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Triphasic vascular effects of thiol compounds and their oxidized forms on dog coronary arteries

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Abstract. The vascular effects of 2-mercaptoethanol, cysteamine, L-cysteine, glutathione (GSH), cystamine and oxidized GSH (GSSG) on the isometric tension of isolated dog coronary arterial strips were examined, and these effects were compared with the triphasic response induced by dithiothreitol (DTT); a rapid and weak contraction (phase A), an intervening slow relaxation (phase B) and a slowly-developing strong contraction (phase C) which we previously reported. The responses of the arteries induced by 2-mercaptoethanol, cysteamine and L-cysteine consisted of phases A, B and C. The order of contractile potency (ED_{50} of phase C) was DTT $\approx$ L-cysteine > 2-mercaptoethanol $\approx$ cysteamine, while the order of relaxant potency (ED_{50} of phase B) was DTT > cysteamine $\approx$ 2-mercaptoethanol. GSSG and cystamine mainly produced relaxation, which corresponded to phase B. The phase C contraction was specific to the reduced forms of thiols, except for GSH, which produced only relaxation. The participation of endothelial cells was not essential for the contracting or relaxing effects of the thiol compounds. The phase C contraction was depressed by W-7, a calmodulin antagonist, while phase A was not. Therefore calmodulin-dependent protein kinases may participate in phase C, not in phase A.
Key words. Thiol compounds; SH-group-containing compounds; disulfides; effects on coronary arteries; vascular smooth muscle; triphasic response.

Since thiol/disulfide exchange reactions modulate functions of certain enzymes and receptors, the intracellular thiol/disulfide status plays an important role in metabolic regulation and cellular signal transductions[1-3]. Furthermore, thiol reacts with nitric oxide to produce nitrosothiol, which is considered to be a stable carrier of nitric oxide or vascular endothelium-derived relaxing factor (EDRF)[4,5]. Nitroglycerin tolerance of arteries is associated with depletion of thiol compounds[6]. In addition, chemical modifiers for SH groups or disulfide bonds, such as dithiothreitol (DTT), 2-mercaptoethanol and L-cysteine, influence physiological and pharmacological responses of vascular systems (e.g., ref. 7 and the references cited therein).

We reported a triphasic response of dog coronary arteries to DTT: an initial, rapid and weak contraction (phase A), an intervening slow relaxation (phase B), and a final, slowly developing, strong contraction (phase C)[7]. A calmodulin antagonist, W-7[8,9], augments phase A and depresses phase C. Dithioerythritol also produces vascular responses similar to those to DTT. In this study, we examined the vascular effects of other thiol compounds such as 2-mercaptoethanol, cysteine, cysteamine and glutathione (GSH), and their oxidized forms, cystamine and oxidized GSH (GSSG), on dog coronary arteries. These responses were compared with DTT-induced response. It is important to investigate the response patterns of arteries induced by thiol compounds, since very little attention has been given to the effects of disulfide-reducing reagents on vascular systems.

Methods

The preparation of arterial strips and the measurement of isometric tension were performed as described[7]. In brief, the procedures were as follows. Helically-cut strips of coronary arteries isolated from mongrel dogs of either sex, sacrificed under anesthesia with sodium pentobarbital (26 mg/kg, i.v.), were used. The arterial strips were fixed vertically between hooks in 20-ml all-glass organ baths, containing modified Krebs-Henseleit solution (pH 7.4, 36.5 °C, bubbled with 95% O_2 and 5% CO_2) under a resting tension of 1.5 g. Isometric tensions were measured with oscillographs and a strip chart recorder. KCl (30 mM) was used to produce a standard contraction (100%). Some strips were partially precontracted with 1–2 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to assess the response of arteries to relaxants. Papaverine hydrochloride (0.1 mM) was used to induce a standard relaxation (−100%). In order to examine the extent of the dependence of drug-induced response on endothelial cells, endothelial cells were removed from some of the arterial strips[7,10]. The results are expressed as mean values ± SEM.

Results

DTT[7]: DL-DTT at high concentrations (1–10 mM) produced the triphasic response in the strips (fig. 1A).

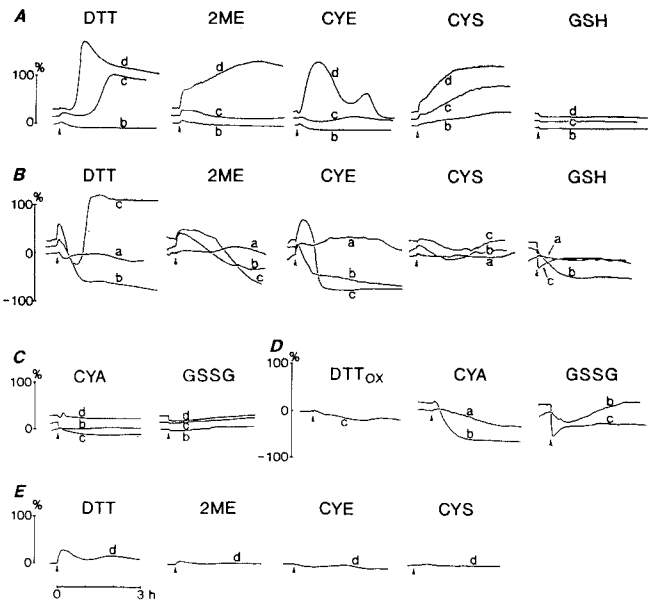

Figure 1. Vascular responses of dog coronary arterial strips to various thiol compounds. DTT, DL-dithiothreitol; 2ME, 2-mercaptoethanol; CYE, cysteamine; CYS, L-cysteine; GSH, glutathione; DTT_{ox}, oxidized DTT; CYA, cystamine; GSSG, oxidized GSH. Arrow heads show the administration of each compound indicated in the figure. The symbols, a, b, c and d, denote the concentrations, 0.01, 0.1 1 and 10 mM, of each compound, respectively. The position of the resting tension of each tracing was vertically shifted, and the tracings were superimposed. Vertical bars indicate the standard contraction and relaxation (see Methods). *A* and *C*, changes in the isometric tensions of the strips under a resting tension of 1.5 g; *B* and *D*, vascular responses induced with the compounds in the strips after partial contraction with 1–2 μM $PGF_{2\alpha}$. *E*, effects of 0.1 mM W-7 on responses of the strips induced with 10 mM of each compound indicated in the figure. W-7 was added to the medium at least 10 min before administration of each thiol compound.

DL-DTT at concentrations less than 0.1 mM, however, produced only phases A and B with no subsequent phase C. These vascular responses were also observed in the absence of the endothelial cells. When the strips were partially precontracted with $PGF_{2\alpha}$, the magnitudes of both phases A and B were increased (fig. 1B).

2-Mercaptoethanol: At concentrations lower than 1 mM, 2-mercaptoethanol produced responses corresponding to phases A and B as observed with DTT (fig. 1A): The magnitude of a transient and weak contraction (phase A) at 1 mM was $16.2 \pm 6.4\%$ ($n = 5$) of that induced by 30 mM KCl. At concentrations higher than 3 mM, 2-mercaptoethanol produced a rapid and weak contraction (phase A) followed by a slowly-developing intense contraction (phase C). Unlike the response produced by DTT, the latent period before phase C was very short. The phase C contraction was maximized at about 2.5 h after administration of 10 mM 2-mercaptoethanol (the maximal magnitude $= 86.5 \pm 6.8\%$, $n = 4$) with the magnitude decreasing slowly with time. The ED_{50} value for phase C was about 10 times higher than that of DTT (fig. 2A). It was difficult to obtain precise ED_{50} values for phase A because of its small magnitude. When 10 mM 2-mercaptoethanol was continuously injected into the bathing solution according to our previously described methods[7] in order to avoid the oxidation of 2-mercaptoethanol, the responses of the arteries to 2-mercaptoethanol were almost the same as those shown in figure 1A, indicating that the vascular effect of 2-mercaptoethanol was due to the reduced form.

In the partially precontracted strips, 2-mercaptoethanol (≥ 0.1 mM) produced an initial weak contraction followed by a slowly-developing relaxation (fig. 1B). The magnitude of the relaxation at 0.3 mM was $-99.0 \pm 0.3\%$ ($n = 3$) of that induced by 0.1 mM papaverine. The ED_{50} value for the relaxation was estimated to be about 0.1 mM (fig. 2B).

The response patterns and their magnitudes were not altered in the absence of endothelial cells. Indomethacin (1 μM) and atropine (1 μM) also did not alter either the time course or the magnitude of the mercaptoethanol-induced responses. However, the phase C contraction was markedly depressed to $3.3 \pm 1.9\%$ by 0.1 mM W-7, but the phase A contraction was still observed ($n = 3$, fig. 1E). The response pattern was very similar to that produced by DTT in the presence of W-7.

Cysteamine: The vascular response induced by cysteamine below 0.1 mM was similar to that caused by DTT or 2-mercaptoethanol. However, 10 mM cysteamine produced an initial weak relaxation followed by a bimodal contraction (fig. 1A). The contraction was

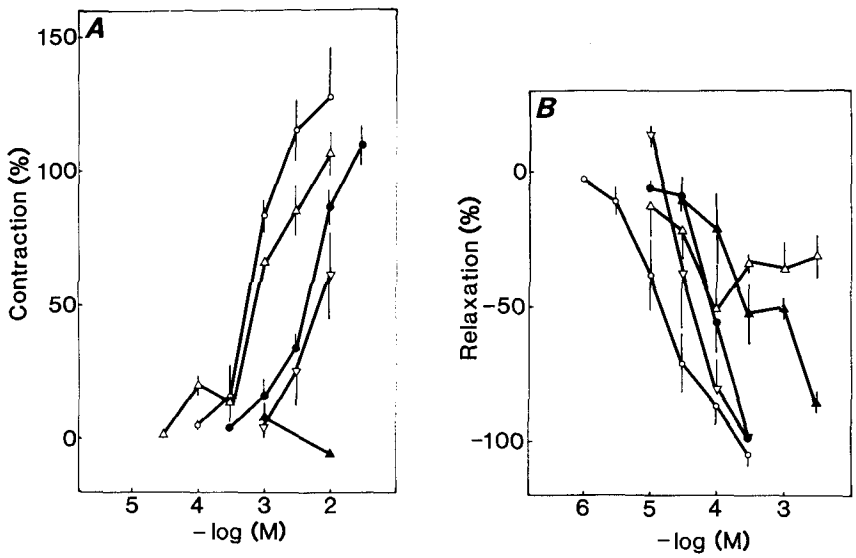

Figure 2. Dose-response curves for responses of dog coronary arterial strips induced with various thiol compounds. Vertical bars represent SEM (n = 3–8). The concentration of each compound is plotted on the abscissa, on a common logarithmic scale. *A*, dose-response curves for phase C contractions. Tension developed during contractions induced by 30 mM KCl was taken as 100%, and resting tension as 0%. *B*, dose-response curves for phase B relaxations of precontracted strips. Relaxation induced by 0.1 mM papaverine was taken as −100%, and contractions induced by 1–2 μM $PGF_{2\alpha}$ as 0%. ○, DTT; ●, 2-mercaptoethanol; △, L-cysteine; ▽, cysteamine; ▲, GSH.

markedly depressed by W-7 (fig. 1E). By the continuous perfusion of 10 mM cysteamine, the arterial strips were initially relaxed and then slowly contracted; after about 1 h, the contraction was maximal and the magnitude was nearly 100%, indicating that the vascular responses shown in fig. 1A were mainly due to the reduced form. The vascular responses were not altered after removal of endothelial cells. In partially precontracted strips, the whole response patterns of the strips resembled those induced by 2-mercaptoethanol (fig. 1B).

L-Cysteine: Exposure of the strips to L-cysteine resulted in rapid and weak contraction followed by a slowly-developing intense contraction without an intervening relaxation (fig. 1A). The maximal magnitude of contraction was 107 ± 8% by 10 mM L-cysteine (n = 4). The response was similar to that by 2-mercaptoethanol and to phases A and C induced by DTT. These contractions were completely suppressed by pretreatment with 0.1 mM W-7 (fig. 1E). The dose-response curve for the slow contraction was almost the same as that for DTT (fig. 2A) in both magnitude and ED_{50} value. The vascular responses were observed without endothelial cells. In the presence of $PGF_{2\alpha}$, relaxation of the arterial strips induced by L-cysteine was observed (fig. 1B); however, the magnitude of the relaxation responses was less than with 2-mercaptoethanol.

GSH: GSH produced no contraction, but a durable relaxation (fig. 1A). In the presence of $PFG_{2\alpha}$ the magnitude of relaxation was increased, but the action was weak, compared with that of other thiol compounds used (figs 1B, 2B). The vascular action of GSH was different from those of other compounds.

Oxidized Forms: Cystamine (0.1 mM, oxidized form of cysteamine) produced a rapid and weak contraction followed by a slow relaxation and no subsequent contraction (fig. 1, C and D). GSSG produced only a rapid and weak relaxation similar to the response induced by GSH (fig. 1C). Patterns of the responses brought about by GSSG in the presence of $PGF_{2\alpha}$ were similar to those brought about by GSH (fig. 1, B and D). Oxidized DTT (*trans*-4,5-dihydroxy-1,2-dithiane, 0.1–1 mM) had no vascular effect (fig. 1D). Experiments with cystine were not feasible because of its low solubility.

Discussion

The present study demonstrates the vascular effects of various thiol compounds. Although each compound had specific action and the vascular responses were somewhat different from each other, the responses could all be described as combinations of the three phases of vascular responses induced by DTT. The phase C contraction was specific for the reduced forms, except for GSH. GSH produced relaxation of the arteries, and this response resembled phase B for DTT. From the dose-response curves (fig. 2), the order of contractile potency (phase C) was DTT ≈ L-cysteine > 2-mercaptoethanol ≈ cysteamine, while the order of relaxant potency (phase B) was DTT > cysteamine ≈ 2-mercaptoethanol > GSH. The slow contraction elicited by L-cysteine overlapped the relaxation (phase B) at high concentrations (fig. 2B). In either event the most potent reagent among the reduced forms used was DTT. Vascular actions of thiol compounds may be attributed to the reduction of disulfide bonds of recep-

tors, channels and enzymes to modulate their functions, the reduction of small disulfide compounds (R-S-S-R′) to produce R-SH and R′-SH, and/or the production of R-S-X (e.g. nitrosothiol). The most potent action of DTT may be due to amplified production of R-SH and R′-SH as well as the reduction of disulfide groups in certain receptors, channels and enzymes. The oxidized forms, cystamine and GSSG, showed mainly the relaxant effect. However, oxidized DTT has no vascular effect. This is probably due to the chemical stability of oxidized DTT[11]. Like the vascular action of DTT[7], the participation of endothelial cells was not essential for the vascular effects of the above reagents, which thus appear to be mainly due to direct actions on smooth muscle cells, although the participation of other cells such as nerve terminals cannot be ruled out.

The delayed contraction (phase C) induced by DTT, 2-mercaptoethanol, L-cysteine or cysteamine was depressed by W-7, indicating the participation of calmodulin-dependent protein kinases in the contraction. The phase C contraction induced by DTT depends on extracellular ionic calcium[7], hence the contraction may be induced by an influx of calcium ions. Furthermore, various thiol compounds such as dithioerythritol, 2-mercaptoethanol and L-cysteine can pass through cell membranes[12–14], therefore phase C may be an intracellular event. Since, unlike phase C, phase A contraction induced by DTT or 2-mercaptoethanol was not suppressed by W-7 (fig. 1E), the mechanism of phase A must be different from that of phase C. Furthermore, phase A contraction induced by DTT was enhanced by pre-exposure to $PGF_{2\alpha}$ or W-7, whereas the contraction induced by 2-mercaptoethanol or L-cysteine was not, suggesting that the mechanism of phase A contraction by DTT may include some components other than those in the case of 2-mercaptoethanol or L-cysteine.

There are few reports on the effects of DTT and L-cysteine on vascular preparations other than dog coronary arteries; a small relaxation (probably our phase B) of rabbit renal and dog tibial arteries was induced by DTT[15], and contractions of rabbit mesenteric arteries were induced by either 1 mM DTT or 10 mM L-cysteine[16,17].

Since thiol compounds have been shown to have vascular actions on arteries, much attention is needed when thiol compounds are used as pretreatment drugs or modulators of vascular action.

The present study indicates that the thiol/disulfide status in the vascular system plays a role in the contraction-relaxation mechanism, since the present compounds mimic both the phasic and tonic responses induced by physiological substances[18–21]. However, at present the possible vascular effects of thiol compounds are almost entirely neglected or forgotten. Since the activities of many enzymes and receptors, including protein kinases, adenylate cyclase and guanylate cyclase, can be modulated by thiol/disulfide exchange reactions[1–3], further investigation on the relationship between the cellular thiol-disulfide status and the contraction-relaxation mechanisms will be needed.

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