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Noradrenaline Stimulation of High-Affinity GTPase Activity in Membranes from Rat Aorta and Caudal Artery

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ABSTRACT. The ability of noradrenaline (NA) to stimulate increases in high-affinity GTPase activity in sarcolemma-enriched rat aorta and caudal artery membranes was examined in the present study. In aortic membranes, NA significantly ($P < 0.05$; N = 5) increased the V_{max} from a basal value of 103 ± 29 to 156 ± 38 pmol P_i/min/mg protein, but did not affect the K_m which was 0.32 ± 0.08 μM in the absence and 0.58 ± 0.16 μM in the presence of NA. However, in caudal artery membranes, NA significantly ($P < 0.05$; N = 6) increased both the V_{max} and the K_m from basal values of 69 ± 12 pmol P_i/min/mg protein and 0.24 ± 0.05 μM, respectively, to 205 ± 54 pmol P_i/min/mg protein and 1.01 ± 0.25 μM, respectively. Removing the endothelium from both artery preparations did not alter significantly basal GTPase activity or the magnitude of the increase stimulated by NA. Prazosin significantly inhibited NA-stimulated increases in GTPase activity in membranes from endothelium-denuded caudal artery and aorta, and in endothelium-intact caudal artery membranes. However, yohimbine significantly inhibited NA-stimulated increases in GTPase activity only in preparations from endothelium-intact caudal arteries. Therefore, in endothelium-intact caudal artery membranes, NA stimulated increases in GTPase activity that were apparently mediated by both α_1-adrenoceptors and α_2-adrenoceptors, while in endothelium-denuded aortic and caudal artery membranes this increase was mediated solely by α_1-adrenoceptors. Western blotting of these arteries confirmed the presence of both $G_{i_\alpha 2,3}$ and $G_{q/11_\alpha}$, which are candidates for mediating the α_1-adrenoceptor-stimulated increases in GTPase activity. BIOCHEM PHARMACOL 52;4:677–684, 1996.

KEY WORDS. artery; G-proteins; noradrenaline; GTPase activity; α_1-adrenoceptor; α_2-adrenoceptor; endothelium

NA† causes vasoconstriction primarily by stimulating α_1-adrenoceptors, although a small contribution may come from post-synaptic α_2-adrenoceptors [1]. The α_1-adrenoceptor is thought to be coupled to heterotrimeric G-proteins, which have not been identified directly in vascular smooth muscle. However, pertussis toxin-sensitive and -insensitive components to the α_1-adrenoceptor-mediated response have been observed in arteries [2, 3], leading to speculation that the α_1-adrenoceptor couples to two different G-proteins, G_q and G_i, respectively [3, 4]. Both G-proteins have been detected with western blots [5–8] and immunoprecipitation [7, 9] in rat aorta and mesenteric artery, leaving the possibility of such coupling open.

When a G-protein is activated by a hormone/receptor complex, GDP is exchanged for GTP, which results in dissociation of the G_α-GTP subunit from the $G_{\beta\gamma}$ subunits, both of which may influence the activity of effectors [10]. The G-protein remains activated until the intrinsic GTPase activity of the G_α-subunit cleaves the bound GTP, freeing a phosphate, P_i [11]. The newly formed G_α-GDP complex has a high affinity for the $G_{\beta\gamma}$ subunits which promotes reassociation of the inactive heterotrimeric G-protein complex [11]. Therefore, measurement of the GTPase activity stimulated by agonists has been used as a reflection of the degree of receptor/G-protein interaction [12]. GTPase activity stimulated by the α_1-adrenoceptor has been measured in rat parotid, and in rabbit atrial and ventricular myocyte membranes [13, 14], but has not yet been measured in vascular smooth muscle membranes to our knowledge.

The aim of the present study was to determine whether NA stimulates high-affinity GTPase activity in membranes prepared from rat aorta and caudal artery, and, if so, to characterize pharmacologically the receptors mediating this increased G-protein activity. Since membranes were prepared from whole arteries, the influence of endothelium on the NA-stimulated GTPase activity was also examined in

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† *Abbreviations:* NA, noradrenaline; AppNHp, 5′-adenylyl-[β,γ]-imidodiphosphate lithium; G-protein, GTP-binding protein; and ECL, enhanced chemiluminescence.

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the present study. Finally, western blots were performed on the membrane preparations with antibodies specific for different G-protein subtypes, to aid in the identification of possible α_1-adrenoceptor-coupled G-proteins.

MATERIALS AND METHODS

Materials

[γ-^{32}P]GTP (≅ 3000 Ci/mmol) was obtained from Amersham (Oakville, Ontario, Canada) or from Dupont NEN (Mississauga, Ontario, Canada). [γ-^{32}P]ATP (≅ 3000 Ci/mmol), ECL western blotting detection kits, and ECL film were obtained from Amersham. Bio-Rad protein dye reagent and BSA standard, acrylamide, bis-acrylamide, dithiothrietol, glycine, β-mercaptoethanol, *N,N,N',N'*-tetramethylethylenediamine (TEMED), ammonium persulfate, and nitrocellulose were obtained from Bio-Rad (Hercules, CA, U.S.A.). Anti-G-protein antisera (AS/7, EC/2, and QL) were obtained from Dupont NEN. All other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Isolation of Arteries

Rats (325–400 g) were given an overdose of pentobarbital sodium (65 mg/kg). Aorta and caudal arteries were removed and placed in Krebs buffer oxygenated with 95% CO_2/5% O_2 (composition in mM: NaCl, 113; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; and dextrose, 11.5). Fat and connective tissue were removed from the arteries, and then the arteries were quick frozen with clamps cooled in liquid nitrogen before storage at −70° until used. Where indicated, the endothelium was removed by inserting a fine wire into the lumen of the arteries, gently rotating the artery around the wire several times, and rinsing the artery in fresh buffer prior to freezing the tissues in liquid nitrogen. This method has been shown in this laboratory to remove the functional endothelium [15].

Preparation of Plasmalemma-Enriched Membrane Fraction

A modification of the method described by Hilf and Jakobs [16] was used to prepare membranes enriched in sarcolemma. Briefly, aorta or caudal arteries were pooled, quickly weighed, and placed in ice-cold homogenization buffer (composition: 20 mM Tris-HCl, 1 mM dithiothrietol, 1 mM EDTA, 100 μg/mL trypsin inhibitor Type II-S, 1 mM phenylmethylsulfonyl fluoride, 3 mM benzamidine-HCl, 1 μM leupeptin, and 1 μM pepstatin A, pH = 8.0). Arteries were minced with scissors, and then homogenized on ice with a Polytron at setting 6 with 4 × 10 sec bursts. The homogenate was then centrifuged at 900 *g* for 10 min, and the resulting post-nuclear supernatant was collected before centrifugation at 105,000 *g* with a TY65 rotor for 1 h at 4°. The pellet (pellet A) was resuspended in 2.5 mL homogenization buffer, layered on a discontinuous gradient composed of 2 mL each of 5, 20, 25, and 30% sucrose, and centrifuged at 70,000 *g* for 2 hr at 4° in an SW41 rotor. The 5/20% interface and the 5% layer were collected, diluted five times with homogenization buffer, and centrifuged at 105,000 *g* for 45 min at 4° in a TY65 rotor; the resulting pellet was resuspended in an appropriate volume of homogenization buffer to yield a protein concentration of 0.3 to 1.0 μg/μL. After assaying for protein content using the BioRad® system which is based upon the method of Bradford [17], the membranes were stored at −70° until assay.

Na^+/K^+-ATPase Assay

The method for measuring ouabain-inhibitable Na^+/K^+-ATPase activity, based upon the measurement of free $^{32}P_i$ from [γ-^{32}P]ATP, was modified from that of Braun and Walsh [14]. The assay was carried out in minimal light to prevent the breakdown of ouabain. A final reaction volume of 100 μL in each tube contained 1–5 μg protein, 50 mM Tris-HCl, 100 mM NaCl, 10 mM KCl, 3 mM $MgCl_2$, 1 mM EGTA, 15 mM NaN_3 and alamethicin (0.1 μg/μg protein); pH = 7.5. Tubes were warmed for 5 min at 37° prior to starting the reaction with [γ-^{32}P]ATP (5 mM; 3000 cpm/nmol) and allowed to react for 15 min at 37°. Tubes were stopped by adding 0.9 mL of ice-cold activated charcoal 5% (w/v) in 20 mM phosphoric acid and kept on ice for 5 min before being centrifuged at 3000 *g* in a swing bucket rotor for 10 min at 4°. An aliquot (0.5 mL) of supernatant was analyzed for free $^{32}P_i$ by liquid scintillation counting. Specific activity was determined by subtracting the activity in the presence of 1 mM ouabain. Membranes prepared from endothelium-denuded aorta and caudal artery did not appear to differ in the level of Na^+/K^+-ATPase activity, or the degree of enrichment in ouabain-inhibitable activity from membranes prepared from endothelium-intact arteries, so the Na^+/K^+-ATPase activity results were combined.

GTPase Assay

The measurement of high-affinity GTPase activity, based upon the release of free $^{32}P_i$ from [γ-^{32}P]GTP, was modified from the method used by Fleming and Watanabe [18]. Briefly, a membrane aliquot was thawed and incubated with alamethacin (0.5 μg/μg protein) for 30 min at 22°. This membrane (2–5 μg protein/tube) was then used to start the GTPase reaction in tubes that had been warmed for 1 min at 37° and were allowed to react for 10 min at 22°. Final reaction composition was 40 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$, 1 mM ATP, 5 mM AppNHp, 5 mM phosphocreatine, 5 U/mL creatine phosphokinase, 0.25 μM [γ-^{32}P]GTP (3000 cpm/pmol; pH = 7.5). Reactions were terminated by the addition of 0.9 mL of ice-cold 5% activated charcoal in 20 mM phosphoric acid, and tubes were incubated on ice for 5 min before being centrifuged at 3000 *g* in a swing bucket rotor for 10 min at 4°. An aliquot of the supernatant (0.5 mL) was counted for free $^{32}P_i$ by liquid scintillation counting. High-affinity GTPase activity was

calculated as the difference between total and non-specific hydrolysis (determined in the presence of 0.1 mM GTP). In the substrate saturation studies, unlabeled GTP was added to give concentrations ranging from 0.01 to 100 μM (0.034 μCi/tube). In preliminary experiments, the high-affinity GTPase activity was found to be dependent on protein concentration and time. Both the protein levels and time used in the assay were on the linear portion of the curves. Also, NA was found to stimulate increases in GTPase activity in a concentration-dependent manner, with 1 μM NA representing a near-maximal concentration in both artery preparations. The concentrations of prazosin and yohimbine used in the pharmacological characterization study (0.1 μM for both) were shown in radioligand binding studies to produce the maximum selective block of the binding of [^{3}H]prazosin and [^{3}H]rauwolscine to α_1-adrenoceptors and α_2-adrenoceptors, respectively, in rat caudal artery [19].

SDS–PAGE and Western Blotting

Proteins from pellet A of rat aorta and caudal arteries were resolved by SDS–PAGE using the method of Laemmli [20] on a 10% acrylamide/0.8% *N,N'*-methylenebisacrylamide gel run overnight at 500 V and 15 mA constant current. For both aorta and caudal artery preparations, 29 μg of membrane protein was added to each lane. Biotinylated molecular weight markers (ECL molecular weight markers, Amersham), ranging from 14,400 to 97,400, were run in parallel with the samples. The resulting gel was then transferred electrophoretically to nitrocellulose membrane according to the method of Towbin *et al.* [21] at 0.2 A for 2 hr at 4° and blocked overnight at 4° in 5% skim milk powder in Tris-buffered saline (pH = 7.5) with 0.1% Tween-20. Specific G-protein α-subunits were identified by incubating the nitrocellulose membrane with 1:5000 anti-G-protein antisera (AS/7, EC/2, or QL) for 1 hr at 37°. A horseradish peroxidase conjugated donkey anti-rabbit secondary antibody (1:25,000), streptavidin-horseradish peroxidase conjugate (1:1500), and enhanced chemiluminescence were used for detection (ECL Western Blotting kit; Amersham).

Data Analysis and Statistics

All assays were performed in triplicate, except for the GTP saturation curves which were performed in duplicate. The results are from (N) different membrane preparations. All results are expressed as means ± SEM. A paired *t*-test or a repeated measures ANOVA, followed by a Bonferroni post-hoc test, was used in the comparisons of the GTPase data. In the GTP saturation curves, data were fit to a one-site binding curve using Graph-Pad Prism software (San Diego, CA, U.S.A.) and used to generate estimates of K_m and V_{max}. These values were then compared, using a paired *t*-test.

Results

In preliminary experiments, no high-affinity GTPase activity was measurable in crude homogenates from rat aorta and caudal arteries, so we purified the homogenates to obtain preparations that were more enriched in sarcolemmal membrane. Ouabain-inhibitable Na^+/K^+-ATPase activity was used as a measure of enrichment of sarcolemmal membrane (Table 1). In aorta, an approximately 16-fold enrichment in sarcolemmal membrane from the post-nuclear supernatant (PNS) to the final preparation was obtained, while the enrichment was approximately 12-fold in caudal artery preparations.

GTP saturation curves in the presence and absence of 1 μM NA revealed high-affinity GTPase activity in rat aorta and caudal artery membranes (a representative curve shown for each in Fig. 1, panels A and B, respectively). In aortic membranes, NA significantly ($P < 0.05$; N = 5) increased the V_{max} from a basal value of 103 ± 29 to 156 ± 38 pmol P_i/min/mg protein, but did not affect the K_m value which was 0.32 ± 0.08 μM in the absence and 0.58 ± 0.16 μM in the presence of NA. However, in caudal artery membranes, NA significantly ($P < 0.05$; N = 6) increased both the V_{max} and the K_m from basal values of 69 ± 12 pmol P_i/min/mg protein and 0.24 ± 0.05 μM, respectively, to 205 ± 54 pmol P_i/min/mg protein and 1.01 ± 0.25 μM, respectively, in the presence of NA. All subsequent GTPase assays were performed using a constant concentration of 0.25 μM GTP (a near K_m value).

The contribution of endothelium to basal and NA-stimulated high-affinity GTPase activity was examined in membrane preparations from both arteries. The basal high-affinity GTPase activity was not different between membranes prepared from endothelium-intact and -denuded aorta and caudal arteries ($P > 0.05$, two-way repeated measures ANOVA; Fig. 2). NA produced an increase in GTPase activity that was statistically significant ($P < 0.05$, Bonferroni post-hoc tests following a two-way repeated measures ANOVA) in all preparations, except in the membranes prepared from endothelium-denuded aorta (Fig. 2). However, overall, removing the endothelium did not alter significantly ($P > 0.05$, two-way repeated measures ANOVA) the magnitude of the increase in GTPase activity produced by NA in either aorta or caudal artery membranes (Fig. 2). This suggests that the inability to detect a

TABLE 1. Enrichment in ouabain-inhibitable Na^+ K^+-ATPase activity in membrane preparations from rat aorta and caudal arteries.

	Activity (μmol P_i/min/mg protein)		
	Post nuclear supernatant	Final preparation	Aproximate fold increase in activity
Aorta (N = 7)	0.04 ± 0.01	0.62 ± 0.14	16
Caudal artery (N = 8)	0.11 ± 0.06	1.34 ± 0.49	12

Results are expressed as means ± SEM.

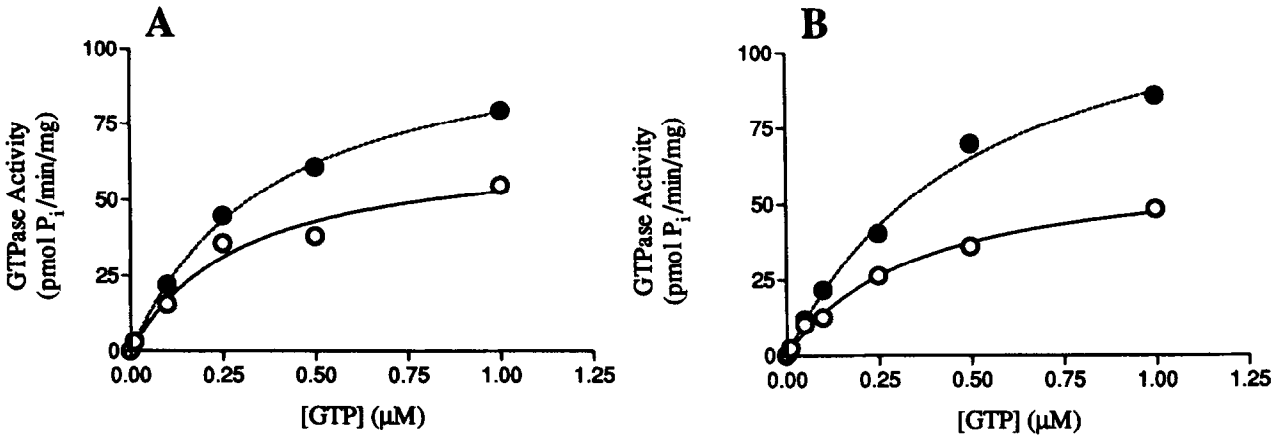

FIG. 1. (A) Substrate saturation curve for high-affinity GTPase activity in membranes prepared from rat aorta in the absence (○) and presence (●) of 1 μM NA. A representative assay of one membrane preparation is shown. Results are the means of replicates. (B) Substrate saturation curve for high-affinity GTPase activity in membranes prepared from rat caudal artery in the absence (○) and presence (●) of 1 μM NA. A representative assay of one membrane preparation is shown. Results are the means of replicates.

significant difference between basal and NA-stimulated levels of GTPase activity in endothelium-denuded aorta membranes in a post-hoc test results from a Type II error, due to the small sample size (N = 3) in this experiment and the limited power of post-hoc tests when many different comparisons are made simultaneously. This is supported by the fact that in the next series of experiments (see below), NA produced a statistically significant ($P < 0.05$, Bonferroni post-hoc test following a one-way repeated measures ANOVA) increase in GTPase activity in endothelium-denuded aorta.

The α-adrenoceptor subtype contributing to the NA-stimulated increase in GTPase activity was investigated in aorta and caudal artery membranes. In endothelium-denuded aorta (Fig. 3A) and caudal artery (Fig. 3B) membranes, a significant ($P < 0.05$) inhibition by 0.1 μM prazosin, but not by yohimbine (0.1 μM), of GTPase activity stimulated by 1 μM NA was observed. Figure 3B also shows that in membranes prepared from endothelium-intact caudal arteries, 0.1 μM prazosin significantly ($P < 0.05$) diminished the increase in high-affinity GTPase activity produced by NA, while 0.1 μM yohimbine apparently abolished the NA-stimulated increase in activity ($P < 0.05$). However, the NA-stimulated GTPase activity remaining in the presence of yohimbine was not significantly different ($P > 0.05$) from that in the presence of prazosin.

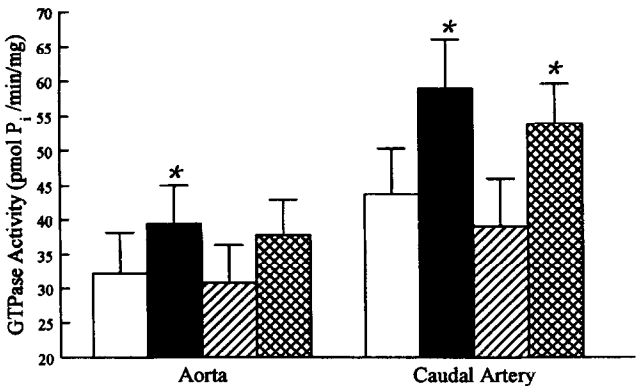

FIG. 2. High-affinity GTPase activity in membranes prepared from endothelium-intact (□,■) and -denuded (▨,▩) aorta (N = 3) and caudal arteries (N = 5–6). Activity in the absence (□,▨) and presence (■,▩) of 1 μM NA is shown as mean ± SEM. Key: (*) significantly different from corresponding basal (absence of NA) value ($P < 0.05$; Bonferroni post-hoc test following a two-way repeated measures ANOVA).

Membranes from rat aorta and caudal arteries were blotted with three different antisera to investigate the presence of G-proteins that are potential candidates for mediating the NA-stimulated increases in high-affinity GTPase activity. The same levels of membrane protein (29 μg) were loaded in each lane, and corresponding bands are shown in Figure 4. In both arteries, one major band at an apparent molecular weight of 39,000 was observed with AS/7 which recognizes $G_{i_\alpha 1}$ and $G_{i_\alpha 2}$, a single band at an apparent molecular weight of 40,000 was observed with EC/2 which recognizes $G_{i_\alpha 3}$ and G_{o_α}, and a single band at an apparent molecular weight of 41,000 was observed with QL which recognizes $G_{q/11_\alpha}$. Since biotinylated molecular weight standards were used, the estimated apparent molecular weights are underestimates of the true weights. No apparent differences in intensity of the bands were observed between aorta and caudal artery with all three antisera, suggesting that levels of the respective G-protein α-subunits do not differ greatly between the two arteries.

Discussion

The high-affinity GTPase activity measured in membranes prepared from rat aorta and caudal artery exhibited characteristics similar to those reported for other tissues, such as cardiomyocytes and erythrocytes. However, the V_{max} values

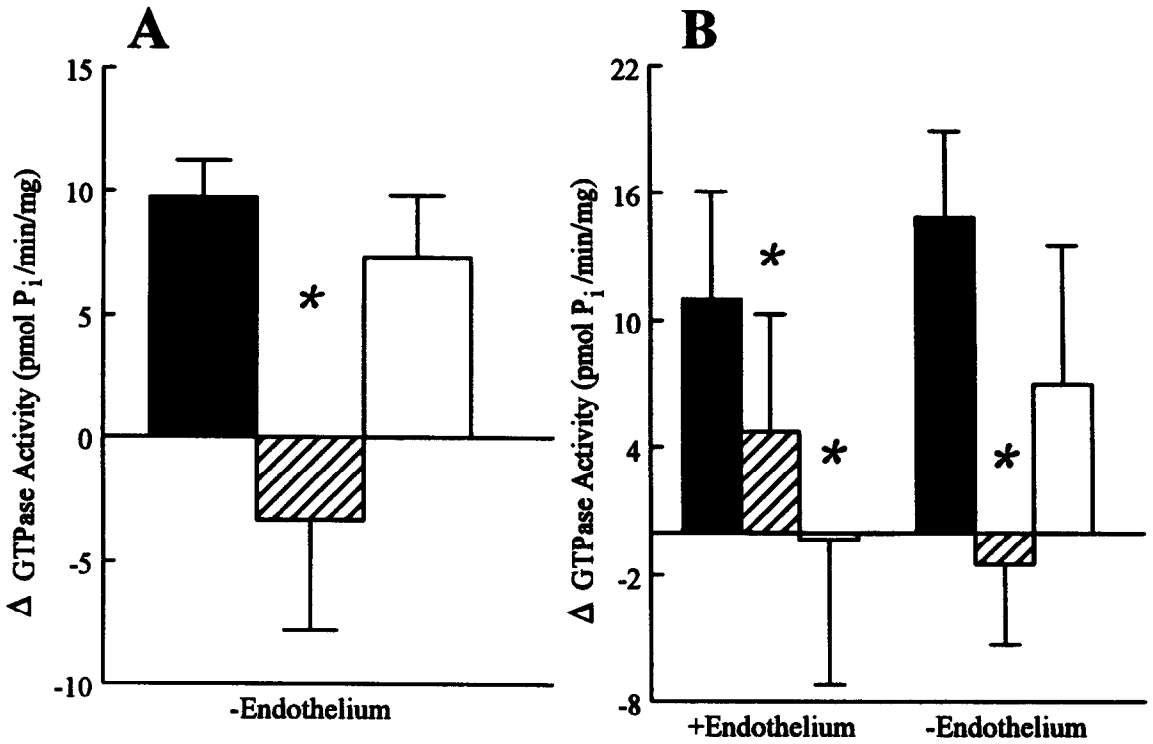

FIG. 3. (A) Change in high-affinity GTPase activity from basal activity in membranes prepared from endothelium-denuded (N = 3) aorta in the presence of 1 μM NA (■); 1 μM NA and 0.1 μM prazosin (▨); and 1 μM NA and 0.1 μM yohimbine (□). Results are expressed as means ± SEM. Key: (*) significantly different from corresponding NA value ($P < 0.05$; one-way repeated measures ANOVA followed by a Bonferroni post-hoc test). (B) Change in high-affinity GTPase activity from basal activity in membranes prepared from endothelium-intact (N = 4) and -denuded (N = 5) caudal arteries in the presence of 1 μM NA (■); 1 μM NA and 0.1 μM prazosin (▨); and 1 μM NA and 0.1 μM yohimbine (□). Results are expressed as means ± SEM. Key: (*) significantly different from corresponding NA value ($P < 0.05$; one-way repeated measures ANOVA followed by a Bonferroni post-hoc test).

for GTPase activity in the arterial membrane preparations used in the present study were an order of magnitude higher than most values reported in these other tissues [14, 16, 18]. While this difference in V_{max} values likely reflects tissue differences, the K_m values estimated for both aorta and caudal artery membranes were similar to reported values for these other tissues [14, 16, 18]. Since we are not aware of any other reports of NA-stimulated high-affinity GTPase activity in vascular smooth muscle, we cannot compare our values directly. However, one notable difference is that while most studies, including the present, have found that agonists significantly stimulate the V_{max}, most have not found a significant increase in the K_m in the presence of agonist [14, 16, 18] as we have in caudal artery membranes. However, a study examining the high-affinity (low K_m) GTPase activity in mouse hippocampus membranes has found a significant increase in the K_m in the presence of agonist [22], and most studies report at least a trend to increase in the presence of agonist [14, 16, 18]. Thus, the estimates of V_{max} and K_m for high-affinity GTPase activity in arterial membranes obtained in the present investigation seem reasonable.

Before examining the results of our pharmacological investigation of the NA-stimulated increase in GTPase activity, we should first discuss what findings are expected. In contractility experiments from our laboratory, the NA-stimulated vasoconstriction in rat aorta was found to be mediated primarily by α_1-adrenoceptors [23]. However, evidence for both α_1-adrenoceptors and α_2-adrenoceptors was found in a study looking at calcium signaling in freshly dispersed single smooth muscle cells from rat caudal artery [24]. Similarly, in contractility studies using canine mesenteric artery, rat cremaster muscle arterioles, rat caudal artery, or perfused rat tail vascular bed, both α_1-adrenoceptors and α_2-adrenoceptors have been found postjunctionally [25–29], although the contribution of α_2-adrenoceptors is small [24–29]. Evidence supporting the presence of both α-adrenoceptor subtypes in vascular tissue also comes from radioligand binding studies that have found both α_1-adrenoceptors and α_2-adrenoceptors in rat caudal and mesenteric artery membranes [19, 30], where presumably the membranes have been prepared from endothelium-intact arteries. It is also interesting to note that the binding study in rat mesenteric artery membranes used arteries that had the adventitia and most nerves removed [30], while the study using rat caudal artery used arteries with intact nerves and adventitia [19], suggesting that the contribution of any membranes from these layers of the

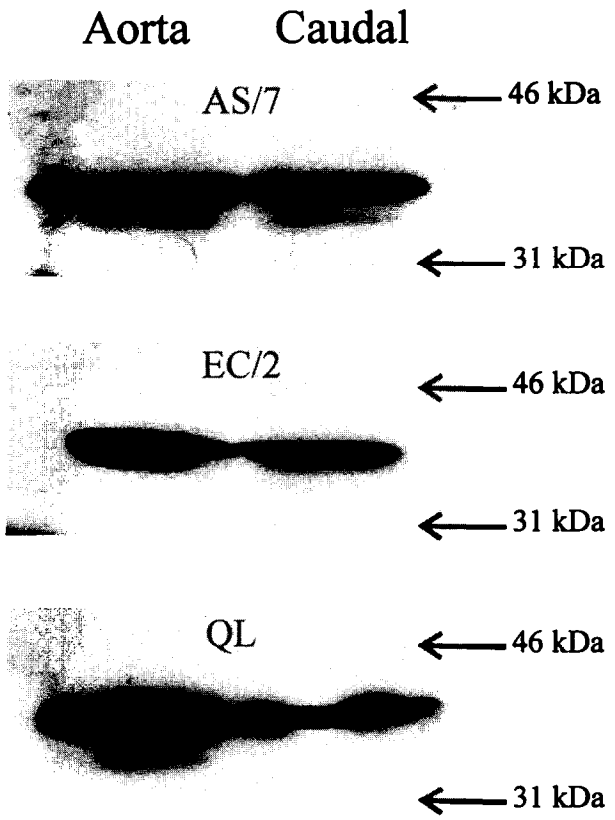

FIG. 4. Western blot of rat aorta and caudal artery membranes. Approximately 29 μg of membrane protein was added to each lane for both aorta and caudal artery preparations, and then blotted with three antisera: AS/7, EC/2, and QL. The blot shown is representative of two different blots.

arteries is small, or at least pharmacologically similar to that found on a mixture of smooth muscle and endothelial cell membranes. Although binding studies have also detected the presence of α_2-adrenoceptors in endothelial cell membranes [31], their contribution to the α-adrenoceptor population in membranes prepared from intact arteries would be expected to be small relative to α-adrenoceptors from smooth muscle. Based on the results of these contractility and binding studies, one would expect to find that α_1-adrenoceptors mediate the NA-stimulated increase in high-affinity GTPase activity in aortic membranes, whereas the NA-stimulated increase in GTPase activity in caudal artery membranes would be expected to be mediated primarily by α_1-adrenoceptors, with perhaps a small contribution from α_2-adrenoceptors.

The results of the present investigation obtained in membranes prepared from endothelium-denuded aorta agree with the expected results since evidence for only α_1-adrenoceptor-mediated increases in GTPase activity was found. Similarly, evidence for only α_1-adrenoceptor-mediated responses was found in endothelium-denuded caudal artery. It is possible that a small NA-stimulated α_2-adrenoceptor-mediated increase in activity was present in caudal artery membranes, but was not detected due to the variability associated with measuring GTPase activity in arterial preparations. Another possible explanation for the lack of α_2-adrenoceptor-mediated increase in high-affinity GTPase activity in endothelium-denuded caudal artery membranes arises from evidence obtained by other investigators that a "permissive agent" is required for functional coupling of the α_2-adrenoceptors [25–28]. Contractility experiments conducted in a variety of arteries, including perfused rat tail vascular bed, have demonstrated that α_2-adrenergic agonists have little or no contractile effect under *in vitro* conditions in which α_1-adrenoceptor responses are maintained [25–28]. α_2-Adrenoceptor-mediated responses can be restored in the presence of subthreshold concentrations of either angiotensin II, endothelin-1, or Bay K 8644, or depolarizing concentrations of KCl [25–28]. It is possible that in the present experiments, the α_2-adrenoceptors became functionally uncoupled in the process of isolating the caudal arteries, due to removal of essential plasma or tissue factors, and a further reduction may have occurred as a result of removing the endothelium. In apparent agreement with the latter proposal, NA-stimulated increases in GTPase activity in membranes prepared from endothelium-intact caudal artery appeared to be mediated by both α_1-adrenoceptors and α_2-adrenoceptors. However, it is surprising that the level of NA-stimulated GTPase activity did not decrease when endothelium was removed, as would be expected if functional uncoupling of the α_2-adrenoceptors were occurring as a result of removing the endothelium.

An unexpected result of the present investigation was the apparent complete block of the NA-stimulated increase in GTPase activity in endothelium-intact caudal artery membranes by the α_2-adrenoceptor antagonist yohimbine, under conditions where a partial sensitivity to the α_1-adrenoceptor antagonist prazosin was also detected. It seems unlikely that the NA-stimulated increases in GTPase activity are mediated exclusively by α_2-adrenoceptors in membranes prepared from endothelium-intact arteries. The inhibitory effect we observed with prazosin and the evidence from radioligand binding and contractility experiments discussed above suggest that even in the presence of endothelium, α_1-adrenoceptors contribute to a large extent to the NA-mediated responses. Since the NA-stimulated GTPase activity remaining in the presence of either yohimbine or prazosin was not significantly different, it is possible that both α_1-adrenoceptors and α_2-adrenoceptors mediate the response in endothelium-intact caudal artery membranes, although studies to confirm this are warranted.

The presence of G-protein α-subunits potentially responsible for mediating the NA-stimulated increase in high-affinity GTPase activity in the rat aorta and caudal arteries was investigated using three different antisera. Although other investigations have demonstrated the presence of these G-proteins in rat aorta, caudal artery has not been investigated to our knowledge. AS/7 has relative selectivity for $G_{i_{\alpha}1}$ (41 kDa) and $G_{i_{\alpha}2}$ (40 kDa), EC/2 has relative selectivity for $G_{i_{\alpha}3}$ (40 kDa) and $G_{o_{\alpha}}$ (39 kDa), and QL is

selective for $G_{q/11_\alpha}$ (42 kDa) [32, 33]. All three antisera produced bands at approximately the expected molecular weights, when the underestimation due to the use of biotinylated molecular weight markers is considered. AS/7 is likely recognizing the $G_{i_\alpha 2}$ subunit, since $G_{i_\alpha 1}$ is not present in arteries [6]. EC/2 may be recognizing $G_{i_\alpha 3}$ as suggested by Anand-Srivastava [5], who used EC/2 in aorta membranes. Finally, because QL recognizes only one class of G-proteins of a single molecular weight, we know that a $G_{q/11_\alpha}$ variant is present in the aorta and caudal artery membranes. Thus, the candidates for mediating the NA-stimulated increases in high-affinity GTPase activity are $G_{i_\alpha 2}$, $G_{i_\alpha 3}$ and $G_{q/11_\alpha}$.

In conclusion, we have demonstrated that NA stimulates increases in high-affinity GTPase activity in aortic and caudal artery membranes enriched in sarcolemma. Endothelium in these preparations does not influence the level of basal activity or the magnitude of the stimulated increase in GTPase activity. However, paradoxically, the presence of endothelium in caudal artery membranes resulted in an NA-stimulated increase in activity that was mediated by a combination of α_1-adrenoceptors and α_2-adrenoceptors, while in the absence of endothelium in both aortic and caudal artery membranes, this increase was mediated solely by α_1-adrenoceptors. Western blotting of these arteries has confirmed the presence of both $G_{i_\alpha 2,3}$ and $G_{q/11_\alpha}$, which are candidates for mediating the α_1-adrenoceptor-stimulated increases in GTPase activity, but this awaits verification with further experiments.

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