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Immunocytochemical Localization of the *Alpha*-1B Adrenergic Receptor and the Contribution of This and the Other Subtypes to Vascular Smooth Muscle Contraction: Analysis with Selective Ligands and Antisense Oligonucleotides[1]

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ABSTRACT

The contribution of the *alpha*-1B adrenergic receptor (AR) to vascular smooth muscle contraction has been assessed using a combination of immunological, molecular biological and pharmacological approaches. A subtype-selective antibody detected *alpha*-1B immunoreactivity in the medial layer of the aorta, caudal, femoral, iliac, mesenteric resistance, renal and superior mesenteric arteries. Receptor protection assays and antisense oligonucleotides were used to assess the contribution of the *alpha*-1B AR to contraction. The *alpha*-1B AR was implicated in mediating the phenylephrine-induced contraction of the mesenteric resistance artery. The *alpha*-1D AR was implicated in mediating the contraction of the aorta, femoral, iliac and superior mesenteric arteries. Similarly, the *alpha*-1A AR was implicated in mediating contraction of the caudal and renal arteries. *In vivo* application of antisense oligonucleotides targeted to the translational start site of the *alpha*-1B AR had no effect on the phenylephrine-induced contraction of the femoral or renal arteries. In contrast, antisense oligonucleotides directed against the *alpha*-1D AR significantly inhibited the phenylephrine response in the femoral artery but had no effect on the renal artery. Application of *alpha*-1A AR antisense oligonucleotides inhibited the contraction of the renal artery without effect on the femoral artery. These data show that (1) *alpha*-1B AR immunoreactivity is widely distributed in the same peripheral arteries in which previous studies detected its mRNA, and (2) despite this distribution, receptor protection and antisense oligonucleotide studies indicate that the *alpha*-1B AR mediates the contraction of only the mesenteric resistance artery.

The *alpha*-1 AR family is a member of the G protein-coupled superfamily of receptors. In analogy to bacteriorhodopsin, these receptors have the now very familiar proposed structure of seven transmembrane spanning domains connected by hydrophilic loops alternately exposed to the extracellular and intracellular environment. The structure of G protein-coupled receptors in general and *alpha*-1 ARs specifically have been the subject of several reviews (Bylund *et al.*, 1995; Graham *et al.*, 1996; Guarino *et al.*, 1996; Minneman and Esbenshade, 1994; Strader *et al.*, 1994). Three genes encoding unique *alpha*-1 AR subtypes (Cotecchia *et al.*, 1988; Lomasney *et al.*, 1991; Perez *et al.*, 1991; Schwinn *et al.*, 1990) have been discovered. Despite early controversy and confusion, a nomenclature consisting of the *alpha*-1A (formerly referred to as the *alpha*-1c), the *alpha*-1B and *alpha*-1D (formerly the *alpha*-1a or *alpha*-1a/d) AR subtypes has been proposed and gained acceptance in the field (Hieble *et al.*, 1995).

There is longstanding evidence that multiple *alpha*-1 AR subtypes participate in the regulation of peripheral vascular function (Bylund *et al.*, 1995; McGrath, 1982; Minneman, 1988). However, the individual contribution of each of the *alpha*-1D AR subtypes has not been established. mRNA for the *alpha*-1A AR is expressed at very high levels in peripheral arteries, ~90% of the total *alpha*-1 AR message pool (Guarino *et al.*, 1996). High-affinity antagonists have been used to implicate the *alpha*-1A AR in the regulation of the caudal and renal arteries (Han *et al.*, 1990; Elhawary *et al.*, 1992; Piascik *et al.*, 1995). Although expressed at low levels compared with the other receptor subtypes, *alpha*-1D AR mRNA can also be detected throughout the peripheral vasculature (Guarino *et al.*, 1996; Piascik *et al.*, 1995). Studies

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ABBREVIATIONS: AR, adrenergic receptor; PSS, physiological saline solution; CEC, chloroethylclonidine; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; PBZ, phenoxybenzamine.

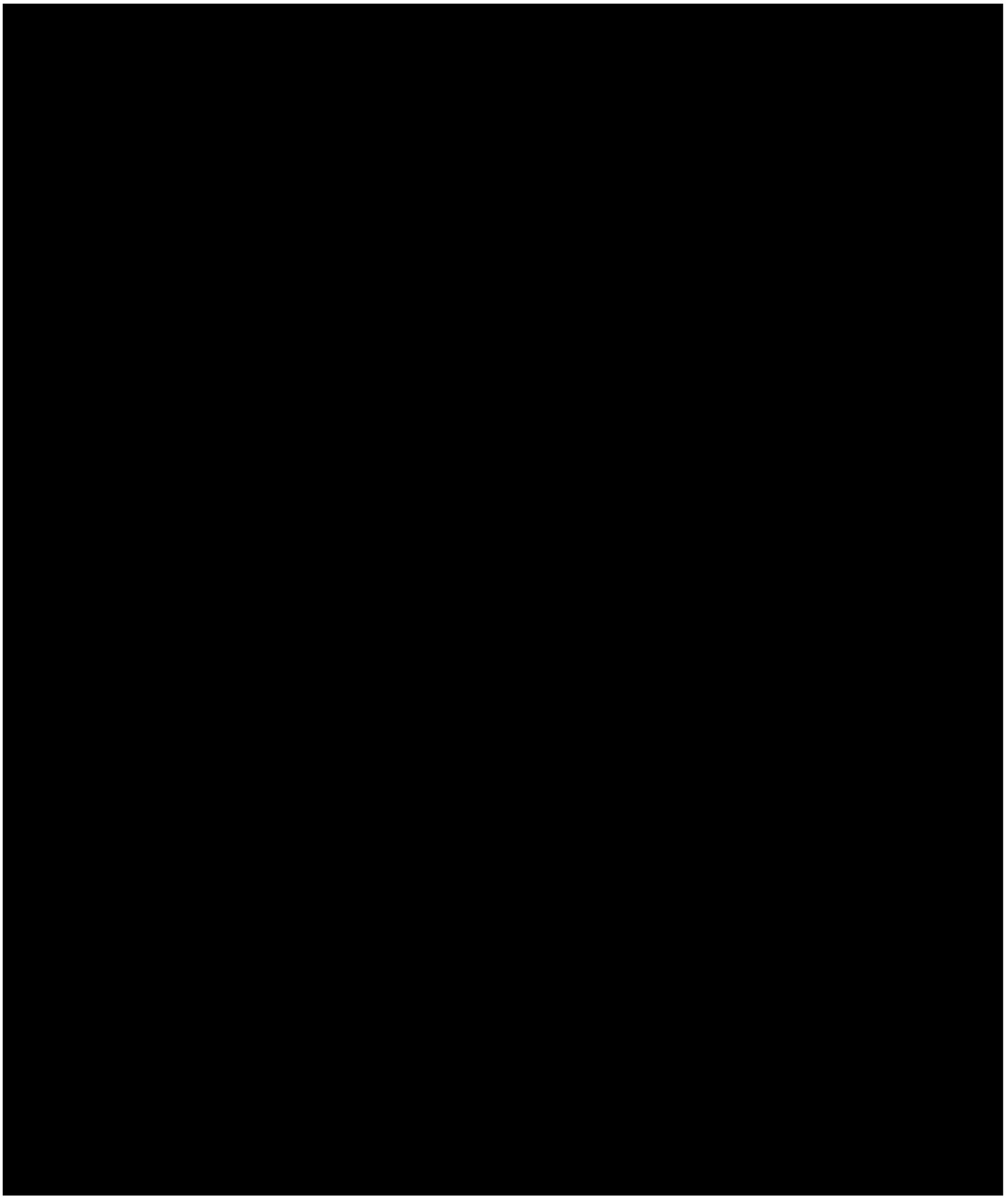

Fig. 1. Immunocytochemical localization of the *alpha*-1B AR in the aorta, caudal and femoral arteries. The experiments were carried out as described in the text. Left, presence of an affinity-purified *alpha*-1B AR antibody. Right, presence of preimmune serum alone.

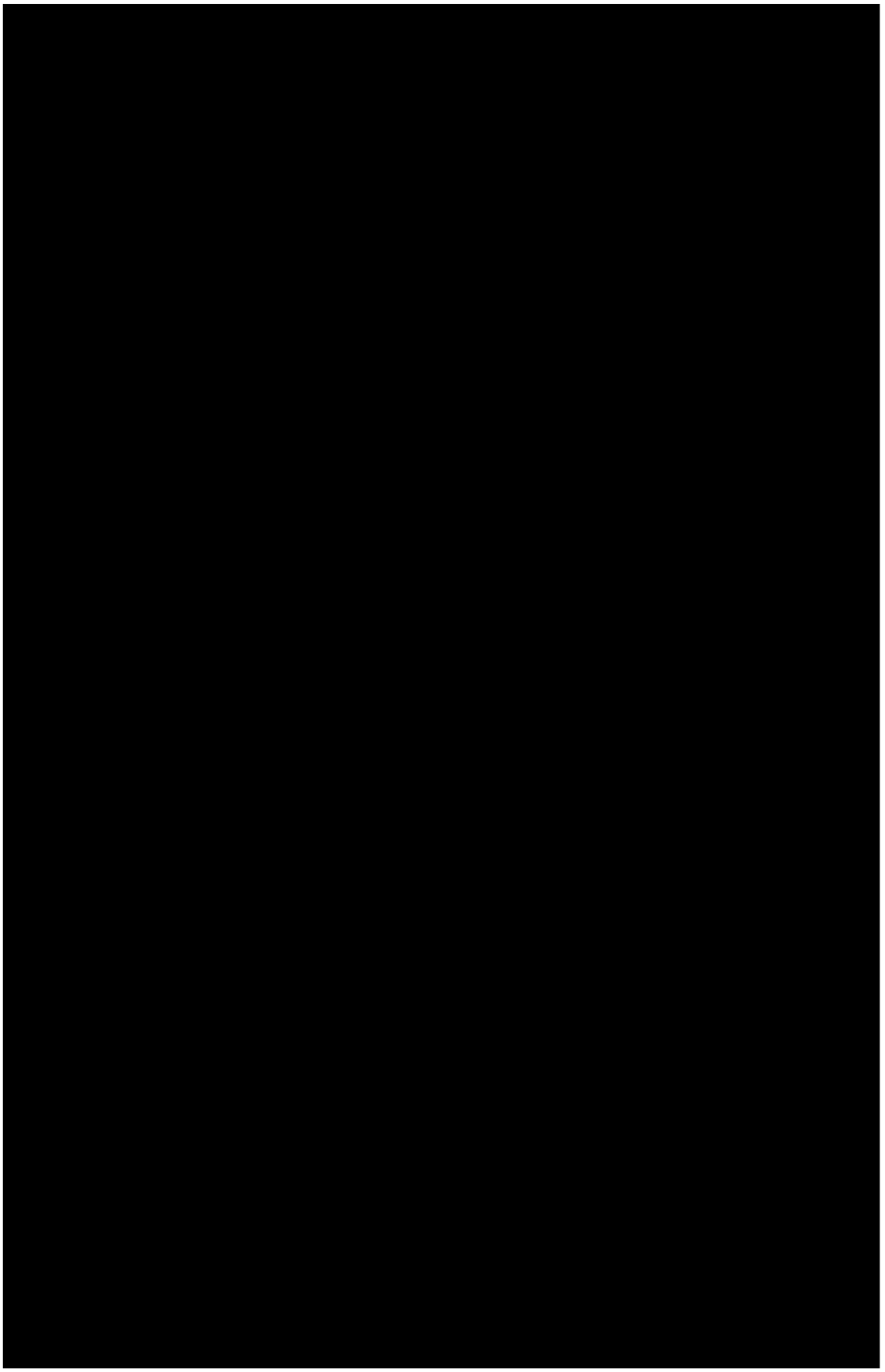

Fig. 2. Immunocytochemical localization of the *alpha*-1B AR in the iliac, mesenteric resistance, renal and superior mesenteric arteries. The experiments were carried out as described in the text. Left, presence of an affinity-purified *alpha*-1B AR antibody. Right, presence of preimmune serum alone.

TABLE 1

Mean fluorescence values obtained from laser scanning confocal microscopy with *alpha*-1B AR immunostaining

Artery	Mean fluorescence			
	Postimmune staining	*n*	Preimmune staining	*n*
	relative units		*relative units*	
Aorta	161 ± 9.9	3	131 ± 17.3	3
Caudal	50 ± 6.9	8	29 ± 3.6	7
Femoral	113 ± 7.8	5	94 ± 5.4	4
Iliac	80 ± 2.5	11	54 ± 6.4	7
Mesenteric resistance	136 ± 19.2	6	88 ± 18.3	6
Renal	137 ± 10.7	3	97 ± 12.1	5
Superior mesenteric	108 ± 11.9	6	62 ± 12.0	7

Each point represents the mean ± S.E.M. All postimmune values are statistically significantly different from preimmune staining as determined by *t* test for unpaired data.

with the high-affinity *alpha*-1D AR antagonist BMY 7378 have shown that the *alpha*-1D AR mediates the contraction of the aorta, iliac artery and skeletal muscle arterioles (Kenny *et al.*, 1995; Leech and Faber, 1996; Piascik *et al.*, 1995; Testa *et al.*, 1995) and is involved in the regulation of systemic arterial blood pressure (Zhou and Varga, 1996).

The role of the *alpha*-1B AR in mediating vascular smooth muscle contraction is less certain. Recently, Leech and Faber (1996) showed that the *alpha*-1B AR mediates constriction of skeletal muscle venules. Early studies with CEC suggested this receptor was involved in the contraction of several blood vessels (Minneman, 1988). However, it is now known that the *alpha*-1A and *alpha*-1D ARs can also be inactivated by CEC to varying degrees (Lomasney *et al.*, 1991; Perez *et al.*, 1991; Schwinn *et al.*, 1990). Therefore, this ligand cannot be considered a subtype-selective alkylating agent that can be used to define *alpha*-1B AR-mediated processes. Furthermore, the *alpha*-1B and *alpha*-1D ARs exhibit similar affinities for most ligands used for receptor characterization (Lomasney *et al.*, 1991; Perez *et al.*, 1991). We are not certain of the extent to which the *alpha*-1B AR mediates vascular smooth muscle contraction. For example, the *alpha*-1B AR was initially thought to regulate the contraction of the aorta (Han *et al.*, 1990; Piascik *et al.*, 1993). However, more recently, several groups of researchers have used BMY 7378 to demonstrate that aortic contraction is mediated by the *alpha*-1D AR (Kenny *et al.*, 1995; Piascik *et al.*, 1995; Testa *et al.*, 1995). Recently, a selective *alpha*-1B AR antagonist was synthesized (Giardina *et al.*, 1996) and may prove useful in dissecting the role of this receptor in the regulation of smooth muscle function.

mRNA for the *alpha*-1B AR is widely distributed in peripheral arteries (Piascik *et al.*, 1994; Guarino *et al.*, 1996). We

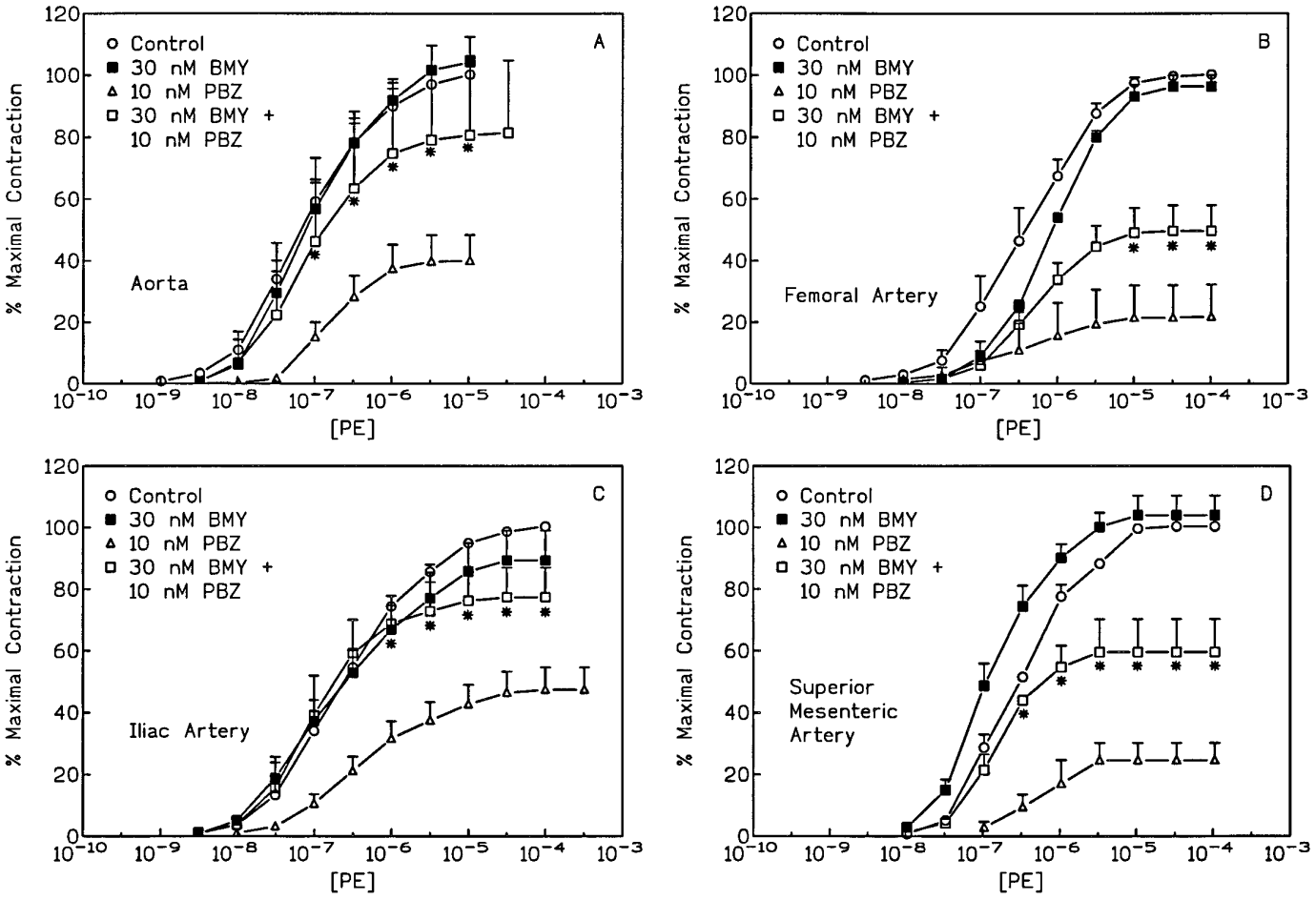

Fig. 3. Effect of BMY 7378 (30 nM) on the inhibitory actions of PBZ. Arteries were incubated in the presence of BMY 7378 alone, PBZ alone, both BMY 7378 and PBZ or vehicle, as detailed in the text. Each point represents the mean ± S.E.M. of eight individual experiments in the aorta, four in the femoral artery, eight in the iliac artery and two in the superior mesenteric artery. *, Statistically ($P > .05$) significant difference from the curve obtained in the presence of PBZ alone.

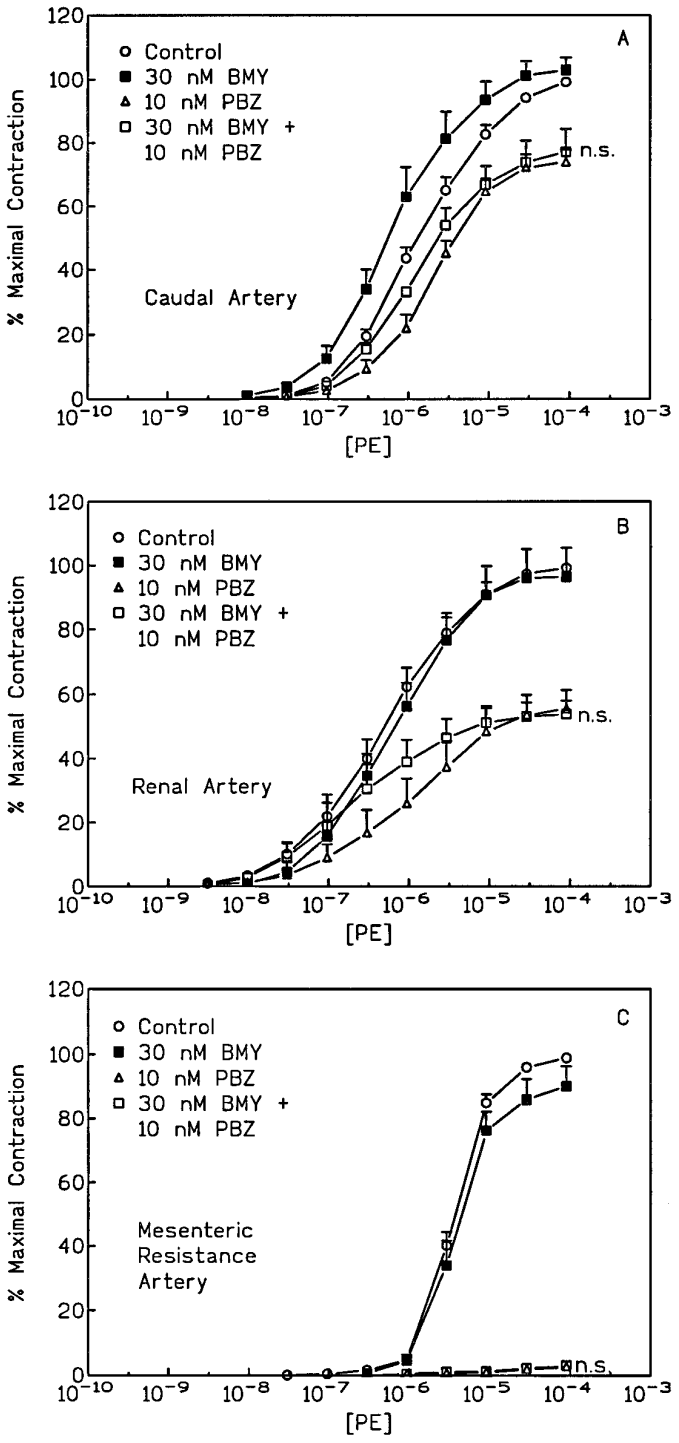

Fig. 4. Effect of BMY 7378 (30 nM) on the inhibitory actions of PBZ. Arteries were incubated in the presence of BMY 7378 alone, PBZ alone, both BMY 7378 and PBZ or vehicle, as detailed in the text. Each point represents the mean ± S.E.M. of three individual experiments in the caudal artery, four in the mesenteric resistance artery and six in the renal artery.

assessed the extent of protein expression of the *alpha*-1B AR with a subtype-selective antibody. We then used pharmacological methods and antisense oligonucleotides to characterize the extent to which the *alpha*-1B AR mediates smooth muscle contraction in the peripheral vasculature. A pluronic gel delivery system was used to apply the antisense oligonucleotides to arteries *in vivo*, and the effect on contractility was assessed 24 hr later. Pluronic gels are liquid at 4°C and solidify at room temperature (Schmolka, 1972). The gels have been used as a system to deliver antisense oligonucleotides into vascular smooth muscle *in vivo* (Bennett *et al.*, 1994; Simons *et al.*, 1992; Villa *et al.*, 1995). It has been shown that antisense c-*myb* oligonucleotides delivered with a pluronic gel inhibit smooth muscle cell growth after vascular injury (Simons *et al.*, 1992; Villa *et al.*, 1995). Our results indicate that the protein expression of an *alpha*-1D AR subtype on peripheral blood vessels cannot be taken as evidence that the receptor is involved in contractile regulation.

Methods

Animals. Male Sprague-Dawley rats weighing between 250 and 350 g were used in all experiments. The aorta, caudal, femoral, iliac, mesenteric resistance, renal and superior mesenteric arteries were removed and processed according to the experimental protocols described below.

Immunocytochemistry. After removal, the blood vessels were cleaned of extraneous tissue and cryoprotected by immersion in increasing amounts of sucrose in PBS (15% for 2 days, 30% for 2 days) at 4°C. The vessels were then placed in O.C.T. mounting media and quick-frozen (−50°C in isopentane cooled with dry ice); 13-μm sections were cut on a cryostat. Sections were placed on poly-L-lysine-coated, circularly etched slides and stored at −80°C until use. Sections were thawed, fixed in cold 4% paraformaldehyde for 10 min and then washed. These sections were incubated in blocking solution (PBS containing 10% normal goat serum, 1% bovine serum albumin and 0.1% Triton X-100) for 2 hr at room temperature and then incubated with *alpha*-1B AR antisera (1:100–150 dilution) for 24 hr at 4°C in the blocking solution without goat serum. Slides were then washed in PBS followed by application of an affinity-purified FITC-conjugated goat anti-rabbit IgG (1:200 dilution; Jackson Immunologicals, West Grove, PA) secondary antibody. After 1 hr at room temperature, the secondary antibody was removed by washing with PBS, and the slides were coverslipped with Vectashield (Vector Laboratories, Burlingame, CA) mounting media and then examined by fluorescence microscopy. The anti-receptor IgG, originally described by Fonseca *et al.* (1995), was affinity-purified against the immunizing peptide immobilized on a solid support matrix (Sulfolink; Pierce Chemicals, Rockford, IL). Preimmune IgG was prepared by fractionating rabbit serum over the same column.

Laser scanning confocal microscopic analysis of immunofluorescent images. Microscopic sections were placed on the stage of an inverted microscope and imaged using a laser-scanning confocal microscope (RCM 8000; Nikon, Melville, NY). FITC fluorescence was excited using the 488-nm wavelength output of an argon laser. Emitted fluorescence was collected using a Nikon 40× water-immersion objective (N.A. 1.15). Image quality was enhanced by averaging 64 scans/image. Images were immediately stored on an optical disk recorder as eight-bit, 512 × 483 pixel TIFF files. The files were later transferred to an 80486 microcomputer for off-line analysis using the program Metamorph (Universal Imaging, West Chester, PA). Fluorescence measurements were confined to the vascular smooth muscle regions between the highly autofluorescent elastin bands. This was facilitated by setting the analysis program threshold settings to exclude the bright elastin autofluorescence. The mean fluorescence in these defined regions was calculated for both preimmune and postimmune arterial sections. Differences in these mean fluorescent values were determined by a *t* test for unpaired data.

***In vitro* assessment of contractile function.** Isolated blood vessel segments were prepared by techniques routinely used in our laboratory (Piascik *et al.*, 1994, 1995). Arterial segments were removed and placed in a cold PSS of the following composition (in mM): NaCl, 130; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.17; $CaCl_2 \cdot 2H_2O$,

TABLE 2
Effect of BMY 7378 pretreatment on the inhibition of developed tension produced by 10 nM PBZ

Tissue	Developed tension				
	Control	BMY 7378 alone	PBZ	30 nM BMY 7378 + PBZ	100 nM BMY 7378 + PBZ
			g		
Aorta	1.25 ± 0.09	1.14 ± 0.19	0.65 ± 0.16	1.12 ± 0.18[a]	N.D.
Caudal	1.32 ± 0.17	1.27 ± 0.24	1.24 ± 0.16	1.33 ± 0.32	1.39 ± 0.22
Femoral	0.83 ± 0.08	0.87 ± 0.06	0.05 ± 0.01	0.25 ± 0.13[a]	0.5 ± 0.1[a]
Iliac	1.29 ± 0.17	0.82 ± 0.19	0.26 ± 0.12	0.47 ± 0.12[a]	0.63 ± 0.07[a]
Mesenteric resistance	1.15 ± 0.15	1.2 ± 0.04	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.02
Renal	0.73 ± 0.14	0.68 ± 0.11	0.29 ± 0.1	0.36 ± 0.05	0.38 ± 0.02
Superior Mesenteric	1.03 ± 0.21	0.94 ± 0.24	0.18 ± 0.02	0.5 ± 0.05[a]	0.62 ± 0.11[a]

[a] Statistically greater than the response to PBZ alone.
N.D., not determined.

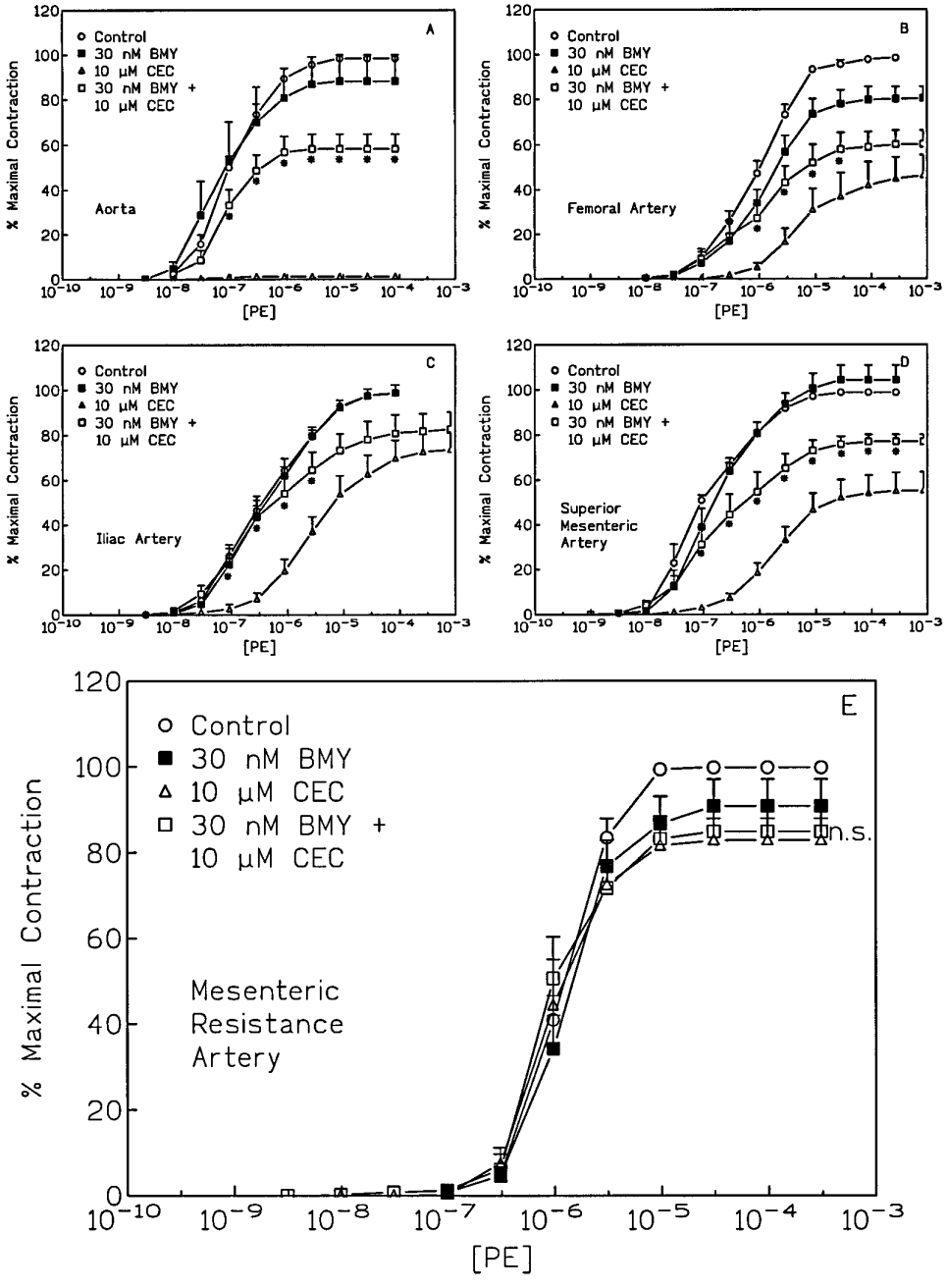

Fig. 5. Effect of BMY 7378 (30 nM) on the inhibitory actions of CEC. Arteries were incubated in the presence of BMY 7378 alone, CEC alone, both BMY 7378 and CEC or vehicle, as detailed in the text. Each point represents the mean ± S.E.M. of four individual experiments in the aorta, six in the femoral artery, seven in the iliac artery, four in the mesenteric resistance artery and nine in the superior mesenteric artery. *, Statistically ($P > .05$) significant difference from the curve obtained in the presence of CEC alone.

TABLE 3
Effect of BMY 7378 pretreatment on the inhibition of developed tension produced by 10 μM CEC

Tissue	Developed tension				
	Control alone	BMY 7378 alone	CEC alone	30 nM BMY 7378 + CEC	100 nM BMY 7378 + CEC
			g		
Aorta	1.18 ± 0.12	1.08 ± 0.26	0.3 ± 0.02	0.73 ± 0.18[a]	N.D.
Femoral	0.73 ± 0.12	0.99 ± 0.18	0.53 ± 0.13	0.66 ± 0.05[a]	0.65 ± 0.03[a]
Iliac	1.35 ± 0.08	1.14 ± 0.1	0.69 ± 0.06	0.86 ± 0.08[a]	1.11 ± 0.19[a]
Mesenteric resistance	1.64 ± 0.13	1.59 ± 0.2	1.26 ± 0.12	1.21 ± 0.07	1.18 ± 0.05
Superior Mesenteric	0.85 ± 0.19	0.93 ± 0.17	0.55 ± 0.08	0.7 ± 0.07[a]	1.2 ± 0.09[a]

[a] Statistically greater response than CEC alone.
N.D., not determined.

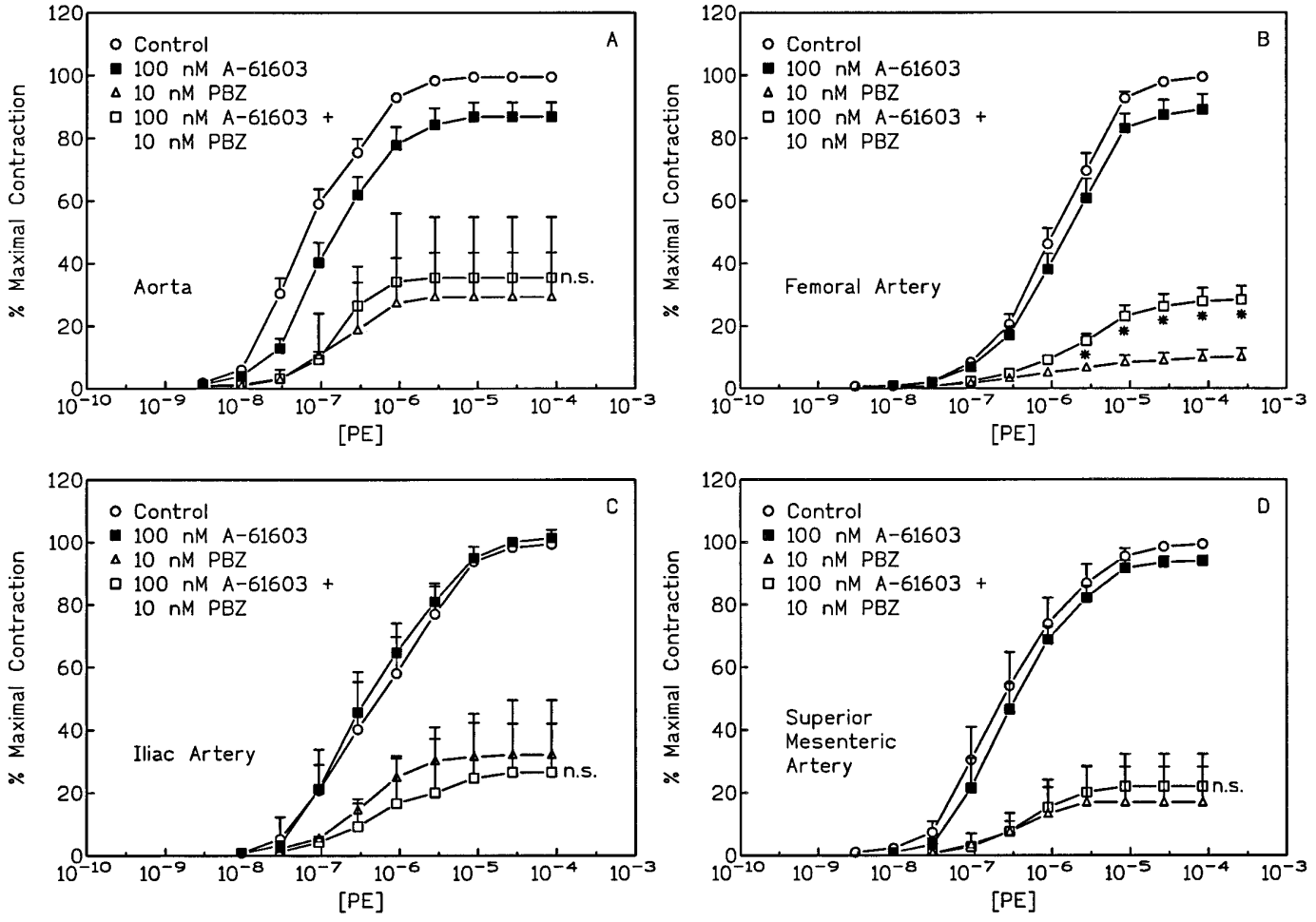

Fig. 6. Effect of A-61603 (100 nM) on the inhibitory actions of PBZ. Arteries were incubated in the presence of A-61603 alone, PBZ alone, both A-61603 and PBZ or vehicle, as detailed in the text. Each point represents the mean ± S.E.M. of eight individual experiments in the aorta, 10 in the femoral artery, two in the iliac artery and two in the superior mesenteric artery.

1.6; $NaHCO_3$, 14. 9; dextrose, 5.5 and Na_2 EDTA, 0.03. Three-millimeter ring segments of aorta, caudal artery, femoral artery, iliac artery, renal artery, superior mesenteric artery and mesenteric resistance vessel (these were cut to 2 mm; ~150–200-μm I.D.) were cut and cleaned of surrounding fat and connective tissue. The mesenteric resistance artery was isolated in the following manner: After removal of the entire mesenteric bed from its primary branching point at the abdominal aorta to the terminal arteriolar/capillary connections at the intestinal wall, a second-generation branch was isolated, cut free and cleaned. Stainless steel or platinum wires of an appropriate diameter were threaded through the lumen of each vessel. One wire was connected to a fixed base and the other to a micrometer clamp to adjust passive force on the tissues. The tissues were mounted in water-jacketed muscle baths containing PSS maintained at 37°C under constant oxygenation (95% O_2/5% CO_2, pH 7.4). Passive forces of $2 \times g$ (aorta), $0.25 \times g$ (mesenteric resistance artery) or $1 \times g$ (all other arteries) were then placed on the vessels. Previous studies have shown that these values for passive force give optimal agonist responses. Changes in force generation were recorded using Grass FT.03 force transducers connected to a Grass model 7 polygraph.

Protection experiments. Cumulative concentration-response curves to phenylephrine (1.0 nM to 100 μM) were generated in all vessels. The tissues were then washed repeatedly for a period of 1 hr

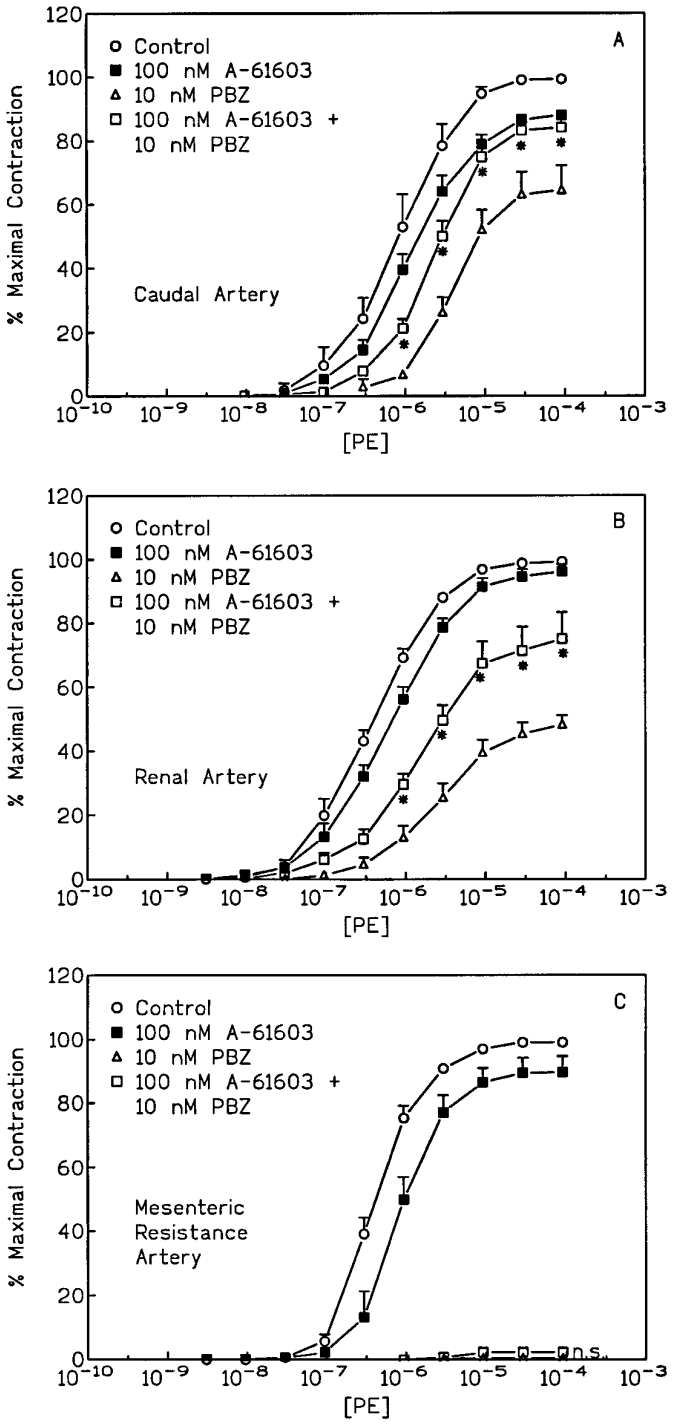

Fig. 7. Effect of A-61603 (100 nM) on the inhibitory actions of PBZ. Arteries were incubated in the presence of A-61603 alone, PBZ alone, both A-61603 and PBZ or vehicle, as detailed in the text. Each point represents the mean ± S.E.M. of four individual experiments in the caudal artery, eight in the renal artery and four in the mesenteric resistance artery. *, Statistically ($P > .05$) significant difference from the curve obtained in the presence of PBZ alone.

to allow the vessels to restabilize at their resting levels of passive force. Pretreatment with protecting agents was carried out as follows: BMY 7378 (30 and 100 nM), A-61603 (100 nM) or vehicle was incubated with the blood vessel segments for 20 min. PBZ (10 nM) or CEC (10 μM) was then added and coincubated in the presence of protecting agent or vehicle for an additional period (10 min for PBZ, 30 min for CEC), after which both ligands were extensively washed from the arteries for 2 hr. Base-line tension was reestablished, and a second phenylephrine concentration-response curve was generated. To determine time-related changes in sensitivity, control rings were run that received only vehicle treatment followed by the washout period. ED_{50} values for the two phenylephrine curves generated in these control vessels were not significantly different. Concentration-response curves are expressed as a percentage of the maximal response obtained in the first phenylephrine concentration-response relationship. Protection from irreversible inactivation was defined as a statistically significant increase in the maximal response to phenylephrine obtained after treatment with a protecting agent compared with the maximal response obtained in the presence of PBZ or CEC alone. A-61603, the optically pure (*R*)-isomer, was a gift from Abbott Laboratories (Abbott Park, IL) and provided to us by Dr. Michael Meyer.

Application of oligonucleotides. Sense and antisense phosphorothioate oligonucleotides were synthesized by Dr. Dianne Perez (Department of Molecular Cardiology, Research Foundation, The Cleveland Clinic). The phosphorothioate backbone makes these nucleotides resistant to breakdown by nucleases. Fluorescently labeled analogs of these oligonucleotides were also made. The sequences for these oligonucleotides are sense, ACC*ATG*GTGCTTCTC AAA*ATG*-AATCCCGAT GAG*ATG*ACTTTCCGA, and antisense, AGAGAGAA-GCACCAT CAGATCGGGATTCAT GTCTCGGAAAGTCAT. The oligonucleotides were received as a dried pellet, resuspended in sterile water, and the concentration was determined spectrophotometrically.

***In vivo* transfection.** A 40% w/v solution of pluronic F-127 gel (Sigma Chemical, St. Louis, MO) was prepared in water at 4°C by mixing on a platform shaker overnight in a cold room. F-127 pluronic gel solutions, which are liquid at 4°C, solidify at room temperature (Schmolka, 1972). Sufficient oligonucleotide was added to the cold gel solution to give the desired concentration (see below). The addition of oligonucleotide reduces the pluronic gel concentration to 30%. Care was taken to ensure that all pipette tips and storage tubes were kept at 4°C. Animals were anesthetized with a mixture of ketamine and acepromazine. After a surgical incision, the femoral or renal artery was located and gently cleaned of adhering tissue. Forty microliters of the pluronic gel/oligonucleotide solution were applied around the artery. After the gel solidified around the artery, it was gently placed back into its original position, and the wound was closed with surgical staples. The animal was then allowed to recover until use in experimentation. To assess the degree of uptake of the oligonucleotide by the smooth muscle, an FITC-labeled oligonucleotide was applied in the gel. The artery was removed 24 hr later and prepared for fluorescence microscopy.

Statistical analysis. Concentration-response curves represent the mean value of a minimum of two experiments on individual blood vessel segments, of which each was from a different animal. Only two concentration-response curves were run on any segment. A two-way analysis of variance followed by Student-Newman-Kuels analysis was used to determine where statistically significant differences existed between the various treatment conditions. In all figures, data are given as mean ± S.E.M. Asterisks indicate statistically significant difference at the $P < .05$ level.

Results

mRNA for the *alpha*-1B AR is widely distributed in the peripheral vascular system (Guarino *et al.*, 1996; Piascik *et al.*, 1995). An anti-peptide antibody prepared against the *alpha*-1B AR (Fonseca *et al.*, 1995) was used to determine the extent to which this mRNA is expressed as receptor protein in a series of peripheral blood vessels. Photomicrographs of the immunofluorescent staining in the aorta, caudal, femoral, iliac, mesenteric resistance, renal and superior mesen-

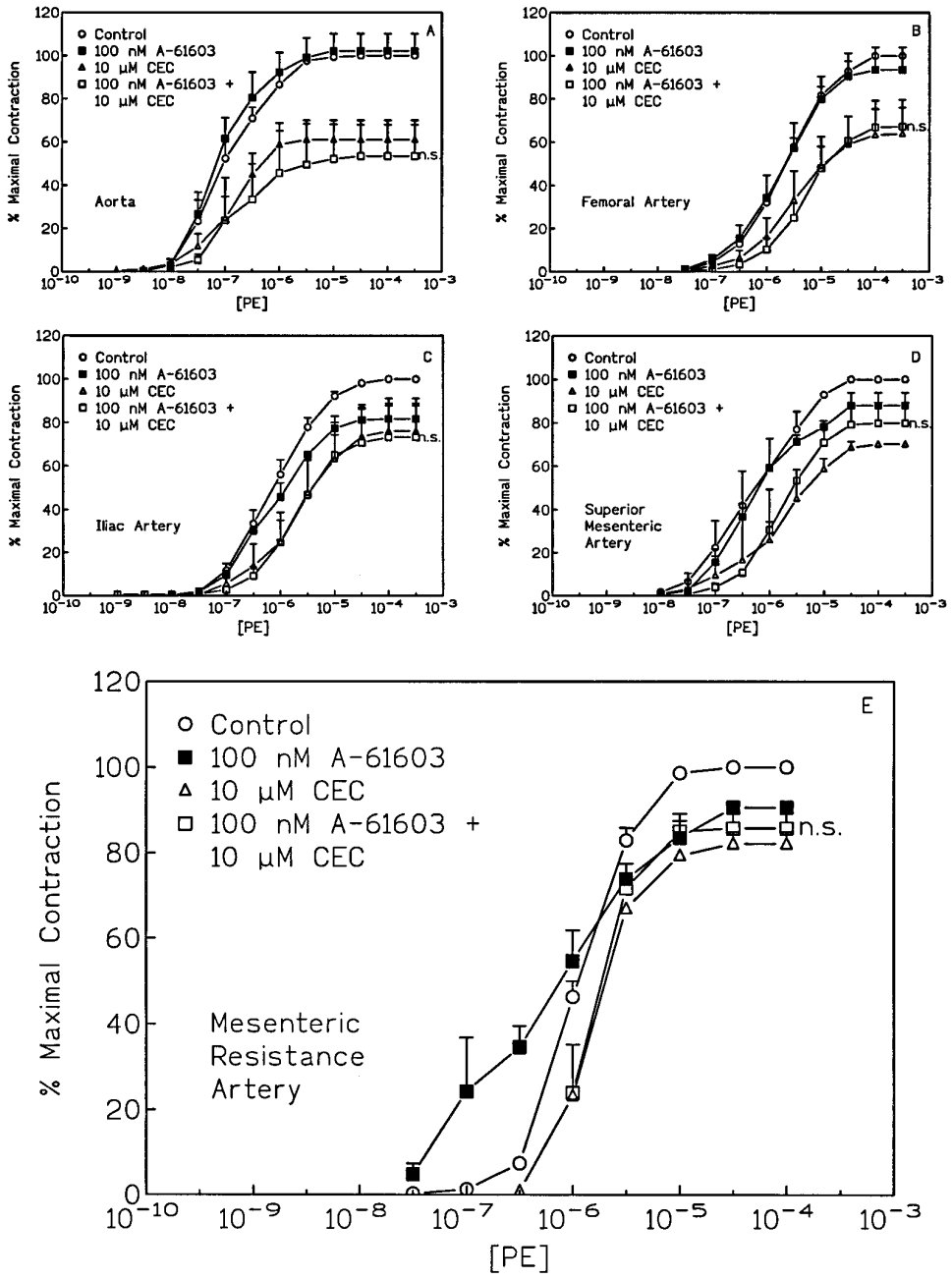

Fig. 8. Effect of A-61603 (100 nM) on the inhibitory actions of CEC. Arteries were incubated in the presence of A-61603 alone, CEC alone, both A-61603 and CEC or vehicle, as detailed in the text. Each point represents the mean ± S.E.M. of three individual experiments in the aorta, four in the femoral artery, three in the iliac artery, two in the superior mesenteric artery and four in the mesenteric resistance artery.

TABLE 4
Effect of A-61603 pretreatment on the inhibition of developed tension produced by 10 nM PBZ

Tissue	Developed tension			
	Control	A-61603 alone	PBZ alone	A-61603 + PBZ
	g			
Aorta	1.21 ± 0.26	0.86 ± 0.12	0.27 ± 0.08	0.4 ± 0.12
Caudal	1.37 ± 0.28	1.47 ± 0.14	0.69 ± 0.12	1.29 ± 0.17[a]
Femoral	1.18 ± 0.11	1.03 ± 0.01	0.12 ± 0.03	0.35 ± 0.06[a]
Iliac	1.11 ± 0.02	1.25 ± 0.35	0.31 ± 0.08	0.35 ± 0.035
Mesenteric resistance	1.85 ± 0.17	1.73 ± 1.8	0.02 ± 0.01	0.04 ± 0.01
Renal	0.95 ± 0.13	1.04 ± 0.1	0.52 ± 0.6	0.7 ± 0.09[a]
Superior Mesenteric	0.88 ± 0.06	1.05 ± 0.09	0.13 ± 0.09	0.2 ± 0.11

[a] Statistically greater response than PBZ alone.

TABLE 5
Effect of A-61603 pretreatment on the inhibition of developed tension produced by 10 μM CEC

Tissue	Developed tension			
	Control	A-61603 alone	CEC alone	A-61603 + CEC
		g		
Aorta	2.03 ± 0.9	1.97 ± 0.27	1.13 ± 0.14	0.9 ± 0.32
Femoral	1.03 ± 0.1	0.98 ± 0.18	0.82 ± 0.18	0.78 ± 0.15
Iliac	1.51 ± 0.24	1.11 ± 0.11	0.97 ± 0.23	0.95 ± 0.16
Mesenteric resistance	1.7 ± 0.13	1.04 ± 0.43	1.44 ± 0.44	1.18 ± 0.25
Superior mesenteric	1.45 ± 0.21	1.64 ± 0.3	1.05 ± 0.67	1.13 ± 0.34

[a] Statistically greater response than CEC alone.

TABLE 6
Summary of protection experiments

Artery	PBZ		CEC		Postulated regulatory *alpha*-1 AR
	BMY	A-61603	BMY	A-61603	
Aorta	+	−	+	−	D
Caudal	−	+	−	N.D.	A
Femoral	+	+	+	−	D
Iliac	+	−	+	−	D
Mesenteric resistance	−	−	−	−	B
Renal	−	+	−	N.D.	A
Superior mesenteric	+	−	+	−	D

+, Statistically significant protection from inactivation by PBZ or CEC; −, no significant protection; N.D., not determined.

teric arteries are presented in figures 1 and 2. An intense immunofluorescent staining was obtained in the medial layer of all blood vessels examined. The fluorescent signal obtained in the tissue exposed to secondary antibody alone was much less than that obtained in the presence of primary *alpha*-1B AR antibody (see figs. 1 and 2). The immunofluorescence signal was quantified with laser scanning confocal microscopy; these data are summarized in table 1. In all cases, the mean fluorescence obtained in the presence of the *alpha*-1B AR antibody was statistically significantly greater than that seen with preimmune serum. Preincubation of the antibody with the immunizing peptide used to generate the antisera significantly reduced *alpha*-1B AR immunoreactivity (data not shown). In contrast, incubation of the *alpha*-1B AR antibody with an immunizing peptide used to generate an *alpha*-1D AR antiserum had no effect on *alpha*-1B AR immunoreactivity (data not shown).

We assessed the effect of PBZ or CEC on vascular smooth muscle contraction alone and after coincubation with either the *alpha*-1D AR-selective antagonist BMY 7378 or the *alpha*-1A AR-selective agonist A-61603 (Knepper *et al.*, 1995). BMY 7378 and A-61603 at the appropriate concentrations would be expected to bind to and protect the *alpha*-1 AR at which they have the highest affinity, whereas the other subtypes would be inactivated by the alkylating agents. PBZ significantly inhibited the spasmogenic response to phenylephrine in all blood vessels examined (figs. 3 and 4). The degree of inhibition differed, with the mesenteric resistance artery being the most sensitive and the caudal being the least sensitive. Incubation with 30 nM BMY 7378 alone resulted in only minimal inhibitory action remaining after the 2-hr washout period. When coincubated in the presence of 30 nM BMY 7378, the inhibitory actions of PBZ were significantly reduced in the aorta, femoral, iliac and superior mesenteric arteries (fig. 3). In contrast, BMY 7378 had no effect on the antagonist actions of PBZ in the caudal, mesenteric resistance or renal arteries (fig. 4). Incubation of 100 nM BMY 7378 produced a greater level of protection in the aorta, femoral, iliac and superior mesenteric arteries, whereas no protective action was noted at this higher dose in the caudal, mesenteric resistance or renal arteries. The maximal level of the contractile response (in grams) for phenylephrine in the various treatment conditions is presented in table 2. The selective protection exerted by BMY 7378 in the aorta, femoral, iliac and superior mesenteric arteries suggests that the phenylephrine response is due to activation of the *alpha*-1D AR.

If this suggestion is correct, then we would expect similar results with CEC. Previous work has shown that neither the caudal nor renal arteries are sensitive to CEC inactivation (Han *et al.*, 1990; Piascik *et al.*, 1995). Therefore, these arteries were not examined in this series of studies. CEC alone produced varying degrees of inhibition in the test arteries. BMY 7378 (30 nM) protected the aorta, femoral, iliac and superior mesenteric arteries from CEC inactivation (fig. 5). Increasing the BMY 7378 concentration to 100 nM produced a greater level of inhibition than that observed at 30 nM. In the presence of either 30 or 100 nM BMY 7378, no protection was noted in the mesenteric resistance artery. The maximal contractile response seen in the various CEC treatment conditions is presented in table 3.

A-61603 is an agonist that has been shown to exhibit a high degree of selectivity for the *alpha*-1A AR (Knepper *et al.*, 1995). At 100 nM, A-61603 had no effect on the inhibitory actions of PBZ in the aorta, iliac or superior mesenteric artery (fig. 6). In contrast, the ligand significantly protected the caudal and renal arteries from inactivation (fig. 7). A modest degree of protection was also noted in the femoral artery (fig. 6). A-61603 had no effect on PBZ inhibition of the mesenteric resistance artery. The fact that this *alpha*-1A AR-selective ligand protected the caudal and renal arteries is consistent with the idea that these arteries are regulated by the *alpha*-1A AR. The protective action in the femoral artery was surprising. The significance of this observation is dealt with in the Discussion.

In results similar to those obtained with PBZ, A-61603 had no effect on the inhibitory actions of CEC in the aorta, femoral, iliac, superior mesenteric and mesenteric resistance arteries (fig. 8). A summary of the level of maximal response for all experiments with A-61603 is presented in tables 4 and 5. A summary of all the protection data and our predictions regarding which receptor is involved in contractile regulation can be found in table 6.

These data indicate that there is limited involvement of the

alpha-1B AR in regulating contraction of the blood vessels examined in this study. To further address this possibility, a novel approach with antisense oligonucleotides was used. We chose the femoral and renal arteries for these studies. The pluronic gel/antisense oligonucleotide mixture can be easily applied to these vessels. To demonstrate that this application technique does in fact deliver antisense oligonucleotides to smooth muscle cells, 150 μM of an FITC-labeled *alpha*-1D AR antisense oligonucleotide was applied to the femoral artery. Twenty-four hours later, an intense fluorescent signal was detected in the medial layer, indicating significant penetration of the oligonucleotide into the arterial smooth muscle (fig. 9). In a similar fashion, application of 250 μM FITC-labeled *alpha*-1A AR antisense oligonucleotide resulted in an intense fluorescence signal detected in the medial layer of the renal artery (data not shown). The application of a nonfluorescent phosphorothioate *alpha*-1D AR antisense oligonucleotide to the femoral artery also had no effect on the response of the artery to KCl or serotonin (fig. 10). Furthermore, an *alpha*-1D AR sense oligonucleotide had no effect on phenylephrine-induced contraction of the femoral artery (fig. 11B).

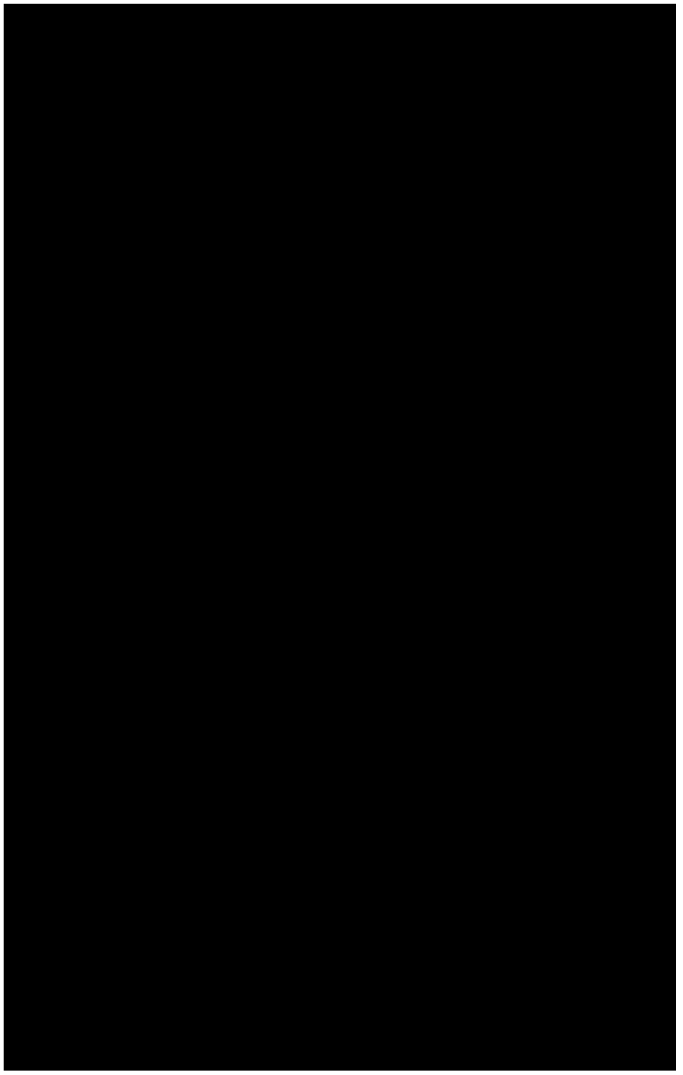

Fig. 9. Uptake of a fluorescent *alpha*-1D AR antisense oligonucleotide in the femoral artery. The oligonucleotide was applied in a F-127 pluronic gel as described in the text. Twenty four hours later, the artery was removed and prepared for microscopy.

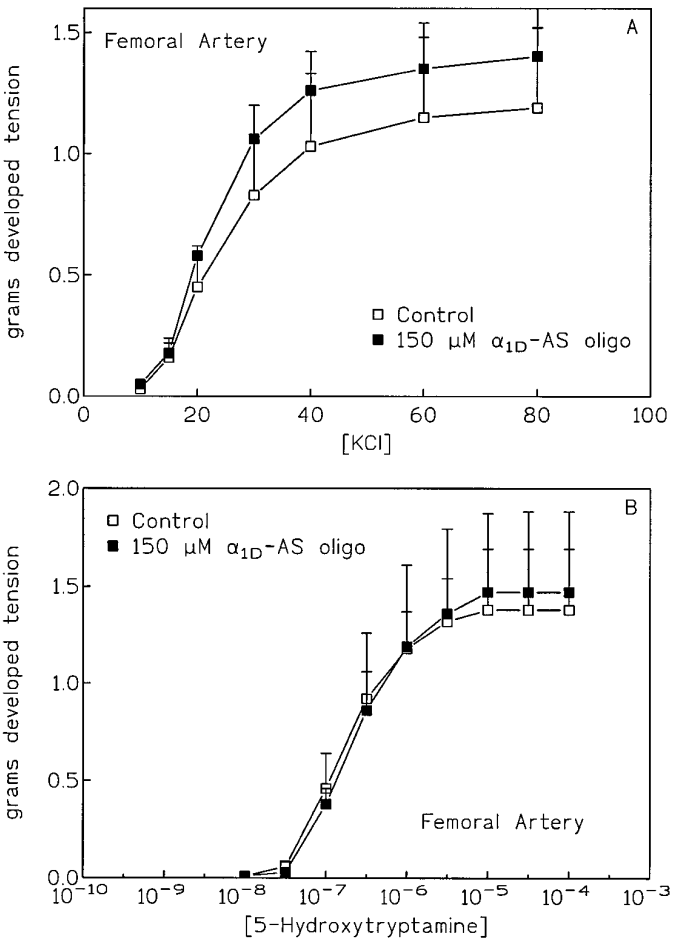

Fig. 10. Effect of antisense *alpha*-1D AR oligonucleotides on the contractile actions of KCl and serotonin. Application of oligonucleotides and contractile function were carried out as described in the text. Each point represents the mean ± S.E.M. of two individual experiments, each of which contained two femoral artery ring segments.

In contrast, an antisense oligonucleotide directed against the translational start site of the *alpha*-1D AR significantly inhibited the femoral artery response to phenylephrine (fig. 11A). The application of 150 μM of an antisense oligonucleotide directed against the translational start site of the *alpha*-1B AR had no effect on the phenylephrine response in the femoral artery (fig. 11D). A similar result was observed when *alpha*-1A AR antisense oligonucleotides were applied to the femoral artery (fig. 11C).

When antisense oligonucleotides directed against the translational start site for the *alpha*-1B AR were applied to the renal artery, no inhibition of the phenylephrine response was observed (fig. 12C). The application of an antisense oligonucleotide directed against the *alpha*-1D AR also had no effect (fig. 12D). Similarly, sense oligonucleotides against the *alpha*-1A AR had no effect on the contractile actions of phenylephrine (fig. 12B). However, treatment with an antisense oligonucleotide directed against the translational start site of the *alpha*-1A AR significantly inhibited the response to phenylephrine in the renal artery (fig. 12A).

The inhibition of contraction produced by either antisense oligonucleotide was concentration dependent, with higher concentrations producing a greater level of inhibition than that observed at lower concentrations (fig. 13).

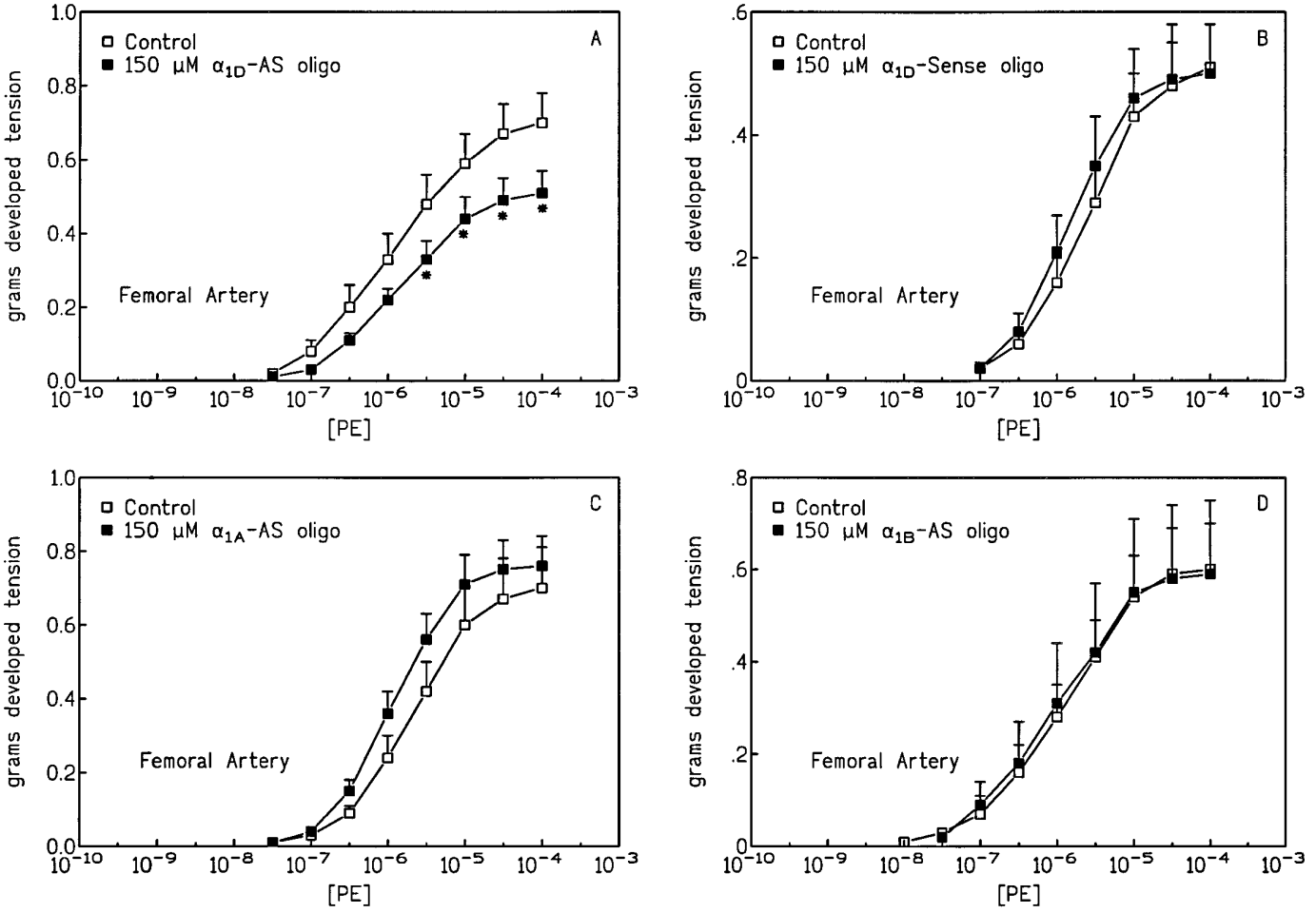

Fig. 11. Effect of a variety of oligonucleotides on the contractile action of phenylephrine in the femoral artery. The femoral artery was transfected with the indicated oligonucleotide and studied 24 hours later as described in the text. Each point represents the mean ± S.E.M. of 15, 30, 14 or 10 individual rings for the *alpha*-1D sense, *alpha*-1D antisense, *alpha*-1B antisense and *alpha*-1A antisense, respectively. *, Statistically significant difference from the control dose-response curve.

Discussion

Despite many significant advances in the regulatory biology of the *alpha*-1D AR subtypes, we have at best an incomplete understanding of the role played by these receptors in modulating physiological processes such as vascular smooth muscle contraction. Of the known *alpha*-1 ARs, *alpha*-1A and *alpha*-1D have most often been implicated in playing a role in regulating vascular smooth muscle tone (Guarino *et al.*, 1996). In this report, we analyzed the contribution of the *alpha*-1B AR to the contraction of a series of peripheral arteries. mRNA for this receptor is widely distributed in peripheral arteries (Guarino *et al.*, 1996). In terms of the level of mRNA expression for the entire *alpha*-1 AR subtype pool, the *alpha*-1B AR is expressed at much lower levels than the *alpha*-1A AR but at higher levels than the *alpha*-1D AR (Guarino *et al.*, 1996; Piascik *et al.*, 1995).

Using an antipeptide antibody, we detected specific *alpha*-1B AR immunoreactivity in the medial layer of all blood vessels examined. This indicates that in all arteries in which we can detect its mRNA, the *alpha*-1B AR is also expressed as protein. It is not known whether the expression of this receptor on vascular smooth muscle cells *a priori* indicates that the subtype plays a role in contraction or whether the *alpha*-1B AR can be expressed on blood vessels and not subserve a contractile function.

Classic and contemporary pharmacological approaches were used to determine the contribution of the *alpha*-1B AR to the contraction of the blood vessels under study. In the first series of experiments, we assessed the ability of subtype-selective ligands (BMY 7378 and A-61603) to protect a specific *alpha*-1D AR from inactivation by PBZ and CEC. The dissociation constant of BMY 7378 estimated in binding studies with a recombinant form of the *alpha*-1D AR or in functional studies is 0.9 to 4 nM (Goetz *et al.*, 1995; Piascik *et al.*, 1995). The dissociation constant for either the *alpha*-1A or the *alpha*-1B AR is >500 nM (Goetz *et al.*, 1995; Piascik *et al.*, 1995). Therefore, at 30 nM, BMY 7378 would occupy >90% of the *alpha*-1D population while occupying <6% of either the *alpha*-1A or *alpha*-1B ARs. BMY 7378 protected the aorta, femoral, iliac and superior mesenteric arteries from inactivation by either PBZ or CEC, supporting our contention that the main *alpha*-1 AR subtype involved in the contractile regulation of these vessels is the *alpha*-1D AR.

The lack of complete protection seen with BMY 7378 could be taken as evidence that in addition to the *alpha*-1D, other *alpha*-1 AR subtypes are involved in the contractile regula-

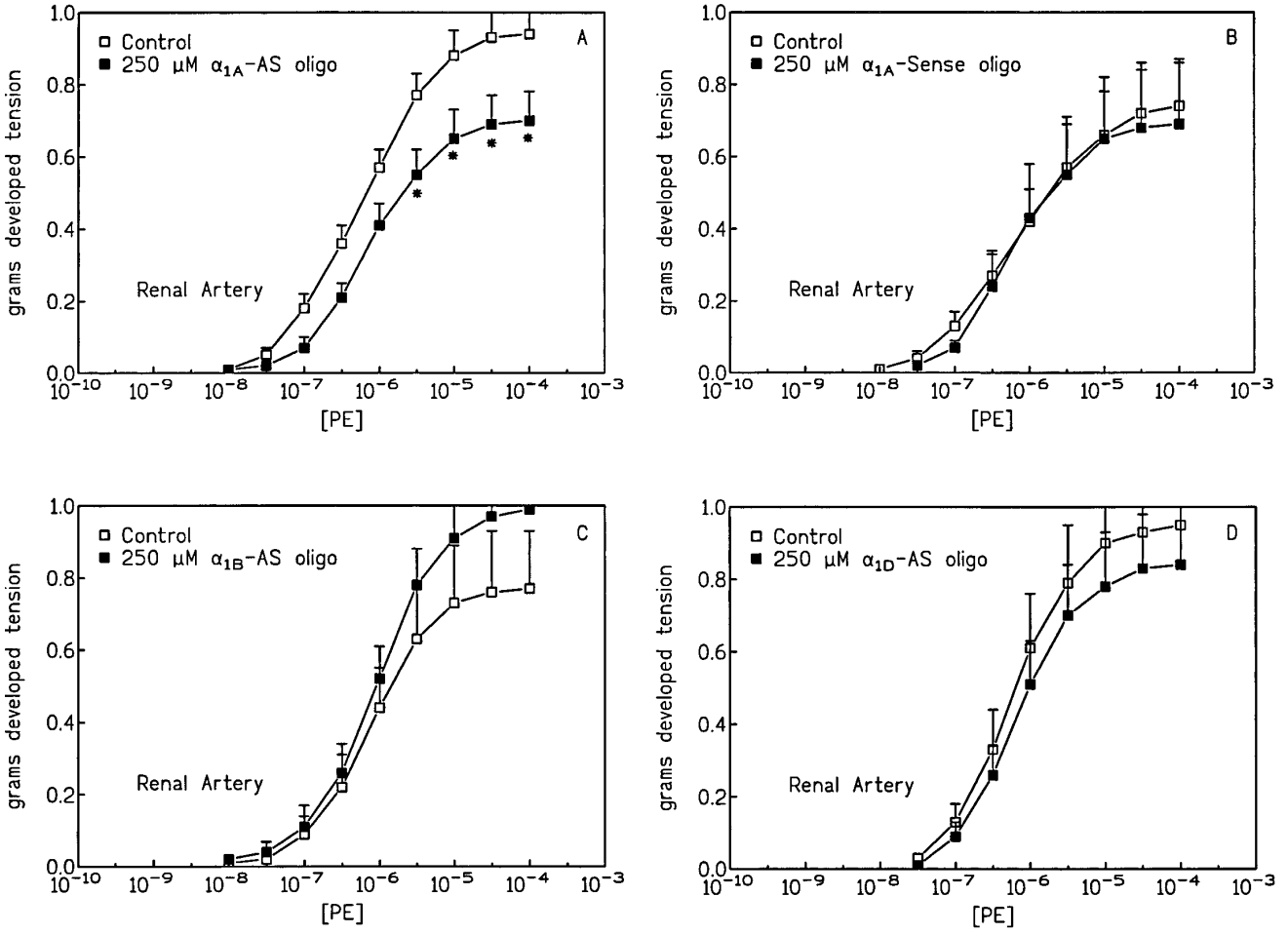

Fig. 12. Effect of a variety of oligonucleotides on the contractile action of phenylephrine in the renal artery. The renal artery was transfected with the indicated oligonucleotide and studied 24 hours later as described in the text. Each point represents the mean ± S.E.M. of eight, 16, six and eight individual rings for the *alpha*-1A sense, *alpha*-1A antisense, *alpha*-1B antisense and *alpha*-1D antisense, respectively. *, Statistically significant difference from the control dose-response curve.

tion of the aorta, femoral, iliac and superior mesenteric arteries. However, there are alternative explanations for the lack of complete protection. There is a dynamic interaction between the ligands and the receptors in these protection experiments. BMY 7378 is added first in concentrations sufficient to nearly saturate the *alpha*-1D AR but have little occupancy at either the *alpha*-1A or *alpha*-1B ARs. The ligand freely dissociates and reassociates with the receptor. When PBZ or CEC is added, it will inactivate the unprotected *alpha*-1A or *alpha*-1B ARs as well as compete with BMY 7378 for the *alpha*-1D AR. Once the alkylating agent acquires a receptor and covalent modification occurs, no dissociation from the receptor will take place. Therefore, the presence of BMY 7378 should be expected to retard, not prohibit receptor inactivation. Indeed, if the incubation period is sufficiently long, either alkylating agent would be expected to completely wipe out the *alpha*-1D AR population. The way to enhance the degree of protection is to use very high concentrations of the protecting agent. This is not practical in this instance because as the concentration of BMY 7378 increases, the chances of *alpha*-1A or *alpha*-1B AR occupation also increase. Therefore, in these experiments, complete receptor protection was difficult to achieve. It should be noted that when the BMY 7378 concentration was increased to 100 nM, there was a greater level of protection in the aorta, femoral, iliac and superior mesenteric arteries without any protection noted in the other arteries (tables 2 and 4). Finally, the fact that two different alkylating agents gave the same results further strengthens the conclusions made with these studies.

In logic similar to that discussed above, we used the *alpha*-1A AR-selective agonist A-61603 in protection studies with PBZ. It has an estimated dissociation constant at the cloned *alpha*-1A AR of 30 nM. Therefore, at 100 nM A-61603, 80% of the *alpha*-1A ARs would be occupied, whereas only 9% and 7% of the *alpha*-1B and *alpha*-1D AR populations, respectively, would be occupied by the agonist. A-61603 showed protective activity in the caudal and renal arteries. This indicates that the *alpha*-1A AR is the major regulatory receptor in these arteries and agrees with previous work using other approaches (Han *et al.*, 1990).

The protective action of A-61603 in the femoral artery was surprising and not consistent with other results discussed above and below. The modest degree of protection could indicate that a component of the response in the femoral artery is mediated by the *alpha*-1A AR. In contrast, protection was not seen when A-61603 was used against inactivation by CEC. This would argue against a role for the *alpha*-1A AR in

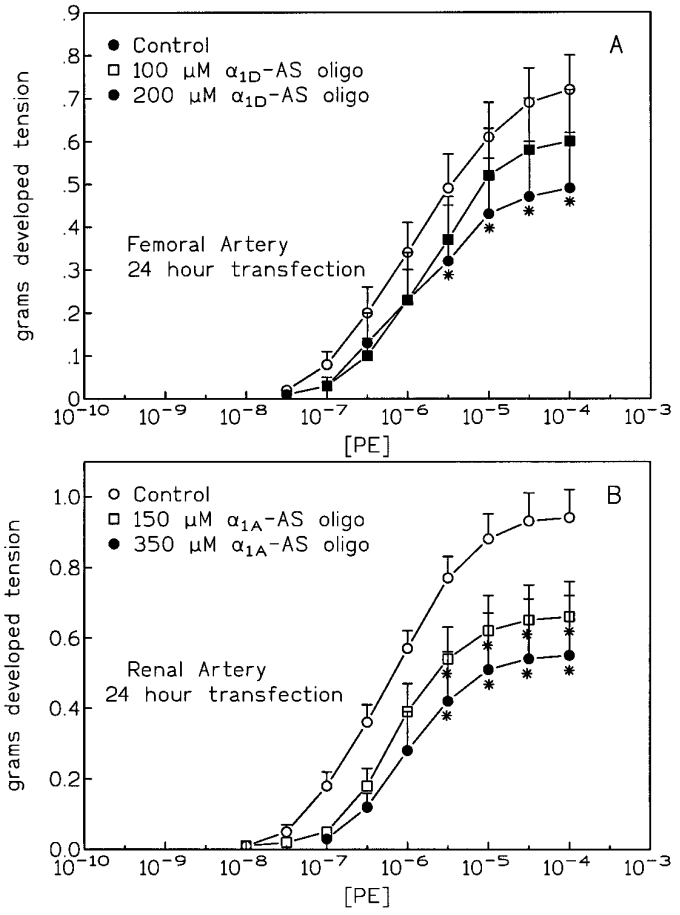

Fig. 13. Effect of various concentrations of *alpha*-1A and *alpha*-1D AR antisense oligonucleotides on the response of the femoral (A) and renal (B) artery. Each point represents the mean ± S.E.M. of 31, eight, four, 16, eight and four individual rings for the control in the femoral, 100 μM *alpha*-1D antisense, 200 μM *alpha*-1D antisense, control in the renal, 150 μM *alpha*-1A antisense, 350 μM *alpha*-1A antisense, respectively. *, Statistically significant difference from the control dose-response curve.

regulating the femoral artery contraction. Furthermore, application of antisense oligonucleotides against the *alpha*-1A AR to the femoral artery also had no effect on phenylephrine-induced femoral artery contraction.

We appreciate that these types of experiments lead to indirect conclusions regarding *alpha*-1 AR regulation. However, certain hypotheses can be made and tested with antisense oligonucleotides. In control experiments, we demonstrated that a fluorescently labeled antisense oligonucleotide can be taken up and retained by either the femoral or renal arteries 24 hr after application in a pluronic gel (fig. 9). We are in the process of quantifying the extent of oligonucleotide uptake using laser scanning confocal microscopy. Our preliminary results indicate that oligonucleotide uptake is very inefficient. The application of 150 μM to the outside of the vessel in pluronic gel yields an estimated 4 μM intracellularly. The surgical procedure and application of antisense directed against the *alpha*-1D AR did not affect the contractile actions of KCl or serotonin. This indicates that neither our application technique nor the mere presence of a 15-residue oligonucleotide accounts for the inhibitory actions observed. If the predictions from the protection studies are correct, then application of antisense constructs directed against the *alpha*-1A or *alpha*-1B AR should have no effect on the response of the femoral artery to phenylephrine. This is exactly what was observed. In contrast, treatment with *alpha*-1D AR antisense oligonucleotides significantly decreased the femoral artery response to phenylephrine. These data support the conclusion that the alpha-1D but not the *alpha*-1A or *alpha*-1B ARs are involved in mediating the contraction of the femoral artery.

In the renal artery, application of antisense oligonucleotides directed against the start site for translation of the *alpha*-1B AR also had no effect on phenylephrine-induced smooth muscle contraction. A similar effect was noted for *alpha*-1D AR antisense oligonucleotides. The application of an *alpha*-1A AR sense oligonucleotide also had no effect on phenylephrine responsiveness. In contrast, the application of an *alpha*-1A AR antisense oligonucleotide significantly inhibited the phenylephrine response. Therefore, the inhibition of contractile function in the renal artery appears to be specific to the *alpha*-1A AR. These data provide independent evidence that this receptor is the dominant *alpha*-1 AR in the renal artery. Antisense oligonucleotide application did not result in a complete inhibition of contractile function. There are several possible reasons for this. The phosphorothioate oligonucleotides, while more stable than those containing phosphodiester linkages, still could be broken down by cellular nucleases, thus reducing the effective concentration. Higher concentrations of oligonucleotides may be necessary to achieve complete inhibition. In this regard, we did note a concentration-dependent inhibition (fig. 13) with either *alpha*-1A or *alpha*-1D AR antisense oligonucleotides.

This is the first demonstration of the use of antisense oligonucleotides delivered *in vivo* to inhibit *alpha*-1 AR responses. It is reasonable to suggest that we merely observed nonspecific effects due to surgery or introduction of foreign nucleotides into the smooth muscle and not specific blockade of the translation of *alpha*-1 AR protein. However, these studies with two different arteries demonstrated inhibition by a specific antisense construct that is predicted from pharmacological studies. A further argument for specificity is the fact that we implanted three antisense constructs each in the femoral and renal arteries. Only one of these, the *alpha*-1D in the femoral and the *alpha*-1A in the renal, inhibited phenylephrine contractility. If the inhibition was simply due to surgical manipulation or introduction of oligonucleotides, then we would expect every antisense construct to inhibit function. Despite a wide distribution of this receptor in the peripheral vasculature, the only artery in which we obtained evidence for an *alpha*-1B AR-mediated contraction was the mesenteric resistance artery. This is interesting because the main mesenteric artery is protected by BMY 7378. Therefore, it appears that the *alpha*-1 AR subtype regulating contraction differs along the mesenteric vascular bed. It may be that the *alpha*-1A and *alpha*-1D AR regulate the larger vessels, whereas the *alpha*-1B AR controls the smaller resistance vessels. In this regard, Leech and Faber (1996) demonstrated that *alpha*-1B AR mediates regulation of skeletal muscle venules. Alternatively, the *alpha*-1B AR may serve other regulatory functions. In recent work, Siwik and Brown (1996) showed that the *alpha*-1B AR regulates protein synthesis in cultured aortic smooth muscle cells. Finally, we have shown the feasibility of the *in vivo* application of antisense oligonucleotides to the femoral and renal arteries and that this

application results in a specific inhibition of *alpha*-1 AR subtype-mediated contractile responses.

References

Bennett, M. R., Anglin, S., McEwan, J. R., Jagoi, R., Newby, A. C. and Evan, G. I.: Inhibition of vascular smooth muscle cell proliferation *in vitro* and *in vivo* by c-myc antisense oligodeoxynucleotides. J. Clin. Invest. **93:** 820–828, 1994.

Bylund, D. B., Regan, J. W., Faber, J. E., Hieble, J. P., Triggle, C. R. and Ruffolo, R. R., Jr.: Vascular α-adrenoceptors: From the gene to the human. Can. J. Physiol. Pharmacol. **73:** 533–543, 1995.

Cotecchia, S., Schwinn, D. A., Randall, R. R., Lefkowitz, R. J., Caron, M. G. and Kobika, B. K.: Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. Proc. Natl. Acad. Sci. USA **85:** 7159–7163, 1988.

Elhawary, A. M., Pettinger, W. A. and Wolff, D. W.: Subtype-selective alpha-1 adrenoceptor alkylation in the rat and its effect on the vascular pressor response. J. Pharmacol. Exp. Ther. **260:2:** 709–713, 1992.

Fonseca, M. I., Button, D. C. and Brown, R. D.: Agonist regulation of α_{1B}-adrenergic receptor subcellular distribution and function. J. Biol. Chem. **270:** 8902–8909, 1995.

Giardina, D., Crucianelli, M., Romanelli, R., Leonardi, A., Poggesi, E. and Melchiorre, C.: Synthesis and biological profile of the enantiomers of [4-(4-amino-6,7-dimethoxyquinazolin-2-yl)-*cis*-octahydroquinoxalin-1-yl]furan-2-yl-methanone (Cyclazosin), a potent competitive α_{1B}-adrenoceptor antagonist. J. Med. Chem. **39:** 4602–4607, 1996.

Goetz, A. S., King, H. K., Ward, S. D., True, T. A., Rimele, T. J. and Saussy, D. L.: BMY 7378 is a selective antagonist of the D subtype of α_1-adrenergic receptors. Eur. J. Pharmacol. **272:** R5–R6, 1995.

Graham, R. M., Perez, D. M., Hwa, J. and Piascik, M. T.: α_1-Adrenergic receptor subtypes: Molecular structure, function, and signaling. Circ. Res. **78:** 737–749, 1996.

Guarino, R. D., Perez, D. M. and Piascik, M. T.: The α_1-adrenergic receptors. Cell. Signal. **8:** 322–323, 1996.

Han, C., Li, J. and Minneman, K. P.: Subtypes of α_1-adrenoceptors in rat blood vessels. Eur. J. Pharmacol. **190:** 97–104, 1990.

Hieble, J. P., Bylund, D. B., Clarke, D. E., Eikenburg, D. C., Langer, S. Z., Lefkowitz, R. J., Minneman, K. P. and Ruffolo, R. R., Jr.: International Union of Pharmacology Nomenclature of Adrenoceptors. Recommendation for nomenclature of α_1-adrenoceptors: Consensus update. Pharmacol. Rev. **47:** 267–270, 1995.

Kenny, B. A., Chalmers, D. H., Philpott, P. C. and Naylor, A. M.: Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br. J. Pharmacol. **115:** 981–986, 1995.

Knepper, S. A., Buckner, S. A., Brune, M. E., DeBernadis, J. F., Meyer, M. D. and Hancock, A. A.: A-61603, a potent *alpha*-1 adrenergic receptor agonist, selective for the *alpha*-1A receptor subtype. J. Pharmacol. Exp. Ther. **274:** 97–103, 1995.

Leech, C. J. and Faber, J. E.: Different α-adrenoceptor subtypes mediate constriction of arterioles and venules. Am. J. Physiol **39:** H710–H722, 1996.

Lomasney, J. W., Cotecchia, S., Lorenz, W., Leung, W.-Y., Schwinn, D. A., Yang-Feng, T. L., Brownstein, M., Lefkowitz, R. J. and Caron, M. G.: Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor. J. Biol. Chem. **266:** 6365–6369, 1991.

McGrath, J. C.: Evidence for more than one type of postjunctional α-adrenoceptor. Biochem. Pharmacol. **31:** 467–484, 1982.

Minneman, K. P.: α_1-Adrenergic receptor subtypes, inositol phosphates and sources of cell calcium. Pharmacol. Rev. **40:** 87–119, 1988.

Minneman, K. P. and Esbenshade, T. A.: α_1-Adrenergic receptor subtypes. Ann. Rev. Pharmacol. Toxicol. **34:** 117–133, 1994.

Perez, D. M., Piascik, M. T. and Graham, R. M.: Solution-phase library screening for the identification of rare clones: Isolation of an α_{1D}-adrenergic receptor cDNA. Mol. Pharmacol. **40:** 876–883, 1991.

Piascik, M. T., Smith,. M. S., Soltis, E. E. and Perez, D. M.: Identification of the mRNA for the novel α_{1D}- and two other α_1-adrenoceptors in vascular smooth muscle. Mol. Pharmacol. **46:** 30–40, 1994.

Piascik, M. T., Smith, M. S., Barron, K. W. and Soltis, E. E.: The regulation of regional hemodynamics by *alpha*-1 adrenoceptor subtypes in the conscious rat. J. Pharmacol. Exp. Ther. **267:** 1250–1255, 1993.

Piascik, M. T., Guarino, R. D., Smith, M. S., Soltis, E. E., Saussy, D. L. and Perez, D. M.: The specific contribution of the novel *alpha*-1D adrenoceptor to the contraction of vascular smooth muscle. J. Pharmacol. Exp. Ther. **275:** 1583–1589, 1995.

Schmolka, I. R.: Artificial skin. I: Preparation and properties of Pluronic F-127 gels for treatment of burns. J. Biomed. Mater. Res. **6:** 571–582, 1972.

Schwinn, D. A., Lomasney, J. W., Loreng, W., Szklut, P. J., Fremeau, R. T., Yang-Feng, T. L., Caron, M. G., Lefkowitz, R. J. and Cotecchia, S.: Molecular cloning and expression of cDNA for a novel α-adrenergic receptor subtype. J. Biol. Chem. **265:** 8183–8189, 1990.

Simons, M., Edelman, E. R., DeKeyser, J.-L., Langer, R. and Rosenberg R. D.: Antisense c-*myb* oligonucleotides inhibit intimal arterial smooth muscle cell accumulation *in vivo*. Nature **359:** 67–70, 1992.

Siwik, D. A. and Brown, R. D.: Regulation of protein synthesis by α_1-adrenergic receptor subtypes in cultured aortic vascular smooth muscle cells. J. Cardiol. Pharmacol. **27:** 508–518, 1996.

Strader, C. D., Fong, T. M., Tota, M. R., Underwood, D. and Dixon, R. A. F.: Structure and function of G-protein-coupled receptors. Annu. Rev. Biochem. **63:** 101–132, 1994.

Testa, R., Destefani, C., Guarneri, L., Poggesi, E., Simonazzi, I., Tadei, C. and Leonardi, A.: The α_{1d}-adrenoceptor subtype is involved in the noradrenaline-induced contractions of rat aorta. Life Sci. **57:** PL159–PL163, 1995.

Villa, A. E., Guzman, L. A., Poptic, E. J., Labhasetwar, V., D'Sousa, S., Farrell, C. L., Plow, E. F., Levy, R. J., DiCorleto, P. E. and Topol, E. J.: Effects of antisense c-*myb* oligonucleotides on vascular smooth muscle cell proliferation and response to vessel wall injury. Circ. Res. **76:** 503–513, 1995.

Zhou, L. and Varga, H.: Vascular α_{1D}-adrenoceptors have a role in the pressor response to phenylephrine in the pithed rat. Eur. J. Pharmacol. **305:** 173–176, 1996.

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