Noninvasive Measurement of Lung Carbon-11-Serotonin Extraction in Man

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The fraction of serotonin extracted on a single passage through the lungs is being used as an early indicator of lung endothelial damage but the existing techniques require multiple arterial blood samples. We have developed a noninvasive technique to measure lung serotonin uptake in man. We utilized the double indicator diffusion principle, a positron camera, ^{11}C-serotonin as the substrate, and ^{11}CO-erythrocytes as the vascular marker. From regions of interest around each lung, we recorded time-activity curves in 0.5-sec frames for 30 sec after a bolus injection of first the vascular marker ^{11}CO-erythrocytes and 10 min later ^{11}C-serotonin. A second uptake measurement was made after imipramine 25–35 mg was infused intravenously. In three normal volunteers, the single-pass uptake of ^{11}C-serotonin was 63.9% ± 3.6%. This decreased in all subjects to a mean of 53.6% ± 1.4% after imipramine. The rate of lung washout of ^{11}C was also significantly prolonged after imipramine. This noninvasive technique can be used to measure lung serotonin uptake to detect early changes in a variety of conditions that alter the integrity of the pulmonary endothelium.

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It is well-established that in addition to its function as the organ of gas exchange, the lung plays a major role in regulating a number of circulating chemical mediators (*1*, *2*). For example, nor-epinephrine and 5-hydroxytryptamine (5-HT serotonin), are both almost completely extracted on a single transit through the pulmonary circulation. The rate-limiting step to serotonin extraction is its active transport across the pulmonary endothelial cell membrane. Once transported into the endothelial cell, serotonin is degraded by monoamine oxidase (MAO).

The extraction of serotonin by the lung is inhibited by a number of drugs such as cocaine, chlorpromazine and the tricyclic antidepressants (e.g., imipramine) (*3*,*4*), and in a number of pathologic conditions such as experimental diffuse lung injury in animals (*5*) and adult respiratory distress syndrome (ARDS) in man (*6*). In the clinical situation, the lung extraction of ^{14}C-serotonin has been developed as a technique to detect early changes in the lungs of patients at high risk for developing ARDS (*6*). However, this technique requires arterial catheterization, which limits its general application in both clinical and experimental situations. In order to make external, noninvasive measurements of the extraction of amines by the lungs, we have synthesized carbon-11-serotonin (^{11}C-5HT) (*7*).

This paper describes the measurement of the fractional extraction of ^{11}C-serotonin on a single pass through the lungs of normal volunteers and the effect of intravenously injected imipramine on this extraction.

METHODS

The fraction of ^{11}C-serotonin extracted on a single pass through the lungs was calculated from a modification of the double-indicator diffusion technique of Crone (*8*). For the reference, nonextracted tracer we used ^{11}C-carbon monoxide labeled erythrocytes (^{11}CO-RBC) produced as detailed below.

Labeled Compounds

Carbon-11. Carbon was produced with 17 MeV protons (35 μA) by the ^{14}N (p, α) ^{11}C nuclear reaction in a nitrogen/hydrogen target.

^{11}C-Hydrogen-Cyanide. This was produced as described elsewhere (*7*). During production, most of the ^{11}C stabilizes as $^{11}CH_4$. Gaseous ammonia was added to this and the mixture passed over platinum wool at 1000°C to produce H^{11}CN.

^{11}C-5 Hydroxytryptamine. We have described the synthesis of ^{11}C-5HT in detail elsewhere (*7*). In brief, this was a four-step procedure starting with nucleophilic substitution of ^{11}C-hydrogen cyanide to 5-methoxygramine to produce ^{11}C-5-methoxy-3-acetonitrile-indole. The methyl ether was cleaved with boron tribromide and the nitrile reduced to give ^{11}C-5 hydroxytryptamine (^{11}C-5HT). This was purified by reverse-phase high pressure liquid chromatography. The synthesis produced 11.1 ± 3.8 mCi of ^{11}C-5HT with a specific activity of 140 mCi/μmole.

^{11}CO-erythrocytes. Labeled carbon monoxide was produced by reduction of $^{11}CO_2$ with zinc at 1000°C. The ^{11}CO was then bubbled through 15 ml of the subject's own blood in a sterile glass bulb. The bulb was rotated gently for 15 min to assure adequate labeling of the erythrocytes with ^{11}CO.

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Lung Extraction Measurements

We measured the fractional lung extraction of ^{11}C-5HT in three normal volunteers before and after intravenous infusion of imipramine (Tofranil-Ciba-Geigy). This procedure was approved by the institute's ethics committee.

In two experiments, the subject lay supine with the model 4200 Positron Camera System (Cyclotron Corporation, Berkeley, CA) positioned to image the lungs. First, 3.0 mCi ^{11}CO-RBC in 3 ml was injected rapidly through a bolus-loop system into a large ante-cubital vein. Counts were recorded from the thorax in 0.5-sec frames for 30 sec followed by 2-sec frames for 270 sec. Fifteen minutes later, after a 10-sec collection period for background counts, 2.5–4.0 mCi ^{11}C-5HT (0.05–0.1 μmole) was injected as a bolus of 3 ml through the same loop system by the same injector. Counts were recorded again in the same sequence of frame rates for 5 min. We then infused imipramine 0.4–0.5 mg/kg body weight (25–35 mg) over a 10-min period. The subject's pulse rate and blood pressure were monitored every minute throughout the infusion. A second 3-ml bolus injection of ^{11}C-5HT was made 10 min from the end of the imipramine infusion (i.e., 25 min after the first injection of ^{11}C-5HT). Again, counts were collected for background subtraction for 10 sec before injection and in the same sequence of frame rates for 5 min after injection.

In one subject, ^{11}CO-RBC and ^{11}C-5HT data were collected on one morning. A second sequence of ^{11}CO-RBC and ^{11}C-5HT data were then collected the next day, 15 min after the infusion of 35 mg of imipramine.

Data Analysis

The double indicator diffusion technique provides a measurement of the single-pass extraction (E) by an organ from measurements of the concentration of test substance (CS) and reference vascular marker (CR) in blood flowing from the organ (*8*):

$$E(T) = \frac{\int_0^T (CR(t) - CS(t))\,dt}{\int_0^T CR(t)\,dt},$$

where E(T) is the extraction integrated to time T and CR and CS have been normalized by dividing the concentration at time t by the amount of R and S injected.

This equation can be modified to measure E from observing the organ itself rather than the draining blood (*9,10*). For each tracer (R and S), the fraction of the amount injected remaining in the organ at time t [RR(t) and RS(t)] is equal to the normalized amount injected (equals 1) minus organ blood flow times the normalized concentration of the tracer that has left the lungs:

$$R(t) = 1 - F\int_0^T C(t)\,dt$$

and

$$E(t) = [R_S(t) - R_R(t)]/1 - R_R(t).$$

We calculated E in regions of interest (ROIs) over each lung for the 25 frames following the peak height. We normalized the external counts in each frame S (5HT) and R (RBC) by dividing by the peak height. If the peak was difficult to identify within one or two frames, the mean of three frames around the assumed peak was used. This assumes that the peak height represents the "injected dose" of each tracer. This technique has been used to measure ^{11}C-chlorpromazine extraction in human lung (*10*).

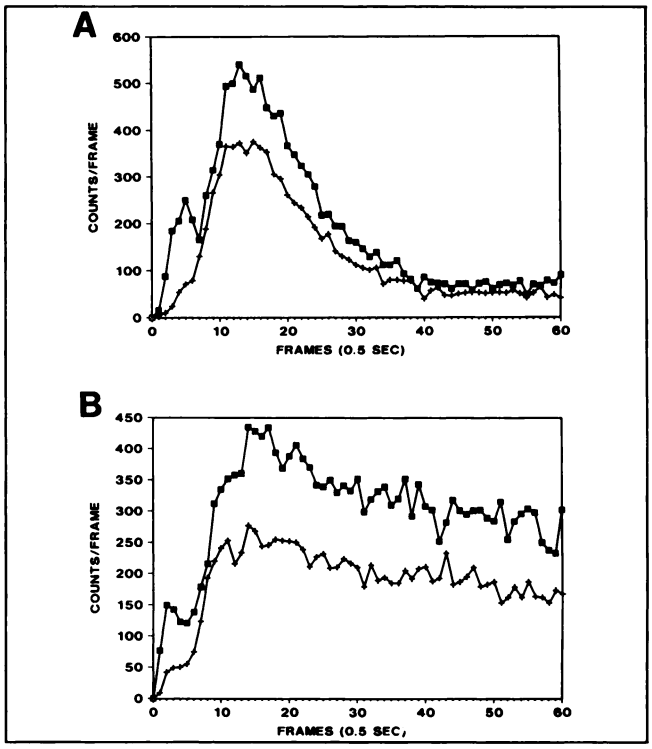

FIGURE 1. Time-activity curves from regions around Rt and left + lungs in subject G.F. during the first pass of ^{11}CO-RBC (A) and ^{11}C-serotonin (B). The early peak in counts in the right lung is contamination from counts in the right heart. The extraction of ^{11}C-serotonin was calculated from the peak of the curve to 25 frames after the peak.

We measured the rate of washout of ^{11}C from the lungs by fitting the data from 30–300 sec to a single-exponential function with a log fit program in a personal computer.

Statistics

A two-tailed paired t-test was used to detect significant differences between means of the single pass extraction and the means of the half times of the isotope washout curves.

RESULTS

Figure 1 shows the raw data obtained from ROIs over both lungs after injecting ^{11}CO-RBC (Fig. 1A) and ^{11}C-5HT (Fig. 1B). These clearly demonstrate retention of ^{11}C-5HT in the lungs. Figure 2 is an image of the lungs

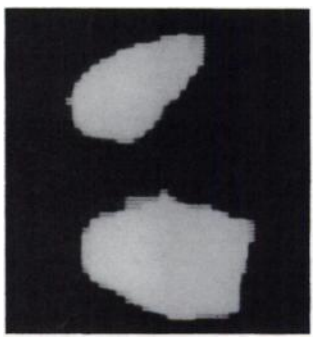

FIGURE 2. Image of ^{11}C-serotonin in the lungs of subject G. F. taken between frames 36 and 60 (12–30 sec).

accumulated during frames 32–60 (12–30 sec). This clearly demonstrates retention of ^{11}C-5HT in the lungs. In the three subjects, the mean extraction of ^{11}C-5HT calculated from the peak to 25 frames after the peak was 63.9% ± 3.6%. This decreased in all subjects to a mean of 53.6% ± 1.4% after infusion of imipramine ($p = 0.046$). Table 1 contains the extraction data for each subject.

This single-pass extraction of ^{11}C-5HT was followed by a gradual washout of ^{11}C from the lungs (Fig. 3). The fraction of the amount extracted that had washed out from the lungs from 0.5 to 5 min was 44.5% ± 3.6% with ^{11}C-5HT alone and 39.9 ± 4.4% with imipramine and ^{11}C-5HT. The washout curves for each lung in each subject were fitted to a single exponential function with a log fit programme on a personal computer. The mean half-life for ^{11}C-5HT alone (corrected for the physical half-life of ^{11}C) was 7.2 ± 0.9 min. The half-life for lung washout after imipramine was 8.8 ± 1 min. These were significantly different ($p = 0.031$). The mean R value for the exponential fit was 0.88 ± 0.18, the range of R value was 0.72–0.98.

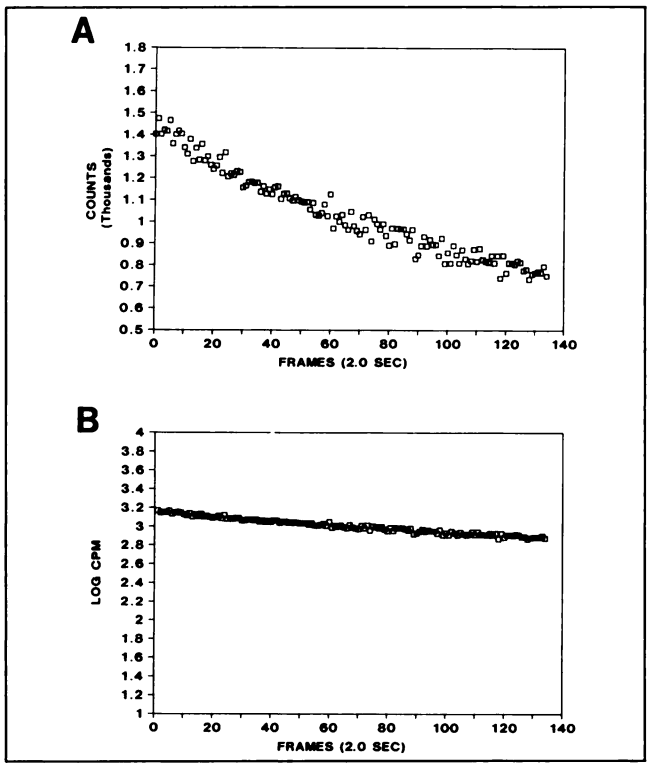

FIGURE 3. Time-activity curves from the right lung of subject G. F. during 30–300 sec of washout of ^{11}C from the right lung. (A) Raw counts uncorrected for the physical half-life of ^{11}C. (B) Excellent fit of the data from Figure 2B to a single-exponential function.

DISCUSSION

We have measured, by a noninvasive technique in man, the fraction of ^{11}C-5HT extracted on a single pass through the lungs (E). We have also demonstrated a reduction in ^{11}C-5HT extraction with intravenously infused imipramine. We obtained a value for E ^{11}C-5HT of 64%, similar to that measured in man by others using ^{14}C-5HT. Gillis et al. (*11*) used ^{14}C-5HT and the double-indicator diffusion technique to measure E in patients undergoing cardiopulmonary bypass. Before bypass, E ^{14}C-5HT was 59.0% ± 11.5% with a range of 44%–72.0%. Dargent et al. (*12*) obtained a much higher value of 97% for E ^{14}C-5HT in a similar group of patients undergoing cardiopulmonary bypass. This same group from Geneva also obtained a value of 91% for E ^{14}C-5HT in patients in an intensive care unit who were at risk of, but did not develop, ARDS (*6*). The difference in E ^{14}C-5HT measured by two groups using the same technique could be explained by a difference in the specific activity of ^{14}C-5HT injected. Gillis et al. (*11*) injected 0.6 μmol ^{14}C-5HT, while the Geneva group (*6,12*) injected 0.1 μmol. The lung extraction of 5HT is a saturable process. The Michaelis constant (Km) for 5HT extraction has been measured in a variety of animal preparations and ranges from 1.65 μ*M* in perfused rabbit lungs to 6.9 μ*M* in rat lungs (*1*). In the isolated lung lobe of a dog, E^{14}C-5HT decreased from 69% when 10 nmol of ^{14}C-5HT were injected to 38% when 100 nmol were injected (*13*). In our experiments, we injected intravenously 0.05–0.1 μmol ^{11}C-5HT [i.e., the same as Dargent et al. and Morel et al. (*6,12*)]. The relatively low extraction in our subjects can be partially explained by the differences in measurement techniques. It could be also due to differences in the chemical form of the ^{11}C-5HT. We injected the hydrochloride while others have used ^{14}C-5HT as the creatinine salt. This may produce differences in E 5HT due to greater protein binding in the case of the hydrochloride or greater lipophilicity and therefore greater lung extraction in the case of the creatinine salt. These factors will be examined further in future studies.

TABLE 1
Percent Extraction of ^{11}C-5HT (Mean of Both Lungs)

Subject	^{11}C-5-HT	Imipramine* + ^{11}C-5HT
G.F.	64.4	52.0
G.M.	60.0	54.3
G.C.	67.2	54.5
mean ± s.d.	63.9 ± 3.6	53.6 ± 1.4†

* Imipramine 0.4–0.5 mg/kg infused over 10 min.
† $p = 0.0463$.

Imipramine caused a significant (16%) reduction in E ^{11}C-5HT in all three subjects. The dose we used (0.4–0.5 mg/kg) has been demonstrated to decrease ^{14}C-5HT extraction in rabbit lungs in vivo from 79% to 60% (*4*). Larger doses (8 mg/kg) in intact dogs caused a reduction in E ^{14}C-5HT from 58% to 23% (*14*). Two subjects had symptoms of insomnia, discoordination, stiffness, and diaphoresis for 2 days after imipramine infusion.

A decrease in pulmonary vascular transit time (in-

creased blood flow) or a reduction in perfused microvascular bed (surface area) causes a decrease in lung 5HT extraction (*15*). Because of these hemodynamic effects on 5HT extraction, the nonextracted vascular marker (^{11}CO-RBC) and the ^{11}C-5HT ideally should be injected simultaneously in the same bolus. With the positron camera and two positron emitters this was not possible and, hence, the 15-min delay between injections of ^{11}CO-RBC and ^{11}C-5HT.

The delay allowed equilibration of ^{11}CO-RBC within the intravascular pool and therefore a constant background. The subjects were supine for 15 min before the start of each study, and we therefore expected no change in pulmonary hemodynamics from the time of ^{11}CO-RBC injection to the end of the measurements. This same procedure has been used successfully to measure lung extraction in dogs of N-isopropyl-p-^{123}I-iodoamphetamine with ^{99m}Tc-sulphide colloid injected in a separate bolus as the intravascular marker (*9*).

An increase in pulmonary capillary permeability and extravascular volume could theoretically cause increased retention of small solutes including serotonin during a single pass through the pulmonary microvascular bed. However, apart from ^{14}C-urea some of which moves directly through cell membranes, the use of single-pass extraction of small solutes to detect increased pulmonary microvascular permeability has not been successful (*16, 17*). This is probably due to the very high blood flow through pulmonary tissue which limits the time for diffusion. A change in distribution volume may change the early wash-out kinetics of serotonin but we do not know to what degree. However, these factors may not limit the usefulness of the ^{11}C-5HT technique since it will be most useful in detecting very early lung damage before changes in permeability or distribution volume have occurred.

The extraction of 5HT by the lung is independent of its subsequent degradation by MAO to 5-hydroxy indole acetic acid (5HIAA). Unlike the platelet or nerve tissue, there is no storage of 5HT in the lung and any 5HT that is not bound to and degraded by MAO effluxes back into the blood (*3*). However, the efflux of 5HT is slower than the diffusion out of the endothelium of 5HIAA (*3*). Thus inhibition of MAO would alter the rate of washout from the lungs of radioactivity after the extraction of labeled 5HT. We observed a greater retention (slower washout) of ^{11}C from the lungs after imipramine infusion compared with ^{11}C-5HT alone.

Pulmonary endothelium contains both MAO-A and MAO-B (*18*). Imipramine inhibits the B fraction of MAO and although serotonin is the major substrate for the A fraction MAO, there is evidence that MAO-B also degrades serotonin in rabbit lung (*19*). Thus inhibiting MAO-B activity with imipramine might effect the rate of degradation of ^{11}C-5HT and thus effect the clearance rate of ^{11}C from the lungs. Clearly, more experiments are required to answer this question.

In summary, we have reported a method to measure the single-pass extraction of ^{11}C-5HT by the lungs and the reduction in extraction after infusion of imipramine. This technique has the potential to measure the extraction of 5HT in a variety of conditions that alter the integrity of the pulmonary vascular endothelium.

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