Scintigraphic Portrayal of Beta Receptors in the Heart

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Myocardial beta adrenergic receptors play important roles in physiology and disease, but the receptors have not before been portrayed. The beta antagonist, iodocyanopindolol (ICYP), was used to develop a scintigraphic method for depicting the receptors in the living heart. Labeled with ^{125}I, ICYP bound firmly to beta receptors in the rat heart; the data conformed to a mathematical model. In vivo saturation kinetics indicated binding sites with two affinities. Inhibition of ICYP binding by beta antagonists of different potency and different selectivity for beta-1 and beta-2 receptors produced the expected pharmacologic effects. Inhibition by lipophilic and hydrophilic antagonists gave no evidence that ICYP was appreciably bound to internalized receptors. Fractional binding by tracer quantities of (−) ICYP and (±) ICYP demonstrated stereospecificity. Labeled with ^{123}I, ICYP bound to the hearts of intact dogs so that scintigraphic tomographs depicted ventricular myocardium. Small doses of beta antagonists selectively reduced the binding of ICYP to lung enabling better visualization of the heart. Thus, ^{123}I-ICYP appears to portray the beta receptors in the living heart, and the characteristics of binding permit the development of mathematical models and lay the basis for quantifying this receptor binding.

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The sympathetic nervous system plays a prominent role in the physiology of the heart, and disturbances in the system have been implicated in the manifestations of heart diseases. For example, myocardial infarction can denervate peri-infarct regions and thereby deprive surviving receptors of neuronal control but leave them under the influence of circulating catecholamines (*1,2*); such a state appears to make for susceptibility to arrhythmias (*3,4*). In addition, heart failure is associated with a loss of beta-1 receptors in the myocardium by mechanisms that are uncertain (*5,6*).

However, in living animals and man measurements of beta adrenergic receptors have been possible only on biopsies from accessible regions of myocardium or in failing hearts removed as part of transplantation therapy (*5,6*). The status of beta receptors throughout the myocardium must then be inferred from the results obtained in limited samples. Direct evidence of the distribution of beta receptors in the living heart should add much to our knowledge of physiology and diseases. Scintigraphic approaches to beta receptors have included radiolabeled beta antagonists, hydroxybenzyl-pindolol (*7*) and pindolol (*8*); neither approach resulted in images of receptors in the heart.

We describe a method that appears to portray scintigraphically the distribution of beta receptors in the living heart using radiolabeled iodocyanopindolol (ICYP), a nonselective beta antagonist (*9,10*). When given intravenously, the binding of ICYP appears to be predominately to beta receptors, and the scintigraphic images portray a diffuse pattern of ^{123}I-ICYP distribution within the myocardium of the left ventricle.

MATERIALS AND METHODS

(−)^{125}I-ICYP (2200 Ci/mmole) was purchased from E.I. duPont deNemours and Company, North Billerica, MA. Both (−) and (±) cyanopindolol (CYP) were gifts from Sandoz Ltd, Basel, Switzerland; the enantiomer (+) CYP was not available. Iodine-125 and ^{123}I were purchased from Nordion International Inc., Kanata, Ontario, Canada. The following were synthesized by a no-carrier-added radioiodination technique (*9*): (−) and (±) ^{125}I-ICYP, each at specific activities exceeding 1550 Ci/mmole and approaching the theoretical limit of 2200 Ci/mmole; the specific activity of (−) ^{123}I-ICYP approached the theoretical limit of 230,000 Ci/mmole. Nonradioactive (±) ^{127}I-ICYP was synthesized in milligram quantities by a modification of the same iodination method and was purified by high-pressure chromatography. (±) Propranolol, (−) timolol, (−) atenolol, and (−) isoproterenol were purchased from Sigma Chemical Company, St. Louis, MO. (±) CGP-12177 was donated by Ciba-Geigy Corporation, Summit, NJ and (±) ICI-118,551 was donated by Imperial Chemical Industry, Macclesfield, UK. Each agent was dissolved in physiologic saline, except ICI-118,551 and the milligram quantities of (±) ^{127}I-ICYP which were dissolved in sterile water and in 25% ethanol in water, respectively.

Procedures

Female Sprague-Dawley rats weighing 180–250 g were purchased from Charles River Breeding Laboratory, Portage, MI.

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Injections of test agents were made over a few seconds into an exposed femoral vein under anesthesia induced by intraperitoneal chloral hydrate. For all but the saturation kinetics experiment, ^{125}I-ICYP as either the (−) or (±) form was injected into rats in 1 μCi (~1.5–2.0 pmole/kg) doses. The animals were killed by quickly opening the chest and removing the heart, lungs, and a sample of blood which were weighed and counted in a gamma counter. The concentrations of radiolabeled ICYP in the lung were assumed to accumulate in the first pass and were subtracted from the administered dose for calculation of concentrations in the heart. Concentrations of ICYP were expressed in nCi/g per 1 μCi per 200 g of animal, which at times was converted to pmoles/g.

For saturation kinetics, milligram quantities of only (±) ICYP were available, so, in two separate experiments, this racemic form was given in seven doses from 2.1–1860 nmole/kg and 12.5–3125 nmole/kg using 2 and 6 μCi of (−) ^{125}I-ICYP as the indicators. For calculations of Bmax and Kd, it was assumed that only the (−) ICYP was bound and that the total ICYP was that delivered by the blood. The delivery of ICYP to the heart was assumed to be 0.0465 of the cardiac output, a value reported for coronary artery blood flow (*11,12*). The free ICYP was the difference between the total ICYP and the ICYP bound to receptors. For the stereospecificity experiment, the binding of (−) ICYP was compared with that of (±) ICYP.

Intravenous injections of radiolabeled ICYP were made over a few seconds into 11 dogs weighing 13.3–22.7 kg. Five dogs received 10 μCi of ^{125}I-ICYP, and the hearts and lungs were removed 3 hr later for radiopharmaceutical concentration measurements. For scintigraphic images, six dogs received 5 mCi doses of ^{123}I-ICYP. Beta antagonists to inhibit variably the binding of the ^{123}I-ICYP were included with most of the doses. Two dogs were injected on three occasions: without an antagonist, with ICI-118,551, and with both ICI-118,551 and propranolol. Two dogs were injected on two occasions in which ^{123}I-ICYP included: ICI-118,551, and ICI-118,551 and propranolol. Two other dogs were injected on two occasions in which ^{123}I-ICYP included a small or large quantity of timolol. Three hours before sacrifice of these dogs, 10 μCi of ^{125}I-ICYP was injected with ICI-118,551, propranolol, or timolol to determine ex vivo the distribution of the radiopharmaceutical in heart and lungs under these circumstances.

Scintigraphy was performed under anesthesia with thiamylal. A GE 400AT scintillation camera (GE Medical Systems, Milwaukee, WI) was used to acquire information. Anterior planar data were acquired for consecutive 10-min periods during the first 2 hr and on four to six occasions in the next 1.5 hr. These planar data (initially 540,000 counts/10 min in the heart) enabled calculation of rates of loss of the radiopharmaceutical from the heart through quantification of ^{123}I-ICYP in a region of interest for the heart and in an appropriate background region of interest in the right lung. Two to 3 hr after injection, scintigraphic tomographs were made of the heart by a method previously described (*13*). The dogs were allowed to recover for additional studies. Sacrifice was performed under anesthesia with an injection of Uthol (Sodium pentobarbital 324 mg/ml, 40% isopropyl alcohol, 2% propylene glycol, 2% benzyl alcohol, 0.03% edetate), 1 ml per 10 pounds, after which the chest was opened and heart and lungs were quickly removed.

To test for deiodination of 125-I-ICYP, samples of myocardium were homogenized in 50% acetonitrile in water on ice (1 g/9 ml) as described for extracting carazolol (*14*), then centrifuged at 20,000 × g for 20 min at 4°C. The supernatants containing all of the radioactivity were placed on a C-18 Sep-Pak cartridge (Waters Associates, Milford, MA), and the radioiodide was eluted with 5 ml of 10 m*M* potassium iodide and counted.

Red cells and heparinized plasma were separated by centrifugation. Plasma proteins were precipitated by 100 mg/ml of sulfosalicylic acid; samples of whole blood, plasma, and plasma proteins were counted for radioactivity in a well counter.

Data Analysis

The kinetic model was constructed by standard modeling methods with parameters estimated by non-linear least squares optimization. Data from the saturation kinetics experiment were analyzed using LIGAND, a computer program (*15*). For calculations of EC-50 and slopes of inhibited binding curves, estimates of the four logistic parameters were by the Statistical Analysis System procedure of non-linear estimation using a weighted least squares criterion: equality of parameters across treatments was tested as described by DeLean et al. (*16*).

General Plan of Experiments

A. Iodine-125-ICYP in rat tissues were experiments designed to demonstrate the nature of ICYP binding in the living heart.
   1. A general pattern of ICYP binding in the heart was obtained over time.
   2. A mathematical model of binding was constructed from the concentrations of ICYP over time in the heart and the aorta, and from data on the metabolism of ICYP.
   3. The binding of ICYP in the heart exhibited characteristics of binding to a receptor (*17*) as demonstrated by:
      a. Saturation kinetics showing saturability of binding;
      b. Inhibition of binding by beta antagonists and agonists in competitive patterns using: (±) propranolol, a commonly-used lipophilic nonselective antagonist, (−) timolol, a more potent lipophilic nonselective antagonist (*18*), (±) CGP-12177, a hydrophilic nonselective antagonist (*19,20*), (±) ICI-118.551, a lipophilic beta-2 selective antagonist (*21*), (−) atenolol, a lipophilic beta-1 selective antagonist, and (−) isoproterenol, a selective beta-1 agonist; and
      c. Stereospecificity of binding using (−) ICYP and (±) ICYP.

B. Iodine-123-ICYP for scintigraphy in the dog heart were experiments designed to demonstrate the feasibility of imaging the beta receptors in the living heart based on the data obtained in rats.

RESULTS

Iodine-125-ICYP in Rat Tissues

General Pattern of Binding to the Heart: Concentrations of ICYP Over Time

Measurements of (−) ^{125}I-ICYP in rat hearts were made over 90 min after injection (Fig. 1); the uninhibited concentrations appeared to become stable by 15 min. The highest concentration of ICYP attained in the heart (before adjustment for that removed by the lung) was 2.4% of the dose. In another experiment (not shown), ICYP exhibited a half-time in the heart of 21.8 hr between 90 min and

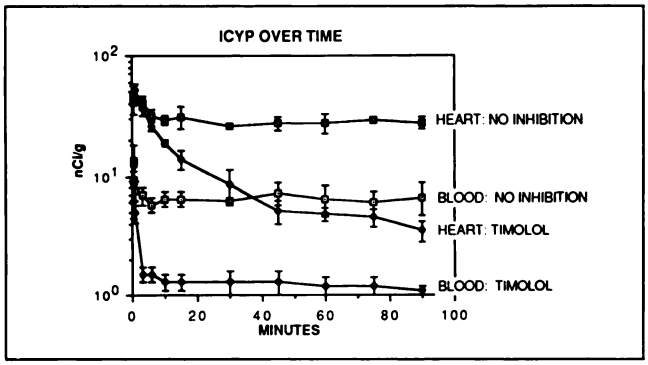


FIGURE 1. Iodine-125-ICYP in rat heart and venous blood over time after intravenous injection of about 1.5 pmole/kg. In some groups of rats, (−) timolol, 34,000 nmole/kg, was given with the ICYP. Means ± s.d.s are shown for groups of four rats.

21 hr after injection. When timolol, 34,000 nmole/kg, was given with the radiopharmaceutical to inhibit binding, the concentration of ICYP in the heart declined continuously, but after 45 min the change was more gradual (Fig. 1) and additional data showed a half-time in the heart of 12.9 hr between 90 min and 21 hr. Because the changes in ICYP binding to the heart, uninhibited and inhibited, were gradual at 75 min after injection, this time was selected to examine the concentrations in the rat heart in most experiments.

At 75 min after injection, lung concentrations of ICYP were 185 ± 17 nCi/g in 15 experiments; this was 19% of the dose in the lungs. As noted above, the ICYP bound to lung was assumed to be unavailable to the heart and therefore was subtracted from the respective dose of ICYP.

When timolol, 34,000 nmole/kg, was given 15 min before the ICYP, binding to the heart was inhibited, albeit to a somewhat lesser degree than when the two agents were given simultaneously (Table 1). However, when given 30 min after ICYP, timolol only slightly inhibited the binding.

TABLE 1
Effects of Timolol on ICYP Binding at Different Times

	Binding (nCi/g)*	Timolol/ vehicle
Addition to ICYP given simultaneously with ICYP		
Vehicle (5)	30.2 ± 2.5	
Timolol† (5)	3.6 ± 1.1	0.12
Addition to ICYP given 15 min before ICYP		
Vehicle (5)	28.1 ± 2.0	
Timolol† (5)	6.5 ± 1.1	0.23
Addition to ICYP given 30 min after ICYP		
Vehicle (5)	26.8 ± 1.8	
Timolol† (5)	24.8 ± 1.5	0.93

* Assay 75 min after ICYP injected; adjusted to 1 μCi, 200 g of body weight, and for radioactivity in lung.
† Timolol 34,000 nmole/kg.

Therefore, inhibition experiments were performed by giving ICYP simultaneously with the inhibiting agent. Residual binding of ICYP in the presence of maximally tolerated beta antagonists was designated "nonspecific" binding and was 10%–20% of total binding in multiple experiments; higher doses were lethal to some animals. The mean of 15% "nonspecific" binding was used in some experiments.

Mathematical Model

Arterial blood sampling was performed at a constant rate from the aorta over the first 20 sec after administration of (−) ^{125}I-ICYP, the concentration was 59 ± 6 nCi/g in four rats. In arterial blood the ^{125}I was 84%–92% in the erythrocytes between 0 and 210 sec after injection (Table 2). The ^{125}I was 72% bound to proteins in plasma obtained between 0–30 sec and 54% bound in plasma from the 180–210-sec interval. The fraction as iodide increased from about 3% to 14% in whole blood between the 0–30- and 180–210-sec intervals, indicating metabolism of ICYP outside of the heart. When deiodination of (−) ^{125}I-ICYP was examined 75 min after injection, the ^{125}I-iodide in nine rat hearts was 3.8% ± 2.4% (mean and s.d.). Therefore, the ^{125}I in the heart was assumed to be (−) ^{125}I-ICYP.

These data and those of concentrations of ICYP in the heart were used to construct a mathematical model of binding of ICYP to the heart (Fig. 2). Analysis of the data were performed four ways, each assuming a different time-dependent percentage of ICYP in blood available for transport to the receptors, since the metabolite fraction in blood was not precisely known. The data shown in Figure 2 were derived from an intermediate level of availability assuming a non-metabolite fraction in the blood that decreases from an initial 100% at the time of injection to 50% at 90 min. Extraction of intravenously administered ICYP by the rat heart was estimated to be approximately 50%. Important to the analyses is that, except for the lowest percentage of radioactivity available for extraction in this model, different fractions of metabolites gave similar values for relative amounts of ICYP distributed to specific and nonspecific

TABLE 2
Distribution of ^{125}I from ICYP in Arterial Blood

	0–30 sec after injection i.v.*		180–210 sec after injection i.v.*	
Fraction of ^{125}I in:				
RBC	(4)	0.84 ± 0.03	(5)	0.92 ± 0.01
Plasma	(4)	0.16 ± 0.038	(5)	0.08 ± 0.01
Fraction of ^{125}I in plasma as:				
Bound to proteins	(4)	0.72 ± 0.02	(5)	0.54 ± 0.02
Unbound	(4)	0.28 ± 0.02	(5)	0.46 ± 0.02
Fraction of ^{125}I as iodide (metabolite) in:				
Whole blood	(4)	0.034 ± 0.003	(5)	0.138 ± 0.046
Plasma	(4)	0.135 ± 0.020	(5)	0.85 ± 0.19

* Total activity in whole blood: 0–30 sec, 45 nCi/g, and 180–210 sec, 3.6 nCi/g.

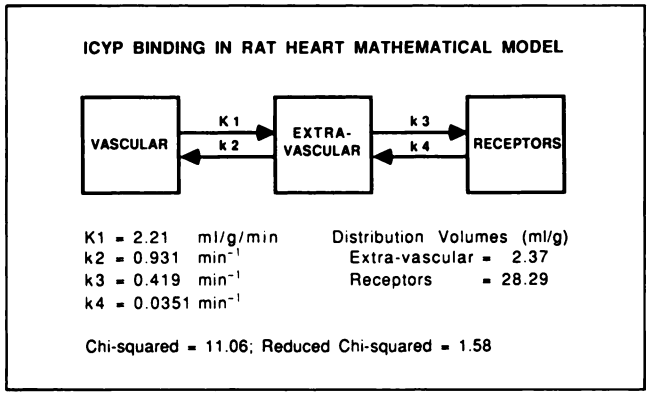


FIGURE 2. Mathematical model of binding of ^{125}I-ICYP in the rat heart.

binding sites. The relatively higher rate for k3 compared to that of k4 (0.419 and 0.035 min^{-1}, respectively, in Fig. 2) indicated that a substantial fraction of ICYP is bound to the receptors.

Characteristics of ICYP Binding in the Heart

Saturation Kinetics. When increasing doses of (±) ICYP were given to rats, a pattern of saturation of receptors was obtained (Fig. 3). Binding was assumed to be the (−) ICYP, and Scatchard analysis of the data indicated two affinity sites of binding (Fig. 3, inset). In this experiment, the Bmax values for the two binding sites were 6.2 and 110 pmole/g; and the means for two experiments gave 5.7 and 310 pmole/g for the two sites. The Kd values for the two binding sites were 25 n*M* and 0.9 μM; and when averaged from two experiments were 22 n*M* and 3.6 μM. The actual ICYP available to the heart may be less than that in the blood. If, for example, only the non-protein bound ICYP in the plasma were available (calculated to be 0.038 of the total blood ICYP in the first 30 sec from the data in Table 2), then the Kd would be proportionally lower.

Inhibition of Binding of ICYP by Beta Antagonists and Agonists. Binding of (−) ^{125}I-ICYP to the rat heart was inhibited in dose-response patterns by a number of beta antagonists and the beta agonist isoproterenol (Fig. 4). The EC-50 of timolol was 33 nmole/kg while that of propranolol was 260 nmole/kg giving timolol an eight-fold greater potency (p = 0.0004).

The inhibition curve of CGP-12177 was of particular interest because this beta antagonist is hydrophilic and not internalized by cells (*19,20*). Therefore, CGP-12177 could be expected to inhibit the binding of ICYP only to beta receptors on the surface of cells. On the other hand, timolol should inhibit binding by surface receptors, and, because of lipophilicity, it may inhibit binding to internalized receptors as well. The slope of the inhibition curve of CGP-12177 was less steep than that of timolol (p = 0.0062) (Fig. 3). With an EC-50 of 10 nmole/kg, CGP-12177 was more potent, but not quite to statistical significance, than timolol (p = 0.07).

The agonist isoproterenol also inhibited the binding of ICYP to the heart (Fig. 4). The slope of the inhibition

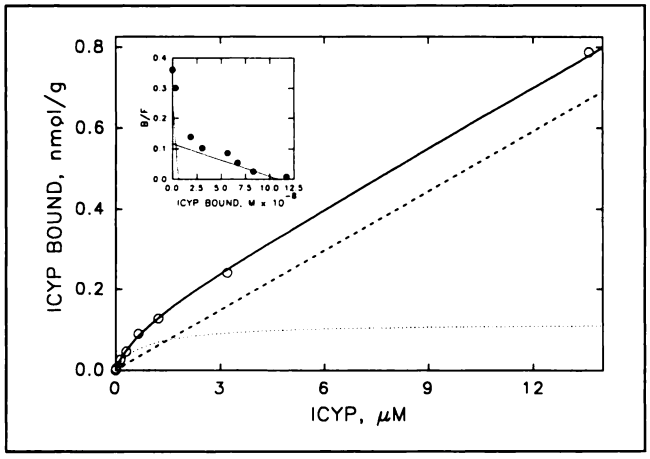


FIGURE 3. Saturation kinetics in rat heart using (±) ICYP and (−) ^{125}I-ICYP as the indicator in the first of two experiments; calculations were based on the binding of the (−) enantiomer only. Four rats were in each group. Saturation of ICYP binding is shown when increasing concentrations of ICYP reached the heart: solid line = total binding; dashed line = "nonspecific" binding (here calculated for best fit by computer but not differing substantially from measured values in the presence of timolol 34,000 nmole/kg); dotted line = "specific" binding derived from subtraction of "nonspecific" from total binding of ICYP. Inset shows Scatchard plot of data in which binding was best fit to two sites: Bmax = 6.2 and 110 pmole/g; and Kd = 25 n*M* and 0.9 μM.

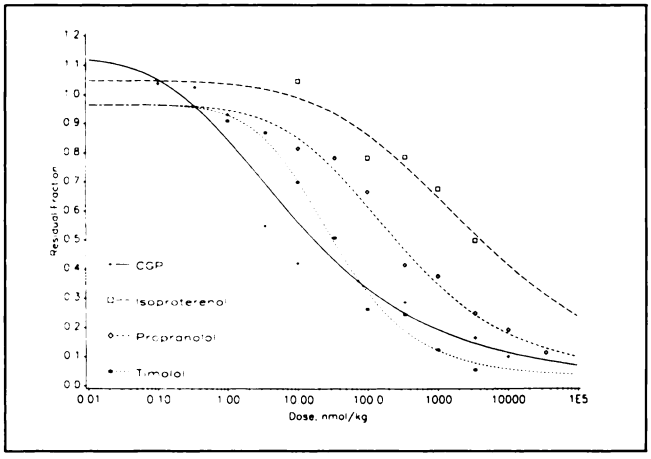


FIGURE 4. Inhibition of ^{125}I-ICYP binding in the rat heart by several beta antagonists and by isoproterenol. Iodine-125-ICYP, 1.5 pmole/kg, and an inhibiting agent were injected together intravenously. Each data point represents results in four or five rats; occasionally experiments were repeated and then the data at individual points were pooled; only the mean values for each dose are shown. EC-50 values (nmol/kg) were: (±) CGP-12177, 10; (−) timolol, 33; (±) propranolol, 260; and (−) isoproterenol, 3790. Timolol was significantly more potent than propranolol (p = 0.0004) but was not significantly less potent than CGP-12177 (p = 0.07). The slope of (−) timolol differed significantly from that of (±) CGP-12177 (p = 0.0062) but not from that of (−) isoproterenol (p = 0.69).

curve of this agent from another class of compounds was not significantly different ($p = 0.69$) from that of timolol.

Binding of ICYP to beta-1 and beta-2 receptors was demonstrated by inhibiting the binding by selective beta antagonists. In the heart, beta-1 receptors predominate (*22–24*), and in this organ inhibition of binding by the selective beta-1 antagonist, atenolol, giving an EC-50 of 459 nmol/kg, was more potent than that of the beta-2 selective antagonist, ICI-118,551, for which the EC-50 was 2670 nmol/kg, ($p < 0.0001$) (Fig. 5A). However, in the lung where beta-2 receptors predominate (*25,26*), ICI-118,551 was more potent than atenolol, EC-50 of 100 and 1556 nmole/kg, respectively, ($p < 0.0001$) (Fig. 5B).

Since the concentration of ICYP in the lung was greater than that in the heart (Figs. 4 and 5), radiolabeled ICYP in the lung would create an impediment to portraying the ICYP in the heart by external scintigraphy. However, at 340 nmole/kg, ICI-118,551 effected a selective inhibition of binding such that ICYP in the heart was over 80% of control value, while ICYP in the lung was at 35% control value. In addition, at 3.4 nmole/kg, timolol inhibited binding of ICYP in the heart to 80%–90% of control and binding to the lung to about 65% of control. These selective effects were employed in the in vivo experiments in dogs (see below).

Stereospecificity. In two experiments, the bindings of (±) ICYP were 66 and 57% of the bindings of (−) ICYP (Table 3). When the bindings of the two forms of ICYP were inhibited by a series of doses of CGP-12177, the resulting curves were comparable in slope and EC-50 values (Fig. 6).

TABLE 3
Stereospecificity of ICYP Binding In Vivo

	Specific binding (nCi/g)*	(±) ICYP/(−) ICYP
Experiment 1		
(−) ICYP† (5)	21.6 ± 2.7	
(±) ICYP‡ (5)	14.3 ± 1.0	0.66
Experiment 2		
(−) ICYP‡ (5)	23.0 ± 1.5	
(±) ICYP‡ (5)	13.1 ± 1.3	0.57

* Adjusted to 1 μCi dose, 200 g of body weight, and for radioactivity in lung, and nonspecific binding.
† From E. I. duPont deNemours Co.
‡ Synthesized at the University of Michigan.

Iodine-123-ICYP for Scintigraphy of The Dog Heart

The information from the rat experiments was applied to the development of satisfactory images of ICYP binding in the dog heart. Using (−) ^{123}I-ICYP alone produced images of the heart that were obscured by radioactivity in the lung (Fig. 7A). When either ICI-118,551, 340 nmole/kg (Fig. 7B-C), or timolol, 3.4 nmole/kg (Fig. 7D), were given with the radiopharmaceutical, lung binding was sufficiently inhibited to obtain well-defined images of ^{123}I-ICYP in the myocardium. Distribution of ^{123}I-ICYP appeared to be diffuse throughout the myocardium of the left ventricle (Fig. 7 B-D) and could be seen in the right ventricle on appropriate images (Fig. 7B) but was not apparent in the atria. The concentration of ^{123}I-ICYP in the dog heart appeared to be stable from 10 min after injection to 3 hr. In two dogs, timolol, 3400 nmole/kg given with the ^{123}I-ICYP, reduced the concentration in the heart to 20% and 40% of the respective values obtained with 3.4 nmole/kg of timolol when radioactivity in regions of interest were compared 2 hr after injection.

Selective inhibition of lung binding of ^{125}I-ICYP was also shown in ex vivo measurements. In the five dogs

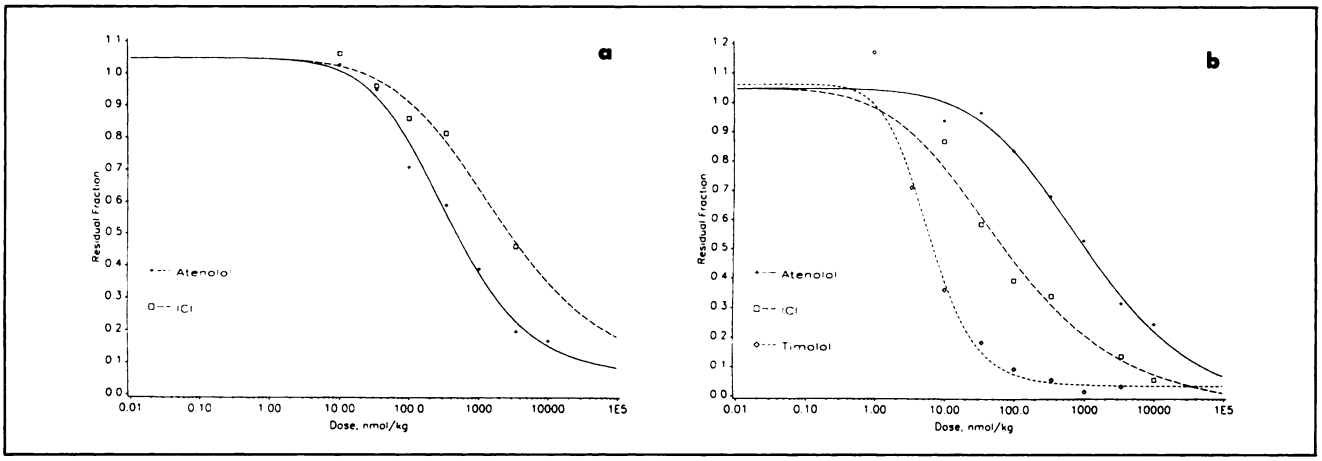
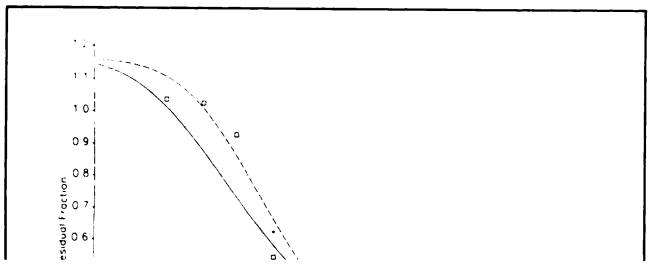


FIGURE 5. Inhibition of ^{125}I-ICYP binding by antagonists, beta-1 selective (−) atenolol and beta-2 selective (±) ICI-118,551. Data were derived as described in Figure 4. (A) In the rat heart, EC-50 values in nmole/kg were: atenolol, 459; and ICI-118,551, 2670; $p = 0.0024$. (B) In the rat lung, EC-50 values in nmol/kg were: atenolol, 1556; and ICI-118,551, 100; $p = 0.0038$. Note that the order of potency for the two agents in the lung is the opposite of that in the heart. Also, note that the inhibition of binding in the lung by ICI-118,551 at 340 nmole/kg gave a residual of less than 35% (Fig. 5B), but in the heart a residual of over 80% (Fig. 5A); and inhibition of binding in the lung by timolol 3.4 nmole/kg gave a residual of 65% (Fig. 5B), but in the heart a residual of over 85% (Fig. 4).



(see above) and approaching the "nonspecific" binding level found in rat heart.

The liver was regularly visualized on images made with ^{123}I-ICYP (Fig. 7A-D). The radiopharmaceutical is concentrated by hepatocytes and excreted through the biliary system, and the gall bladder can be seen on images of the abdomen. Excretion as %ID was 5%/3 hr and 12%/24 hr in the urine and 15%/24 hr in the feces. Absorbed radia-

by small doses of ICI-118,551 or of timolol. After this selective inhibition of ICYP in the dog lung, binding of ICYP in the heart was inhibitable by either propranolol or timolol; the latter beta antagonist reduced the heart concentration to 18% of total binding, a level comparable to that attained in the rat. These results indicated that the ICYP portrayed in the heart was largely bound to beta receptors. The distribution of ICYP, which reflected the sites of beta receptors in the left ventricle, appeared to be diffuse.

The highly selective inhibition of lung binding of ICYP should not impair future measurements aimed at quantifying the receptors in the myocardium. The method of producing tomographic images can be adopted in any Nuclear Medicine laboratory with a scintillation camera. Moreover, an instrument capable of acquiring single photon emission data in dynamic and tomographic modes,

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Postinjection L-Phenylalanine Increases Basal Ganglia Contrast in PET Scans of 6-^{18}F-DOPA

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The sensitivity of ^{18}F-DOPA positron emission tomography for imaging presynaptic dopamine systems is limited by the amount of specific-to-nonspecific accumulation of radioactivity in brain. In rhesus monkeys, we have been able to increase this ratio by taking advantage of the lag time between ^{18}F-DOPA injection and the formation of its main metabolite, the amino acid ^{18}F-fluoromethoxydopa, the entrance of which into brain is responsible for most of the brain's nonspecific radioactivity. By infusing an unlabeled amino acid, L-phenylalanine, starting 15 min after ^{18}F-DOPA administration, we preferentially blocked the accumulation of ^{18}F-fluoromethoxydopa by preventing its entrance into brain through competition at the large neutral amino acid transport system of the blood-brain barrier. This method appears as reliable as the original and more sensitive, as demonstrated by the comparison of normal and MPTP-treated animals under both conditions.

ine (^{18}F-3-OM-DOPA), one of the major metabolites of ^{18}F-DOPA. This amino acid, primarily produced in the liver, can readily cross the blood-brain barrier (BBB), probably using the same large neutral amino acid (LNAA) transport system as L-DOPA. 3-OM-DOPA appears to have a uniform distribution throughout the brain in rodents (*7*) and in primates (*8*). Administration of ^{14}C- or ^{3}H-labeled L-DOPA or ^{18}F-DOPA to rats or primates leads to significant concentrations of 3-OM-DOPA in plasma and a substantial background of 3-OM-DOPA in brain (*9–11*). The other metabolites of L-DOPA produced in the periphery and found in plasma, mainly dopamine (DA), homovanillic acid, 3,4-dihydroxyphenylacetic acid, and their sulfated conjugates, are not likely to cross the BBB to any significant degree.