Receptor-Binding Radiotracers: A Class of Potential Radiopharmaceuticals

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To date no radiopharmaceutical is routinely used to study changes in receptor concentration. Frequently changes in receptor concentration, or the appearance of receptors in tumors, indicates a specific pathologic state. With a receptor-binding radiotracer, in vivo studies of these changes will be possible. A reversible bimolecular model and in vitro tests were used to determine equilibrium constants and maximal target-to-blood ratios for new derivatives. Theoretical calculations showed that derivatives binding to the estrogen receptor, the beta adrenoceptor, or the cholinergic receptor are capable of achieving satisfactory target-to-blood ratios. Using in vitro tests, the apparent affinity constant was determined for five iodinated estrogen derivatives and five derivatives of beta blockers. Results of the in vitro study with derivatives of beta blockers, and in vivo displacement studies using propranolol, indicated that the high heart-to-blood ratios (5 to 20) obtained with the new derivatives were not the result of a specific interaction with the receptor. In this instance factors other than receptor binding control the in vivo distribution. The in vitro assay using estrogen receptors showed that of the five derivatives, iodohexestrol and 17-alpha-iodoethynylestradiol bind to the receptor with the highest affinity. In vivo studies confirmed these results; iodohexestrol gave a uterus-to-blood ratio of 10 in immature rats when plasma-protein binding was blocked. With a tritiated muscarinic cholinergic blocking agent, heart-to-blood ratios near the theoretical maximum were obtained. This compound most closely follows the mechanism described by the model. Use of the theoretical model in conjunction with in vitro assays can greatly aid in the design of this new class of receptor-binding radiopharmaceuticals.

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Many biochemicals and drugs cause highly specific changes in the body when given in relatively low doses. Evidence has come from a variety of sources to suggest that the primary event in the action of many peptide and steroid hormones and drugs is the binding to a specific site on the plasma membrane or in the cytosol. Those proteins that mediate the specific interaction are termed *receptors*. This concept is not new—it was first suggested by Lucretius in 50 B.C.—and has been broadened to include specific interactions that do not cause a known physiologic effect (*1*). Since receptors are involved in the action of the drug or biochemical, one might expect changes in the concentration of receptor as a function of disease state. The insulin receptor is one of the better-defined receptor systems in this regard (*2,3*). Changes in the concentrations of receptors in the central nervous system are also well documented in Huntington's chorea, parkinsonism, and Alzheimer's dementia disease (*4*–

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TABLE 1. CONCENTRATION OF RADIOTRACER FOR TYPICAL RADIOPHARMACEUTICAL DOSES IN HUMANS

Radionuclide	$T_{1/2}$	Dose (mCi)	Ci/mmol*	Concentration (p*M*) PV†	Concentration (p*M*) ECF‡	μCi/Kg
C-11	20.4 m	20	9.5×10^6	0.7	0.2	286
Tc-99m	6 hr	20	5.3×10^5	12.6	3.1	286
I-123	13.1 hr	5	2.4×10^5	6.8	1.7	71
Br-77	56 hr	5	5.6×10^4	29.5	7.4	71
In-111	2.82 d	5	4.7×10^4	34.2	8.5	7.1
I-131	8.06 d	0.5	1.6×10^4	10.2	2.5	7.1
I-125	59.7 d	0.5	2.2×10^3	75.9	19.0	7.1
H-3	12.33 y	(0.5)	2.9×10^1	5670	1417	7.1

* Assuming one radionuclide atom per molecule.
†Plasma volume of 3 liters in 70-Kg man.
‡Extracellular fluid volume of 12 liters in 70-Kg man.

6). In order to study the radiotracer interaction with receptors in vivo and to provide information on the relationship of receptor concentration to disease, new radiotracers must be designed that are capable of carrying a gamma-emitting radionuclide to the receptor.

6-[^{131}I]iodomethylnorcholesterol (I-131 CH) and 2-[^{18}F]fluoro-2-deoxyglucose (F-18 DG) interact with enzymes. It has been shown, however, that both these radiotracers concentrate in the target organ to a greater extent than the parent compound. Iodine-131 CH concentrates in the adrenals to a greater extent than C-14-labeled cholesterol and the adrenal-to-liver ratios are higher (*7*). Likewise F-18 DG achieves higher heart-to-blood and brain-to-blood ratios than glucose itself (*8*). The differential concentration cannot be due entirely to interaction with a "receptor enzyme" in an uncomplicated reversible equilibrium situation; rather, "metabolic trapping" is involved (*8*). The equilibrium constant for glucose-hexokinase is small and could not sustain the observed target-to-blood ratios (*9*). Analogous studies have not been reported for I-131 CH. Likewise [^{11}C]norepinephrine has been synthesized and shown to have a heart-to-blood ratio of 11 at ½ hr in the dog (*10*). Again, the uptake of norepinephrine in the heart is probably not due to interactions with the beta adrenoceptor alone, since it has been shown that norepinephrine is taken up in the heart by the Uptake I system (*11*).

MATERIALS AND METHODS

The synthesis of the iodinated estrogens and the iodinated beta-adrenoceptor blocking agents have been described elsewhere (*12,13*). Tritiated quinuclidinyl benzilate (QNB) was purchased commercially*, with specific activity 30 Ci/millimol. Tritiated N-methyl quinuclidinyl benzilate (MQNB) is prepared by mixing excess methyl iodide with [^{3}H]QNB. After evaporation of the excess methyl iodide, the radiochemical purity was checked in two systems and found to be >95%. Distribution data in Table 4 are taken from Ref. 13. Distribution data in Tables 5 and 6 were carried out under the same experimental conditions as those used in Ref. 13. The distribution was determined in either Sprague-Dawley rats or guinea pigs. The thyroid was not blocked. Under light halothane anesthesia, 0.1 ml of the radiopharmaceutical was injected into an exposed femoral vein. At 15 min or 2 hr postinjection, the animals were killed and samples of blood, heart, liver, lung, and thyroid were taken. The tissue samples and diluted standards of the radiopharmaceutical were counted in a scintillation counter. The results were expressed as the percentage of the injected dose per gram of tissue. Each group consisted of at least five animals.

RESULTS AND DISCUSSION

Model of receptor binding. In order to define better the receptor system required to achieve target-to-blood ratios that will allow external imaging, we have used a simple reversible bimolecular model:

$$\begin{array}{ccccc} & & & \overset{K}{} & \\ & R & + & L & \rightleftharpoons RL \\ & (R_o - B) & & (L_o - B) & B \end{array}$$

$$B = \frac{(1 + KR_o + KL_o) \pm \sqrt{(1 + KR_o + KL_o)^2 - 4K^2L_oR_o}}{2K}$$

With information on the original receptor concentration (R_o), the original concentration of radiotracer (L_o), and the affinity constant (K) between the two, the concentration of the receptor-bound radioactivity (B) can be determined. From this and the concentration of the free unbound radioactivity ($F = L_o - B$), the maximum theoretical target-to-blood ratio can be determined by assuming that a simple reversible equilibrium is set up between the bound and free radioactivities.

Determination of the concentration of radiotracer. This is calculated assuming carrier-free material and distribution in either the plasma volume (PV) or the extracellular fluid (ECF) of man (Table 1). The assumption of carrier-free tracer must be checked carefully, not only for well-known cases such as I-131 that contains I-127, but also for cyclotron-produced radionuclides such as C-11 (*14*). If the radioactivity is injected in animals on a per-kilogram basis, the same concentration of radiotracer will be obtained in the plasma volume and ECF in all species immediately after injection. The H-3 number was set from the usual 5-μCi injection into a 0.5-kg animal, since it is rarely used in humans.

Determination of the receptor concentration and equilibrium constants. Most receptor concentrations are of the order of 10^{-7} to 10^{-9} *M* in the target organ (*1*). Often the concentration of the receptor can be found in the literature but rarely is the affinity constant available for a derivative containing a nonisotopic substitution. In vitro tests using isolated receptor systems allow the determination of both these parameters. The in vitro radioreceptor assay may take two forms (*15*). The first type measures the ability of the nonradioactive tracer to block the binding of a high-affinity, tritium-labeled molecule from the receptor. This is useful when the new derivative cannot be prepared easily at high specific activity or when the screening of a large number of candidates for radiolabeling is necessary. The second method of carrying out the in vitro assay involves the use of the new derivatives in the radiolabeled form. This approach gives more information because the amount of nonreceptor binding can be determined directly, as can the binding constant. With the new derivatives in the nonlabeled form, these determinations may not be possible.

In addition, the presence of nonreceptor protein binding can be determined. This is usually carried out by equilibrium dialysis or by adding plasma proteins to the radioreceptor assay (*16*). Finally, the new radiolabeled derivative can be studied by use of a sucrose gradient to determine which proteins are binding the radiotracer. With the proper controls and standards, the receptor and nonreceptor binding of the new derivative can be determined. This technique is applicable only in situations where the dissociation rate of the receptor complex is slow at the assay temperature. Using the simple two-compartment model, we have calculated the necessary combination of receptor concentration and equilibrium constant to give a target-to-blood ratio greater than 10. The ratio of the concentration of bound-to-free radioactivity (B/F) calculated from the quadratic equation will equal the target-to-blood ratio if protein binding and metabolism are minimal. Since they are generally not minimal, the B/F ratios represent ideal maxima (Fig. 1). As a first approximation, the neglecting of protein binding and metabolism results in a model that is most helpful in eliminating substrate-receptor combinations that will not yield sufficient target-to-blood ratios.

Maximum bound-to-free ratios. As can be seen from Fig. 1, the affinity constant must be at least 10^8 to achieve B/F ratios of greater than 10 if the receptor concentration is 10^{-7} *M*. If it is only 10^{-8} *M*, the affinity constant must be 10^9 for the usual doses of carrier-free radiopharmaceuticals. Tritium-

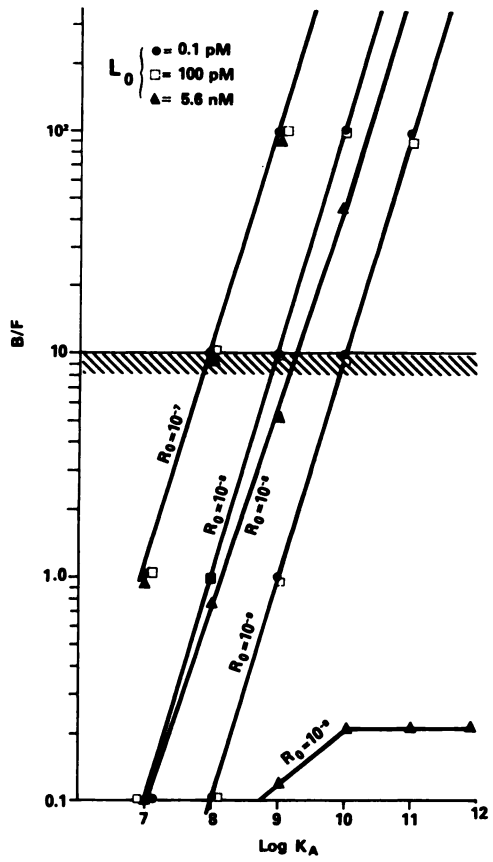

FIG. 1. The variation of the bound to free ratio (B/F) as a function of the affinity constant (K_A), the original concentration of radiotracer (L_o) and the original receptor concentration (R_o).

TABLE 2. EFFECT OF SPECIFIC ACTIVITY ON B/F RATIO IN HUMANS

Specific activity	L_0 concentration in ECF*	K_A required for B/F = 10		
Ci/mmol	pM	$R_0 = 10^{-7}M$	$R_0 = 10^{-8}M$	$R_0 = 10^{-9}M$
10	83000	4×10^8	NP+	NP
30	27000	1.3×10^8	NP	NP
10^2	8300	10^8	4×10^9	NP
10^3	830	10^8	$\sim 10^9$	4×10^9
10^4	83	10^8	10^9	$\sim 10^{10}$
10^5	8.3	10^8	10^9	10^{10}
10^6	0.83	10^8	10^9	10^{10}
10^7	0.083	10^8	10^9	10^{10}

* Calculated on the basis of an injected dose of 10 mCi of radiolabeled ligand (L_0) distributed in biospace of 12 L of extracellular fluid (ECF) in a 70-Kg man equivalent to 36 μCi distributed in 0.04 L of ECF in a 0.25-Kg animal. Use of 1 mCi of radioligand is equivalent to use of a radiochemical that has ten times the specific activity.

+ Not possible (NP) to achieve B/F ratio of 10 with stated concentration of receptor (R_0) and radioligand (L_0) with K_{ass} of $\leq 10^{10}$.

TABLE 3. AFFINITY CONSTANTS FOR RECEPTOR-BINDING DERIVATIVES

Receptor Type	Compounds	Abbreviation	Apparent affinity constant $K_A \times 10^{-9}$	
Estrogen	2-Iodoestradiol	I-E	< 0.01	
	17α-Iodoethynyl estradiol	I-EE	2.5	
	3-Iodohexestrol	I-HEX	< 0.01	
	3-[^{125}I]iodohexestrol	I-HEX	0.8	
	Estradiol 17β succinyl iodotyrosine methyl ester	I-EST	< 0.01	
	Estradiol 6-(O-carboxymethyl)-oxime-iodotyrosine methyl ester	I-ECMO	< 0.01	
			TEM† $K_A \times 10^{-6}$	RLM* $K_A \times 10^{-6}$
Beta adrenoceptor	1-[(4-hydroxy) phenethylamino[-3-[2-allyl phenoxy]-propan-2-ol	TYR-ALP	33.3	4.0
	1-[(4-hydroxy) phenethylamino]-3-[4-capramido phenoxy]-propan-2-ol	TC-PRAC	2.0	0.03
	1-[(4-hydroxy) phenethylamino]-3-[(2-chloro-4-capramido) phenoxy]-propan-2-ol	TCC-PRAC	0.5	0.04
	1-[(4-hydroxy) phenethylamino]-3-[(4-acetamido) phenoxy]-propan-2-ol	T-P	0.06	0.01
	1-[(4-hydroxy-5-methoxy) phenethylamino]-3-(m-tolyloxy)-propan-2-ol	PD-3	33.3	0.76
			Apparent affinity constant $K_A \times 10^{-9}$	
Muscarinic-cholinergic	[^{3}H]quinuclidinyl benzilate	H-QNB	3.3[23]	
	[^{3}H]-N-methylquinuclidinyl benzilate	H-MQNB	3.3	

† Apparent affinity constant for derivative with turkey erythrocyte membrane, a source of beta-1 adrenoceptor (22).

* Apparent affinity constant for derivative with rat lung microsomal fraction, a source of beta-2 adrenoceptor (unpublished data).

labeled compounds at 30 Ci/millimol would require a slightly higher affinity constant to achieve the same bound-to-free (i.e., target-to-blood) ratio because of the high concentration of labeled compound relative to receptor. At $10^{-9}M$ receptor concentration the affinity constant would have to be 10^{10} to achieve a B/F ratio of 10 with most radionuclides. The use of tritium compounds at this estimated in vivo concentration of receptor gives no indication of the results likely to be achieved with other radionuclides. Likewise, the use of other derivatives with low specific activity can appreciably reduce the maximal B/F ratio (Table 2). Although a maximum bound-to-free ratio could conceivably be reached when enough radioligand has cleared from the blood, this will severely limit the percent-

age of the radiotracer that will be bound by the receptor.

The B/F ratio is independent of the species if the concentrations of radiotracer and receptor—and the equilibrium constant between the two—are the same in the species concerned. That is not true of the %dose/g calculation. The concentration of the bound radiotracer will be the same in all species, as will the concentration in the blood, if the injection is on a per-kilogram basis (Table 1); but since the %dose/g is obtained by multiplying the concentration of the bound radiotracer by the weight of the organ (≈ volume) and dividing by the dose and the weight of the organ in appropriate units, the % dose/g decreases with increasing total body weight. The %dose per organ should be similar in all species if the concentration of unmetabolized derivative is the same in the blood.

$$\%\text{dose/g} = 100 \times \frac{[\text{B}] \times \text{organ wt (kg)}}{\text{radiotracer injected (moles)}} \div \text{weight of organ in grams} = \frac{100 \times [\text{B}] \times 10^{-3}}{\text{moles injected}}$$

Therefore,

$$\frac{\%\text{dose/g in 70-kg man}}{\%\text{dose/g in 1-kg animal}} = \frac{1}{70}$$

As proposed earlier (*17*), consideration of the change in %dose/g is important during analysis of preliminary small-animal data.

Examples of candidates for receptor-binding radiotracers. Three types of ligand-receptor systems look especially interesting as candidates for radiotracer design: a) the estrogen receptor, b) the beta adrenoceptor, and c) the muscarinic-cholinergic receptor. The presence of estrogen receptors in breast malignancies can radically alter the mode of treatment. Currently the presence of estrogen receptors is determined in the primary neoplasm (*18*). Metastases may or may not have the same estrogen dependency and therefore may respond to ablative therapy to a different extent (*18,19*). A survey of all metastases by external imaging can greatly aid in the choice of therapy.

Both beta adrenoceptors and muscarinic receptors are present in the heart muscle, including the left ventricle. The concentration of receptors in heart may reflect a certain disease state or may reflect cellular membrane changes (*20*); therefore, a radiotracer known to bind to either receptor would be most useful for external mapping of these processes.

Using the appropriate in vitro receptor assays, the apparent affinity constant was determined for five iodinated estrogen derivatives (*12,21*) and five derivatives of beta-adrenoceptor blockers (*13,22*). The affinity constants for [^{3}H] quinuclidinyl benzilate (QNB) and [^{3}H]-N-methylquinuclidinyl benzilate (MQNB) (*23*) were obtained from the literature (Table 3).

Given these affinity constants and the receptor concentration from the literature, (*23-25*), a maximal target-to-blood ratio (B/F) can be obtained (Table 4). We have attempted to validate this model by studying the target-to-blood ratio in animals.

Radiolabeled beta-adrenoceptor blockers. For the radioiodinated beta adrenoceptors the model predicted that PD-3 should be the most cardioselective drug (largest heart-to-lung ratio) and that none of the radiotracers should have heart-to-blood ratios in excess of 10 (Table 4). As shown in Table 5, I-PD-3 was the least cardioselective in vivo and I-TP had the highest heart-to-lung ratio (*12*). An unex-

TABLE 4. MAXIMAL TARGET-TO-BLOOD RATIOS CALCULATED FROM RECEPTOR CONCENTRATIONS AND APPARENT AFFINITY CONSTANTS

Compound	Target organ	Receptor concentration in target organ (n*M*)	Apparent affinity constant $\times 10^{-6}$	Maximal B/F
I-E	immature rat uterus	100[24]	<10	<1
I-EE	immature rat uterus	100	2500	250
I-HEX	immature rat uterus	100	<10	<1
[^{125}I]HEX	immature rat uterus	100	~800	~80
I-EST	immature rat uterus	100	<10	<1
I-ECMO	immature rat uterus	100	<10	<1
TYR-ALP	heart	10[25]	33.3	<1
TC-PRAC	heart	10	2.0	<1
TCC-PRAC	heart	10	0.5	<1
T-P	heart	10	0.06	<1
PD-3	heart	10	33.3	<1
QNB	heart	15[23]	3300	49
MQNB	heart	15	3300	49

TABLE 5. DISTRIBUTION OF IODINATED BETA ADRENOCEPTOR BLOCKERS IN RATS*

	Time (hr)	H/B	H/L
[^{125}I]TYR-ALP	2	1.8	0.1
[^{125}I]TC-PRAC	2	4.0	0.6
[^{125}I]TCC-PRAC	2	6.2	0.6
[^{125}I]TP	2	18.7	2.0
[^{125}I]PD-3	2	0.8	0.1

* Data from Ref. 13; see reference for experimental method.

pectedly high heart-to-blood ratio was also obtained with I-TP. Since the model did not predict this, we tested whether receptor binding was indeed taking place by coinjecting a potent beta blocker, propranolol, into the rat in order to displace any receptor-bound I-TP (Table 6). Since the propranolol could not displace the I-TP, the radioiodinated derivative of practolol must be binding to a nonreceptor protein. Distribution studies in dogs (Table 6) and (not shown) guinea pigs and rabbits gave low heart uptake and low heart-to-blood ratios, thus confirming our hypothesis that the I-TP was binding to nonreceptor proteins in the rat heart. The model correctly predicted the low B/F ratio caused by receptor binding. Further efforts to design higher-affinity derivatives of beta blockers are under way.

Radiolabeled estrogens. In the case of the radiolabeled estrogen, the theoretical model predicts high target-to-blood ratios for tritium-labeled estrogens. Distribution data in immature rats showed that these ratios can be achieved experimentally (*15*). The apparent equilibrium constants for the nonradioactive iodinated derivatives were not sufficient, in general, to give high target-to-blood ratios. The exception to this is 17-alpha-iodoethynyl estradiol, which had an apparent affinity constant equal to that of estradiol itself. By sucrose-gradient centrifugation analysis, iodohexestrol was shown to be specifically bound to the estrogen receptor to the extent of 15-20% in an unpurified receptor preparation (*12*). From these data it appears that protein binding effectively decreased the concentration of iodohexestrol available for receptor binding. As discussed previously, one of the shortcomings of the radioreceptor assay using the nonradioactive derivative is the inability to determine whether the derivative has a weak affinity constant for the receptor or is bound to nonreceptor protein to a large extent. Use of the radioactive form in both the radioreceptor assay and in the sucrose-gradient centrifugation assay indicated that the affinity of the hexestrol derivative is higher than estimated because extensive nonreceptor binding decreased the available steroid. Iodohexestrol could be displaced from nonreceptor protein by thyroxine both in vitro and in vivo, and in fact uterus-to-blood ratios rose to 10 in immature rats when the nonreceptor binding was eliminated by coinjection of thyroxine (*12*). The other derivatives showed low uterus-to-blood ratios with or without thyroxine (*12*).

The estrogen derivatives illustrate another facet of the equilibrium model. The B/F ratio is based on unchanged derivative in the blood. The estrogens are metabolized rapidly, so that the percentage of unchanged steroid in the blood is small at 2 hr after injection. In this situation the theoretical maximum will be larger than the experimental value because metabolites cannot be differentiated from parent compound when the blood radioactivity is measured by external imaging. Even with the tritium-labeled steroids, the theoretical maximum is not achieved because of metabolism. This has been described in detail elsewhere (*26*). Based on the theoretical model, the experimental data obtained with H-3-labeled estrogens, and the preliminary data with iodinated derivatives, (especially 17-alpha-iodoethynyl estradiol), it appears that gamma-emitting radiotracers that bind to estrogen receptors with high affinity can be prepared. Clinical trials in breast-cancer patients are now in progress.

TABLE 6. DISTRIBUTION OF IODINATED TP IN RATS AND DOGS

		% Dose/g				
Species	Time (hr)	Heart	Blood	Lung	H/B	H/L
Rat	2	0.57 ± 0.07	0.028 ± 0.002	0.19 = 0.00	22.6	3.5
Rat/PLP*	2	0.60 ± 0.04	0.029 ± 0.006	0.25 = 0.01	21.5	3.1
Rat	2	0.57 ± 0.13	0.025 ± 0.005	0.29 = 0.17	22.9	2.3
Dog†	2	0.032	0.034	—	1	—

* Rats were injected ½ hr before [^{125}I]TP with sufficient propranolol (PLP) to give a blood concentration of 10^{-6} *M*, assuming a 20 ml plasma volume as the distribution space. Each group contained at least five animals.

† Average of multiple samples in a single dog. Scan at 2 hr showed localization of radioiodine in the gallbladder with no other area of concentration apparent.

TABLE 7. DISTRIBUTION OF TRITIUM RADIOACTIVITY AFTER INJECTION OF 4 μ Ci (0.13 PMOL) OF [^{3}H]MQNB IN RATS AND GUINEA PIGS*

Animal	Compound	Time (hr)	%Dose/g ± s.d.† Heart	Blood	Lung	H/B	H/L
Guinea pig	MQNB	¼	1.61 ± 0.24	0.050 ± 0.004	0.55 ± 0.14	32.2	3.0
		2	0.82 ± 0.24	0.036 ± 0.015	0.32 ± 0.15	28.4	3.0
Rat	MQNB	¼	2.35 ± 0.49	0.075 ± 0.015	0.184 ± 0.017	31.5	12.7
		2	0.60 ± 0.06	0.064 ± 0.005	0.103 ± 0.018	9.3	6.0

* MQNB = N-methyl quinuclidinyl benzilate, 30 Ci/mmol.
† Each group contained at least five animals.

Radiolabeled muscarinic cholinergic blockers. 3-Quinuclidinyl benzilate (QNB) is a potent muscarinic antagonist in the central and peripheral nervous systems. H-3 QNB and H-3 MQNB bind to heart tissue with a high affinity and sufficiently high capacity to achieve high heart concentrations. Distribution data for H-3 MQNB in guinea pigs and rats show high heart-to-blood ratios in agreement with those predicted by the theoretical model (Table 7). In addition, the radioactivity seems to be specifically bound in the heart. Injection of 1 mg/kg atropine i.m. ½ hr before the injection of H-3 MQNB eliminates the high heart-to-blood ratio by blocking the muscarinic receptors in the heart (*26*).

These preliminary data indicate that derivatives of QNB and other muscarinic receptor antagonists can be designed as probes for the detection and quantification of muscarinic receptors.

CONCLUSION

Many of the radiopharmaceuticals in use today are based on active transport. The best example is the simple anion iodide, which is taken up by the thyroid. No currently available radiopharmaceutical is based on the interaction of a radiotracer with a receptor under equilibrium conditions. This specific interaction is of interest because of the documented presence of altered levels of receptors in various disease states.

A number of factors such as protein binding, metabolism, excretion, etc., will affect the target-to-nontarget ratio. However, if a radiotracer does not bind to the receptor with the same affinity as the parent, these other problems are secondary. On the other hand, it follows that, although a high affinity constant is certainly necessary, it is not sufficient in all cases. In fact, in the case of iodohexestrol, the secondary problem of protein binding decreased the B/F ratio significantly.

The proposed simple model, based on the interaction of the radiotracer and the receptor under equilibrium conditions, can be used to eliminate a number of candidate systems and radiolabeled derivatives that have either too low a concentration of receptor or too low an affinity constant. For example, of the compounds listed in Table 4, nine out of 13 were eliminated by the model. Just as for tritiated compounds of low specific activity, calculations also can be made for other low-specific-activity compounds (such as F-18 or Br-77 radiotracers prepared using carrier halogen) to determine the maximal B/F ratio. All this can be done without extensive animal studies or preliminary clinical trails.

In the case of the receptor systems examined in this paper, the muscarinic cholinergic receptor and the estradiol receptor are the most promising. By the use of in vitro tests to determine the affinity constant of the new radiotracers, we have been able to judge which derivatives will give the highest target-to-blood ratios. For the estradiol and muscarinic derivatives on one hand and for the beta-adrenoceptor derivatives on the other, the in vitro tests and the simple equilibrium model have indicated what is needed: in the estradiol case, that 17-alpha-iodoethynylestradiol and iodohexestrol are the derivatives to pursue, and in the case of the muscarinic derivatives, that N-methyl-quinuclidinyl benzilate is an important parent structure. Likewise, in the beta-adrenoceptor blockers we learned that we cannot use cardioselective drugs as model compounds because of their low affinities. Both conclusions are important and are difficult to reach without the simple model and in vitro tests. The use of in vitro tests to reduce the number of variables that have to be taken into account has long been a practice of pharmaceutical research. The same approach seems to be just as important in the design of receptor-binding radiopharmaceuticals.

Radiotracers that bind to receptors appear to be a potential source of radiopharmaceuticals that could give important information about the changes in receptor concentration or the appearance of receptors as a function of a specific pathologic state.

If the receptor is on the plasma membrane of the cell, the radiotracer can also indicate changes in membrane structure. Use of the equilibrium model in conjunction with in vitro assays can greatly aid in the design of receptor-binding radiopharmaceuticals.

FOOTNOTE

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