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Lack of involvement of pertussis toxin-sensitive G-proteins in norepinephrine-induced vasoconstriction of rat aorta smooth muscle

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Abstract

Several studies have shown that stimulation of pertussis toxin (PTX)-sensitive G-proteins amplified α-adrenoceptor (α-AR) agonist-induced vasoconstriction in small muscular and resistance arteries. The aim of this study was to assess the potential involvement of PTX-sensitive G-proteins in norepinephrine (NE)-induced constriction in a large diameter artery, the rat aorta. PTX (1 μg/mL, 2 hr; 3 μg/mL, 4 hr) did not modify concentration–response curves to NE in endothelium-denuded aortic rings. However, several lines of evidence suggested that aortic smooth muscle cells (SMC) had a PTX-sensitive G-protein pathway. [α-^{32}P]ADP-ribosylation of $G_{i/o}$-proteins by PTX (3 μg/mL, 4 hr) was demonstrated *in situ* in the intact aorta without endothelium. $\alpha_{i/o}$ subunits were identified *in vitro* by both immunoblotting and ADP-ribosylation experiments in rat aorta SMC membranes. The measurement of $G_{i/o}$-specific GTPase activity evidenced an effective coupling between NE receptors and $G_{i/o}$-proteins, as NE induced an increase in basal $G_{i/o}$-specific GTPase activity (20.7 ± 2.8 vs 7.2 ± 2.2 pmol P_i/mg protein at 5 min; $P < 0.05$ vs basal). Co-immunoprecipitation revealed the *in vitro* coupling between α_{1D}-ARs and G_i-protein in rat aorta SMC membranes. In conclusion, we identified a PTX-sensitive $G_{i/o}$-protein pathway in rat endothelium-denuded aorta. We showed an effective coupling between NE receptors and G_i-proteins via α_{1D}-ARs. Since PTX has no effect on NE-induced vasoconstriction, the PTX-sensitive G_i-protein pathway does not play a predominant role in NE-induced responses in rat aorta SMC in contrast to small diameter muscular and resistance arteries.

Keywords: Smooth muscle; Pertussis toxin; $G_{i/o}$-proteins; Vasoconstriction; Norepinephrine; Aorta

1. Introduction

PTX catalyses ADP-ribosylation of the α subunits of G_i- and G_o-proteins [2], resulting in inhibition of the coupling of G-protein to the receptor [3]. PTX diminishes the effects of a wide range of vasoconstrictor agents such as α-AR agonists. The effect of PTX on α-AR agonist-mediated vasoconstriction was shown *in vivo* [4] as well as *in vitro* in resistance mesenteric arteries [5,6] and in small diameter muscular tail artery [7,8]. These results suggest that, in vascular SMC, a PTX-sensitive G-protein intracellular pathway is involved in the contractile responses induced by α-AR agonists. However, conflicting observations in large vessels have cast doubt on the universal involvement of this PTX-sensitive pathway in α-AR-mediated vasoconstriction. Some authors have shown that PTX diminished α-AR-mediated contractions [6,9,10], whereas others have shown that PTX had no effect [11,12].

To assess the universal involvement of a PTX-sensitive G-protein pathway in α-AR-mediated contraction of SMC, we wanted to compare the results that we obtained earlier on small diameter muscular rat tail artery [8] with a model of large vessel. We chose the rat aorta because little information was available regarding the function of PTX-sensitive G-proteins in aorta SMC in comparison to aorta endothelial cells [13]. The objective of the present study was to investigate whether rat aorta SMC had a functional PTX-sensi-

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Abbreviations: α-AR, α-adrenoceptor; DTT, DL-dithiothreitol; NE, norepinephrine; PSS, physiological salt solution; PTX, pertussis-toxin; and SMC, smooth muscle cells.

Preliminary results of this study were presented at the January 1999 Meeting of the British Pharmacological Society [1].

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tive G-protein pathway and whether it was involved in vasoconstriction induced by NE, a full α-AR agonist.

2. Materials and methods

2.1. Materials

Tris(hydroxymethyl)-aminomethane was purchased from Merck. Benzamidine, DTT, ethylenediaminetetraacetate, leupeptin, NAD, NE, PTX, phenylmethylsulphonyl fluoride, and SDS were purchased from Sigma Chemical Co. Materials used for immunoblotting were obtained from Bio-Rad. [α-^{32}P]NAD (30 Ci/mmol), [^{3}H]prazosin (77 Ci/mmol), and [γ-^{32}P]GTP (30 Ci/mmol) were obtained from NEN Life Sciences. PTX was activated extemporaneously by incubation with 25 mM DTT (37°, 1 hr). Solutions were then dialysed (Biotech dispodialyser Spectra/Por®, molecular weight cut-off = 8, Spectrum Laboratories Inc.) for 3 hr against PSS (containing in mM: 140 NaCl, 5 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 6 glucose) (pH 7.4) with a change of bath at 15, 30, 60, and 120 min.

2.2. Animals

Adult, male, outbred Wistar rats (Harlan; Iffa-Credo) were housed under controlled temperature and illumination and had access to food and water *ad lib*. The procedures followed in the care and euthanasia of the animals were in accordance with the legislation of the European Community.

2.3. Concentration–response curve to NE in endothelium-denuded aortic rings

Thoracic aortas were dissected out under sodium pentobarbital anaesthesia (60 mg/kg, i.p.). Rats were killed with an overdose of sodium pentobarbital. Aortic rings (2 mm long) were denuded of endothelium by rubbing the intimal surface and mounted between two stainless steel wire hooks, the lower fixed to the bottom of the organ bath and the upper to an isometric force transducer (World Precision Instruments). The organ bath was filled with PSS (pH 7.4, aerated with 100% O_2, 37°). Rings were incubated with activated PTX (1 μg/mL for 2 hr or 3 μg/mL for 4 hr) or DTT (25 mM) dialysed in PSS (control), under a passive tension of 2 g. Following wash-out of PTX, segments were stimulated twice with modified PSS containing 80 mM KCl (composition in mM: 65 NaCl, 80 KCl, 1.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 6 glucose) (pH 7.4) with a wash-out of 10 min after the first stimulation. At the plateau of the second KCl stimulation, the absence of endothelium was confirmed by the absence of carbachol-induced (10 μM) relaxation of segments (results not shown). Following equilibration (20 min), cumulative concentration–response curves to NE (1 nM to 10 μM) were performed. Results (mean ± SEM) are expressed in g, and pEC_{50} and E_{max} were determined. Significant differences ($P < 0.05$ vs control) were determined by ANOVA plus Bonferroni test.

2.4. Isolation of rat endothelium-denuded aorta SMC membrane fraction

Rats were killed by cervical dislocation and the thoracico-abdominal aortas were dissected out. Endothelium was removed by opening up the aorta lengthwise and rubbing the intimal surface as described above. Fractions containing membrane proteins were prepared as described by Abebe et al. [14]. Aortas were homogenised in ice-cold buffer (containing in mM: 15 Tris(hydroxymethyl)-aminomethane (pH 7.4), 1 ethylenediaminetetraacetate, 0.1 phenylmethylsulphonyl fluoride, 0.1 benzamidine, and 0.001 leupeptin) with a glass Dounce homogeniser (Kontes). Following differential centrifugation, the pelleted membrane fractions were resuspended in the same buffer as above and stored at −80° until use. Protein concentration was determined by the method of Lowry et al. [15].

2.5. $G_{i/o}$-protein identification

2.5.1. Identification by immunoblotting

Membrane fractions solubilised in Laemmli buffer were resolved into 16.5% SDS–polyacrylamide gel and then transferred to a 0.4-μm thick nitrocellulose membrane (Bio-Rad). Equal amounts of membrane fractions (10 μg) were loaded onto each lane. Blots were incubated with polyclonal rabbit antibodies directed against G_i α subunits 1–2 (1:1000 dilution, Calbiochem), G_i α subunit 3 (1:1000 dilution, Calbiochem), or monoclonal mouse antibody directed against G_o α subunits (1:3000 dilution, Chemicon). Alkaline phosphatase and its substrate, the 5 bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium, was used as the detection system (Bio-Rad). Positive control was a mixture of bovine brain purified G-protein subunits (Bio-Rad). Densitometry was performed using the NIH Image® program (v.1.58, Wayne Rasband, National Institutes of Health), which gave results as total integrated O.D. values. Since the same amount of membrane fractions was loaded onto each well of a gel, relative proportions were compared by using band densities within a blot. As we did not have an external calibrated reference, we could not compare band densities between two different blots.

2.5.2. Identification by ADP-ribosylation

PTX-catalysed transfer of ADP-ribose from [α-^{32}P]NAD to G-protein α subunits was undertaken in rat aorta SMC membranes. Membrane fractions (40 μg) were mixed with an ADP-ribosylation mixture (in mM: 200 KH_2PO_4 (pH 7.6), 5 $MgCl_2$, 2 ATP, 2 ethylenediaminetetraacetate, 20 thymidine, 2 DTT, 1 GTP, 0.02 NAD (30 Ci/mmol, 10 μCi/60 μL of assay)), and activated PTX (1 μg/mL) [16]. Control was membrane fractions (40 μg) incubated with the

ADP-ribosylation mixture and DTT (25 mM) dialysed in PSS or with activated PTX (1 μg/mL). Following incubation for 1 hr at 30°, samples were separated in a SDS–polyacrylamide gel and blotted as described above. Labelled blots were dried and apposed to an x-ray film (Fuji Medical NIF).

Using a corresponding quantity of aortas, the same technique was applied to endothelium-denuded aortic rings. The rings were incubated *in situ* at 37° with the ADP-ribosylation mixture and activated PTX (1 μg/mL, 2 hr or 3 μg/mL, 4 hr) as described above in "Concentration–response curve to NE in rat endothelium-denuded aortic rings".

2.6. NE receptor/$G_{i/o}$-protein coupling studies

2.6.1. Measurement of $G_{i/o}$-specific GTPase activity upon NE stimulation

The method for the measurement of GTPase activity was based upon the release of free $^{32}P_i$ from [γ-^{32}P]GTP. Briefly, the assay was performed in a buffer (containing in mM: 100 NaCl, 50 HEPES (pH 8.0), 1 ethylenediaminetetraacetate, 2 $MgCl_2$, 1 DTT) with NE (1 μM) and [γ-^{32}P]GTP (0.1 μM, 30 Ci/mmol). Membrane fractions were preincubated for 1 hr at 30° with activated PTX (1 μg/mL) or with DTT (25 mM) dialysed in PSS (control). Ten micrograms of membrane fraction was used to start the reaction at 30°. Different incubation times were utilised (30 sec–30 min). The reaction was stopped by addition of 5% ice-cold activated charcoal in 50 mM KH_2PO_4 (pH 7.4). Tubes were then centrifuged to separate $^{32}P_i$ from nucleotide-bound phosphate. An aliquot of the supernatant (0.4 mL) was counted by liquid scintillation in Aquasol-2 (NEN) with a Beckman LS6000 Counter (Beckman Instruments Inc.). Results are expressed as pmol P_i/mg protein. Specific GTPase activity was calculated by subtracting non-enzymatic hydrolysis (determined in the absence of membrane fractions) and contamination of substrate with $^{32}P_i$ (determined by zero time blank). Non-enzymatic $^{32}P_i$ release and $^{32}P_i$ contamination of the substrate during the assay were less than 2% of the total amount of radioactivity. GTPase activity produced specifically by $G_{i/o}$-proteins was defined as the difference in GTP hydrolysis between untreated and PTX-treated membrane fractions. All assays were performed in triplicate and results are expressed as means ± SEM. Significant differences were determined by ANOVA plus Scheffé test.

2.6.2. Detection of α_1-ARs by measuring [^{3}H]prazosin binding

Total binding was determined by incubation of membrane fractions (10 μg) with the radioligand [^{3}H]prazosin (3–30,000 pM) in an assay buffer (in mM: 50 Tris(hydroxymethyl)-aminomethane (pH 7.4), 200 NaCl, 0.5 ethylenediaminetetraacetate) containing 0.1% (w/v) ascorbic acid. After 1 hr at room temperature, assay mixtures were filtered under vacuum on GF/C Whatman filters (Whatman) presoaked with 0.3% polyethylenimine using a cell harvester (Brandel®). Filters were washed 3 times with ice-cold Tris(hydroxymethyl)-aminomethane buffer (50 mM, pH 7.4) and dried. Filter-bound radioactivity was determined by liquid scintillation in Aquasol-2 (NEN) using a Beckman Scintillation LS6000 Counter (Beckman Instruments Inc.). Non-specific [^{3}H]prazosin binding was defined as that occurring in the presence of NE (0.1 mM). Binding data were analysed by the Radlig® program (v. 4, Biosoft) [17]. Assays were carried out in triplicate and results are means ± SEM of three separate experiments.

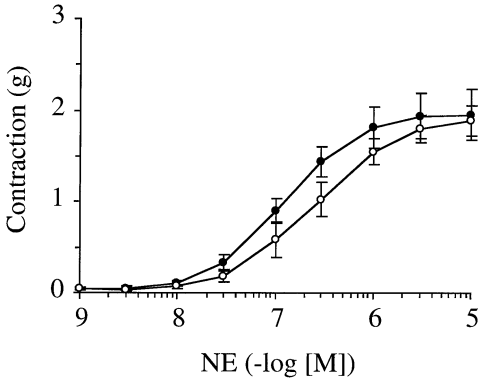
2.6.3. Coupling of G-protein α subunits with α_1-AR subtypes

Membrane fractions (400 μg) were solubilised as described by Gurdal *et al.* [10] and incubated at 4° for 3 hr with polyclonal goat antibodies directed against α_{1A}-, α_{1B}-, or α_{1D}-AR subtypes (1:250 dilution, Santa Cruz Biotechnology). Non-immune serum at the same dilution was used as a control. Following incubation, 100 μL of a 10% suspension of protein A from *Staphylococcus aureus* cells (Pansorbin® cells, Calbiochem) was added, and incubation was continued for a further 1 hr at 4°. Samples were centrifuged, washed, and resuspended in Laemmli buffer. Proteins were separated into a 10% SDS–polyacrylamide gel, immunoblotted with polyclonal goat anti-G_q α antibody (1:500 dilution, Santa Cruz Biotechnology), monoclonal mouse anti-G_o α antibody (1:3000 dilution, Chemicon), or polyclonal rabbit anti-G_i α subunit 1–2 and 3 antibodies (1:1000 dilution each, Calbiochem). Blots were incubated for 1 hr at room temperature with corresponding species-specific horseradish peroxidase-labelled immunoglobulin G (Santa Cruz and Amersham Pharmacia Biotech) and exposed to an x-ray film with enhanced chemoluminescent reagents (SuperSignal West Pico Chemiluminescent substrate, Pierce Chemical Co.). The selectivity of the antisera directed against α_{1A}-, α_{1B}-, or α_{1D}-ARs and the effectiveness of the immunoprecipitation was tested: the three immunocomplexes were separated into an SDS–polyacrylamide gel and immunoblotted with antibodies directed against α_{1A}-, α_{1B}-, or α_{1D}-ARs. A single band was observed with each subtype of α_1-AR, and data indicated that there was no cross-reactivity among the three antibodies tested (results not shown).

3. Results

3.1. Effect of PTX on concentration–response curve to NE in rat endothelium-denuded aortic rings

In rat endothelium-denuded aortic rings, NE produced concentration-dependent contractions (Fig. 1, upper panel, filled circles). PTX (1 μg/mL, 2 hr) had no effect on agonist-induced vasoconstriction (Fig. 1, upper panel, open circles) even at a higher concentration and longer incubation time (3



PTX (1 μg/ml, 2 h)	-	+	-
PTX (3 μg/ml, 4 h)	-	-	+
pEC_{50}	6.9 ± 0.1	6.5 ± 0.2	7.1 ± 0.1
E_{max} (g)	2.0 ± 0.3	2.1 ± 0.2	2.8 ± 0.3

Fig. 1. Concentration–response curves to NE (upper panel) in rat endothelium-denuded aortic rings in the absence (filled circles, N = 6) or presence of PTX (1 μg/mL, 2 hr) (open circles, N = 5). pEC_{50} and E_{max} were determined for two different treatments with PTX (1 μg/mL, 2 hr or 3 μg/mL, 4 hr) (lower panel). Data are means ± SEM.

μg/mL, 4 hr) (Fig. 1, lower panel). KCl-induced contraction was not altered by PTX treatment (results not shown).

3.2. Identification of $G_{i/o}$-proteins

Immunoblotting of G_i-proteins using a combination of two antibodies directed against α_i subunits 1–2 and subunit 3 revealed a band at 40–41 kDa in rat aorta SMC membrane fractions. The presence of the three subunits was confirmed by using the two antibodies separately. G_o-proteins were revealed by immunoblotting using an antibody directed against α_o subunits. A band around 40 kDa was detected in rat aorta SMC membrane fractions (results not shown).

To further identify $G_{i/o}$-proteins in rat aorta SMC membrane, PTX-catalysed transfer of ADP-ribose from [α-^{32}P]NAD to G-protein α subunits was assessed *in vitro* using rat aorta SMC membranes (Fig. 2). A single band was observed in the PTX-treated membrane fractions (lane 1) but was absent in the controls (lanes 2 and 3). Its molecular weight was consistent with the molecular weight of $\alpha_{i/o}$ subunits. This indicated that in aorta SMC membrane, PTX [α-^{32}P]ADP-ribosylated $G_{i/o}$-protein α subunits.

3.3. Demonstration of a PTX-sensitive intracellular pathway

To demonstrate the existence of a PTX-sensitive intracellular pathway in rat aorta SMC, PTX-catalysed transfer of ADP-ribose from [α-^{32}P]NAD to G-protein α subunits was assessed using rat endothelium-denuded aortic rings incubated *in situ* with PTX and [α-^{32}P]NAD. No labelling

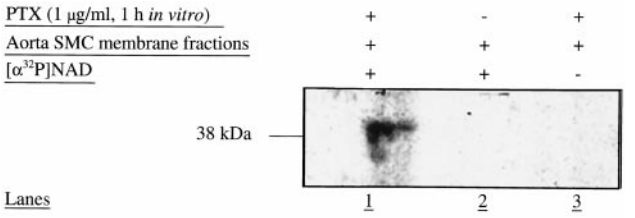


Fig. 2. Identification of $G_{i/o}$-proteins by ADP-ribosylation. Rat aorta SMC membrane fractions (40 μg) were incubated with activated PTX (1 μg/mL) and NAD (0.02 mM, 30 Ci/mmol, 10 μCi/60 μL of assay) (lane 1). Controls were rat aorta SMC membrane fractions (40 μg) incubated with NAD (0.02 mM, 30 Ci/mmol, 10 μCi/60 μL of assay; lane 2) or activated PTX (1μg/mL; lane 3).

with ^{32}P was detected in membrane fractions prepared from endothelium-denuded aortic rings treated with PTX (1 μg/mL, 2 hr, 37°) (result not shown). The same experiment was performed using a higher concentration and longer incubation time of PTX (3 μg/mL, 4 hr, 37°) (Fig. 3). A band was observed in membrane fractions prepared from PTX-treated endothelium-denuded aortic rings (lane 1), but was absent in the controls (lanes 2 and 3). Its molecular weight was consistent with the molecular weight of $\alpha_{i/o}$ subunits. This indicated that in rat endothelium-denuded aortic rings PTX [α-^{32}P]ADP-ribosylated $G_{i/o}$-protein α subunits *in situ*.

3.4. NE receptor and $G_{i/o}$-protein coupling studies

3.4.1. Measurement of NE-induced GTPase activity produced by $G_{i/o}$ α subunits

Measurement of the $G_{i/o}$-specific GTPase activity at basal level and upon NE stimulation was used as an indicator of $G_{i/o}$-activation by NE receptors. Indeed, both basal and NE-stimulated total GTPase activities were reduced by a treatment with PTX (1 μg/mL). NE (1 μM) was found to stimulate the basal GTPase activity level of $G_{i/o}$-proteins in rat aorta SMC membrane fractions (Fig. 4).

3.4.2. Detection of α_1-ARs by measuring [^{3}H]prazosin binding

[^{3}H]Prazosin (3–30,000 pM) bound to rat aorta SMC membrane fractions in a specific and saturable manner.

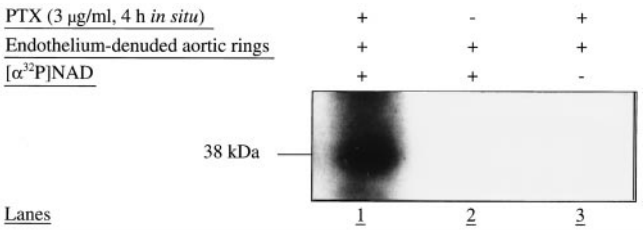


Fig. 3. Identification of a PTX-sensitive pathway. PTX-induced [α-^{32}P]ADP-ribosylation was assessed *in situ* in rat endothelium-denuded aortic rings incubated with NAD (0.02 mM, 30 Ci/mmol, 10 μCi/60 μL of assay) and activated PTX (3 μg/mL, 4 hr) (lane 1). Controls were rat endothelium-denuded aortic rings incubated with NAD (0.02 mM, 30 Ci/mmol, 10 μCi/60 μL of assay; lane 2) or activated PTX (3 μg/mL, 4 hr; lane 3).

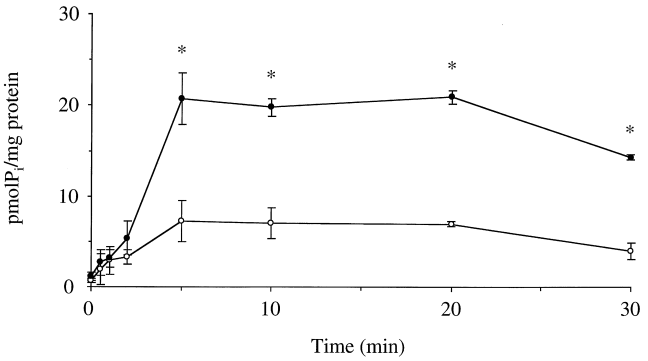


Fig. 4. GTPase activity of $G_{i/o}$-proteins in rat aorta SMC membrane fractions. Membrane fractions (10 μg) were incubated for the time shown in the presence (filled circles) or absence (open circles) of NE (1 μM). $G_{i/o}$-specific GTPase activity (pmol P_i/mg protein) was defined as the difference in GTP hydrolysis between untreated and PTX (1 μg/mL)-treated membrane fractions. All assays were performed in triplicate and are expressed as means ± SEM. Significant differences (*: $P < 0.05$ vs control) were determined by ANOVA plus Scheffé test.

Analysis of the binding parameters revealed the presence of two binding sites, the first with high affinity ($pK_{d\,high}$ = 9.0 ± 0.3 and $B_{max\,high}$ = 92.9 ± 38.7 fmol/mg protein) and the second with lower affinity ($pK_{d\,low}$ = 7.2 ± 0.2 and $B_{max\,low}$ = 407.3 ± 95.1 fmol/mg protein).

3.4.3. Coupling of G-protein α subunits with α_1-ARs

Blots presented in Fig. 5 indicated that the three subtypes of α_1-ARs coupled to the α subunit of G_q (Fig. 5A). None of them coupled to a G_o-protein (Fig. 5B). Only the α_{1D}-AR subtype was co-immunoprecipitated with G_i α subunit (Fig. 5C).

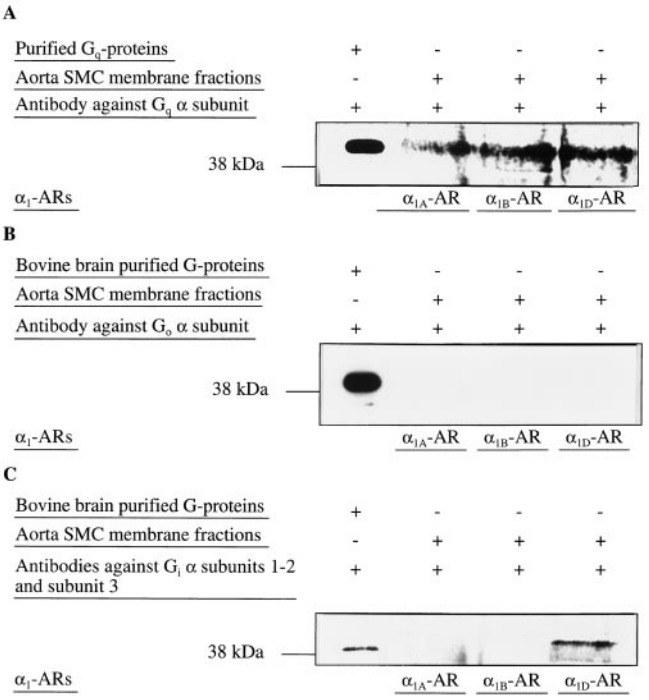


Fig. 5. Coupling between α_1-AR subtypes and G-protein α subunits. Rat aorta SMC membrane fractions (400 μg) were immunoprecipitated with antibodies directed against α_{1A}-, α_{1B}-, or α_{1D}-ARs. The three immunocomplexes were separated by SDS–polyacrylamide gel and immunoblotted with antibodies directed against G_q (A), G_o (B), or G_i (C) α subunits.

4. Discussion

Functional studies revealed that treatment of rat endothelium-denuded aortic rings with PTX did not modify the concentration–response curves to NE. Thus, NE-induced vasoconstriction is not affected by PTX in rat aorta SMC in contrast to rat tail artery SMC [8,18]. This indicates that variations in the PTX-sensitive intracellular component of α-AR agonist-induced vasoconstriction exist between the two vessels.

The lack of effect of PTX could not be explained by the absence of $G_{i/o}$-proteins, as these proteins were evidenced by immunoblotting and ADP-ribosylation experiments in rat aorta SMC membranes. The existence of a PTX-sensitive intracellular pathway was also observed, since $\alpha_{i/o}$ subunits were ADP-ribosylated *in situ* by PTX in endothelium-denuded aortic rings. Thus, the ineffectiveness of PTX in modifying NE-induced vasoconstriction was postulated to be the consequence of an impaired coupling between NE receptors and $G_{i/o}$-proteins. However, the NE-induced increase in $G_{i/o}$-specific GTPase activity ruled out this possibility. The coupling between NE receptors and $G_{i/o}$-proteins is therefore efficient in rat aorta SMC. In rat endothelium-denuded aorta, Weber and MacLeod have shown that the increase in GTPase activity induced by NE is mediated solely by α_1-ARs [19]. Furthermore, previous *in vitro* studies indicate that α-AR agonists contract rat aorta by activating α_1-ARs with no obvious involvement of α_2-ARs [20–22]. Recently, subdivision of α_1-ARs into three subtypes (α_{1A}-, α_{1B}-, and α_{1D}-ARs) through gene cloning, radioligand binding, and functional studies [23] led to the conclusion that contractions induced by α-AR agonists in the rat aorta occur predominantly via the α_{1D}-AR subtype [24–27], although expression of mRNAs for all three α_1-AR subtypes has been detected in this tissue [28,29]. For these reasons, α_1-ARs may be effectively coupled to $G_{i/o}$-proteins.

Complementary binding experiments with [^{3}H]prazosin as a selective α_1-AR antagonist in the concentration range used [30] suggested the presence of a heterogeneous population of α_1-ARs in rat aorta SMC [28,31]. The dissociation constant for the high-affinity site is consistent with that obtained for the α_{1D}-AR subtype by functional and binding experiments [24,25,27]. The prazosin low-affinity site may represent a particular conformational state of the α_{1A}-AR subtype [32]. Alternatively, it may be related to the controversial α_{1L}-AR subtype whose cDNA has not been isolated yet [31,33]. The co-immunoprecipitation experiments confirmed the expression of the three α_1-AR subtypes in rat aorta SMC and their coupling to a G_q-protein [34]. An *in*

vitro coupling was found only between α_{1D}-ARs and G_i-protein in rat aorta SMC. Since PTX has no effect on NE-induced vasoconstriction in rat endothelium-denuded aortic rings, the α_{1D}-AR/G_i-protein-mediated pathway may not play a significant role in NE-induced responses. This is in contrast with previous results in rat tail artery SMC [8,18]. Thus, the PTX-sensitive G-protein pathway does not seem to be universally involved in NE-mediated vasoconstriction of SMC.

In conclusion, we identified a PTX-sensitive $G_{i/o}$-protein pathway in rat endothelium-denuded aorta. We showed an effective coupling between NE receptors and G_i-proteins via α_{1D}-ARs. Since PTX has no effect on NE-induced vasoconstriction, the PTX-sensitive G_i-protein pathway does not play a predominant role in NE-induced responses in rat aorta SMC in contrast to small diameter muscular and resistance arteries.

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