RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

In Vivo Receptor Binding of Iodinated Beta-Adrenoceptor Blockers

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Six radiolabeled beta-adrenoceptor blocking agents with a range of affinity constants were evaluated as radioindicators for adrenoceptors in guinea-pig heart and lung. All concentrated in the heart and lung at levels in excess of 0.1% dose/g tissue. On the basis of displacement studies using propranolol, two of the six compounds showed beta-adrenoceptor binding in the lung, and one, H-3 carazolol, showed receptor binding in the heart. These results agree qualitatively with a bimolecular reversible equilibrium model, and suggest that the beta-adrenoceptor blockers as a group will not be useful in vivo probes of receptor concentration in the heart because of the low affinity constants and high levels of nonreceptor binding associated with the present-day clinical beta blockers. Beta-adrenoceptor blocking agents with affinity constants in excess of 10^9 will be needed to give heart-to-blood ratios of 10.

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In an earlier publication describing iodinated beta-adrenoceptor blockers, one radiotracer, a practolol derivative, was shown to have a heart-to-blood ratio (H/B) of 20:1 in rats (*1*). This localization was later showed to be species-specific, prompting further study of the binding characteristics of this class of potential radiopharmaceuticals (*2*). This paper reports the determination of the level of receptor and nonreceptor binding in vivo for a series of beta-adrenoceptor blockers that have a range of affinity constants for the receptor in heart and lung. From such a determination we hope to delineate the physical and chemical characteristics necessary to give high concentration in the heart and at least 10:1 H/B ratios as a result of beta-adrenoceptor binding.

METHODS

Preparation of compounds. 4-hydroxyphenethylamino-3-(4-acetamido) phenoxypropan-2-ol (TYR-PRAC), 4-hydroxyphenethylamino-3-(2-allyl)phenoxypropan-2-ol (TYR-ALP), and 4-hydroxyphenethylamino-3-[1-oxotetrahydronaphthaloxy]-propan-2-ol (TYR-BUN) were prepared as previously described (*1*). The structures were determined by standard chemical methods, including elemental analysis. The chloramine-T method of iodination (*3*) was used to label these compounds with carrier-free I-125 in the substrate to an iodine ratio of >1000. This ratio favors monoiodination. The carrier-free iodinated radiotracer was isolated on high-pressure liquid chromatography (HPLC) using acetonitrile:0.01 *M* ammonium formate ($\simeq$ 1:1) or ethanol:water ($\simeq$ 1:1) as solvent. I-125 TYR-PRAC was separated as previously described (*1*). In this case the I-127 compound was prepared and shown by elemental analysis to be the monoiodinated tyrosine derivative. In addition, all radioiodinated products were analyzed for radiochemical purity in at least two chromatographic systems. The radiolabeled substrates were assayed, diluted to a concentration of 333 μCi/ml, and immediately frozen at −70°C until used. The radiochemical purity was reanalyzed at the time of injection and found to be greater than 95%. H-3 dihydroalprenolol (DHA), H-3 carazolol (CAR) and I-125-iodinated hydroxybenzylpindolol (I-125 HYP) were obtained com-

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mercially.* The radiochemical purity was assayed before use and found to be greater than 95%.

Binding assay. The procedure for the determination of the affinity constants by in vitro radioreceptor assay has been described in detail previously (*4*).

Biological distribution studies. The in vivo distribution of the radiolabeled beta-adrenoceptor blockers was determined in male guinea pigs weighing 400–600 g at the time of study. The thyroid was not blocked. Under light halothane anesthesia, 0.1 ml of the radiopharmaceutical ($\simeq$ 5 to 10 μCi) was injected into an exposed femoral vein. At selected times after injection, the animals were killed and samples of blood, ventricular muscle, lung, and thyroid were taken. The tissue samples and diluted standards of the radiopharmaceutical were counted in a scintillation counter. The results are expressed as the percentage of the injected dose per gram wet tissue. Each group consisted of at least five animals.

The rat experiments with two compounds, I-125 TYR-PRAC and I-125 TYR-ALP, were repeated in the guinea pig to determine the effects of species differences and specific activity. Since all iodinated derivatives were separated from the noniodinated precursor by HPLC in these experiments, they can approach the maximum specific activity of 2200 Ci/millimol, whereas those reported in the earlier publication (*1*) were separated by TLC procedures that did not guarantee maximum specific activity.

Theoretical calculations. The theoretical bound-to-free ratios were calculated using a simple reversible bimolecular equilibrium model (*5*). The form of the equation is:

$$r^2 + r(1 + KL_0 - KR_0) - KR_0 = 0, \qquad (1)$$

which has a solution:

$$r = \frac{-(1 + KL_0 - KR_0) \pm \sqrt{(1 + KL_0 - KR_0)^2 + 4\,R_0K}}{2}, \qquad (2)$$

where r = [B]/[F] = concentration of bound/concentration of free radioligand, K = affinity constant, L_0 = initial concentration of radioligand in body, R_0 = total concentration of receptor in target organ.

This equation has been derived for a single compartment, but the same r values are obtained for compartments of nonequivalent size in the case of the heart and extracellular fluid, using I-125-labeled compounds. The derivation of the equations for compartments of unequal size (*6*) will be the subject of another report.

The equation for competitive inhibition in a single compartment has been taken from Ekins (*7*), and is:

$$r^2 + r\,(1 + KL_0 - KR_0) - KR_0 + \frac{hK_h(r + 1)r}{(K_h/K)r + 1} = 0 \qquad (3)$$

where h = concentration of nonradioactive inhibitor, K_h = affinity constant for the nonradioactive inhibitor and the receptor.

RESULTS AND DISCUSSION

Binding in guinea-pig heart and lung. On the basis of calculations of the maximal bound-to-free ratio using the quadratic equation describing the interaction between a single radioligand and a single receptor (*5*), one would expect heart-to-blood ratios of approximately two or less for all beta-adrenoceptor blockers in this series (Table 1). However, all should produce a detectable level of radioactivity in heart tissue. All radiotracers should also show a detectable level of radioactivity in the lung, and I-125-HYP, DHA, and CAR should have lung-to-blood ratios greater than 1.

These theoretical results were obtained using affinity constants determined by an in vitro method using rat ventricular and lung tissue and the noniodinated precursor (*8*). The last was used because of its ready availability and the recent report that iodination does not affect the binding affinity significantly in a small series of tyramine derivatives (*9*). This extrapolation from rat to guinea pig is based on a small series of compounds

TABLE 1. MAXIMAL TARGET-TO-BLOOD RATIOS FOR SELECTED BETA ADRENOCEPTOR BLOCKERS

	K_A (RVM*)	K_A (RLM†)	H/B (theor)‡	L/B (theor)‡
TYR-PRAC	0.05×10^6	0.01×10^6	<1	<1
TYR-BUN	3×10^6	2×10^6	<1	<1
TYR-ALP	5×10^6	3.7×10^6	<1	<1
HYP	83×10^6	169×10^6	<1	3.9
DHA	130×10^6	50×10^6	<1	1.2
CAR	1600×10^6	800×10^6	1.9	18.5

* RVM = rat ventricular muscle.
† RLM = rat lung microsomal fraction.
‡ Theoretical B/F ratios calculated according to Ref. *5*.

TABLE 2. DISTRIBUTION OF RADIOLABELED BETA-ADRENOCEPTOR BLOCKERS IN GUINEA PIGS

	Time (hr)	% dose/g ± s.d.*			% dose/g ± s.d.		
		Blood	Heart	Lung	Thyroid	H/B	L/B
I-125 TYR-PRAC	1/4	0.09 ± 0.02	0.25 ± 0.14	0.22 ± 0.10	0.07 ± 0.04	2.65	2.43
	2	0.04 ± 0.01	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.05	2.22	1.73
I-125 TYR-BUN	1/4	0.10 ± 0.07	0.19 ± 0.10	0.49 ± 0.35	—	2.04	4.74
	2	0.02 ± 0.00	0.06 ± 0.01	0.47 ± 0.17	—	3.19	24.5
I-125 TYR-ALP	1/4	0.11 ± 0.02	0.35 ± 0.14	1.70 ± 1.00	0.11 ± 0.05	3.07	12.8
	2	0.04 ± 0.00	0.16 ± 0.03	1.47 ± 0.32	0.10 ± 0.06	3.65	33.2
I-125 HYP	1/4	0.50 ± 0.21	0.39 ± 0.09	1.85 ± 0.28	0.11 ± 0.02	0.83	3.95
	2	0.14 ± 0.05	0.15 ± 0.05	0.79 ± 0.23	0.35 ± 0.15	1.05	5.83
H-3 DHA	1/4	0.05 ± 0.01	0.21 ± 0.06	4.60 ± 1.92	—	3.74	74.8
	2	0.05 ± 0.01	0.08 ± 0.01	1.34 ± 0.36	—	1.43	23.8
H-3 CAR	1/4	0.05 ± 0.01	0.31 ± 0.10	3.09 ± 1.25	—	5.71	57.1
	2	0.04 ± 0.01	0.21 ± 0.05	2.62 ± 0.43	—	5.08	63.5
Iodide	1/4	0.29 ± 0.02	0.14 ± 0.01	0.22 ± 0.02	0.45 ± 0.14	0.47	0.73
	2	0.12 ± 0.01	0.06 ± 0.01	0.10 ± 0.01	0.74 ± 0.13	0.48	0.80

* Each group contained at least five animals.

covering three orders of magnitude in affinity; it showed similar affinity constants in the two species (*4*).

The quantity of receptor was estimated from literature sources to be 2.4 n*M* in the rat heart (*9*) and ten times that in the rat lung (*12*). Barnett et al. have determined that rat heart contains >80% beta-1 receptors, whereas the rat lung contains 25 and 75% of beta-1 and beta-2, respectively (*12*). Fluorescent studies using a beta-blocker derivative indicate that the receptor sites may not be evenly distributed in the rat heart (*13*). The assumption of a homogeneous distribution of receptor gives lower theoretical B/F ratios.

As predicted by the model (Table 2), at 0.25 and 2 hr after injection none of the radiolabeled derivatives showed H/B ratios equal to or greater than 10. On the other hand, the L/B ratios were greater than 10 for I-125 TYR-ALP, H-3 DHA, and H-3 CAR (Table 2). The values obtained for I-125 TYR-PRAC differ substantially from those obtained in rats (*1*). Apparently a high-affinity or high-capacity nonadrenoceptor site bound the I-125 TYR-PRAC in the rat heart and gave the reported ratio (H/B) of 20. The values for I-125 TYR-ALP are similar to those obtained in the rat. A bound-to-free ratio (i.e., heart-to-blood) of 10 was arbitrarily chosen as one that will allow detection of focal abnormalities by external imaging. With the advent of single-photon computerized tomography, external detection may be achieved with lower ratios.

All three tyramino derivatives had relatively low affinity constants. In general, it has been reported that a tyramino derivative decreases the K_A value for beta-adrenoceptor blockers, relative to the parent isopropylamino derivative in rat ventricular muscle (*8*).

We note that I-125 HYP would give a theoretical H/B ratio of 125 using the equilibrium constant ($5 \times 10^{10}\ M^{-1}$), determined for I-125 HYP using turkey erythrocytes as the source of beta adrenoceptor (*10*). However, differences in the accessory site of the beta adrenoceptor result in different K_A values when various sources of beta adrenoceptors are used (*4*). Harms has

TABLE 3. DISTRIBUTION OF H-3 PLP AS A FUNCTION OF DOSE

	Dose of PLP					
	20 min			120 min		
	1.1 μg/kg*	1.5 mg/kg†	7.5 mg/kg†	1.1 μg/kg*	1.5 mg/kg†	7.5 mg/kg†
Heart % dose/g	0.02	0.29	0.59	0.01	0.09	0.24
μg/g	0.006	1.18	2.36	0.003	0.35	0.95
Lung % dose/g	0.14	3.2	3.1	0.02	0.43	2.1
μg/g	0.039	13.0	12.4	0.006	1.71	8.4

* Taken from Ref. *1*.

† Taken from Ref. *17*. Weight of rats was assumed to be 250 g.

TABLE 4. EFFECT OF COMPETITIVE ANTAGONIST ON THE DISTRIBUTION OF I-125 HYP AT 2 HR IN GUINEA PIGS

Competitive antagonist	Quantity (μg)	% dose/g ± s.d. Blood	Heart	Lung	% dose/g ± s.d. Thyroid	H/B	H/L
HYP*	60	0.16 ± 0.03	0.16 ± 0.05	0.48 ± 0.22‡	0.17 ± 0.09	0.98	0.35‡
Control†	—	0.11 ± 0.01	0.13 ± 0.01	0.84 ± 0.24	0.28 ± 0.03	1.22	0.17
HYP	350	0.17 ± 0.04	0.13 ± 0.05	0.57 ± 0.16‡	0.38 ± 0.22	0.77	0.23
Control	—	0.18 ± 0.04	0.16 ± 0.03	1.20 ± 0.29	0.56 ± 0.25	0.88	0.13
PLP	550	0.10 ± 0.08	0.09 ± 0.08	0.26 ± 0.20‡	0.12 ± 0.08	0.88	0.34
Control	—	0.11 ± 0.08	0.09 ± 0.07	0.42 ± 0.27	0.21 ± 0.22	0.78	0.20
PLP	50	0.20 ± 0.08	0.18 ± 0.06	0.66 ± 0.18‡	0.27 ± 0.11	0.91	0.27
Control	—	0.13 ± 0.08	0.13 ± 0.10	0.89 ± 0.72	0.27 ± 0.19	0.96	0.16
PLP	250	0.17 ± 0.06	0.18 ± 0.06	0.62 ± 0.20‡	0.25 ± 0.17	1.05	0.28
Control	—	0.20 ± 0.05	0.19 ± 0.02	1.23 ± 0.09	0.48 ± 0.18	0.97	0.15
PLP	100	0.12 ± 0.04	0.13 ± 0.04	0.42 ± 0.17‡	0.13 ± 0.10	1.10	0.31
Control	—	0.18 ± 0.08	0.21 ± 0.09	1.12 ± 0.45	0.14 ± 0.04	1.18	0.19

* Competitive antagonist was injected 1–30 min before injection of I-125 HYP.

† Control experiments were carried out on same day. Each group contained at least five animals.

‡ Displacement experiment value is significantly different from control value (using the two-way Friedman's analysis) at the level of P = 0.001. The H/L value is also significantly different. No significant difference was detected for the % dose/g in the heart, blood, thyroid and the H/B ratio.

shown that the guinea-pig heart (GPH) and the human heart have similar affinities for a small number of beta-adrenoceptor blocking agents (*11*). We and others have reported similar binding affinities in rat and guinea-pig hearts for various beta blockers (*4*). It therefore appears that the rat ventricle is the appropriate model and, as a result, the significantly lower K_A for this muscle (Table 1) must be applied in light of the intended use of these radiotracers.

Competitive inhibition of binding. *Calculation of the concentration of inhibitor.* In vivo displacement experiments using known receptor binders is one method of proving that a specific receptor interaction is the cause of the organ uptake (*14–16*). Propranolol (PLP) is a potent beta-adrenoceptor blocker. Studies using 5 μCi of H-3 PLP at 5 Ci/millimol (*1*), or pharmacologic amounts of PLP, gives similar distribution in rats although the absolute amounts are larger for the latter case (*17*). The concentration in both the heart and the lung increases as the injected dose increases (Table 3).

In the present series, 50 to 500 μg of PLP was preinjected to block the beta-adrenoceptor binding of the radioiodinated derivative (Table 4). From the data in Table 3 we calculate that the concentration of PLP should be greater than 0.1 μM in the heart and 0.6 μM in the lung. Since the simple bimolecular equilibrium model is based on free PLP, these numbers may not give an accurate picture of the situation. Schneck et al. (*17*) indicate that the PLP is distributed in various cellular fractions and is bound to high-capacity, low-affinity nonadrenoceptor sites. This alters the binding to receptor as discussed in detail previously (*14*).

Calculations using the cubic equation for competitive inhibition described by Ekins et al. (*7*) show that greater than 95% of the radioiodinated beta blocker I-125 HYP should be displaced at the levels of PLP employed if the PLP is neither metabolized nor bound by other proteins (Table 5). If the free concentration of PLP in the ECF is only 10^{-10} M, the B/F ratio in the lung is not reduced from an original value of 4.1. As the free concentration rises to 10^{-8} and 10^{-6}, the B/F ratio decreases to 3.2 and 0.07, respectively. Likewise the B/F ratio would be reduced in the heart to a similar extent for I-125 HYP, but starting with a maximum B/F ratio of 0.20. Similar calculations can be performed for other radioligands.

In vivo inhibition studies. Our data (Table 4) for the beta adrenoceptor blocker I-125 HYP show that PLP displaces HYP from the lung but not from the heart. This is similar to the results obtained by Strauss et al. (*18*). Several additional experiments were carried out in an attempt to assure that a large concentration of unbound PLP would be present, since the relative time of injection of inhibitor and the radiolabel may be important if the inhibitor is degraded rapidly. We injected PLP immediately before injection of I-125 HYP and immediately before sacrifice of the animal at 2 hr after injection of I-125 HYP. No significant decrease in concentration in the heart was observed under various conditions. To substantiate further that we are measuring receptor binding of I-125 HYP in the lung, we used the inactive D form of PLP as an inhibitor. D-PLP did not reduce the binding of I-125 HYP in the lung to the same extent as the DL mixture used under the same conditions in the experiments described in Table 4.

TABLE 5. CALCULATION OF IN VIVO DISPLACEMENT OF RADIOLABELED TRACERS FROM THE BETA ADRENOCEPTOR AS A FUNCTION OF PLP DOSE*

Compound	Organ	K†	R_0‡	L_0‖	B/F ratio 10^{-6} M PLP	B/F ratio 10^{-8} M PLP	B/F ratio 10^{-10} M PLP	B/F ratio None
I HYP	Heart	8.3×10^7	2.4×10^{-9} M	40×10^{-12} M	0.002	0.110	0.197	0.20
	Lung	1.7×10^8	24×10^{-9} M	40×10^{-12} M	0.073	3.200	3.980	4.11
DHA	Heart	1.3×10^8	2.4×10^{-9} M	2.8×10^{-9} M	0.003	0.146	0.227	0.24
	Lung	5.0×10^7	24×10^{-9} M	2.8×10^{-9} M	0.021	0.885	1.120	1.13
CAR	Heart	1.6×10^9	2.4×10^{-9} M	2.8×10^{-9} M	0.040	0.915	1.298	1.30
	Lung	0.8×10^9	24×10^{-9} M	2.8×10^{-9} M	0.335	13.265	17.041	17.08

* Calculated according to Eq. 3 (*7*).

† K = affinity constant for interaction of radiotracer with beta adrenoceptor; K_h for heart 9×10^7 M^{-1}; K_h for lung 5.6×10^7 M^{-1}.

‡ R_0 = total concentration of beta adrenoceptor.

‖ L_0 = original concentration of radiotracer.

Neither H-3 DHA, I-125 TYR-PRAC, I-125 TYR-ALP, nor I-125 TYR-BUN showed decreased uptake in the heart or lung in the presence of PLP. However, H-3 CAR showed decreased uptake in both heart and lung in the presence of PLP (Table 6). It is therefore specifically bound to some extent in both the heart and lung and indicates the affinity constant needed to achieve high H/B ratios.

It appears that the amount of PLP used and the relative times of injection and sacrifice are not crucial. The experiments in this and other laboratories indicate that I-125 HYP is a borderline example of a material that can be specifically bound to beta adrenoceptors. Apparently the larger concentration of receptor in the lung allows some receptor binding to occur, whereas the receptor concentration in the heart—decreased by a factor of 10—is not sufficient to cause specific binding. This could also be caused by differences in the concentration of nonreceptor proteins that effectively bind the radiotracer. H-3 DHA—which has an affinity constant for rat ventricle and lung like that of HYP—shows no adrenoceptor binding in the heart or lung. Likewise the low-affinity radiotracers I-125 TYR-PRAC and I-125 TYR-ALP show no beta-adrenergic receptor binding. On the other hand, H-3 CAR shows beta-adrenoceptor binding in both the heart and the lung, and represents the first example in vivo of detectable beta-adrenoceptor binding in the heart. Only in this latter case are the heart-to-blood ratios indicative of receptor binding.

Metabolism of iodinated beta blockers. In 1965 Stock and Westermann reported that a 1-(3-methyl-phenoxy)-3-isopropylaminopropanol was metabolized rapidly in rats (*19*). The enzymatic degradation of this compound by microsomal enzymes of the liver was studied indirectly by injecting a known microsomal enzyme inhibitor, diethylaminoethyldiphenylpropylacetate (SKF 525A), before the injection of the beta blocker. The concentration of the beta blocker was significantly higher in organs of SKF-525A-treated rats, compared with the untreated animals. The concentration doubled

TABLE 6. EFFECT OF COMPETITIVE ANTAGONIST ON THE DISTRIBUTION OF RADIOLABELED BETA-ADRENOCEPTOR BLOCKERS AT 2 HR IN GUINEA PIGS

Radiotracer	Competitive antagonist	Quantity (μg)	% dose/g ± s.d.* Blood	Heart	Lung	% dose ± s.d. Thyroid	H/B	H/L
I-125 TYR-PRAC	PLP	250	0.02 ± 0.01	0.10 ± 0.02	0.07 ± 0.02	0.06 ± 0.03	5.49	1.62
Control	—	—	0.03 ± 0.00	0.09 ± 0.01	0.09 ± 0.03	0.06 ± 0.04	3.57	1.04
I-125 TYR-BUN	PLP	100	0.04 ± 0.01	0.08 ± 0.02	0.56 ± 0.17	—	2.01	0.15
Control	—	—	0.04 ± 0.01	0.06 ± 0.02	0.47 ± 0.11	—	1.47	0.13
I-125 TYR-ALP	PLP	100	0.04 ± 0.01	0.30 ± 0.06	1.92 ± 0.25	0.10 ± 0.04	6.96	0.16
Control	—	—	0.05 ± 0.02	0.22 ± 0.07	1.92 ± 0.44	0.14 ± 0.01	4.48	0.12
H-3 DHA	PLP	250	0.08 ± 0.01	0.13 ± 0.03	2.28 ± 0.50	—	1.60	0.06
Control	—	—	0.04 ± 0.00	0.12 ± 0.01	1.71 ± 0.16	—	1.97	0.07
H-3 CAR	PLP	100	0.07 ± 0.01	0.07 ± 0.01	0.41 ± 0.12	—	1.03	0.18
Control	—	—	0.04 ± 0.01	0.21 ± 0.05	2.62 ± 0.43	—	5.01	0.08

* Each group contained at least five animals. Control experiments were carried out on same day.

TABLE 7. DISTRIBUTION OF I-125 IN GUINEA PIGS AT 2 HR AFTER INJECTION, WITH AND WITHOUT SKF-525A TREATMENT

	% dose/g ± s.d.			% dose ± s.d.*		
	Blood	Heart	Lung	Thyroid	H/B	H/L
I HYP	0.12 ± 0.02	0.12 ± 0.03	0.91 ± 0.34	0.24 ± 0.08	0.96	0.14
+ SKF-525A	0.13 ± 0.05	0.12 ± 0.05	0.77 ± 0.27	0.20 ± 0.10	0.96	0.16
I TYR-PRAC	0.02 ± 0.00	0.06 ± 0.01	0.05 ± 0.01	0.02 ± 0.02	3.66	1.11
+ SKF + 525A	0.01 ± 0.00	0.07 ± 0.03	0.06 ± 0.02	0.04 ± 0.02	4.37	1.07

* Groups of six guinea pigs were injected subcutaneously with 50 mg/kg SKF-525A 30 min before i.v. injection of iodinated derivative.

in the heart and increased 900% in the liver. Similar studies using I-125 HYP and I-125 TYR-PRAC did not show increased concentrations in the heart (Table 7), showing that metabolism was not a major deterrent to high H/B ratios.

The percent dose in the thyroid is given in Table 2. Although this does not necessarily reflect deiodination, because the uptake in the thyroid seems to be related to the lipophilicity of the beta blocker (*1,18*), it does give a maximum value of deiodination. At the level of 20% deiodination, the iodide in the blood would add approximately 0.02% dose/g to the value obtained for the iodinated beta-adrenoceptor blocker alone. This would decrease the H/B ratio significantly. However, it would not affect the tests for specific binding (Tables 4 and 6).

CONCLUSION

None of the iodinated derivatives in this study has the proper combination of high affinity constant and low protein binding to give H/B ratios greater than 10. From the competitive displacement studies it is obvious that the concentration of iodinated derivatives in the heart is not a reflection of the receptor concentration, and these radiotracers could not be used to monitor changes in concentration as a function of disease. In the lung it appears that part of the I-125 HYP is bound to receptor, so in this case the change in receptor concentration as a function of disease could be monitored.

H-3 CAR is bound to beta adrenoceptor in both heart and lung, although the heart-to-blood ratio is still low. These data suggest that K_A values of greater than 10^9 will be required to detect specific binding in the heart.

Because no present-day clinical or experimental beta-adrenoceptor blocking agent has an affinity constant greater than that obtained with H-3 CAR, radioiodinated beta-adrenoceptor blocking agents seem to be unsuitable for external imaging, due to the high level of nonadrenoceptor binding and the relatively low affinity constants. This is in contrast to the muscarinic blocking agents, which have higher affinities in combination with higher receptor concentration, and consequently give higher H/B ratios (*20*).

FOOTNOTE

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