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^-5 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.1 In fact, DuP 753 does not inhibit either the pressor effects of norepinephrine or vasopressin or the depressor effect of bradykinin in anesthetized rats.2 The compound has also been shown to reduce blood pressure in furosemide-treated normotensive rats2 or in animals affected by various forms of experimental or spontaneous hypertension.3 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, and 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.;4 the rat portal vein was prepared according to Mastrangelo et al5; strips of the rat stomach as well as strips of the rat urinary bladder were prepared according to Vane.6 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.7 The longitudinal strips of human ileum and colon were prepared by the method described by Crema et al8 and modified by Couture et al.9 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 portalmesenteric 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.
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</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></td>
<td></td>
<td></td>
<td></td>
<td>BK (2.4)</td>
<td>9.0±1.0</td>
<td>8.5±0.9</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></td>
<td></td>
<td></td>
<td></td>
<td>NE (72.9)</td>
<td>23.6±5.3</td>
<td>23.7±5.5</td>
</tr>
<tr>
<td>Rat portal vein</td>
<td>8.66</td>
<td>7.66</td>
<td>1.00</td>
<td>NKB (20.7)</td>
<td>15.3±1.7</td>
<td>13.3±1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NE (486.4)</td>
<td>15.4±3.1</td>
<td>10.8±1.7</td>
</tr>
<tr>
<td>Rat stomach</td>
<td>8.66</td>
<td>7.63</td>
<td>1.03</td>
<td>BB (6.2)</td>
<td>16.0±1.5</td>
<td>14.8±1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ach (138.0)</td>
<td>45.5±4.4</td>
<td>54.7±5.9</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>26.8±5.1</td>
<td>31.5±3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ach (55.0)</td>
<td>42.0±7.3</td>
<td>40.3±5.5</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></td>
<td></td>
<td></td>
<td></td>
<td>NKA (88.2)</td>
<td>56.0±1.2</td>
<td>54.0±6.1</td>
</tr>
<tr>
<td>Human colon</td>
<td>8.36</td>
<td>7.49</td>
<td>0.87</td>
<td>Ach (550.4)</td>
<td>14.4±2.9</td>
<td>13.2±2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BK (8.1)</td>
<td>78.3±13.2</td>
<td>80.8±12.3</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>92.5±11.6</td>
<td>90.0±12.7</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. Δ, 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 obtained with DuP 753 against Ang II are 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. 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 stimulants (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, and on the α-adrenergic receptor, 2) in the rabbit jugular vein on the kinin B2 and the substance P (NK-1) receptors, 3) on the rabbit pulmonary artery on the neurokinin A (NK-2) and the α-adrenergic receptors, 4) in the rat portal vein on the neurokinin B (NK-3) and the α-adrenergic receptors, 5) in the rat stomach on the bombesin (BB3) and the muscarinic receptors, 6) in the rat urinary bladder on the bombesin (BB3) 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 (un-
published observations from our laboratory). Inter-
restingly 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 concen-
trations (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–re-
sponse 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 dis-
placed 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 signifi-
cantly 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.\(^2\) in anesthetized rats against BK and noradrenaline; they extend the characterization of DuP 753 to other receptor systems such as the B\(_1\) 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.\(^{12,15-17}\)

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.\(^{5,12,17,18}\) 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.\(^{19}\) 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\(^{17}\) appear to be due to the activation of the same intracellular mechanism, that is, phosphatidylinositol (PI\(_3\)) turnover.\(^{17}\) 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 pA\(_2\), are very similar; pA\(_2\) 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. 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
DuP 753 is a specific antagonist for the angiotensin receptor.
N E Rhaleb, N Rouissi, F Nantel, P D'Orléans-Juste and D Regoli

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
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/17/4/480

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/