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Effects of α_1-acid glycoprotein on isometric tension of mouse aorta

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Abstract

We examined the effects of human α_1-acid glycoprotein on isometric tension of mouse aortic rings. α_1-Acid glycoprotein (7.5–75 μM) produced a transient, concentration-dependent relaxation of the phenylephrine-precontracted preparation. Although N^G-nitro-L-arginine methyl ester or removal of endothelium rarely affected the α_1-acid glycoprotein-induced relaxation, extracellular heparin inhibited the α_1-acid glycoprotein-induced relaxation. In 10 mM Ca^{2+}-containing external solutions, the α_1-acid glycoprotein-induced relaxation was significantly potentiated. In the 60 mM KCl-precontracted preparation, α_1-acid glycoprotein produced weaker relaxation than in the phenylephrine-precontracted preparation. These results suggest that the vasorelaxant effect of α_1-acid glycoprotein is mainly achieved by block of Ca^{2+} entry in the vascular smooth muscle cells. The interaction between α_1-acid glycoprotein molecules and plasmalemmal Ca^{2+} entry channels may be modified by extracellular Ca^{2+} and heparin.

Keywords: α_1-Acid glycoprotein; Aorta, mouse; Isometric tension; Ca^{2+} entry; Smooth muscle cell, vascular

1. Introduction

α_1-Acid glycoprotein is one of the major acute phase reactants in plasma during inflammation (Kremer et al., 1988). The plasma level of human α_1-acid glycoprotein (10–25 μM under physiological conditions) increases several-fold in inflammation and cancer, after surgery and during pregnancy. Although many biological activities of α_1-acid glycoprotein have been described (Bennett and Schmid, 1980; Fournier et al., 2000), most of the underlying mechanisms remain uncertain. Both α_1-acid glycoprotein and its mRNA were constitutively expressed in primary cultures of human microvascular endothelial cells from dermal tissue (Sorensson et al., 1999). Maeda et al. (1980) demonstrated that human α_1-acid glycoprotein promoted the passage of erythrocytes through a membrane-filter of 3 μm in pore size and exhibited a protecting effect against hemolysis during filtration. In terms of protection against tissue damage, such as inflammation, cancer, surgery and pregnancy, the facility of circulation of blood in the tissue is an important issue.

The present study was designed to determine whether α_1-acid glycoprotein has possibility to influence the circulation of blood at the site of acute phase reaction and to know the underlying mechanism accordingly. Thus, we tested the effects of α_1-acid glycoprotein on isometric tension of mouse aortic rings.

2. Materials and methods

Descending thoracic aortae were excised from BALB/c mice (6–8 weeks old) immediately after decapitation under ether anaesthesia. The aorta was trimmed free of loosely adhering connective tissue and fat and cut into 2-mm-long rings. The ring was mounted on two horizontal tungsten wires (100 μm diameter) connected to a force displacement transducer and a movable device, respectively, in 5 ml organ bath of a small vessel myograph (MTOB-1, LaboSupport, Osaka, Japan). The organ bath was filled with normal physiological salt solution (Karaki et al., 1991) of the following composition (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, glucose 5.5 and ethylenediamine tetraacetic acid 0.01. The solution was maintained at 37 ± 0.5 °C, and aerated with 95% O_2–5% CO_2, which maintained a pH of ~7.4. The preparations were equili-

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brated for 60 min under 0.5 g of passive tension with rinsing every 15 min, and repeatedly exposed to phenylephrine (0.1 μM) until responses became stable. The isometric tension was monitored on a pen recorder (FBR-251A, Shimadzu, Japan) and stored on a digital data recorder (VR-10B, Instrutech, New York, USA).

Endothelium was present in all experiments except when indicated. For experiments with denuded aortic rings, the endothelium was removed by gently rubbing the luminal surface of the aortic rings with a stainless-steel rod. The endothelial function was assessed by testing the relaxatory effect of acetylcholine (1 μM) in the aortic rings precontracted with phenylephrine (0.1 μM). All peak relaxations to acetylcholine and α_1-acid glycoprotein were measured as percent reversal of the maximal phenylephrine-induced contraction prior to application of each relaxatory agent. The organ bath solution, containing high concentration (60 mM) of K^+, was prepared by replacing NaCl with equimolar KCl in normal solution. In the experiments with extracellular Ca^{2+} concentration of 10 mM, a 1 M stock solution of $CaCl_2$ was added directly to the organ bath.

Human α_1-acid glycoprotein (purified from Cohn Fraction VI) and L-phenylephrine were purchased from Sigma (St. Louis, MO). Acetylcholine, N^G-nitro-L-arginine methyl ester (L-NAME), N^G-monomethyl-L-arginine (L-NMMA) and heparin were from Wako (Osaka, Japan).

Experimental data are given as mean ± S.E.M., and the statistical significance was estimated by Student's *t*-test for comparisons between two groups and analysis of variance (ANOVA) with Dunnett's test for multiple comparisons. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Concentration-dependent relaxation with α_1-acid glycoprotein

Since the plasma level of α_1-acid glycoprotein is known to increase up to about 75 μM during acute phase reactions (Kremer et al., 1988), we tested the effects of human α_1-acid glycoprotein at 7.5–75 μM on isometric tension of isolated mouse aortic rings. Fig. 1A is a set of representative traces, showing the effects of α_1-acid glycoprotein on the tension of the endothelium-intact aorta precontracted with phenylephrine at 0.1 μM. α_1-Acid glycoprotein produced transient relaxations in a concentration-dependent manner. The concentration–response relation for the α_1-acid glycoprotein-induced relaxation is shown in Fig. 1B.

In 4 out of 36 aortic rings, the 75 μM α_1-acid glycoprotein-induced relaxation was followed by augmented contraction (see Fig. 2A), while the rest of rings tested revealed only relaxation. The augmentation in contraction was 5–13% of amplitude of the tonic precontraction before application of α_1-acid glycoprotein, although the values varied from ring to ring of aorta.

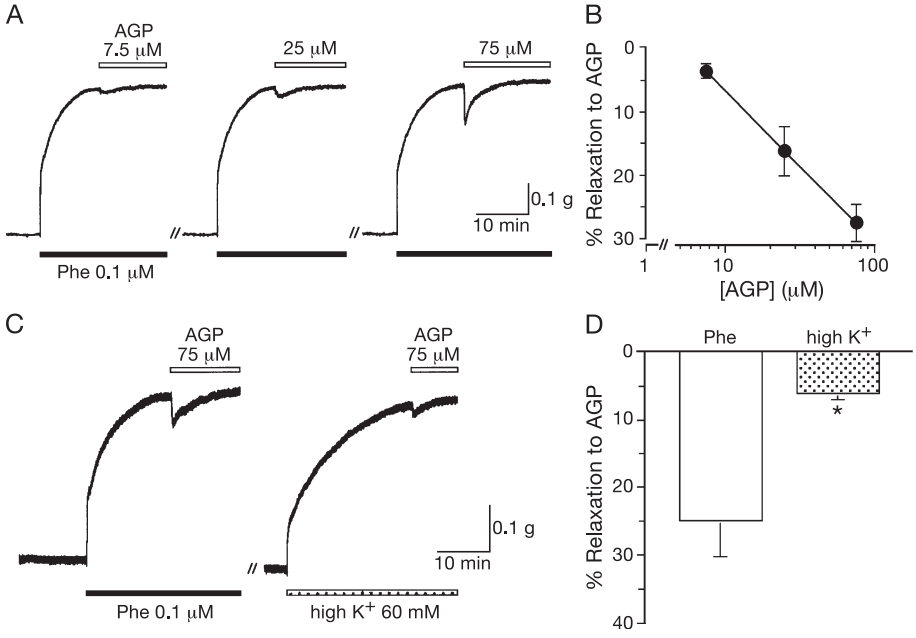

Fig. 1. (A) Concentration-dependent relaxation with α_1-acid glycoprotein. Representative traces showing the α_1-acid glycoprotein-induced relaxation at indicated concentrations in phenylephrine (0.1 μM)-precontracted rings of mouse aorta. AGP: α_1-acid glycoprotein. Phe: phenylephrine. (B) Average concentration–relaxation curves to α_1-acid glycoprotein from experiments as depicted in A. The relaxations are expressed in percent reversal of the maximal phenylephrine-induced contraction prior to application of α_1-acid glycoprotein. $N = 8–28$. (C) Representative traces showing the α_1-acid glycoprotein-induced relaxation in phenylephrine- and high K^+ (60 mM)-precontracted aorta. (D) Average relaxations to α_1-acid glycoprotein (75 μM) in the phenylephrine-precontracted (open column) and high K^+-precontracted (dotted column) aorta from experiments as depicted in C. Values are expressed as percent reversal of the maximal phenylephrine-induced contraction prior to application of α_1-acid glycoprotein. *$P < 0.05$ vs. control.

α_1-Acid glycoprotein at 75 μM produced neither relaxation nor contraction when applied alone to the aortic rings at basal tone without any contractile stimulants ($n=5$). Human serum albumin (Fraction V) at 75 μM revealed neither relaxation nor contraction in all aortic rings tested ($n=5$) in the absence and presence of phenylephrine (data not shown).

3.2. Comparison of the effects of α_1-acid glycoprotein in phenylephrine- and high K^+-precontraction

It is important to know whether the α_1-acid glycoprotein-induced relaxation depends upon the mode of precontraction. We tested the effects of α_1-acid glycoprotein in aortic rings precontracted with a high K^+ (60 mM) external solution and compared the effects with those in phenylephrine-precontraction. As shown in Fig. 1C, α_1-acid glycoprotein elicited relaxations to smaller extent in the aortic rings precontracted with high K^+ solution than those with phenylephrine (5.9 ± 1.1% vs. 24.7 ± 5.4%, $n=8$, $P<0.05$; Fig. 1D). These results suggest that α_1-acid glycoprotein preferentially inhibit the phenylephrine-induced contraction.

3.3. Involvement of nitric oxide-dependent pathways in α_1-acid glycoprotein-induced relaxation

To determine whether the vasorelaxant effect of α_1-acid glycoprotein involves nitric oxide (NO)-dependent pathways, we tested the effects of α_1-acid glycoprotein in the aortic rings treated with a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) and in the aortic rings of which the endothelia were removed. When L-NAME at 1 mM, which is the concentration that prevented the relaxations to acetylcholine at 1 μM, was administered to the aortic rings prior to application of phenylephrine, the α_1-acid glycoprotein-induced relaxation was rarely affected (25.1 ± 7.8%, $n=9$, vs. 27.6 ± 2.9% in controls; Fig. 2A and D). Similarly, N^G-monomethyl-L-arginine (L-NMMA, 0.1 mM) did not affect the α_1-acid glycoprotein-induced relaxation (data not shown). Consistently, removal of the endothelium revealed no significant change in the α_1-acid glycoprotein-induced relaxation (22.1 ± 5.3%, $n=8$; Fig. 2D).

3.4. Effects of increased extracellular calcium on α_1-acid glycoprotein-induced relaxation

It is known that mammalian oligosaccharides bearing sialic acid and fucose, such as sialyl-Lewis X (sLe^X), are parts of the functional carbohydrate moiety of α_1-acid

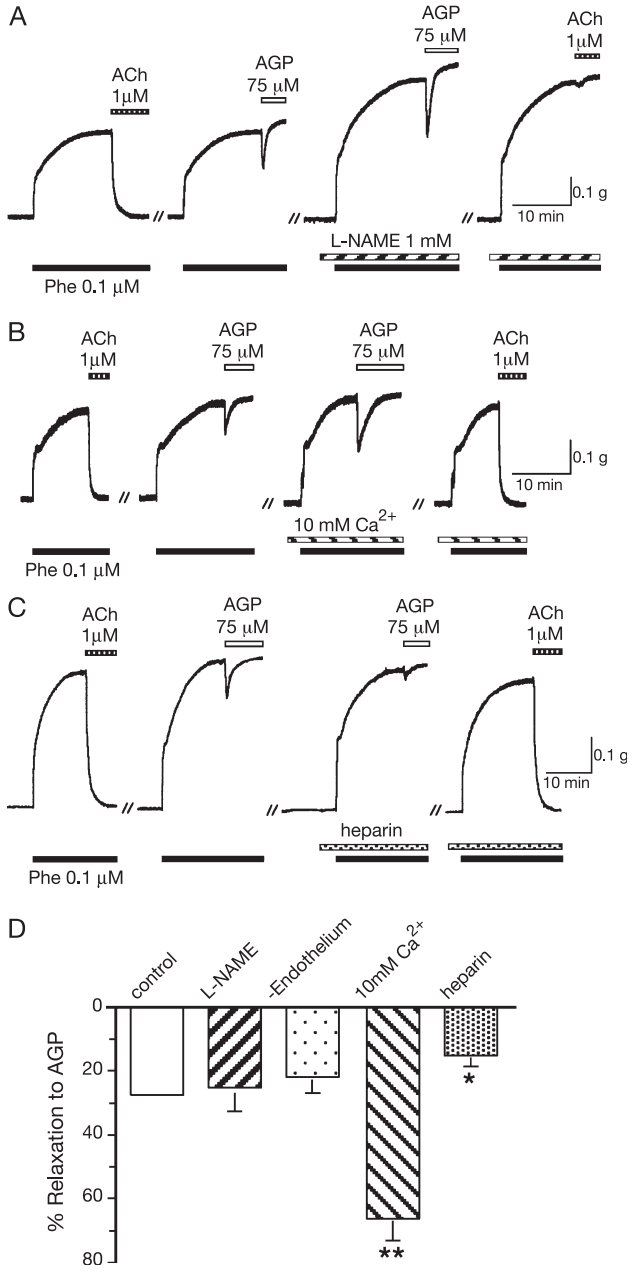

Fig. 2. (A) Involvement of NO-dependent pathways in the α_1-acid glycoprotein-induced relaxation. Representative traces showing the acetylcholine- or α_1-acid glycoprotein-induced relaxation in phenylephrine-precontracted aorta in the absence and presence of N^G-nitro-L-arginine methyl ester. AGP: α_1-acid glycoprotein. ACh: acetylcholine. Phe: phenylephrine. L-NAME: N^G-nitro-L-arginine methyl ester. (B) Effects of increased extracellular Ca^{2+} on the α_1-acid glycoprotein-induced relaxation. Representative traces showing the acetylcholine- or α_1-acid glycoprotein-induced relaxation in phenylephrine-precontracted aorta in control (1.5 mM) and high concentrations (10 mM) of extracellular Ca^{2+}. (C) Effects of heparin on the α_1-acid glycoprotein-induced relaxation. Representative traces showing the acetylcholine- or α_1-acid glycoprotein-induced relaxation in phenylephrine-precontracted aorta in the absence and presence of heparin (100 U/ml). (D) Average relaxations to α_1-acid glycoprotein (75 μM) in the phenylephrine-precontracted aorta under control (open column), N^G-nitro-L-arginine methyl ester-treated (hatched column) and endothelium-removed (dotted column) conditions, in high concentration of extracellular Ca^{2+} (hatched column), and under heparin-treated conditions (dotted column). Values are expressed as percent reversal of the maximal phenylephrine-induced contraction prior to application of α_1-acid glycoprotein. *$P<0.05$ and **$P<0.01$ vs. control.

glycoprotein (Bennett and Schmid, 1980) and are recognized by selectin family of cell adhesion molecules in a Ca^{2+}-dependent manner (Geng et al., 1990). To determine whether the vasorelaxant effects of α_1-acid glycoprotein in mouse aorta are Ca^{2+}-dependent, we compared the effects of α_1-acid glycoprotein in normal (1.5 mM) and high (10 mM) Ca^{2+}-containing external solutions. When extracellular Ca^{2+} concentration was raised to 10 mM, the α_1-acid glycoprotein-induced relaxation was potentiated in all phenylephrine-precontracted aortic rings tested ($66.6 \pm 6.7\%$, $n=8$, $P<0.01$; Fig. 2B and D). The α_1-acid glycoprotein-induced relaxation immediately recovered to the control level when extracellular Ca^{2+} concentration was returned to the normal level.

In contrast to those effects of elevated Ca^{2+} on the α_1-acid glycoprotein-induced relaxation, monovalent cations did not seem to be important modulators of the α_1-acid glycoprotein action, since the α_1-acid glycoprotein-induced relaxation was rarely affected when extracellular $Na^+$was replaced by Li^+ (data not shown).

3.5. Effects of heparin on α_1-acid glycoprotein-induced relaxation

We tested the effects of unfractionated heparin (100 U/ml) on the vasorelaxation induced by α_1-acid glycoprotein (Fig. 2C), since heparin is known to inhibit selectin binding to sLe^X in a Ca^{2+}-dependent manner (Nelson et al., 1993; Koenig et al., 1998). Heparin applied alone at concentrations up to 100 U/ml produced neither contraction nor relaxation in all aortic rings tested ($n=8$). Heparin also did not influence the phenylephrine-precontraction. Pretreatment of the aortic rings with heparin prior to application of phenylephrine resulted in a decrease in the α_1-acid glycoprotein-induced relaxation ($15.2 \pm 3.7\%$, $n=8$, $P<0.05$; Fig. 2D). This indicates that heparin (100 U/ml) was able to inhibit the relaxant effect of α_1-acid glycoprotein by 45%. The inhibitory effect of heparin on the α_1-acid glycoprotein-induced relaxation was immediately recovered when heparin was removed ($n=8$). These results suggest that the α_1-acid glycoprotein-induced relaxation may involve heparin-sensitive intermolecular interaction between α_1-acid glycoprotein and its binding sites on the vascular smooth muscle cells.

4. Discussion

In the present study, we have demonstrated that human α_1-acid glycoprotein produces a transient, concentration-dependent relaxation in the phenylephrine-precontracted aortic rings (Fig. 1). The transient relaxation with α_1-acid glycoprotein was rarely affected by L-NAME, L-NMMA, or removal of endothelium (Fig. 2), suggesting that production of endothelial NO does not play crucial roles in the development of the α_1-acid glycoprotein-induced relaxation. It is likely that α_1-acid glycoprotein acts more effectively at the stage of contraction, at which the contractility is governed by activation of α_1-adrenoceptor rather than voltage-dependent Ca^{2+} channels (VDCCs) on the vascular smooth muscle cells, as shown in Fig. 1C. α_1-Adrenoceptor activation in vascular smooth muscle cells is known to increase the intracellular free Ca^{2+} level by both inositol trisphosphate-dependent Ca^{2+} release and Ca^{2+} entry through nonselective cation channels (NSCCs) (Karaki et al., 1997). It is suggested that the α_1-acid glycoprotein-induced relaxation is achieved by inhibition of both VDCCs and NSCCs to a distinct extent in the smooth muscle cells.

The augmented contraction following the α_1-acid glycoprotein (75 μM)-induced relaxation, which was observed in 4 out of 36 aortic rings, might involve another source of Ca^{2+}, such as capacitative Ca^{2+} entry (Karaki et al., 1997), during blockade of VDCCs and NSCCs with α_1-acid glycoprotein, although the augmentation in contraction could not be evaluated quantitatively because of large variance of the extent of augmentation and its low rate of occurrence.

The potentiated relaxation with α_1-acid glycoprotein at high concentrations of extracellular Ca^{2+} (10 mM) may inform us about the selective recognition by its binding sites on the vascular smooth muscle cells, since the α_1-acid glycoprotein-induced relaxation was rarely affected when extracellular $Na^+$was replaced by Li^+. The immediate recovery from the potentiation of α_1-acid glycoprotein-induced relaxation by washing out the high Ca^{2+}-containing external solution with the normal external solution may favor the external site of action for the Ca^{2+}-dependent potentiation of the α_1-acid glycoprotein-induced relaxation. The effects of extracellular Ca^{2+} on the α_1-acid glycoprotein-induced relaxation might be due to the Ca^{2+}-dependent recognition of the carbohydrate moiety of α_1-acid glycoprotein by selectin family (Bennett and Schmid, 1980; Geng et al., 1990).

It has been reported that heparin fragments as small as tetrasaccharides specifically block the interactions of selectin with sLe^X-containing ligands (Nelson et al., 1993; Koenig et al., 1998). Heparin is also known to inhibit inositol trisphosphate-dependent Ca^{2+} release from the sarcoplasmic reticulum when applied intracellularly. Our results show that the α_1-acid glycoprotein-induced relaxation was moderately, but significantly, inhibited in the presence of heparin and that the inhibition of the relaxation was immediately recovered by removal of heparin. The immediate recovery of the α_1-acid glycoprotein-induced relaxation suggested that the action of heparin occurs extracellularly. The functional lectin-like domain which may interact with sLe^X moieties on α_1-acid glycoprotein might be expressed at the surface of vascular smooth muscle cells and heparin might inhibit the interaction of the lectin-like domain with sLe^X on α_1-acid glycoprotein.

In conclusion, we have demonstrated that human α_1-acid glycoprotein produces a transient relaxation in the mouse aorta precontracted with phenylephrine. It is more likely that the α_1-acid glycoprotein-induced relaxation is due to block of Ca^{2+} entry through VDCC or NSCC in the smooth

muscle cells rather than activation of endothelial NO pathway. The interaction between α_1-acid glycoprotein molecules and plasmalemmal Ca^{2+} entry channels may be modified by extracellular Ca^{2+} and heparin.

The physiological significance of the α_1-acid glycoprotein-induced transient relaxation of mouse aorta is at present a matter for speculation. The transient relaxation induced by α_1-acid glycoprotein might occur as prolonged relaxation in the peripheral blood vessel, which may result in improvement of the blood circulation in tissues with inflammation. Testing additional biological parameters, further investigations are needed to fully illustrate the physiological roles of α_1-acid glycoprotein.

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