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# Pharmacology of A-216546: a highly selective antagonist for endothelin $ET_A$ receptor

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**Abstract**

Endothelins, 21-amino acid peptides involved in the pathogenesis of various diseases, bind to endothelin $ET_A$ and $ET_B$ receptors to initiate their effects. Here, we characterize the pharmacology of A-216546 ([2*S*-(2,2-dimethylpentyl)-4*S*-(7-methoxy-1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl) aminocarbonylmethyl)-pyrrolidine-3*R*-carboxylic acid), a potent antagonist with > 25,000-fold selectivity for the endothelin $ET_A$ receptor. A-216546 inhibited [$^{125}$I]endothelin-1 binding to cloned human endothelin $ET_A$ and $ET_B$ receptors competitively with $K_i$ of 0.46 and 13,000 nM, and blocked endothelin-1-induced arachidonic acid release and phosphatidylinositol hydrolysis with $IC_{50}$ of 0.59 and 3 nM, respectively. In isolated vessels, A-216546 inhibited endothelin $ET_A$ receptor-mediated endothelin-1-induced vasoconstriction, and endothelin $ET_B$ receptor-mediated sarafotoxin 6c-induced vasoconstriction with $pA_2$ of 8.29 and 4.57, respectively. A-216546 was orally available in rat, dog and monkey. In vivo, A-216546 dose-dependently blocked endothelin-1- induced pressor response in conscious rats. Maximal inhibition remained constant for at least 8 h after dosing. In conclusion, A-216546 is a potent, highly endothelin $ET_A$ receptor-selective and orally available antagonist, and will be useful for treating endothelin-1-mediated diseases. 

*Keywords:* A-216546; Antagonist; Endothelin $ET_A$ receptor

## 1. Introduction

Following the discovery of endothelin (Yanagisawa et al., 1988), functional characterization of the three endothelin isoforms (endothelin-1, endothelin-2 and endothelin-3) predicts that at least two mammalian receptor subtypes are present: endothelin $ET_A$ receptor that is selective for endothelin-1, and endothelin $ET_B$ receptor that has equal affinity for the three isoforms (Opgenorth, 1995). The existence of the two distinct high-affinity endothelin receptor subtypes has been confirmed by cloning. Unique cDNAs that code for proteins belonging to the G-protein-linked heptahelical receptor superfamily are identified in bovine and rat tissues (Arai et al., 1990; Sakurai et al., 1990). Human endothelin $ET_A$ and $ET_B$ receptors have also been cloned and found to have ~90% deduced amino acid homology with the bovine or rat receptor and ~60% identity with each other (Arai et al., 1993; Elshourbagy et al., 1993). While pharmacological studies suggest that there may be more endothelin receptor subtypes (Bax and Saxena, 1994), no additional homologous mammalian cDNAs have been identified.

In tissues and cells, endothelin binding initiates a complex signal transduction cascade (Simonson, 1993). Endothelin-1 binding activates phospholipases C and D, causing increases in inositol 1,4,5-trisphosphate and neutral 1,2-diacylglycerol which are associated with a biphasic increase in the intracellular $Ca^{2+}$ concentration and activation of various kinase-mediated pathways involved in mitogenic responses (Wu-Wong et al., 1997b; Wu-Wong and Opgenorth, 1998). Recent evidence suggests that endothe-

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lin may play a role in modulating apoptosis of endothelial and smooth muscle cells (Wu-Wong et al., 1997b; Shichiri et al., 1998). Thus, in addition to the potent vasoconstricting activity and its involvement in cardiovascular diseases, endothelin-1 may play a pivotal role in the pathogenesis of cell growth disorders such as cancer, restenosis, and benign prostatic hyperplasia (Webb et al., 1998).

A major advance was made in the endothelin field with the development of endothelin receptor antagonists. In particular, BQ-123 (cyclo (D-Trp–D-Asp–Pro–D-Val–Leu)) (Ihara et al., 1991) and FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4 + + + methylpentanoyl] amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl) propionic acid) (Sogabe et al., 1993) have been important tools in the investigation of endothelin-mediated pathophysiology. Both are endothelin $ET_A$ receptor-selective, but are peptidic compounds and have poor pharmacokinetics with limited utility as therapeutic agents. Following the peptidic compounds, many pharmaceutical companies reported the discovery of a number of non-peptide antagonists with greatly improved pharmacokinetics, such as Ro 47–0203 (4-*tert*-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2-bipyrimidin-4-yl]-benzenesulfonamide, bosentan), SB 217242 ((+)-(1*S*,2*R*,3*S*)-3-[2-(2-hydroxyeth-1-yloxy)-4-methoxyphenyl]-1-(3,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indan-2-carboxylic acid), PD 156707 (sodium 2-benzo(1,3ioxol-5-yl-4-(4-methoxy-phenyl)-4-oxo-3-(3,4,5-trimethoxybenzyl)-but-2-enoate), etc. (see Table 1). Some of these antagonists are being investigated in human clinical trials.

Although it remains an unsettled issue, evidence favors that the endothelin $ET_A$ receptor may play a more important pathological role. While both endothelin $ET_A$ and $ET_B$ receptors are found in most tissues, endothelin $ET_A$ receptor is the predominant receptor responsible for the vasoconstricting and mitogenic/anti-apoptotic effects of endothelin-1 (Davenport et al., 1995; Maguire and Davenport, 1995; Opgenorth, 1995; Wu-Wong et al., 1997b). Although endothelin $ET_B$ receptor is also involved in vasoconstriction in some tissues (Pollock and Opgenorth, 1993; Davenport and Maguire, 1994), endothelin $ET_B$ receptor seems to play a pivotal role in endothelin-1-mediated vasodilation via nitric oxide release (Ishikawa et al., 1994), and is involved in clearing endothelin from the circulation (Fukuroda et al., 1994a). The endothelin $ET_B$ receptor has also been shown to mediate the inhibitory effect of endothelin-1 on platelet aggregation (McMurdo et al., 1993). More recently, the evidence suggests that blocking endothelin $ET_A$ receptor alone is as effective as blocking both endothelin $ET_A$ and $ET_B$ receptors in several disease models (Schiffrin et al., 1995; Burke et al., 1997; Chen et al., 1997; Borgeson et al., 1998). Taken together, these findings support the concept that a highly endothelin $ET_A$ receptor-selective antagonist may be the preferred modality for treating vascular diseases.

Previously, we have reported the characterization of A-127722 (2-(4-methoxyphenyl)-4-(1,3-benzodioxol-5-yl)-1-[[(dibutyl amino)carbonyl]methyl]-pyrrolidine-3-carboxylic acid), a potent, orally available, non-peptide antagonist that is ~1000-fold more selective for endothelin $ET_A$ receptor (Opgenorth et al., 1996). Because of a concern that, under conditions of acute overdosing and/or chronic dosing, endothelin receptor antagonists with an $ET_A/ET_B$ selectivity of ~1000-fold may 'cross-over' to block endothelin $ET_B$ receptor and thus confound the interpretation of data from in vivo studies, we feel that there is a need to develop a highly endothelin $ET_A$ receptor-selective antagonist. Such an antagonist will be very useful for identifying the roles of endothelin $ET_A$ receptor in a more precise manner, without a concern for whether endothelin $ET_B$ receptor is affected. Based on the above rationale, we have developed A-216546. In this study, we report the in vitro and in vivo characterization of A-216546, a non-peptide

Table 1
ET receptor antagonists: comparison of potency ($K_i$, nM)

| Compound | Company | $ET_A$ | | *N* | $ET_B$ | | *N* | Selectivity | Reference |
|---|---|---|---|---|---|---|---|---|---|
| A-216546 | Abbott | 0.46 | – | 3 | 13,000 | – | 1 | 28,261 | – |
| PD 156707 | Parke-Davis | 0.38 | (0.17) | 4 | ND | (139) | – | (818) | Reynolds et al. (1995) |
| FR 139317 | Fujisawa | 0.80 | (1.0) | 3 | ND | (7,300) | – | (7,300) | Aramori et al. (1992) |
| TBC11251 | Texas Biotech | ND | (0.43) | – | ND | (> 4,300) | – | (> 10,000) | Wu et al. (1997) |
| SB 217242 | SmithKline Beecham | ND | (1.1) | – | ND | (111) | – | (1,000) | Ohlstein et al. (1996) |
| Ro 61–1790 | Roche | ND | (0.13) | – | ND | (> 130) | – | (> 1,000) | Roux et al. (1997) |
| TAK–044 | Takeda | ND | (1.3) | – | ND | (590) | – | (454) | Masuda et al. (1996) |
| SB 209670 | SmithKline Beecham | ND | (0.20) | – | ND | (18) | – | (90) | Ohlstein et al. (1994) |
| LU 127043 | BASF/Knoll | ND | (6) | – | ND | (960) | – | (160) | Raschack et al. (1995) |
| Ro 47–0203 | Roche | 3.67 | (6.5) | 3 | 423.3 | (343) | 3 | (53) | Clozel et al. (1994) |
| L–749329 | Merck | 33.6 | (0.13) | 3 | 1,418[a] | (5.4) | – | (42) | Walsh (1995) |

[a] $K_i$ for $ET_B$ estimated from the $IC_{50}$ value.
() = Published data.
ND = no data.

antagonist that is >25,000-fold more selective for the endothelin $ET_A$ receptor than for the endothelin $ET_B$ receptor.

## 2. Materials and methods

### 2.1. Materials

Endothelin-1 and -3 were purchased from American Peptide (Santa Clara, CA). [$^{125}$I]endothelin-1 (2200 Ci/mmol), [$^{125}$I]endothelin-3 (2200 Ci/mmol), *myo*-[$^{3}$H]inositol (22 Ci/mmol) and [$^{3}$H]arachidonic acid (210 Ci/mmol) were purchased from DuPont New England Nuclear (Boston, MA). Fig. 1 shows the structure of A-216546 ([2*S*-(2,2-dimethylpentyl)-4*S*-(7-methoxy-1,3-benzodioxol-5-yl)-1-(N,*N*-di(*n*-butyl) aminocarbonylmethyl)-pyrrolidine-3*R*-carboxylic acid) which was synthesized at Abbott Laboratories. A-216546 is the (+)-enantiomer of a chiral molecule. All other reagents were of analytical grade.

### 2.2. Animals

Male Sprague–Dawley rats were purchased from Charles River (Kingston, NY). New Zealand White rabbits, male, 2.0–2.5 kg, were purchased from Covance (Kalamazoo, MI). Male and female dogs used in pharmacokinetic studies were purchased from Marshall Research Animals (North Rose, NY). Female cynomolgus monkeys were obtained from the Abbott Drug Analysis Colony. All protocols utilizing live animals were approved by Abbott Laboratories' Institutional Animal Care and Use Committee and were conducted in American Association for Accreditation of Laboratory Animal Care (AAALAC)-accredited facilities.

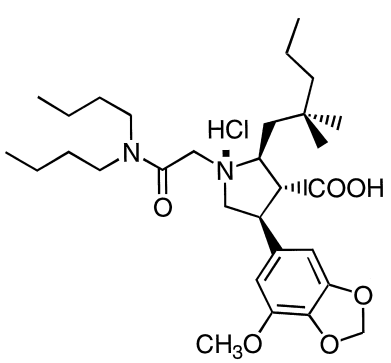


Fig. 1. Chemical structure of A-216546 ([2*S*-(2,2-dimethylpentyl)-4*S*-(7-methoxy-1,3-benzodioxol-5-yl)-1-(*N*,*N*-di(*n*-butyl) aminocarbonylmethyl)-pyrrolidine-3*R*-carboxylic acid]). A-216546 is the (+)-enantiomer of a chiral molecule. The (+)-enantiomer is 200-fold more potent than the (−)-enantiomer in inhibiting ET-1 binding to human $ET_A$ receptor.

### 2.3. Cell culture

Chinese hamster ovary (CHO) cells were acquired from American Type Culture Collection (Rockville, MD). Human pericardial smooth muscle cells were obtained from Dr. Maria J. Vidal at the Instituto Scientifico, San Raffaele, Milan, Italy. MMQ cells, a cell line derived from rat pituitary, were licensed from the University of Virginia. We have previously reported the endothelin-binding characteristics of the human pericardial smooth muscle and MMQ cells (Wu-Wong et al., 1994b). CHO cells were grown in Ham's F-12 nutrient medium, human pericardial smooth muscle cells in Dulbecco's modified minimal essential medium (DMEM), and MMQ cells in RPMI-1648 containing 10% fetal bovine serum. All growth media and supplements were purchased from Gibco/BRL (Grand Island, NY). CHO cells were transfected with the human endothelin $ET_A$ and $ET_B$ receptor expression constructs by the Lipofectin method (Gibco/BRL) and stable transformants were selected with 500 μg/ml geneticin.

### 2.4. Receptor binding

Membranes were prepared from porcine cerebellum, rat pituitary MMQ cells, or CHO cells as previously described (Wu-Wong et al., 1996, 1997a). Briefly, cerebella or cells were homogenized in 25 volumes (w/v) of 10 mM HEPES (pH 7.4) containing 0.25 M sucrose and protease inhibitors (3 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 5 μg/ml Pepstatin A) by 3–10 s polytron at 13,500 rpm with 10 s intervals (for cerebella) or by a micro-ultrasonic cell disruptor (Kontes) (for cells). The mixture was centrifuged at $1000 \times g$ for 10 min. The supernatant was collected and centrifuged at $30{,}000 \times g$ for 30 min (for cerebella) or at $60{,}000 \times g$ for 60 min (for cells). The precipitate was resuspended in Buffer A-1 (20 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$, pH 7.4) containing the aforementioned protease inhibitors and centrifuged again. The final pellet was resuspended in Buffer A-1 containing protease inhibitors and stored at −80°C until used. Protein content was determined by the Bio-Rad dye-binding protein assay.

### 2.5. Radioligand binding to membranes

Binding assays were performed in 96-well microtiter plates precoated with 0.1% bovine serum albumin unless otherwise indicated. Membranes were diluted in Buffer A (Buffer A-1 with the aforementioned protease inhibitors plus 0.025% bacitracin and 0.2% bovine serum albumin) to a final concentration of 0.05 mg/ml of protein. In competition studies, membranes were incubated with 0.1 nM of [$^{125}$I]endothelin-1 or -3 in Buffer A (final volume:

0.2 ml) in the presence of increasing concentrations of unlabeled test ligands for 4 h at 25°C. In saturation studies, membranes were incubated with increasing concentrations of [$^{125}$I]endothelin-1 or -3 in Buffer A (final volume: 0.2 ml) in the presence or absence of unlabeled test ligands for 4 h at 25°C. After incubation, unbound ligands were separated from bound ligands by vacuum filtration using glass-fiber filter strips in PHD cell harvesters (Cambridge Technology, Watertown, MA), followed by washing the filter strips with saline (1 ml) for three times. Non-specific binding was determined in the presence of 1 μM endothelin-1 or -3.

For $K_i$ calculation, $K_d$ values for endothelin-1 binding in the presence of an antagonist at different concentrations were determined by Scatchard analysis and were designated as $K_{d'}$. $K_{d'}$ was plotted against the concentrations of antagonists ($[I]$) for determining the slopes. $K_{d'} = K_d$ when $[I] = 0$. Since $K_{d'} = (1 + ([I])/(K_i))K_d$, $K_i = (K_d/\text{slope})$.

### 2.6. Receptor specificity

The specificity of A-216546 was examined by measuring the ability of A-216546 to compete with radioligand binding in 73 different ligand binding assays. A-216546 was tested at $10^{-5}$ M, in duplicate. These assays were performed by Cerep (Celle L'Evescault, France).

### 2.7. Phosphatidylinositol hydrolysis and arachidonic acid release

Evaluation of A-216546 in antagonizing endothelin-1-induced phosphatidylinositol hydrolysis and arachidonic acid release was done according to methods previously described (Wu-Wong et al., 1994a). Briefly, MMQ cells ($0.4 \times 10^6$ cells/ml) were labeled with 10 μCi/ml of *myo*-[$^3$H]inositol in RPMI for 16 h. Cells were washed with PBS, incubated with Buffer B (Earle's solution: 140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, buffered with 25 mM HEPES, pH 7.4) containing protease inhibitors (3 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 5 μg/ml Pepstatin A) and 10 mM LiCl for 60 min, and then challenged with 1 nM endothelin-1. The endothelin-1 challenge was terminated by the addition of 1.5 ml of chloroform/methanol (1:2, v/v). Total inositol phosphates were extracted after adding chloroform and water to give final proportions of chloroform/methanol/water of 1:1:0.9 (v/v/v). The upper aqueous phase (1 ml) was retained and a small portion (100 μl) counted. The rest of the aqueous sample was analyzed by batch chromatography using the anion-exchange resin AG1-X8 (Bio-Rad, Hercules, CA).

To assay arachidonic acid release, human pericardial smooth muscle cells in 48-well culture plates at 80% confluence were labeled with 0.4 μCi/well of [$^3$H]arachidonic acid in DMEM with 10% fetal bovine serum for 16–24 h. Cells were then incubated with DMEM plus 0.2% bovine serum albumin (0.5 ml/well) for 30 min. After the incubation, the medium was removed and 0.3 ml DMEM with 0.2% bovine serum albumin plus 10 nM BQ-788 ((*N-cis*-2,6-dimethylpiperidinocarbonyl L-gamma–MeLeu–D-Trp ($COOCH_3$)-Nle), an endothelin $ET_B$ receptor-selective antagonist (Ishikawa et al., 1994) was added to each well. A-216546 at different concentrations was added, and finally, 1 nM endothelin-1 was added. Cells were incubated at 37°C for another 30 min, and the incubation medium was collected for radioactivity determination and calculation of arachidonic acid release.

### 2.8. Vessel contraction

Antagonism of endothelin-1 or sarafotoxin 6c-induced vasoconstriction by A-216546 was evaluated using isolated rat aortic rings (mediated by endothelin $ET_A$ receptors) or rabbit pulmonary artery rings (mediated by endothelin $ET_B$ receptors) (Panek et al., 1992). Male Sprague–Dawley rats (350–500 g) or New Zealand White rabbits were anesthetized with sodium pentobarbital (50 mg/kg, i.p.; Abbott Laboratories, North Chicago, IL), and the thoracic aorta or the pulmonary artery was quickly removed and placed in Krebs–Henseleit (KH) buffer gassed with 95:5 $O_2$:$CO_2$ to maintain pH at 7.4. The vessel was cleared of extraneous tissue and segmented into 4–5 mm wide rings with care taken to preserve the endothelium. Vessel segments (rings) were suspended in 2 ml jacketed tissue baths (siliconized) maintained at 37°C. Rings were attached via gold chain to an isometric force transducer (Harvard Bioscience, South Natick, MA) linked to a physiographic recorder (Linseis, Princeton Junction, NJ) for monitoring tension changes. Baseline tension was set at 2.0 g (rat aortic rings) or 1.0 g (rabbit pulmonary artery rings) and the tissues were allowed to equilibrate for 2.5 h. During this period, the tissues were first washed every 5 min with fresh KH buffer and the tension continually adjusted to establish a stable baseline. After 30 min, tissues were maximally constricted with norepinephrine (1 μM) followed by a challenge with acetylcholine (3 μM). A positive relaxant response to acetylcholine confirmed the presence of intact endothelium. After washout of norepinephrine and acetylcholine responses and equilibration at baseline tension, agonist concentration–response curves ($10^{-11}$ M to $10^{-6}$ M) were performed in the presence or absence of A-216546. A-216546 was added 15 min prior to the onset of the agonist concentration–response curve.

In any given experimental set, tissues from the same animal were treated with vehicle (KH buffer) or one of the concentrations of A-216546. Agonist-induced concentration–response curves from vehicle- and antagonist-treated curves were calculated, normalized against the maximal contraction achieved in each tissue, and the effective concentration of endothelin-1 causing 50% maximum re-

sponse ($EC_{50}$) was determined using the curve-fitting program ALLFIT in the GraphPad Prism software (GraphPad Software, San Diego, CA). Schild analysis of the antagonist-induced $EC_{50}$ shifts was performed, yielding a $pA_2$ value as the comparative index of antagonism for the vessel types tested (Arunlakshana and Schild, 1956). $pA_2$ = log(concentration ratio − 1) − log[antagonist], where the concentration ratio is the ratio of $EC_{50}$ values with and without antagonist.

### 2.9. Pharmacokinetics

The pharmacokinetic behavior of A-216546 was first evaluated in male Sprague–Dawley rats (250–300 g). A-216546 was prepared as a 10 mg/ml solution in a 20% ethanol, 30% propylene glycol, 50% of a 5% dextrose in water vehicle containing 1 M equivalent of sodium hydroxide, at concentrations to provide a 0.5 ml/kg volume for intravenous (i.v.) and a 1.0 ml/kg volume for oral (gavage) dosing. Heparinized blood samples were obtained from a tail vein of each animal at 0.1 (i.v. only), 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 9, and 12 h after dosing. The parent drug concentrations were determined by reverse-phase high-performance liquid chromatography (HPLC) with UV detection following liquid–liquid extraction of the plasma samples. Initial estimates of the pharmacokinetic parameters for NONLIN84 (Statistical Consultants, 1986) were obtained with the program CSTRIP (Sedman and Wagner, 1976). Area under the curve (AUC) values were calculated by the trapezoidal rule over the time course of the study. The terminal-phase rate constant ($\beta$) was used in the extrapolation of the AUC from 0 h to infinity. A comparison of the dose-normalized AUC following oral administration with that obtained following an i.v. dose provided an estimate of the bioavailability ($F$). Similar studies were performed utilizing male or female beagle dogs and female cynomolgus monkeys. All animals were fasted overnight prior to dosing, but were permitted free access to water; food was returned to the animals $\sim$4 (monkeys) or $\sim$12 (dogs) h after dosing.

### 2.10. In vivo pseudoefficacy

Male Sprague–Dawley rats (250–350 g) were anesthetized via inhalation of methoxyflurane (Penthrane, Abbott Laboratories, North Chicago, IL). The right femoral artery and vein were catheterized for determination of blood pressure and heart rate, and for i.v. administration of endothelin-1 (i.v. bolus, 0.3 nmol/kg in 0.1% bovine serum albumin-saline). The catheters (Micro-Renethane, Braintree Scientific, Braintree, MA) were tunneled subcutaneously, exteriorized at the ventral neck and plugged. After recovery from the anesthesia (45–60 min), rats were dosed (2 ml/kg) with A-216546 or vehicle (20% ethanol, 30% propylene glycol, 50% of 5% dextrose in water, 1 Eq. NaOH) by oral gavage and placed in individual cages without additional restraint. The arterial catheter was unplugged and coupled to a strain-gauge pressure transducer (Transpac II, Abbott Laboratories, North Chicago, IL). Heart rate, systolic, diastolic and mean arterial pressures were sampled (200 Hz for 5 s) at 2 min intervals via a data acquisition system ($Mi^2$, Modular Instruments, Malvern, PA) starting 30 min prior to the first administration of endothelin-1 and continuing until 4 h after oral dosing of A-216546. Arterial blood samples were taken at 4 h after dosing and placed in tubes containing EDTA (5 mg/ml blood) and placed on ice. After centrifugation, plasma was removed by pipetting and stored at −20°C for later determination of plasma drug concentration by HPLC.

In additional groups of rats, instrumented as above but 1 day prior to dosing, a single oral dose of A-216546 (30 mg/kg) or vehicle was administered and followed by endothelin-1 (0.3 nmol/kg, i.v.) challenges at 4, 8 and 24 h post-dosing. Blood pressure responses were monitored for 1 h before and after each challenge. Blood samples were obtained at 25 h and treated as described above.

Since i.v. administration of endothelin-1 produces a transient vasodepressor response followed by a prolonged vasopressor response, the two components were analyzed by different methods. The maximal depressor effect was determined by direct analysis of the stripchart recording. The vasopressor response was quantified by calculation of the area under the mean arterial blood pressure curve ($AUC_{bp}$) expressed as percent change from the baseline value (average of the 30 min period prior to the endothelin-1 challenge) in each animal: $AUC_{bp}$ = percent change mean arterial blood pressure × time. The $AUC_{bp}$ includes the time points when the endothelin-1-induced positive deflection in mean arterial blood pressure first occurred until the mean arterial blood pressure returned to within 2% of the control baseline value.

### 2.11. Statistics

The radioligand binding analysis program 'EBDA and LIGAND' (Biosoft, MO, USA) was used to analyze data for the calculation of $IC_{50}$ and $K_i$. Unless noted, values are expressed as mean ± S.E.M. and $n$ represents the number of animals or separate experiments in each group. Statistical analysis of data was performed using StatView II software (Abacus Concepts, Berkeley, CA). Group comparisons were determined by an analysis of variance (ANOVA) followed by the Fisher's protected least significant difference test. $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Receptor binding

The potency of A-216546 for inhibiting endothelin-1- or endothelin-3 binding to endothelin $ET_A$ and $ET_B$ recep-

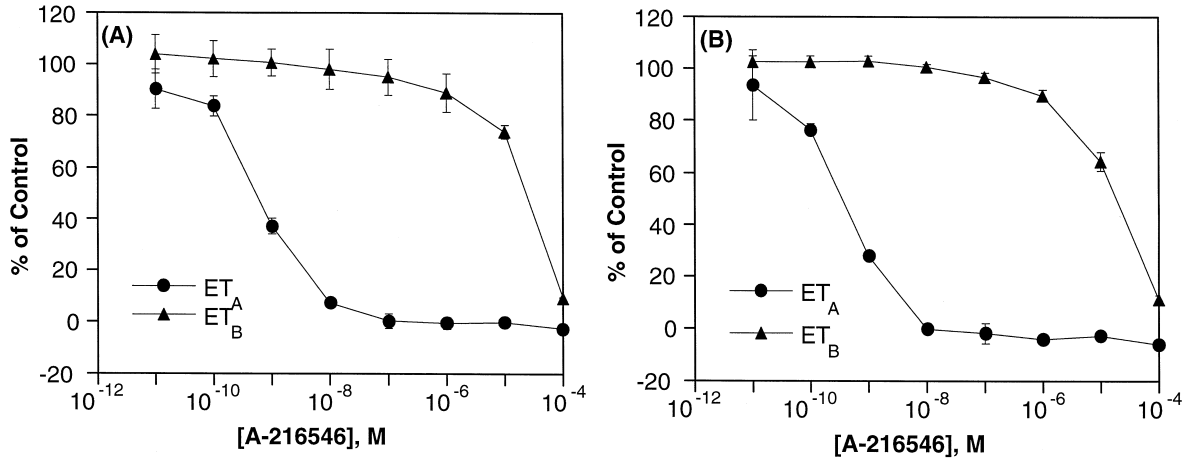


Fig. 2. Competition studies. Membranes were incubated with 0.1 nM [$^{125}$I]endothelin-1 (●) or -3 (▲) in the presence of increasing concentrations of A-216546 for 3 h at 25°C. (A) Membranes (20 μg) were prepared from MMQ cells or porcine cerebellum (PC). (B) Membranes (20 μg) were prepared from CHO cells stably transfected with the human endothelin $ET_A$ or $ET_B$ receptors. Results are expressed as percentage of control (specific binding in the absence of unlabeled ligand). Non-specific binding, determined in the presence of 1 μM of endothelin-1 or endothelin-3, was subtracted from total binding to give specific binding. Data shown are mean ± S.E.M. ($n = 4$; $n$: four separate experiments).

tors was evaluated using membranes prepared from human and non-human tissues and cells. Fig. 2 shows the results from competition binding studies. A-216546 effectively inhibited specific [$^{125}$I]endothelin-1 binding to endothelin $ET_A$ receptor in membranes prepared from rat pituitary MMQ cells with an $IC_{50}$ value of 0.56 ± 0.11 nM ($n = 4$) (Fig. 2A). A-216546 was much less effective in inhibiting specific [$^{125}$I]endothelin-3 binding to endothelin $ET_B$ receptor in membranes prepared from porcine cerebellum with an $IC_{50}$ value of 16,700 ± 3400 nM ($n = 4$) (Fig. 2A). In membranes prepared from CHO cells stably transfected with the human endothelin $ET_A$ and $ET_B$ receptors, A-216546 again effectively inhibited specific [$^{125}$I]endothelin-1 binding to endothelin $ET_A$ receptor with an $IC_{50}$ value of 0.49 ± 0.04 nM ($n = 4$), but was less effective in inhibiting specific [$^{125}$I]endothelin-3 binding to endothelin $ET_B$ receptor with an $IC_{50}$ value of 15,400 ± 1600 nM ($n = 4$) (Fig. 2B).

To further examine the nature of the interaction between A-216546 and endothelin receptor, [$^{125}$I]endothelin-1 saturation binding studies were performed using membranes prepared from CHO cells stably transfected with the human endothelin $ET_A$ and $ET_B$ receptors. Fig. 3 shows the Scatchard analysis of the binding data. For the

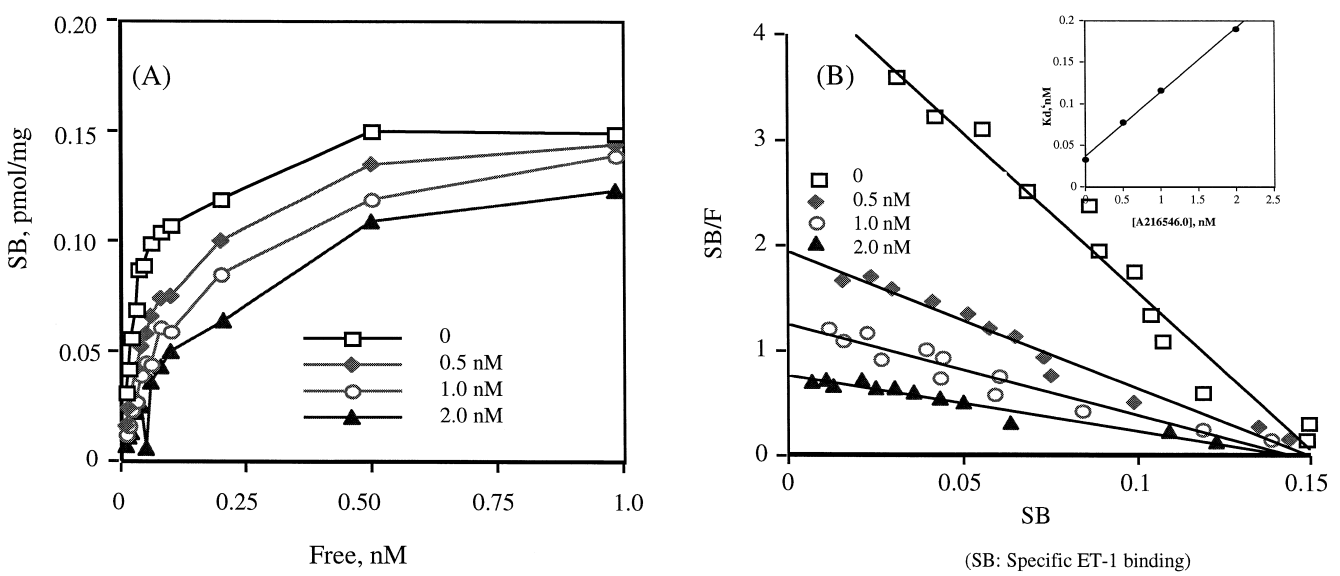


Fig. 3. [$^{125}$I]endothelin-1 saturation binding studies. Membranes (20 μg) from CHO cells stably transfected with the human endothelin $ET_A$ receptors were incubated with increasing concentrations of [$^{125}$I]endothelin-1 in the presence of A-216546 (concentrations as indicated) for 4 h at 25°C (A). Non-specific binding was determined by adding 1 μM unlabeled endothelin-1 and was subtracted from total binding to give specific binding. Scatchard plots are shown in (B). $K_i$ values were determined from the inset in (B) as described in Section 2. The results shown are representative of three separate experiments.

Table 2
Effect of 10 μM Abbott-216546.1 on 73 radioligand binding assays[a]

| Binding site | Percentage of inhibition |
|---|---|
| Adenosine $A_1$ receptor (human) | 23 |
| Adenosine $A_{2A}$ receptor (human) | –[b] |
| Adenosine $A_3$ receptor (human) | 32 |
| $\alpha_1$-adrenoceptor (non-selective) | – |
| $\alpha_2$-adrenoceptor (non-selective) | 18 |
| $\beta_1$-adrenoceptor (human) | – |
| $\beta_2$-adrenoceptor (human) | – |
| Norepinephrine uptake | – |
| Angiotensin $AT_1$ receptor (human) | – |
| Angiotensin $AT_2$ receptor (human) | – |
| Benzodiazepine (central) | – |
| Benzodiazepine (peripheral) | – |
| Bombesin receptor | – |
| Bradykinin $B_2$ receptor (human) | – |
| Calcitonin gene-related peptide | – |
| Cannabinoid $CB_1$ receptor (human) | – |
| Cannabinoid $CB_2$ receptor (human) | 11 |
| $Cholecystokinin_A$ receptor (human) | – |
| $Cholecystokinin_B$ receptor (human) | – |
| Dopamine $D_1$ receptor (human) | 14 |
| Dopamine $D_2$ receptor (human) | – |
| Dopamine $D_3$ receptor (human) | – |
| Dopamine $D_4$ receptor (human) | – |
| Dopamine $D_5$ receptor (human) | – |
| Dopamine uptake (human) | – |
| Endothelin $ET_A$ receptor (human) | 98 |
| Endothelin $ET_B$ receptor (human) | 56 |
| GABA receptor (non-selective) | – |
| Galanin receptor (human) | – |
| Platlet-derived growth factor receptor | – |
| Interleukin-1β | – |
| Tumor necrosis factor-alpha | – |
| Histamine $H_1$ receptor (central) | – |
| Histamine $H_2$ receptor | – |
| Melatonin ML1 receptor | 12 |
| Muscarinic M1 receptors (human) | – |
| Muscarinic M2 receptors (human) | |
| Muscarinic M3 receptors (human) | |
| Muscarinic M4 receptors (human) | – |
| Muscarinic M5 receptors (human) | – |
| Neurokinin-1 receptor (human) | – |
| Neurokinin A receptor (human) | – |
| $NK_3$ tachykinin receptor (human) | – |
| Y1 receptor | – |
| Y2 receptor | – |
| Neurotensin receptor (human) | – |
| δ-Opioid receptor (human) | 62 |
| κ-Opioid receptor (human) | 28 |
| μ-Opioid receptor (human) | 17 |
| PCP receptor | – |
| Thromboxane $A_2$ /prostaglandin $H_2$ receptors | 16 |
| Prostaglandin $I_2$ receptor | 15 |
| $P_{2X}$-purinergic receptor | 37 |
| $P_{2Y}$-purinergic receptor | |
| $5\text{-Hydroxytryptamine}_{1A}$ (human) | – |
| $5\text{-Hydroxytryptamine}_{1B}$ | – |
| $5\text{-Hydroxytryptamine}_{2A}$ (human) | – |
| $5\text{-Hydroxytryptamine}_{2C}$ (human) | – |
| $5\text{-Hydroxytryptamine}_3$ | – |
| $5\text{-Hydroxytryptamine}_{5A}$ (human) | – |
| $5\text{-Hydroxytryptamine}_6$ (human) | – |
| $5\text{-Hydroxytryptamine}_7$ (human) | – |
| σ-Receptor (non-selective) | – |
| Somatostatin receptor | – |
| Vasoactive intestinal peptide receptor | – |
| Vasopresine $V_{1a}$ receptor (human) | – |
| Vasopresine $V_2$ receptor | – |
| $Ca^{2+}$ channel (L, verapamil site) | 29 |
| $K^+$ channel (volt-dependent) | – |
| $K^+$ channel ($Ca^{2+}$-dependent) | – |
| $Na^+$ channel (site 1) | 22 |
| $Na^+$ channel (site 2) | – |
| $Cl^-$ ionophore | 61 |

[a]For details about the various assays, please contact Cerep (Celle L'Evescault, France).
[b]The symbol '–' indicates an inhibition of $< 10\%$.

endothelin $ET_A$ receptor (Fig. 3A), it is evident that increasing the concentration of A-216546 causes successive decreases in the slopes of the lines, indicating a change in the $K_d$ value (the equilibrium dissociation constant) of endothelin-1 binding. A-216546 did not show a significant effect on the $B_{max}$ (maximal [$^{125}$I]endothelin-1 binding). The $K_i$ value of A-216546 for endothelin $ET_A$ receptor was determined to be $0.46 \pm 0.03$ nM ($n = 3$; $n$: three separate experiments). Similar studies were performed in the human endothelin $ET_B$ receptor and the $K_i$ value was 13,000 nM. These data indicate that A-216546 decreased the binding affinity of endothelin without affecting the

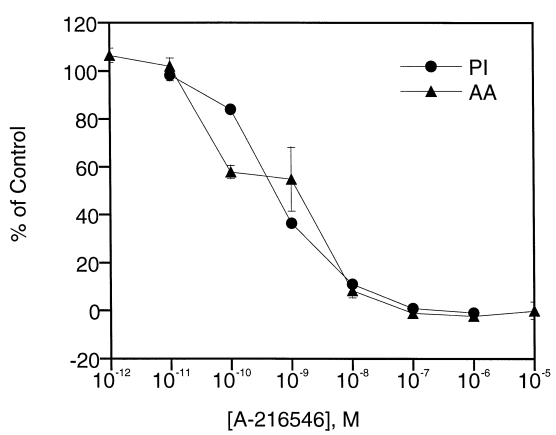


Fig. 4. A-216546 inhibited endothelin-1-evoked phosphatidylinositol hydrolysis and arachidonic acid release. Phosphatidylinositol hydrolysis: MMQ cells prelabeled with *myo*-[$^3$H]inositol were challenged with 1 nM endothelin-1 in the presence of increasing concentrations of A-216546. Results were calculated by normalizing the increase in signal stimulated by endothelin-1 in the presence of test agents to control (the increase stimulated by endothelin-1 in the absence of A-216546). Arachidonic acid (AA) release: human pericardial smooth muscle cells in 48-well plates prelabeled with [$^3$H]arachidonic acid were challenged with 1 nM endothelin-1 in the presence of increasing concentrations of A-216546 for 30 min at 37°C. Radioactivity released was expressed as percentage of control (cells not treated with endothelin-1 or test agents). Data shown are mean ± S.E.M. ($n = 3$; $n$: three separate experiments).

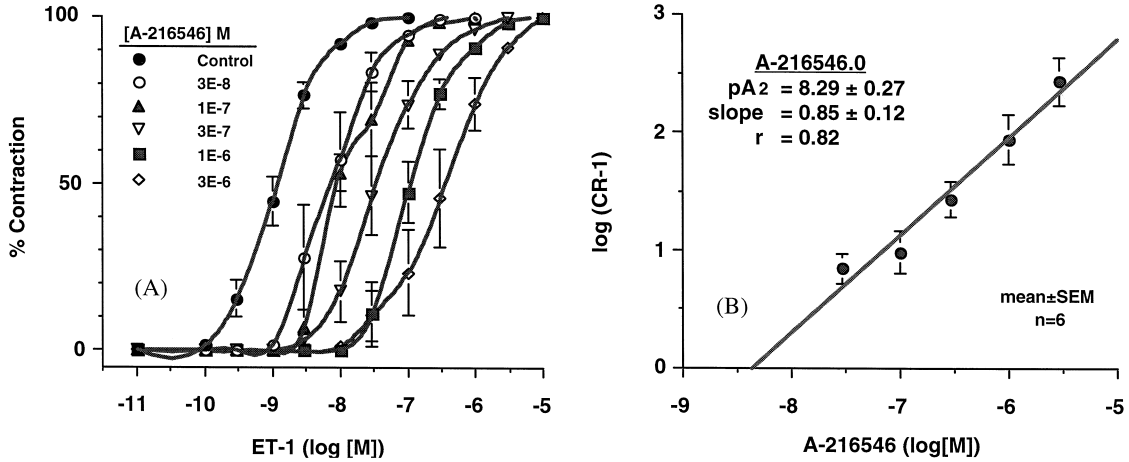


Fig. 5. Inhibition of endothelin $ET_A$ receptor-mediated vasoconstriction in isolated rat aorta by A-216546: (A) Endothelin-1 alone or in the presence of A-216546 (concentrations as indicated). (B) The Schild analysis of A-216546 inhibition of the endothelin-1-induced response. Data shown are mean ± S.E.M. ($n = 6$; $n$: number of tissues used at each concentration, a total of 36 pieces of aorta rings were prepared from six animals).

receptor density, suggesting that A-216546 is a strictly competitive inhibitor of endothelin-1 binding. Furthermore, A-216546 is a potent inhibitor of endothelin binding with > 25,000-fold selectivity toward the endothelin $ET_A$ receptor. The $K_i$ values for various endothelin receptor antagonists are compared (Table 1).

### 3.2. Receptor specificity

The specificity of A-216546 was determined in 73 various radioligand binding assays covering receptors, ion transporters, and other membrane proteins. As shown in Table 2, A-216546 at 10 μM inhibited [$^{125}$I]endothelin-1 binding to endothelin $ET_A$ and $ET_B$ receptors by 98% and 56%. A-216546 showed no significant activity in any of the other assays at concentrations up to 10 μM, except for 62% inhibition of [$^3$H]DPDPE ([D-Pen$^{2,5}$] enkephalin) binding to δ-opioid receptor, and 61% inhibition of [$^{35}$S]TBPS (*t*-butylbicyclophosphorothionat) binding to $Cl^-$ ionophore. As a point of reference, a 10 μM concentration is > 20,000-fold A-216546's $K_i$ for binding at the endothelin $ET_A$ receptor and is a concentration which greatly exceeds the plasma concentrations that would be achieved with oral administration of A-216546 in vivo at efficacious doses. These results indicate that A-216546 is specific for the endothelin $ET_A$ receptor.

### 3.3. Phosphatidylinositol hydrolysis and arachidonic acid release

A-216546 effectively inhibited endothelin-1-evoked phosphatidylinositol hydrolysis in MMQ cells with an $IC_{50}$

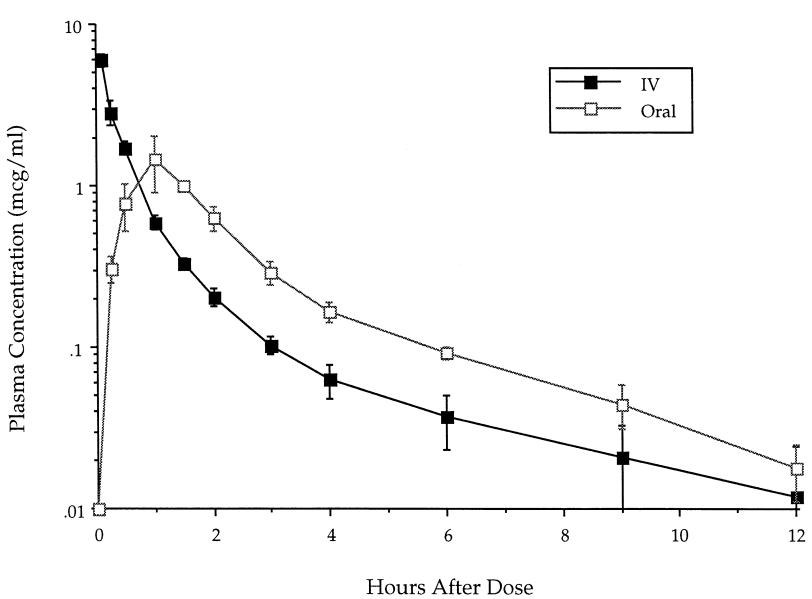


Fig. 6. Plasma concentrations of A-216546 after a 5 (i.v.) or 10 (oral) mg/kg dose in rat (mean ± S.E.M., $n = 4$; $n$: number of animals).

Table 3
Pharmacokinetics of a single oral dose of A-216546

| Species | Dose (mg/kg) | $t_{1/2}$ (h)[a] | $C_{max}$ (μg/ml) | $F$ (%) |
|---|---|---|---|---|
| Rat | 10 | 2.5 | 1.69 | 47.7 |
| Dog | 5 | 3.3 | 1.97 | 19.9 |
| Monkey | 5 | 1.0 | 1.10 | 18.1 |

[a] Plasma elimination half-life following oral dosing.

value of $0.59 \pm 0.01$ nM ($n = 3$) (Fig. 4). A-216546 alone did not show any agonist or antagonist effects on basal phosphatidylinositol hydrolysis. Similarly, A-216546 effectively inhibited endothelin-1-evoked arachidonic acid release in human pericardial smooth muscle cells with an $IC_{50}$ value of $3.03 \pm 0.2$ nM ($n = 3$), but by itself did not show any agonist activity (Fig. 4).

### 3.4. Vessel ring contraction

A-216546 produced concentration-dependent, parallel rightward shifts in the endothelin-1 concentration–response curve, as illustrated in Fig. 5A. For the endothelium-intact rat aortic rings utilized in these studies, the endothelin-1 $EC_{50}$ was determined to be 1.1 nM ($n = 6$). Schild analysis of the concentration–response curves in the presence of various A-216546 concentrations yielded a $pA_2$ of $8.29 \pm 0.27$ ($n = 6$). The slope of the regression lines was not significantly different from unity ($0.85 \pm 0.12$, $r = 0.82$). The endothelin-1 maximal contraction in the tissue was not significantly affected by A-216546. A-216546 (up to concentrations of 10 μM) was devoid of agonist activity.

To examine the endothelin $ET_A$ receptor selectivity of A-216546, antagonism of A-216546 was then evaluated in isolated endothelium-denuded rings of rabbit pulmonary artery in which sarafotoxin-6c, an endothelin $ET_B$ receptor-selective receptor agonist, -induced vasoconstriction is mediated principally by the vasoconstrictor-type endothelin $ET_B$ receptors (not shown). The $EC_{50}$ of sarafotoxin 6c-induced vasoconstriction was 1.57 nM ($n = 4$, $n$: number of tissues). A-216546 shifted the sarafotoxin 6c concentration–response relationship to the right in a concentration-dependent manner, and Schild analysis yielded a $pA_2$ of $4.57 \pm 0.12$ ($n = 18$; $n$: number of animals), with a slope of $1.72 \pm 0.32$ and $r = 0.81$. A-216546 alone also did not exhibit any intrinsic agonist activity on baseline contractile tone of rabbit pulmonary artery. Therefore, A-216546 exhibits $>$ 5000-fold selectivity for the endothelin $ET_A$ receptor in this assay system.

### 3.5. Pharmacokinetics

The pharmacokinetic profile of A-216546 was examined in three species. In male Sprague–Dawley rats, the pharmacokinetic behavior of A-216546 following a 5 mg/kg i.v. dose was characterized by an apparent plasma elimination half-life of 1.6 h, with volume of distribution values of 0.56 and 3.7 l/kg for $V_c$ and $V_\beta$, respectively. The plasma clearance of A-216546 in rat was 1.5 l/h kg. Parent compound was slowly absorbed from the 10 mg/kg oral dose, with peak plasma concentrations recorded 1.3 h after oral (solution) administration. Peak plasma concentrations averaged 1.69 μg/ml, declining with an apparent elimination half-life of 2.5 h. The bioavailability of A-

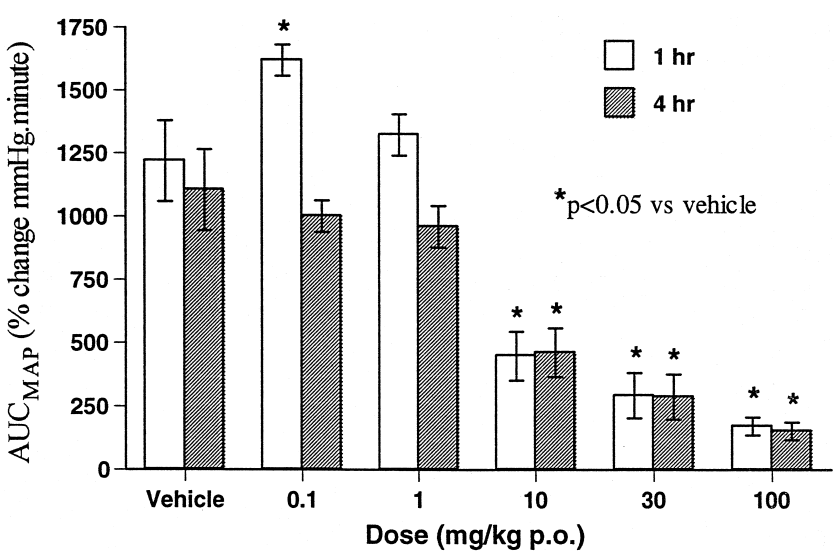


Fig. 7. Dose-response data for the in vivo antagonism in conscious rats of endothelin-1-induced (0.3 nmol/kg, i.v. bolus) increases in arterial blood pressure by a single oral dose of A-216546. Blood pressure responses to exogenous endothelin-1 were determined at 1 and 4 h after dosing (vehicle-treated group: $n = 16$; drug-treated group: $n = 6$; $n$: number of animals). Area under the curve ($AUC_{bp}$) calculations were made to account for the unique time element of the endothelin-induced pressure response in vivo. Statistical analysis was performed by an ANOVA followed by the Fisher's protected least significant difference test. $* P < 0.05$.

216546 from the solution formulation was estimated to be 47.7% in the rat (Fig. 6; Table 3).

Similar studies were performed in the beagle dog and in the cynomolgus monkey, and the results are summarized in Table 3.

### 3.6. In vivo pseudoefficacy

Measurement of the endothelin-1-induced changes in mean systemic arterial blood pressure in conscious, normotensive rats was used to evaluate the in vivo oral efficacy of A-216546. A-216546 (1 to 100 mg/kg) or vehicle was administered orally by gavage. Endothelin-1 (0.3 nmol/kg, i.v.) was administered as a bolus 1 and 4 h after dosing. To account for the fact that endothelin-1 produces a prolonged vasoconstriction, the pressor response was quantified by calculation of the $AUC_{MAP}$ of mean arterial blood pressure (MAP) measured over time, where MAP was expressed as the percent change from baseline pressure (i.e., average pressure measured over 30 min prior to the endothelin-1 challenge).

A-216546 exhibited a dose-dependent inhibition of the peak pressor response to endothelin-1 (Fig. 7), and statistically significant inhibition was achieved at doses of 3 to 100 mg/kg. The duration of inhibition of the endothelin-1 pressor response exceeded 4 h, since the degree of inhibition 4 h after dosing was similar to that observed 1 h after dosing.

A-216546 at 10 and 30 mg/kg had no effect on the transient depressor response to endothelin-1, and A-216546 at 100 mg/kg exhibited a modest inhibition on endothelin-1-induced transient depressor response. These results indicate that A-216546 is well-absorbed following oral dosing and is efficacious for inhibition of endothelin-1-induced pressor responses with the duration of inhibition exceeding 4 h. The lack of an effect on the endothelin-1-induced depressor response is consistent with endothelin $ET_A$ receptor selectivity.

To further study the biological duration of effect for A-216546, a single oral dose of A-216546 (30 mg/kg) on endothelin-1-induced pressor activity are compared (Fig. 8). Significant suppression of the endothelin-1-induced vasopressor response by A-216546 was evident at 4 and 8 h post-dosing. However, no significant suppression was observed at 24 h. A-216546 was present in plasma at 25 h at a concentration of $0.051 \pm 0.008$ μg/ml ($n = 6$). These results suggest that A-216546 remains efficacious for at least 8 h after dosing.

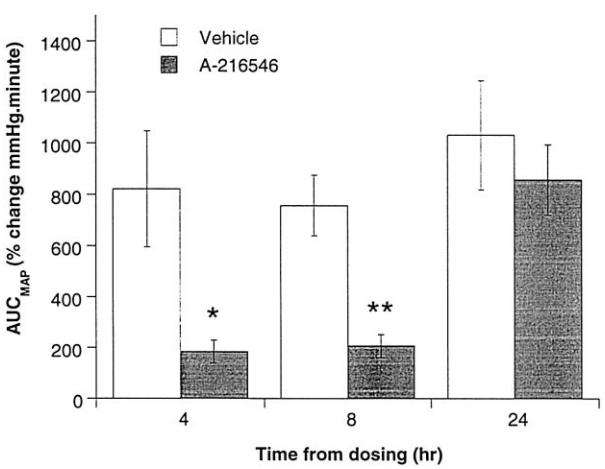


Fig. 8. The biological duration of effect for A-216546. The experimental protocol was similar to that in Fig. 7, except that a single oral dose of A-216546 (30 mg/kg) was administered, followed by endothelin-1 (0.3 nmol/kg, i.v.) challenges at 4, 8 and 24 h post-dosing dosing (vehicle-treated group: $n = 5$; drug-treated group: $n = 6$; $n$: number of animals). Blood pressure responses were monitored for 1 h before and after each challenge. Statistical analysis was performed by an ANOVA followed by the Fisher's protected least significant difference test. $*\ P < 0.05$ vs. vehicle; $**\ P < 0.01$ vs. vehicle.

## 4. Discussion

The availability of potent antagonists for endothelin has sparked keen interest in further developing these agents for clinical utilization. However, it continues to be a matter of debate and great interest as to what kind of antagonists against which subtype of endothelin receptor may be more clinically beneficial. Emerging evidence suggests that a highly endothelin $ET_A$ receptor-selective antagonist may be the choice for therapeutic treatment. First, endothelin $ET_B$ receptor is known to mediate the release of NO, which results in a beneficial vasodilation effect (Parris and Webb, 1997). Secondly, endothelin $ET_B$ receptor is involved in the clearance of endothelin-1 from circulation, and chronic endothelin $ET_B$ receptor blockage will result in an elevation in the plasma endothelin-1 level (Fukuroda et al., 1994a), which may incur adverse effects. Recently at the Fifth International Conference on Endothelin, Willette et al. (1998) showed that systemic administration of equivalent doses of endothelin receptor antagonists with different potencies for endothelin $ET_B$ receptor produced elevations in the plasma endothelin-1 level, which correlated linearly with their affinities at the endothelin $ET_B$ receptor, further confirming the role of endothelin $ET_B$ receptor as a clearance receptor for circulating endothelin-1. Thirdly, endothelin $ET_A$ receptor-selective antagonists have been shown to be equally to or more efficacious than non-selective antagonists in various animal disease models (Opgenorth, 1995).

We have previously reported the characterization of A-127722, a novel, non-peptide endothelin antagonist that displays very high affinity binding ($K_i = 69$ pM) with a more than 1000-fold greater affinity for human endothelin $ET_A$ than $ET_B$ receptor. Although A-127722 is $>$ 1000-fold more selective for endothelin $ET_A$ receptor, it exhibits a $K_i$ of 63.3 nM for endothelin $ET_B$ receptor. There is a concern that A-127722, at high doses and/or during condi-

tions of chronic dosing, may cause a partial inhibition of the endothelin $ET_B$ receptor. To further improve the selectivity of A-127722 towards the endothelin $ET_A$ receptor, A-216546 was synthesized. A-216546 retains a high affinity ($K_i = 0.46$ nM) for the human endothelin $ET_A$ receptor with a $>25{,}000$-fold greater affinity for endothelin $ET_A$ than $ET_B$ receptor. Similar binding characteristics are apparent from studies utilizing membranes prepared from MMQ cells (rat pituitary cell line) and porcine cerebellum. Verification that A-216546 is a functional antagonist was demonstrated by the concentration-dependent inhibition of endothelin-1-stimulated arachidonic acid release and phosphatidylinositol hydrolysis, and by the lack of any agonist activity in these assays at concentrations up to 1 μM. A comparison of the in vitro potencies between A-216546 and other benchmark endothelin receptor antagonists is shown in Table 1. Clearly, A-216546 is as potent as, or more potent than, most of the endothelin receptor antagonists in the literature, and exhibits a selectivity for endothelin $ET_A$ receptor not surpassed by any other known endothelin receptor antagonist.

The hallmark activity of endothelin-1 is potent constriction of vascular smooth muscle. The putative pathogenic roles that have been suggested for endothelin-1 usually involve a vasoconstrictive component (Opgenorth, 1995). In this regard, we examined the ability of A-216546 to inhibit endothelin-1-induced contraction of vascular tissue utilizing isolated rat aorta and rabbit pulmonary artery. It is known that, in the rat aorta, the endothelin $ET_A$ receptor is the predominant mediator of endothelin-1 activity (Panek et al., 1992), while in the rabbit pulmonary artery, the endothelin $ET_B$ receptor mediates the endothelin-1 responses (Fukuroda et al., 1994b). A-216546 produces a parallel and rightward shift of the endothelin-1 concentration–response curve without affecting maximal force generated to yield $pA_2$ values of 8.29 and 4.57 for the aorta and the artery, respectively. These results suggest that A-216546 is highly selective for the endothelin $ET_A$ receptor, and, as in the binding and arachidonic acid release assays, A-216546 appears to act as a fully competitive receptor antagonist.

In addition to being highly potent and endothelin $ET_A$ receptor-selective, the data presented here indicate that A-216546 is orally available in the rat, dog and monkey. In the conscious rat, A-216546 exhibits a dose-related inhibition of the blood pressure response to exogenous endothelin-1 yielding an $ED_{50}$ of approximately 10 mg/kg for a single oral dose. At 30 mg/kg, nearly 80% of the endothelin-1-induced blood pressure response was antagonized by A-216546, yet no effect was observed on the transient depressor activity of endothelin-1.

The in vivo $ED_{50}$ for the orally administered A-216546 is higher than expected for a compound with a $pA_2$ value of 8.29. The observed disparity between in vitro and in vivo potencies seems to be a general phenomenon for most of the endothelin receptor antagonists, and may be related to the lipophilic nature of these compounds. The carboxylic acid residue of A-216546 is likely to make it susceptible to protein binding, as well. Our previous studies show that most endothelin receptor antagonists exhibit strong binding to plasma proteins, especially serum albumin (Wu-Wong et al., 1996). As a result, when the concentrations of plasma proteins, such as serum albumin, increase, the potency of an antagonist tends to decrease (Wu-Wong et al., 1997a). Reports in the literature on endothelin receptor antagonists utilize various serum albumin concentrations in binding assays. For example, 0.01% human serum albumin or bovine serum albumin was used in the binding assay by Williams et al. (1995) or Sogabe et al. (1993). Bovine serum albumin at 0.1% was used in the assays by Webb et al. (1995) and Reynolds et al. (1995), while 0.5% bovine serum albumin was used by Clozel et al. (1994). In this report, 0.2% bovine serum albumin was used in the receptor binding studies. In comparison, the protein concentration in human blood is 7% and 55–60% of that is serum albumin. When the different protein concentrations in vitro and in vivo are taken into consideration, a higher-than-expected in vivo $ED_{50}$ for A-216546 may not be too surprising.

In summary, A-216546 is a potent, orally available endothelin receptor antagonist with a high selectivity for the endothelin $ET_A$ receptor. The potency and bioavailability of A-216546 suggest that it will have important utility for preclinical evaluation of the pathophysiology of the endothelin system. In particular, the $>25{,}000$-fold selectivity towards the endothelin $ET_A$ receptor makes A-216546 a useful tool in studying the pathophysiological role of endothelin $ET_A$ receptor in an unequivocal manner. Furthermore, A-216546 is being considered for clinical development as a therapeutic agent for chronic treatment of human diseases involving endothelin-1 mediated by the endothelin $ET_A$ receptor. The high selectivity of A-216546 for the endothelin $ET_A$ receptor may provide a potential advantage in that a higher dose may be used under chronic dosing conditions to achieve a greater tissue penetration, resulting in a greater local and selective antagonism of the endothelin $ET_A$ receptor without a concern over blocking the endothelin $ET_B$ receptor.

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