POTENTIATION IN VARIOUS AGONISTS-INDUCED CONTRACTIONS OF RABBIT MESENTERIC ARTERY BY SULFHYDRYL REAGENTS

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Abstract—The role of sulfhydryl and disulfide groups as determinants of rabbit mesenteric arterial responses to various contractile agonists were determined. The addition of $1 \times 10^{-3}$ M or of $1 \times 10^{-2}$ M 2-mercaptoethanol (2-MEt), a sulfhydryl reagent, produced a leftward displacement (potentiation) of the concentration-response curves of mesenteric arterial strips for KCl. Dithiothreitol (DTT), a reagent that reduces disulfide bonds to sulfhydryl groups, also potentiated the contractile response to KCl in this strip. In mesenteric arterial strips treated with 14 mM KCl after exposure to Ca2+-free Krebs’ bicarbonate solutions containing 0.1 mM EGTA, the addition of CaCl2 in a concentration of 2.5 mM caused a contraction (14 mM KCl-induced Ca2+-contraction). The presence of 2-MEt or DTT expectedly potentiated this 14 mM KCl-induced Ca2+-contraction. Verapamil, a calcium antagonist, inhibited the 14 mM KCl-induced Ca2+-contraction both in the presence and the absence of these sulfhydryl reagents. 2-MEt also potentiated the contractile responses of mesenteric arterial strips to histamine, norepinephrine, angiotensin II and prostaglandin F2α suggesting that the potentiation by the sulfhydryl reagent is a nonspecific effect. This sulfhydryl reagent potentiated the each agonist-induced Ca2+-contraction. It is concluded that reduction of a disulfide bridge to a sulfhydryl group at Ca2+-channels increases transmembrane influx of Ca2+ in strips of rabbit mesenteric artery and the increased Ca2+ influx in turn accompanied the contractile responses to various agonists.

Pharmacological receptor systems can be modified in situ by various procedures including exposure to enzymes, sulfhydryl reagents, chelating agents, urea, lipid solvents, protein denaturants and changes in pH or temperature (1). It is conceivable that the modification might occur either at the receptor level or somewhere along the hypothetical chain of events that links the receptor with the response mechanism. Thus for these studies, it is more realistic to attribute changes observed in terms of modification of either component parts or the total receptor system. In the case of muscles, the determination of parameters other than contraction, e.g., membrane permeability or electrical properties, might limit the changes measured to only a part of the total receptor system. Among the more group-specific reagents are those which cause oxidation of sulfhydryl groups or reduction of disulfide bridges. Sulfhydryl groups and disulfide bridges have been implicated in the oxytocin and vasopressin (2, 3), acetylcholine (4-6) α-adrenergic (7), and prostaglandin receptors (8) of various tissues.
Fleisch et al. (9) showed that dithiothreitol (DTT), a reagent that reduces disulfide bridges to sulfhydryl groups (10), markedly potentiated the responses of rabbit thoracic aorta to histamine and abolished the responses to angiotensins without greatly affecting responses to potassium chloride (KCl), norepinephrine, or serotonin. They also demonstrated 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 (11). In the present study, we examined the role of sulfhydryl and disulfide groups as determinants of rabbit mesenteric arterial responses to various contractile agonists.

Materials and Methods

Preparation of mesenteric 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) was quickly excised. After removal of excess fat and adventitial connective tissue, the artery was helically cut at an angle of approximately 45° to the longitudinal axis into strips of 1.5 mm in width and 20 mm in length according to the method of Lewis and Koessler (12) or Furchgott and Bhadrakom (13). 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 arterial strips. Before the experiments were commenced, strips were allowed to equilibrate for 1 hr in the bathing solution. During the equilibration period, the strip relaxed with time, so the resting tension was always readjusted, and the solutions were replaced every 15 min.

After the equilibration time of 1 hr under the resting tension, maximally effective concentration of KCl (40 mM) was administered twice or three times until successive responses remained constant. The cumulative concentration-response curve for a contractile agonist was obtained by a stepwise increase in concentration of agonist as soon as a steady response was obtained to the preceding concentration. The concentration of the agonist in the bath was increased by a factor of about 3 until the maximum response was obtained (14). Concentrated stock 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 concentration-response curves for an agonist were determined simultaneously on paired arterial strips with an interval of 60 min between each of the determinations (15, 16). Usually paired strips from the same animal received different treatments. One strip was subjected to various agents at the third trial; 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 (17). Effects of various agents such as 2-mercaptoethanol and dithiothreitol on the contractile response to various agonists were determined with these agents prior to the addition of the agonist.

Experiments using Ca2+-free solutions were performed in the following way. Arterial
strips were contracted with an agonist such as KCl, histamine, norepinephrine, angiotensin II or prostaglandin F₂α, two 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 a Ca²⁺-free Krebs' bicarbonate solution for 30 min with successive washings by this solution every 10 min. After the latter 30 min period, the arterial strips were then contracted with their respective agonists, i.e., KCl, histamine, norepinephrine, angiotensin II and prostaglandin F₂α, twice or three times until the responses were almost abolished. When CaCl₂ in a final concentration of 2.5 mM was added to a Ca²⁺-free solution after the agonist-induced small contractions had been stabilized, an additional increase in the tension developed (agonist-induced Ca²⁺-contraction; Ca²⁺-induced contraction in the muscle treated with various agonists, see Fig. 3). Some preparations were treated for 20 min with sulfhydryl reagents before the addition of each agonist. Ca²⁺-free Krebs' bicarbonate solutions were prepared by omitting the CaCl₂ from normal Krebs' bicarbonate solution. Additional experiments employed Ca²⁺-free solutions containing 0.1 mM ethylene glycol bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) brought to pH 7.4 with NaOH. Osmotic adjustment was not made when K⁺ was added or external Ca²⁺ was removed.

Whenever an ED50 value was determined, responses to the agonist 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 were 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 (18). Statistical significance was assumed when P<0.05.

**Drugs and chemicals:** These included potassium chloride (KCl, Nakarai), histamine diphosphate (Nakarai), /-norepinephrine bitartrate (Wako), angiotensin II (Protein Research Foundation), prostaglandin F₂α (Prostarmon F, Ono Pharmaceutical), 2-mercaptoethanol (Nakarai), dithiothreitol (Nakarai), phentolamine mesylate (Regitine mesylate, Ciba), pyrilamine maleate (Sigma) and verapamil hydrochloride. All drugs were prepared daily in Krebs' bicarbonate solution and kept on ice during the course of the experiment.

**Results**

**Potentiation by sulfhydryl reagents of the contractile response of mesenteric artery to KCl:** The addition of KCl in concentrations ranging from 5 to 50 mM caused a concentration-dependent contraction in mesenteric arterial strips (Fig. 1). The maximum contraction was attained at 40 mM; the mean value of the maximum contractile tension was 2132±144 mg (N=18). The concentration-response curve for KCl in strips of mesenteric artery was shifted to the left in the presence of various agonists, see Fig. 3). Some preparations were treated for 20 min with sulfhydryl reagents before the addition of each agonist. Ca²⁺-free Krebs' bicarbonate solutions were prepared by omitting the CaCl₂ from normal Krebs' bicarbonate solution. Additional experiments employed Ca²⁺-free solutions containing 0.1 mM ethylene glycol bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) brought to pH 7.4 with NaOH. Osmotic adjustment was not made when K⁺ was added or external Ca²⁺ was removed.

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**Drugs and chemicals:** These included potassium chloride (KCl, Nakarai), histamine diphosphate (Nakarai), /-norepinephrine bitartrate (Wako), angiotensin II (Protein Research Foundation), prostaglandin F₂α (Prostarmon F, Ono Pharmaceutical), 2-mercaptoethanol (Nakarai), dithiothreitol (Nakarai), phentolamine mesylate (Regitine mesylate, Ciba), pyrilamine maleate (Sigma) and verapamil hydrochloride. All drugs were prepared daily in Krebs' bicarbonate solution and kept on ice during the course of the experiment.
Cleland's reagent) (10), a reagent that reduces disulfide bonds to sulfhydryl groups, also potentiated the contractile responses to KCl in this strip (Fig. 1 B). In a few cases, the strip was contracted to a tension of 0.3–1.8 g by 1 x 10^{-3} M DTT alone, we excluded these cases. The concentration-response curve of mesenteric artery for KCl was not affected by the 20-min treatment with 1 x 10^{-4} M DTT (Fig. 1 B).

The concentration-response curve for KCl was determined after the pretreatment with these sulfhydryl reagents and then washing them out by exchanging the solution three times; similar leftward displacement of the concentration-response curve to that obtained in the presence of the sulfhydryl reagent was also observed (data not shown).

Verapamil antagonized the potentiated contractile response of mesenteric artery to KCl in a concentration-dependent manner both in the presence and the absence of 2-MEt (Fig. 2). The antagonistic action of verapamil against the KCl-contraction has been attributed to an inhibition of the transmembrane influx of Ca^{2+} since verapamil is known as a specific calcium antagonist.

Potentiation by sulfhydryl reagents of KCl-induced Ca^{2+}-contraction: In mesenteric arterial strips treated with 14 mM KCl after exposure to Ca^{2+}-free Krebs' bicarbonate solutions containing 0.1 mM EGTA, the addition of 2.5 mM CaCl_{2} caused a contraction (14 mM KCl-induced Ca^{2+}-contraction, Fig. 3A left). The 14 mM KCl-induced Ca^{2+}-contraction in mesenteric artery was very markedly potentiated by the treatment for 20 min with 2-MEt or with DTT (Fig. 3A, center and right). Verapamil inhibited this Ca^{2+}-contraction in the mesenteric artery both in the absence and the presence of these sulfhydryl reagents (Fig. 3B). The contractile effect of CaCl_{2} alone without treatment of KCl in the absence or the

Fig. 1. Effects of 2-mercaptoethanol (2-MEt, A) and dithiothreitol (DTT, B) on the concentration-response curve for KCl in strips of rabbit mesenteric artery. Three sequential concentration-response curves for KCl were obtained from a single preparation. 2-MEt or DTT was added 20 min before the third concentration-response curve. These sulfhydryl reagents were still present during exposure of the arterial strips to KCl. Concentration-response curves were determined simultaneously on three individual arterial strips. Two of the strips were subjected to 1 x 10^{-3} M and 1 x 10^{-2} M 2-MEt (or 1 x 10^{-4} M and 1 x 10^{-3} M DTT); 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, concentration-response curves were corrected accordingly. In each preparation, the tension developed by KCl in the third concentration-response curve in the absence or the presence of the sulfhydryl reagent was taken as 100%. Mean values of the maximum contractile tensions developed by KCl in the absence or the presence of 1 x 10^{-3} M 2-MEt were 2186 ± 206 mg (control, N=9) and 2322 ± 200 mg (2-MEt, N=9), respectively. Vertical bars represent the S.E. Figures in parentheses indicate the number of preparations used.
The presence of the sulfhydryl reagents on mesenteric arterial strips was negligible (Fig. 3C).

The potenizations by sulfhydryl reagents and the inhibition by verapamil of 14 mM KCl-induced Ca\(^{2+}\)-contraction in mesenteric artery are listed in Table 1. Verapamil inhibited this Ca\(^{2+}\)-contraction in the presence or the absence of the sulfhydryl reagent in a concentration-dependent manner. Potentiaged Ca\(^{2+}\)-contraction in mesenteric artery in the presence of 2-MET was not attenuated by 1×10\(^{-6}\) M phentolamine, thereby suggesting that the 2-MET-produced potentiation is not
mediated through the release of endogenous norepinephrine or through the direct stimulation of α-adrenergic receptors.

Effect of 2-Met on the contractile response of mesenteric arterial strips to various agonists: The concentration-response curves of mesenteric arterial strips for various contractile agonists such as histamine, norepinephrine, angiotensin II and prostaglandin F2α were shifted to the left in the presence of 2-Met (Fig. 4). In the presence of 1×10⁻³ M 2-Met, the pD₂ values of the third curves were increased by 0.07±0.01 (histamine, N=13), 0.02±0.004 (norepinephrine, N=9), 0.10±0.02 (angiotensin II, N=9) and 0.07±0.01 (prostaglandin F2α, N=8) log units with respect to the second curve. When the third concentration-response curves for these agonists were determined in the absence of 2-Met, the third pD₂ values were decreased by 0.07±0.01 (histamine, N=13), 0.02±0.004 (norepinephrine, N=9), 0.10±0.02 (angiotensin II, N=9) and 0.07±0.01 (prostaglandin F2α, N=8) log units with respect to the second.

Each agonist-induced Ca²⁺-contraction of the mesenteric arterial strips was then determined in the presence of the sulphydryl reagent. The addition of CaCl₂ in a concentration of 2.5 mM caused a phasic contraction in mesenteric arterial strips treated with each agonist (1.5×10⁻⁶ M histamine, 9.1×10⁻⁸ M norepinephrine, 1.8×10⁻⁹ M angiotensin II or 2.0×10⁻⁶ M prostaglandin F2α, N=8) log units with respect to the second.

Table 1. Effects of verapamil on KCl-induced Ca²⁺-contraction in rabbit mesenteric arterial strips in the presence or the absence of sulphydryl reagent

| Treated with² | Sulphydryl reagents Antar | Conditions³ | N | Tension developed by 2.5 mM CaCl₂⁴ |
|--------------|--------------------------|-------------|---|----------------------------------|
| KCl 14 mM    | 2-Met 1×10⁻³ M           | Control     | 10 | 1158±124 (100.0)                 |
|              | +Verapamil                | 5×10⁻⁶ M    | 10 | 650±101 (56.1)                   |
|              | +Verapamil                | 2×10⁻⁷ M    | 10 | 317±67 (27.4)                    |
|              | +Verapamil                | 1×10⁻⁶ M    | 10 | 95±33 (8.2)                      |
|              | +Phentolamine             | 1×10⁻⁶ M    | 10 | 1197±131 (103.4)                 |
| DTT 1×10⁻³ M | Control                  | 8           | 1844±133 (100.0)                 |
|              | +Verapamil                | 5×10⁻⁸ M    | 8  | 1210±126 (65.6)                  |
|              | +Verapamil                | 2×10⁻⁷ M    | 8  | 671±98 (36.4)                    |
|              | +Verapamil                | 1×10⁻⁶ M    | 8  | 173±46 (9.4)                     |
| None         | Control                  | 8           | 587±72 (100.0)                   |
|              | +Verapamil                | 5×10⁻⁸ M    | 8  | 300±49 (51.1)                    |
|              | +Verapamil                | 2×10⁻⁷ M    | 8  | 103±26 (17.5)                    |
|              | +Verapamil                | 1×10⁻⁶ M    | 8  | 21±10 (3.6)                      |

² CaCl₂ in a concentration of 2.5 mM was added to the mesenteric arterial strips treated with 14 mM KCl after exposure to EGTA (0.1 mM)-added Ca²⁺-free Krebs' bicarbonate solutions in the presence or the absence of sulphydryl reagent. Contractile tension developed by 2.5 mM CaCl₂ was expressed as mg, and figures in parentheses indicate the percentage of each control.

³ Verapamil or phentolamine was added 25 min before KCl; after the first 5 min, each sulphydryl reagent was added.

See Fig. 3 and "Materials and Methods" for details. N indicates the number of preparations used. Data are expressed as the mean±S.E. **Significantly different from the control (P<0.01). ***Significantly different from the control (P<0.001).
Fig. 4. Effects of 2-mercaptoethanol (2-MEt) on the concentration-response curves for histamine (A), norepinephrine (B), angiotensin II (C) and prostaglandin F2α (D) in strips of rabbit mesenteric artery. Three sequential concentration-response curves for each agonist were obtained from a single preparation. 2-MEt in a concentration of $1 \times 10^{-3}$ M was added 20 min before the third concentration-response curve. In each preparation, the tension developed by the agonist in the third concentration-response curve in the absence or the presence of 2-MEt was taken as 100%. Mean values of the maximum contractile tensions developed by the agonists in the presence of $1 \times 10^{-3}$ M 2-MEt were 2329±230 mg (histamine, N=13), 2249±175 mg (norepinephrine, N=9), 1845±302 mg (angiotensin II, N=9) and 2570±332 mg (prostaglandin F2α, N=8), respectively. On the other hand, mean values of the maximum contractile tensions developed by the agonists in the absence of 2-MEt were 2477±241 mg (histamine, N=13), 2463±164 mg (norepinephrine, N=9), 1498±286 mg (angiotensin II, N=8) and 2162±312 mg (prostaglandin F2α, N=8), respectively. Vertical bars represent the S.E. N indicates the number of preparations used.

Table 2. Potentiating effect of 2-mercaptoethanol on each agonist-induced Ca²⁺-contraction in rabbit mesenteric arterial strips

| Agonist treated with | Conc.     | N   | Tension developed by 2.5 mM CaCl₂ | Control | +2-MEt 10⁻³ M |
|----------------------|-----------|-----|---------------------------------|---------|----------------|
|                      |           |     | mg                              | mg      | mg             |
| KCl                  | $1.4 \times 10^{-2}$ M | 17  | 618±69                         | 1184±105*** |
| Histamine            | $1.5 \times 10^{-6}$ M | 13  | 361±58                         | 1310±128*** |
| Norepinephrine       | $9.1 \times 10^{-8}$ M | 10  | 448±72                         | 985±140*** |
| Angiotensin II       | $1.8 \times 10^{-9}$ M | 13  | 561±88                         | 1405±196*** |
| Prostaglandin F₂α    | $2.0 \times 10^{-6}$ M | 9   | 699±91                         | 1575±213*** |

CaCl₂ in a concentration of 2.5 mM was added to the mesenteric arterial strips treated with each contractile agonist after exposure to EGTA (0.1 mM)-added Ca²⁺-free Krebs' bicarbonate solutions in the absence or the presence of 2-mercaptoethanol (2-MEt). 2-MEt was added 20 min before each agonist. Contractile tension developed by 2.5 mM CaCl₂ was expressed as mg. See Fig. 3 and "Materials and Methods" for details. N indicates the number of preparations used. Data are expressed as the mean±S.E. ***Significantly different from the control (P<0.001).

Histamine-induced Ca²⁺-contraction of mesenteric arterial strips was expectedly antagonized by pyrilamine, a histamine H₁-receptor blocking agent in the presence and the absence of 2-MEt (Table 3). Norepinephrine-induced Ca²⁺-contraction of the strips was also expectedly antagonized by phentolamine, and α-adrenergic receptor blocking agent (Table 3). A relatively high concentration of verapamil ($1 \times 10^{-6}$ M), which almost completely inhibited the KCI-induced Ca²⁺-contraction, exhibited a weak effect on histamine- or norepinephrine-induced Ca²⁺-contraction (Table 3).
Table 3. Effects of pyrilamine and phentolamine on agonist-induced Ca\textsuperscript{2+}-contraction in mesenteric arterial strips in the presence and the absence of 2-mercaptoethanol

| Treated with\textsuperscript{a} | Conc. of 2-ME\textsubscript{t}\textsuperscript{a} | Conditions\textsuperscript{b} | N  | Tension Developed by 2.5 mM CaCl\textsubscript{2}\textsuperscript{a} |
|-----------------|-----------------|-----------------|----|-------------------|
| His 1.5×10\textsuperscript{-8} M | 1×10\textsuperscript{-3} M | Control | 15 | 1447±120 (100.0)  |
|                 |                 | +Pyrilamine 1×10\textsuperscript{-6} M | 15 | 271±122*** (18.7) |
|                 |                 | +Verapamil 1×10\textsuperscript{-6} M | 15 | 1129±137* (78.0)  |
| His 2.2×10\textsuperscript{-5} M | 0 | Control | 8  | 1170±195 (100.0)  |
|                 |                 | +Pyrilamine 1×10\textsuperscript{-8} M | 8  | 135±60*** (11.5)  |
|                 |                 | +Verapamil 1×10\textsuperscript{-6} M | 8  | 764±132* (65.3)   |
| NE 9.1×10\textsuperscript{-8} M | 1×10\textsuperscript{-3} M | Control | 8  | 987±147 (100.0)   |
|                 |                 | +Phentolamine 1×10\textsuperscript{-6} M | 8  | 32±14*** (3.2)    |
|                 |                 | +Verapamil 1×10\textsuperscript{-6} M | 8  | 668±89* (67.7)    |

\textsuperscript{a} CaCl\textsubscript{2} in a concentration of 2.5 mM was added to the mesenteric arterial strips treated with histamine (His) or norepinephrine (NE) after exposure to EGTA (0.1 mM) added Ca\textsuperscript{2+}-free Krebs\textsuperscript{b} bicarbonate solutions in the presence or the absence of 2-mercaptoethanol (2-MEt). Contractile tension developed by 2.5 mM CaCl\textsubscript{2} was expressed as mg and figures in parentheses indicate the percentage of each cont ol.

\textsuperscript{b} Pyrilamine, phentolamine or verapamil was added 25 min before each agonist (histamine or norepinephrine); after the first 5 min, 2-MEt was added. See Fig. 3 and "Materials and Methods" for details. N indicates the number of preparations used. Data are expressed as the mean±S.E. *Significantly different from the control (P<0.05). **Significantly different from the control (P<0.001).

Discussion

Our present study provides new observations concerning the nature of pharmacological receptor activity in vascular smooth muscle. Sulfhydryl reagents such as 2-mercaptoethanol (2-MEt) and dithiothreitol (DTT) potentiate the contractile responses of rabbit mesenteric artery to KCl (Fig. 1). These sulfhydryl reagents reduce disulfide bonds to form reduced sulfhydryl groups. Therefore, these reagents-induced potentiations of the contractile response of mesenteric artery to KCl suggest that the intact sulfhydryl group, and not the disulfide bond, is necessary for the potentiation of potassium contraction of mesenteric artery. The contractile response of vascular smooth muscle to KCl is accompanied by a depolarization-dependent increase in membrane permeability to calcium ion (19, 20). 2-MEt and DTT potentiated the KCl-induced Ca\textsuperscript{2+}-contraction of mesenteric artery (Fig. 3). This KCl-induced Ca\textsuperscript{2+}-contraction of mesenteric artery was concentration-dependently inhibited by low concentrations of verapamil (Table 1). Verapamil is known as a calcium antagonist. This agent, employed clinically as a coronary vasodilator, has a relaxing effect on cardiac and many smooth muscle preparations (21–23). This action of verapamil has been attributed to an inhibition of the transmembrane influx of Ca\textsuperscript{2+}, which may act as an activator for the contractile system, into the cell during excitation or depolarization of the cell membrane (24, 25). These evidences taken together suggest that potentiations by sulfhydryl reagents of the contractile response of mesenteric artery to KCl might be produced by enhanced membrane permeability to calcium ion.

Furthermore, the sulfhydryl reagents potentiated the contractile response of mesenteric artery to norepinephrine, histo-
mine, angiotensin II and prostaglandin F₂α. These poten-
tiations appear to be a relatively
nonspecific effect on the receptor-linked Ca²⁺
channels for these contractile agonists since
the response to KCl was also potentiated by
the reagent. The contractile response of
vascular smooth muscle to norepinephrine
is mediated by stimulation of \( \alpha \)-adrenergic
receptors which results in an enhanced
membrane permeability to calcium ion and
the release of calcium from intracellular
stores. Since the contractile responses to KCl
and norepinephrine were essentially poten-
tiated by disulfide bond reduction, it is likely
that nonspecific potentiating effects account
for the actions of sulfhydryl reagents.

Fleish et al. have demonstrated the various
effects of DTT on the contractile responses
of thoracic aorta isolated from rabbits, guinea-
pigs and rats (9, 11). They showed that
there were species differences in these
effects since 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 (11).
They also demonstrated that DTT markedly
potentiated the responses of rabbit aorta to
histamine and abolished the responses to
angiotensins without greatly affecting re-
 sponses to KCl, norepinephrine and serotonin
(9). However, the regional differences of
rabbit arteries in the responses to sulfhydryl
reagents have never been described. It is
well known that the vasculature exhibits a
marked heterogeneity (26–28), therefore there
is a need for caution when extrapolating
data on the aorta to other vascular tissues.
In the present study, we demonstrated that
at least in the rabbit mesenteric artery, the
contractile responses to all the agonists used
were potentiated by sulfhydryl reagents.
From all these lines of evidence, it is con-
cluded that sulfhydryl reagents have both
potentiating and antagonizing effects on
vascular smooth muscle contraction and that
there are regional and species differences in
the response of arterial preparations to the
reagents.

The present study was designed to extend
the understanding of the potentiating effects
of sulfhydryl reagents on the contraction of
vascular smooth muscle. We found that
sulfhydryl reagents (2-MEt and DTT) non-
specifically increased the contractile response
of rabbit mesenteric artery. The present
observations suggest that the sulfhydryl
group plays an important role in potentiating
the contractions of rabbit mesenteric artery.
Reduction of disulfide bonds to sulfhydryl
groups results in potentiation of the responses
to various contractile agonists. With regard to
these pharmacological receptor systems,
another possibility exists: the pharmacological
receptor systems including Ca²⁺ channels,
normally in a reduced state, are oxidized in
the artificial environment of the tissue bath
and need to be reduced to regain full activity.

Additional biochemical and receptor iso-
lution studies are now required to evaluate
the role of the sulfhydryl group-dependent
sites as primary or secondary binding sites of
pharmacological receptors.

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