CHAPTER IV

RESULTS

Part A. Effect of GSH on vasorelaxation

1. Influence of endothelium

The effects of GSH on vasorelaxation in endothelium-intact and -denude rat thoracic aorta which were pre-contracted with PE were demonstrated in Fig. 5A and 5B. The result showed that GSH caused vasorelaxation in concentration-dependent manner on both intact and denude rat aortic rings, but with different degree of relaxation (Fig. 6). Interestingly, the vascular tension of the endothelium-denude rat aortic rings in the presence of GSH dropped below the initial resting tension when the concentration of GSH increased to 8mM (Fig. 5A). This phenomenon was not observed in experiments with the endothelium-intact rat aortic rings (Fig. 5B). In addition, removal of endothelium from aortic preparations affected the characteristic of the GSH-vasorelaxation profiles. As shown in Fig. 5B, GSH induced the transient and non-sustainable relaxation of endothelium intact preparations. On the contrary, in endothelium-denuded preparations, the vasorelaxation effects of GSH were sustained for longer period (Fig. 5A).

There was a concern whether high concentration of GSH might affect muscle contractility by changing the osmolarity in the experiment. In this study, L-valine at the equimolar concentration as GSH was applied as a control group. The data demonstrated that L-valine could not induce relaxation even at the highest concentration (Fig. 6). Hence, the changes in osmolarity were irrelevant to the vasorelaxation effects of GSH in this study. GSH at the low concentrations of 2 and 4 mM induced relaxation of the intact preparations at higher degree than those of the denude preparations. However, the

different outcomes were observed at high concentration of GSH (8 mM). The endothelium denude preparation appeared to be more sensitive to vasorelaxant effect of GSH (8 mM) than the intact preparations. These findings suggested an important role of endothelium in maintaining vascular tone in response to high antioxidative environment. From these results, the concentration of GSH at 5 mM was selected for further investigation.

2. Endothelium-dependent mechanisms of GSH-induced vasorelaxation

2.1. Effects of various vasorelaxant inhibitors

In this experiment, various inhibitors of vasorelaxation, including ibuprofen, L-NAME, methylene blue, glibenclamide, propranolol and atropine were used to examine the mechanisms of GSH-induced relaxation in the intact aortic rings. At the concentration of 5 mM, GSH caused approximately 70-80% relaxation in endothelium-intact aortic rings treated with PE 1μM (Fig. 7). In addition, the presence of L-NAME, methylene blue and glibenclamide resulted in significant reduction of the relaxant effect of GSH (Fig. 7, 8 and Fig. 34 and 35 in Appendices). These findings suggested that GSH mediated the endothelium-dependent relaxation via NO-cGMP and hyperpolarizing signaling pathway.

2.2. Influence of calcium

An increase of intracellular Ca^{2+} in the endothelium activates the NO synthase activity and increases production of NO, resulting in vasorelaxation (Karaki and Hori, 1998). Hence, this study was to examine the influence of intracellular Ca^{2+} on the GSH-induced relaxation by applying Ca^{2+}-free environment to the experiment condition. In this study, EGTA was used to chelate extracellular Ca^{2+} whereas BAPTA-AM was used to

chelate intracellular Ca^{2+} in both endothelium and smooth muscle cells. Upon changing medium from Ca^{2+}-containing solution to Ca^{2+}-free medium containing EGTA, the contractile response to PE decreased by 80%. However, the PE-induced contraction was still sustainable (Fig. 9). Under this condition, the vasorelaxation effects of GSH were compromised significantly (Fig. 10). On the contrary, BAPTA–AM (10μM) had no markedly influence on either PE-induced contraction or the degree of GSH-induced relaxation (Fig. 11 and 12). These finding suggested that extracellular Ca^{2+} was more critical than intracellular Ca^{2+} in GSH-induced vasorelaxation in the endothelium intact preparation. It was likely that the endothelium-dependent vasorelaxation effect of GSH depended on extracellular Ca^{2+}. GSH might affect processes of Ca^{2+} influx, without any interference on activation of Ca^{2+} release from the cellular storage.

Although, the potency of GSH on vasorelaxation did not alter in the presence of BAPTA-AM, the relaxation profiles were affected. As shown in Fig. 11A, the relaxation induced by GSH did not sustain in the normal Ca^{2+}-containing solution. The presence of BAPTA-AM prolonged the relaxation time of the aortic rings treated with GSH (Fig. 11B). It was likely that the deprivation of intracellular Ca^{2+} stabilized the vasorelaxant effect of GSH. Moreover, the GSH-induced relaxation profiles of endothelium-intact aortic tissue and those of endothelium-denude preparations were found to be similar in the presence of BAPTA-AM. Hence, Ca^{2+} content in endothelium cells was crucial for control the characteristic of GSH-induced transient relaxation.

2.3 Potentiation effects of GSH on the Ach- or SNP-induced relaxation

From previous experiments, GSH exerted its vasorelaxant effect mainly through NO-cGMP pathway. However, it was possible that another mechanism involved. If there

were more than one mechanism involved in the relaxation effect of GSH, the potentiation may occur. Hence, in this study, the cumulative concentration of Ach (0.01-100 μM) or SNP (0.001-10 μM) were applied to induce relaxation in the presence of GSH in endothelium-intact rat aortic rings. As known, Ach induced relaxation through the NO-cGMP pathway by increase production of NO in endothelium cells whereas SNP induced relaxation via direct stimulation of guanylate cyclase (GC) to produce cGMP in smooth muscle cells. As shown in Fig.13, 14 and 15, GSH significantly potentiated the relaxation effects of Ach, but not those of SNP.

Another interesting finding was that the potentiative effects of GSH on Ach-induced relaxation depended on the amount of intact endothelium cells. The more endothelium intacted, the less potentiative effect of GSH on Ach-induced relaxation was observed. In the preparations with 80-90% of intact endothelium, GSH had no significant potentiative effect of Ach-induced relaxation. However, in those with less than 70% of intact endothelium, GSH elicited its potentiative relaxation significantly. These results suggested that GSH could enhance the function of endothelium in production of NO. The lack of potentiating effect in SNP-induced vasorelaxation might reflect that GSH had no influence on GC or contractile elements.

3. Endothelium-independent mechanism of GSH-induced vasorelaxation

3.1 Vasorelaxation effects of GSH

As shown in Fig. 16A, GSH caused relaxation of endothelium-denude aortic rings precontracted with PE in concentration-dependent manner. The similar results were also observed when the aortic rings were precontracted with either KCl or Bay K8644 (L-type Ca^{2+} opener). However, the aortic tissues precontracted with PE produced more sensitive

response to GSH than those precontracted with either Bay K8644 or KCl (Fig. 16A and B). The apparent EC_{50} values of vasorelaxant effects of GSH (in mM) were summarized in Table 1. Moreover, GSH at high concentration (8 mM) markedly reduced the aortic tension to less than the initial resting tension if the aortic tissues were precontracted with PE. The "below-baseline" vasorelaxant effects of GSH were not seen in the aortic tissue precontracted with either KCl or Bay K8644.

Table 1. Vasorelaxant potency of GSH and NAC in endothelium-denude aortic rings precontracted with PE, KCl and Bay K8644

	% Relaxation*	
Contractants	GSH	NAC
Phenylephrine (PE)	138.56±7.43	146.89±6.22
Potassium chrolide (KCl)	98.08±4.37	92.87±8.17
Bay K8644	94.76±6.6	ND

Data are expressed as mean ± S.E.M. from 6-8 separated experiments

* % Relaxation was calculated as the percentage of the maximal vascular tension induced by each contractants

ND; Not determined

In this study, L-valine was also applied as a control group. The results demonstrated that high concentration of L-valine at the equivalent concentration as GSH and NAC had no effect on vascular tension. Hence, high concentration of GSH and NAC in this experiment had no influence on the results of GSH or NAC on vascular tension. The findings in the experiments suggested that GSH or NAC had direct vasorelaxant effects on aortic smooth muscle.

3.2. Effects of various vasorelaxant inhibitors.

At 5 mM, GSH was able to induce endothelium-independent vasorelaxation about 20-30% of the sustained contraction induced by PE (1 μM). This experiment was to determine the endothelium-independent mechanisms of GSH on relaxation by using various known inhibitors of vasorelaxation including ibuprofen, L-NAME, methylene blue, glibenclamide, propranolol and atropine. The results indicated that only glibenclamide showed significant inhibitory effects against GSH-induced relaxation in denude rat aortic rings (Fig. 17 and 18). These findings suggested that the endothelium-independent mechanisms may involve the activation of K^+ channel-mediated hyperpolarizing effect. However, the vasorelaxant inhibitors in this study even at high concentration could not inhibit GSH-induced relaxation completely, suggesting several mechanisms might involve in the relaxation. It was possible that GSH might affect the disruption of Ca^{2+} mobilization and signaling in activation of contraction.

Part B. Effect of GSH on contractility of smooth muscle

1. Influence of endothelium

In order to investigate the influence of endothelium on GSH action, endothelium intact- and denude-aortic rings were used for comparison of the inhibitory potency of GSH on PE-induced contraction. As shown in Fig. 19, the presence of endothelium could hinder the contractile response to PE, especially at the low concentration (0.001 μM–0.01 μM). The maximum tension caused by PE (10 μM) in this study was 1.56 g ± 0.117 (n=6) for endothelium intact preparation and 1.38 g ± 0.14 (n=6) for endothelium denude preparation. As shown in Fig. 20A, the presence of GSH (5 mM) could significantly suppress the PE-induced contraction of either endothelium-intact or -denude aortic rings when the concentrations of PE were 0.01 μM – 10 μM. When the concentration of PE was 0.001 μM, GSH did not significantly suppress the PE-induced contraction of the intact rat aortic rings but significantly suppress those of denude rat aortic rings.

Similarly to the effects of GSH (5 mM), NAC (5 mM) could significantly suppress the contraction induced by PE in either intact or denude rat aortic rings (Fig. 20B). However, the inhibitory potencies of these 2 compounds against PE-induced contraction were different. The result showed that, at the equimolar concentration of 5 mM, NAC was more potent than GSH in inhibiting the contraction of denude rat aortic rings. This discrepancy was not observed when the aortic preparations still had endothelium intact of 70-80%. Furthermore, NAC elicited its inhibitory action against PE-induced contractions in the endothelium-denude aortic preparation, better than those in the endothelium intact preparation. Hence, it appeared that the presence of endothelium hindered the inhibitory action of NAC. On the contrary, when GSH was

applied in place of NAC, its inhibitory action against PE-induced contraction of the endothelium intact and denude preparations was similar. These observations implied the different intrinsic property of NAC and GSH.

2. Direct effects of GSH on the aortic contraction

2.1. Inhibitory effects of GSH

In order to investigate the direct effects of GSH on aortic smooth muscle, the endothelium-denude aortic rings were used in these experiments. As seen in Fig. 21A, GSH was able to shift the concentration-response curve of PE-induced contraction rightward. At the concentration of 5 mM, GSH appeared to effectively inhibit the contraction induced by PE. However, the inhibitory effect of GSH at 5 mM decreased gradually when the concentration of PE increased. By contrast, this reverse relationship was not observed when the concentration of GSH was at 8 mM. As shown in Fig. 21A, GSH at the concentration of 8 mM appeared to shift the concentration-response curve of PE-induced contraction rightward in parallel to those of the control group. In addition, GSH at 8 mM was able to significantly suppress PE-induced contraction greater than GSH at 5 mM. These findings suggested that the inhibitory effects of GSH against PE-induced contraction were concentration-dependent.

The inhibitory effect of NAC was also investigated in this study. The results demonstrated that, like GSH, NAC could inhibit PE-induced contraction. However, it appeared that NAC was more potent than GSH in inhibiting PE-induced contraction (Fig. 21B).

2.2. Comparative effects of GSH, NAC, homocysteine and captopril

2.2.1. Inhibition against PE-induced contraction

In addition to GSH, various compounds containing sulfhydryl group were determined for their inhibitory effects against PE-induced contraction. These compounds included GSH, NAC, homocysteine and captopril. As shown in Fig. 22, GSH at concentration of 5 mM was able to inhibit the aortic contraction provoked by single treatment of PE at the concentration of less than 0.1 μM. In this study, NAC, homocysteine and captopril at the equimolar as GSH also elicited their inhibitory effects against PE-induced contraction in similar pattern to those of GSH, but with different potency (Fig. 22). The inhibitory effects of the tested thiol compounds were prominent only when the low concentration of PE was used to contract the aortic tissues. These inhibitory effects were not observed when the concentration of PE was more than 0.1 μM. In this study, captopril was the least potent compound in suppression of PE-induced contractions, especially when the low concentration of PE (0.001 and 0.01 μM) were applied (Fig. 22). It was possible that the sulfhydryl group of each inhibitor contributed to its intrinsic inhibitory action. The attribution of sulhydryl group on inhibitory action needs further investigation.

2.2.2. In vitro Antioxidant activity

In previous section, the compounds containing sulhydryl group elicited their influence on the responsiveness of aortic smooth muscle toward PE treatment. It was possible that these compounds exerted its actions through sulhydryl action and/or its antioxidant capacity. Hence, this study was to compare the antioxidant activity of the tested compounds including GSH, NAC, homocysteine and captopril, by using DPPH

free radical scavenging assay. The result showed that the capability of all tested compounds to reduced DPPH free radical was indifferent when their concentrations were 0.5 and 5 mM. Upon increasing the concentration of these thiol containing compound to 50 and 500 mM, their antioxidant activity increased in concentration-dependent manner. Furthermore, the results showed that the antioxidative effects of NAC at 50 and 500 mM were higher than those of GSH, homocysteine and captopril (Fig. 23).

2.3 Inhibitory effects of GSH against various contractants

In addition to PE, the direct inhibitory effects of GSH were tested against contraction-induced by various contractants including serotonin (5-HT; 1 μM), histamine (1 mM), PE (1 μM), PMA (1 μM), TEA (1 mM) and KCl (60 mM). At the concentration used in this study, all of these contractants produced submaximum contraction (EC ≈ 80-90%) of denude aortic rings which could sustain for at least 15 minutes. The contraction of endothelium denude aortic preparation in response to these contraction was shown in Table 2. As shown in Fig. 24A, GSH (5 mM) could not significantly suppress the aortic contraction induced by PE, PMA, TEA and KCl. By contrast, GSH significantly inhibited the contraction induced by 5-HT and histamine. The results also showed that the contraction induced by histamine was more sensitive to GSH action than those induced by 5-HT (Fig. 24A). These findings suggested that the direct inhibitory effect of GSH on vasoconstriction were determined by the responsiveness of aortic muscle toward specific contractants. In addition, GSH was unlikely to interfere membrane integrity involving the binding and signaling of α_1-adrenoceptor as well as membrane depolarization and the inhibition of potassium channel.

This study also aimed to investigate the inhibitory effects of NAC on the contraction induced by 5-HT, histamine, PE, KCl and TEA (Fig. 24B). Similarly to GSH treatment, the result showed that the effects of NAC pretreatment significantly inhibited the contraction induced by 5-HT and histamine, but not by PE, KCl and TEA.

Table 2. The percentage of maximum contraction in endothelium-denude aortic rings precontracted with PE, KCl, TEA, 5-HT, His and PMA

Contractants	% contraction*
Phenylephrine (PE, 1 μM)	87.89±6.22
Potassium chrolide (KCl, 60 mM)	89.87±8.17
Tetraethylammonium chloride (TEA, 1 mM)	88.37±6.37
Serotonin (5-HT, 1 μM)	86.18±7.02
Histamine (His, 1 mM)	84.52±5.16
Phorbal-12-myristate-13-acetate (PMA, 1 μM)	81.93±8.69

Data are expressed as mean ± S.E.M. from n=6-8 separated experiments

* % contraction was calculated as the percentage of the maximal vascular tension induced by each contractants

3. Inhibitory effects of GSH in Ca^{2+}-free condition

3.1 Influence on Ca^{2+} release from internal stores

These experiments aimed to investigate the effects of GSH on the Ca^{2+}-release from internal storage in the vascular smooth muscle cells. In this study, two contractants with known mechanisms were used to release Ca^{2+} from its internal stores. They were PE (an α_1 agonist) to activate IP_3 receptor and caffeine to activate ryanodine receptor on sarcorplasmic reticulum (SR) (Baran *et al.*, 2008; Ji *et al.*, 1998; Watanabe *et al.*, 1992).

In Ca^{2+}- free solution, PE (1 μM) and caffeine (1 mM) were able to provoke transient aortic contractions as shown in Fig. 25A and B. All of the sulhydryl containing compounds in this study significantly inhibited PE-induced contraction in Ca^{2+}-free solution (Fig. 26A). The results suggested that these compounds were able to inhibit an increase of intracellular Ca^{2+} through α_1-mediated IP_3 receptor mechanism. The descending order of the inhibition potency was GSH > captopril > NAC > homocysteine. GSH and NAC were further investigated for its inhibitory actions against caffeine-induced contraction in Ca^{2+}-free solution. The results showed that neither GSH nor NAC could inhibit aortic contraction in this condition (Fig. 26B), suggesting that both compounds did not interfere Ca^{2+}-release from internal storage through activation of ryanodine receptor. Hence, GSH and other sulfhydryl containing compounds in this study selectively affected intracellular Ca^{2+}-release mechanisms. These compounds may affect the mechanism coupling with plasma membrane activation or signaling of α_1-adrenoceptor. Since GSH and NAC were hardly transported or diffuse through plasma membrane into the cells (Kugiyama *et al*, 1998), it was likely that the primary targets of these compounds were located at plasma membrane.

3.2 Influence on Ca^{2-} influx

These experiments aimed to investigate the effects of GSH on the Ca^{2+}-influx through voltage-gated Ca^{2+} channels. The investigations were performed by cumulative addition of $CaCl_2$ into high K^+, Ca^{2+}- free depolarizing solution in which the aortic muscle were suspended. Interestingly, this experiment showed that GSH induced transient contraction of the aortic muscles in the resting state when they were suspended in high K^+, Ca^{2+}-free depolarizing solutions, but not in normal Kreb solutions. This

effect was not observed when L-valine was used in place of GSH (Fig. 27A and B). As shown in Fig. 28A, GSH and NAC significantly inhibited the contraction and shifted the concentration-response curve rightward. In addition, the inhibitory effects of GSH and NAC were irreversibly. Addition of $CaCl_2$ at high concentration could not restore the maximum contraction (Fig. 28A).

The inhibitory effects of GSH on $CaCl_2$–induced contraction in high K^+, Ca^{2+}-free solution were concentration-dependent (Fig. 28B). At the concentration of 2 mM, GSH was unable to suppress the contractile response of the aortic tissue significantly. In this study, the significant inhibitory effects of GSH were observed at the concentration of 5 mM and 8 mM. These findings suggested that the extracellular Ca^{2+} influx through Ca^{2+} channel could be suppressed by GSH.

The actions of GSH on Ca^{2+} channel were further investigated by using various types of contractants to provoke contraction, followed by addition of $CaCl_2$. As shown in Fig. 29A, the contractile responses were originated from initial release of intracellular Ca^{2+} on initial phase, followed by subsequent influx of extracellular Ca^{2+} on second phase. The results indicated that GSH significantly reduced both phases of contraction when PE and 5-HT were used as contractants (Fig. 29A and Fig. 30). On the other hand, KCl could not provoke contraction in Ca^{2+}-free condition because it had no effect on Ca^{2+} release from internal store, only the second phase of contraction which was mediated by KCl-induced influx of extracellular Ca^{2+} was observed (Fig. 29B). Furthermore, the second phase contraction with experiments using KCl and Bay K8644 as contractants significantly decreased in the present of GSH (Fig. 30). In this study, verapramil which was a selective L-type Ca^{2+} channel blocker as well as Bay K8644 which was L-type

Ca^{2+} channel opener were also applied as positive control groups. Data were shown in Fig. 36 and 37 (in Appendices).

In addition to GSH, the effect of homocysteine was also investigated in this study. Results showed that homocysteine provided inhibitory profiles similarly to those of GSH, but with a lesser degree when Bay K8644 and KCl were contractants (Fig. 30).

Taken together, these findings demonstrated that GSH and homocysteine were able to disrupt Ca^{2+} release mediated by PE and 5-HT as well as Ca^{2+} influx through receptor operated Ca^{2+} channel (ROC) and voltage operated Ca^{2+} channel (VOC).

3.3 Effects on spontaneous contraction of Ca^{2+}-deprived aorta

Under normal physiology conditions, Ca^{2+} influx to the vascular smooth muscle cell cannot occur spontaneously. However, it has been demonstrated that aortic rings with PE-mediated depletion of intracellular Ca^{2+} could generate spontaneous contraction upon addition of Ca^{2+}. This phenomenon suggested the spontaneous replenishment of Ca^{2+} to the cells through store-operated Ca^{2+} channel (SOC). In addition, it can be observed as an increase in the resting tone (IRT) of the aortic preparation. As shown in Fig. 31, a spontaneous contraction or IRT of the rat aortic rings pretreated with PE repetitively was reproduced as stated in several previous reports (Noguera *et al*, 1998; Jackson, 2000). The initial step started with the contraction induced by PE in KHS, followed by repetitive addition of PE in Ca^{2+}-free environment until the transient contraction response to PE was not observed. The spontaneous contraction or IRT was produced by replacing Ca^{2+} free solution with KHS. The results showed that GSH (5 mM) significantly decreased the IRT response (Fig. 32), suggesting that GSH may interfere Ca^{2+} entry through SOC.

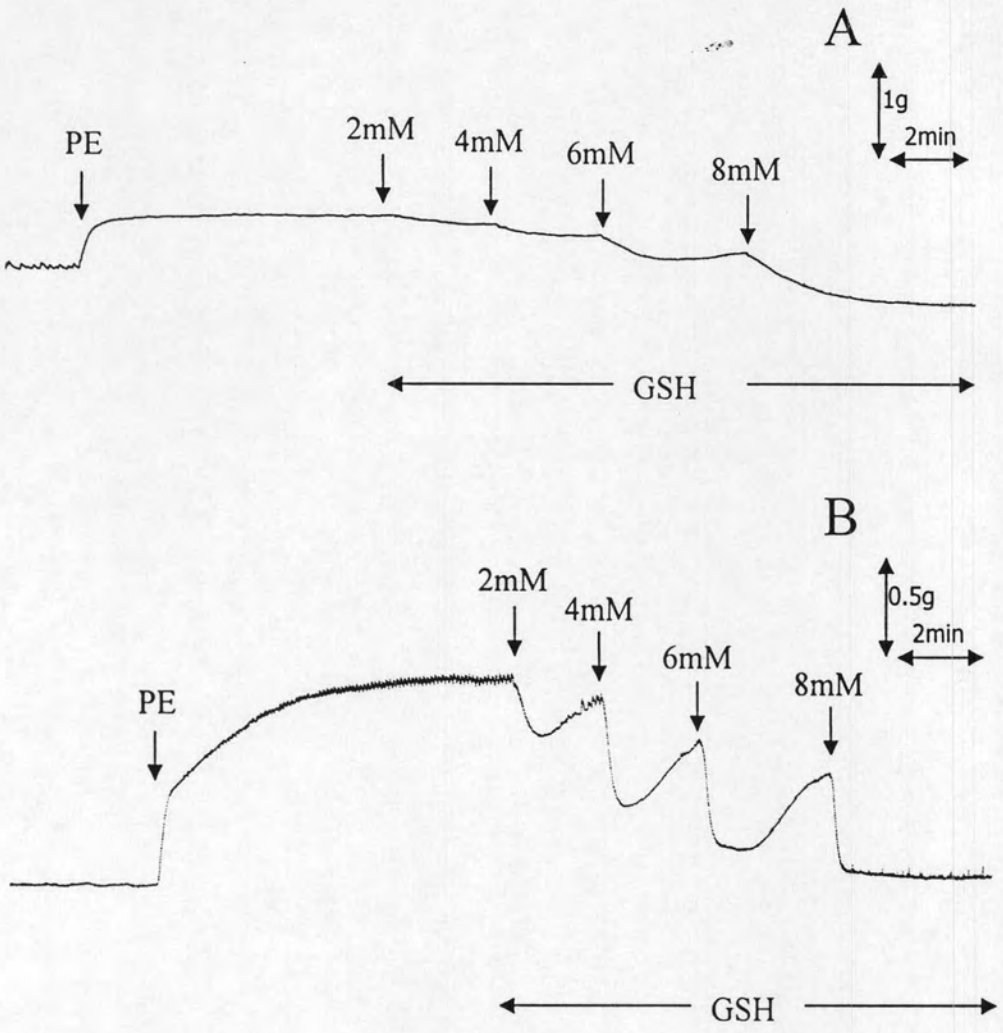


Fig. 5 The representative tracing of GSH induced relaxation in endothelium-denude (A) and endothelium-intact (B) rat aortic rings. The aortic rings were precontracted with PE (1 μM), followed by addition of GSH cumulatively (2, 4, 6 and 8 mM).

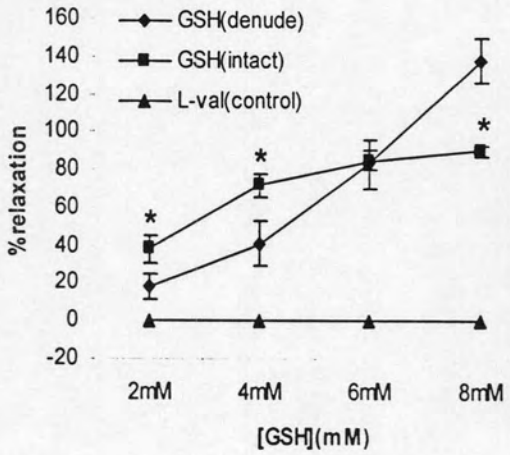


Fig. 6 Concentration-dependent relaxant effects of GSH in endothelium-denude and endothelium-intact rat aortic rings. The tissues were precontracted with PE (1 μM) prior to cumulative addition of GSH. L-valine was also used in place of GSH as a control. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *$P<0.05$ showed significant difference between intact and denude preparation.

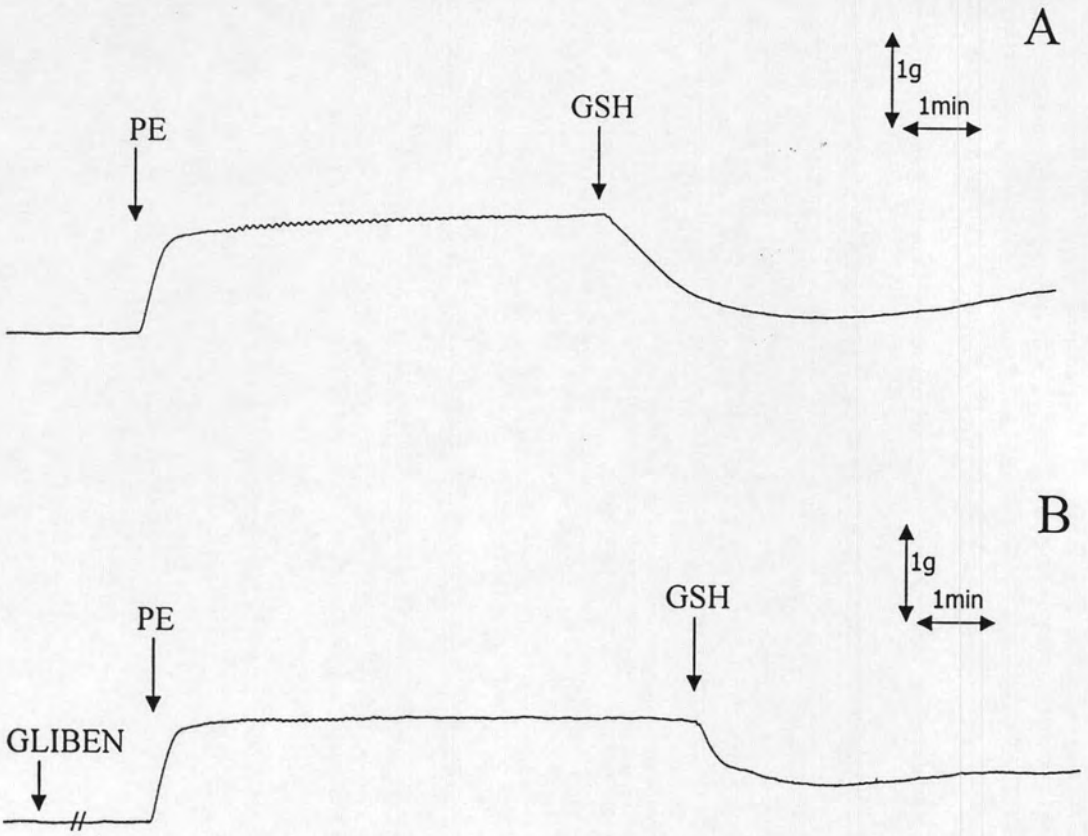


Fig. 7 The representative tracing of the relaxation induced by GSH (5 mM) on endothelium-intact rat aortic rings which were preincubated in the absence (A) or presence (B) of glibenclamide (10 μM) for 30 minutes prior to addition of PE (1 μM) and GSH (5 mM).

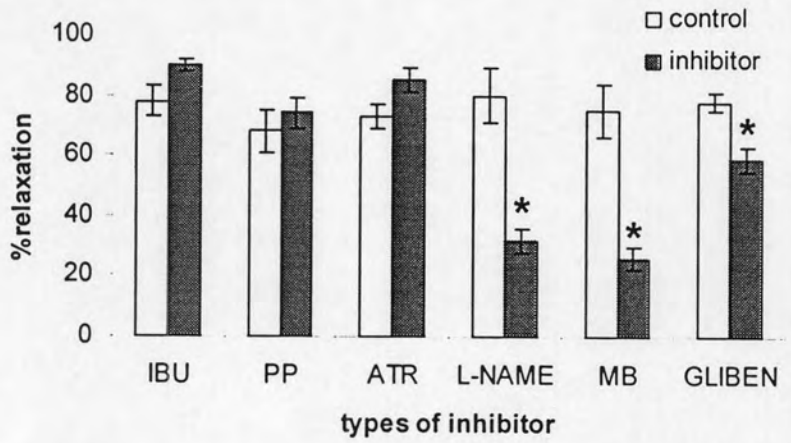


Fig. 8 Effects of various inhibitors [ibuprofen (IBU, 10 μM), propranolol (PP, 10 μM), atropine (ATR, 10 μM), L-NAME (10 μM), methylene blue (MB, 10 μM), glibenclamide (GLIBEN, 10 μM)] on the GSH-induced relaxation in endothelium-intact rat aortic rings. The tissues were preincubated with each inhibitor for 30 min prior to addition of PE (1 μM). When the PE-induced contraction reached to maximum and sustainable stage, GSH (5 mM) was added to induce relaxation. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *$P<0.05$ showed significant difference from control group.

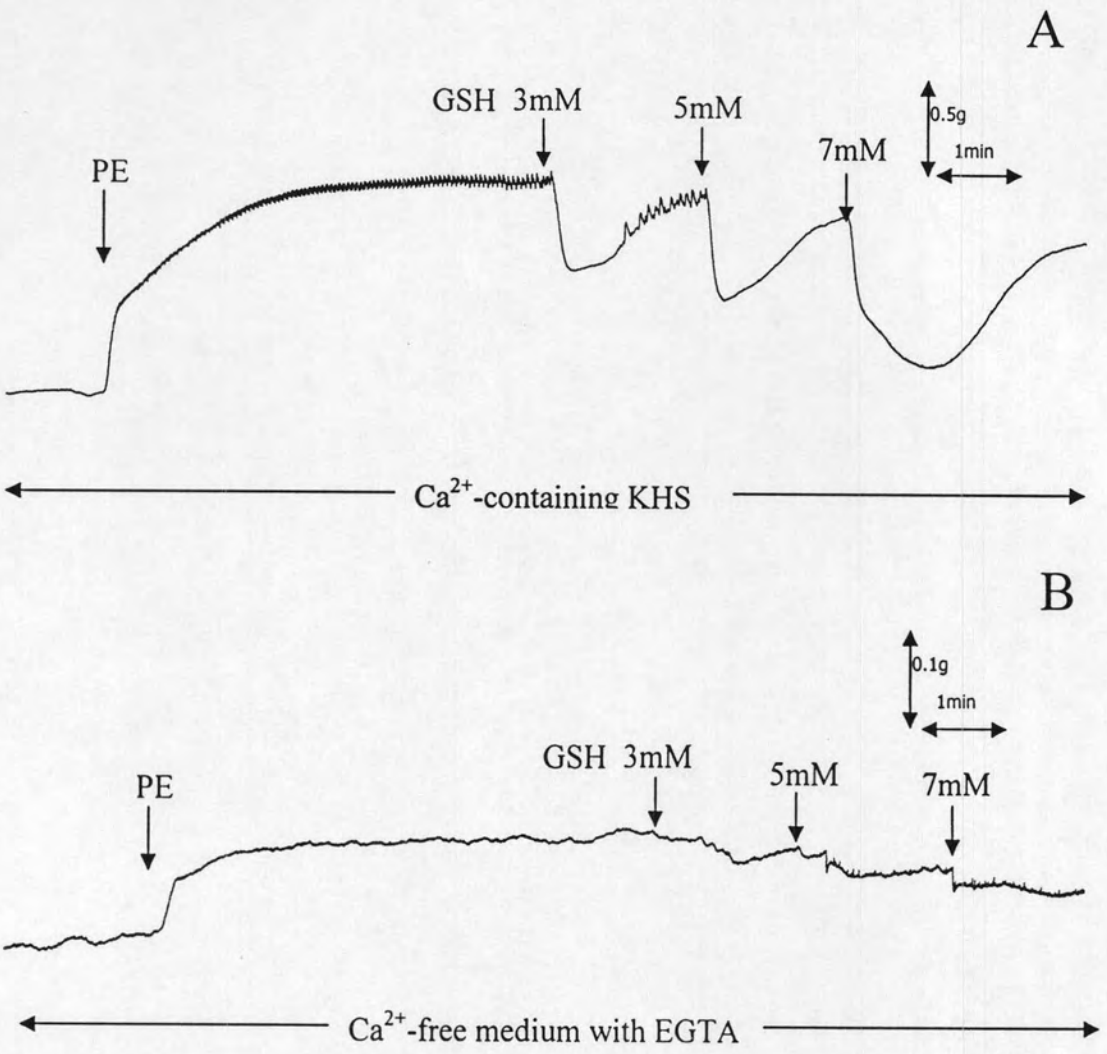


Fig. 9 The representative tracing of GSH-induced relaxation in endothelium-intact rat aortic rings. GSH-induced vasorelaxation were performed in normal KHS (A) and in Ca^{2+}-free KHS containing of EGTA (0.2 mM) (B). GSH-induced relaxation was calculated as the percentage of maximum contraction caused by PE (1 μM) under each condition.

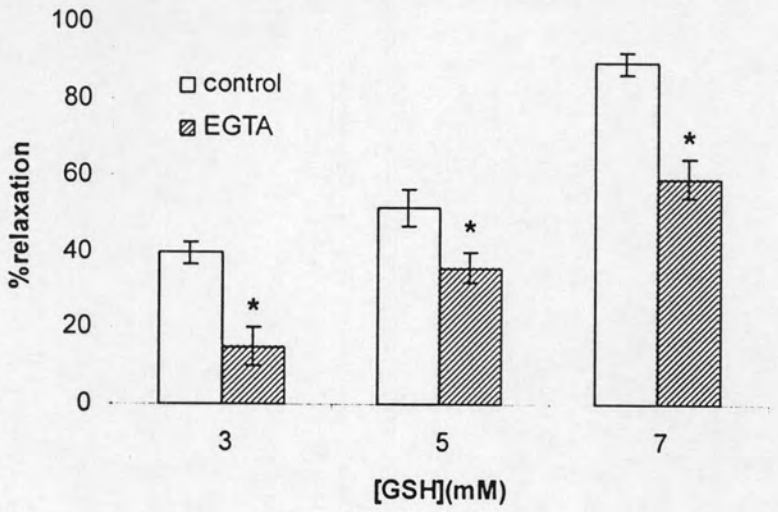


Fig. 10 Concentration-dependent relaxant effects of GSH (3, 5 and 7 mM) obtained in the presence and in the absence of extracellular Ca^{2+} in endothelium-intact rat aortic rings. GSH-induced relaxation was calculated as the percentage of maximum contraction caused by PE (1 μM) under each condition. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group in normal KHS.

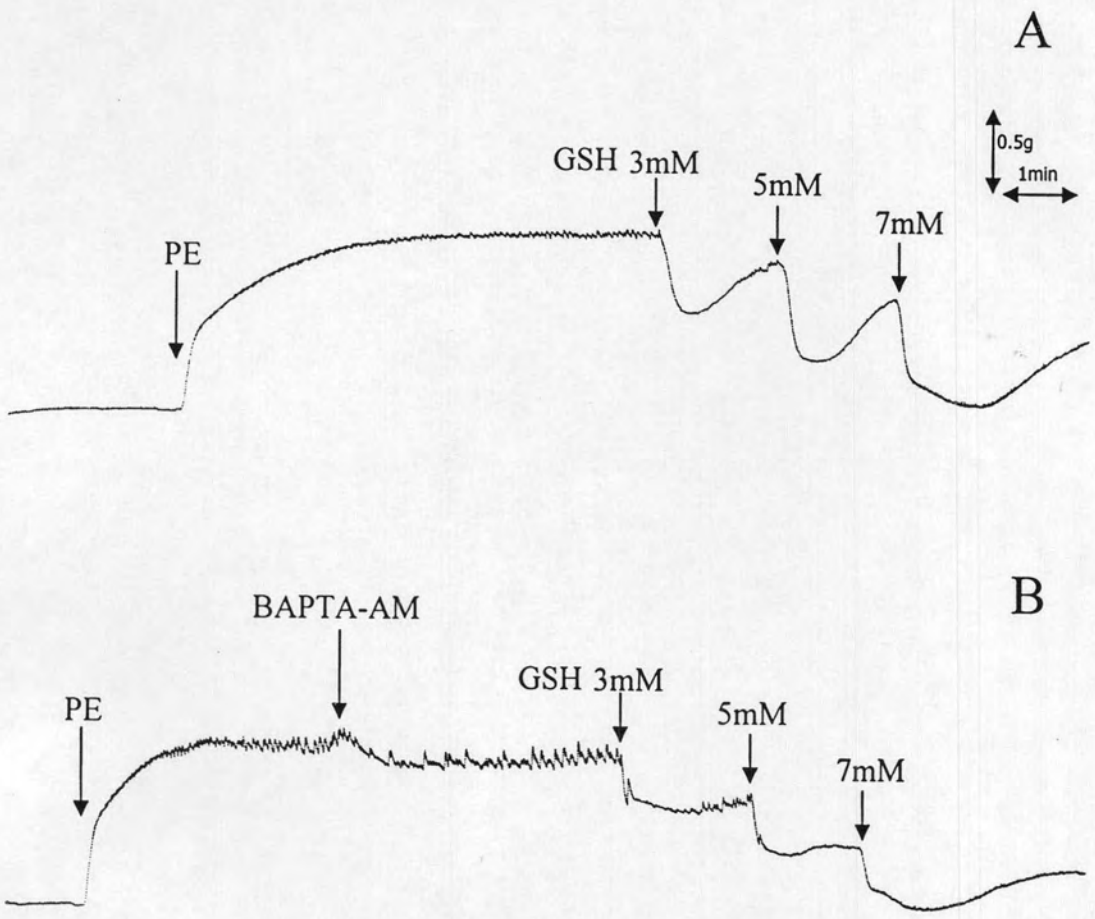


Fig. 11 The representative tracing of GSH-induced relaxation in the absence (A) and presence (B) of BAPTA-AM (10 μM).

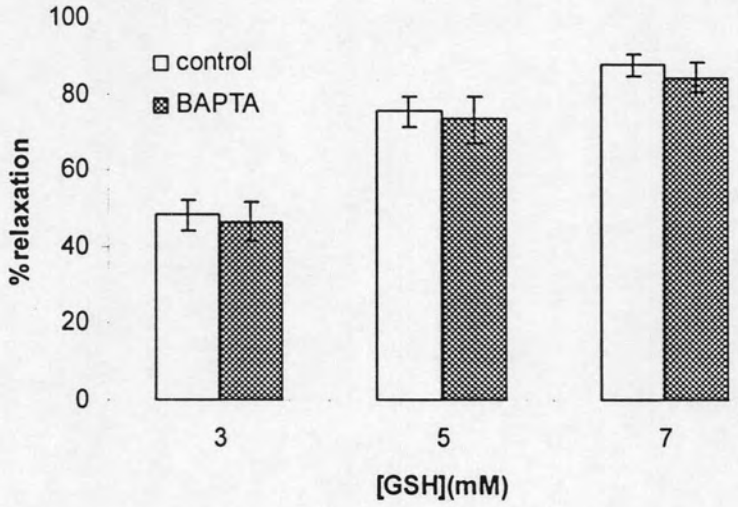


Fig. 12 Concentration-dependent relaxant effects of GSH (3, 5 and 7 mM) obtained in the presence and in the absence of intracellular Ca^{2+} in endothelium-intact rat aortic rings. The tissues were precontracted with PE (1 μM). Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group.

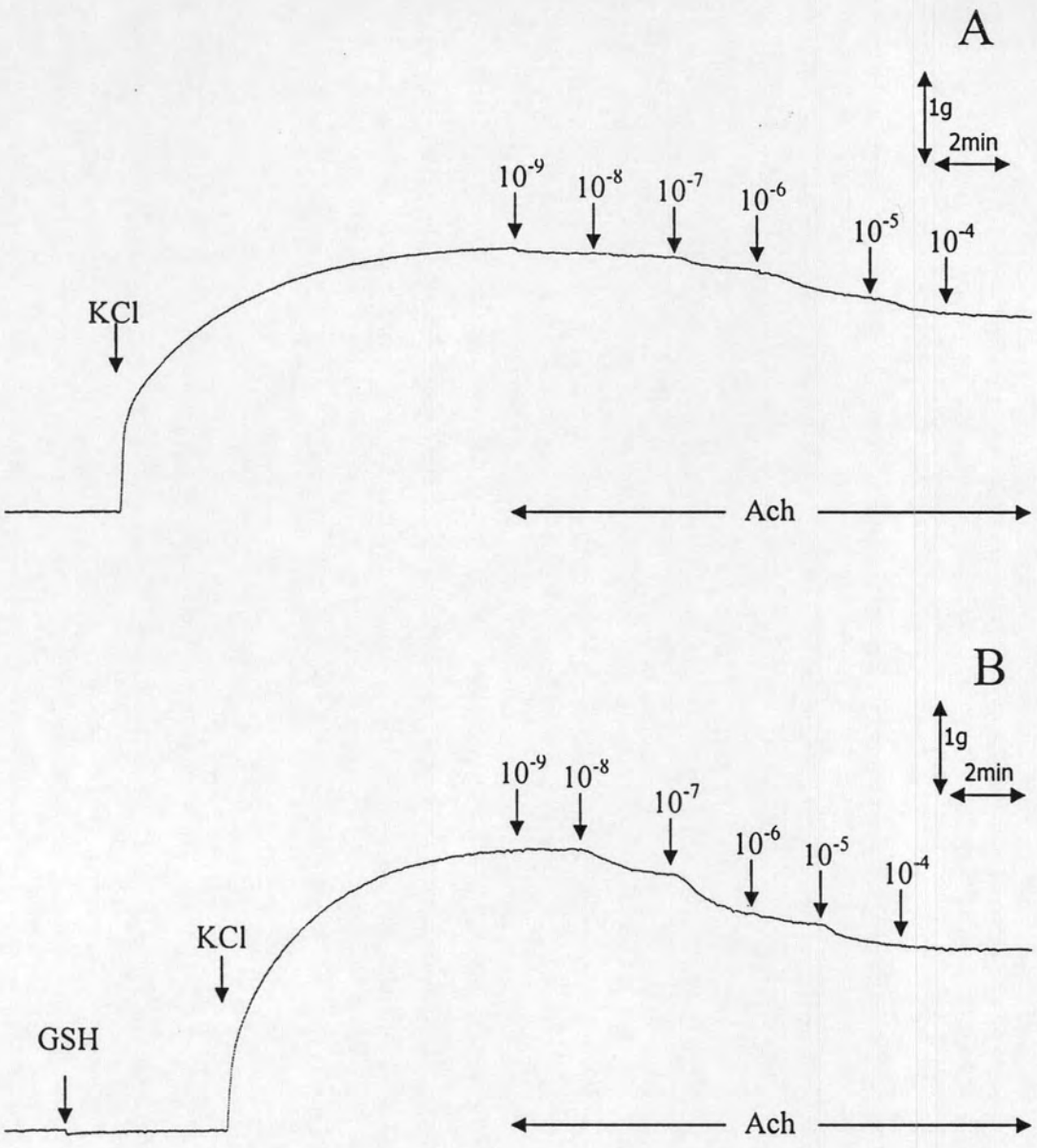


Fig. 13 The representative tracing of Ach-induced vasorelaxation of endothelium-intact rat aortic rings in the absence (A) and presence of GSH (B). GSH (5 mM) were preincubated with tissues for 5 min prior to addition of KCl (60 mM), followed by cumulative addition of Ach (0.01-100 μM) to induce relaxation

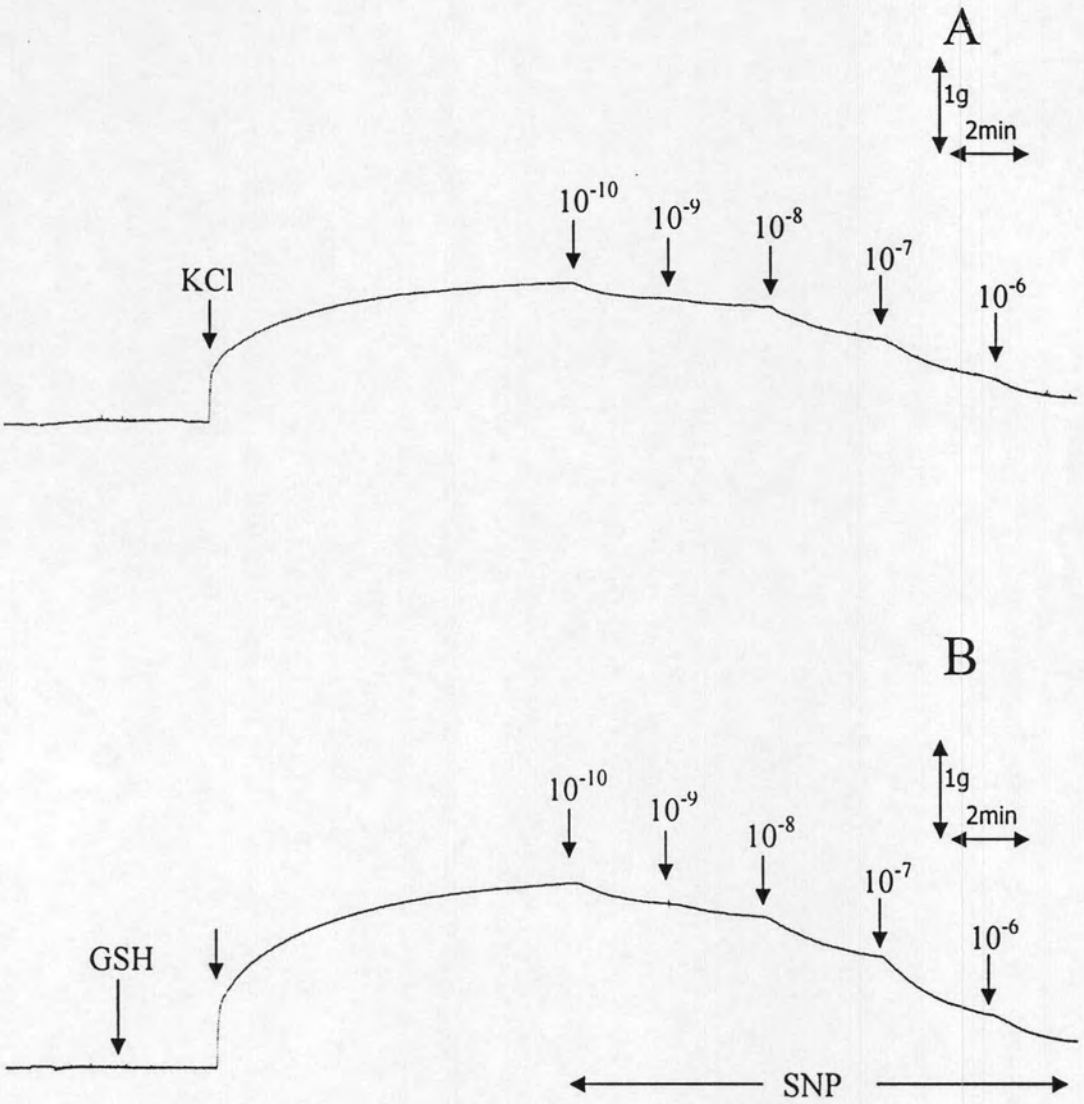
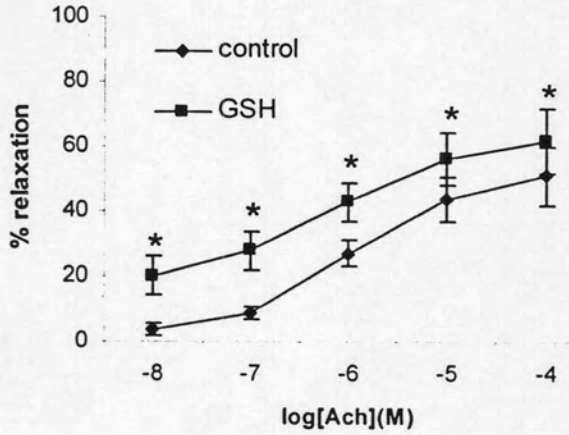


Fig. 14 The representative tracing of SNP-induced vasorelaxation of endothelium-intact rat aortic rings in the absence (A) and presence of GSH (B). GSH (5 mM) were preincubated with tissues for 5 min prior to addition of KCl (60 mM), followed by cumulative addition of SNP (0.01– 1 μM)

A



B

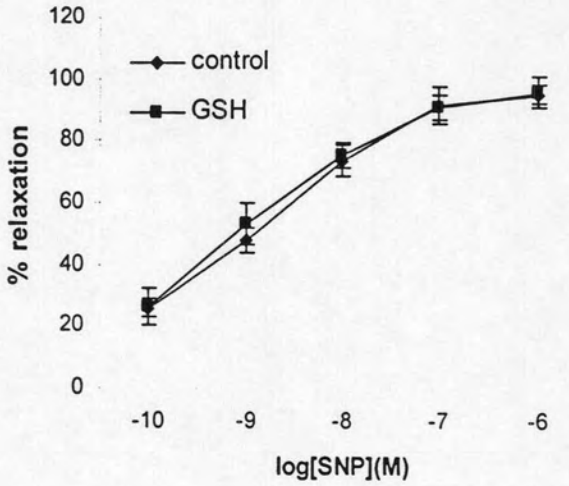


Fig. 15 Potentiative effects of GSH on vasorelaxation-induced by Ach (A) or SNP (B). Experiments were performed in endothelium-intact rat aortic rings. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group.

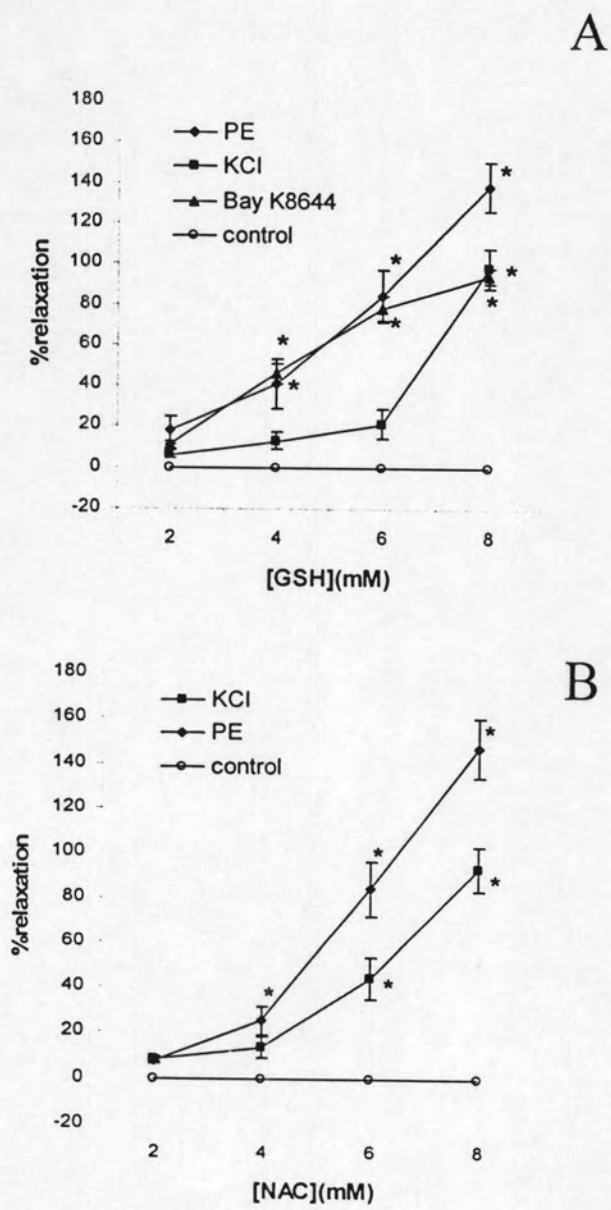


Fig. 16 Endothelium-independent vasorelaxant effect of GSH (A) or NAC (B) The aortic tissues were precontracted with PE (1 μM), KCl (60 mM) and BayK8644 (1 μM), followed by cumulative addition of GSH and NAC (5 mM). Data are mean ± S.E.M. of 6-8 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group.

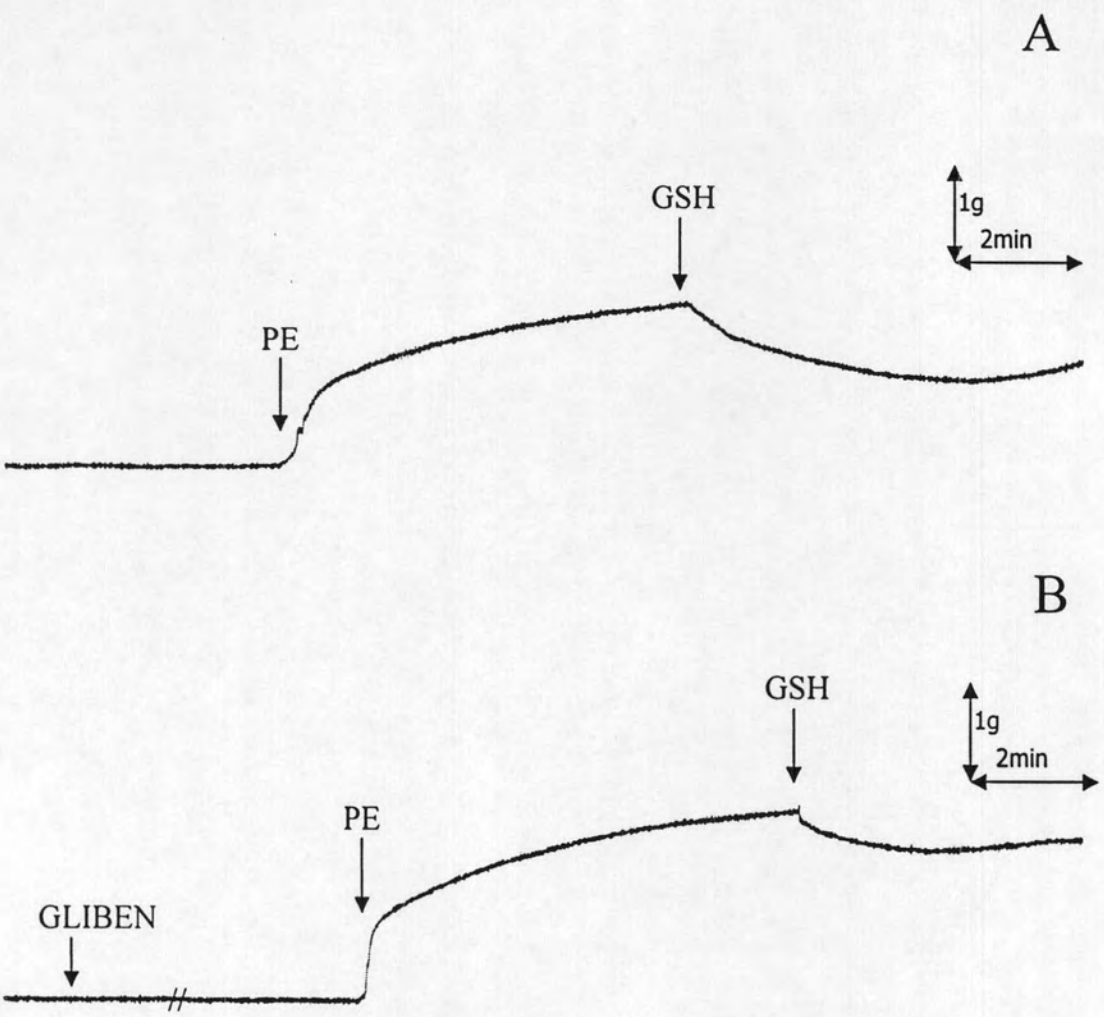


Fig. 17 The representative tracing of the relaxation induced by GSH (5 mM) on endothelium-denude rat aortic rings which were preincubated in the absence (A) or presence (B) of glibenclamide (10 μM) for 30 minutes prior to addition of PE (1μM) and GSH (5 mM).

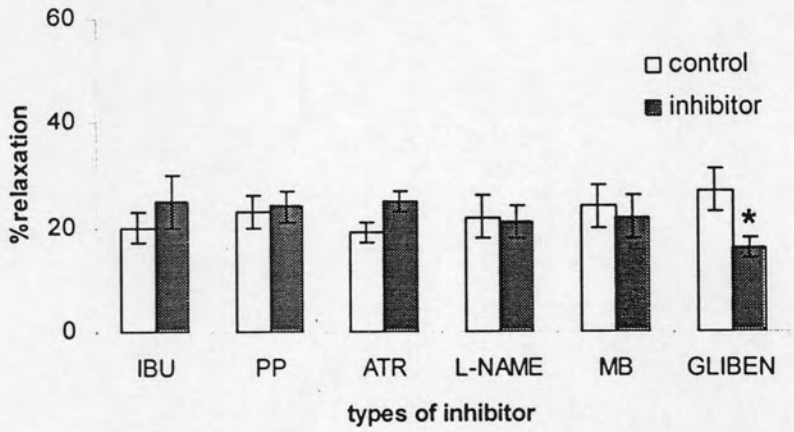


Fig. 18 Effects of various inhibitors [ibuprofen (IBU, 10 μM), propranolol (PP, 10 μM), atropine (ATR, 10 μM), L-NAME (10 μM), methylene blue (MB, 10 μM), glibenclamide (GLIBEN, 10 μM)] on the GSH-induced relaxation in endothelium-denude rat aortic rings. The tissues were preincubated with each inhibitor for 30 min prior to addition of PE (1 μM). When the PE-induced contraction reached to maximum and sustainable stage, GSH (5 mM) was added to induce relaxation. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *$P<0.05$ showed significant difference from control group.

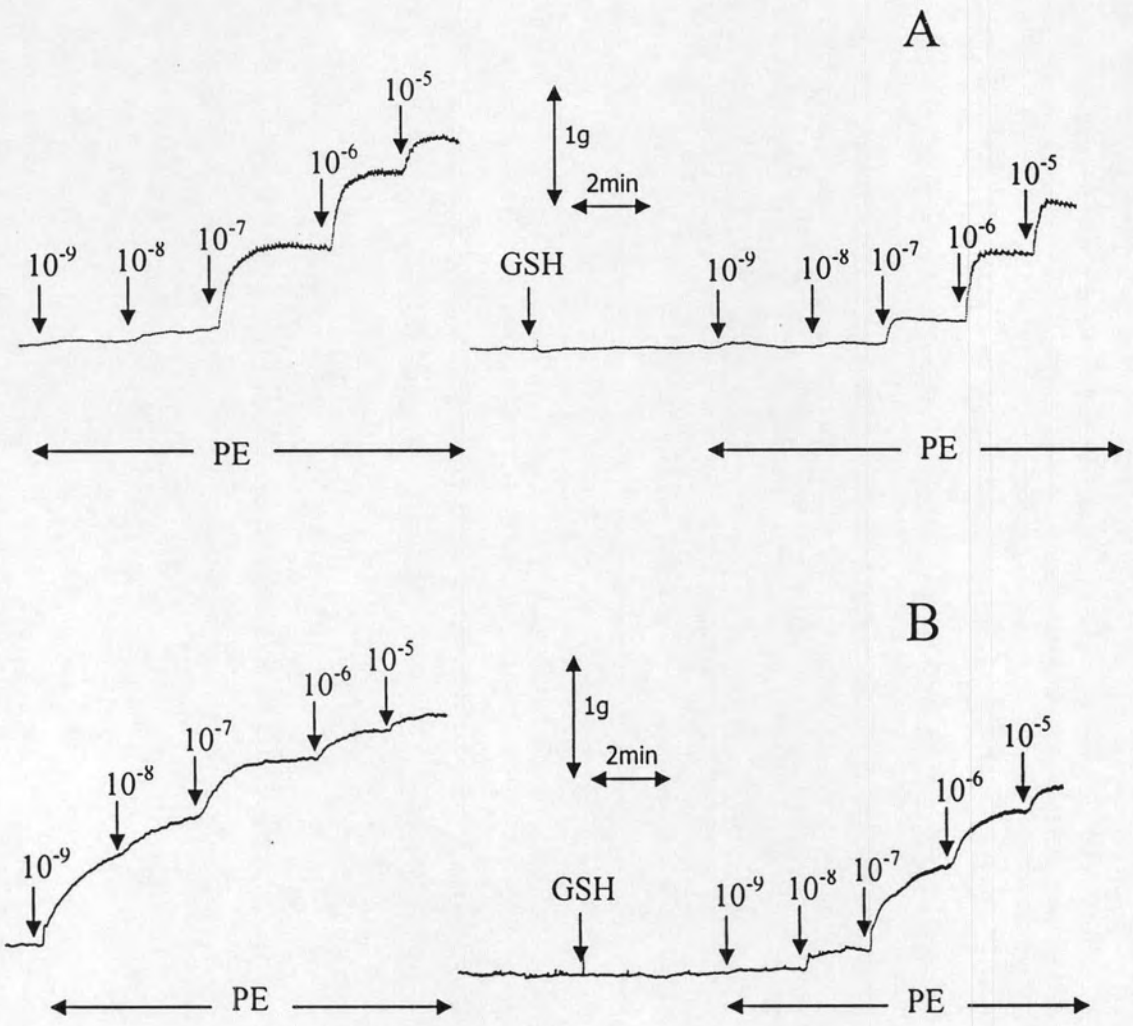
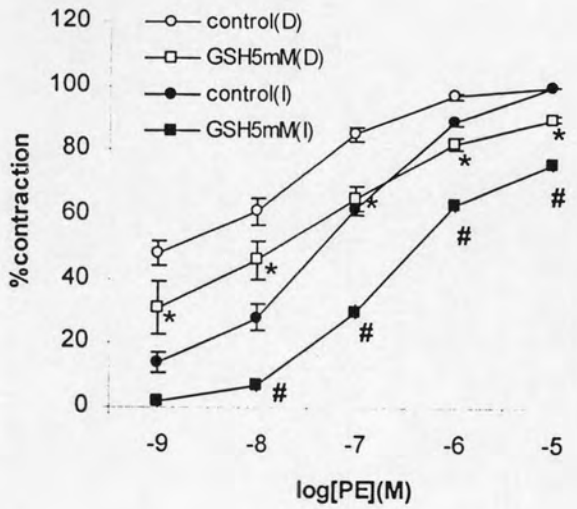


Fig. 19 The representative tracing of PE-induced contraction in the absence (left) and presence (right) of GSH (5 mM) was preincubated with endothelium-intact (A) or endothelium-denude (B) aortic rings 5 min prior to addition of PE (0.001–10 μM) cumulatively to induce contraction.



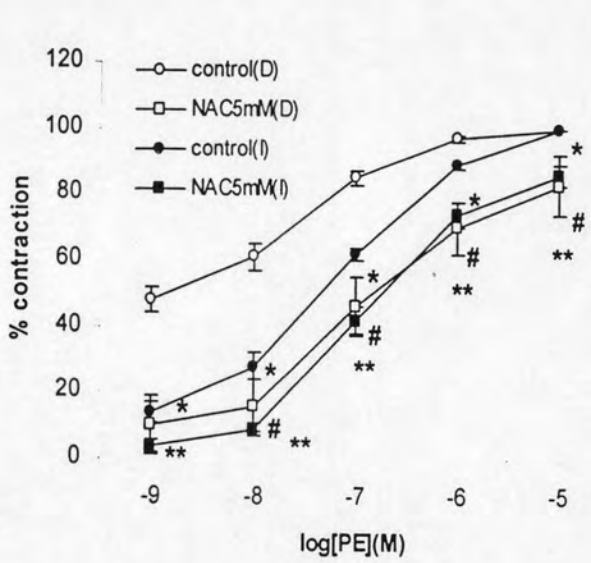
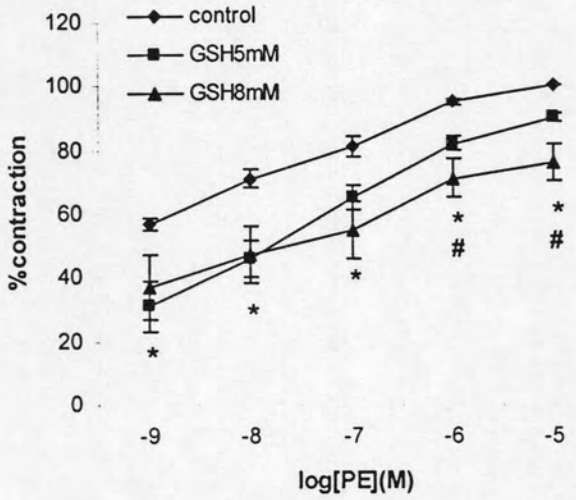


Fig. 20 The effects of GSH (5 mM) (A) and NAC (5 mM) (B) on the contraction-induced by PE (0.001–10 μM) in endothelium-intact and endothelium-denude rat aortic ring. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group of endothelium-denude preparation (D). # P<0.05 showed significant difference from control group of endothelium-intact preparation (I). **P<0.05 showed significant difference from control group between endothelium-intact and endothelium-denude preparation

A



B

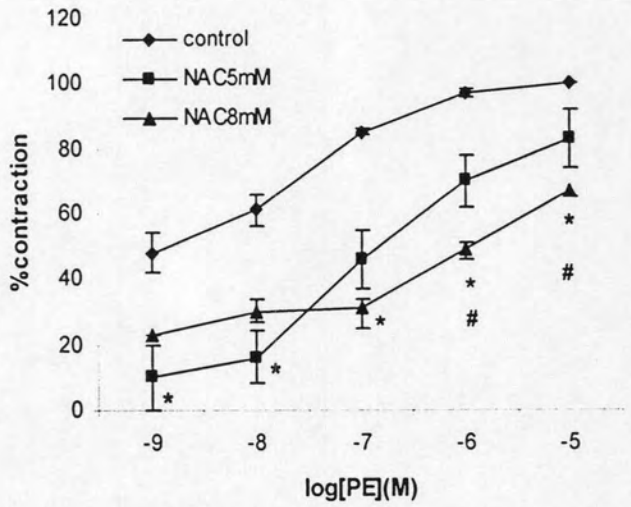


Fig. 21 The effects of GSH (A) or NAC (B) at concentration 5 and 8 mM on the contraction induced by PE (0.001–10 μM) in endothelium-denude rat aortic ring. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group. # P<0.05 showed significant difference from the effect of GSH (5 mM)

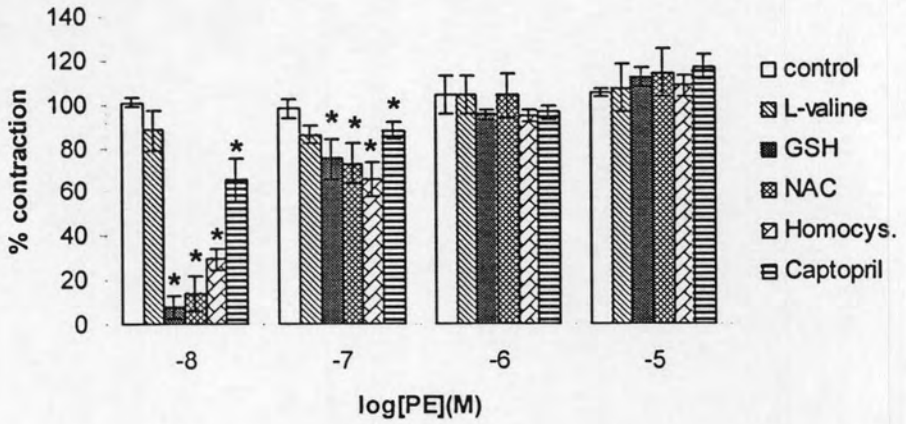


Fig. 22 Comparative inhibitory effects of various sulfhydryl containing compounds on the contraction of endothelium-denude rat aorta. The equiconcentration of GSH, NAC, homocysteine and captopril (at 5 mM) were incubated with the aortic tissues 5 minutes prior to addition of PE to provoke the contraction. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *$P<0.05$ showed significant difference from control group.

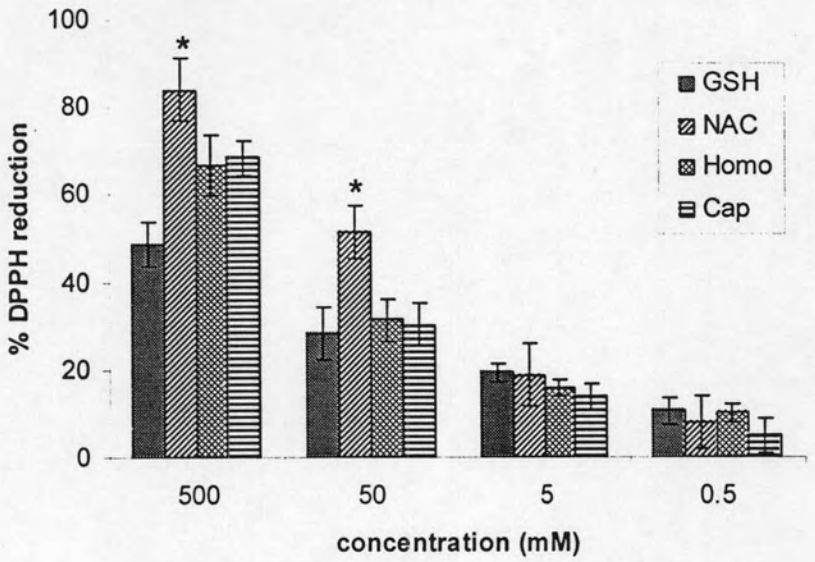


Fig. 23 The effects of various sulfhydryl containing compounds on DPPH free radical scavenging assay. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference between various sulfhydryl containing compounds.

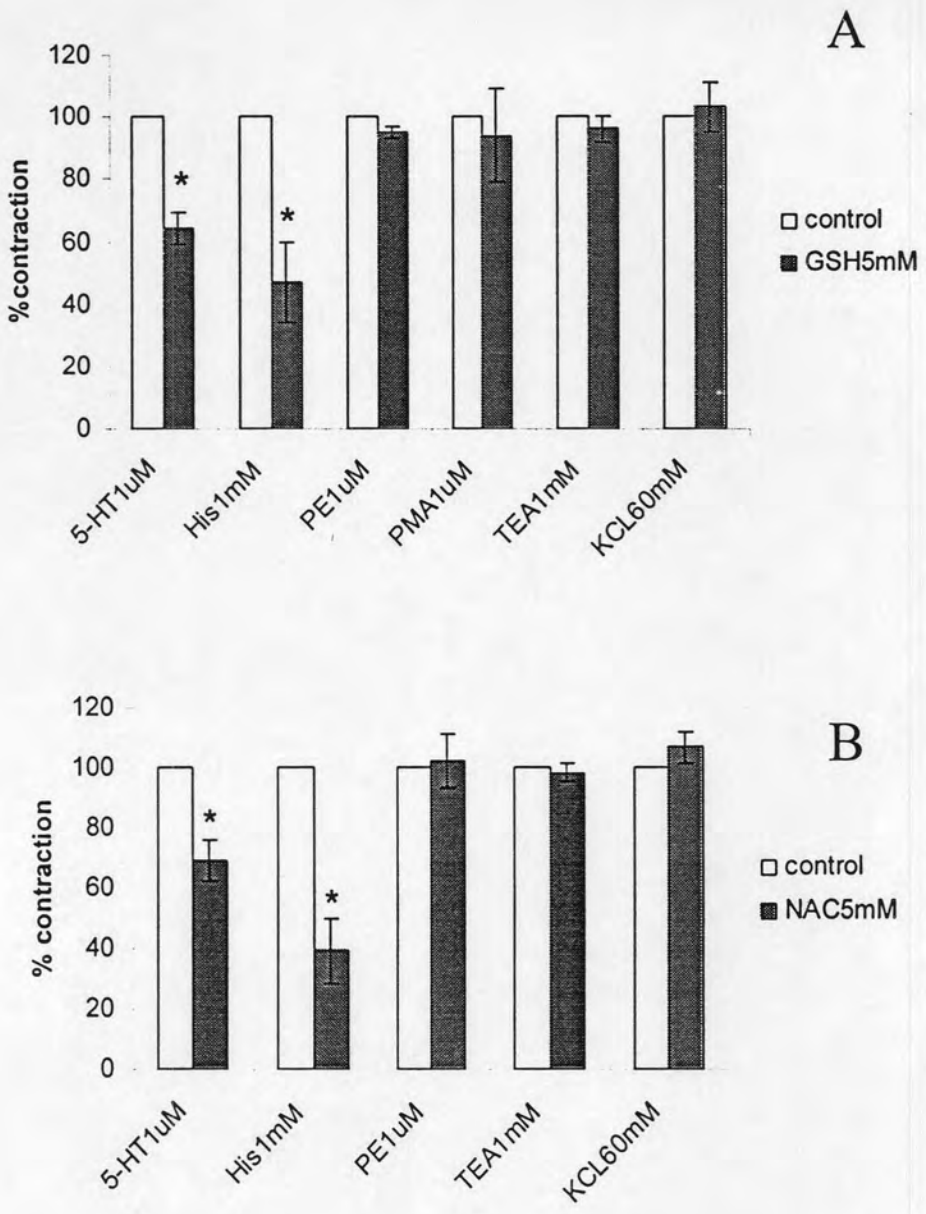


Fig. 24 Effects of GSH (A) or NAC (B) on the aortic contraction induced by serotonin (5-HT) (1 μM), histamine (His) (1 mM), phenylephrine (PE) (1 μM) KCl (60 mM) tetraethylammonium (TEA) (1 mM) and phorbal ester (PMA) (1 μM), Endothelium-denude aortic rings were treated with GSH (5 mM) 5 min prior to addition of contractants. Data are mean ± S.E.M. of 6 separated experiments performed preparations obtained from different animals. *P<0.05 showed significant difference from control group.

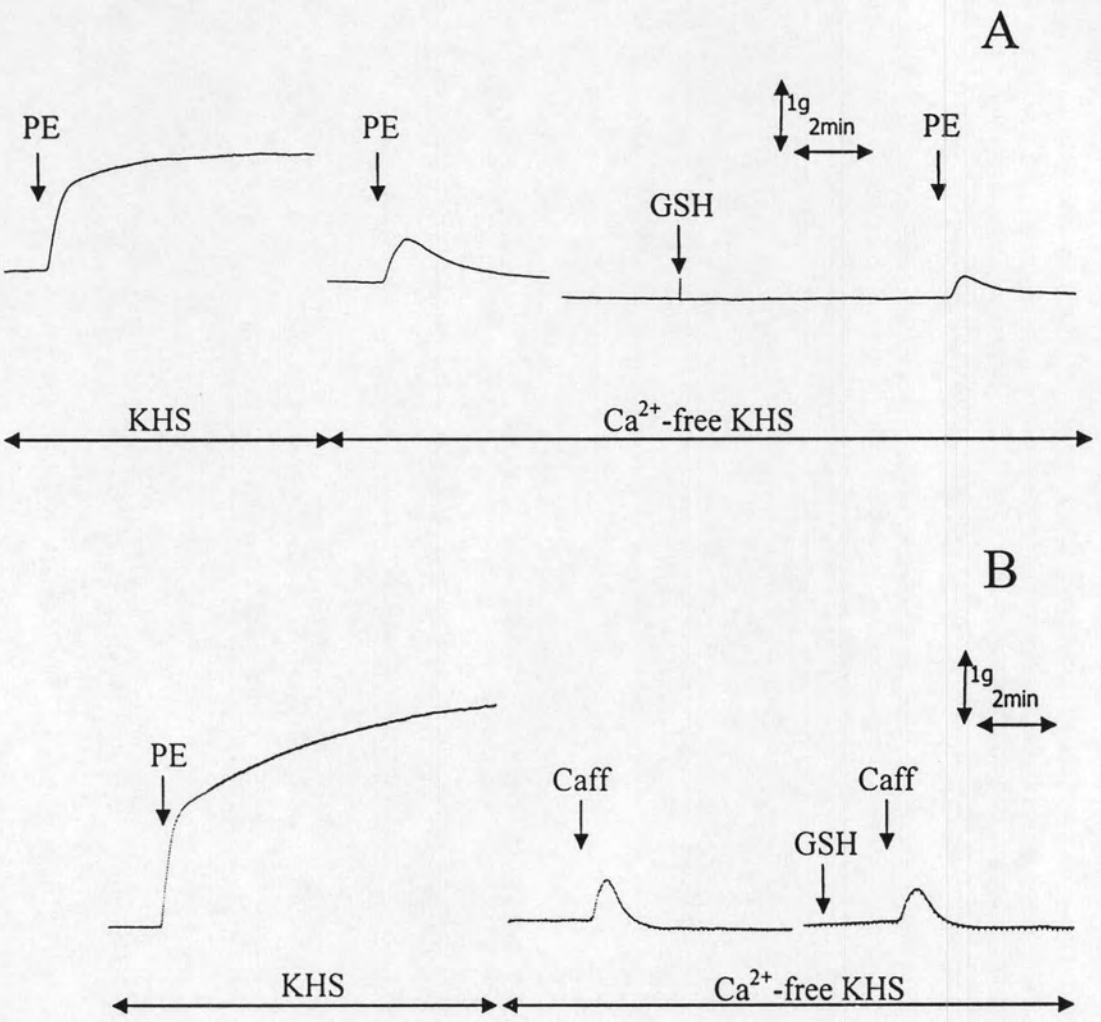
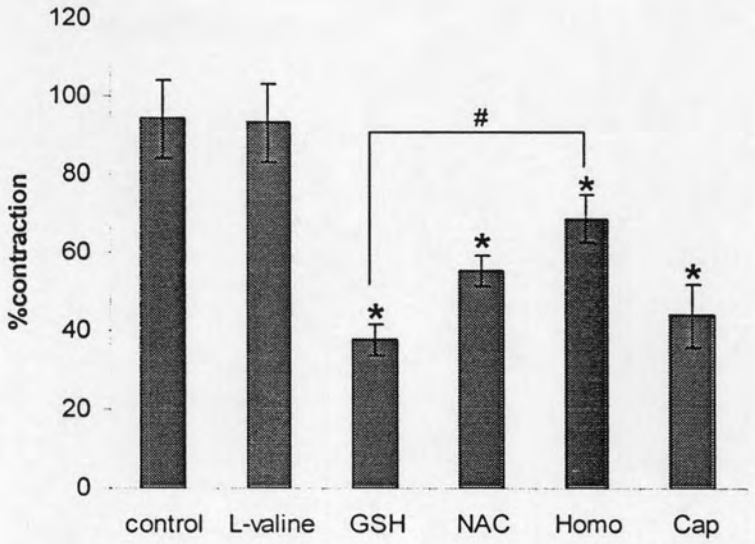


Fig. 25 The representative tracing of the contraction induced by PE (1 μM) (A) or caffeine (10 mM) (B) of endothelium-denude aortic rings in the absence and presence of GSH (5 mM).

A



B

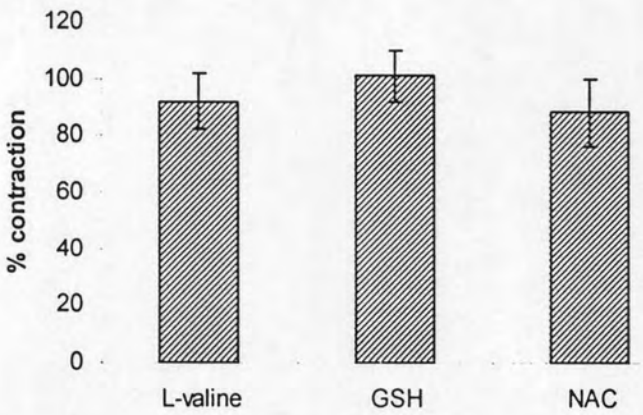


Fig. 26 Comparative effects of certain sulfhydryl containing compounds and L-valine on the endothelium-independent contraction induced by PE (A) or caffeine (B) in Ca^{2+}-free medium. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from L-valine group. # P<0.05 showed significant difference from the effect of homocysteine.

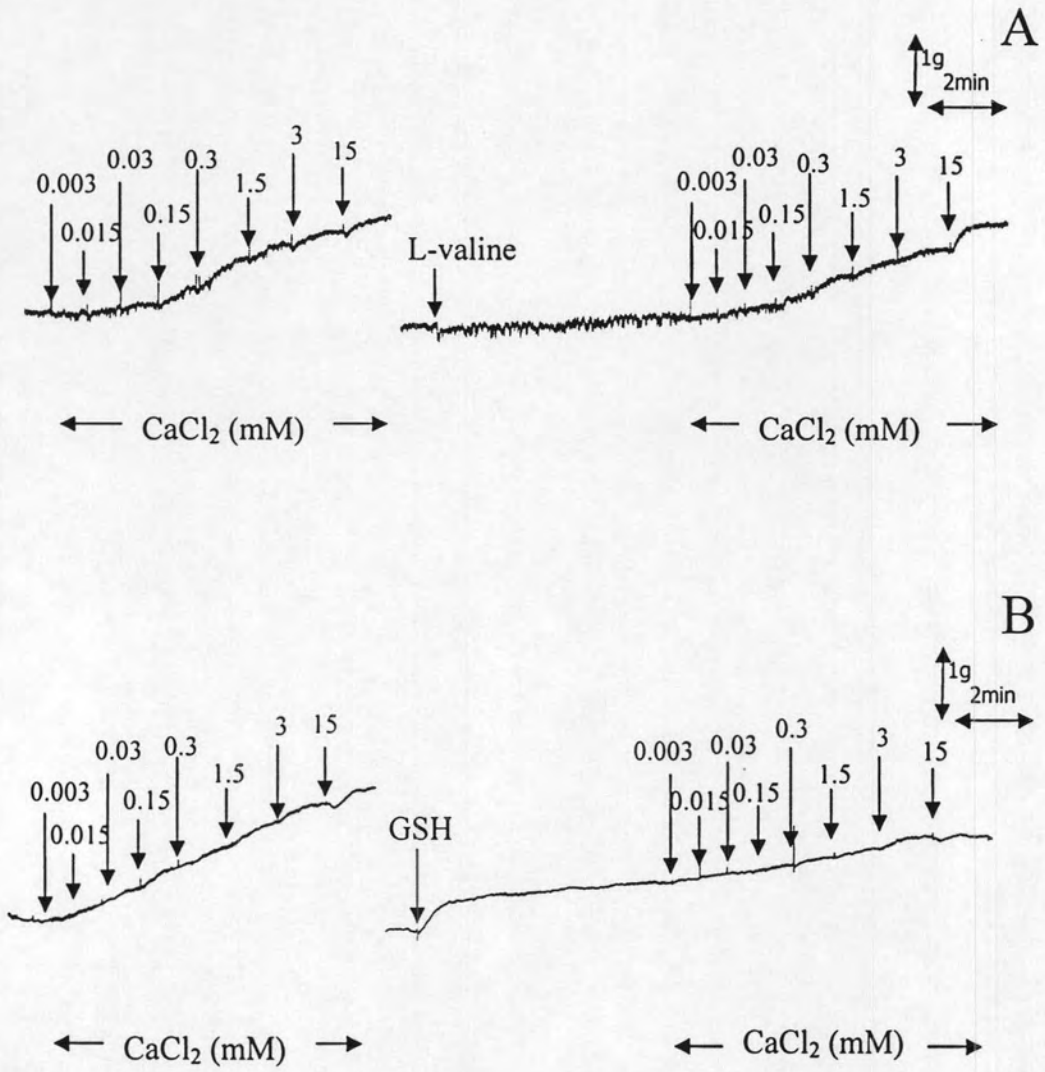
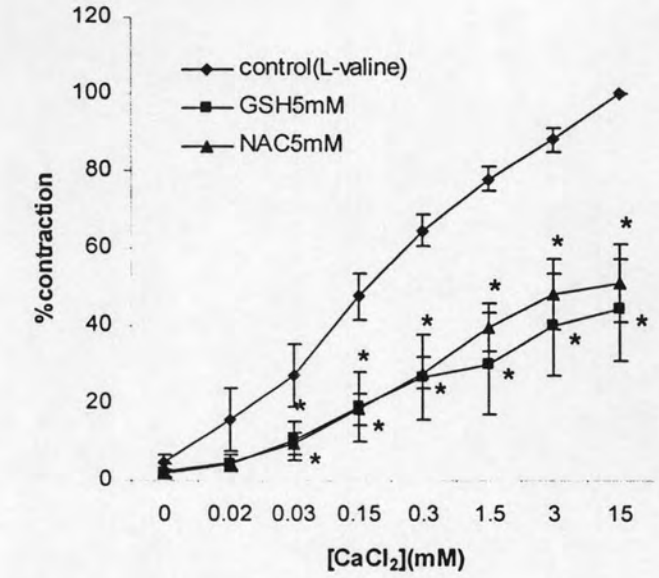


Fig. 27 The representative tracing of endothelium-denude aortic rings in the absence and presence of 5 mM L-valine (A) or GSH (B). The contraction induced by cumulative addition of $CaCl_2$ (0.003, 0.015, 0.03, 0.15, 0.3, 1.5, 3 and 15 mM) in high K^+, Ca^{2+}-free solution.



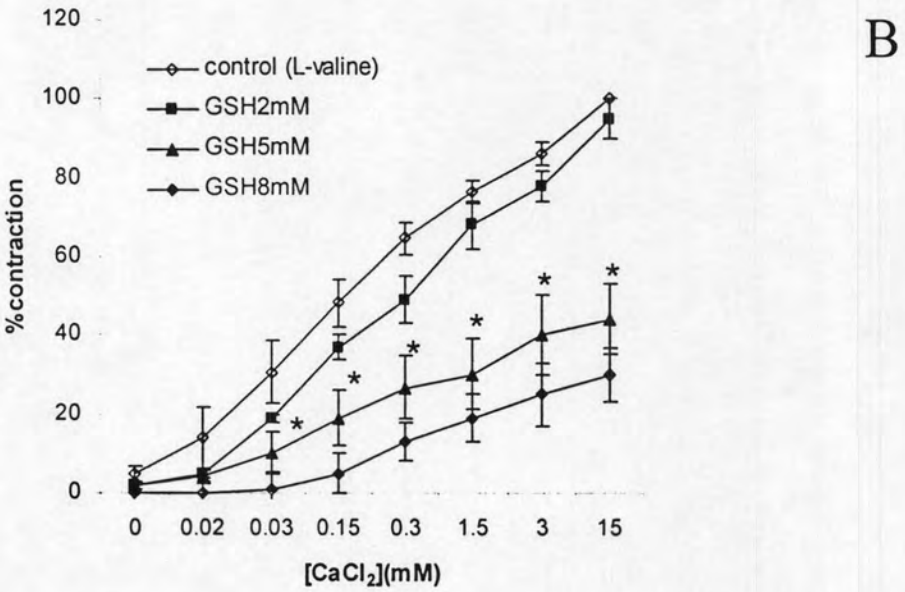


Fig. 28 The inhibitory effect of L-valine, GSH and NAC against contraction induced by $CaCl_2$. The endothelium-denude aortic ring were suspended in high K^+, Ca^{2+}-free condition, followed by cumulative addition of $CaCl_2$ cumulatively. Either L-valine, GSH or NAC (5 mM) were added 5 min prior to $CaCl_2$ treatment. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from L-valine group.

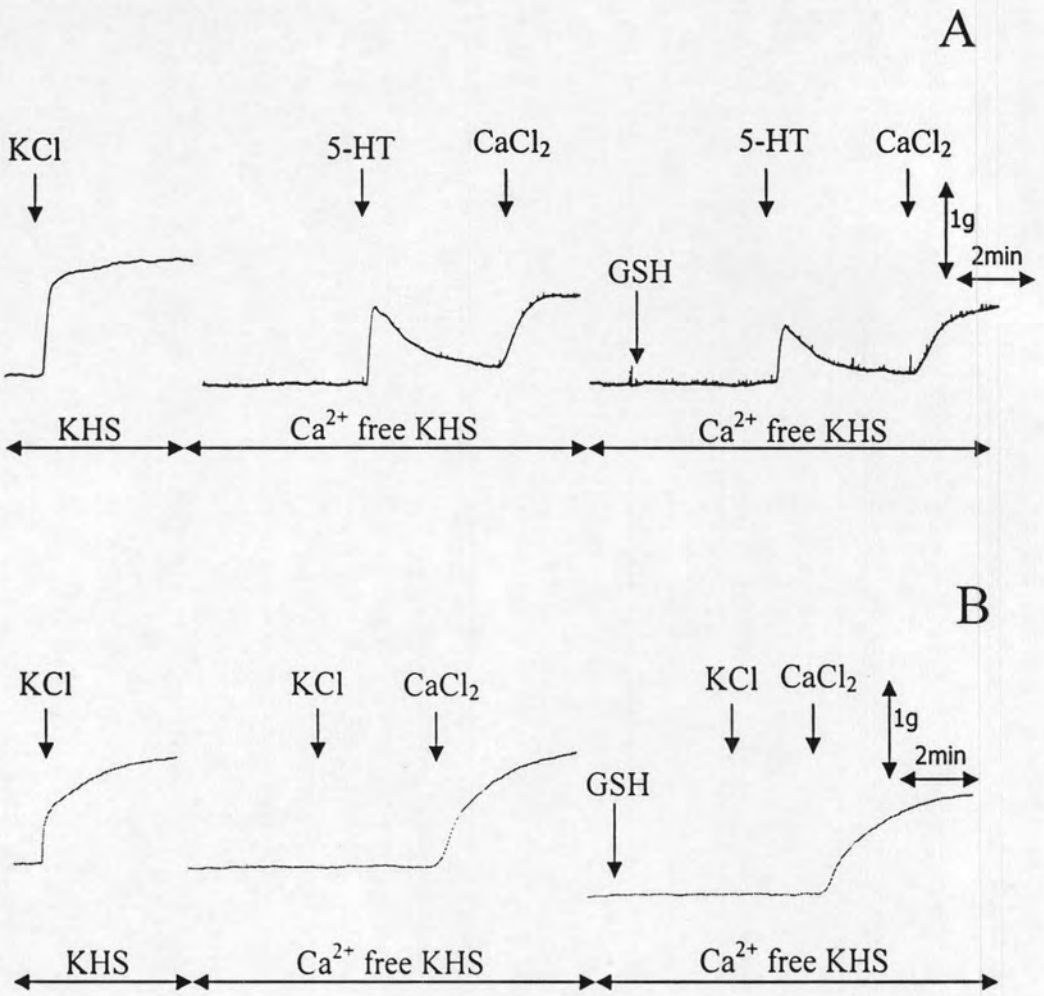


Fig. 29 The representative tracing showing the agonist induced contraction followed by addition of $CaCl_2$ in Ca^{2+}-free condition. The transient contraction in Ca^{2+}-free condition could be observed when 5-HT (1 μM) (A) and KCl (30 mM) (B) were contractants in endothelium-denude aortic rings. The second responses were observed after adding $CaCl_2$ (1 mM) into medium. GSH (5 mM) was preincubated with aortic tissues for 5 min before addition of 5-HT or KCl.

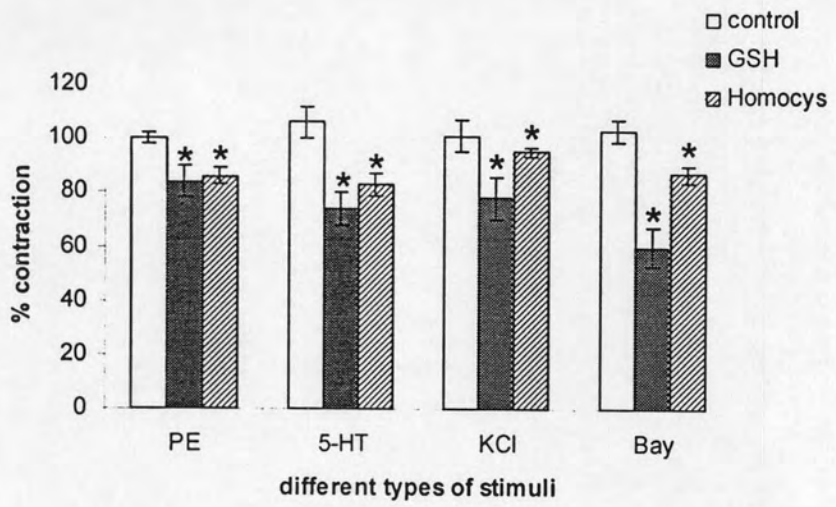


Fig. 30 Inhibitory effects of GSH and homocysteine (5 mM) contraction induced by addition of $CaCl_2$ (1 mM) in the presence of various contractants in Ca^{2+}-free KHS. Various contractants include PE (1 μM), 5-HT (1 μM), KCl (30 mM) and Bay K8644 (10 μM) induced contraction in endothelium-denude aortic rings. Data are mean ± S.E.M. of 6-8 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group.

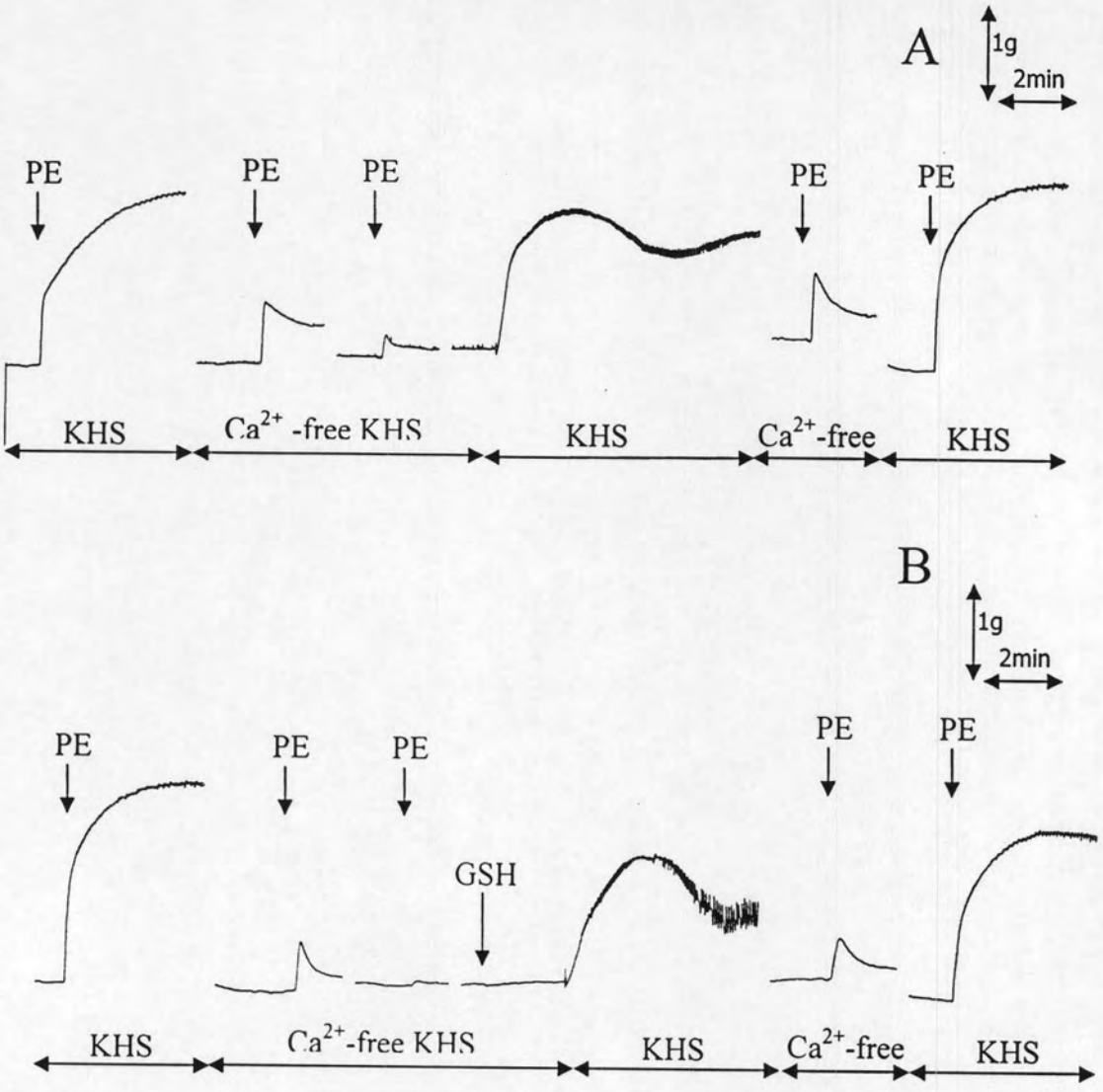


Fig. 31 The representative tracing of the spontaneous contraction of Ca^{2+}-deprived aortic tissues upon addition of Ca^{2+} (A). The endothelium-denude aortic tissues were treated with PE repetitively in Ca^{2+}-free KHS to deplete intracellular Ca^{2+}. Upon changing the medium from Ca^{2+}-free KHS to KHS, replenishment of cytosolic Ca^{2+} caused spontaneous contraction or the increase resting tone (IRT). The inhibitory effect of GSH at 5 mM (B).

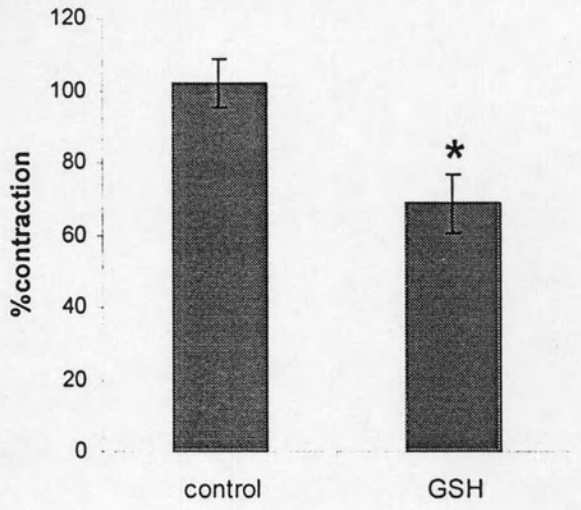


Fig. 32 Effects of GSH on spontaneous contraction of Ca^{2+}-depleted aortic ring upon addition of Ca^{2+} into medium. Data are mean ± S.E.M. of 6 separated experiments performed in preparations obtained from different animals. *P<0.05 showed significant difference from control group.