cysteine, Sigma) on GTN-induced relaxation was examined, these agents (100 μM in final concentration) were administered 30 min before the first cumulative dose of GTN. However, because of the extremely high reactivity of NO to atmospheric oxygen (after opening of the air tight cap of the tubing) only one concentration of NO solution (prepared by the method of Shikano et al. (8)) was administered to each preparation. In the case of 8-Br-cGMP (8-bromoguanosine 3'5' cyclic monophosphate sodium salt, Sigma) only one concentration was also applied to each preparation because of an extremely slow development of relaxation by this compound (relaxation was evaluated after 1 hr). The degree of relaxation was expressed as a percent of the contraction induced by high K+ solution. Data were presented as the mean±S.E.M., and the difference between means was evaluated by the unpaired Student's t-test or that among groups was made by one way analysis of variance followed by Tukey's method. All animals were dealt with in a humane way according to the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

As is shown in Fig. 1, cumulative administration of GTN preferentially relaxed large-CCA, in accordance with general agreement (4). Estimated mean ED50 values were 7.16×10⁻⁷ M for large-CCA and 7.44×10⁻⁶ M for small-CCA. The relaxation by NO was of a similar pattern, the estimated mean ED50 being 1.13×10⁻⁶ M for large-CCA and 2.65×10⁻⁵ M for small-CCA. Thus, the vasodilatroy effect of NO is 20.3 times more potent in large-CCA than in small-CCA, while that of GTN was 10.4 times more potent in large-CCA. These findings suggest that diversity in conversion of GTN to the active

![Graph showing relaxation (%)](image)

**Fig. 1.** Effects of glyceryl trinitrate (GTN), nitric oxide (NO) and 8-Br-cGMP on large- and small-CCA. Only GTN was administered in a cumulative manner to large-CCA (open circle; tension before administration was 2.05±0.37 g, n=5) and small-CCA (closed circle; tension before administration was 1.37±0.26 g, n=5). In the case of NO or 8-Br-cGMP, only one concentration was administered to each preparation. Tension before administration of 3.3×10⁻⁷, 3.3×10⁻⁶ and 3.3×10⁻⁵ M of NO to large-CCA (open circle) was 3.71±1.05 (n=4), 2.74±0.98 (n=5) and 2.20±0.60 g (n=4), respectively, and that to small-CCA (closed circle) at corresponding concentration was 1.11±0.40 (n=4), 1.37±0.26 (n=4) and 0.93±0.20 g (n=5), respectively. Tension before administration of 8-Br-cGMP in large-CCA (open circle) at 10⁻⁴, 10⁻⁵ and 10⁻⁶ M was 1.76±0.49 (n=5), 2.33±0.41 (n=5) and 2.40±0.57 g (n=4), respectively. That in small-CCA (closed circle) at the corresponding concentration was 1.01±0.24 (n=5), 1.33±0.57 (n=6) and 1.25±0.59 g (n=5), respectively. Each point represents a mean±S.E.M. (*P<0.05, vs large-CCA). For the characterization of each relaxation, some parameters of relaxation with each agent evoke about 50% relaxation was evaluated. Time to the maximum relaxation with 4.4×10⁻⁵ M of GTN in large-CCA was 25.4±6.6 min and that with 4.4×10⁻⁵ M of GTN in small-CCA was 29.8±13 min, the respective half time (T1/2) was 9.2±1.1 and 12.8±2.0 min. With NO, maximum relaxation (T1/2 in parenthesis) was observed after 7.0±2.8 min (0.6±0.1 min) in large-CCA at 3.3×10⁻⁶ M and 13.0±6.0 min (0.5±0.1 min) in small-CCA at 3.3×10⁻⁶ M. Observation for relaxant effect of 8-Br-cGMP was limited to 60 min in both large- and small-CCA, and T1/2 was 19.6±2.8 min (at 10⁻⁴ M) and 22.8±1.6 min (at 10⁻⁵ M), respectively. In a separate series of experiments, relaxant effects of 8-Br-cGMP at 3×10⁻⁴ and 3×10⁻⁵ M were examined. The degree of relaxation at 3×10⁻⁴ M was 19.7±3.6% (n=6) in large-CCA and 24.0±3.4% (n=4) in small-CCA, and that at 3×10⁻⁵ M was 50.3±2.8% (n=6) in large-CCA and 59.2±6.2% (n=5) in small-CCA. However, no statistically significant difference was found between large- and small-CCA.
metabolite NO, either enzymatic or non-enzymatic (9), could not be the major cause of the heterogeneous responses of the large-CCA to this compound.

In contrast, 8-Br-cGMP, a substance that can activate the cGMP-kinase directly (10), was found to be a more potent relaxant of small-CCA than large-CCA. The estimated mean ED_{50} values of 3.02 × 10^{-5} M for large-CCA and 6.19 × 10^{-6} M for small-CCA indicate that the substance is 4.87 times more potent in relaxing small-CCA. Thus, the activation of cGMP-kinase by cyclic GMP seems to be more efficient in inducing the relaxation of small-CCA.

Various kinds of evidence on the microvessel level show that the preferential relaxation of large microvessels (>200 μm) as against small microvessels (<100 μm) is due to a difference in the rate of biotransformation of GTN to its active metabolite NO, which is attributable to differences in the amount of intracellular glutathione formed from l-cysteine or N-acetylcysteine (2). However, in larger sizes of small- and large-CCA used in study, supplementation of l-cysteine (100 μM) or N-acetylcysteine (100 μM) had no effect on GTN-induced relaxation (Fig. 2), in good accordance with a report by De la Lande et al. (11). Therefore, the mechanism for the preferential dilatation does not depend on the different thiol contents in these sizes of coronary arteries.

From these findings, it may be concluded that not the processes operative downstream to activation of cGMP-kinase inclusive of the activation of the enzyme but the processes downstream to activation of guanylate cyclase inclusive of activation of the enzyme by NO would contribute to cause preferential dilatation of large-CCA induced by GTN and NO.

Some investigators have implicated electrophysiological difference to explain the preferential dilatation. The difference in transmembrane and/or intracellular Ca^{2+} kinetics (12) was assumed to play an important role. However, in the present study, high-K^{+}-depolarization (127.7 mM) was used to induce the contraction of coronary artery. Thus, K^{+} channel opening (7) may be excluded as the main cause of preferential relaxation of large-CCA by GTN in this study.

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