A Subtype-Selective Thyromimetic Designed to Bind a Mutant Thyroid Hormone Receptor Implicated in Resistance to Thyroid Hormone

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Two recent reports have demonstrated that small organic compounds (MW < 800) can act to restore function to forms of p53 and the human growth hormone receptor complex that are functionally impaired by specific genetic mutations.[1−2] These examples of "pharmacological rescue" of genetically impaired proteins suggest that it may be possible to design new drugs to recover activity from many proteins known to be mutated in a number of genetically based diseases. However, thus far, compounds used to restore function to proteins bearing natural mutations associated with human disease are of too low potency (micromolar activity) to act as practical therapeutics.[1] Mutations to the family of nuclear and steroid hormone receptors are implicated in a diverse set of genetic diseases.[3−4] In many cases these mutations have been shown to reside within or around the hormone-binding pocket of the receptor and disrupt normal transactivation function.[5−7] In this work we demonstrate that by using a known receptor agonists as a structural scaffold, potent (nanomolar active) hormone analogues can be rationally designed to complement a mutant form of the human thyroid hormone receptor beta (hTRβ) implicated in the genetic disease resistance to thyroid hormone (RTH) (Figure 1).

The thyroid hormone receptor (TR) functions as a ligand-dependent transcriptional regulator that controls the expression of a specific set of genes involved in development and homeostasis in response to triiodothyronine (T3).[8−9] There are two known TR subtypes: TRα which has been found in high concentration in skeletal muscle and brain and is closely linked to cardiac function, and TRβ which is undetectable in kidney and heart tissues.[10] Many RTH-associated mutations to TRβ are known to impair or abolish ligand-dependent transactivation function which can lead to a range of clinical presentations such as goiter, learning disabilities, impaired bone maturation, and mental retardation.[9,11] Although many mutant receptors show only reduced activity toward T3, clinical treatment of RTH with supraphysiological concentrations of T3 to recover TRβ activity can lead to over-stimulation of TRα which is implicated with undesirable side effects such as tachycardia and heart arhythmia.[12−14]

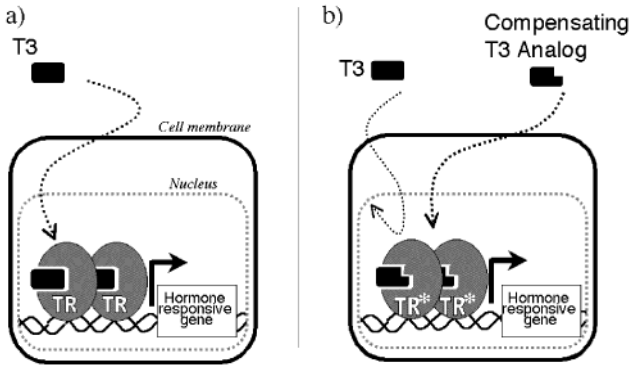

Figure 1. (a) Normally, T3 binds to the thyroid hormone receptor (TR) and regulates expression of specific genes. (b) Mutations to TR* prevent normal T3 binding; however, appropriate analogues can recover normal TR function.

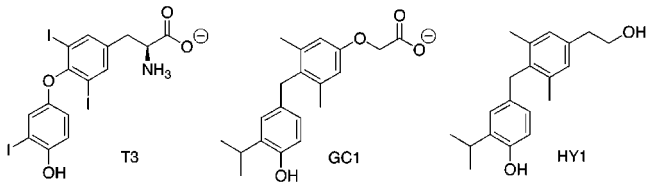

Figure 2. Thyroid hormone (T3) and analogues.

The RTH-associated mutation, TRβ(R320C) exhibits a reduced affinity for T3.[15−16] Ligand-dependent transactivation assays performed with HEK293 cells transiently transfected with mutant or "wild-type" (Wt) receptor, show that T3 is 7-fold less active with the mutant hTRβ(R320C) ($EC_{50} = 4.3 \pm 0.1$ nM) than the "wild-type" hTRβ(Wt) ($EC_{50} = 0.66 \pm 0.02$ nM).[17] Furthermore, concentrations of T3 required to significantly activate the mutant TRβ(R320C), impart an undesirable saturating response to TRα-mediated transactivation ($EC_{50} = 0.14 \pm 0.24$ nM) (Figure 3). Clinically, treatment of RTH patients harboring the hTRβ(R320C) mutation with supraphysiological levels of T3 was indeed observed to affect cardiac function in some patients.[16] Therefore, compounds having high affinity and selectivity for mutant forms of TRβ over the α-subtype are sought for RTH therapy.

In some instances thyroid hormone analogues (Figure 2) have been used in RTH therapy; however, their use is largely empirical, and in some instances treatment is also associated with cardiotoxicity.[18−20] The potent nonhalogenated thyromimetic GC1 is therefore of particular interest because it preferentially binds TRβ ($K_d = 67$ pM) over TRα ($K_d = 440$ pM).[12] However, GC1 shows a significantly reduced activity toward the mutant receptor TRβ-(R320C) ($EC_{50} = 37.7 \pm 10.8$ nM) than to the TRβ(Wt) (EC_{50} 3.67 ± 1.1 nM) in cultured cells and is therefore no longer selective for the *mutant* β-subtype over TRα(Wt) ($EC_{50} = 6.6 \pm 1.0$ nM)(Figures 3 and 5).

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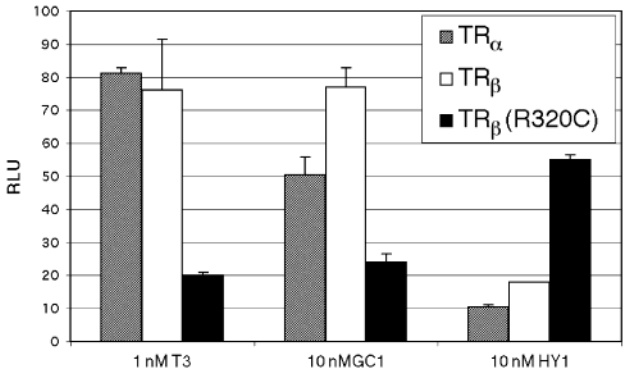

Figure 3. Luciferase reporter gene activity reported as relative light units (RLU) induced by various ligand−receptor pairs.

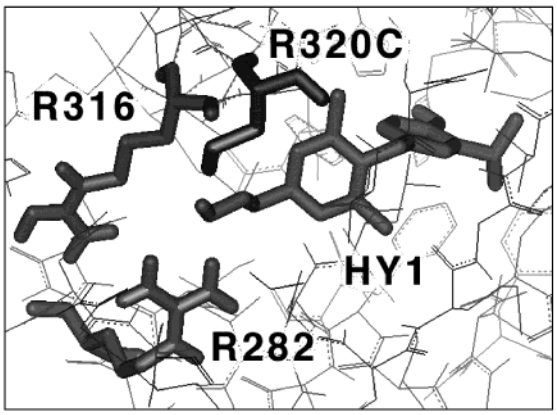

Figure 4. Model of HY1 bound to hTRβ(R320C) generated in Flo98/QXP based on the reported T3−TRβ cocrystal structure.

The TR(R320C) mutant is similar to mutations to charged (basic) residues of the retinoic acid receptor that we and others have shown to alter ligand-binding specificity to favor binding neutral ligands.[21−22] On the basis of site-models generated from the coordinates of the T3/TRβ crystal structure,[5] we designed the neutral alcohol HY1 as a potential subtype-selective ligand for the mutant receptor hTRβ(R320C) (Figures 2 and 4).[23] This ligand receptor design is chemically related to a study by Soprano et al., who showed that an artificial mutation in the retinoic acid receptor, hRARβ(R269A), binds the neutral ligand retinol in preference to its natural ligand retinoic acid,[22]

Assays of transactivation function show that HY1 (EC50 = 7.01 ± 3.0 nM) is 5-times more potent an agonist toward TRβ-(R320C) than the parent compound GC1, indicating that our designed ligand was indeed more potent than GC1 (Figure 5). Importantly, HY1 is also capable of eliciting substantial transactivation response from the mutant TRβ at concentrations that show only partial activation of TRα (EC_{50} = 37.69 ± 10.4 nM) and TRβ (EC_{50} = 32.05 ± 8.7 nM).(Figure 3). Although even greater levels of subtype-selectivity may be desirable, these data suggest that HY1 may have unique potential as a therapeutic capable of recovering activity from the mutant form of TRβ while potentially avoiding the undesirable side effects associated with TRα over stimulation.

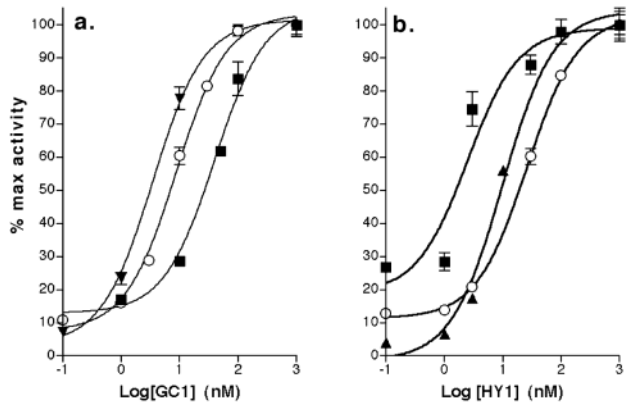

Figure 5. (a) GC1 shows selectivity for TRβ over TRα but is not selective for the RTH mutant TRβ·R320C). (b) HY1 has improved activity and selectivity for the mutant TRβ(R320C). TRα, (○); TRβ, (▼); TRβ-(*R*320C), (■).

This work demonstrates that by making compensatory modifications to known hormone agonists, new, highly potent ligands can be made which are selective for mutant receptors implicated in human disease. This paper focuses on one particular mutation associated with the relatively rare genetic disease RTH. However, many genetic diseases including Cushings disease (GR), rickets (VDR), androgen insensitivity syndrome (AR), adrenal hypoplasia congenita (DAX1),[4] as well as certain forms of diabetes (PPAR),[7] leukemia (RAR),[24] and prostate cancer (AR),[25] are also associated with mutations to hormone receptor ligand-binding domains.[26] Although in principle this general strategy may require a unique drug to be designed for each mutation associated with a particular disease, as demonstrated by this work on hTRβ and the related work of Soprano et al. on RAR,[22] similar design strategies may be used to complement structurally similar mutations in related receptors.

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Supporting Information Available: Synthesis and characterization of HY1, experimental details of cell-culture experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) GR, glucocorticoid receptor; VDR, vitamin D3 receptor; AR, androgen receptor; Dax1, an orphan receptor; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor.