RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Tc-99m Galactosyl-Neoglycoalbumin: In Vitro Characterization of Receptor-Mediated Binding

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Hepatic binding protein (HBP) is a membrane receptor that binds and transports plasma glycoproteins from hepatic blood to hepatocellular lysosomes. We have characterized the in vitro binding of Tc-99m galactosyl-neoglycoalbumin (Tc-NGA), a synthetic HBP ligand, to liver membrane. Structural modifications of NGA resulted in the alteration of the equilibrium constant, K_A, and the forward-binding rate constant, k_b. Binding was second-order; the relative amount of membrane-bound NGA depended on the initial concentrations of ligand and membrane. Membrane displacement studies, using carrier ligands in contrast to previously bound Tc-NGA or I-NGA, correlated with the binding characteristics of a native HBP ligand, asialo-orosomucoid. We used computer simulation to study the detectability of the changes in HBP concentration at different values of k_b. The simulations indicated that radiopharmacokinetic sensitivity to alterations in [HBP] should be possible using a neoglycoalbumin preparation with a carbohydrate density within the range of 15 to 25 galactose units per albumin molecule.

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The process by which receptor molecules bind ligand substrates exhibits two unique properties; molecular specificity and saturability. Each relates a significant chemical parameter to the kinetics of the binding reaction. The first property, molecular specificity, identifies the contribution of chemical structure to the reaction rate, and is quantified by the forward and reverse rate constants (k_b and k_{-b}). The saturability of a receptor-ligand interaction is the result of a finite number of ligand binding sites provided by the receptor molecule.

Because measurements of equilibrium affinity constant and kinetic rate constants relate chemical structure and stoichiometry to chemical kinetics, the development of receptor-binding radiopharmaceuticals has benefited from assays of in vitro binding. The approach has been to screen various ligand derivatives for the appropriate parameters that will permit adequate accumulation at a target with a given receptor concentration. For the most part, these studies have emphasized thermodynamic (equilibrium) measurements. For example, Eckelman developed a biphasic model by which the maximum theoretical target-to-background ratio could be calculated (*1*). Data for the model were supplied by in vitro measurements of equilibrium binding constants. After screening several labeled muscarinic derivatives, he selected 3-quinuclidinyl-4-iodo-benzilate (4-Iodo QNB) as the most promising candidate for the mapping of muscarinic receptors in heart and brain (*2*). As another example, design of a neuroleptic agent utilized both equilibrium and transient-state binding data (*3*) to compare the binding rate constants of haloperidol and spiroperidol. The higher affinity of the latter compound was a prominent reason for choosing C-11 spiroperidol (*4*) as the optimal tracer.

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A second use of in vitro experiments has been the measurement of nonspecific binding exhibited by a labeled derivative. Katzenellenbogen used in vitro techniques to search for an estrogenic analog with low nonspecific binding. After formulating an index of binding selectivity (*5*) based on the relationship of ligand lipophilicity to nonspecific binding, 16α-[Br-77]bromoestradiol-17β (*6*) was selected for the imaging of estrogen-dependent mammary tumors.

Our laboratory has been interested in a class of receptors called lectins (*7*), which bind specific carbohydrate residues of glycoproteins. Hepatic binding protein (HBP) resides on the cell surface of mammalian hepatocytes (*8*), where it binds and transports galactose-terminated glycoproteins to the hepatic lysosomes. Extensive studies (*9*) have implicated HBP as the normal mechanism for the homeostasis of plasma glycoproteins. Based on the significance of HBP in hepatic physiology, we proposed the use of this receptor as the basis for a diagnostic agent capable of routine measurement of liver function (*10*).

This paper presents results that characterize a synthetic HBP radioligand, Tc-99m galactosyl-neoglycoalbumin (Tc-NGA), as a receptor-binding radiopharmaceutical, in terms of saturability, nonspecific binding, tissue specificity, and molecular specificity. Lastly, we present the concept of kinetic sensitivity, which measures the ability of a physiochemical parameter to alter the in vivo uptake of a radiopharmaceutical. To illustrate this concept, we simulated the biokinetics of the Tc-NGA-HBP system.

MATERIALS AND METHODS

Radiopharmaceutical synthesis and quality control. Synthesis of the analog ligand, galactosyl-neoglycoalbumin (NGA), was accomplished by the covalent coupling of a bifunctional reagent, 2-imino-2-methoxyethyl-1-thio-β-D-galactopyranoside (IME-thiogalactose) to the primary amino groups of human serum albumin (HSA). The IME-thiogalactose was synthesized in four steps (*11–13*) and repeatedly recrystallized (methanol) and decolorized (activated charcoal in warm methanol). The coupling reaction was carried out in fresh borate buffer (Clarke's, pH 8.6, 0.2*M*). With a 20 mg/ml solution of HSA and an incubation temperature of 25°C, the reaction reached its maximum yield within 1 hr. Unreacted IME-thiogalactose was removed from the product by diafiltration* with five exchange volumes of isotonic saline. The NGA solution was then concentrated to 125 mg/ml. A colorimetric analysis (*14*) was used to measure the number of galactose groups attached to each albumin molecule.

The albumin derivative was labeled with either iodine-125 or technetium-99m. Chloramine-T (*15*) produced I-NGA yields in excess of 95%. The electrolytic method of Benjamin (*16*) produced technetium-NGA labels with yields also in excess of 95%. The labeled products were passed through a polydextran column (120 × 1.5 cm). Quality control, which included polyacetate electrophoresis (250 V, 20 min) and polyacrylamide gel-filtration chromatography (20 × 1.0 cm) (*10*), showed the chromatographically purified NGA to be free of multimeric forms of albumin and of free technetium or iodine. The technetium label of all neoglycoalbumin preparations was stable for at least 4 hr. Concentration of the pooled eluate was measured by uv absorbance (280 nm, $a_m = 2.49 \times 10^{-5}$ l/mole).

Membrane receptor preparation and binding assay. Rabbit hepatocyte membrane was isolated using the method described by Ray (*17*). The binding assays were carried out under the conditions outlined by Van Lenten (*18*). For the equilibrium studies, four initial concentrations and eight neoglycoalbumin preparations of labeled NGA were used: 4, 20, 100, and 200 p*M*; and 4, 6, 7, 13, 21, 25, 37, and 44 average galactose groups per albumin molecule. Studies that followed the time course of the binding reaction showed that equilibrium was reached within 30 min (*19*). As a result, the filtration step was carried out after a 1-hr incubation. The filters bearing the membrane-bound NGA, and standards containing the initial amount of ligand, were counted in an automatic gamma counter using a 10–80 keV window for I-125 NGA and a 100–200 keV window for Tc-99m NGA. Binding displacement experiments were performed using NGA-10, NGA-25, and NGA-44. After incubation with labeled NGA, a 1000-fold molar excess of either cold NGA, N-acetylgalactosamine, or D-glucose was added to the reaction tube. The latter carbohydrate is not capable of inhibiting HBP binding or of displacing previously bound HBP ligands (*20*). Displacement of the previously bound NGA was followed by filtering sets of reaction tubes at various times after the addition of the displacement reagent. Unless noted, all assays used 250 μg of membrane per assay tube. Data analysis used standard regression techniques (*21*).

In vivo simulations. Using the results of the in vitro measurements, we simulated the NGA kinetics to determine whether physiologic changes in receptor concentration or blood flow would alter the liver's time-activity curves. Stimulation of NGA kinetics was performed by a FORTRAN-coded program (*19*) that utilized a numeric integrator (*22*). The program was executed in a standard nuclear medicine computer using the video terminal for graphic output. The kinetic model (Fig. 1) (*23*) used three compartments: extrahepatic blood, hepatic blood, and hepatocyte-bound NGA. The first two compartments communicate by hepatic blood flow. The formation of the receptor-ligand complex depends on the number of receptors and the forward binding rate constant, k_b.

The simulation required values for each of the above

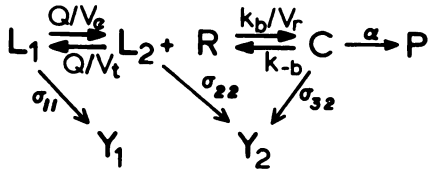

FIG. 1. Model for Tc-NGA kinetics incorporates three processes: hemodynamic delivery of ligand, receptor binding of ligand to hepatocyte membrane, and catabolism of ligand by hepatic lysosomes.

parameters and the volumes of each compartment. The volumes and the hepatic blood flow for a 70-kg human were taken from a standard textbook (*24*). The number of receptors within the liver was estimated from in vitro binding data using isolated rat hepatocytes (*25*). The k_b for NGAs with various galactose-coupling ratios were those calculated from our in vitro data. The model also contains a metabolic rate constant, α, whose value was taken from rabbit in vivo data (*10*). The simulations used a molar dose of ligand that would occupy 1% of the receptor sites. Table 1 lists the symbols for the model and the values of each parameter.

RESULTS

The affinity of labeled NGA could be synthetically controlled. The molar ratio of the reactants used in the coupling reaction (IME-thiogalactose and HSA) controlled the number of galactose groups coupled to each albumin molecule (Fig. 2). The carbohydrate density of the neoglycoalbumin determined the equilibrium constant for binding of the NGA preparation to hepatic membrane. Casting the equilibrium binding data of NGAs with different carbohydrate densities into the

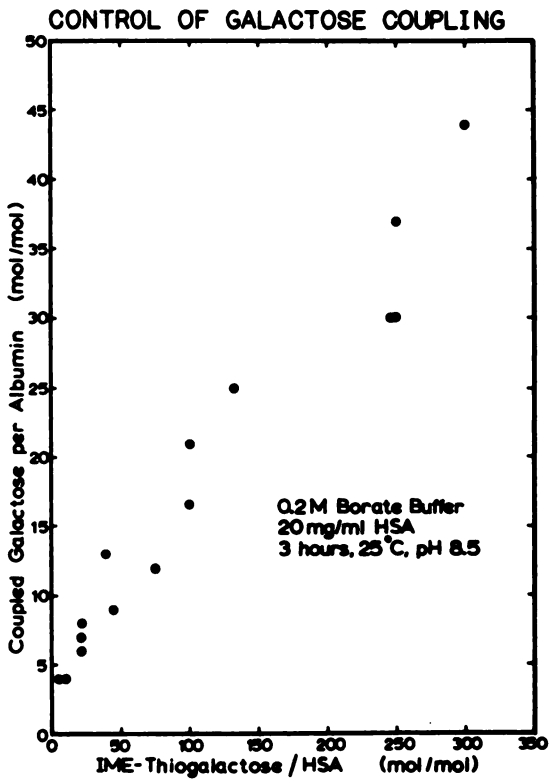

FIG. 2. Number of galactose groups per albumin molecule could be controlled by molar ratio of reactants, IME-thiogalactose, and human serum albumin (HSA).

form of Scatchard plots produced monophasic lines with different slopes. Figure 3 is a representative plot in which initial ligand concentrations ranging from 4 to 200 p*M* produced bound-to-free ratios of 0.22 to 0.45. The regression coefficient for this plot was 0.91.

TABLE 1

Symbol	Representation	Units	Simulation value
Q	hepatic blood flow	L sec^{-1}	2.9×10^{-2}
V_e	extrahepatic blood volume	L	4.6
V_h	hepatic blood volume	L	0.41
V_r	sinusoidal plasma volume	L	0.18
k_b	forward binding rate constant	M^{-1} sec^{-1}	varied
k_{-b}	reverse binding rate constant	sec^{-1}	1.0×10^{-5}
α	lysosomal catabolic rate constant	sec^{-1}	1.0×10^{-4}
L_0	molar dose of NGA	mole	varied
R_0	HBP (free) at time zero	mole	varied
L_1	NGA in extrahepatic blood	mole	
L_2	NGA in hepatic blood	mole	
R	HBP (free) in hepatocytes	mole	
C	HBP-NGA complex in hepatocytes	mole	
P	labeled metabolic products	mole	
Y_1	Time-activity data from precordial region-of-interest	cts	
Y_2	Time-activity data from liver region-of-interest	cts	
σ's	detector coefficients	cts $mole^{-1}$	

TABLE 2. IN VITRO I-125-NGA MEMBRANE* BINDING DATA

Galactose density (M/mole)	Equilibrium constant, K_A (M^{-1})	B/F† range	Regression coefficient
44	$7.0 \pm 1.4 \times 10^9$	0.09–0.23	0.94
	$6.7 \pm 0.7 \times 10^9$	0.21–0.52	0.96
	$8.8 \pm 2.6 \times 10^9$	0.13–0.49	0.77
37	$5.3 \pm 0.8 \times 10^9$	0.2–0.4	0.92
	$7.7 \pm 3.1 \times 10^9$	1.0–2.8	0.85
	$5.3 \pm 1.5 \times 10^9$	2.3–3.7	0.85
25	$5.4 \pm 1.3 \times 10^9$	1.0–1.4	0.84
	$5.2 \pm 1.0 \times 10^9$	2.2–3.5	0.69
21	$2.5 \pm 1.7 \times 10^9$	1.0–1.4	0.84
	$4.1 \pm 1.4 \times 10^9$	2.2–3.5	0.69
	$5.3 \pm 0.9 \times 10^9$	2.2–3.0	0.91
13	$1.2 \pm 0.7 \times 10^9$	0.08–0.14	0.84
	$2.3 \pm 0.5 \times 10^9$	0.13–0.28	0.97
	$1.3 \pm 0.8 \times 10^9$	0.11–0.30	0.63
7	No binding	0.02–0.03	
6	No binding	0.03–0.05	
4	No binding	0.04–0.01	
	No binding	0.01–0.01	

* 250 μg lyophilized membrane.
† Range of L_0 = 4 to 200 p*M*.

Equilibrium constants (K_A), the range of the bound-to-free values, and the coefficient of the regression line for each plot are listed in Table 2 for neoglycoalbumins having different galactose densities. The Scatchard plots of all NGA preparations produced sloped lines that were composed of clusters of data points (as in Fig. 3), each cluster representing different initial amounts of ligand. Neoglycoalbumins with less than seven sugars per albumin exhibited low binding ratios (B/F < 0.05) and produced Scatchard plots with scattered data points and no slope. Increasing the amount of membrane (50, 100, 250, and 500 μg) at a constant [L_0] (100 p*M*) resulted in a linear increase in the amount of bound I-NGA ($r = 0.99$, $p<0.001$, Fig. 4).

Measurements of the reverse binding rate constant, k_{-b}, revealed a statistically insignificant difference between three neoglycoalbumins with different densities of galactose (NGA-10, NGA-25, and NGA-37, Fig. 5). The forward binding rate constant for each NGA (Fig. 6) was calculated by multiplying the value of the reverse binding rate constant common to all NGA preparations (3.0×10^{-5} sec^{-1}) by the equilibrium constant, K_A, of

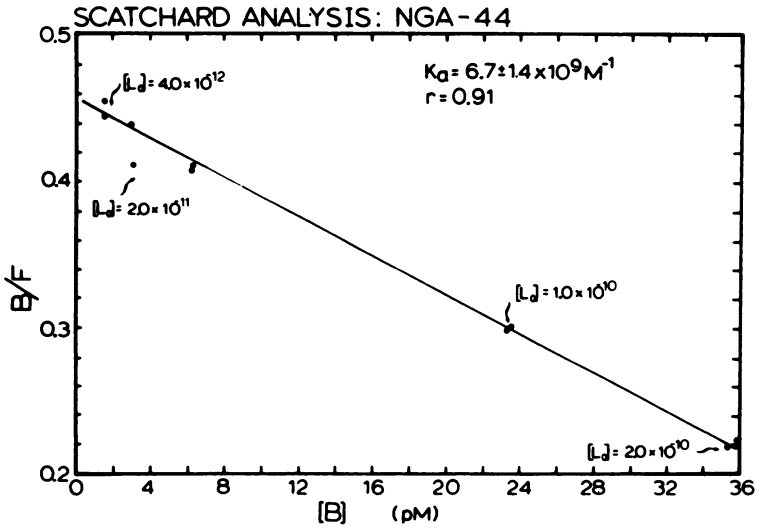

FIG. 3. Scatchard analysis was used to calculate equilibrium constant, K_A, for NGA binding. Monophasic plots indicated second-order binding reaction to single site. Initial concentration of ligand ranged over two orders of magnitude.

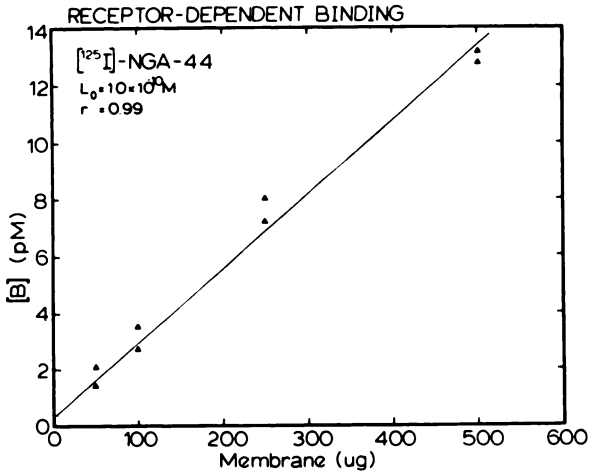

FIG. 4. Amount of membrane-bound I-125 NGA varied linearly with amount of membrane within each reaction tube ($r = 0.99$, $p < 0.001$).

each NGA. Both N-acetylgalactosamine and cold NGA displaced labeled NGA that was previously bound to the receptor (Fig. 7), but D-glucose did not displace the bound ligand. The rate of displacement, when challenged by N-acetylgalactosamine, was the same as for the displacement of unlabeled NGA. The radioactive label (iodine or technetium) did not affect the displacement rates.

Simulations of NGA-13 ($k_b = 0.3 \times 10^5\ M^{-1}\ sec^{-1}$) uptake by the liver produced a spectrum of curves corresponding to a normal receptor concentration and 15 and 25% decrease (Fig. 8A). The difference between the curves produced by a normal and a 15% decrease in receptor concentration was reduced when a higher k_b (2.7 $\times 10^5\ M^{-1}\ sec^{-1}$, NGA-44) was used (Fig. 8B). At the high k_b, a 25% decrease in hepatic blood flow produced a detectable shift in the simulated uptake curve.

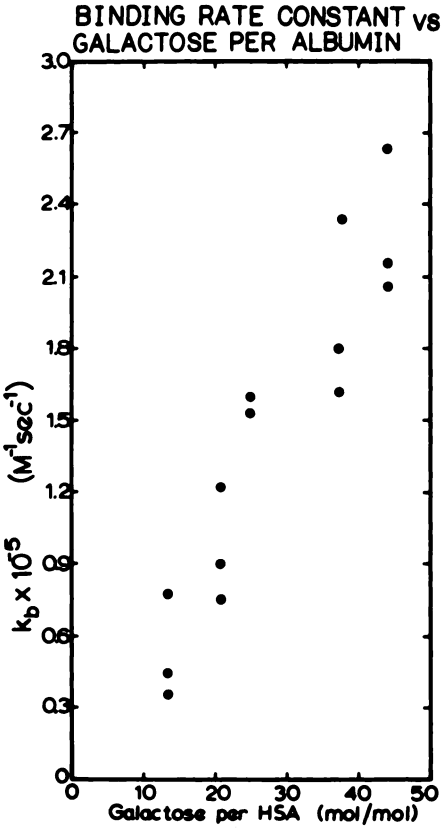

FIG. 6. Number of galactose residues per albumin molecule determined forward rate constant of binding, k_b. Because galactose density can be controlled easily during synthesis, tracer uptake can be preselected to optimize kinetic sensitivity to alterations in receptor concentration.

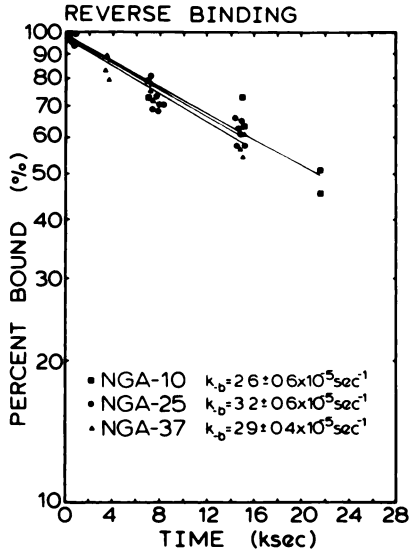

FIG. 5. Reverse binding rate constant, k_{-b}, was required to calculate forward binding rate constant, k_b. Three neoglycoalbumins tested had different galactose densities (10, 25, and 37), but displayed similar reverse binding rate constants. Individual regression coefficients were 0.86, 0.79, and 0.82.

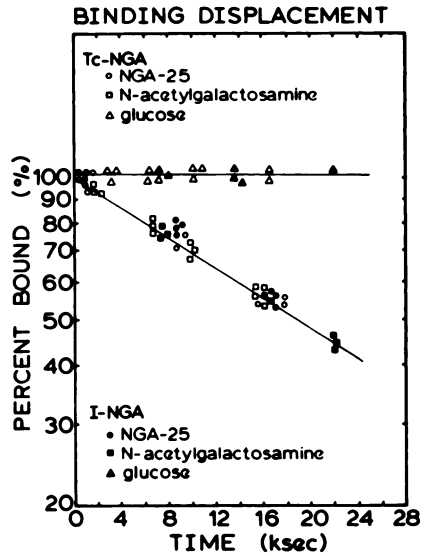

FIG. 7. Binding site of NGA is HBP. Inability of D-glucose, and ability of N-acetylgalactosamine, to replace previously bound NGA correlates with reported monosaccharide displacement of native HBP ligand, asialo-orosomucoid.

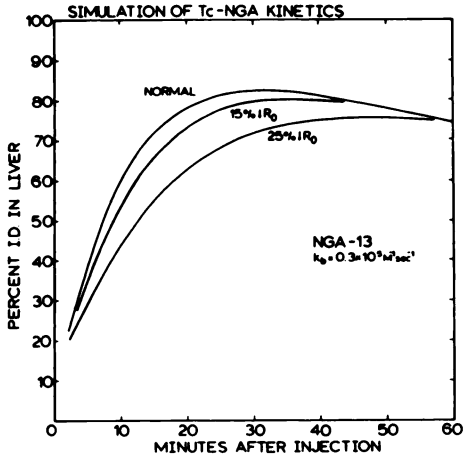

FIG. 8. Top: Hepatic accumulation of NGA was simulated to investigate ability of different receptor concentrations to change liver uptake curves. Neoglycoalbumin having 13 galactose groups per albumin exhibited uptake curves that were altered by different hepatic receptor concentrations.

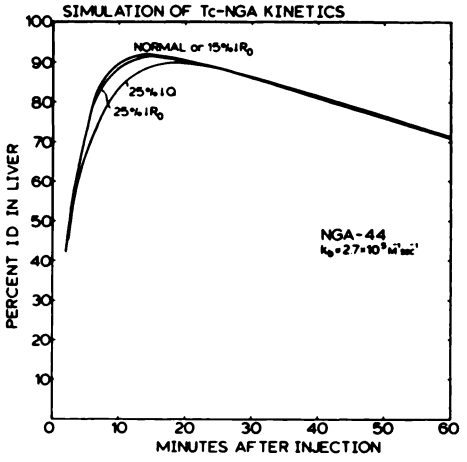

FIG. 8. Bottom: Simulations using forward binding rate constant of $2.7 \times 10^5\ M^{-1}\ sec^{-1}$ indicated that changes in receptor concentration would have little effect on hepatic accumulation of an NGA having 44 galactose groups per albumin. 15% decrease in receptor produced curve similar to normal curve. A 25% decrease produced only slight variation. Uptake curve was sensitive, however, to 25% decrease in hepatic blood flow.

DISCUSSION

Receptor-binding agents provide new perspectives to the design of radiopharmaceuticals. Because it is possible to characterize both chemically and physically the process responsible for tissue binding, quantifiable criteria for radiopharmaceutical performance can be established. We will describe the receptor binding of NGA in terms of saturability, nonspecific binding, tissue specificity, and molecular specificity. A fifth criterion, kinetic sensitivity, forms a useful predictor of the in vivo performance of a radiopharmaceutical.

Saturability. A principal characteristic of receptor-ligand binding is the dependence of the reaction rate on the amount of initial ligand and receptor. Although the term saturability is commonly used to describe this dependence, the saturation of an entire receptor population can occur only under the extreme condition of vastly high k_b and ligand-receptor ratio. This is especially the case in vivo when receptor recycling or turnover occurs.

Second-order reactions, if allowed to reach equilibrium and operated under conditions that prevent catabolism of the ligand-receptor complex, will fit the Scatchard model. Thus, the relative amount of ligand bound to the membrane preparation (B/F) when the reaction has reached equilibrium will depend on the initial amounts of ligand and receptor. In vitro assays using plasma-membrane preparations assume a direct relationship between the weight of membrane (which is usually lyophilized) and the number of receptors. Because the hepatic membrane preparation is devoid of lysosomal enzymes, the NGA-HBP complex is not catabolized. As a result, the increased B/F values produced by increasing the initial NGA concentration, ($[L_0]$, Fig. 3), and the linear dependence of bound NGA with differing amounts of membrane (Fig. 4), confirm the second-order nature of NGA binding. This second-order behavior is also exhibited by HBP binding to native ligands where the reaction rate depends upon the concentration of ligand (*26*) and receptor (*27*). This also suggests that in vivo hepatic accumulation of Tc-NGA will be controlled by the number of HBP molecules that communicate with hepatic blood.

Specificity: nonspecific binding. The monophasic Scatchard plots from our equilibrium binding studies indicate a lack of nonspecific binding (NSB) of NGA to the membrane preparation. The plots, which covered a range of two orders of magnitude in initial NGA concentration, were similar to those obtained from the binding of asialo-orosomucoid (*25,28*) and asialofetuin (*28*). Nonspecific binding, which is usually attributed to lipophilic interaction with membrane structures, superimposes an additional line on a Scatchard plot. Although the slope of the NSB line, and hence the binding affinity, is lower in magnitude than that for binding to the specific receptor, the number of nonspecific sites can be extremely high. As a result, these high-concentration, low-affinity binding sites can compete effectively against much lower concentrations of high-affinity receptor sites, and artificially elevate the binding capacity for the ligand. The low NSB exhibited by NGA was not unexpected; unlike the neuroleptic and estrogenic agents—which are moderately lipophilic and thus exhibit NSB (*4,5*)—Tc-NGA is a carbohydrate derivative of albumin and hence is highly hydrophilic.

Specificity: tissue. An important factor governing the performance of a receptor-binding radiopharmaceutical is the tissue distribution of the receptor. Optimal tissue specificity exists when the receptor resides in a single cell type within a single organ. Eliminating competition from

other organs maximizes accumulation of ligand by the target organ. Such competition may be extremely detrimental if the competing tissue receives a higher percentage of cardiac output. Hepatic binding protein is present only in a single cell type, the hepatocytes (*29,30*), of a single organ, the liver (*31*).

Other receptor-binding radiopharmaceuticals are much less tissue-specific. For example, estrogen receptors are present not only in mammary tumors, but also in the uterus (*32*) and liver (*33*). Muscarinic receptors have been found in the brain (*34*), ileum (*35*), myocardium (*36*), and pancreas (*37*). Dopamine receptors not only provide synaptic transfer of information in the central nerve system (*38*), but also mediate nervous control of vascular smooth muscle (*39*). Dopaminergic receptors have been characterized in coronary (*40*), renal (*41*), and mesenteric (*41*) arteries. The high tissue specificity of HBP ligands was a major factor in our selection of the HBP-NGA system as a model for the development of receptor-binding radiopharmaceuticals.

Specificity: molecular. The unique feature of receptors and their ligands is the complementarity of their molecular structures. One measure of receptor-ligand specificity is the rate at which the reaction proceeds. The forward binding rate constant, k_b, of a given ligand provides a quantitative link between chemical structure and molecular specificity. An increase in the galactose-to-albumin ratio of NGA is an alteration of ligand structure in the sense that an increase of carbohydrate density in a ligand increases the probability of substrate-receptor interaction based on a simple geometric model.

The displacement studies established HBP as the receptor responsible for the binding of NGA to liver membrane. Native HBP ligands (asialo-orosomucoid, for example) can be displaced from membrane (*20,18*) or isolated receptor (*42*) by a molar excess of N-acetygalactosamine, but not by D-glucose (Fig. 7).

The rate of displacement, and hence the k_{-b}, were the same for NGA preparations with different carbohydrate densities (Fig. 5). Using isolated rat hepatocytes, similar values of k_{-b} have also been measured for asialo-orosomucoid and asialofetuin (*43*) [$(5.4 \pm 1.5) \times 10^{-6}$ and $(6.3 \pm 1.5) \times 10^{-6}$ sec^{-1}, respectively, at 10°C], these being two native HBP ligands that have different blood clearance half-times (*44*) and different forward binding rate constants (*26*).

Kinetic sensitivity. Another, and more critical, question regarding radiopharmaceutical performance is the sensitivity of target-organ pharmacokinetics to alterations in specific physiological parameters that are diagnostically significant. The most important parameters are blood flow and receptor concentration. If a change in receptor concentration is not capable of producing an observable alteration in the time-activity data, one cannot estimate the value of that parameter.

Using computer simulation of our kinetic model, we were able to investigate the influence of k_b upon the kinetic sensitivity of the receptor concentration and hepatic blood flow. The dependence of k_b on the number of galactose groups per albumin allows us to modify k_b synthetically while keeping all other radiopharmaceutical properties constant. By quantifying binding in terms of the fundamental units of receptor-ligand function, k_b and R_0, we were able to simulate the performance of receptor-ligand reaction within the context of two other processes: hemodynamic delivery of the ligand to the liver, and lysosomal metabolism. The equilibrium constant, K_A, is not a kinetic parameter and thus cannot provide a measure by which the hemodynamic delivery, receptor binding, and metabolic fluxes may be compared.

The relative magnitudes of these three rate processes determine the ability of the radiopharmaceutical uptake measurements to reflect changes in receptor concentration. This was illustrated by the simulations (Fig. 8). Using an injection of NGA having 13 galactose groups per albumin (NGA-13), and a carbohydrate density that exhibited a k_b of 0.3×10^5 M^{-1} sec^{-1}, we found the hepatic uptake sensitive to alterations in receptor concentration: decreases in HBP concentration shifted the curves downward and to the right (Fig. 8A). At a higher carbohydrate density (44 galactose groups per albumin, NGA-44), and forward binding rate constant $k_b = 2.7 \times 10^5$ M^{-1} sec^{-1}, the kinetic sensitivity to HBP concentration was severely diminished: a 25% decrease in concentration was required to produce a minimally different curve (Fig. 8B). However, a 25% decrease in hepatic blood flow significantly altered the uptake curve. Under real conditions the ability to distinguish between each curve will be limited by the amount of noise in the time-activity data. Thus, if time-activity data are to enable us to observe a change in kinetic parameters produced by a ligand with a high forward binding rate constant, we will need high levels of activity and minimal organ motion.

A high forward binding rate constant is similar to a high extraction coefficient, and indicates a radiopharmaceutical that will accumulate within a target organ at a rate limited by the flow of blood into the organ. Prevention of this condition can be achieved if the first-order parameter of the binding reaction, $k_b[R]_0$, is of the same magnitude as the parameter for target-organ perfusion, Q/V, where Q is blood flow in l/sec and V is the distribution volume of the tracer (*19*).

Our simulations indicate a kinetic insensitivity to HBP concentration when k_b is greater than 2.7×10^5 M^{-1} sec^{-1}. The ability to control the carbohydrate density synthetically has enabled us to prepare various HBP ligands with a range of forward binding rate constants. Using healthy rabbits, we then injected and followed the time-course of Tc-NGAs of differing k_b and molar doses (*45*). The observation of affinity-dependent uptake and

dose-dependent uptake of the radiopharmaceutical confirmed our simulations and the concept of pharmacokinetic sensitivity.

Although all receptor-ligand systems differ in their biochemistry, each will require as a fundamental kinetic structure: (a) delivery of the ligand to the target tissue, and (b) binding of the ligand to the receptor. As a result, these tracers may display delivery-limited kinetics. Due to the fenestrated structure of the hepatic sinusoids, macromolecules such as Tc-NGA can communicate freely with the cell surface. Thus, liver blood flow, rather than interstitial diffusion, governs the delivery of Tc-NGA to the receptor. As a result, the Tc-NGA-HBP system provides an excellent opportunity to study the influence of radiopharmaceutical delivery (hepatic blood flow) upon the ability to observe changes in receptor biochemistry (HBP affinity and concentration).

FOOTNOTE

* Amicon; PM30; Danvers, MA.

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