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Nonpeptide Angiotensin II Receptor Antagonists: Insurmountable Angiotensin II Antagonism of EXP3892 is Reversed by the Surmountable Antagonist DuP 753

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ABSTRACT

Effects of 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole, potassium salt (DuP 753), a surmountable angiotensin II (AII) receptor antagonist, on the insurmountable AII antagonism induced by 2-*n*-propyl-4-trifluoromethyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid (EXP3892) were examined. In the rabbit aorta, EXP3892 exhibited selective and insurmountable AII antagonism. DuP 753 at 10^{-6} M, added before or after EXP3892, reversed partially the depressed AII maximal response caused by 10^{-9} M EXP3892. Repeated washing of the rabbit aorta treated with DuP 753 at 10^{-6} M or EXP3892 at 10^{-9} M did not restore completely the sensitivity to AII for at least 2 hr. In the pithed rat, EXP3892 showed selective and insurmountable AII antagonism. DuP 753 at 0.1 to 3 mg/kg i.v., given before or after EXP3892, reversed the reduced AII-maximal response induced by EXP3892 at 0.1 mg/kg i.v. We propose that DuP 753 by binding to the AII receptor induces conformational changes resulting in a reduction of the affinity of the receptor for coupling factors/transducer proteins, which causes surmountable antagonism. EXP3892 would diminish the binding capacity for coupling factors accounting for insurmountable antagonism. As DuP 753 and EXP3892 compete for the same AII receptor, the reduced AII-maximal response by EXP3892 may be reversed by DuP 753.

Effects of antagonists on agonist dose-response curves are usually categorized as either surmountable (*i.e.*, parallel shifts to the right of the agonist dose-response curves with no alteration of the agonist-maximal response) or insurmountable antagonism (*i.e.*, antagonism with a depression of the agonist-maximal response), a classification scheme first introduced by Gaddum and his associates (1955). The early nonpeptide AII receptor antagonists, including DuP 753, all exhibited surmountable AII receptor antagonism (Wong *et al.*, 1988, 1989, 1990a,d; Chiu *et al.*, 1990). In renal hypertensive rats with high renin and spontaneously hypertensive rats, DuP 753 has been shown to be a potent and p.o.-active antihypertensive agent (Wong *et al.*, 1990b, c and d). Subsequently, insurmountable AII receptor antagonists, such as 2-*n*-butyl-4-chloro-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid (EXP3174) (Wong *et al.*, 1990e), have been identified. EXP3174 is an active metabolite of DuP 753 (Wong *et al.*, 1990e).

Recently, Kaumann and Frenken (1985) reported that methysergide reduced the maximal contractile response to serotonin in calf coronary arteries. Interestingly, ketanserin, a competitive serotonin receptor antagonist, reversed the methysergide-induced decrease in the maximal response to serotonin (Kaumann and Frenken, 1985). A similar interaction between methysergide and ketanserin was also observed in other tissues (Lemoine and Kaumann, 1986; Frenken and Kaumann, 1987). Based on these results, Kaumann proposed a two-state model of allosteric regulation of serotonin receptors (Kaumann and Frenken, 1985; Lemoine and Kaumann, 1986; Frenken and Kaumann, 1987; Purdy, 1988). In this model, methysergide, by acting at the allosteric site, induces the serotonin receptor into a low efficacy state causing insurmountable antagonism. Ketanserin by competing with methysergide at the allosteric site converts the serotonin receptor from a low efficacy state to a high efficacy state resulting in a reversal of insurmountable antagonism. Likewise, Xu and Purdy (1989) reported that another insurmountable antagonist, 2-brom-*d*-lysergic acid diethylamide, exerted allosteric blockade of serotonin receptors in rabbit aorta. In light of the availability of surmountable and insurmountable nonpeptide AII receptor antagonists, it was of interest to examine whether surmountable and insurmountable nonpeptide AII receptor antagonists exhibit a similar allosteric interaction. The surmountable antagonist DuP 753 and the insurmountable antagonist EXP3892 were chosen for this study. Their structures are shown in figure 1.

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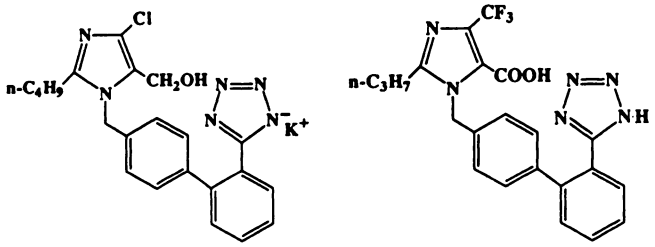
ABBREVIATIONS: AII, angiotensin II; DuP 753, 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole, potassium salt; EXP3892, 2-*n*-propyl-4-trifluoromethyl-1-[(2′-(1H-tetrazol-5yl)biphenyl-4-yl)methyl]imidazole-5-carboxylic acid; sarile, [Sar^1,Ile^8]AII.



DuP 753 EXP3892

Fig. 1. Chemical structures of DuP 753 and EXP3892.

Methods

Effects on AII-Induced Contractile Response in Rabbit Aorta

Rabbit thoracic aorta helical strips were prepared as described previously (Wong *et al.*, 1989). Briefly, the strips were mounted with resting tension of 2.5 g in tissue baths containing Krebs-bicarbonate solution of the following composition (millimolar): NaCl, 118.4; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; $NaHCO_3$, 25; dextrose, 10.1; and disodium EDTA, 0.01. The Krebs' solution was kept at 37°C and bubbled continuously with 5% CO_2 in oxygen. A control cumulative concentration-contractile response curve for AII was obtained for each tissue. The tissue was washed several times until the base line was reached.

Group 1. In testing for AII antagonism, EXP3892 at 10^{-10}, 10^{-9}, 10^{-8} or 10^{-7} M was incubated with the tissue for 15 min. The concentration-response curve for AII was then repeated. Concentration-contractile response curves for norepinephrine and KCl were also examined in the presence or absence of EXP3892 at 10^{-7} M to test the specificity of this antagonist.

Group 2. To study the interaction between DuP 753 and EXP3892, the tissue was first incubated with DuP 753 at 10^{-6} M or its vehicle for 30 min. EXP3892 at 10^{-9} M or its vehicle was then added in the presence of DuP 753 and was incubated with the tissue for an additional 15 min. The concentration-response curve for AII was then repeated in the presence of test compounds. In the second series of experiment, the same protocol was repeated except that the tissue was incubated first with EXP3892 or its vehicle and then with DuP 753 or its vehicle. In the third series of experiments, the tissue was treated with EXP3892 and DuP 753-vehicle, DuP 753 and EXP3892-vehicle or the combination of EXP3892 and DuP 753 for 15 min.

Group 3. To examine the interaction between prazosin, a competitive *alpha*-1 adrenergic receptor antagonist, and phenoxybenzamine, a noncompetitive *alpha* adrenergic receptor antagonist, the protocol described above (Group 2) was followed except that norepinephrine, prazosin (3×10^{-8} M) and phenoxybenzamine (3×10^{-8} M) were used instead of AII, DuP 753 and EXP3892, respectively.

Group 4. To test the reversible nature of DuP 753 and EXP3892, the protocol described above (Group 1) was used except that each tissue was incubated with vehicle, DuP 753 (10^{-6} M) or EXP3892 (10^{-9} M) for 15 min. After the determination of the concentration-response curve for AII in the the presence of test compounds, the tissues were washed and rechallenged with increasing concentrations of AII 60 min later. This procedure was repeated again 120 min later.

The analog contraction signal was recorded with a force-displacement transducer (model FT03, Grass Instrument Co., Quincy, MA) connected to a polygraph (model 7D, Grass Instrument Co.) and analyzed with a digital computer (Buxco Electronics, Inc., Sharon, CT). Responses were expressed as a percentage of the AII-maximal response. As EXP3892 exhibited insurmountable AII antagonism, the dissociation constant (K_B) for EXP3892 was derived from the double-reciprocal regression as described by Kenakin (1987).

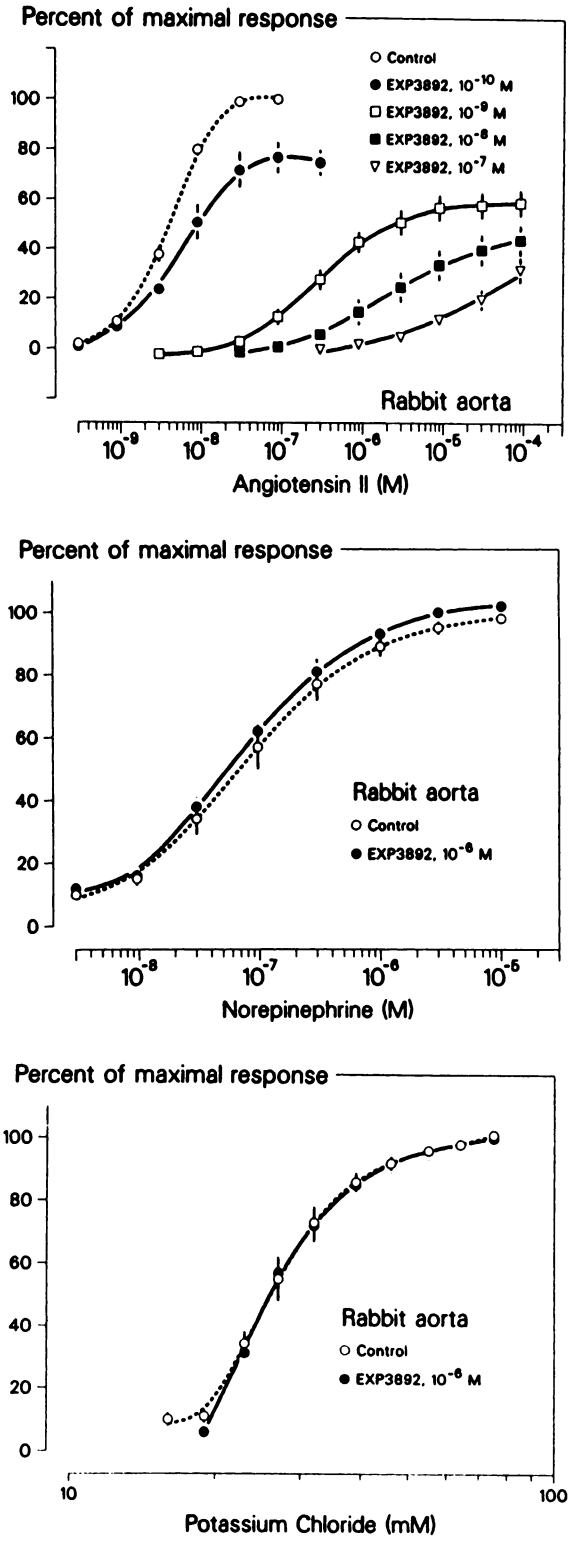


Fig. 2. Effects of EXP3892 on log concentration-contractile response curves for AII (upper panel), norepinephrine (middle panel) and potassium chloride (lower panel) in isolated rabbit aorta. Maximal responses to AII, norepinephrine and KCl averaged 1.86 ± 0.09, 2.89 ± 0.24 and 2.42 ± 0.26 g, respectively. Values represent means ± S.E.M. and n = 6–8 per group.

Effects on AII-Induced Pressor Response in Pithed Rats

Male CD Sprague-Dawley rats (300–400 g) (Charles River Laboratories, Kingston, NY) were anesthetized, cannulated and pithed as

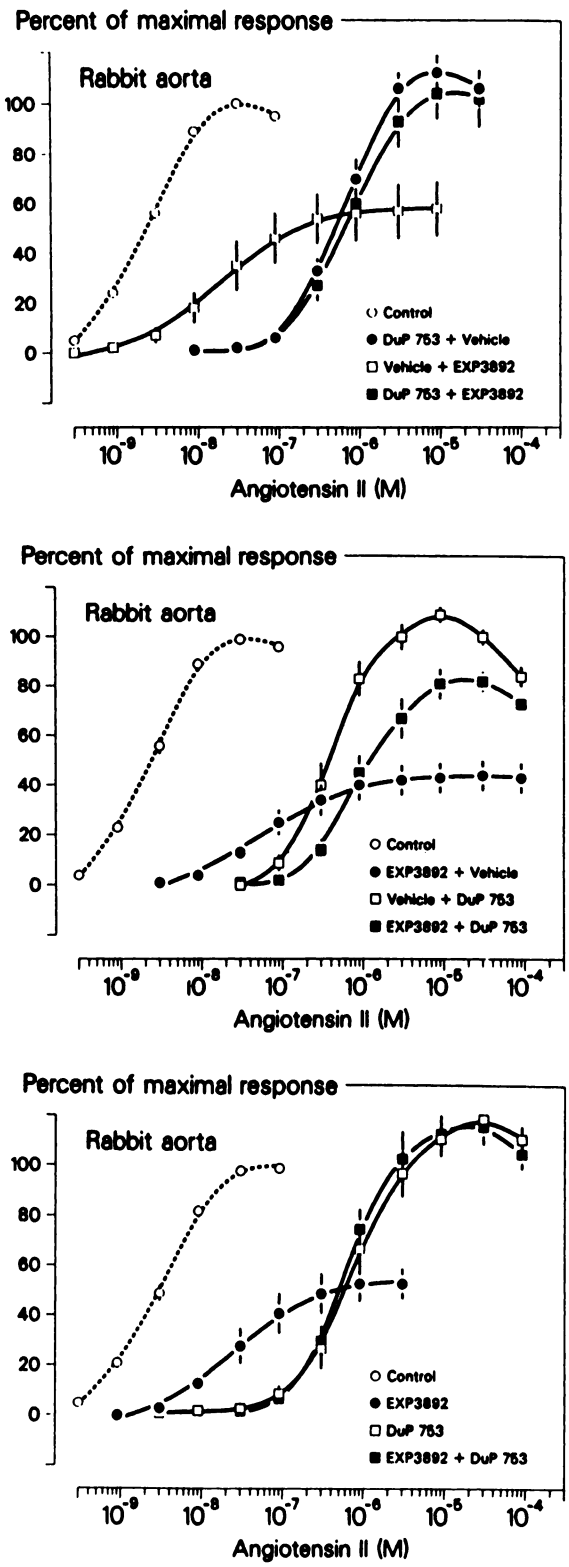


Fig. 3. Effects of EXP3892 (10^{-9} M), DuP 753(10^{-6} M) and the combination of EXP3892 (10^{-9} M) and DuP 753(10^{-6} M) on the log concentration-contractile response curve for AII in isolated rabbit aorta. Upper panel, the aorta was treated first with DuP 753 or its vehicle for 30 min followed by EXP3892 or its vehicle for another 15 min. Middle panel, the aorta was treated first with EXP3892 or its vehicle for 30 min followed by DuP 753 or its vehicle for another 15 min. Lower panel. the aorta was treated with EXP3892, DuP 753 or the combination of EXP3892 and DuP 753 for 15 min. Maximal response to AII averaged 3.18 ± 0.27, 2.74 ± 0.23 and 1.91 ± 0.11 g. Values represent means ± S.E.M. and n = 6–9 per group.

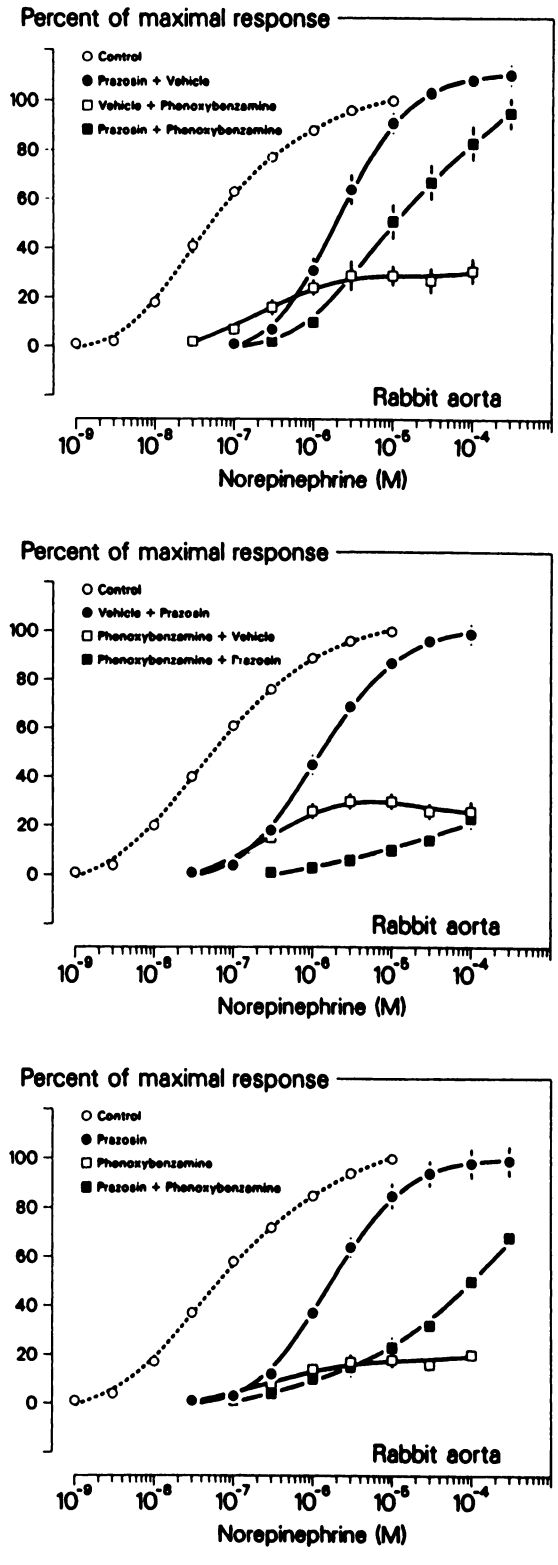


Fig. 4. Effects of prazosin (3×10^{-8} M), phenoxybenzamine (3×10^{-8} M) and the combination of prazosin (3×10^{-8} M) and phenoxybenzamine (3×10^{-8} M) on the log concentration-contractile response curve for norepinephrine in isolated rabbit aorta. Upper panel, the aorta was treated first with prazosin or its vehicle for 30 min followed by phenoxybenzamine or its vehicle for another 15 min. Middle panel, the aorta was treated first with phenoxybenzamine or its vehicle for 30 min followed by prazosin or its vehicle for another 15 min. Lower panel, the aorta was treated with prazosin, phenoxybenzamine or the combination of prazosin and phenoxybenzamine for 15 min. Maximal response to norepinephrine averaged 4.77 ± 0.14, 5.16 ± 0.12 and 4.6 ± 0.15. Values represent means ± S.E.M. and n = 6 per group.

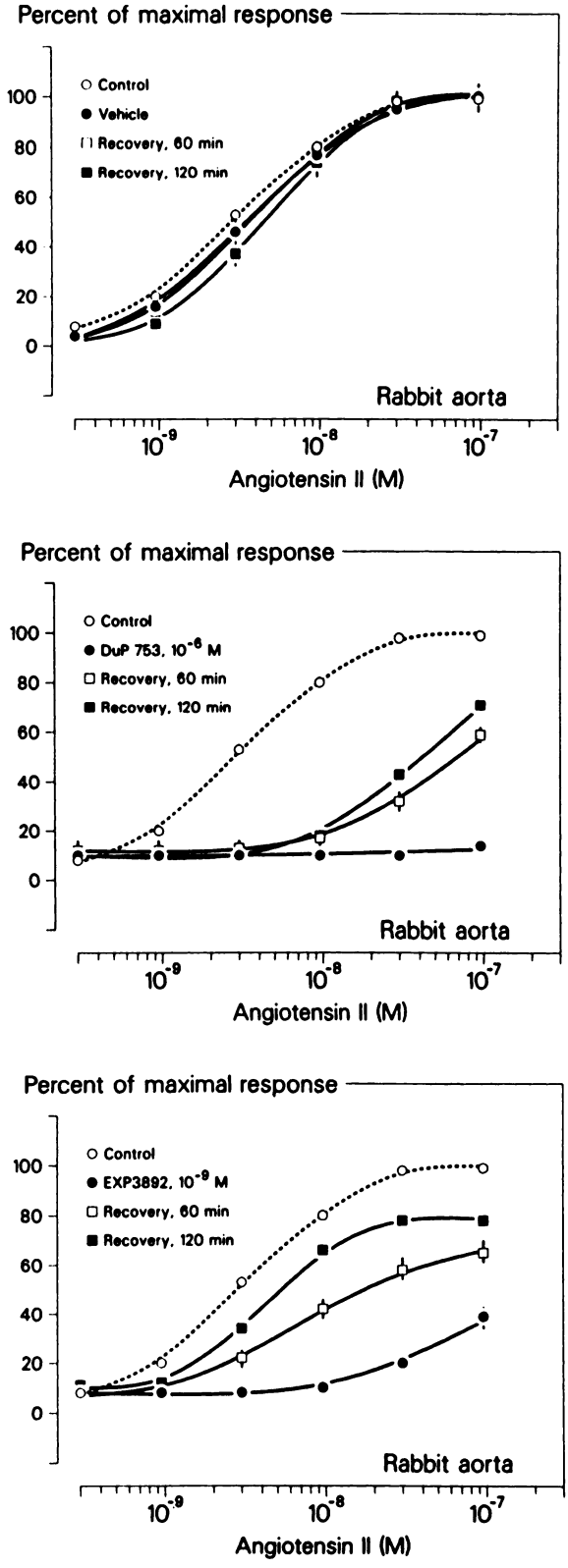


Fig. 5. Reversibility of the effects of vehicle, DuP 753(10^{-6} M) and EXP3892(10^{-9} M) on the log concentration-contractile response curve for AII in isolated rabbit aorta. A control concentration-response curve for AII was first determined. Each tissue was then washed, incubated with vehicle (upper panel), DuP 753 (middle panel) or EXP3892 (lower panel) for 15 min and restimulated with AII. Maximal response to AII averaged 2.04 ± 0.12 g. The tissues were washed and restimulated with AII at 60 min later. This procedure was repeated again 120 min later. Values represent means ± S.E.M. and n = 7–8 per group.

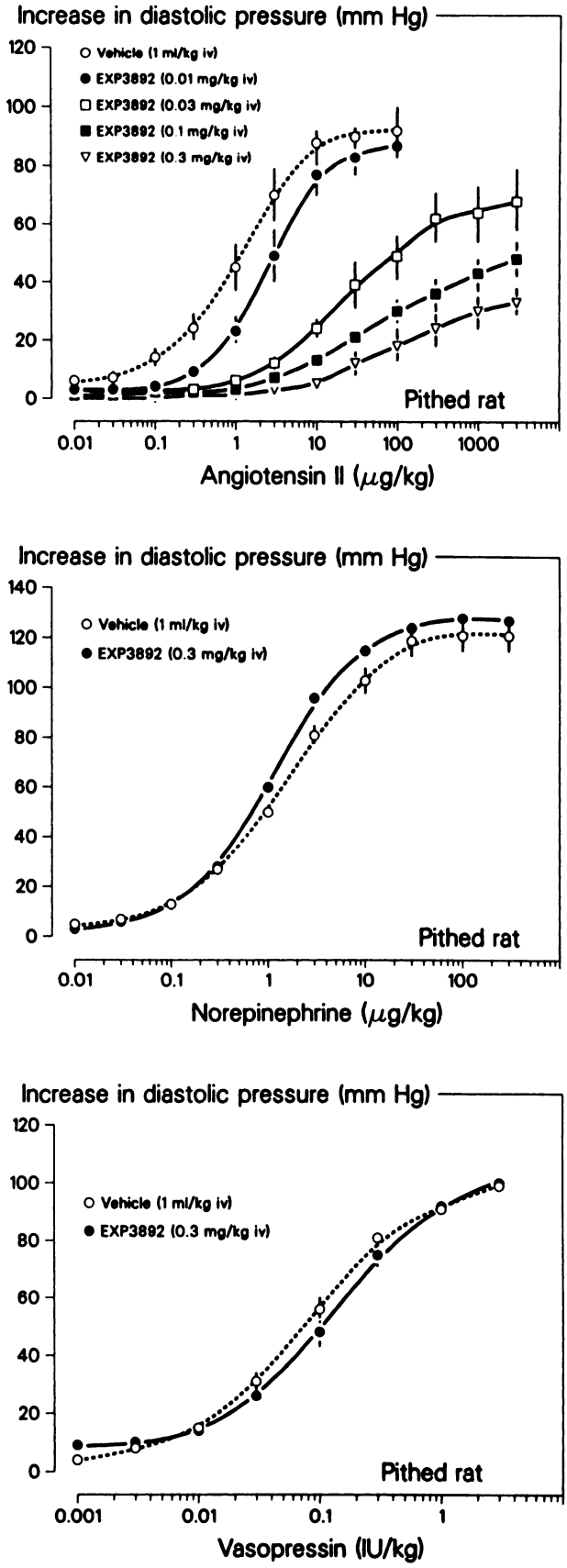
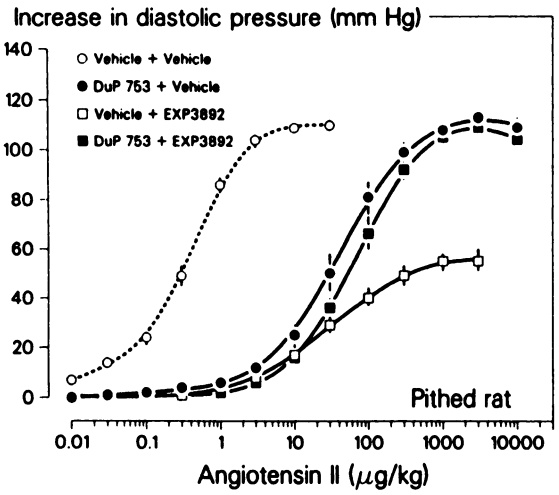
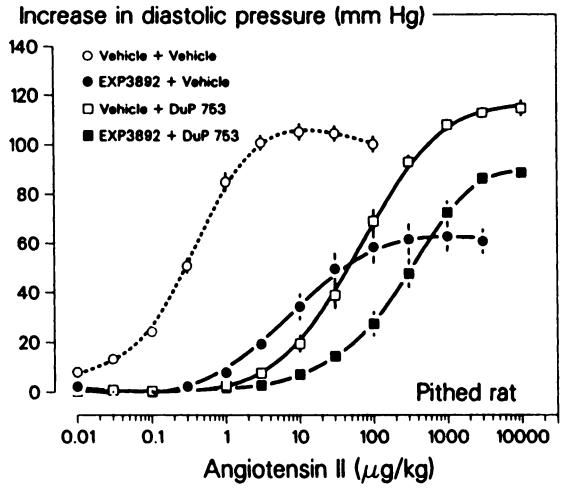


Fig. 6. Effects of EXP3892 on log dose-pressor response curves for AII (upper panel), norepinephrine (middle panel) and vaospressin (lower panel) in pithed rats. Values represent means ± S.E.M. and n = 4–6 per group.





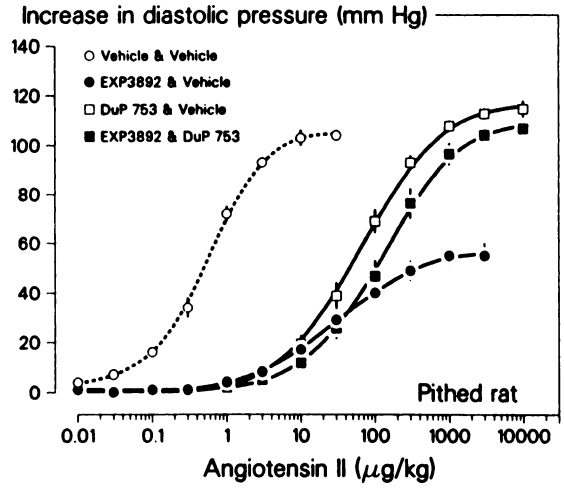
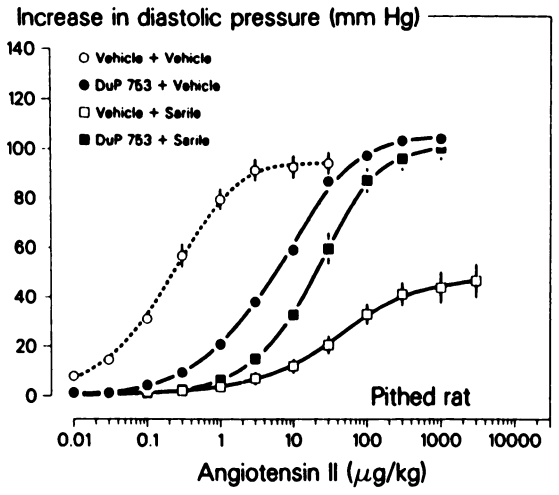
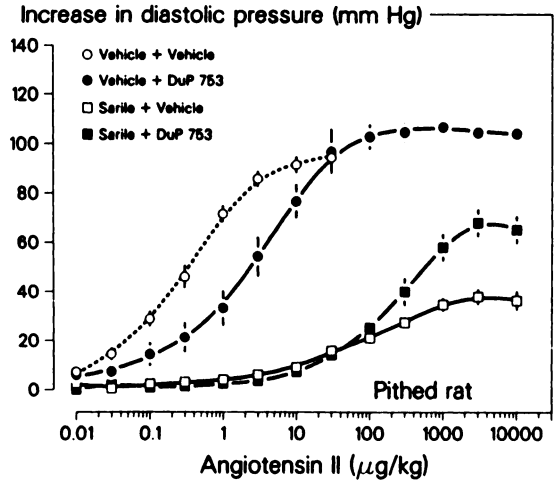


Fig. 7. Effects of DuP 753 (10 mg/kg i.v.), EXP3892 (0.1 mg/kg i.v.) and the combination of DuP 753 (10 mg/kg i.v.) and EXP3892 (0.1 mg/kg iv.) on the log dose-pressor response curve for AII in pithed rats. Upper panel, the rat was treated first with DuP 753 or its vehicle for 15 min followed by EXP3892 or its vehicle for another 15 min. Middle panel, the rat was treated first with EXP3892 or its vehicle for 15 min followed by DuP 753 or its vehicle for another 15 min. Lower panel, the rat was pretreated with the combinations of vehicle and vehicle, EXP3892 and vehicle, DuP 753 and vehicle or EXP3892 and DuP 753 for 15 min. Values represent means ± S.E.M. and n = 5–6 per group.





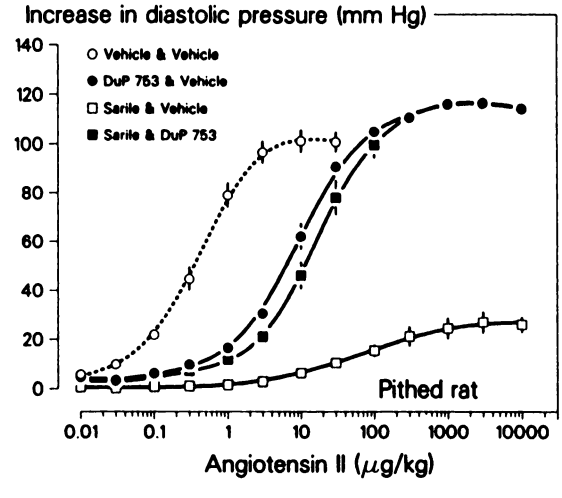


Fig. 8. Effects of DuP 753 (3 mg/kg i.v.), sarile, AII (10 μg/kg/min i.v.) and the combination of DuP 753 (3 mg/kg iv.) and sarile (10 μg/kg/min iv.) on the log dose-pressor response curve for AII in pithed rats. Upper panel, the rat was treated first with DuP 753 or its vehicle for 15 min followed by sarile or its vehicle for another 15 min. Middle panel, the rat was treated first with sarile or its vehicle for 15 min followed by DuP 753 or its vehicle for another 15 min. Lower panel, the rat was pretreated with the combinations of vehicle and vehicle, sarile and vehicle, DuP 753 and vehicle or sarile and DuP 753 for 15 min. Values represent means ± S.E.M. and n = 5–6 per group.

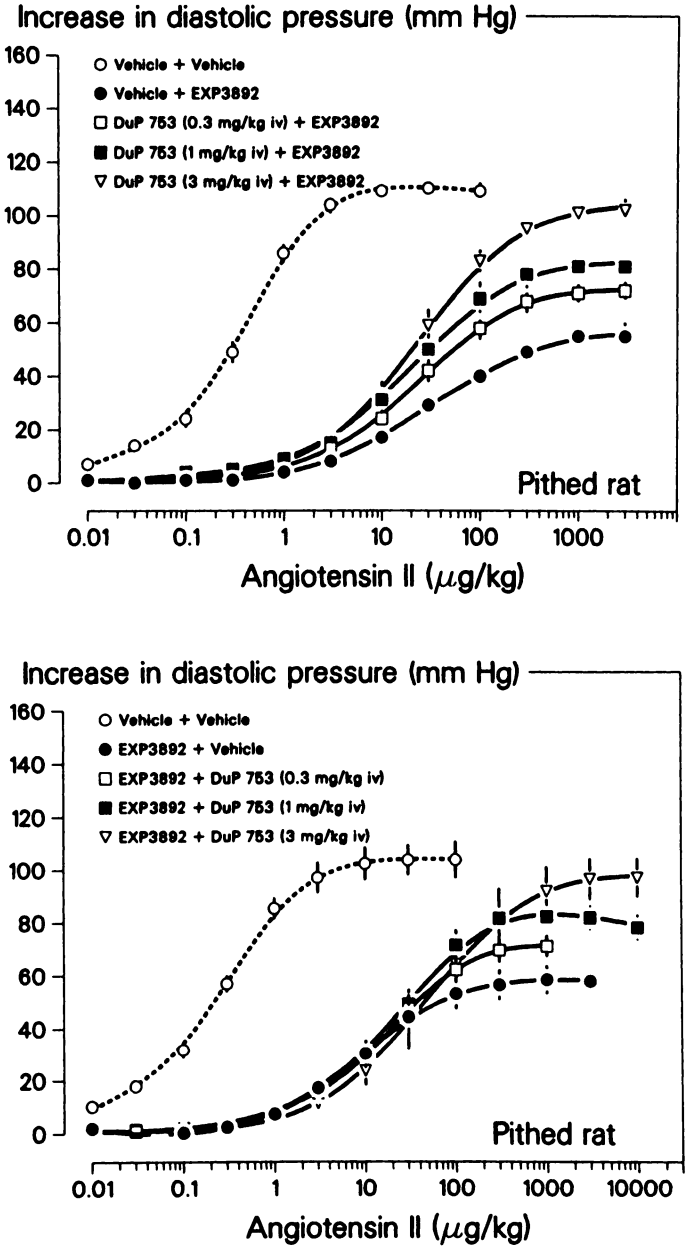


Fig. 9. Effects of the combinations of vehicle and vehicle, vehicle and EXP3892 (0.1 mg/kg i.v.) and the combination of DuP 753 (0.3 to 3 mg/kg i.v.) and EXP3892 (0.1 mg/kg i.v.) on the log dose-pressor response curve for AII in pithed rats. Upper panel, the rat was treated first with DuP 753 or its vehicle for 15 min followed by EXP3892 or its vehicle for another 15 min. Lower panel. the rat was treated first with EXP3892 or its vehicle for 15 min followed by DuP 753 or its vehicle for another 15 min. Values represent means ± S.E.M. and n = 5–6 per group.

described previously (Wong *et al.*, 1989). The carotid artery and the jugular vein were cannulated for arterial pressure measurement and i.v. administration of drug, respectively. Blood pressure was measured using a pressure transducer (model P23ID, Gould Inc., Oxnard, CA) coupled to a polygraph (model 7D, Grass Instrument Co.) and analyzed with a digital computer (Buxco Electronics, Inc.). Dose-pressor response curves for AII were generated as described previously (Wong *et al.*, 1989). Only one full dose-response curve was obtained in each rat.

Group 1. In testing for AII antagonism, 15 min before the injection of AII the animal was pretreated with vehicle or EXP3892 at 0.01, 0.03, 0.1 or 0.3 mg/kg i.v. To determine the specificity of EXP3892, dose-response curves for norepinephrine and vasopressin were also determined in pithed rats pretreated with vehicle or EXP3892 at 0.3 mg/kg i.v.

Group 2. To determine the interaction between DuP 753 and EXP3892, the animal was pretreated with DuP 753 (10 mg/kg i.v.) or its vehicle and EXP3892 (0.1 mg/kg i.v.) or its vehicle at 30 and 15 min before injection of AII, respectively. In the next series of experiments, the same protocol was repeated except that the order of administration of DuP 753(10 mg/kg i.v.) or its vehicle and EXP3892 (0.1 mg/kg i.v.) or its vehicle was reversed. In the third series of experiments, the animal was pretreated with DuP 753-vehicle and EXP3892-vehicle, EXP3892 (0.1 mg/kg i.v.) and DuP 753-vehicle, DuP 753 (10 mg/kg i.v) and EXP3892-vehicle and the combination of DuP 753 (10 mg/kg i.v.) and EXP3892 (0.1 mg/kg i.v).

Group 3. To study the interaction between DuP 753 and the noncompetitive peptide AII receptor antagonist, sarile, the same protocol used above (Group 2) was followed except that DuP 753 at 3 mg/kg i.v. and sarile at 10 μg/kg/min i.v. were used instead of DuP 753 at 10 mg/kg i.v. and EXP3892 at 0.1 mg/kg i.v., respectively.

Group 4. The same protocol used above (Group 2) was followed except that the effects of various doses of DuP 753 (0.1 to 3 mg/kg) given either before or after EXP3892 at 0.1 mg/kg i.v. were examined.

Statistics. Statistical analyses used were linear regression, analysis of variance and Duncan's new multiple-range test for multiple comparison (Cody and Smith, 1987). These analyses were carried out by a computer package, Statistical Analysis System (SAS Institute Inc., Cary, NC), in a VAX 8800 computer. The level of significance was taken at $P < .05$. All data were expressed as means ± S.E.M.

Drugs. AII, norepinephrine, phenoxybenzamine, sarile and vasopressin were obtained from Sigma Chemical Company (St. Louis, MO). Prazosin was obtained from Pfizer Inc. (Groton, CT). DuP 753 and EXP3892 (Carini *et al.*, 1989) were synthesized at the Du Pont Merck Pharmaceutical Company (Wilmington, DE). AII, DuP 753, norepinephrine, phenoxybenzamine and prazosin were dissolved in Krebs' buffer for *in vitro* experiments or in saline for *in vivo* experiments. EXP3892 was dissolved in a mixture of 5% $NaHCO_3$-5% dextrose (50:50) at 1 mg/ml and diluted to the desired concentration with Krebs' buffer for *in vitro* experiments and with 5% dextrose for *in vivo* experiments. Sarile was dissolved in saline.

Results

Effects on AII-Induced Contractile Response in Rabbit Aorta

In the rabbit aorta, EXP3892 at 10^{-10} to 10^{-7} M caused nonparallel shifts to the right of the log concentration-contractile response curve for AII and reduced the maximal response to AII by 50 to 60% (fig. 2). The calculated K_B of EXP3892 was 2.6×10^{-11} M. At 10^{-6} M, EXP3892 did not change the concentration-response curves for norepinephrine or KCl (fig. 2).

As shown in figure 3, DuP 753 at 10^{-6} M shifted the concentration-contractile response curve for AII to the right in a parallel fashion without altering the maximal response to AII. EXP3892 at 10^{-9} M caused the expected nonparallel shift of the concentration-response for AII to the right and depressed the AII-maximal response. Pretreatment of the aorta with DuP 753 at 10^{-6} M restored totally the reduced AII maximal response induced by EXP3892 at 10^{-9} M (fig. 3, upper panel). When DuP 753 was added after EXP3892, the reduced AII-maximal response was restored partially (fig. 3, middle panel). Total reversal of the reduced AII-maximal response was also observed when DuP 753 was added concomitant with EXP3892 (fig. 3, lower panel).

Pretreatment of the aorta with prazosin at 3×10^{-8} M for 30 min before the addition of phenoxybenzamine at 3×10^{-8} M or addition of both antagonists at the same time reduced the

phenoxybenzamine-induced depressed norepinephrine-maximal response (fig. 4). However, prazosin added 30 min after phenoxybenzamine did not restore the reduced norepinephrine-maximal response (fig. 4, middle panel).

The reversible nature of the inhibitory effects of DuP 753 at 10^{-6} M and EXP3892 at 10^{-9} M on the contractile response to AII is shown in figure 5. Compared to the vehicle, the inhibitory effects of DuP 753 and EXP3892 on the response to AII were still evident after the tissue had been washed for 60 to 120 min (fig. 5).

Effects on AII-Induced Pressor Response in Pithed Rats

In the pithed rat, EXP3892 at 0.01 to 0.3 mg/kg i.v. shifted the log dose-pressor response curve for AII dose-dependently to the right and reduced the maximal pressor response to AII (fig. 6). At 0.3 mg/kg i.v., EXP3892 did not alter the dose-response curves for norepinephrine and vasopressin (fig. 6). The mean diastolic blood pressures for the vehicle group and the 0.01, 0.03, 0.1 and 0.3 mg of EXP3892 per kg were 39 ± 2, 38 ± 2, 25 ± 1, 25 ± 1 and 22 ± 1 mm Hg, respectively. Compared to the vehicle-group, EXP3892 significantly decreased diastolic blood pressure at 0.03 to 0.3 mg/kg i.v. ($P < .05$).

As shown in figure 7, DuP 753 at 10 mg/kg i.v. shifted the pressor-response curve for AII to the right in a parallel fashion without altering the maximal response to AII. EXP3892 at 0.1 mg/kg i.v. caused the expected nonparallel shift of the pressor-response for AII to the right and depressed the AII-maximal response. Depending on the sequence of the injections of DuP 753 and EXP3892, the reduced AII-maximal response induced by EXP3892 was either restored, partially or totally, by DuP 753 (fig. 7). The mean diastolic blood pressure in the groups of vehicle and vehicle, DuP 753 and vehicle, vehicle and EXP3892 and DuP 753 and EXP3892 were 42 ± 1, 28 ± 2, 32 ± 3 and 28 ± 2 mm Hg, respectively (fig. 7, upper panel). The mean diastolic blood pressure in the groups of vehicle and vehicle, EXP3892 and vehicle, vehicle and DuP 753 and EXP3892 and DuP 753 were 46 ± 2, 29 ± 2, 32 ± 3 and 32 ± 2 mm Hg, respectively (fig. 7, middle panel). The mean diastolic blood pressure after coadministration of vehicle and vehicle, vehicle and EXP3892, vehicle and DuP 753 and EXP3892 and DuP 753 were 44 ± 1, 32 ± 3, 32 ± 3 and 28 ± 1 mm Hg, respectively (fig. 7, lower panel). The diastolic blood pressures of all the antagonist-treated groups described above were significantly different from that of the corresponding vehicle-treated group ($P < .05$).

Sarile at 10 μg/kg/min also reduced the maximal pressor effect of AII (fig. 8). DuP 753 at 3 mg/kg i.v., injected before or after sarile, restored partially or totally the depressed AII-maximal pressor effect induced by sarile (fig. 8). The mean diastolic blood pressures in groups treated with vehicle and vehicle, DuP 753 and vehicle, vehicle and sarile and DuP 753 and sarile (fig. 8, upper panel) were 50 ± 2, 31 ± 2, 32 ± 2 and 32 ± 4 mm Hg, respectively. The mean diastolic blood pressures in groups treated with vehicle and vehicle, vehicle and DuP 753, sarile and vehicle and sarile and DuP 753 (fig. 8, middle panel) were 47 ± 2, 35 ± 2, 38 ± 3 and 27 ± 2 mm Hg, respectively. The mean diastolic blood pressures in groups treated with vehicle and vehicle, DuP 753 and vehicle, sarile and vehicle and sarile and DuP 753 (fig. 8, lower panel) were 42 ± 1, 30 ± 2, 29 ± 2 and 32 ± 2 mm Hg, respectively. The diastolic blood pressures in all the antagonist-treated groups described above were significantly different from that of their corresponding vehicle-treated group ($P < .05$).

The depressed maximal response induced by EXP3892 at 0.1 mg/kg was dose-dependently reversed by DuP 753 at 0.3, 1 and 3 mg/kg i.v., which was given either before or after EXP3892 (fig. 9). The mean diastolic blood pressures in the groups treated with vehicle and vehicle, vehicle and EXP3892, DuP 753 (0.3 mg/kg) and EXP3892, DuP 753 (1 mg/kg) and EXP3892 and DuP 753 (3 mg/kg) and EXP3892 (fig. 9, upper panel) were 42 ± 1, 32 ± 3, 30 ± 1, 34 ± 23 and 30 ± 2 mm Hg, respectively. The mean diastolic blood pressures in the groups treated with vehicle and vehicle, EXP3892 and vehicle, EXP3892 and DuP 753 (0.3 mg/kg), EXP3892 and DuP 753 (1 mg/kg) and EXP3892 and DuP 753 (3 mg/kg) (fig. 9, lower panel) were 46 ± 1, 34 ± 3, 28 ± 1, 27 ± 2 and 27 ± 1 mm Hg, respectively. The diastolic blood pressures of all antagonist-treated groups described above were significantly different from that of their corresponding vehicle-treated group ($P < .05$).

Discussion

The present study demonstrates that EXP3892 caused nonparallel shifts to the right of the log dose-response curve for AII and reduced the maximal response to AII in the rabbit aorta and in pithed rats, suggesting insurmountable antagonism. In contrast, DuP 753 shifted the AII dose-response curve to the right in a parallel fashion and did not alter the AII-maximal response (Chiu *et al.*, 1990; Wong *et al.*, 1990a). By using the double-reciprocal regression for K_B determination as described by Kenakin (1987) for insurmountable antagonists, we calculated that the K_B for EXP3892 in the rabbit aorta was 2.6×10^{-11} M, indicating that this compound is about 4- and 127-fold more potent than the previously reported insurmountable AII antagonist EXP3174 and the surmountable AII antagonist DuP 753, respectively (Wong *et al.*, 1990b,e). It should be noted that the inhibitory effect of EXP3892 on the response to AII is specific, because even at a high concentration or dose EXP3892 did not change the concentration-contractile response curves for norepinephrine and KCl *in vitro* and the dose-response curves for norepinephrine and vasopressin *in vivo*. Similar to our previously reported nonpeptide AII receptor antagonists, EXP3892 also lacks agonistic activity *in vitro* as well as *in vivo* (data not shown).

A variety of mechanisms may contribute to the insurmountable antagonism exerted by EXP3892. Insurmountable antagonism may be attributed to the kinetics of the antagonists interacting with receptors, *i.e.*, forming a covalent bond with the receptor (irreversible antagonism) or dissociating very slowly from the receptor (pseudoirreversible antagonism). It is not likely that EXP3892 is covalently linked to the AII receptors because DuP 753 reversed its reduced AII-maximal response *in vitro* as well as *in vivo*. In contrast, prazosin, a competitive *alpha*-1 adrenergic receptor antagonist, added after the aorta was treated with phenoxybenzamine, an *alpha* adrenergic-receptor-alkylating agent, did not restore the reduced norepinephrine-maximal response in rabbit aorta. Pseudoirreversible antagonism may account for the insurmountable antagonism inasmuch as repeated washing of the rabbit aorta treated with EXP3892 at 10^{-9} M did not restore the sensitivity to AII completely for at least 2 hr, suggesting that EXP3892 is a slowly dissociating antagonist. Paradoxically, repeated washing of the rabbit aorta treated with DuP 753 at 10^{-6} M also did not

restore totally the sensitivity to AII for at least 2 hr. The observations that DuP 753 and EXP3892 are both slowly dissociating antagonists and that EXP3892, but not DuP 753, produced insurmountable AII antagonism indicate that pseudoirreversible inhibition is not the explanation for the insurmountable antagonism by EXP3892.

This study shows that DuP 753, EXP3892 and sarile lowered blood pressure in the pithed rat, which is attributed to the blockade of the vasoconstrictor effect of AII by these antagonists as the plasma renin activity is high in the pithed rat (Wong *et al.*, 1990a). Because DuP 753, EXP3892 or the combined treatment of DuP 753 and EXP3892 decreased basal diastolic blood pressure to a similar degree in the pithed rat, it is not likely that vasodilatation *per se* accounts for the insurmountable AII antagonism induced by EXP3892. Furthermore, the insurmountable AII antagonism was also observed in isolated rabbit aortic helical strips whose basal tension was not altered by the antagonists. Besides acting as a direct vasoconstrictor, AII also stimulates the synthesis of vasodilating substances such as prostaglandin and endothelium-derived relaxing factor (Peach, 1988). Thus, the vasoconstrictor effect of AII possibly represents a net effect of vasoconstriction and vasodilatation. Consequently, it is conceivable that EXP3892 blocks the AII receptors mediating vasoconstriction greater than those mediating synthesis of vasodilating substances with a resultant insurmountable antagonism. However, the removal of endothelium does not affect the contractile response to AII in the rabbit aorta (Saye *et al.*, 1984). As the EXP3892-mediated insurmountable AII antagonism was observed in the rabbit aorta, the endothelium-derived relaxing factor is not likely to be involved in this insurmountable AII antagonism. Like EXP3892, sarile also induced insurmountable AII antagonism which was reversed by DuP 753. As sarile inhibits both the direct vasoconstriction and prostaglandin release induced by AII in rabbit mesentery artery (Blumberg *et al.*, 1977), the AII-induced vasodilating prostaglandin release is not likely to be involved in the insurmountable AII antagonism induced by sarile and possibly EXP3892.

Kaufmaun and colleagues (Kaumann and Frenken, 1985; Lemoine and Kaumann, 1986; Frenken and Kaumann, 1987) attempted to explain the insurmountable antagonism induced by methysergide and its reversal by ketanserin with a two state-allosteric model of serotonin receptor regulation (see Introductory section). Because they observed a persistent residual response to serotonin, regardless of the concentration of methysergide, they proposed that the allosteric site is distinct from the serotonin recognition site. This view is supported by Xu and Purdy (1988) who reported that the serotonin dose-response curves in the presence of ketanserin alone and in the presence of ketanserin as well as 10^{-9} M of 2-brom-*d*-lysergic acid diethylamide, a methysergide-like compound, were superimposable, implying that 2-brom-*d*-lysergic acid diethylamide does not bind to the serotonin receptor. On the contrary, when the rabbit aorta or pithed rat was treated first with EXP3892 followed by DuP 753, there was a further rightward shift of the AII dose-response curve than that treated with DuP 753 alone (the middle panels of figs. 3 and 7). Our results, therefore, cannot exclude that EXP3892 does not bind to the AII receptor.

de Chaffoy de Courcelles and associates (1986) proposed a receptor-transducer coupling model to explain the insurmountable antagonism, which appears to be more consistent with our data than the Kaumann model. According to this model, two binding sites are postulated: one (R_{out}) is located at the outer side of the plasma membrane for the agonist and antagonist, and the other (R_{in}) is located at the inner side of the membrane for the coupling factor of the receptor. By binding to R_{out}, a surmountable antagonist such as DuP 753 would induce conformational changes resulting in a reduction of the affinity of R_{in} for the coupling factor, whereas an insurmountable antagonist such as EXP3892 would diminish the binding capacity for the coupling factor. This model assumes that the conformational changes induced by antagonists are slowly reversible. Furthermore, the surmountable and insurmountable antagonists can displace each other competitively and reverse the conformation changes induced by each other. That DuP 753 and EXP3892 compete for a common site is supported by the receptor protection experiment that pretreatment with DuP 753 protected against insurmountable antagonism induced by EXP3892 in a dose-dependent manner and *vice versa*. Although this model assumes an agonist and an antagonist can displace each other competitively at R_{out}, addition of AII after pretreatment with EXP3892 cannot immediately reverse the insurmountable antagonism because the conformational changes in R_{in} induced by EXP3892 are slowly reversible.

In conclusion, considerable progress has been made in developing surmountable and insurmountable nonpeptide AII receptor antagonists. We observed that insurmountable antagonism to AII by EXP3892 can be reversed by a surmountable antagonist DuP 753. Similarly, we as well as others (Wienen *et al.*, 1990) have also observed that insurmountable AII antagonism by the peptide AII receptor antagonist sarile is reversed by DuP 753. To account for this paradox, we adapted the model proposed by de Chafoy de Courcelles *et al.* (1986) to explain insurmountable antagonist effects. Although this model is consistent with our findings, conceivably there may be other explanations accounting for this phenomenon. Furthermore, insurmountable antagonism may not be limited to a single mechanism and may be influenced by factors such as the agonist/antagonist used, tissues, species and experimental conditions (Bond *et al.*, 1989).

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