In Vivo Labeling of Endothelin Receptors with [^{11}C]L-753,037: Studies in Mice and a Dog

Sasa Aleksic†, Zsolt Szabo, Ursula Scheffel, Hayden T. Ravert, William B. Mathews, Levente Kerenyi, Paige A. Rauseo, Raymond E. Gibson, H. Donald Burns, and Robert F. Dannals

Division of Nuclear Medicine, Department of Radiology, The Johns Hopkins Medical Institutions, Baltimore, Maryland; and Department of Pharmacology, Merck Research Laboratories, West Point, Pennsylvania

Endothelin (ET) is a potent mammalian vasoconstrictive peptide and a pressor agent. Its 3 isoforms, ET-1, ET-2, and ET-3, mediate several physiologic actions in several organ systems, binding to 2 major receptor subtypes: ET_A and ET_B. This study was undertaken to evaluate [^{11}C]L-753,037 [(+)-(5*S*,6*R*,7*R*)-2-butyl-7-[2-((2*S*)-2-carboxy-propyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopenteno [1,2-β]pyridine-6-carboxylate), a new mixed ET receptor A and B antagonist, as a tracer for in vivo labeling of ET receptors in mice and a dog. **Methods:** [^{11}C]L-753,037 was synthesized, purified, and formulated from a normethyl precursor, L-843,974, and [^{11}C]H_3I. The tracer was studied for its in vivo kinetics, biodistribution, and ET receptor binding characteristics in mice. In the dog, PET imaging was performed to evaluate binding of [^{11}C]L-753,037 to ET receptors in the heart. Specificity of binding was studied in the heart with the selective ET_A antagonist L-753,164. **Results:** Kinetic studies in mice showed highest tracer uptake at 5 min after injection in liver (25.0 percentage injected dose per gram [%ID/g]), kidneys (18.7 %ID/g), lungs (15.2 %ID/g), and heart (5.6 %ID/g). Initial high uptake in liver, lungs, and kidneys was followed by rapid washout during the next 10 min and a very slow clearance during the time of observation (2 h after injection). By contrast, the radioactivity in the heart remained constant over 2 h. Administration of both ET_A (L-753,164) and mixed ET_A/ET_B (L-753,137) receptor antagonists resulted in dose-dependent inhibition of [^{11}C]L-753,037 binding in mouse heart, lungs, and kidneys but not in the liver. Radioactivity in the brain was very low, indicating that the tracer does not cross the blood–brain barrier. In the dog, a dynamic PET study of the heart showed high tracer accumulation at 55–95 min after injection. Injection of L-753,164 at 30 min before [^{11}C]L-753,037 administration led to a significant reduction in tracer binding. [^{11}C]methyl triphenyl phosphonium was used as a tracer for reference images of the dog heart muscle. **Conclusion:** The results suggest that [^{11}C]L-753,037 binds to ET receptors in vivo and is, therefore, a promising candidate for investigation of these receptors and their occupancy by ET receptor antagonists using PET.

Key Words: endothelin receptors; PET; dogs

J Nucl Med 2001; 42:1274–1280

Received Nov. 20, 2000; revision accepted Apr. 9, 2001.

†Dr. Aleksic died Apr. 7, 2000.

For correspondence or reprints contact: Zsolt Szabo, MD, PhD, Division of Nuclear Medicine, The Johns Hopkins Outpatient Center, Room Nr. 3233, 601 Caroline St., Baltimore, MD 21287.

Endothelin (ET) is a novel 21–amino acid peptide with potent vasoactive properties. It was first described in 1985 by Hickey et al. (*1*) and purified and cloned by Yanagisawa et al. (*2*) in 1988. In 1989, 3 distinct genes encoding 3 ET isoforms (ET-1, ET-2, and ET-3) were discovered (*3*). ET-1 is believed to play an important role in regulating vascular function. It is synthesized in a series of proteolytic cleavages with the last step mediated by ET-converting enzyme (ECE), in which the last 18 amino acids are removed from a precursor referred to as big ET-1 (*4*).

The hemodynamic response after intravenous administration of ET-1 has a biphasic nature: a transitory hypotension followed by sustained increase in arterial blood pressure. At least 2 receptor subtypes are responsible for this effect: ET_A and ET_B (*2*). ET_A receptors are located on vascular smooth muscle cells and are responsible for vasoconstriction, whereas ET_B receptors are located on both smooth muscle cells and endothelial cells and play a role in both vasoconstriction and vasodilatation, respectively. Both receptors are members of the G-protein–coupled superfamily of receptors.

There is evidence to suggest that ET is involved in several pathologic states, including pulmonary, renal, and cardiovascular disease. In the human organism, ET receptors are expressed in the heart, brain, lung, kidney, and aorta (*5*). The heart is an organ of particular interest because myocardiocytes have high concentrations of both ET_A and ET_B receptors (*6*) and ET has been suggested to play a role in hypertension (*7*), congestive heart failure (*8*), atherosclerosis (*9*), myocardial ischemia (*10*), and infarction (*11*).

There is increasing evidence from both in vitro and in vivo studies that ET is mitogenic to tumor tissue and is capable of inducing angiogenesis in human cancers (*12*). Expression of the ET receptors has been found in cancer of the breast (*13*), ovary (*14*), and lung (*15*) and in gliomas and meningiomas (*16*). ET may initiate or support the growth and progression of these tumors. Thus, there is a strong motivation to investigate the ET receptors with PET or SPECT.

In the past decade, several ligands have been developed with antagonistic properties to ET receptors, binding either

to ET_A or ET_B or to both (*17–25*). Selective ET receptors have been labeled with ^{18}F and ^{11}C (*26*) for use with PET. This study focuses on the pharmacologic characterization of [^{11}C]L-753,037 and the PET images obtained from a dog.

Nishikibe et al. (*27*) described the compound (+)-(5*S*, 6*R*,7*R*)-2-butyl-7-[2-((2*S*)-2-carboxy-propyl)-4-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)cyclopenteno [1,2-β]-pyridine-6-carboxylate (J-104,1134, also named L-753,037) as a potent, selective inhibitor of both A and B ET receptors. In this study, L-753,037 was labeled with ^{11}C and its biodistribution and binding to ET receptors were investigated in mice and a dog. The results suggest that [^{11}C]L-753,037 is indeed binding to ET receptors in several organs, including the heart. This nonselective tracer appears to be a promising agent for studying ET receptors using PET.

MATERIALS AND METHODS

Synthesis and Purification of [^{11}C]L-753,037

[^{11}C]L-753,037 was synthesized, purified, and formulated in 17 min with an average specific radioactivity of 93.8 GBq/μmol, as has been described (*28*).

Unlabeled Antagonists

L-753,037 and L-753,164 were gifts from Banyu Pharmaceutical, Tsukuba, Japan.

In Vivo Kinetics and Biodistribution in Mice

Animals were housed and cared for according to standards recommended by the National Institutes of Health. The experimental protocol was approved by the Animal Care and Use Committee of The Johns Hopkins Medical Institutions. Male CD-1 mice (weight range, 23–28 g) received intravenous injections of approximately 0.2 mL 7.4 MBq (1–2 μg/kg) [^{11}C]L-753,037 through a tail vein. The animals were killed by cervical dislocation at different time points (5, 15, 30, 60, and 120 min) after injection. Organs of interest (heart, lungs, kidneys, liver, brain, and blood) were removed and weighed, and the radioactivity of the tissue samples was measured in an automated γ-counter. Standards were prepared as aliquots of the injectate and counted together with the tissue samples. The percentages of the injected dose per organ (%ID/organ) and per gram of tissue (%ID/g) were calculated.

Inhibition Studies in Mice

Inhibition studies were performed using 2 drugs: L-753,164, a selective ET_A receptor antagonist (affinity for ET_A/affinity for $ET_B \geq 10{,}000$), and L-753,037, a mixed ET_A/ET_B receptor antagonist (affinity for ET_A/affinity for $ET_B = 3$) (Fig. 1). Increasing concentrations of the blockers (0.001–10 mg/kg L-753,164 [0.0018–18.0 μmol/kg] or 0.1–50 mg/kg L-753,037 [0.188–94.1 μmol/kg]) were injected intraperitoneally 30 min before tracer administration. Thirty minutes after [^{11}C]L-753,037 injection, the animals were killed by cervical dislocation. Organ samples were prepared and counted as described above.

PET Imaging in the Dog

Two PET imaging studies were performed on a male beagle dog (weight, 13 kg), 1 control study and 1 study after blocking ET_A receptors with unlabeled L-753,164. In each of the 2 studies, serial images were obtained using a PET scanner (4096+; General Electric Medical Systems, Milwaukee, WI). The dog was anesthetized intravenously with sodium pentobarbital (25 mg/kg) and additional pentobarbital was administered during the experiment at a rate of 3 mg/kg/h. [^{11}C]L-753,037 (349 MBq in 4.3 mL saline) was injected intravenously into a leg vein. Eighteen consecutive PET images of increasing scan length (4 × 15 s, 3 × 1 min, 3 × 2 min, 3 × 6 min, 3 × 10 min, and 2 × 20 min) were obtained starting at the time of the intravenous injection of the radiotracer.

The second dynamic PET scan was obtained after intravenous preinjection of 1 mg/kg (1.8 μmol/kg) L-753,164 dissolved in saline (ET_A selective antagonist) 30 min before the tracer injection (356 MBq in 4.5 mL saline).

To obtain reference images of the heart muscle in the dog, [^{11}C]methyl triphenyl phosphonium iodide (MTP) (*29*) was used, which was prepared by methylation of triphenylphosphine with [^{11}C]methyl iodide. [^{11}C]MTP (321 MBq) was injected intravenously and 17 images with scan times similar to those used with [^{11}C]L-753,037 were obtained.

The dog was monitored for oxygen saturation and heart rate throughout the study. None of the parameters showed a significant change during the experiment.

Statistical Analysis

Data were analyzed for significant differences by ANOVA and a post hoc Dunnett test. Differences were considered significant at $P < 0.05$. The effective dose for blocking [^{11}C]L-753,037 binding

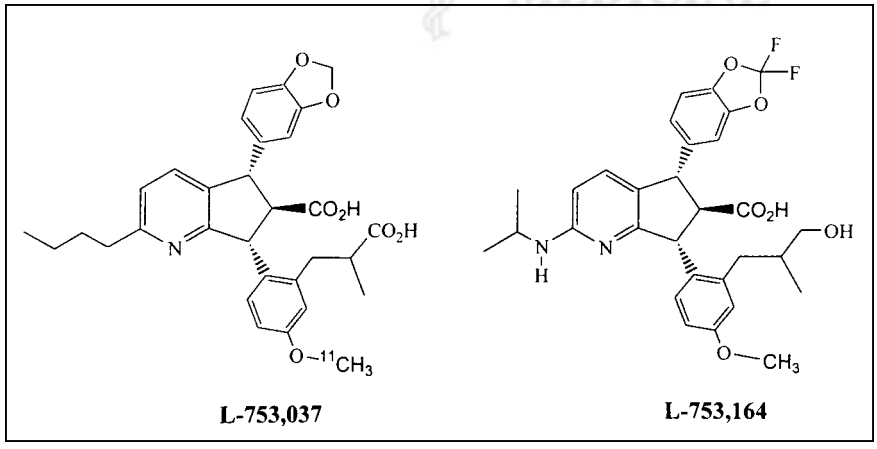

FIGURE 1. Structures of ET receptor antagonists. L-753,037 exhibits 7-fold higher affinity for ET_A receptor than for ET_B receptor. L-753,164 is profoundly ET_A selective ($ET_A/ET_B = >10{,}000$).

by 50% (ED_{50}) was calculated from total binding in heart and kidneys using log-logit transformation.

RESULTS

In Vivo Kinetics and Biodistribution in Mice

After [^{11}C]L-753,037 was injected intravenously into mice, the biodistribution of the tracer was determined as a function of time after injection (Fig. 2). Highest tracer uptake (mean %ID/g) was observed in the liver (25.0), kidneys (18.7), lungs (15.2), and heart (5.6) at 5 min after injection, whereas brain radioactivity (data not shown) was low (0.25). Initial uptake in the liver, kidneys, and lungs was followed by a rapid washout during the next 10 min and then by a very slow clearance during the rest of the observation period (2 h). At 2 h after injection, 27%, 19%, and 41% of the peak activity remained in the liver, kidneys, and lungs, respectively. By contrast, the radioactivity in the heart remained constant over 2 hrs. Blood radioactivity was low at 5 min (1.1 %ID/g) and fell to <1 %ID/g within 15 min after injection.

Inhibition of In Vivo [^{11}C]L-753,037 Binding

The saturability and selectivity of [^{11}C]L-753,037 binding to ET receptors was investigated by pretreatment of mice with increasing doses of unlabeled L-753,037, as well as with the ET_A-specific antagonist L-753,164. The blockers were injected intraperitoneally 30 min before tracer administration, and the animals were killed either 30 or 60 min after injection of the radioactive tracer.

The effect of 1 mg/kg L-753,164 and 1 mg/kg L-753,037 on the binding of [^{11}C]L-753,037 in different organs is shown in Figure 3. Tracer accumulation was significantly reduced by both inhibitors in the heart, kidneys, and adrenals and by L-753,037 in the lungs, whereas liver uptake of [^{11}C]L-753,037 was not affected by either of the 2 inhibitors. Although mean inhibition of tracer binding in the heart, lungs, and kidneys by the ET_A/ET_B blocker L-753,037 was more pronounced than that by the selective blocker L-753,164, statistical analysis using ANOVA and the post hoc Dunnett test revealed no statistically significant differences between the effects of the 2 inhibitors.

A dose-dependent inhibition of [^{11}C]L-753,037 binding to ET receptors in the mouse heart was observed after preinjection of increasing doses of the unlabeled, ET_A-specific ligand L-753,164 (Fig. 4). The ED_{50} of total tracer binding at 30 min after administration in the heart was calculated to be 0.35 mg/kg (0.63 μmol/kg). In the kidneys (data not shown), the ED_{50} was 0.73 mg/kg (1.31 μmol/kg). [^{11}C]L-753,037 radioactivity in the blood (data not shown) increased from 0.17 %ID/g in the control group to 0.34 %ID/g after injection of 10 mg/kg (18.0 μmol/kg) L-753,164.

Preinjection of the mixed ET_A/ET_B antagonist L-753,037 resulted in even larger, dose-dependent reductions in [^{11}C]L-753,037 accumulation in the heart and kidneys than observed with the ET_A-specific ligand L-753,164. At 0.1 mg/kg (0.19 μmol/kg) L-753,037, inhibition of tracer accumulation in the mouse heart was 65.1%; at 1.0 mg/kg, 93.1%; and at 10 mg/kg (18.8 μmol/kg), 94.7%. A comparison of the effects of the 2 blockers (L-753,164 and L-753,037) on tracer concentrations in the heart at 30 min after injection (expressed as percentage of saline controls) is presented in Figure 5.

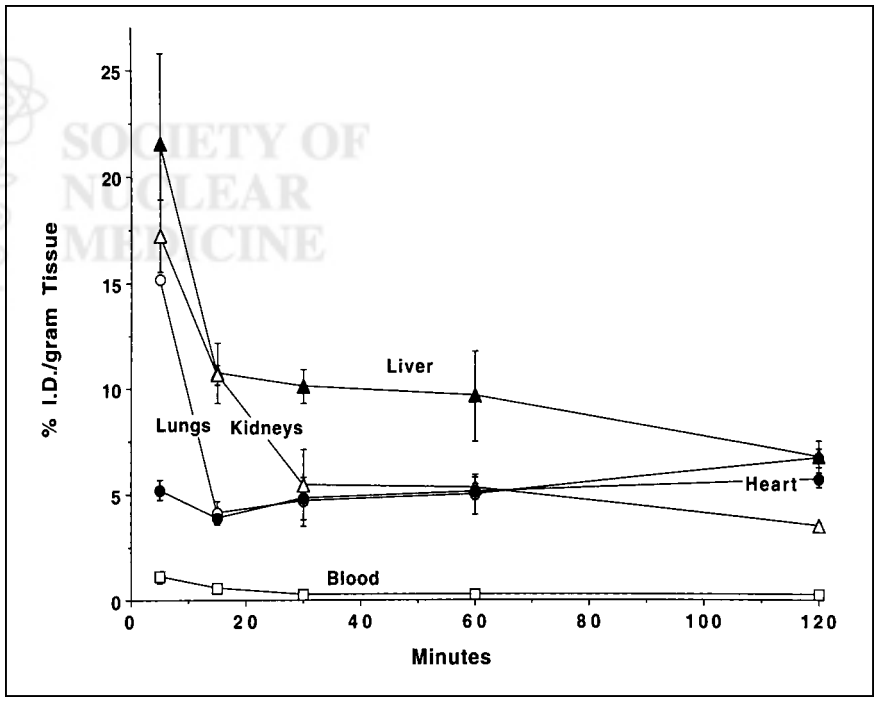

FIGURE 2. Distribution of [^{11}C]L-753,037 in various organs of mice at different times after intravenous injection. Data are presented as mean values of %ID/g tissue ± SEM; $n = 3$ mice.

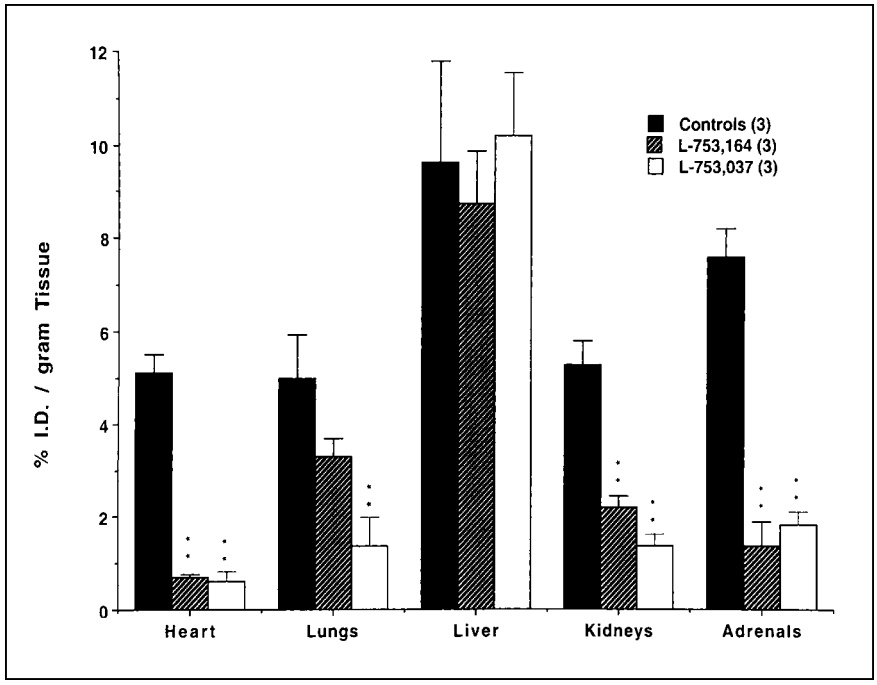

FIGURE 3. Inhibition of [^{11}C]L-753,037 binding in different mouse organs by 1 mg/kg L-753,164, an ET_A receptor inhibitor, and L-753,037, a mixed ET_A/ET_B receptor blocker, 60 min after intravenous tracer administration. Drugs were injected intraperitoneally 30 min before tracer injection. Data are mean ± SEM; numbers of animals are in parentheses. $^{**}P < 0.001$.

PET Imaging

PET scans showed high accumulation in the wall of the dog heart at 55–95 min after injection of [^{11}C]L-753,037 (Fig. 6A) and a significant reduction in binding ($P < 0.001$) after administration of the selective ET_A inhibitor L-753,164 (Fig. 6B). PET images obtained after intravenous injection of [^{11}C]MTP (Fig. 6C) compared well with the images generated with [^{11}C]L-753,037. The high tracer accumulation seen in the [^{11}C]L-753,037 images at the dorsal part of the dog's chest (Figs. 6A and 6B) remains unexplained.

Time–activity curves of [^{11}C]L-753,037 radioactivity were derived from regions of interest placed on the lateral wall, left ventricle (blood content), and lungs. Shown in Figure 7 are the time–activity curves of the ventricular cavity, the lateral wall, and the lungs before (baseline) and

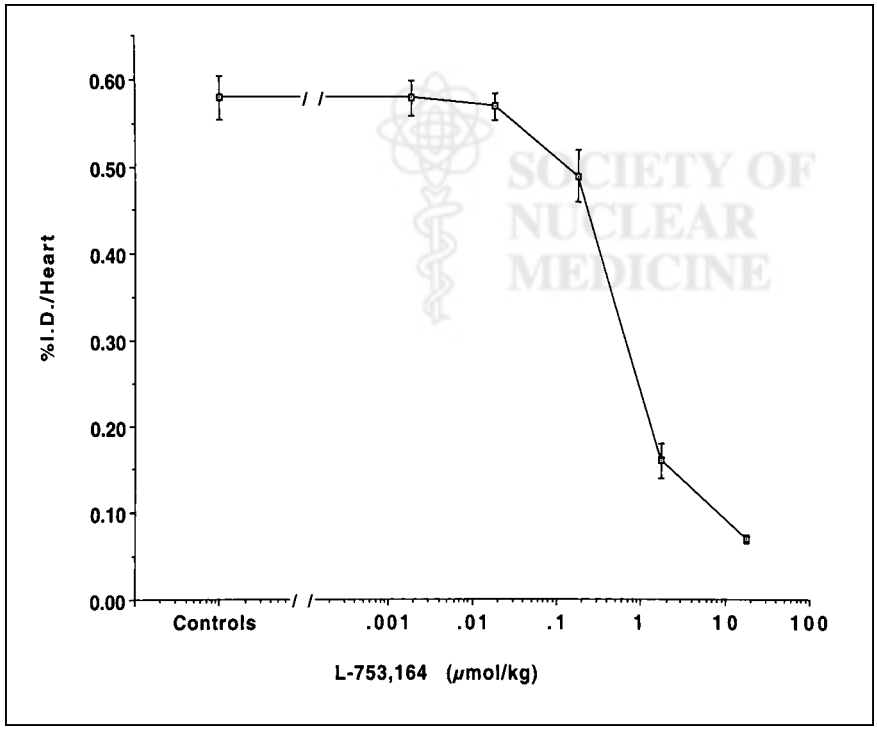

FIGURE 4. Dose-dependent inhibition of [^{11}C]L-753,037 binding to ET receptors in mouse heart by selective ET_A antagonist L-753,164. Antagonist was injected intraperitoneally 30 min before tracer injection; ^{11}C radioactivity was measured 30 min after intravenous administration. $n = 3$ mice for each dose level. Data are presented as mean ± SEM.

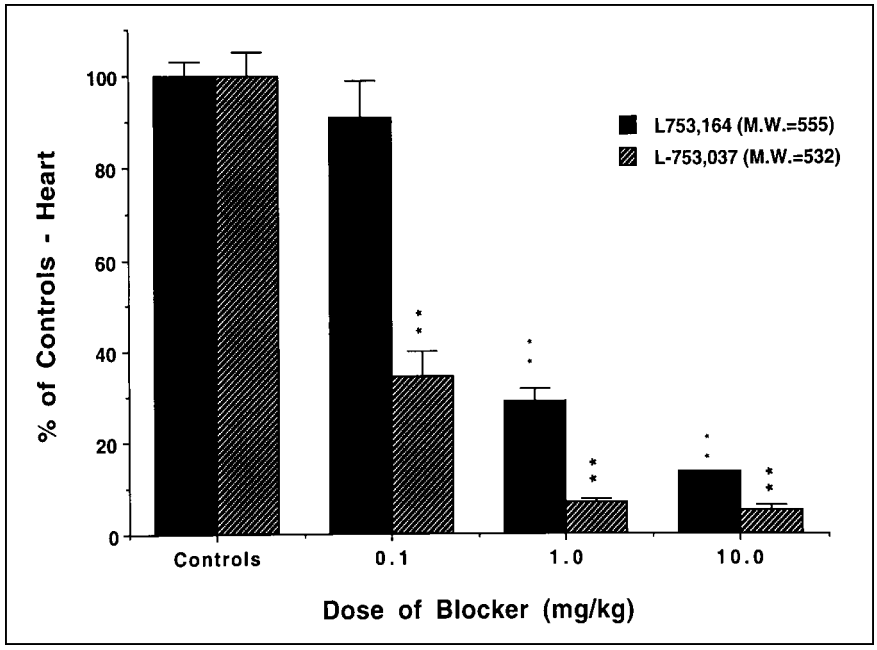

FIGURE 5. Comparison of effects of different doses (0.1–10 mg/kg, administered 30 min before tracer injection) of ET_A-specific inhibitor L-753,164 and mixed ET_A/ET_B inhibitor L-753,037 on radioactivity concentrations in mouse heart 30 min after intravenous injection of [^{11}C]L-753,037. Data are percentages (±SEM) of saline controls; $n = 3–5$. $^{**}P < 0.001$.

after injection of the L-753,164 inhibitor. There was a significant reduction in radioactivity in the lateral heart wall after pretreatment with L-753,164. By contrast, radioactivity in the cavity of the left ventricle did not show any reduction. Activity in the lungs was not reduced after receptor blocking with L-753,164 but was slightly elevated, which was probably caused by an increase in blood activity.

DISCUSSION

Recently, L-753,037 has been described as a potent, selective inhibitor of ET_A and ET_B receptors, with K_i values of 0.034 and 0.104 nmol/L, respectively, using [^{125}I]ET-1 competition binding assays in cloned human ET_A and ET_B receptor preparations (*27*). The binding of L-753,037 to ET receptors in vitro was shown to be reversible and competitive (*27*). In the study presented here, L-753,037 was radiolabeled with the positron-emitting radionuclide ^{11}C and the new radiotracer was evaluated for its binding to ET receptors in mice and a dog.

Special attention was given to the in vivo binding of [^{11}C]L753,037 to ET receptors in the heart because myocardiocytes are known to contain high concentrations of ET receptors, and ET may be involved in diseases such as congestive heart failure, hypertension, atherosclerosis, and myocardial infarction (*8,9,11,30–32*). In an animal model of chronic heart failure, both ET-1 plasma concentrations and ET-1 binding sites have been reported to be elevated (*33,34*). Recently, densities of ET-1 receptors have been measured in human heart tissue from patients with idiopathic dilative cardiomyopathy and chronic heart failure (CHF) (*35,36*). Although increases in ET_A receptor density in these conditions were found by some groups of investigators (*36,37*), no increase was reported by another group (*35*). PET scanning of ET_A/ET_B receptors in vivo may afford a more accurate estimate of receptor densities in a patient's heart muscle and may provide a means to follow the progression of CHF in various stages of the disease.

The binding of [^{11}C]L-753,037 to ET receptors in various organs was determined in mice. At 30 and 60 min after intravenous injection, the liver showed the highest tracer accumulation, followed by the adrenals, kidneys, heart, and lungs. [^{11}C]L-753,037 did not cross the blood–brain barrier,

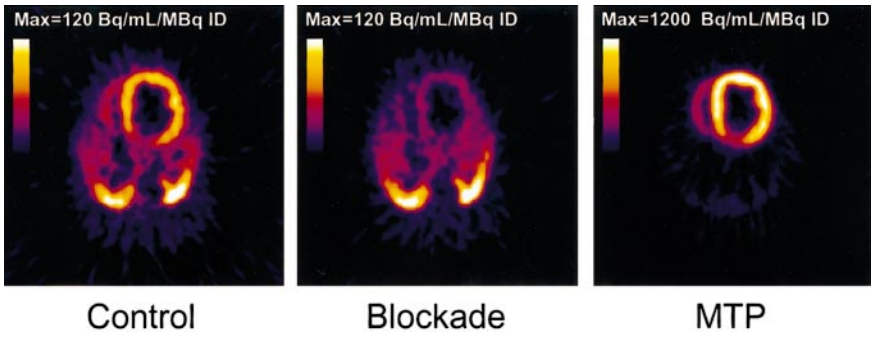

FIGURE 6. PET images of dog heart 55–95 min after injection of [^{11}C]L-753,037 without (left) and with (middle) preinjection of 1 mg/kg unlabeled L-753,164, a selective ET_A antagonist. There is significant reduction of [^{11}C]L-753,037 accumulation in wall of heart ($P < 0.001$) at 30 min after tracer administration. For comparison, perfusion scan with [^{11}C]MTP is also shown (right).

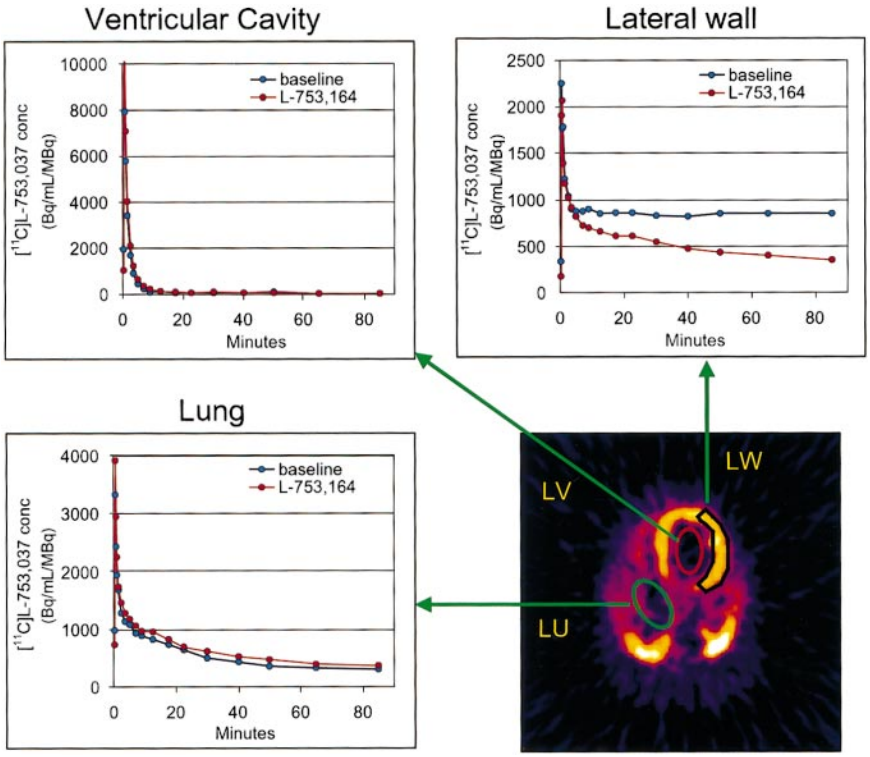

FIGURE 7. Time–activity curves of [11C]L-753,037 in ventricular cavity and lateral wall of dog heart and lungs before and after intravenous injection of 1 mg/kg L-753,164 (ET_A antagonist).

as indicated by low radioactivity counts in the brain. ET binding sites previously have been studied in vitro in rat tissue (*38*) and in vivo in rats (*39*) using [125I]ET-1, a tracer with a high affinity for both ET_A and ET_B receptors. Highest densities of [125I]ET-1 binding sites were reported in the lungs, with progressively lesser concentrations in the kidneys, adrenals, and heart, whereas the liver showed relatively low binding. In 1996, BQ-123, an ET_A-selective receptor antagonist, was used by Shin et al. (*40*), who showed ET_A receptor concentrations in rat lungs about 7 times larger than those in the heart and adrenals and no detectable receptor sites in the kidneys and liver. Because in this study the high accumulation of [11C]L-753,037 in the liver could not be inhibited by either the ET_A-specific ligand L-753,164 or the mixed ET_A/E_B antagonist L-753,037 (Fig. 3), it has to be concluded that [11C]L-753,037 binding in the liver is not specific for ET receptors. The possibility remains that a considerable portion of the 11C radioactivity in the liver is caused by metabolic products and not the parent compound. A high-performance liquid chromatography analysis could be used to confirm this explanation.

Clearance times of the tracer from different organs varied widely (Figs. 2 and 7). The apparent binding of the [11C]L-753,037 tracer in the heart as early as 5 min after administration and the lack of significant washout from heart tissues over the entire observation period (2 h for mice, 90 min for the dog) were remarkable. By contrast, tracer clearance from the lungs was much faster than from the heart, resulting in favorable heart-to-lung ratios in the dog PET study of 2.5 and 2.7 at 65 and 85 min, respectively, after injection.

Interestingly, inhibition of [11C]L-753,037 binding in the mouse heart (Fig. 5) was greater with the mixed ET_A/ET_B inhibitor than with the selective ET_A blocker. The affinities for these 2 antagonists for the ET_A receptor differ by less than 2-fold in vitro but by as much as 10-fold in vivo. This may reflect differences in metabolism or the volume of distribution for the 2 antagonists. The apparent difference between the degree of inhibition in the heart caused by the 2 blockers presented in Figures 3 and 5 is, most likely, because inhibition in Figure 3 was measured at 60 min after tracer injection, whereas in Figure 5 it was measured at 30 min after injection. This finding emphasizes the importance of radioligand clearance for the evaluation of specific binding.

Visualization of ET binding sites in cardiac muscle using [11C]L-753,037 and PET was successful (Fig. 6). Images of the left ventricle compared well with images obtained with [11C]methyl triphenyl phosphonium, a tracer for measuring myocardial blood flow during ischemia (*29*). Preinjection of the ET_A-selective inhibitor L-753,164 (1 mg/kg = 1.8 μmol/kg) led to a significant reduction in [11C]L-753,037 accumulation in cardiac muscle, indicating specific binding of the tracer to ET receptors in the myocardium. By contrast, [11C]L-753,037 uptake in the lungs was not affected by the antagonist L-753,164, an indication that accumulation of this tracer in the lungs is, apparently, not caused by binding to ET_A receptors. The same conclusion can be drawn from results obtained in the lungs of the mice (Fig. 3), which showed no significant decrease in tracer uptake after L-753,164 pretreatment.

CONCLUSION

The results of this study indicate that the new PET tracer [^{11}C]L-753,037 binds to ET_A and ET_B receptors in vivo in mice and dogs. [^{11}C]L-753,037 should be useful for noninvasive imaging of the ET receptors in healthy individuals and patients with cardiovascular diseases and cancer. [^{11}C]L-753,037 could also be applied to determine the potency of new ET receptor antagonists in vivo.

REFERENCES

1. Hickey KA, Rubanyi G, Paul RJ, Highsmith RF. Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am J Physiol.* 1985;248: C550–C556.
2. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature.* 1988;332:411–415.
3. Inoue A, Yanagisawa M, Kimura S, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci USA.* 1989;86:2863–2867.
4. Xu D, Emoto N, Giaid A, et al. ECE-1: a membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. *Cell.* 1994;78:473–485.
5. Webb DJ. Endothelin: from molecule to man. *Br J Clin Pharmacol.* 1997;44:9–20.
6. Hemsen A, Franco-Cereceda A, Matran R, Rudehill A, Lundberg JM. Occurrence, specific binding sites and functional effects of endothelin in human cardiopulmonary tissue. *Eur J Pharmacol.* 1990;191:319–328.
7. Douglas SA, Gellai M, Ezekiel M, Ohlstein EH. BQ-123, a selective endothelin subtype A-receptor antagonist, lowers blood pressure in different rat models of hypertension. *J Hypertens.* 1994;12:561–567.
8. Kiowski W, Sutsch G, Hunziker P, et al. Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure. *Lancet.* 1995;346:732–736.
9. Bacon CR, Cary NR, Davenport AP. Distribution of endothelin receptors in atherosclerotic human coronary arteries. *J Cardiovasc Pharmacol.* 1995; 26(suppl):S439–S441.
10. Salomone OA, Elliott PM, Calvino R, Holt D, Kaski JC. Plasma immunoreactive endothelin concentration correlates with severity of coronary artery disease in patients with stable angina pectoris and normal ventricular function. *J Am Coll Cardiol.* 1996;28:14–19.
11. Setsuta K, Seino Y, Tomita Y, Nejima J, Takano T, Hayakawa H. Origin and pathophysiological role of increased plasma endothelin-1 in patients with acute myocardial infarction. *Angiology.* 1995;46:557–565.
12. Kurbel S, Kurbel B, Kovacic D, et al. Endothelin-secreting tumors and the idea of the pseudoectopic hormone section in tumors. *Med Hypotheses.* 1999;52:329–333.
13. Alanen K, Deng DX, Chakrabarti S. Augmented expression of endothelin-1, endothelin-3 and the endothelin-B receptor in breast carcinoma. *Histopathology.* 2000;36:161–167.
14. Bagnato A, Salani D, Di Castro V, et al. Expression of endothelin 1 and endothelin A receptor in ovarian carcinoma: evidence for an autocrine role in tumor growth. *Cancer Res.* 1999;59:720–727.
15. Ahmed SI, Thompson J, Coulson JM, Woll PJ. Studies on the expression of endothelin, its receptor subtypes, and converting enzymes in lung cancer and in human bronchial epithelium. *Am J Respir Cell Mol Biol.* 2000;22:422–431.
16. Pagotto U, Arzberger T, Hopfner U, et al. Expression and localization of endothelin-1 and endothelin receptors in human meningiomas: evidence for a role in tumoral growth. *J Clin Invest.* 1995;96:2017–2025.
17. Ihara M, Noguchi K, Saeki T, et al. Biological profiles of highly potent novel endothelin antagonists selective for the ETA receptor. *Life Sci.* 1992;50:247–255.
18. Kobayashi M, Ihara M, Sato N, et al. A novel ligand, [^{125}I]BQ-3020, reveals the localization of endothelin ETB receptors. *Eur J Pharmacol.* 1993;235:95–100.
19. Clozel M, Breu V, Gray GA, et al. Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. *J Pharmacol Exp Ther.* 1994;270:228–235.
20. Williams DL, Murphy KL, Nolan NA, et al. Pharmacology of L-754,142, a highly potent, orally active, nonpeptidyl endothelin antagonist. *J Pharmacol Exp Ther.* 1995;275:1518–1526.
21. Ohlstein EH, Nambi P, Lago A, et al. Nonpeptide endothelin receptor antagonists. VI. Pharmacological characterization of SB 217242, a potent and highly bioavailable endothelin receptor antagonist. *J Pharmacol Exp Ther.* 1996;276: 609–615.
22. Wu C, Chan MF, Stavros F, et al. Discovery of TBC11251, a potent, long acting, orally active endothelin receptor-A selective antagonist. *J Med Chem.* 1997;40: 1690–1697.
23. Maguire JJ, Kuc RE, Davenport AP. Affinity and selectivity of PD156707, a novel nonpeptide endothelin antagonist, for human ET(A) and ET(B) receptors. *J Pharmacol Exp Ther.* 1997;280:1102–1108.
24. Hoshino T, Yamauchi R, Kikkawa K, Yabana H, Murata S. Pharmacological profile of T-0201, a highly potent and orally active endothelin receptor antagonist. *J Pharmacol Exp Ther.* 1998;286:643–649.
25. Ohlstein EH, Nambi P, Hay DW, et al. Nonpeptide endothelin receptor antagonists. XI. Pharmacological characterization of SB 234551, a high-affinity and selective nonpeptide ETA receptor antagonist. *J Pharmacol Exp Ther.* 1998;286: 650–656.
26. Johnstrom P, Aigbirhio FI, Clark JC, Downey SP, Pickard JD, Davenport AP. Synthesis of the first endothelin-A- and -B-selective radioligands for positron emission tomography. *J Cardiovasc Pharmacol.* 2000;36(suppl):S58–S60.
27. Nishikibe M, Ohta H, Okada M, et al. Pharmacological properties of J-104132 (L-753,037), a potent orally active, mixed ET_A/AT_B endothelin receptor antagonist. *J Pharmacol Exp Ther.* 1999;289:1262–1270.
28. Ravert HT, Mathews WB, Hamill TG, Burns HD, Dannals RF. Radiosynthesis of a potent endothelin receptor antagonist. *J Labelled Compounds Radiopharm.* 2000;43:1205–1210.
29. Krause BJ, Szabo Z, Becker LC, et al. Myocardial perfusion with [^{11}C]methyl triphenyl phosphonium: measurements of the extraction fraction and myocardial uptake. *J Nucl Biol Med.* 1994;38:521–526.
30. Wei CM, Lerman A, Rodeheffer RJ, et al. Endothelin in human congestive heart failure. *Circulation.* 1994;89:1580–1586.
31. Dashwood MR, Barker SG, Muddle JR, Yacoub MH, Martin JF. [^{125}I]-endothelin-1 binding to vasa vasorum and regions of neovascularization in human and porcine blood vessels: a possible role for endothelin in intimal hyperplasia and atherosclerosis. *J Cardiovasc Pharmacol.* 1993;22(suppl):8:S343–S347.
32. Omland T, Lie RT, Aakvaag A, Aarsland T, Dickstein K. Plasma endothelin determination as a prognostic indicator of 1-year mortality after acute myocardial infarction. *Circulation.* 1994;89:1573–1579.
33. Miyauchi T, Sakai S, Ihara M, et al. Increased endothelin-1 binding sites in the cardiac membranes in rats with chronic heart failure. *J Cardiovasc Pharmacol.* 1995;26(suppl):S448–S451.
34. Sakai S, Miyauchi T, Sakurai T, et al. Endogenous endothelin-1 participates in the maintenance of cardiac function in rats with congestive heart failure: marked increase in endothelin-1 production in the failing heart. *Circulation.* 1996;93: 1214–1222.
35. Ponicke K, Vogelsang M, Heinroth M, et al. Endothelin receptors in the failing and nonfailing human heart. *Circulation.* 1998;97:744–751.
36. Zolk O, Quattek J, Sitzler G, et al. Expression of endothelin-1, endothelin-converting enzyme, and endothelin receptors in chronic heart failure. *Circulation.* 1999;99:2118–2123.
37. Pieske B, Beyermann B, Breu V, et al. Functional effects of endothelin and regulation of endothelin receptors in isolated human nonfailing and failing myocardium. *Circulation.* 1999;99:1802–1809.
38. Koseki C, Imai M, Hirata Y, Yanagisawa M, Masaki T. Autoradiographic distribution in rat tissues of binding sites for endothelin: a neuropeptide? *Am J Physiol.* 1989;256:R858–R866.
39. Gibson RE, Fioravanti C, Francis B, Burns HD. Radioiodinated endothelin-1: a radiotracer for imaging endothelin receptor distribution and occupancy. *Nucl Med Biol.* 1999;26:193–199.
40. Shin HC, Kato Y, Shitara Y, et al. The endothelin receptor is a major determinant for the nonlinear tissue distribution of the endothelin antagonist BQ-123. *J Pharmacol Exp Ther.* 1996;278:607–613.