Molecular Complexes of Thyroid Hormone Tyrosyl Rings with Aromatic Donors. Possible Relationship to Receptor Protein Interactions

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Several lines of evidence have indicated that thyroid hormones share common molecular properties (accessible planar face and lateral halogenation) with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds of environmental importance and can modulate their toxicity. Binding of dioxin to a soluble intracellular protein (dioxin or Ah receptor) appears to be the initial step in their mechanism of toxicity and a stacking interaction model has been proposed at the molecular level. It has also been recognized that the Ah receptor and the triiodothyronine nuclear receptor share certain physical and chemical properties important in their binding interactions. In this work, we examined the possibility that thyroid hormones might also be able to bind by a stacking complexation mechanism. By use of methods based on nuclear magnetic resonance spectroscopy, selected, structurally distinct thyroid hormone analogues with widely different hormonal activities were shown to function as electron acceptors in molecular complexes with aromatic donors involving the nonphenolic or tyrosyl ring. Binding free energies for these complexes correlated well with those previously reported for the triiodothyronine (L-T_3) nuclear receptor binding interaction with the same compounds. This included preference for L-T_3 over thyroxine (L-T_4), very favorable binding of 3,5,3′-triiodothyroacetic acid (Triac), and marked preference for L-T_3 over D-T_3. These results suggest that a considerable part of the structural specificity in thyroid hormone action may be mediated by the tyrosyl ring interaction. Binding ligands for the triiodothyronine nuclear receptor and the Ah receptor may share common molecular parameters in the expression of their binding activities.

Thyroid hormone receptors have been found in the nuclei of nearly all mammalian tissues, but are not found in certain lower species that are known not to respond to these hormones. A number of lines of evidence implicate a role for these binding proteins in thyroid hormone action.[1] Of importance is an excellent correlation between the binding of triiodothyronine (T_3), thyroxine (T_4), and a large number of other iodothyronine analogues to these receptors and the analogue's biological potency.

We have recently[2,3] pointed out that these T_3 receptors have physical properties in common with the Ah (dioxin) receptors. A theoretical binding model for Ah receptor–halogenated aromatic hydrocarbon interactions has recently been described.[4] The important element in the model appears to be the availability of an accessible planar face with highly polarizable groups attached, which can undergo a dispersion interaction with protein. Such a stacking model is reminiscent of those drawn for charge-transfer complexes, and polarization of the effector molecule by the electronic environment of the binding site would be relevant. Evidence that these halogenated aromatic hydrocarbons may act as electron acceptors in charge-transfer complexes with the Ah receptor has been provided.[5,6] In view of the possibility that the Ah receptor may be a thyroid hormone binding protein involved in regulating thyroid hormone action, it was of interest to investigate the potential of thyroid hormone analogues to function as electron acceptors in molecular complexes with donor aromatic systems. In addition, we hope to see if such a binding mechanism could account for the relative binding affinities of these analogues to known thyroid hormone binding proteins such as the T_3 nuclear receptor thought to be involved in regulating thyroid hormone activity. This result would provide additional evidence that the toxicity of TCDD and related compounds may be associated with their ability to regulate thyroid hormone activity.

The potential of thyroid hormone analogues to form charge-transfer complexes involving the substituted phenolate ion and electron acceptors was recognized some time ago,[7] but the physiological importance of such complexes remains unclear. It is known[8] that the conformation (skewed rings as in Figure 1) brought about by the 3,5-iodine substituents is characteristic of all active thyroid hormones and that active hormones are further characterized by the presence of polarizable groups in these positions. Thus, the nonphenolic (tyrosyl in the case of alanyl-substituted analogues) ring of thyroid hormones appeared to be suitable (sterically accessible planar face that is polarizable) for undergoing a stacking interaction of the type described for the Ah receptor interaction.[4] In the context of this hypothesis, it was recently recognized[9] that the nonphenolic ring protons in nuclear magnetic resonance (NMR) analysis are, in general, shifted downfield (deshielded) with respect to those from the phenolic ring protons, and the only protein side chain resonances appearing in this region of the spectrum are from histidine C-2 protons. Tryptophan and histidine contain the most effective donor side chains in charge-transfer complexes among the amino acids in proteins.[10] Since the nonphenolic ring protons in 3,5-substituted thyroid hormone analogues are sharp singlets in the spectrum, it was convenient to use this peak to monitor the potential for molecular interaction with donor molecules.

Six thyroid hormone analogues (Figure 1) that maintain the 3,5-diiodo substitution pattern in the nonphenolic ring were selected for study for consistency in conformational properties and ease of study by NMR. These six analogues can also be subdivided into two groups, one of which represents successive loss of iodine from the phenolic ring and the other representing simple carbon extension of the free acid side chain. In addition, two other analogues were

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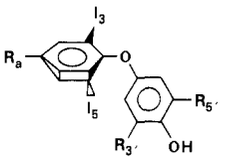

L-T_4 $R_{3'} = R_{5'} = I$; $R_a = -CH_2-CH(NH_3^{\oplus})-COO^{\ominus}$

L-T_3 $R_{3'} = I, R_{5'} = H$; $R_a = -CH_2-CH(NH_3^{\oplus})-COO^{\ominus}$

L-T_2 $R_{3'} = R_{5'} = H$; $R_a = -CH_2-CH(NH_3^{\oplus})-COO^{\ominus}$

Triform $R_{3'} = I, R_{5'} = H$; $R_a = -COOH$

Triac $R_{3'} = I, R_{5'} = H$; $R_a = -CH_2COOH$

Triprop $R_{3'} = I, R_{5'} = H$; $R_a = -CH_2CH_2COOH$

Figure 1. Structures for thyroid hormone analogues studied in this work.

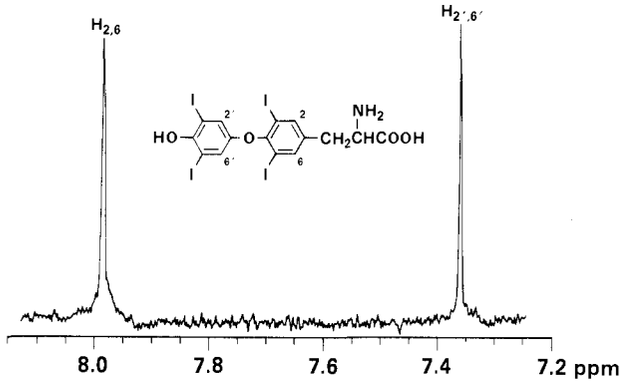

Figure 2. Proton NMR spectrum of the aromatic region of L-T_4 in acetone-d_6.

studied for their predictive value. D-3,5,3′-Triiodothyronine (D-T_3) represents a special case in which the reported[11] binding activity to the T_3 nuclear receptor is in disagreement with its known hormonal activity. The 3,5-dichloro-3′,5′-diiodothyronine (dichloro analogue of L-T_4) was studied as an example of an analogue for which hormonal activity has been reported,[12] but its binding activity with the T_3 nuclear receptor has not. These analogues also represent a wide range of hormonal activity and, therefore, should provide a good test of the possible importance of the nonphenolic ring in receptor binding and the expression of biological activity. Methylated benzenes were selected as donor molecules since their usefulness for this purpose and their varying donor strengths have been clearly demonstrated.[13] We recognize that compounds that contain structural elements characteristic of donor side chains in amino acids would be more relevant donors to study, but we felt that the methylated benzenes would be simpler donors to study initially to establish the importance of the π-donor–acceptor mechanism and its relationship to the T_3 nuclear binding interaction. The utility of NMR in the study of π-complex formation has been demonstrated[14] by the close agreement between results obtained by the NMR method and those obtained by other spectroscopic techniques.

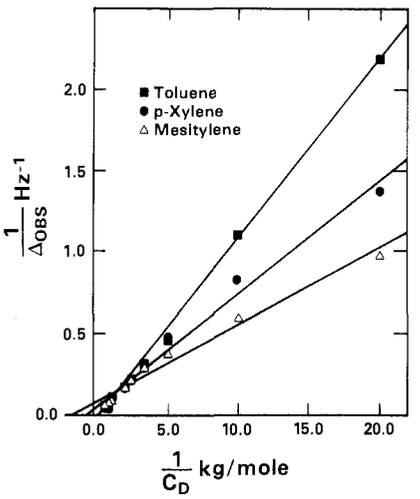

Figure 3. Plots of the reciprocal of the observed shifts of tyrosyl protons in L-T_4 versus the reciprocal of the concentration of three different donor aromatic compounds. The equilibrium constant (K) and ΔG for toluene and mesitylene are, respectively, 0.196 and 1.349 kg/mol and 0.96 and −0.18 kcal/mol. The p-xylene data are given in Table I.

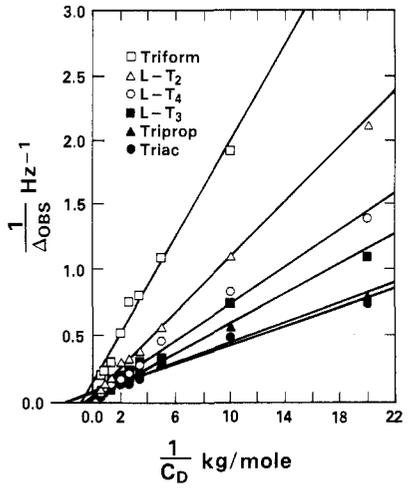

Figure 4. Plots of the reciprocal of the observed shifts of tyrosyl or nonphenolic ring protons in thyroid hormone analogues versus the reciprocal of the concentration of added p-xylene. The properties of these complexes are given in Table I.

Results

Figure 2 shows the aromatic region of a proton NMR spectrum of L-T_4 in acetone-d_6. Consistent with the results of others,[9] the lower field singlet at 7.984 ppm can be assigned to the tyrosyl protons, $H_{2,6}$, and the singlet at 7.359 ppm to the phenolic protons, $H_{2'6'}$. Addition of electron donor to the system was observed in all cases to shift the nonphenolic ring protons to higher field. Similar changes were seen for the phenolic protons but the shifts were significantly smaller (about half). The phenolic protons were observed to overlap with the peaks from the donor compounds and in most cases were multiplets. This made it difficult to follow changes in chemical shifts for these protons throughout the study.

Figure 3 shows linear plots of the reciprocal of the observed shifts of tyrosyl protons in L-T_4 versus the reciprocal of the concentration of three different donor molecules. The magnitude of the shift was seen to increase with increasing donor strength (mesitylene > p-xylene > toluene). Figure 4 shows linear plots of the reciprocal of the observed chemical shifts of nonphenolic ring protons in thyroid

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Table I. Properties of Complexes between Thyroid Hormone Analogues and *p*-Xylene Related to T_3 Nuclear Receptor Binding

compd[a]	Δ_{max} obsd,[b] Hz	$\Delta_{AD}{}^{A}$, Hz	equilibrium constant, K,[c] kg/mol	ΔG_{NMR},[d] kcal/mol	ΔG_{Rec},[e] kcal/mol
L-T_2	11.9	44.8	0.208	0.93	-8.24
DiCl-T_4	10.2	33.6	0.249	0.82	-9.09[f]
L-T_4	18.3	27.9	0.515	0.39	-11.23
Triform	5.0	7.7	0.700	0.21	-10.91
L-T_3	13.8	17.0	0.766	0.16	-12.38
Triac	20.4	20.1	1.379	-0.19	-12.99
Triprop	19.8	15.1	1.791	-0.34	-12.88

[a] Structures are shown in Figure 1. [b] From NMR measurements and used for determining K as described in the Experimental Section. [c] From linear regression analysis. [d] Calculated from K at 298 K. [e] Calculated free energy of binding (ΔG) from nuclear receptor binding study reported in ref 15 (pH 7.6, T = 298 K). [f] Predicted value from eq 3.

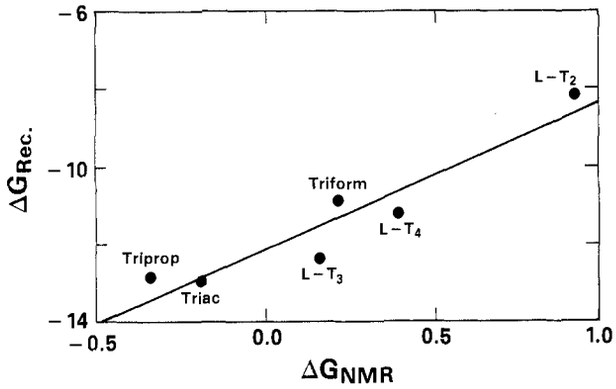

Figure 5. Plot of the binding free energies (ΔG_{NMR}) of *p*-xylene–thyroid hormone analogue complexes from this study versus the binding free energies (ΔG_{Rec}) of the receptor–thyroid hormone analogue interactions from the reference work.[15]

hormone derivatives versus the reciprocal of the concentration of the intermediate strength donor system, *p*-xylene. The properties of these various complexes are given in Table I.

The strongest binding was found for Triac and the weakest for Triform. Further extension of the side-chain length as in Triprop had little effect on binding activity. L-T_4 binding was intermediate for this range of compounds but somewhat weaker than L-T_3. The absence of both iodines in the phenolic ring (L-T_2) significantly lowers the complexing activity (relative to L-T_4 or L-T_3 with two or one iodines in the phenolic ring, respectively); however, measurable binding activity was maintained. The equation relating NMR experimental (this work) and receptor experimental[15] free energies is

$$\Delta G_{Rec} = 3.75\Delta G_{NMR} - 12.16 \quad (1)$$

with correlation coefficient = 0.895, standard deviation of fit = 0.647, and n = 6 (Figure 5).

The result with D-T_3 (K = 0.166 kg/mol, ΔG_{NMR} = 1.06 kcal/mol) is in interesting contrast to the much stronger binding of L-T_3. The dichloro analogue of L-T_4 also showed rather low (K = 0.249 kg/mol, ΔG_{NMR} = 0.823 kcal/mol) binding activity relative to L-T_4 itself.

Figure 6 shows a representative computer graphics model from molecular mechanics minimization of the interaction between T_3 and *p*-xylene. The amino acid side chain in T_3 has been replaced with an ethyl group to simplify the calculation. The minimization procedure was initialized with the molecules separated at distances substantially larger than those to which they minimized. The approximate separation distance between planes (nonphenolic and *p*-xylene rings) was 3.49 Å, which is close to the value of 3.54 Å reported[16] between the planes in layered aromatic hydrocarbons and the value of 3.50 Å reported[4] in our coplanar PCB–porphine interaction model for the Ah receptor. This result supports the steric and energetic accessibility of the planar face of the tyrosyl ring for a stacking interaction.

Discussion

The NMR method used in this work permits convenient monitoring of the extent of molecular interactions with simple spectral changes while enabling more precise characterization of the molecular nature of the interaction between donor and acceptor. These studies show that thyroid hormone analogues can function as electron acceptors in molecular complexes with donor molecules and that the stereochemically accessible nonphenolic ring is the preferred site for such interactions. Implicit in this model is a stacking type of geometry as might be expected in charge-transfer complexation although our model does not explicitly account for charge transfer. Figure 6 provides a stereoview of one possible mode of interaction between the planar faces of *p*-xylene and the nonphenolic ring of a thyroid hormone analogue based on energy minimization procedures. Although the charge-transfer complexing ability of thyroid hormones has been previously considered,[7] this has been mainly with regard to their potential to function as donor molecules involving the phenolic ring. However, the potential of thyroid hormones and polynuclear hydrocarbons to have large electron affinities has been recognized.[17]

Thyromimetic activity is directly related to the ability of the sterically large 3,5-substituents to constrain the diphenyl ether thyronine nucleus to the two approximately energetically equal, readily interconvertible, proximal and distal conformers.[8,18] Further inspection of the structures studied (Figure 1) in this work based on X-ray crystallographic measurements[8] shows that Triform, Triac, and Triprop tend to have the skewed diphenyl ether conformation whereas the iodothyronines are twist-skewed. While this difference could affect the electronic condition of the nonphenolic ring, both groups of compounds show similar [similar ϕ(swing) angle about diphenyl ether linkage] steric accessibility of their nonphenolic ring in a stacking interaction. In such a steric arrangement, the 3′- and 5′-positions are nonequivalent with respect to the alanine-bearing nonphenolic ring. This draws attention to the possible differences in steric accessibility of the nonphenolic ring in the resulting distal versus proximal conformers in T_3.

The possibility exists that the bulky 3,5-substituents, in addition to positioning the phenolic ring, together with the methylene group of the alanine side chain provide enhancement of the binding characteristics of the inner ring to some biologic receptor through their electronic contributions.[18] Because of the near collinearity of the hydrophobic property (π) and group size for 3,5-substituents, however, it has not been possible to rule out or confirm an intrinsic hydrophobic effect of 3,5-substituents. The results of this study show that the 3,5-substituents in addition to determining the conformational properties

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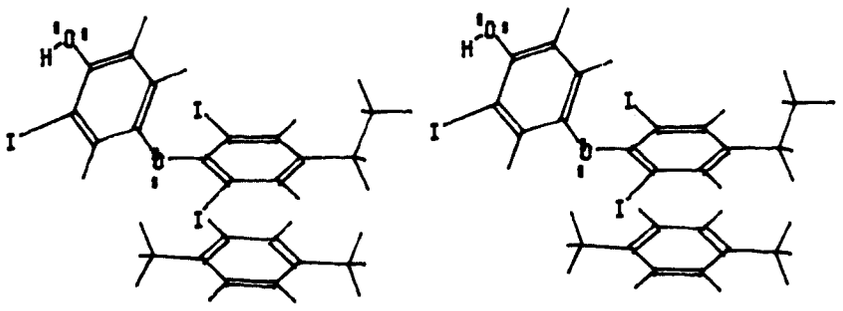

Figure 6. Computer-generated stereopair representation of the complex between L-T_3 and *p*-xylene based on molecular mechanics energy minimization as described in the Experimental Section. The side chain was replaced with an ethyl group to simplify the calculation (ΔE was −7.39 kcal/mol).

and accessibility of the planar face of the nonphenolic ring can also affect the electron-acceptor strength of the ring through electronic interactions [compare 3,5-diiodo (in T_4) and 3,5-dichloro-T_4 results]. It is evident that the nature of the phenolic ring and side chain flanking the nonphenolic ring is also important in determining the strength of the complex. There is also evidence of a complex interplay of all three of these structural components, viz. the phenolic and nonphenolic rings and side chains, in the expression of binding activity. Thus, a great deal of structural specificity is brought about through the nonphenolic ring interaction.

It is possible that this type of molecular interaction with thyroid hormones may play an important role in regulating their biological activities. There may be a need to reevaluate the relative importance of structural differences in the tyrosyl and phenolic rings of thyroid hormones in relation to their biological potency. Of particular interest in understanding the structural basis of thyroid hormone activity is that this binding model shows a preference for L-T_3 over L-T_4, which is characteristic of the T_3 nuclear receptor in tissue (L-T_4 binding activity is approximately 14% of L-T_3) and the approximately 3–5-fold greater biological potency of L-T_3 in vivo. In this model, it is not necessary to invoke a steric blockage of the additional phenolic ring iodine in order to explain the preference for T_3.[19,20] The additional iodine in T_4 may serve to weaken (intramolecularly) the electron acceptor ability of the tyrosyl ring through a buttressing (polarizing) effect on the adjacent ortho hydrogen atom, which lies beneath the plane of the tyrosyl ring (see Figure 1). Buttressing effects of iodine substituents on chemical reactivity in related systems have been previously noted.[21] This explanation is also consistent with the distal conformer (5′-iodine in Figure 1) of T_3 being the active form[18] since the proximal conformer (3′-iodine in Figure 1) would be similar to, if not identical (both have buttressed condition) with, T_4 in this regard.

Furthermore, this preference for T_3 is ostensibly stereospecific, showing a marked preference for the L optical isomer (over the D form). In separate and independent experiments, we repeated these measurements and obtained similar results (L-T_3, K = 0.650 kg/mol and ΔG_{NMR} = 0.26 kcal/mol; D-T_3, K = 0.137 kg/mole and ΔG_{NMR} = 1.18 kcal/mol). In addition, racemic T_3 (DL-T_3) gave an intermediate result (K = 0.584 kg/mol, ΔG_{NMR} = 0.32 kcal/mol). These results may again reflect the preference for equilibrium binding to the distal conformer[22] in which case the D-T_3 complex is apparently destablized (with respect to L-T_3) because of differences in stereochemistry of the alanine side chain. However, these interesting and somewhat surprising results deserve further study. If such a mechanism does operate, it would differ fundamentally from those that invoke a unique three-point attachment of the amino acid side chain to some biological receptor in order to explain the observed lower (6–7-fold) biological activity of the D form.[8]

The binding results with L-T_2 show that substitution of iodine in the 3′- and 5′-positions while increasing the free energy of binding somewhat appears to have no major effect on the ability of the analogue to form a molecular complex of this type. This result along with the results of the other analogues discussed above is consistent with previous thermodynamic analyses[23] of the T_3–nuclear receptor interactions, which indicate that the phenyl ring structure of the thyroid hormone molecule is primarily responsible for the hydrophobic binding and that halogens substituents can enhance this property.

With regard to affinity for the T_3 nuclear receptor, Triac has been shown[15] to bind with significantly greater affinity (283%) than L-T_3 (defined as 100%) with similar results for Triprop (235%). Triform, on the other hand, showed significantly less affinity (9%). Our measurements in this work are compatible with these results as shown in Table I. The greater acceptor ability of the nonphenolic ring in Triac and Triprop is remarkable and probably also a function of more favorable steric accessibility. However, in the absence of a saturated carbon link between the nonphenolic ring and the carboxyl group (as in Triform), the effect on binding is in the opposite direction. The low potency–binding ratio for compounds of this type appears to be related to the rapid metabolism and elimination rate of the compound in tissue.[24]

Thus, for these two structurally distinct subclasses (amino and desamino) of thyroid hormone analogues with wide differences in receptor binding activity, we find a good correlation (Figure 5) of the binding free energies for the *p*-xylene–nonphenolic ring model binding complexes with the binding free energies for the receptor–hormone analogue binding interactions. Further evidence that the tyrosyl ring may be important in the nuclear receptor binding event comes from examining the relative binding affinities of closely related thyroid hormone analogues.[25] For ex-

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ample, in 3′-*i*-Pr-3,5-T_2 replacement of the 3,5-iodine substituents with bromine and methyl groups is seen to result in a decrease in relative binding affinity from 1 (3,5-diiodo) to 0.36 (3,5-dibromo) to 0.007 (3,5-dimethyl), respectively. Similarly, in L-T_3 successive losses of iodine in the 3,5-positions to form *r*-T_3 and 3′,5′-T_2 are also seen to result in a decrease in a relative binding affinity from 1 to 0.002 to negligible binding activity. In the case of the 3′-*i*-Pr-3,5-T_2 analogues, the changes reflect presence of less polarizable atoms.[26] In the case of the L-T_3 analogues, the changes reflect both a loss in electron-acceptor capability and increases in conformational flexibility[8] due to the loss of one or both 3,5-iodine atoms.

As an additional test for the model, we examined the complexation properties of 3,5-dichloro-3′,5′-diiodothyronine (dichloro analogue of L-T_4) with *p*-xylene. On the basis of these measurements (see Table I), this thyroid hormone analogue is predicted to show a significantly poorer binding activity than L-T_4 itself to the nuclear receptor and would probably also have weaker hormonal activity. The hormonal activity of the dichloro analogue has been reported[12] to be about 100 times lower than L-T_4.

Although the binding activity of this compound was not reported in the reference work[15] used in this study, it has been reported[25] that 3,5-dichloro substitution is similar in effect on hormonal activity to 3,5-dimethyl substitution. The 3,5-dimethyl analogue of L-T_3 has a binding free energy of −9.28 kcal/mol,[15] in close agreement with our predictive value (−9.09 kcal/mol) for the 3,5-dichloro analogue of L-T_4. This somewhat more positive ΔG predicted for the L-T_4 analogue is also consistent with the pattern seen in comparing 3′-monosubstituted analogues with the corresponding 3′,5′-disubstituted analogues in general.[25] In preliminary studies,[27] we have also compared the nuclear receptor binding activities of Triac and biphenyl Triac (Triac without the ether oxygen bridge). The approximate K_d for Triac was $\sim 10^{-10}$ M whereas for biphenyl Triac it was $\sim 10^{-7}$ M. The much lower binding affinity for biphenyl Triac suggested by this result is also consistent with the stacking model proposed in this work since the ortho iodines[4] in the linear biphenyl structure hinder access to the planar face of the nonphenolic ring system because of the resulting twist conformation. This would also provide an explanation for the apparent lack[25] of hormonal activity of the biphenyl analogues in general.

In conclusion, the interaction model that we have developed here deserves further study to investigate its potential to serve as a simple experimental binding model for the nuclear T_3 receptor in tissue. Obviously, other factors must be considered in a full accounting of T_3 receptor interactions particularly with conformationally dissimilar analogues. The mode of interaction between the planar faces of *p*-xylene and the nonphenolic ring of a thyroid hormone analogue presented in Figure 6 may suggest an important interactive force in the binding of thyroid hormone analogues with the triiodothyronine nuclear receptor, but it must be viewed as an oversimplication of a complex binding interaction. Although more rigorous theoretical methods exist for calculating equilibrium geometries and interaction energies between aromatic systems,[28] the large size of these systems imposes a severe restriction on the type of approach that may be followed. In addition, we have previously[4] used molecular mechanics calculations to provide simple qualitative interaction models for TCDD and related compounds. Nevertheless, the model provides alternative and simpler explanations for the apparent nuclear receptor preference for L-T_3 over L-T_4, Triac over L-T_3, and possibly L-T_3 over D-T_3.

We pursued a reasonable approach based on interactions we have found[4] to be important in explaining the binding behavior of toxic halogenated aromatic compounds to Ah receptor preparations. A consistent picture has emerged from these studies that supports our proposal[29] that the Ah receptor may play a role in modulating thyroid hormone action. Several lines of evidence suggest that the thyroid hormone receptor and the Ah receptor may be identical or bear a close structural relationship. This includes a strong dependence of binding activity on hydrophobic properties[23,30] of the binding ligands and on dilution, temperature, and pH properties of the assay itself.[31,32] Also, tissue distribution[33,34] of the two receptors in the rat is similar, and the relative levels of receptors in the various tissues are consistent with the known thyroid hormone responsiveness of these tissues.[33] In addition, as supported by this work, theoretical models[4] that appear to account for structure–binding relationships are based on similar molecular parameters. Finally, certain toxic effects of both thyroid hormones and TCDD segregate with the Ah gene locus (for which the Ah receptor is the gene product) in cell culture systems.[35] Future studies should address the structural relationship of the Ah and T_3 receptors.

Further studies are also necessary to clearly establish the importance of the tyrosyl ring interaction in nuclear receptor binding of thyroid hormones. Of fundamental importance would be recognition that iodine is not directly required but facilitates binding by increasing the electron-acceptor strength of the tyrosyl ring through its polarization properties. This in turn would suggest that thyroid hormone binding proteins may play a role in mediating the toxicity and carcinogenicity of many halogenated and nonhalogenated, highly polarizable, aromatic compounds of environmental concern.

Experimental Section

Materials. L-Thyroxine (L-T_4), L-3,3′,5-triiodothyronine (L-T_3), D-3,3′,5-triiodothyronine (D-T_3), L-3,5-diiodothyroxine (L-T_2), and 3,3′,5-triiodothyropionic (Triprop) free acids were purchased from Sigma Chemical Co. (St. Louis, MO). 3,3′,5-Triiodothyroacetic (Triac) free acid was generated from the diethanolamine salt (from Sigma) by neutralization with HCl and extraction into ether. 3,3′,5-Triiodothyroformic (Triform) free acid was available from U.S. Biochemicals Corp. (Cleveland, OH). 3,5-Dichloro-3′,5′-diiodothyronine was available from Calbiochem-Behring (La Jolla, CA). Biphenyl Triac was a gift from Dr. M. Bolger, School of Pharmacy, University of Southern California, Los Angeles. Toluene, *p*-xylene, and mesitylene aromatic solvents were from Aldrich Chemical Co. (Milwaukee, WI), gold label 99% pure. Acetone-d_6 and tetramethylsilane were also from Aldrich. It is important that acetone-d_6 be free of aromatic impurities.

NMR Methods. All NMR samples were prepared in acetone-d_6 (99.5% purity) in a 5-mm NMR tube. An appropriate amount (nine concentrations between 0.05 and 1.4 mol/kg) of donor

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solvent was added, and the final volume was adjusted to 1 mL with acetone-d_6. All spectra were recorded on a GE QE-300 superconducting spectrometer operating at 300 MHz in the fourier transform mode. One pulse with presaturation mode for the solvent peak was used. For each measurement, the sweep width was 3 kH_z with 200 scans and 16K data size collected. Chemical shifts were reported in hertz downfield from the internal tetramethylsilane standard. Spectra were collected at ambient temperature (298 K).

Calculations. A modification of the Benesi-Hildebrand equation[36] for determining equilibrium constants for complex equilibria has been derived for NMR applications. By considering the chemical shift of protons on molecules undergoing rapid exchange between complexed and uncomplexed states and by following treatments used in NMR studies of hydrogen-bonding equilibria, the equation shown below may be derived[37,38] where

$$1/\Delta_{obsd}{}^{A} = 1/K\Delta_{AD}{}^{A}c_D + 1/\Delta_{AD}{}^{A} \quad (2)$$

$\Delta_{obsd}{}^{A}$ is the observed shift of acceptor protons in the complexing medium, $\Delta_{AD}{}^{A}$ is the shift of acceptor protons in the pure complex, c_D is the concentration of donor in moles per kilogram of solvent; K is the equilibrium constant. This equation requires that $c_D K(\Delta_{AD}{}^{A}/\Delta_{A}{}^{A}) \gg 1$. When $c_D \gg c_A$ (as used in this work), the shift of pure acceptor protons ($\Delta_{A}{}^{A}$) is very small relative to $\Delta_{AD}{}^{A}$; thus, for reasonable K values (≥ 0.1), this condition is usually met. It also assumes that solutions are ideal or that the quotient $\gamma_{AD}/(\gamma_A)(\gamma_D)$ remains constant over the range of solutions studied.

Equilibrium constants (K) were calculated from the NMR data by least-squares regression analysis. The correlation coefficients were analyzed for statistical significance as a test for the linearity of the plot. All coefficients were significantly different from zero, $P < 0.001$. The standard free energy (ΔG) was calculated from K by the equation

$$\Delta G = -RT(\ln K) \quad (3)$$

where R is the gas constant and T the absolute temperature.

The molecular mechanics program MODEL 1.3 kindly provided to the University of North Carolina, Chapel Hill, (UNC-CH) by W. Clark Still (Columbia University) was used to perform approximate force field calculations on representative complexes with a VAX780 computer. This program was modified in our laboratory to utilize a Tektronix 4107 color display terminal with graphics tablet input and the MM2p force field.[39] Parameters were estimated from Meyer et al.[40] A program for matching local coordinates was used for approximately locating the molecular planes of interest before MM2p optimization of the complex. The structure of p-xylene is based on standard geometries and that for the thyroid hormone is based on X-ray studies.[8]

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6-[^{18}F]Fluorometaraminol: A Radiotracer for in Vivo Mapping of Adrenergic Nerves of the Heart[1]

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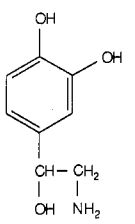
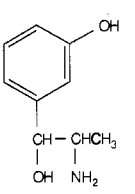
The false neurotransmitter metaraminol has been ^{18}F labeled and evaluated as a possible heart imaging agent on the basis of its selective accumulation in adrenergic nerves. Reaction of 6-(acetoxymercurio)-*N*-*t*-BOC-metaraminol with acetyl hypofluorite followed by removal of the BOC group provides a regiospecific synthesis of 6-fluorometaraminol (4). Use of acetyl hypo[^{18}F]fluorite gives [^{18}F]-4 in 60 min in 20–42% radiochemical yield. Systemic blockade of the neuronal uptake-1 carrier with desmethylimipramine or systemic destruction of the adrenergic nerves with 6-hydroxydopamine lowers [^{18}F]-4 accumulation ≥85% in all four regions of the rat heart. These preliminary findings suggest that [^{18}F]-4 could be used to assess neuronal damage in various heart diseases by positron emission tomography.

Metaraminol is a substitute adrenergic transmitter that stoichiometrically displaces norepinephrine (NE) from its storage sites within the neuron.[2] Like NE, metaraminol is transported into the adrenergic neuron by the uptake-1 carrier protein, sequestered within storage vesicles, and released by nerve impulse.[3] Metaraminol is less potent than NE in activating postsynaptic adrenergic receptors.[4] Although structurally similar to NE, metaraminol is metabolized by neither catechol-*O*-methyltransferase nor monoamine oxidase.[5] The metabolic stability of metar-



NOREPINEPHRINE



METARAMINOL

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