ALTERATIONS IN PHARMACOLOGICAL RECEPTOR ACTIVITIES OF RABBIT ARTERIES BY SULFHYDRYL REAGENTS

Masahisa ASANO* and Hiroyoshi HIDAKA**
Department of Pharmacology, Mie University School of Medicine, Edobashi, Tsu 514, Japan
Accepted October 18, 1982

Abstract—The addition of 1×10⁻³ M or 1×10⁻² M 2-mercaptoethanol (2-MEt), a sulfhydryl reagent, produced a leftward displacement (potentiation) of the dose-response curves of mesenteric arterial strips for histamine, norepinephrine, serotonin, angiotensin II, prostaglandin F₂α and KCl. N-ethylmaleimide abolished the 2-MEt-induced potentiation of the arterial responses. Dithiothreitol (DTT) and cysteine also potentiated the contraction by these agonists in mesenteric arterial strips, suggesting that the sulfhydryl group plays a role in the arterial responses to various contractile agonists. In contrast to the mesenteric arterial strips, 2-MEt abolished the contraction by angiotensin II without greatly affecting contraction by norepinephrine in the thoracic aorta, femoral, renal and carotid arterial strips. These data suggest that there are regional differences in the responsiveness of rabbit arteries to sulfhydryl reagents. In mesenteric arterial strips treated with 1×10⁻⁶ M histamine, 6×10⁻⁸ M norepinephrine or 1×10⁻⁹ M angiotensin II after exposure to Ca²⁺-free Krebs' bicarbonate solutions containing 0.1 mM EGTA, the addition of 2.5 mM CaCl₂ caused a contraction (agonist-induced Ca²⁺-contraction). Sulfhydryl reagents potentiated each agonist-induced Ca²⁺-contraction in this artery. Moreover, in thoracic aortic strips, sulfhydryl reagents enhanced only histamine-induced Ca²⁺-contraction and attenuated norepinephrine- and angiotensin II-induced Ca²⁺-contractions. It is concluded that the reduction of a disulfide bond of the arterial strips to a sulfhydryl group affects the pharmacological receptor activities of the strips and that the changes in the receptor activities may be related to the changes in the transmembrane influx of calcium.

It is well known that pharmacological receptor systems can also be modified by sulfhydryl reagents (1). Fleisch et al. (2) showed that dithiothreitol (DTT), a reagent that reduces disulfide bridges to sulfhydryl groups (3), markedly potentiated the responses of rabbit thoracic aorta to histamine and abolished the responses to angiotensins without greatly affecting responses to KCl, norepinephrine or serotonin. They also demonstrated the species difference that DTT, in concentrations that potentiated histamine-induced contractions of rabbit aorta, depressed such responses of guinea-pig aorta and abolished the feeble action of histamine on the rat aorta (4). However, the regional difference of rabbit arterial strips in the response to the sulfhydryl reagents has never been described. It is well known that the vascular tissue exhibits a marked heterogeneity (5-7); therefore, there is a need for caution when extrapolating data on the aorta to other vasculatures. It is conceivable that the potentiation and the attenuation by
sulfhydryl reagents might occur either at the receptor level or somewhere along the hypothetical chain of events that links receptor with response mechanism. In this study, the term "receptor system" refers not only to the specific macromolecules that directly interact with agonists or antagonists, but also to those components, if any, that intervene in the steps linking the receptor to the final cellular component whose changes of state directly gives rise to the response (8, 9). If competitive antagonists are used as test drugs, changes in pA₂ values (10–12) and in apparent dissociation constants (K_b) (13, 14) might provide evidence for modification of the receptor itself. Freisch et al. have determined the K_b and pA₂ values for pyrilamine in rabbit thoracic aorta in the presence of DTT to ascertain whether histaminergic H₁-receptors represent one homogenous receptor population, and they found that enhancement of the histamine response by DTT is not accompanied by an alteration in the K_b and pA₂ values for pyrilamine (2). Therefore, it is likely that the potentiation would occur at a receptor-linked process rather than at the receptor macromolecules. Thus, we attempted to define the effects of sulfhydryl reagents on agonist-induced Ca²⁺-contraction.

In the present study, we examined the role of sulfhydryl and disulfide groups as determinants of arterial responses to various contractile agonists in mesenteric, femoral, renal, carotid arteries and thoracic aorta of rabbits.

Materials and Methods

Preparation of arterial strips and experimental procedures: Albino rabbits of either sex weighing 2.2–2.6 kg were sacrificed by bleeding from the carotid artery. The superior mesenteric artery (0.8–1.8 mm outside diameter), femoral artery (0.5–1.2 mm), renal artery (0.6–1.5 mm), carotid artery (1.5–2.5 mm) and thoracic aorta (3.0–4.5 mm) were quickly excised. After removal of excess fat and adventitial connective tissue, the arteries were helically cut resulting in strips of 1.5 mm in width and 20 mm in length (mesenteric artery and other arteries) according to the method of Lewis and Koessler (15) or Furchgott and Bhadrakom (16). The size of the aortic strip was somewhat larger (2.5×25 mm). The helical strip was fixed vertically between hooks in a water jacketed (37±0.5°C) tissue bath containing 40 ml of modified Krebs' bicarbonate solution (pH 7.5). The composition of the bathing solution used was as follows (in millimolar concentration): NaCl, 115.0; KCl, 4.7; CaCl₂·2H₂O, 2.5; MgCl₂·6H₂O, 1.2; NaHCO₃, 25.0; KH₂PO₄, 1.2; and dextrose, 10.0. The tissue bath solutions were maintained at 37±0.5°C and bubbled with a mixture of 95% O₂ and 5% CO₂. The upper end of the strip was connected to the lever of a force-displacement transducer (TB-611T, Nihon Kohden Kogyo, Co., Tokyo, Japan) by a silk thread. An initial resting tension of 1 g was applied to the mesenteric and other arterial strips, and an initial resting tension of 2 g applied to the thoracic aortic strips. Before the experiments were commenced, strips were allowed to equilibrate for 1 hr in the bathing solution. During the equilibration period, the solutions were replaced every 15 min.

After the equilibration time of 1 hr under the resting tension, a submaximally effective concentration of KCl (40 mM) was administered twice or three times with a 40-min interval of each response until successive responses remained constant. Cumulative dose-response curves for a contractile agonist were obtained by a stepwise increase in concentration of an agonist as soon as a steady response was obtained to the preceding dose. The concentration of the agonist in the bath was increased by a factor of about 3 until the maximum response was obtained (17). Concentrated solutions of
Drugs were added directly to the bathing solution in a volume of 0.2 ml to give the final concentrations desired. Three sequential dose-response curves for an agonist were determined simultaneously on paired arterial strips with an interval of 60 min between the each determination (18, 19). Usually paired strips from the same animal received different treatments. One strip taken at random was subjected to various agents at the third curve; another strip was a control serving as an indicator of changes in tissue sensitivity during the course of the experiment. If such changes were noted, maximum contractile tensions and pD2 values (negative logarithm of the molar concentration of ED50) were corrected accordingly (20). Effects of various agents such as 2-mercaptoethanol (2-MEt), dithiothreitol (DTT), cysteine and ascorbic acid on the contractile response to several agonists were determined by treatment for 20 min with these agents prior to the addition of the agonist.

Whenever an ED50 value was determined, responses to these agonists were calculated as a percentage of the maximum contraction obtained with that agonist. The ED50 value was obtained visually from a plot of percent contraction vs. log concentration of agonist and expressed as the negative logarithm (pD2 value). Results shown in the text, Tables and Figures are expressed as the mean value±S.E. Comparison of the results was accomplished with the Student's t-test, paired t-test or analysis of variance (21). Statistical significance was assumed when P<0.05.

**Agonist-induced Ca2+-contraction**: Experiments using Ca2+-free Krebs’ bicarbonate solutions containing 0.1 mM ethylene glycol bis (β-aminoethyl)ether)-N,N,N’,N’-tetraacetic acid (EGTA) were performed in the following way: Arterial strips were contracted with an agonist such as histamine, norepinephrine and angiotensin II, twice or three times each, in normal Krebs’ bicarbonate solution. After the successive responses to each agonist remained constant, these strips were washed and relaxed in normal solution. All the latter strips were then exposed to the Ca2+-free Krebs’ bicarbonate solutions containing 0.1 mM EGTA for 30 min with successive washings in this Ca2+-free solution every 10 min. After the latter 30 min period, the arterial strips were then contracted with their respective agonists, i.e., histamine, norepinephrine and angiotensin II, twice or three times until the responses were almost abolished. When CaCl2 in a final concentration of 2.5 mM was added to the Ca2+-free Krebs’ bicarbonate solutions containing 0.1 mM EGTA after the agonist-induced small contractions had been stabilized, additional increase in the tension was developed (agonist-induced Ca2+-contraction). Some preparations were treated for 20 min with sulfhydryl reagents before the addition of the agonist. Ca2+-free Krebs’ bicarbonate solutions were prepared by omission of the CaCl2 from normal Krebs’ bicarbonate solution, and additional experiments employed Ca2+-free Krebs’ bicarbonate solutions containing 0.1 mM EGTA brought to pH 7.4 with NaOH. Osmotic adjustment was not made when K+ was added or external Ca2+ was removed.

**Drugs and chemicals**: These included histamine diphosphate (Nakarai), l-norepinephrine bitartrate (Wako), serotonin creatinine sulfate (Merck), angiotensin II (Protein Research Foundation), prostaglandin E2 (Prostarmon F, Ono Pharmaceutical), 2-mercaptoethanol (Nakarai), dithiothreitol (Nakarai), l-cysteine hydrochloride (Nakarai), N-ethylmaleimide (Nakarai), ascorbic acid (Wako), phentolamine mesylate (Regitine mesylate, Ciba) and pyrilamine maleate (Sigma). All the concentrated solutions of drugs were prepared daily in Krebs’ bicarbonate solution and kept on ice during the
course of the experiment.

Results

Potentiation by 2-mercaptoethanol of the contractile response of mesenteric artery:
The dose-response curves for various contractile agonists such as histamine, nor-epinephrine, serotonin, angiotensin II, prostaglandin F$_{2\alpha}$ and KCl in the strips of rabbit mesenteric artery were shifted to the left in the presence of 2-mercaptoethanol (2-MEt) (Fig. 1). 2-MEt in a concentration of $1 \times 10^{-3}$ M or of $1 \times 10^{-2}$ M was added 20 min before the third dose-response curve for the agonist and was still present during exposure of the strip to each agonist. In the presence of $1 \times 10^{-2}$ M 2-MEt. the pD$_2$ values of the third curves were increased by 1.05±0.08 (histamine, N=13), 0.69±0.05 (norepinephrine, N=9), 0.08±0.04 (serotonin, N=11), 0.25±0.04 (angiotensin II, N=8), 0.53±0.04 (prostaglandin F$_{2\alpha}$, N=8) and 0.22±0.03 (KCl, N=7) log units with respect to the second curve. When the third dose-response curves for these agonists were determined in the absence of 2-MEt, the third pD$_2$ values were decreased by 0.08±0.01 (histamine, N=13), 0.01±0.01 (norepinephrine, N=9).

![Fig. 1. Effects of 2-mercaptoethanol (2-MEt) on the dose-response curves for histamine (His), nor-epinephrine (NE), serotonin (5-HT), angiotensin II (Angio II), prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) and KCl in strips of rabbit mesenteric artery. Three sequential dose-response curves for an agonist were obtained from a single preparation. 2-MEt at a concentration of $1 \times 10^{-3}$ M or of $1 \times 10^{-2}$ M was added 20 min before the third dose-response curve. 2-MEt was present during exposure of arterial strips to the agonist. Dose-response curves were determined simultaneously on three individual arterial strips. Two of the strips were subjected to $1 \times 10^{-3}$ M and $1 \times 10^{-2}$ M 2-MEt; the third strip was a control serving as an indicator of changes in tissue sensitivity during the course of the experiment. If such changes were noted, dose-response curves were corrected accordingly. In each preparation, the tension developed by the agonist in the third dose-response curve in the absence or the presence of 2-MEt was taken as 100%. Vertical bars represent the S.E. Numbers in the figure indicate the numbers of preparations used.](image-url)
0.25±0.04 (serotonin, N=11), 0.12±0.02 (angiotensin II, N=8), 0.09±0.01 (prostaglandin F2\alpha, N=8) and 0.03±0.01 (KCl, N=7) log units with respect to the second. This represents a small but highly significant (P<0.01) potentiating effect of 2-Met.

Maximum contractile tensions and pD2 values of these agonists at the third dose-response curves in the absence or the presence of 1 \times 10^{-3} M 2-Met are shown in Table 1.

![Figure 2](image_url)  
**Fig. 2.** Effects of N-ethylmaleimide (NEM) and 2-mercaptoethanol (2-MEt), present separately or in combination, on the dose-response curves for histamine in strips of rabbit mesenteric artery. The strips were preincubated with 1 \times 10^{-6} M NEM for 20 min and were then thoroughly washed out. Vertical bars represent the S.E. Figures in parentheses indicate the number of preparations used. See text for details.

### Table 1. Comparison of maximum contractile tensions and pD2 values of various agonists in the absence and the presence of 2-mercaptoethanol (2-MEt)

| Agonists       | Control \(^a\) | 2-MEt 1-10^{-3} M \(^b\) |
|----------------|---------------|--------------------------|
|                | N  | Maximum Tension | pD2 | N  | Maximum Tension | \(d\)pD2 |
|----------------|----|-----------------|-----|----|-----------------|--------|
| Histamine      | 13 | 2477±241 mg     | 5.56±0.09 | 13 | 2329±230 mg     | 0.87±0.07 |
| Norepinephrine | 9  | 2463±164 mg     | 6.70±0.12 | 9  | 2249±175 mg     | 0.43±0.05 |
| Serotonin      | 11 | 2235±217 mg     | 6.41±0.29 | 11 | 2284±175 mg     | 0.33±0.04 |
| Angiotensin II | 8  | 1498±286 mg     | 8.91±0.10 | 8  | 1845±302 mg     | 0.38±0.04 |
| Prostaglandin F2\alpha | 8  | 2126±312 mg     | 5.48±0.14 | 8  | 2570±332 mg     | 0.30±0.04 |
| KCl            | 7  | 2132±306 mg     | 1.91±0.05 | 7  | 2322±300 mg     | 0.15±0.03 |

\(^a\) The sequential dose-response curves for each agonist were obtained from a single preparation. 2-Met at a concentration of 1 \times 10^{-3} M was added 20 min before the third dose-response curve. One strip was used to obtain the control dose-response curve.

\(^b\) The maximum contractile tension was attained at 1 \times 10^{-3} M histamine, 1 \times 10^{-4} M norepinephrine, serotonin, 1 \times 10^{-7} M angiotensin II, 3 \times 10^{-5} M prostaglandin F2\alpha or 40 mM KCl, respectively.

\(^c\) The ED50 value was obtained visually from a plot of percent contraction vs. log concentration of each agonist and expressed as the negative logarithm (pD2 value).

\(^d\) The shift in pD2 value of each agonist by the addition of 1 \times 10^{-3} M 2-Met was expressed as \(d\)pD2. These shifts in pD2 value were significantly different when compared to the shifts in pD2 value of each agonist in the absence of 2-Met (P<0.01).

N indicates the number of preparations used. Data are expressed as the mean±S.E.

Since N-ethylmaleimide (NEM) covalently binds sulfhydryl groups, the arterial strip was exposed to varying concentrations for a standard 20-min interval and was then thoroughly washed by exchanging the bathing solution three times. After this procedure, the reaction was essentially irreversible. Effect of NEM and effect of NEM plus 2-MEt combination on the dose-response curve for histamine were determined in the following way (Fig. 2): On 32 mesenteric arterial strips, two dose-response curves for histamine from a single pre-
response curves for histamine were determined in the absence of either NEM or 2-MEt. The pD₂ value of the second curve was decreased by 0.12±0.02 log units with respect to the first curve (curve A). In the second group (eight arterial strips), the second dose-response curve was determined in the presence of 1×10⁻³ M 2-MEt. In this group, the second pD₂ value was increased by 0.73±0.08 log units with respect to the first (curve B). In the third group (eight arterial strips), the second curve was determined after the 20-min pretreatment with 1×10⁻⁵ M NEM. In this group, the second pD₂ value was decreased by 0.78±0.10 log units, and the maximum contraction by histamine was decreased to 69% (curve C). In the fourth group (eight arterial strips), the second curve was determined in the presence of 1×10⁻³ M 2-MEt after the 20-min pretreatment with 1×10⁻⁵ M NEM. In this group, the second pD₂ value was decreased by 0.58±0.07 log units (curve D). This experiment shows that NEM not only decreased the maximum response to histamine, but also caused a parallel rightward displacement of the dose-response curve, and NEM abolished the 2-MEt-induced potentiation (Fig. 2).

Effects of dithiothreitol, cysteine and ascorbic acid on the contractile responses of mesenteric artery: Dithiothreitol (DTT), an agent that reduces disulfide bonds to sulfhydryl groups, and cysteine, a thiol agent, also potentiated the contractile responses of various agonists in the strips of the mesenteric artery (Fig. 3). The addition of 1×10⁻³ M DTT or of 1×10⁻² M cysteine caused a slight

Fig. 3. Effects of dithiothreitol (DTT) and cysteine on the dose-response curves for histamine (His), norepinephrine (NE) and angiotensin II (Angio II) in strips of rabbit mesenteric artery. The addition of 1×10⁻³ M DTT or 1×10⁻² M cysteine caused a slight contraction (broken horizontal lines of the figure); thereupon, His, NE or Angio II was administered. Vertical bars represent the S.E. Numbers in the figure indicate the number of preparations used.
contraction (0.02–0.4 g) in the strips (broken horizontal lines of Fig. 3); in a few cases, the strip was contracted to a tension of 1.8–2.0 g by 1×10⁻³ M DTT, and we excluded these cases. The arterial strip was exposed to either 1×10⁻³ M DTT or 1×10⁻² M cysteine until the small response to this sulfhydryl reagent had reached a plateau (about 20–30 min later); thereupon, histamine, norepinephrine or angiotensin II was administered, and the result is shown as Fig. 3. The difference between these two dose-response curves in the absence and the presence of the sulfhydryl reagent may be due to the potentiation of the response to agonist by the reagent. In order to determine whether the effect of the sulfhydryl reagent on agonist-induced contraction was a “potentiation” or an “additive effect”, analysis by the method of Trendelenburg (22–25) was performed. The effects of the sulfhydryl reagents on the contraction of mesenteric arterial strips were revealed to be a potentiation (Fig. 3).

The reaction with ascorbic acid, a commonly used reducing agent, unlike what was observed with the sulfhydryl reagent, had no potentiating effect on the contractile agonist in these mesenteric arterial strips. The dose-response curves for histamine and angiotensin II in the strips of mesenteric artery were shifted to the right in the presence of 1×10⁻³ M ascorbic acid (Fig. 4). Ascorbic acid did not produce a potentiation in the contractile responses of mesenteric arteries to other agonists such as norepinephrine, serotonin, prostaglandin F₂α and KCl (data not shown).

**Effect of 2-mercaptoethanol on the contractile response of thoracic aorta:** The dose-response curves of thoracic aortic strips for various contractile agonists in the presence of 2-MEt was varied (Fig. 5). Only the dose-response curve for histamine was shifted to the left by 2-MEt in aortic strips. 2-MEt produced a rightward displacement of the dose-response curve for norepinephrine, serotonin, angiotensin II, prostaglandin F₂α and KCl. The addition of 1×10⁻² M 2-MEt not only caused a rightward displacement of the dose-response curve for angiotensin II, but also decreased the maximum response to angiotensin II.

In order to determine whether the potentiating effect of 2-MEt on various contractile agonists were observed only in the mesenteric artery, studies of the effect of 2-MEt were performed on strips of femoral, renal and carotid arteries. In the strips of

---

**Fig. 4.** Effect of ascorbic acid on the dose-response curves for histamine (His) and angiotensin II (Angio II) in strips of rabbit mesenteric artery. Ascorbic acid at a concentration of 1×10⁻³ M was added 20 min before the third dose-response curve. Vertical bars represent the S.E. Numbers in the figure indicate the number of preparations used.
femoral, renal and carotid arteries, 2-MEt produced a potentiation only for histamine. The contractile responses to other agonists such as norepinephrine, serotonin, angiotensin II, prostaglandin F$_2$-$\alpha$ and KCl were slightly attenuated or not affected by 10$^{-3}$ M 2-MEt. Typical experiments of these arteries are shown in Fig. 6. This shows that 2-MEt causes a potentiation for all contractile agonists only in the mesenteric artery, and 2-MEt produces a potentiation only for histamine in other arteries and the aorta. DTT also produced the same effects as 2-MEt on these arteries and the aorta.

Potentiation by the sulfhydryl reagents of agonist-induced Ca$^{2+}$-contraction: In mesenteric arterial strips treated with 1$\times$10$^{-6}$ M histamine after exposure to EGTA (0.1 mM) added Ca$^{2+}$-free Krebs bicarbonate solutions, the addition of CaCl$_2$ in a concentration of 2.5 mM caused a phasic contraction (Fig. 7A, left). Treatment with 1$\times$10$^{-3}$ M 2-MEt or with 1$\times$10$^{-3}$ M DTT caused a slight persistent relaxation. The contractile response of mesenteric artery to finally added CaCl$_2$ was very markedly potentiated by the 20 min treatment with the sulfhydryl reagents (Fig. 7A, center and right).

The addition of CaCl$_2$ also caused a phasic contraction in mesenteric arterial strips treated with 6$\times$10$^{-3}$ M norepinephrine (Fig. 7B, left) or with 1$\times$10$^{-9}$ M angiotensin II (Fig. 7C, left) after exposure to EGTA (0.1 mM) added Ca$^{2+}$-free solutions. In these mesenteric arterial strips, 2-MEt and DTT potentiated each agonist-induced Ca$^{2+}$-contraction (Fig. 7B and C). The contractile effect of CaCl$_2$ itself without treatment with histamine, norepinephrine or angiotensin II in the absence or of the presence of sulfhydryl reagents on mesenteric arterial strips was negligible (Fig. 7D).

Agonist-induced Ca$^{2+}$-contraction was also determined in thoracic aortic strips of the rabbit. In aortic strips treated with 1$\times$10$^{-6}$ M histamine, 3$\times$10$^{-5}$ M norepinephrine or 1$\times$10$^{-5}$ M angiotensin II after exposure to EGTA (0.1 mM) added Ca$^{2+}$-free solutions, the addition of CaCl$_2$ in a concentration of 2.5 mM also caused a phasic contraction.
Histamine-induced Ca²⁺-contraction of aortic strips was significantly potentiated by 1 × 10⁻³ M 2-MEt or 1 × 10⁻³ M DDT (Fig. 8A). However, these sulfhydryl reagents attenuated the norepinephrine- and angiotensin II-induced Ca²⁺-contractions (Fig. 8B and C).

Effects of 2-MEt and DTT on various concentrations of histamine-induced Ca²⁺-contractions in mesenteric arterial strips are shown in Fig. 9. These sulfhydryl reagents produced a parallel leftward displacement of the histamine-induced Ca²⁺-contractions in mesenteric arterial strips. The potency of the sulfhydryl reagents for producing a leftward displacement agreed well to the results obtained in Figs. 1 and 3.

Discussion
Our present study provides new obser-
Fig. 7. Potentiation by sulfhydryl reagents of agonist-induced Ca\(^{2+}\)-contraction in mesenteric arterial strips. The mesenteric arterial strips were exposed to a Ca\(^{2+}\)-free Krebs' bicarbonate solution containing 0.1 mM EGTA for 30 min. In the 0.1 mM EGTA added Ca\(^{2+}\)-free solution, the arterial strips were contracted with their respective agonists (A: 1 \times 10^{-6} M histamine, B: 6 \times 10^{-8} M norepinephrine and C: 1 \times 10^{-9} M angiotensin II) twice or three times until the responses were almost abolished. Then, CaCl\(_2\) in a concentration of 2.5 mM was added to the 0.1 mM EGTA added Ca\(^{2+}\)-free solution after the agonist-induced small contractions had been stabilized (ca 5 min) in the absence and the presence of 1 \times 10^{-3} M 2-mercaptoethanol (2-MEt) and of 1 \times 10^{-3} M dithiothreitol (DTT). 2-MEt and DTT were added 20 min before the administration of the respective agonists. See "Materials and Methods" for details.

Fig. 7. Potentiation by sulfhydryl reagents of agonist-induced Ca\(^{2+}\)-contraction in mesenteric arterial strips. The mesenteric arterial strips were exposed to a Ca\(^{2+}\)-free Krebs' bicarbonate solution containing 0.1 mM EGTA for 30 min. In the 0.1 mM EGTA added Ca\(^{2+}\)-free solution, the arterial strips were contracted with their respective agonists (A: 1 \times 10^{-6} M histamine, B: 6 \times 10^{-8} M norepinephrine and C: 1 \times 10^{-9} M angiotensin II) twice or three times until the responses were almost abolished. Then, CaCl\(_2\) in a concentration of 2.5 mM was added to the 0.1 mM EGTA added Ca\(^{2+}\)-free solution after the agonist-induced small contractions had been stabilized (ca 5 min) in the absence and the presence of 1 \times 10^{-3} M 2-mercaptoethanol (2-MEt) and of 1 \times 10^{-3} M dithiothreitol (DTT). 2-MEt and DTT were added 20 min before the administration of the respective agonists. See "Materials and Methods" for details.

Observations concerning the nature of pharmacological receptor systems in isolated rabbit arteries. Sulfhydryl reagents such as 2-MEt, DTT and cysteine have various effects on the contraction of rabbit arteries. These agents reversely potentiate the contractile responses of rabbit mesenteric artery to histamine, norepinephrine, serotonin, angiotensin II, prostaglandin F\(_{2\alpha}\) and KCl (Figs. 1 and 3, Table 1). These sulfhydryl reagents reduce disulfide bonds to form reduced sulfhydryl groups. Therefore, these reagent-induced potentiations of the contractile responses of mesenteric artery to various agonists suggest that the intact sulfhydryl group, and not the disulfide bond, is necessary for the expression of the contractions of mesenteric artery. These potentiations in the mesenteric artery appear to be a relatively nonspecific effect on the receptor sites for
these agonists since the contractile response to KCl was also potentiated by these sulfhydryl reagents.

N-ethylmaleimide (NEM), a disulfide- and sulfhydryl group-alkylating and oxidizing reagent, inhibits the contractile responses of rabbit mesenteric artery to various agonists. NEM also reverses the potentiating effect of the sulfhydryl reagents in mesenteric artery (Fig. 2). The ability of NEM to reverse the potentiating effect of sulfhydryl reagents on agonist-induced contraction of mesenteric artery is related to the formation of an oxidative state of sulfhydryl groups of the arterial smooth muscle. Unlike the sulfhydryl reagents, ascorbic acid, a commonly used reducing agent, produced only an attenuation in the contractile responses of mesenteric arterial strips to histamine, norepinephrine, serotonin, angiotensin II, prostaglandin F$_2$α and KCl (Fig. 4). This phenomenon may have been related to the difference in their reducing potencies. It is impossible that sulfhydryl reagents (2-MEt, DTT, cysteine), which contain sulfhydryl groups, form a nonspecific complex with a contractile agonist, increase the effective concentration of these substances at the receptor site, and thereby result in apparent potentiation of the response to these agonists which is independent of
any effect on smooth muscle disulfide bonds. From these evidences taken together, it is concluded that sulfhydryl reagents potentiated the contraction of rabbit mesenteric artery through the reduction of a disulfide bond of the receptor system and/or the incorporation of these reagents to the receptor system.

The data, presented in this study, also demonstrate marked regional differences in the reactivity of rabbit arteries to sulfhydryl reagents. In mesenteric artery, sulfhydryl reagents potentiated the contractions by various agonists as described above. However, these reagents potentiated the contraction only by histamine in femoral, renal, carotid arteries and thoracic aorta. The vascular contractile effects of norepinephrine, angiotensin II and KCl are highly variable. 2-MEt clearly induced the potentiation in norepinephrine-, angiotensin II- and KCl-induced contractions of mesenteric artery, whereas the reagent produced the attenuation or no effect in other arteries (Fig. 6). DTT, another sulfhydryl reagent, also produced the same effects as observed with 2-MEt on these arteries. This evidence may suggest that both the potentiation and the attenuation are related to the intact sulfhydryl groups.

The role of sulfhydryl groups or of disulfide bridges in the action of drugs on nerve and muscle membranes has received considerable attention (2, 26–28). Reduction of disulfide bonds to sulfhydryl groups causes profound changes in drug action which are dependent on both the tissue selected for analysis and the species from it was obtained. As with many contractile agonists, there are species and regional variations in response to DTT. Histamine-induced contractions of rabbit aorta are potentiated by DTT; those in guinea pig aorta are inhibited, whereas the small responses of rat aorta to high concentrations of histamine are abolished. Moreover, dose-response curves of guinea pig aorta for norepinephrine are shifted further to the right than similar responses of rabbit aorta. This is in contradistinction to the responses produced by angiotensin in rabbit aorta and guinea pig aorta that are both abolished by DTT (2, 4).

We investigated the mechanism of the alteration of the contraction by sulfhydryl reagents by agonist-induced Ca2+-contraction which is used to determine the change in transmembrane influx of calcium. 2-MEt and DTT markedly potentiated the agonist-induced Ca2+-contraction of mesenteric artery (Fig. 7). In thoracic aorta, however, these sulfhydryl reagents potentiated only histamine-induced Ca2+-contraction and attenuated other agonist-induced Ca2+-contractions (Fig. 8). From the observations obtained in mesenteric artery and thoracic aorta concerning the agonist-induced Ca2+-contractions, it is concluded that potentiations and attenuations by sulfhydryl reagents of the contractions of arteries might be the result of changes in the transmembrane permeability to calcium ion.

With regard to these pharmacological
receptor systems demonstrated in the present study, another possibility may exist: the pharmacological receptor systems, normally in a reduced state in mesenteric artery, are oxidized in the artificial environment of the tissue bath and need to be reduced to regain their full activities.

Acknowledgment: The authors wish to thank Ms. Mieko Mizukoshi and Ms. Sachiko Uga for their skillful technical assistance.

References

1) Ehrenpreis, S., Fleisch, J.H. and Mittag, T.W.: Approaches to the molecular nature of pharmacological receptors. Pharmacol. Rev. 21, 131-181 (1969)
2) Fleisch, J.H., Krzan, M.C. and Titus, E.: Pharmacological receptor activity of rabbit aorta. Effect of dithiothreitol and N-ethylmaleimide. Circ. Res. 33, 284-290 (1973)
3) Cleland, W.W.: Dithiothreitol: A new protective reagent for sulfhydryl groups. Biochemistry 3, 480-482 (1964)
4) Fleisch, J.H., Krzan, M.C. and Titus, E.: Alterations in pharmacologic receptor activity by dithiothreitol. Am. J. Physiol. 227, 1243-1248 (1974)
5) Bevan, J.A. and Osher, J.V.: Relative sensitivity of some large blood vessels of the rabbit to sympathomimetic amines. J. Pharmacol. Exp. Ther., 160, 370-374 (1965)
6) Bevan, J.A., Hosmer, D.W., Ljung, B., Pegram, B.L. and Su, C.: Norepinephrine uptake, smooth muscle sensitivity, and metabolizing enzyme activity in rabbit veins, Circ. Res., 34, 541-547 (1974)
7) Bevan, J.A., Hosmer, D.W., Ljung, B., Pegram, B.L. and Su, C.: Innervation pattern and neurogenic response of rabbit veins. Blood Vessels 11, 172-182 (1974)
8) Fleisch, J.H. and Ehrenpreis, S.: Thermal alteration in receptor activity of the rat fundal strip. J. Pharmacol. Exp. Ther., 162, 21-29 (1968)
9) Fleisch, J.H. and Ehrenpreis, S.: A study of the alteration in receptor activity of the rat fundal strip by urea. J. Pharmacol. Exp. Ther., 163, 363-366 (1968)
10) Schmid, H.O.: pA, a new scale for the measurement of drug antagonism. Br. J. Pharmacol. 2, 189-206 (1947)
11) Schmid, H.O.: pA and competitive drug action. Br. J. Pharmacol. 4, 277-290 (1949)
12) Arunlakshana, O. and Schild, H.O.: Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48-58 (1959)
13) Furchgott, R.F.: The pharmacological determination of adrenergic receptors. Ann. N.Y. Acad. Sci., 139, 553-570 (1967)
14) Furchgott, R.F.: The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In Catecholamines, Edited by Blaschko, H. and Muscholl, E., p. 283-335, Springer-Verlag, Berlin (1972)
15) Lewis, J.H. and Koessler, K.K.: Demonstration of arterial contraction in vitro. Arch. Intern. Med., 39, 182-187 (1927)
16) Furchgott, R.F. and Bhadrakom, S.: Relations of strips of rabbit aorta to epinephrine, isopropylartenol, sodium nitrite and other drugs. J. Pharmacol. Exp. Ther., 108, 129-143 (1953)
17) van Rossum, J.N.: Cumulative dose-response curves. II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. Ther., 143, 299-330 (1963)
18) Hidaka, H. and Asano, M.: Relaxation of isolated rabbit arteries by fusaric (5-butylicolic) acid. J. Pharmacol. Exp. Ther., 199, 620-629 (1976)
19) Hidaka, H., Asano, M., Iwadare, S., Matsumoto, I., Totsuka, T. and Aoki, N.: A novel vascular relaxing agent, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide which affects vascular smooth muscle actomyosin. J. Pharmacol. Exp. Ther., 207, 8-15 (1978)
20) Asano, M. and Hidaka, H.: Contractile response of isolated rabbit aortic strips to unsaturated fatty acid peroxides. J. Pharmacol. Exp. Ther., 208, 347-363 (1979)
21) Finney, D.J.: Statistical Method in Biological Assay. p. 99-138, Charles Griffin and Co., London (1964)
22) Trendelenburg, U.: The action of acetylcholine on the nictitating membrane of the spinal cat. J. Pharmacol. Exp. Ther., 135, 39-44 (1962)
23) Lagner, S.Z. and Trendelenburg, U.: The onset of denervation supersensitivity. J. Pharmacol. Exp. Ther., 151, 73-86 (1966)
24) Draskoczy, P.R. and Trendelenburg, U.: The uptake of L- and D-norepinephrine by the isolated perfused rabbit heart in relation to the stereospecificity of the sensitizing action of cocaine. J. Pharmacol. Exp. Ther., 159, 66-73 (1968)
25) Asano, M. and Hidaka, H.: Potentiation of the contractile response to acetylcholine in aortic
strips by low concentrations of vascular contractile agonists. Br. J. Pharmacol. 69, 639-646 (1980)

26) Karlin, A.: Molecular interactions of the acetylcholine receptor. Fed. Proc. 32, 1847-1852 (1973)

27) Karlin, A. and Bartels, E.: Effect of blocking sulfhydryl groups and of reducing disulfide bonds on the acetylcholine activated permeability system of electroplax. Biochim. Biophys. Acta 126, 525-535 (1966)

28) Needleman, P., Jakschik, B. and Johnson, E.M., Jr.: Sulfhydryl requirement for relaxation of vascular smooth muscle. J. Pharmacol. Exp. Ther. 187, 324-331 (1973)