Original Contributions

DuP 753 Is a Specific Antagonist for the Angiotensin Receptor

Nour-Eddine Rhaleb, Noureddine Rouissi, François Nantel, Pedro D'Orléans-Juste, and Domenico Regoli

2-n-Butyl-4-chloro-5-hydroxy-methyl-1-[(2'-1H)-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazol potassium salt (DuP 753) is a nonpeptide angiotensin II receptor antagonist that inhibits the contractile effects of angiotensin II competitively and shows pA2 values of 8.27 on the rabbit aorta and jugular vein, 8.66 on the rat portal vein and stomach, 8.19 on the rat urinary bladder, and 8.36 on human colon, ileum, and urinary bladder. This agent (more than 10-7 M) exhibits no agonistic activity and does not affect the contractile effects of norepinephrine, acetylcholine, bradykinin, desArg9-bradykinin, substance P, neurokinin A, neurokinin B, or bombesin in the various tissues. The present results demonstrate that DuP 753 is a potent nonpeptide antagonist with high affinity, specificity, and selectivity for the angiotensin receptor. (Hypertension 1991;17:480-484)

DuP 753 (2-n-butyl-4-chloro-5-hydroxy-methyl-1-[(2'-1H)-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazol potassium salt) is a nonpeptide antagonist of angiotensin II (Ang II) that is orally active and fairly specific for Ang II. DuP 753 shows a similar pharmacological spectrum as EXP 6803 and EXP 6155, two other antagonists previously reported. In fact, DuP 753 does not inhibit either the pressor effects of norepinephrine or vasopressin or the depressor effect of bradykinin in anesthetized rats. The compound has also been shown to reduce blood pressure in furosemide-treated normotensive rats or in animals affected by various forms of experimental or spontaneous hypertension. In the present study, DuP 753 was tested in vitro on selective peptide monoreceptor systems to determine the extent of its specificity for the angiotensin receptor and exclude all possible interference with receptors for kinins (B1 and B2), neurokinins (NK-1, NK-2, NK-3), bombesin (BB1 and BB2), and the α-adrenergic and the muscarinic receptors.

Methods

Male Sprague-Dawley rats (150-200 g) and Albino New Zealand rabbits (1-1.5 kg) were killed by stunning and exsanguination. The aorta, jugular vein, and pulmonary artery of the rabbits, the stomach, urinary bladder, and the portal vein of the rats were rapidly removed from the animals and kept in oxygenated 95% O2-5% CO2 Tyrode's (for the stomach and urinary bladder) or Krebs' (for the isolated vessels) solutions. Strips of the rabbit veins were prepared according to Gaudreau et al; the rat portal vein was prepared according to Mastrangelo et al; strips of the rat stomach as well as strips of the rat urinary bladder were prepared according to Vane. The human tissues were taken from different donors of both sexes (age range, 40-65 years). The human urinary bladder was prepared according to Dion et al. The longitudinal strips of human ileum and colon were prepared by the method described by Crema et al and modified by Couture et al. The strips were suspended in 10-ml organ baths filled with oxygenated Krebs' (at 37°C) or Tyrode's (at 32°C) solutions under a tension of 4.0 g (human urinary bladder), 2.0 g (rabbit aorta, rat stomach, human ileum, human colon), 1.0 g (rabbit pulmonary artery, rat urinary bladder), or 0.5 g (rabbit jugular and rat portal-mesenteric vein). Changes of tension produced by various agents were recorded with isometric transducers (model FT 03C, Grass Instrument Co., Quincy, Mass.) calibrated as to 1 g=30 mm on polychannel recorders (model 7D, Grass). After an equilibration period of 60-120 minutes, during which the tissues were washed and the tension adjusted.

From the Department of Pharmacology, Medical School University of Sherbrooke, Sherbrooke, Quebec, Canada.

The work was performed with the financial support of the Medical Research Council of Canada (M.R.C.C.). N.E.R. and P.D.J. are, respectively, student and scholar of the Heart and Stroke Foundation of Canada. F.N. is a student of the Georges Phe'nix Foundation and D.R. is a Career Investigator of the M.R.C.C.

Address for correspondence: Dr. Domenico Regoli, Department of Pharmacology, Medical School, University of Sherbrooke, Sherbrooke, Quebec, Canada J1H 5N4.

Received October 1, 1990; accepted November 16, 1990.
TABLE 1. Antagonist Effects of DuP 753 Against Angiotensin II in Six Isolated Organs and Absence of Antagonistic Effect by DuP 753 Against Other Peptide or Nonpeptide Agonists

<table>
<thead>
<tr>
<th>Tissue</th>
<th>pA2</th>
<th>pA10</th>
<th>Δ</th>
<th>Agonist (nM)</th>
<th>Control</th>
<th>+DuP 753 (21.7 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit aorta</td>
<td>8.27</td>
<td>7.19</td>
<td>1.08</td>
<td>DesArg²-BK (27.7)</td>
<td>17.2±2.8</td>
<td>21.3±3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NE (12.2)</td>
<td>46.8±4.3</td>
<td>48.0±2.1</td>
</tr>
<tr>
<td>Rabbit jugular vein</td>
<td>8.27</td>
<td>7.33</td>
<td>0.94</td>
<td>SP (6.5)</td>
<td>6.8±0.7</td>
<td>6.6±0.7</td>
</tr>
<tr>
<td>Rabbit pulmonary artery</td>
<td>8.27</td>
<td>7.33</td>
<td>0.94</td>
<td>NKA (4.4)</td>
<td>26.3±5.5</td>
<td>24.4±4.5</td>
</tr>
<tr>
<td>Rat portal vein</td>
<td>8.66</td>
<td>7.66</td>
<td>1.00</td>
<td>NE (72.9)</td>
<td>23.6±5.3</td>
<td>23.7±5.5</td>
</tr>
<tr>
<td>Rat stomach</td>
<td>8.66</td>
<td>7.63</td>
<td>1.03</td>
<td>NKB (20.7)</td>
<td>15.3±1.7</td>
<td>13.3±1.9</td>
</tr>
<tr>
<td>Rat urinary bladder</td>
<td>8.19</td>
<td>7.27</td>
<td>0.92</td>
<td>BB (6.2)</td>
<td>16.0±1.5</td>
<td>14.8±1.9</td>
</tr>
<tr>
<td>Human urinary bladder</td>
<td>8.36</td>
<td>7.49</td>
<td>0.87</td>
<td>Ach (550.4)</td>
<td>351.3±57</td>
<td>350.0±60</td>
</tr>
<tr>
<td>Human colon</td>
<td>8.36</td>
<td>7.49</td>
<td>0.87</td>
<td>NKA (88.2)</td>
<td>56.0±1.2</td>
<td>54.0±6.1</td>
</tr>
<tr>
<td>Human ileum</td>
<td>8.36</td>
<td>7.41</td>
<td>0.95</td>
<td>Ach (550.4)</td>
<td>14.4±2.9</td>
<td>13.2±2.9</td>
</tr>
</tbody>
</table>

pA2 and pA10 are log of the molar concentration of antagonist that reduces the effect of a double (pA2) or 10 times (pA10) higher concentration of agonist (angiotensin II) to that of a single one. Values in parentheses are the nanomolar concentration of the agonists. \( \Delta \) is the difference between pA2 and pA10. Values of response are mean±SEM of at least five determinations and indicate contraction in millimeters (30 mm=1 g). BK, bradykinin; NE, norepinephrine; SP, substance P; NKA, neurokinin A; BB, bombesin; Ach, acetylcholine.

Results

Specificity of DuP 753 for the Angiotensin Receptor

Under the experimental conditions described above, DuP 753 exerted a potent antagonistic effect against Ang II in the nine isolated organs without influencing the effects of the other peptide or nonpeptide stimulators (Table 1). Thus, DuP 753 was found to be inactive as stimulant and as antagonist 1) in the rabbit aorta on the B1 receptor, which is activated by desArg²-BK,11 and on the \( \alpha \)-adrenergic receptor, 2) in the rabbit jugular vein on the kinin B211 and the substance P (NK-1) receptors, 3) on the rabbit pulmonary artery on the neurokinin A (NK-2) and the \( \alpha \)-adrenergic receptors, 4) in the rat portal vein on the neurokinin B (NK-3) and the \( \alpha \)-adrenergic receptors, 5) in the rat stomach on the bombesin (BB2)12 and the muscarinic receptors, 6) in the rat urinary bladder on the bombesin (BB3)12 and the muscarinic receptors, and 7) in human preparations, namely human urinary bladder, colon, and ileum on the muscarinic receptors. As shown in Table 1, no significant differences were observed between the effects of the various agents other than obtained in the absence (control) and in the presence of DuP 753.

Activity of DuP 753 on Angiotensin Receptor in Various Organs

The pA2 values measured with DuP 753 against Ang II in the various organs were very similar, in fact

every 15 minutes, the contractile agents were applied in increasing concentrations to build complete concentration–response curves. Thereafter, average concentrations (producing approximately 70% of the maximal response) of each agent were tested in the absence and in the presence of DuP 753, which had been applied 10 minutes before. In this way, pA2 and pA10 values were measured. In the rabbit aorta, cumulative concentration–response curves were recorded for Ang II in the absence and in the presence of increasing concentrations of the antagonist (from 10.8 nM to 54.2 μM) to build a Schild plot.

Results obtained with DuP 753 against Ang II are presented in terms of pA2 and pA10, whereas for the other agonists the mean±SEM of the contractions recorded in the absence and in the presence of the antagonist are compared. Significance of differences between controls and experimental (recorded in the presence of DuP 753) values are evaluated with Student’s t test for paired samples. Probability values lower than 0.05 were considered significant.

Bradykinin (BK), desArg²-bradykinin (desArg²-BK), substance P, neurokinin A, and neurokinin B were prepared in our laboratory by the solid-phase method10; Ang II and bombesin were purchased from Bachem, acetylcholine and norepinephrine from Sigma Chemical Co., St. Louis, Mo., ascorbic acid and sulfolane from Anaehemica, Lachine, Quebec, Canada. DuP 753 was a generous gift of Dr. R.D. Smith, Du Pont Co., Wilmington, Del. Concentrated solutions (1–5 mg/ml) of peptides, acetylcholine, and DuP 753 were prepared in water and that of neurokinin B in 10% sulfolane. Solutions of norepinephrine were made in water containing 0.5% ascorbic acid.

Results

Specificity of DuP 753 for the Angiotensin Receptor

Under the experimental conditions described above, DuP 753 exerted a potent antagonistic effect against Ang II in the nine isolated organs without influencing the effects of the other peptide or nonpeptide stimulators (Table 1). Thus, DuP 753 was found to be inactive as stimulant and as antagonist 1) in the rabbit aorta on the B1 receptor, which is activated by desArg²-BK,11 and on the \( \alpha \)-adrenergic receptor, 2) in the rabbit jugular vein on the kinin B211 and the substance P (NK-1) receptors, 3) on the rabbit pulmonary artery on the neurokinin A (NK-2) and the \( \alpha \)-adrenergic receptors, 4) in the rat portal vein on the neurokinin B (NK-3) and the \( \alpha \)-adrenergic receptors, 5) in the rat stomach on the bombesin (BB2)12 and the muscarinic receptors, 6) in the rat urinary bladder on the bombesin (BB3)12 and the muscarinic receptors, and 7) in human preparations, namely human urinary bladder, colon, and ileum on the muscarinic receptors. As shown in Table 1, no significant differences were observed between the effects of the various agents other than obtained in the absence (control) and in the presence of DuP 753.

Activity of DuP 753 on Angiotensin Receptor in Various Organs

The pA2 values measured with DuP 753 against Ang II in the various organs were very similar, in fact
almost identical (between 8.19 and 8.27) for four of the nine preparations, slightly higher (8.66) for the rat stomach and portal vein, where active metabolic degradation of the peptide agonist might favor the metabolically resistant nonpeptide antagonist (unpublished observations from our laboratory). Interestingly enough, the three human tissues investigated in the present experiment showed pA2 values very similar to those of the animal preparations. These findings indicate that the receptor for angiotensin in arterial and venous vessels as well as in the rat and human gastrointestinal and urinary systems is the same. The data shown in Table 1 also suggest that DuP 753 exerts a competitive antagonism against Ang II since the difference between pA2 and pA10 values is near to 1.0 in all preparations according to Schild.13 It is also worthy of mention that no agonistic effect was observed in any organ with high concentrations (10^-5 M) of DuP 753 (Figure 1). Moreover, such high concentrations of DuP 753 completely blocked the myotropic effects of Ang II on the rabbit aorta and jugular vein without interfering with the responses of these tissues to desArg9-BK or BK and substance P, respectively (Figure 1).

**Competitiveness of DuP 753 for Angiotensin**

To demonstrate that DuP 753 is indeed a competitive antagonist for angiotensin, concentration–response curves were measured with Ang II on the rabbit aorta, in the absence and in the presence of five concentrations (from 10.8 nM to 54.1 μM) of DuP 753. The results are illustrated in Figure 2 by showing the curves on panel A and the Schild plot on panel B. Thus, the curves of angiotensin are displaced to the right by the antagonist; the curves are parallel to the control and the maximum effect of angiotensin is obtained in the presence of antagonist at the five concentrations used. Figure 2A illustrates the data plotted according to Schild.14 Individual values fit on a straight line with a slope not significantly different from a unity (0.96) and a pA2 value of 8.33 extrapolated at the intersection of the line with the abscissa, which corresponds to the pA2 (8.27) of DuP 753 measured experimentally.

The antagonistic effect of DuP 753 is completely reversible in 20–30 minutes for the low (5.5 nM) and 60–90 minutes for the high (0.54 μM) concentration of antagonist (results not shown).

**Discussion**

The results presented above demonstrate that the nonpeptidic angiotensin antagonist developed by the Du Pont Company is selective for the angiotensin receptor and does not interact with the receptors for...
other peptides and biologically active amines. These results confirm the findings obtained by Wong et al in anesthetized rats against BK and noradrenaline; they extend the characterization of DuP 753 to other receptor systems such as the B₁ for the kinins, the three receptors for the neurokinins, the two receptors for bombesin, and the cholinergic muscarinic receptor. The present data have all been obtained in vitro, using monoreceptor systems that have been identified and characterized in recent years. The contractile responses of the various preparations (the arteries, the veins, the stomach, and urinary bladder) appear to be due to the direct activation of receptors by the various agents, since the potential contributions of a large number of other endogenous stimulants have been excluded by systematic specificity studies. It is therefore suggested that the competition between Ang II and DuP 753 occurs, in the various tissues, primarily at the level of the smooth muscle fibers, where the angiotensin receptor appears to be localized. The majority of the preparations used in the present study are isolated vessels, which are stimulated by Ang II and by at least one of the other peptides. One interesting example is the rabbit jugular vein, whose contractions in response to very similar concentrations of Ang II, bradykinin, and substance P appear to be due to the activation of the same intracellular mechanism, that is, phosphatidylinositol (PI₃) turnover. Thus, the selective antagonist exerted by DuP 753 against angiotensin must occur at the receptor site and may not involve any postreceptor mechanism, even if DuP 753 would enter into the cells.

Affinities of DuP 753 in the various preparations, evaluated in terms of pA₂, are very similar; pA₂ values varied between 8.19 (the rat urinary bladder)
and 8.66 (the rat stomach and the rat portal vein), were the same (8.27) in three of the nine preparations, and 8.36 in the three human tissues. There is no doubt that the angiotensin receptor is the same in large arteries, large veins, and in the gastrointestinal and urinary systems from both humans and other mammals. It is therefore concluded that DuP 753 reduces or blocks the actions of angiotensin in several peripheral organs; DuP 753 is, however, inactive on one of the two angiotensin binding sites in the adrenal glands.20 Despite the multiplicity of peripheral effects, the major target of DuP 753 is the arterial resistance vessels, where angiotensin exerts its major physiological role in the regulation of blood pressure. Similarities of pA2 values in the various organs suggest that diffusion barriers, metabolism, and the local redistribution of DuP 753 may not play any major role for the interaction of the antagonists with the receptor. Apparent affinities for angiotensin and its antagonist expressed by the pD2 and pA2 values, may therefore be closed to the real affinities.21 pD2 values of Ang II and pA2 of DuP 753, as measured in two of the most sensitive preparations, the rabbit jugular vein and the rabbit aorta (Table 1), indicate that the affinity of the antagonist is very similar to that of the naturally occurring peptide. It is therefore suggested that angiotensin and DuP 753 interact in a similar way with the receptor site. The analysis of the antagonism, performed on the rabbit aorta by using a wide range of concentrations, indicates that DuP 753 interacts with angiotensin at the receptor level in a competitive manner. Indeed, the difference between pA2 and pA10 and the slope of the Schild plot presented in Figure 2 are near to unity. Concentration–response curves obtained with angiotensin in the presence of increasing concentrations of DuP 753 are parallel to the control curve, and the maximum effect is reached even in the presence of very high concentrations of DuP 753. These data and the slope of the Schild plot indicate that DuP 753 exerts a competitive antagonism against angiotensin in vitro, despite the fact that a prolonged duration of action has been demonstrated for DuP 753 in anesthetized animals.2 This difference in the duration of antagonism of DuP 753 between in vitro and in vivo experiments may be explained at least in part by the metabolism, which is very active for Ang II but not for the nonpeptide antagonist.

Acknowledgments

We acknowledge the secretarial work of C. Théberge and the technical assistance of M. Boussougou.

References


Key Words: angiotensin II • vascular smooth muscle • antihypertensive agents • renin-angiotensin system
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Hypertension. 1991;17:480-484
doi: 10.1161/01.HYP.17.4.480

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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