Radioreceptor assay of an endothelin A receptor antagonist in plasma and urine

Peter Cernacek,1* Luigi Franchi,2 Jocelyn Dupuis,1 Jean-Lucien Rouleau,1 and Mortimer Levy2,3

Orally active nonpeptide antagonists of endothelin (ET) receptors may prove beneficial in the treatment of cardiovascular and renal disease. The pharmacodynamics and pharmacokinetics of these drugs are not sufficiently known, and practical methods for their analysis have not been developed. We describe a simple, sensitive, and reproducible radioreceptor assay (RRA) for LU135252, a selective antagonist of the ET$_A$ receptor, using porcine aortic smooth muscle membranes as the acceptor and $^{125}$I-endothelin-1 as the ligand. With methanol extraction of plasma and urine samples, recovery of LU135252 ranged from 79% to 91% at 60–1000 nmol/L. The logit-log transformed calibration curves constructed with LU135252 added to plasma or to urine were linear ($r = 0.993 \pm 0.005, n = 11$) in the range from 18.7 to 2400 nmol/L. The detection limit with plasma- and urine-based calibration curves was 19 nmol/L. The inter assay coefficient of variation was 12.6% at 70 nmol/L ($n = 9$) and 6.5% at 590 nmol/L ($n = 9$). Endothelin-1 did not interfere in the RRA at pathophysiologically and clinically relevant concentrations [up to 15 pmol/L (40 pg/mL)]. When LU135252 was added to plasma, the signal was completely stable after storage for 1 week at 4 °C, although there was a modest loss of the signal after 24 h at room temperature. The practical performance of this RRA was then tested in plasma samples obtained from (a) rats after a single oral administration of LU135252, (b) from coronary-ligated rats chronically treated with LU135252, and (c) in plasma and urine samples obtained from dogs during intrarenal infusion of LU135252.

The family of potent vasoactive and mitogenic peptides, the endothelins (ETs), includes at least three isoforms, termed endothelin-1, -2, and -3 (ET1, ET2, and ET3) and has been thought to play a role in a variety of cardiovascular, renal, and other disorders (1, 2). An increased plasma concentration and/or local expression of ET1 in disease states have suggested that this peptide may be of pathophysiological importance and that the inhibition of its effects may be of therapeutic benefit.

The effects of ETs are mediated by two types of cell-surface receptors, termed ET$_A$ and ET$_B$ (3, 4). Although ET$_B$ is nonselective, exhibiting a similar affinity to all three isoforms, ET$_A$ is selective in that it binds only ET1 and ET2, whereas its affinity to ET3 is very low. From the functional point of view, ET$_A$ mediates the vasoconstrictive effect of ETs, whereas ET$_B$ expressed on the vascular endothelium (5) is a vasodilator. More recently, an ET$_B$ receptor subtype present on vascular smooth muscle cells has been shown to mediate vasoconstriction as well (6). In a number of cardiovascular and renal pathologies, such as posts ischemic renal failure (7), cyclosporine nephrotoxicity (8), certain types of hypertension (9), neointimal proliferation (10), ischemic myocardial damage, congestive heart failure (11–13), and pulmonary hypertension (14), blockade of ET receptors by the selective (ET$_A$) or nonselective (ET$_A$/ET$_B$) antagonists has been shown or suggested to be beneficial. Thus, ET receptor antagonists appear to have opened new therapeutic avenues.

The first generation of ET receptor antagonists included cyclic pentapeptides isolated from Streptomyces misakienisis (15) and synthetic peptide analogs of ET1, such as BQ788 (16), TAK-044 (17), and FR139317 (18). The second generation has included orally active nonpeptide compounds derived from benzene sulfonamide, such as bosentan (19), from naphthalenesulfonamide (20), such as BMS-182874 (21), from indane-carboxylic acid (SB209670, (22)), or from butenolide (23). Quantitating these drugs in

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1 Montreal Heart Institute, Department of Laboratory Medicine, 5000 Belanger Street East, Montreal, Quebec, H1T 1C8 Canada.
Departments of Medicine and Physiology, McGill University, Montreal, Quebec, H1T 1C8 Canada.
* Author for correspondence. Fax (514) 593-2577; e-mail jhunter@is.RVH.McGill.CA.
Received October 7, 1997; revision accepted May 14, 1998.

4 Nonstandard abbreviations: ET, endothelin; RRA, radioreceptor assay; and LU, LU equivalents.
plasma, urine, or in other body fluids is essential to the elaboration of appropriate dosing regimens and the study of their in vivo toxicity, pharmacokinetics, and pharmacodynamics. HPLC methods have been described for measurement of the butenolide-based ET<sub>A</sub> antagonist (24) and of the nonselective ET<sub>A</sub>/ET<sub>B</sub> antagonist bosentan (25). This specific and accurate approach has, however, important disadvantages: complex sample preparation, the need for expensive equipment and highly qualified labor, and, because of the complexity of the procedure, a low sample throughput.

Here we present a simple radioreceptor assay (RRA) to quantitate plasma and urine concentrations of LU135252, a recently discovered orally active selective antagonist of the ET<sub>A</sub> receptor (26). Using this RRA, we measured LU135252 concentrations reached after oral administration in the rat and after intrarenal infusion in the dog.

**Materials and Methods**

**CHEMICALS**
LU135252 [(+)-(S)-2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid] synthesized by Knoll AG, Ludwigshafen, Germany, was a generous gift of Dr. M. Kirchengast of that company. <sup>125</sup>I-ET1 (specific activity, 2000 Ci/mmol) was purchased from Amersham. HPLC grade methanol was obtained from Fisher, RIA grade bovine serum albumin from Sigma-Aldrich, and ET1 from Peninsula Laboratories. All other reagents were of analytical grade.

**PREPARATION OF PORCINE AORTIC MEMBRANES**
Aortae were taken from anesthetized pigs, immediately frozen in liquid nitrogen, and stored at −80 °C. Before further processing, the endothelium was removed by gentle scrubbing of the internal vascular surface. The tissue (5–10 g) was placed in an ice-cold buffer (100 g of tissue per liter of buffer, containing 50 mmol/L Tris, 0.1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L EDTA, pH 7.4) and homogenized in a cold room (4 °C) with a tissue blender, using three strokes of 1 min with cooling periods of 30 s between each stroke. The homogenate was centrifuged at 1800g for 20 min at 4 °C. The supernatant was then centrifuged at 35 000g for 30 min at 4 °C. The final pellets (crude membranes) were reconstituted in the binding buffer (see below) at 1–1.5 g protein/L and stored at −80 °C. Rat cerebellar membranes were obtained from guillotined adult male Sprague-Dawley rats by the same method.

**RRA**
Binding reactions were performed at room temperature in a total volume of 200 μL; 50 μL of the radioligand (<sup>125</sup>I-ET1, ~10 000 cpm) was mixed with 50 μL of the calibrator or sample in the binding buffer (the same as the homogenization buffer, supplemented with 5 g/L bovine serum albumin, pH 7.4). The reaction was started by addition of 100 μL of the aortic membranes and 5–7 μg of protein per tube. It was terminated 3 h later by addition of 1 mL of cold 5 g/L bovine serum albumin in phosphate-buffered saline, pH 7.4, immediately followed by a rapid centrifugation (3 min at 13 000g). The supernatant was carefully aspirated, and the radioactivity of pellets was counted in an automated gamma counter (RiaStar, Canberra Packard Canada) with 78% efficiency. Radioactivity data were processed (logit/log transformation) with the built-in software of the gamma counter.

**SAMPLE PREPARATION**
Aliquots of plasma or urine (0.1 mL) were thawed, 1 mL of methanol was added, and the mixture was thoroughly vortex-mixed. After centrifugation to separate the precipitated protein (15 min at 2800g), the supernatant was carefully decanted into 12 × 75-mm plastic tubes and evaporated to dryness under a stream of air. Before the assay, the dry residue was reconstituted in 150 μL of the binding buffer.

**CALIBRATION CURVE**
To construct the calibration curve, 1.87–240 pmol of LU135252 was added to 0.1-mL aliquots of rat, human, or dog plasma, or dog urine. Each aliquot was then processed in the same fashion as the plasma or urine samples.

**SAMPLE STABILITY**
Three pools of plasma with added LU135252 (150 or 1500 nmol/L) were prepared and aliquoted (0.1 mL). The aliquots were then processed as described above, either immediately or after standing for 24 h at room temperature or for 24, 48, 72, or 168 h at 4 °C.

**RECOVERY**
To estimate the recovery of LU135252 during the extraction procedure, plasma and urine samples with added LU135252 (60, 200, or 1000 nmol/L) were compared with aqueous calibration curves prepared in the binding buffer.

**INTERASSAY PRECISION AND INTERFERENCES**
Two pools of plasma with added LU135252 (150 or 1500 nmol/L) were aliquoted at 0.1 mL, kept frozen at −20 °C, and used as internal quality-control material. NaCl and urea were added to three pools of urine at concentrations specified below. LU135252 (150 or 1500 nmol/L) was added to three pools of urine; the pH was adjusted with NaOH or HCl to 4, 5, 6, 7, or 8; and aliquots were immediately extracted with methanol as described above. ET1, 2–80 pmol/L (5–200 pg/mL), was added to four pools of plasma and two pools of urine.

**LU135252 CONCENTRATIONS IN RAT PLASMA**
To estimate the achieved plasma concentrations and their time course after intragastric administration, a catheter (PE60) was installed in the left jugular vein of a rat under halothane anesthesia and tunneled subcutaneously to exit...
in the interscapular region. When the rat awakened from anesthesia, LU135252 (50 mg/kg) was administered by gavage (time 0). Blood was obtained via a heparinized needle at time 0 and at 1, 3, 6, 9, and 24 h after the administration. To assess the concentrations achieved during chronic oral treatment, rats with congestive heart failure postmyocardial infarction induced by coronary ligation as previously described (27), as well as sham-operated rats, were treated with 100 mg of LU135252/kg of body weight administered by gavage once daily. After 5 weeks, blood was obtained from the aorta and from the pulmonary artery under ether anesthesia, 42–46 h after the last gavage. To confirm the presence of heart failure, left ventricular end-diastolic pressure was measured using a Millar catheter with a pressure sensor at its tip, introduced via the left carotid artery and advanced into the left ventricle.

**LU135252 concentrations in dog plasma and urine**

To explore the plasma LU135252 concentration, its urinary excretion, and its renal effects during intrarenal infusion, mongrel dogs of either sex (body weight, 14.0 ± 2.6 kg) were anesthetized, and a catheter was introduced into the left renal artery, using the techniques described elsewhere (28). Urine was collected separately from each ureter. Left and right kidney glomerular filtration rates were assessed as inulin clearance, and renal plasma flow was assessed as clearance of p-amino hippuric acid. Serum and urine sodium was measured by flame photometry. All of these techniques are well established in our laboratory (28). After the dog was stabilized and control urine was collected, LU135252 was infused at 0.1, 0.2, or 0.5 mg·kg⁻¹·min⁻¹ in the left renal artery for 45 min; the right kidney received the vehicle (9 g/L NaCl). Thereafter, with the LU135252 infusion still running, ET1 was infused in the left femoral vein at 15 ng·kg⁻¹·min⁻¹ during 45 min. In preliminary experiments, this dose of ET1 elicited a marked pressor effect as well as a marked decrease in the glomerular filtration rate, renal plasma flow, diuresis, and natriuresis. All protocols in the dog and in the rat were approved by the McGill University Animal Care Committee. All results of LU135252 measurement performed on the samples obtained in vivo from rats and dogs are reported as LU equivalents (LU, nmol/L), because the interference of LU135252 metabolites cannot be excluded with certainty at the present time.

**Statistical evaluation**

The calibration curves were evaluated by linear regression analysis (logit/log transformation of the data) by the least-squares method. The recovery of LU135252 and its stability were evaluated by the paired Student’s t-test. The effects of intrarenal LU135252 infusion in the dog were submitted to ANOVA for repeated measurements, whereas plasma LU concentrations in the rat were evaluated by unpaired t-test, or when indicated by a significant F-test result, by the nonparametric test (Mann–Whitney) for independent means. A P value of 0.05 was taken as the limit of statistical significance.

**Results**

**Calibration curve, precision, and recovery**

In competition binding experiments, LU135252 competed with ₁₂⁵I-ET1 for the ETA receptor with high affinity, characterized by a K_d value of 28.5 ± 7 nmol/L (n = 10). In preliminary experiments testing the affinity of LU135252 to ETB receptor, using the rat cerebellar membranes (a >95% pure preparation of ETB), we found a K_d value of 1.56 × 10⁻³ mol/L (n = 4), indicating ∼550-fold selectivity to ETA vs ETB receptor, in good agreement with the manufacturer’s data (26). To construct practically useful calibration curves, we used the linear part of the logit/log relationship, from 3.12 to 400 nmol of LU135252 per liter of binding mixture. In this range, the inhibition of binding of ₁₂⁵I-ET1 (B/Bo × 100%) was from 86.4% ± 5.2% to 17.8% ± 2.5% (Fig. 1). With the design of the assay, this corresponds to plasma concentrations of 18.7–2400 nmol/L. The calibration (logit/log-transformed) curve was stable and linear in this range, with a correlation coefficient of 0.993 ± 0.005, a slope of −1.42 ± 0.14, and an x-intercept (midpoint) of 196 ± 13 nmol/L (n = 11 in all instances). Solvent (methanol) blanks did not exhibit any effect on the binding of the radioligand. Blank (zero-LU) rat and human plasma exhibited B/Bo × 100% equal to 99.1% ± 6.3% (n = 11), whereas the canine urine samples gave a value of 96.2% ± 3.6% (n = 11). Thus, the limit of detection calculated as the blank value minus 2 SD was not different from the mean B/Bo obtained with the lowest point of the calibration curve (18.7 nmol/L, 86.4% ± 5.2%). The precision data at two concentrations, obtained with frozen aliquots of plasma with added LU135252, are presented in Table 1. The intraassay CV at

![Fig. 1. Typical competition curve with binding of 125I-ET1 to porcine aortic membranes as found with LU135252 (○), in nmol/L, plasma with added ET1 (●), in pmol/L, and with serial dichotomic dilutions (undiluted to 64-fold dilution) of plasma with added LU135252 (▼).](image-url)
the midpoint of the calibration curve was 7.8%. Interassay variation was 12.6% at the low concentration (70 nmol/L) and 6.5% at the high concentration (Table 1). The recovery of LU135252 from plasma was 79.3% ± 5.6% at 60 nmol/L, 88.6% ± 3.6% at 200 nmol/L, and 91.2% ± 3.2% at 1000 nmol/L (n = 5 in each instance).

**SAMPLE STABILITY**

Samples of plasma with two concentrations of added LU135252 exhibited a modest but significant loss of signal after 24 h at room temperature (−11% at 150 nmol/L and −14.4% at 1500 nmol/L, n = 3 in both instances; Table 2). In plasma stored at 4 °C, LU135252 was, however, completely stable for at least 1 week.

**INTERFERENCES**

The most important potential interferent both in plasma and in urine is endogenous ET1, because of its high affinity to ET receptors. We previously found Kd values of 50–250 pmol/L for ET1 binding in various rat and dog tissues (29, 30). In plasma, ET1 (extracted from 0.1 mL of the sample), had no effect at the binding of the radioligand at concentrations up to 15 pmol/L (40 pg/mL; B/Bo × 100% at 15 pmol/L = 91.5% ± 1.4%, n = 4; Fig. 1). Addition of ET1 to urine produced no inhibition of binding even at 40 pmol/L (100 pg/ML) of ET1 (not shown). Hence, ET1 does not interfere in this RRA at clinically and pathophysiologically relevant concentrations. In urine, the measured values were independent of sample pH from pH 4 to pH 8 (Table 3). Neither urea nor NaCl at 200–800 mmol/L affected the binding of the radioligand to the membranes. In urine with added LU135252 (150 or 1500 nmol/L, n = 3), there was no effect of either urea or NaCl (both at 800 mmol/L) on the measured LU concentrations (not shown).

**LU IN RAT PLASMA**

After a single dose of 50 mg/kg administered by gavage, plasma LU peaked after 1 h at 21 ± 5 μmol/L, in good agreement with the manufacturer’s data obtained by HPLC analysis (M. Kirchengast, personal communication). Twenty-four hours later, there was still a measurable LU concentration, at 3.2 ± 0.9 μmol/L (Fig. 2), well compatible with the 12-h half-life of this compound in the rat. In chronically treated rats (100 mg kg−1 · day−1 by mouth), there was no difference in plasma LU in the mixed venous blood of control rats compared with rats suffering from myocardial infarction-induced heart failure. In addition, LU concentrations in aortic and mixed venous blood (pulmonary artery) were equivalent: controls: aorta, 773 ± 374 nmol/L, and pulmonary artery, 823 ± 421 nmol/L; infarcted rats: aorta, 937 ± 496 nmol/L, and pulmonary artery, 894 ± 456 nmol/L (Fig. 3).

**LU IN DOG PLASMA AND URINE AFTER INTRARENAL INFUSION**

Continuous unilateral intrarenal infusion of LU135252 led to much higher plasma LU concentrations (Fig. 4) than those anticipated from the available data on the LU135252 dose/concentration relationships obtained previously in the rat and on the basis of a bolus administration (M. Kirchengast, personal communication). Measured systemic plasma LU concentrations were between 60 μmol/L at an infusion rate of 0.1 mg · kg−1 · min−1 and 400 μmol/L at 0.5 mg · kg−1 · min−1. At these concentrations, LU135252 had no effect on renal hemodynamics or on the excretory variables (Fig. 5), except for the mild decrease in renal plasma flow at 0.5 mg · kg−1 · min−1. Paradoxically, this lack of renal effects contrasted with a mild systemic hypotensive effect, which was significant at 0.2 and 0.5

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### Table 1. Intraassay and interassay precision of LU RRA.

<table>
<thead>
<tr>
<th>LU concentration</th>
<th>n</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraassay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>192 nmol/L</td>
<td>10</td>
<td>7.8%</td>
</tr>
<tr>
<td>80 nmol/L</td>
<td>9</td>
<td>12.6%</td>
</tr>
<tr>
<td>590 nmol/L</td>
<td>9</td>
<td>6.5%</td>
</tr>
</tbody>
</table>

### Table 2. Stability of LU in plasma.

<table>
<thead>
<tr>
<th>LU added, nmol/L</th>
<th>Fresh</th>
<th>24 h/room temperature</th>
<th>4 °C/24 h</th>
<th>4 °C/48 h</th>
<th>4 °C/72 h</th>
<th>4 °C/7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>137 ± 22</td>
<td>1228 ± 16</td>
<td>144.7 ± 29.3</td>
<td>153.6 ± 7.3</td>
<td>142.2 ± 16</td>
<td>140.6 ± 16.4</td>
</tr>
<tr>
<td>1500</td>
<td>1514 ± 89</td>
<td>12956 ± 184</td>
<td>1550 ± 85</td>
<td>1518 ± 161</td>
<td>1347 ± 141</td>
<td>1496 ± 111</td>
</tr>
</tbody>
</table>

* Three pools of plasma were supplemented with 150 or 1500 nmol/L and assayed immediately or after the specified conditions. Means ± SD are shown.

* P = 0.094 vs fresh plasma (paired t-test).

* P = 0.043 vs fresh plasma (paired t-test).

### Table 3. Effect of urinary pH on LU RRA.

<table>
<thead>
<tr>
<th>LU added, nmol/L</th>
<th>pH 4</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1500 (n = 3)</td>
<td>1376 ± 40</td>
<td>1434 ± 55</td>
<td>1356 ± 83</td>
<td>1426 ± 68</td>
<td>1520 ± 50</td>
</tr>
</tbody>
</table>

* Three pools of urine were supplemented with 1500 nmol/L and assayed after adjustment of pH to the specified values. Means ± SD are presented.
Urinary excretion of LU was modest, corresponding to 1–3.5% of the infused dose (Fig. 5).

Discussion

In recent years, RRAs have gained popularity compared with immunoassays, especially for the analysis of those drugs that undergo extensive metabolism and give rise to a number of metabolites that may or may not be bioactive. In such instances, cross-reactivity of the antibodies with various metabolites, in addition to that with the parent compound, can substantially affect the accuracy of immunoassays (31). RRAs offer the advantage of measuring, in addition to the parent drug, only those metabolites that interact with the respective receptor, hence those that are of pharmacodynamic importance.

To establish a RRA of LU135252, we took advantage of the high affinity of this compound to the ET<sub>A</sub> receptor (K<sub>d</sub> = 28 nmol/L in our hands). When porcine aortic membranes obtained after de-endothelialization of the vessels are used, it is possible to obtain a virtually pure preparation (~95%) of the ET<sub>A</sub> receptor. This preparation can be used as a practical and inexpensive substitute for receptor-transfected cells or for the cloned receptor protein. With 5–10 g of aortic tissue, we could obtain an amount of membrane protein sufficient to perform the assay in >1500 tubes, i.e., to analyze >600 samples in duplicate. Our previous work with membranes obtained from cultured rat vascular smooth muscle cells indicated that the latter source of the receptor preparation had been much less economical (P. Cernacek and D. Blais, unpublished). In the present study, there was no degradation of the labeled ligand during the assay, and the membranes retained their binding characteristics for at least 6 months when stored at −80 °C. Furthermore, with preparations obtained from three different animals, the slopes, correlation coefficients, and x-intercepts of the calibration curves were similar. The assay is stable and precise, as shown by the analysis of pools of plasma and urine with
added LU135252. The analytical recovery assessed at three concentrations varied from 79% to 91%. Because the binding of the radioligand was consistently lower in the RRA buffer than in plasma- or urine-based curves, we decided to use the latter ones for routine assays. This approach also obviates the problem of recovery of LU135252 from the sample. Linearity of the calibration curves constructed with LU135252 added to plasma or urine confirms that there was no substantial difference in the recovery of the compound at lower and higher concentrations. In addition, there was no difference in the slopes, correlation coefficients, and x-intercepts of calibration curves obtained with human, dog, or rat plasma, or human or dog urine.

Another advantage of this RRA lies in its simplicity, which translates to low labor intensity and high sample throughput. We could analyze 50 samples within 6 h, including the incubation time (3 h). In contrast, the HPLC method for a similar ET antagonist requires considerably longer sample preparation, and a 30-min run per sample (24).

We carefully explored the potential interference by ET1, which is known to be present in both plasma and urine, and which has ~100-fold higher affinity to the ET$_A$ receptor than the LU compound. Our experiments made clear that, when extracted from 0.1-mL samples, the amount of ET1 is so low that it does not inhibit the binding of $^{125}$I-ET1 in concentrations up to 15 pmol/L (40 pg/mL). Our usual values of plasma ET1 are in the range of $\leq$0.4 pmol/L (1 pg/ml) in healthy man (32), and $<$1.1 pmol/L (3 pg/mL) in the dog (33). Most other laboratories find reference values in this range (34). Even in conditions of an extreme activation of the ET system, such as in sepsis, plasma ET1 does not exceed 7.5 pmol/L (20 pg/mL) (35). Thus, because of its very low circulating concentrations, endogenous ET1 does not interfere in this assay. In urine, the results were not affected by high concentrations of NaCl or urea, nor were they affected by the pH of the sample in a broad range from pH 4 to pH 8.

LU135252 is primarily metabolized in the liver and excreted via the bile, whereas urinary excretion is modest (M. Kirchengast, personal communication). It is metabolized by the hydroxylation of the pyrimidine ring and
demethylation of the methoxy group (M. Kirchengast, personal communication). None of the metabolites characterized to date exhibit a measurable binding activity to ET<sub>A</sub> receptor. Therefore, it can be assumed that our RRA would measure the parent compound, LU 135252, specifically. Yet in principle, our assay has the characteristics of pharmacodynamic drug monitoring, measuring the effect of the drug and of those potential metabolites that may bind to the receptor rather than the characteristics of an analytical method specifically measuring the parent compound.

Plasma concentrations of LU in the orally treated rats, either after a single administration or during chronic treatment, have indicated a high oral bioavailability of the compound. The LU concentrations, both 44 h after the last dose in chronically treated rats and 24 h after a single intragastric administration, are in good agreement with the terminal half-time of 12 h stated by the manufacturer (M. Kirchengast, personal communication). The lack of difference between the arterial and mixed venous LU concentrations can mean either a lack of pulmonary extraction of the drug or, more likely, that the pulmonary receptor-mediated removal has been saturated during chronic treatment.

In the experiments in the dog, we were surprised by very high plasma LU concentrations, in the range of 10<sup>-5</sup> to >10<sup>-4</sup> mol/L, achieved after a relatively short (45 min) intrarenal administration. Even at the lowest infusion rate (0.1 mg·kg<sup>-1</sup>·min<sup>-1</sup>), the LU concentrations in plasma were in the high micromolar range, compatible with inhibition of not only ET<sub>A</sub> receptor, but also of ET<sub>B</sub> receptor. Regardless of the type of the ET receptor blocked, there was a conspicuous absence of any effect on renal hemodynamics or excretory function, except for the mild decrease in renal plasma flow after the highest dose (0.5 mg·kg<sup>-1</sup>·min<sup>-1</sup>). In contrast, the mean arterial blood pressure decreased, giving further support for a role of ET1 in the regulation of blood pressure and vascular tone (36). Of importance is that LU135252 infusion at any dose assured a full protection against the pressor and renal effects of the high-dose (15 ng·kg<sup>-1</sup>·min<sup>-1</sup>) infusion of ET1.

This RRA method has the potential to be used to measure other ET receptor antagonists. In preliminary studies with SB209607, a nonselective ET<sub>A</sub>/ET<sub>B</sub> antagonist (22), we have obtained results compatible with a reliable quantification of this compound as well.

We gratefully acknowledge excellent technical assistance by Alison Taylor, Emma Resurreccion, and Nathalie Ruel. This work was funded by the Medical Research Council of Canada (M.L.), the Pharmaceutical Manufacturers Association of Canada (P.C.), and the Kidney Foundation of Canada (P.C.).

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