A Noninvasive Procedure for In Vivo Assay of a Lung Amine Endothelial Receptor

Juan J. Touya, Javad Rahimian, David E. Grubbs, Howard F. Corbus, and Leslie R. Bennett

UCSF—Fresno Central San Joaquin Valley Medical Education Program, Department of Nuclear Medicine, Saint Agnes Medical Center; and Department of Biology, California State University, Fresno, California

Lung endothelial N-isopropyl-p-iodoamphetamine (IMP) binding sites were assessed applying principles of competitive binding assay adapted for in vivo measurements obtained by digital imaging. Data were acquired following the method published by Rahimian et al., a modification of the dual indicator dilution technique of Chinard and Crone. Iodine-123 (^{123}I) IMP, the test cellular tracer, and technetium-99m (^{99m}Tc) dextran, the reference vascular tracer were imaged during their first pass through the superior vena cava, right heart, lungs, and left heart in West African dwarf goats. The lung fractional extraction of IMP diminished progressively from 0.96 to 0.20 as the amount of IMP in the test tracer boluses was gradually increased from 0.6 to 150 mg. This demonstrated that lung extraction of IMP is by way of a saturable binding system, presumably receptors. The dissociation constant of IMP-lung binding sites reaction was calculated by Scatchard plot and found to be 11.7 mg. The amount of IMP bound at saturation (R), was found to be 30 mg. Assuming that a single molecule of IMP bound a single receptor, the total number of free receptors was computed as the Avogadro's number times R, divided by the IMP molecular weight, and found to be 6.04×10^{19}. Using a computer model, it was determined that the 20 mg per bolus isotherm was the most sensitive for measuring the number of total free receptors (binding sites). This is the first time, to our knowledge, that noninvasive in vivo assessment of receptors in lung has been accomplished. Basically, the method used can be applied in humans and, also, to assess receptors in organs other than the lungs.

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In addition to its function in the exchange of gases, the pulmonary circulation carries out an important metabolic function through the modulation of the chemical composition of the arterial blood, indicating that the lung is a significant metabolic organ. In man, the entire blood volume passes through the lungs every 20 sec and its composition is modified by several mechanisms: (a) uptake of plasma substances and subsequent partial or total enzymatic inactivation; (b) partial or total enzymatic inactivation of plasma substrates, without previous uptake; (c) formation of active materials from lung cell constitutents; and (d) formation of active materials from blood components. Through these mechanisms the lungs control the blood concentration of some hormones, biogenic amines, peptides, fatty acid derivatives, and drugs (*1*). The lung is also capable of accumulating, without metabolic interaction, nonbiogenic amines due to the existence of saturable binding sites of limited specificity in the endothelial cells (*2*).

Previous studies of lung metabolic functions have been carried out using invasive procedures in tissue slices, homogenates or minces, isolated lung and endothelial cells, subcellular fractions, cells in culture, isolated perfused lung preparations, in situ perfused lungs, and intact animals or human subjects (arterial-venous differences) (*3*).

In 1982, (*4,5*) we demonstrated the feasibility of measuring noninvasively in dogs, lung metabolic functions using a technique based on the dual indicator dilution method for analysis of the transport of substances through the capillary membranes, developed by Chinard and Crone (*6,7*). We used N-isopropyl-[^{123}I]-*p*-iodoamphetamine ([^{123}I]IMP), a synthetic amine, as cellular test tracer and [^{99m}Tc]sulfur colloid as a vascu-

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For reprints contact: J. J. Touya, Dept. of Nucl. Med., St. Agnes Medical Center, 1303 E. Herndon Ave., Fresno, CA 93710.

lar reference tracer, assessing the [^{123}I]IMP lung extraction during its first pass through the lungs.

The purpose of the present research is to demonstrate: (a) that the lung uptake of N-isopropyl-*p*-iodoamphetamine (IMP) is by way of a saturable mechanism; (b) that by combining modern emission imaging techniques with the principles of displacement analysis used in radioimmunoassay, it is possible to measure, in vivo and noninvasively, the total amount of lung endothelial binding sites (receptors) for a determined amine.

MATERIALS AND METHODS

Eight West African dwarf goats, four females and four males, weighing between 20 and 30 pounds each, were used in 15 experiments. After fasting 24 hr, animals were anesthetized with xylazine (0.30 mg/kg b.w.) administered intramuscularly 20 min before each experiment. A sterile nonpyrogenic 20G teflon catheter was placed in the jugular vein and connected to a continuous saline drip. N-isopropyl-[^{123}I]-*p*-iodoamphetamine ([^{123}I]IMP) and technetium-99m (^{99m}Tc) dextran, the test and reference tracers, were injected as boluses through this catheter. The activity and volume of the test tracer were kept constant, 3 mCi and 3 ml per bolus, respectively, while the amount of IMP was varied. In the different experiments, the amount of IMP per bolus was 0.6, 5, 10, 20, 40, 60, 80, 100, or 150 mg. Technetium-99m sulfur colloid, which was used in the earlier dog experiments as reference tracer (*5*), was replaced by [^{99m}Tc]dextran (molecular weight 87,000) because a high uptake of sulfur colloid was observed in the goat lungs making it unsuitable as a vascular tracer. The boluses of [^{99m}Tc]dextran consisted of 10 mCi in 1 ml. Both the test and the reference tracer boluses were successively injected at an interval of 10 min and each one was flushed through the i.v. line with 10 ml of saline. A minimum of 1 wk free interval between measurements in the same animal was observed for the pharmacologic washout of the previously injected drugs.

The tracers were imaged during their first pass through the superior vena cava (SVC), right heart, lungs, and left heart using a scintillation camera* on line with a modified clinical acquisition module.† A low-energy, all-purpose collimator was used. The system deadtime, as determined by the two sources method, was measured several times and found to be always less than 3 μsec. The sequential images were acquired for 40 sec in 64 × 64 matrices using 1.5 zoom and a time resolution of 0.16 sec per frame. After acquisition, images were transferred to an ADAC 3300 computer and processed. During processing, regions of interest were drawn over the SVC and the left lung to create the corresponding time-activity curves. The SVC and lung time-activity curves of the test and reference tracers were deconvolved to determine the impulse response functions for each tracer. The fractional extraction of the test tracer was computed as the difference between the test tracer and the reference tracer normalized impulse response functions, divided by one minus the normalized impulse response function of the reference tracer. The extraction fraction of the test tracer was determined at the time that 90% of the reference tracer had left the lung compartment.

DATA ANALYSIS AND RESULTS

Lung extraction of IMP as a function of amount injected

The lung fractional extraction of IMP decreased with the increased amount of IMP in the test tracer bolus. As shown in Fig. 1, 96% of the injected IMP was extracted by the lung during its first pass through the pulmonary circulation, when the amount of IMP in the bolus was 0.6 mg. When the amount of IMP in the bolus was increased to 150 mg, the extraction dropped to 20%; and 80% of the injected IMP passed through the lung without being removed.

Dissociation constant of the binding reaction of IMP in the lungs

The amount of IMP extracted by the lungs was considered to be the bound ligand (Z) and the amount

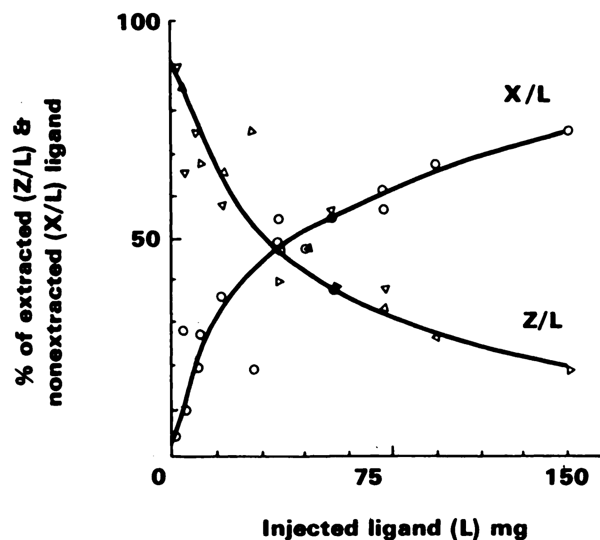

FIGURE 1
Percent of [^{123}I]IMP extraction as function of total amount of IMP injected. Triangles are percent of extracted IMP by lung and line through triangles represents curve of bound ligand (Z) divided by total ligand (L) as function of injected ligand (L). Circles are percent of IMP injected which passes through lungs. Therefore, line through circles represents curve of free ligand (X) over total ligand (L) as function of total ligand (L)

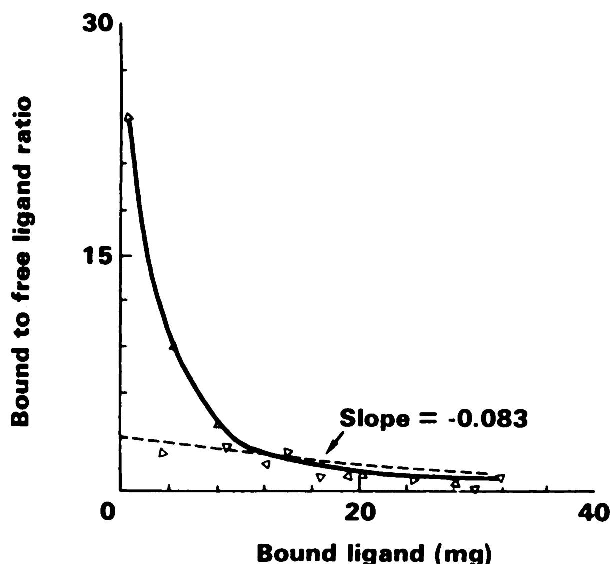

FIGURE 2
Scatchard plot. In abscissas ratio of bound IMP (Z) over free IMP (X) has been plotted. In ordinates amount of bound IMP (Z) in mg has been plotted. Curve shows two slopes, first is unresolvable. Second was used to calculate dissociation constant. Dissociation constant, 11.7 mg is equal to inverse of slower slope

of IMP which passed through the lungs was considered to be the free ligand (X). A Scatchard plot of the ratio of bound ligand over free ligand (Z/X) versus the bound ligand in mg (Z), was constructed to calculate the dissociation constant (Kd) of the IMP-binding site reaction. Following Schafer (*8*), the inverse of the slope of this curve (Fig. 2) is equal to the dissociation constant of the binding reaction at equilibrium. The dissociation constant of the IMP-binding sites reaction in the lung of the West African goats was calculated to be 11.7 mg.

Saturability of IMP lung binding sites

The amount of bound IMP in mg (Z) was plotted against the amount of injected IMP in mg (L) and against the amount of free IMP in mg (X). These curves (Fig. 3) showed a rapid rise followed by a plateau which began at the level of 80 mg of total injected IMP and 52 mg of free IMP. The presence of a plateau in these two curves demonstrated that the lung uptake of IMP was saturable and, therefore, that the uptake took place in discrete binding sites.

Total number of IMP binding sites in the lungs

Knowing the amount of IMP needed to saturate the binding sites, the total number of binding sites was calculated using the following formula:

$$NR = R \times No/MW$$

where NR is the total number of binding sites, R is the amount of bound IMP at the plateau of the curve in Fig. 3 (30.4 mg); No is the Avogadro number (6.025×10^{23}) and MW is the molecular weight of IMP (303). For this calculation it was assumed that each binding site bound a single molecule of IMP; therefore, the number of bound IMP molecules at saturation is equal to the number of available binding sites in the lung. The total number of IMP binding sites in the West African dwarf goats was 6.04×10^{19}.

Dose response curves

The percent of bound IMP was plotted against the logarithm of the number of moles of injected IMP (Fig. 4). This curve showed that when small amounts of IMP were injected, the sensitivity of the measurements deteriorated. A computer simulation was used (Fig. 5) to determine the binding isotherm with highest sensitivity for measuring the number of free binding sites. Isotherms were computer-created using as variables the amount of injected IMP and the percent of free binding sites; and as constants the previously computed dissociation constant and the total number of binding sites in the lung. The isotherm which best approached the diagonal was obtained for simulated injections of ~20 mg of IMP per bolus.

DISCUSSION

Although the word "receptor" or "binding site" has been used with various connotations, we use the term in this paper in a broad sense—to designate any biomole-

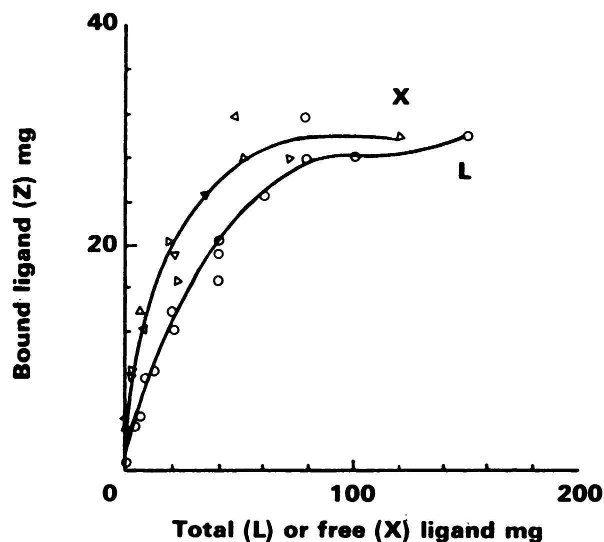

FIGURE 3
Dependence of bound ligand concentration (Z) on concentration of free ligand (X) and total ligand (L). Since L is sum of X and Z, curve of Z versus L is shifted to right, with respect to curve of Z versus X, by amount of Z

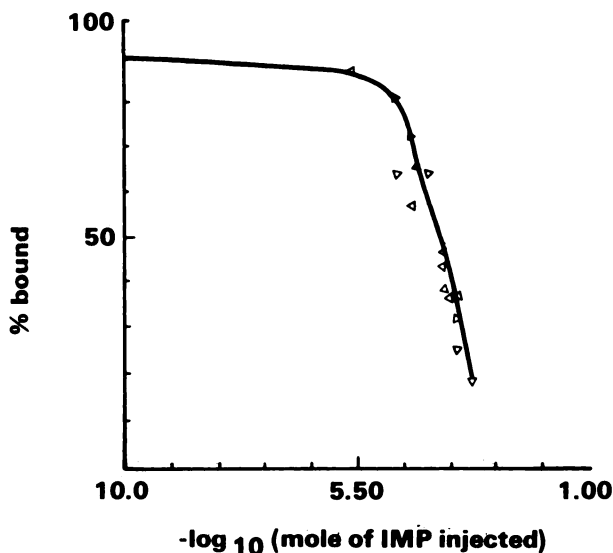

FIGURE 4
Percent of bound ligand as a function of number of molecules of ligand injected. This curve shows that small amounts of ligand in test tracer bolus offer low sensitivity to assess binding sites. Amounts larger than 150 mg were not used because pharmacological effect of free ligand in central nervous system

cule which specifically binds a chemical agent (ligand), from either inside or outside the cell (*8*). Under this definition a specific cellular recognition site which binds a drug, hormone, or amine is a receptor (*9*). The existence of receptors has been theorized for the past century; however, it is only within the past two decades that they have been studied directly, principally through the development of the remarkably simple technique of radioligand binding. Receptors have, up until this time, been measured in samples of the extracellular fluid, cell membranes, cytosol, cell organelles, cells and tissues, but they have not been assessed in vivo in a total organ. Motulsky et al. predicted in 1982 (*10*) the possibility of noninvasive procedures to measure in vivo the beta-adrenergic receptors by using appropriately labeled ligands and either single photon emission scans, positron emission tomography or nuclear magnetic resonance.

N-isopropyl-*p*-iodoamphetamine (IMP), a synthetic amine which can easily be labeled by isotopic exchange with ^{123}I, was developed by Winchell, Baldwin, and Lin for the purpose of evaluating regional brain blood flow in humans (*11*). It is a lipophilic amine and the mechanism of its brain accumulation is not definitely known. It is postulated that it passes through the blood-brain barrier because of its lipophilic properties; and once in the brain, it is either bound to nonspecific receptor sites or is converted to nonlipophilic metabolites (*12*). In studies of the biodistribution of [^{123}I]IMP a highly significant early accumulation of IMP within the lungs was noticed. Winchell et al. (*11*) observed in rats that at 5 min postintravenous administration, 12% of the dose was in the lungs, while Holman et al. (*13*) observed in monkeys that at 15 min, 11% of the dose was in the lungs. Both groups of investigators noticed that the amount of [^{123}I]IMP accumulated in the brain increased with time as the amount in the lungs decreased.

Stimulated by these findings, we studied the uptake of IMP in the lung immediately after its arrival to the pulmonary circulation (*5*). To measure the uptake or accumulation of IMP during its first pass through the pulmonary circulation, we developed a procedure based on the dual tracer dilution technique of Chinard and Crone (*6,7*) for measuring the transfer of molecules through membranes. Using this technique, we demonstrated that ~96% of the i.v. bolus of [^{123}I]IMP was cleared by the lung during its first pass through the

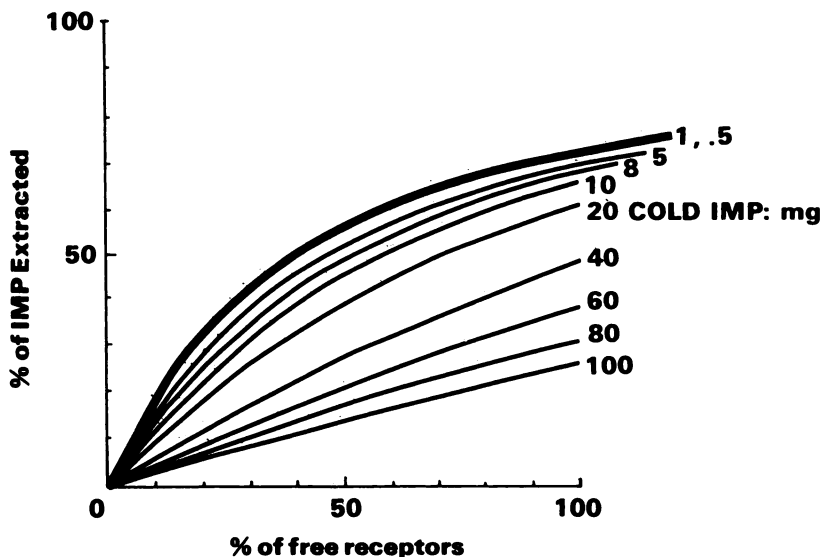

FIGURE 5
Computer simulation of binding isotherms. Variables were amount of injected IMP (L), and percent of free lung endothelial receptors (binding sites). Constants were dissociation constant of IMP-receptor binding (11.7 mg) and number of total receptors in endothelium of pulmonary circulation (6.04 × 10^{19})

pulmonary circulation, and later slowly released from the lung into the systemic circulation (*4,5*).

Oldendorf (*14*) has postulated that the uptake of substances in an organ is initiated by either a nonsaturable lipid-mediated or a saturable carrier-mediated capillary membrane transport mechanism. With the purpose of determining which of these two mechanisms was involved in the lung uptake of [^{123}I]IMP, we progressively increased the amount of IMP in the test tracer boluses by adding unlabeled IMP. Results of the experiments presented here definitely demonstrated that the lung uptake of [^{123}I]IMP is by way of a saturable mechanism. The curve of lung extraction as a function of the total amount of IMP injected showed a progressively decreasing down slope and became asymptotic at a dose of ~80 mg of IMP per bolus. If the transport of the IMP from the plasma to the lung were due exclusively to its lipophilic properties, the amount of IMP taken by the lung should linearly increase with the amount injected, the lung uptake should be a function of the partitioning or ratio of solubility of IMP in the plasma and in the phospholipids of the cell membranes (*12*). The fact that the lung uptake of IMP in goats is saturable validates mathematically the presence of IMP binding sites in the lung. Our results agree with the findings of Philpot et al. (*15*) who, using invasive methods, demonstrated that amphetamine, a similar molecule to IMP, is removed by lung endothelial cell through carrier-mediated transport mechanisms, while other basic amines are removed by both receptors and lipid-mediated transport mechanisms. We have not yet studied the behavior of IMP and of its binding site after the initial binding; nor have we investigated the specificity of this reaction. Akber et al. (*16*) found propranolol to decrease the lung uptake of IMP in a dose-related ratio and they suggest that propranolol competes with IMP for the same lung endothelial binding sites. Our objectives were to determine only the uptake mechanism and at the same time to develop a procedure for in vivo assay of the binding sites.

Here we are presenting a technique and results of the first in vivo measurements of the total amount of a determined receptor (endothelial binding sites) in the lung. To do that we have combined algorithms of displacement analysis with processing techniques of gamma photon emission images. This technique can easily be applied to humans since the used radioligand and the reference tracer are radiopharmaceuticals approved for human use, and the instrumentation is commonly found in any nuclear medicine department of a community hospital.

The principles of this technique might be applied to measure receptors in other organs. The lung is the organ where assessment of receptors can be accomplished with greater ease because the radioligand can be injected intravenously and arrives as an intact bolus to the lungs. To measure receptors in other organs it will be necessary to inject the radioligands by a catheter placed in the input artery of the organ or to develop a technique to compute the organ input, which is within the capability of today's emission imaging technology. The binding reaction between IMP and lung receptors occurs so rapidly that measurements can be made during the first pass through the pulmonary ciculation. In situations where the binding reactions take place more slowly it will be necessary to adjust the time of image sampling to the reaction rate allowing equilibrium to occur, or to mathematically compute assay values prior to equilibrium.

Further investigation is necessary to refine the procedure, but we predict that this technique will bring new knowledge about the lung metabolic functions and that its future applications will allow the in vivo assay of receptors in other organs.

FOOTNOTES

* Pho/Gamma V Siemens scintillation camera, Siemens Medical Systems, Iselin, N.Y.

† ADAC Laboratories, San Jose, CA.

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