Membrane Receptor for Thyroid Hormone: Physiologic and Pharmacologic Implications

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Annu. Rev. Pharmacol. Toxicol. 2011. 51:99–115

First published online as a Review in Advance on September 20, 2010

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

This article's doi: 10.1146/annurev-pharmtox-010510-100512

0362-1642/11/0210-0099$20.00

Keywords

tetrac, nanoparticulate tetrac, integrin αvβ3, cancer cell proliferation, angiogenesis, ion transport, protein trafficking, growth factors

Abstract

Plasma membrane integrin αvβ3 is a cell surface receptor for thyroid hormone at which nongenomic actions are initiated. L-thyroxine (T_4) and 3,3′,5-triiodo-L-thyronine (T_3) promote angiogenesis and tumor cell proliferation via the receptor. Tetraiodothyroacetic acid (tetrac), a deaminated T_4 derivative, blocks the nongenomic proliferative and proangiogenic actions of T_4 and T_3. Acting at the integrin independently of T_4 and T_3, tetrac and a novel nanoparticulate formulation of tetrac that acts exclusively at the cell surface have oncologically desirable antiproliferative actions on multiple tumor cell survival pathway genes. These agents also block the angiogenic activity of vascular growth factors. Volume and vascular support of xenografts of human pancreatic, kidney, lung, and breast cancers are downregulated by tetrac formulations. The integrin αvβ3 receptor site for thyroid hormone selectively regulates signal transduction pathways and distinguishes between unmodified tetrac and the nanoparticulate formulation. The receptor also mediates nongenomic thyroid hormone effects on plasma membrane ion transporters and on intracellular protein trafficking.

INTRODUCTION

A cell surface receptor for thyroid hormone was first described in 2005 and was linked to hormonal modulation of angiogenesis (1–3). The receptor was identified on integrin αvβ3, a structural protein of the plasma membrane of dividing endothelial and vascular smooth muscle cells. Because this integrin is also widely expressed on cancer cells, it was not surprising that thyroid hormone has also been found to cause proliferation of a variety of human cancer lines via the cell surface receptor (4–6). The receptor domain is complex (6) and is near the arginine-glycine-aspartate (RGD) recognition site on the integrin that is important to the latter's interactions with a variety of extracellular matrix proteins and growth factors (7). More than a decade ago, an RGD peptide was shown to interfere with the action of thyroid hormone on the interaction of laminin and integrins (8), and a small-molecule inhibitor of integrin αvβ3 was also shown to block the bone-resorbing effect of thyroid hormone (9). Both of these interesting observations implicated certain integrins in the molecular basis of thyroid hormone action at the cell surface and anticipated the identification of the receptor site for iodothyronines on integrin αvβ3.

Identification of an integrin receptor for thyroid hormone provides a molecular basis for certain nongenomic actions of the hormone. Such actions do not require the intranuclear binding of 3,3′,5-triiodo-L-thyronine (T_3) by nuclear thyroid hormone receptor (TR) isoforms that define the well-described and extensively studied genomic actions of the hormone (10, 11). Nongenomic effects of thyroid hormone that begin at the plasma membrane integrin receptor may lead to specific changes in gene transcription; therefore, the distinction between nongenomic and genomic actions lies not at the level of whether gene expression occurs but rather at the site of initiation of the action. Furthermore, other nongenomic actions of T_3 may apparently begin in cytoplasm and culminate in gene transcription (12, 13) and can involve TR isoforms that reside in the extranuclear compartment.

Nongenomic actions of thyroid hormone have also been described as "rapid-onset." This is not a useful descriptive term because such actions are usually demonstrated in the artificially defined, thyroid hormone–deprived environment to which hormone is then added; in the intact organism, however, thyroid hormone levels in tissues and blood are relatively constant. Rather than being rapid in onset, many nongenomic actions of thyroid hormone appear to contribute to basal levels of activity of a variety of proteins, including ion pumps [Ca^{2+}-ATPase (14), Na,K-ATPase (13), Na^+/H^+ antiporter (15)], and contribute to intracellular protein trafficking (16) and protein turnover (17). In the realms of both genomic and nongenomic actions, thyroid hormone contributes to rates of specific gene expression.

It is also necessary to point out that the thyroid hormone analog L-thyroxine (T_4), widely viewed in genomic mechanisms of hormone action as a prohormone antecedent to T_3 through tissue deiodinases (18), is biologically active at the integrin receptor. That is, T_4 is proangiogenic at physiologically relevant hormone concentrations (19, 20), can foster cancer cell proliferation (5, 21), causes platelets to aggregate (22), and can stimulate fibroblast migration toward a vitronectin cue (S.A. Mousa, H. Cui, P.J. Davis, and F.B. Davis, unpublished observations).

Thyroid hormone analogs in addition to T_4 and T_3 have been shown to act at the integrin. Other agonist analogs at the receptor are diiodothyropropionic acid (DITPA) (3) and the noniodinated analog GC-1 (2). Tetraiodothyroacetic acid (tetrac), the deaminated derivative of T_4, inhibits binding and actions of T_4 and T_3 at the integrin receptor (1), as does triac (23, 24), which is derived from T_3. In the absence of T_4 and T_3, as discussed below, tetrac has distinctive effects on angiogenesis, gene expression, and tumor cell proliferation that are initiated at the integrin. There are differences in structure among T_4, T_3, DITPA, GC-1, and tetrac (**Figure 1**). For their activities at the integrin, however, the hormone analogs share the following structural attributes:

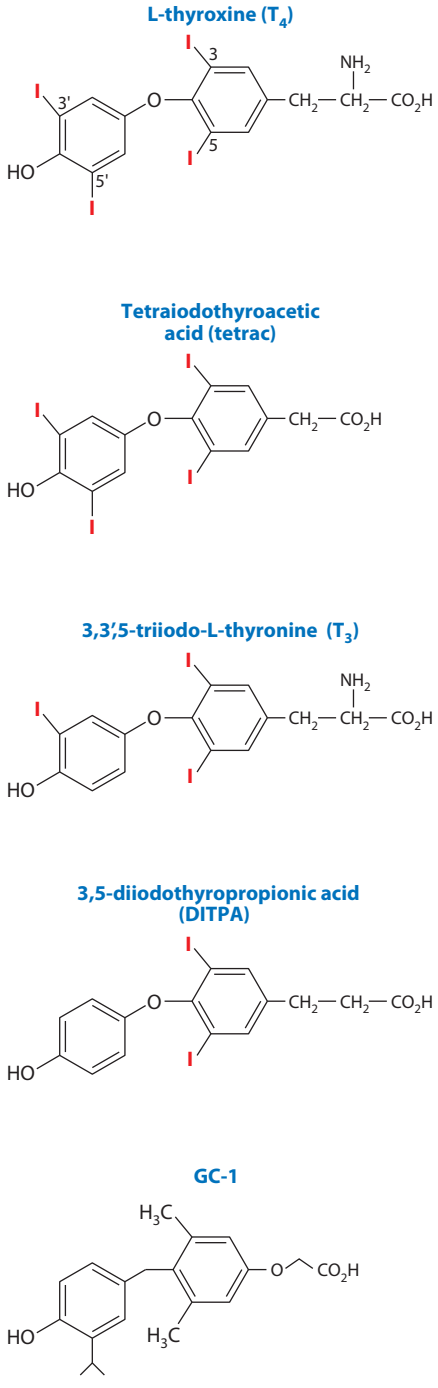


Figure 1

Structures of principal thyroid hormone analogs known to act at the cell surface receptor for iodothyronines on integrin $\alpha v\beta 3$: L-thyroxine (T_4) and 3,3′,5-triiodo-L-thyronine (T_3); tetraiodothyroacetic acid (tetrac), the deaminated derivative of T_4; diiodothyropropionic acid (DITPA); and GC-1. GC-1 lacks iodides but mimics the actions of agonist thyroid hormone analogs at the integrin in selected assay systems.

(*a*) a diphenyl structure, but not necessarily a diphenylether (i.e., GC-1 lacks the ether bond between the rings); (*b*) inner-ring bulky substituents at the 3 and 5 positions; (*c*) a side chain at position 1 of the inner ring that is of specific length and that terminates in a carboxyl group; and (*d*) an outer-ring hydroxyl at position 4′. Shortening the side chain on the inner ring—by removal of a carbon, e.g., conversion of propionic acid to acetic acid—converts agonism to antagonism. This is archetypically seen with the conversion of T_4 to tetrac. Nominally, this conversion may be interpreted as a result of the loss of the side-chain amine. However, DITPA is an agonist and has no amine, but it does retain a propionic-acid side chain. That bulky substituents on the outer ring are unnecessary for bioactivity is shown by GC-1 and DITPA.

This review is concerned with the biological and chemical activities of the receptor for thyroid hormone on integrin αvβ3 (25–27). Because the integrin engages in cross talk with adjacent growth factor receptors, because it can generate intracellular signals that lead to changes in expression of differentially regulable genes, and because it can alter the activities of a variety of plasma membrane ion pumps, the receptor has extensive, complex pharmacology. We have described tetrac as a specific antagonist for the integrin receptor, but inside the cell, this metabolite of T_4 is a thyroid hormone agonist (thyromimetic) (28). Covalent binding of tetrac to a nanoparticle that is excluded from the cell interior provides a probe for agonist thyroid hormone actions initiated at the integrin receptor—that is, tetrac is an antagonist that blocks the effects of T_4 and T_3 at the receptor. However, nanoparticulate tetrac has effects on gene expression that are distinct from those of unmodified tetrac in tumor cells (see Modulation of Growth of Human Tumor Xenografts via the Integrin Receptor for Thyroid Hormone, below). The pharmacology of iodothyronines at the cell surface also includes analogs such as DITPA (3) and the 3,5-methylated, rather than iodinated, synthetic thyroid hormone analog GC-1 (2) (**Figure 1**).

CHARACTERIZATION OF THE THYROID HORMONE RECEPTOR ON INTEGRIN αvβ3

The binding of radiolabeled thyroid hormone by integrin αvβ3 was initially demonstrated through the use of integrin purified from plasma membranes and the use of the recombinant protein (1) subjected to nondenaturing polyacrylamide gel electrophoresis (PAGE). Plasma membrane protein preparations revealed only a single protein capable of binding the labeled hormone, which was verified by immunoprecipitation to be αvβ3. Displacement studies revealed that the K_d for T_4 and T_3 are 10^{-10} M and 10^{-8} M free hormone concentration, respectively; tetrac at 10^{-7} M inhibited the binding of both analogs to the receptor. It was assumed that a single binding site that favored T_4 accounted for the binding of both hormones. Recent analyses of the pharmacokinetics and pharmacodynamics of T_4 and T_3 at the integrin receptor have revealed a more complex domain that contains two binding sites (**Figure 2**) (6). One of these sites binds T_3 exclusively and activates Src kinase and phosphatidylinositol 3-kinase (PI3K), specifically driving TRα from cytoplasm to the nucleus and promoting transcription of the *hypoxia-inducible factor 1α* (*HIF-1α*) gene. The latter is a participant in a cell survival mechanism for many cancer cells. The second receptor site (S2) binds both T_4 and T_3 and activates extracellular regulated kinase (ERK) 1/2; the latter transduces the hormone signals into cancer cell proliferation. This receptor and downstream pathways result in the importation of TRβ1 from cytoplasm into the nucleus. Unmodified tetrac blocks all actions at both sites. RGD peptide inhibits the T_3 site (S1) and T_4 action at the combined T_4/T_3 site but does not affect the action of T_3 on cell proliferation (6, 27).

Because an RGD peptide inhibited the actions of thyroid hormone analogs that were initially described at the integrin receptor, the latter was projected to be at or near the RGD recognition site on αvβ3 (1). Computer modeling of the site and the RGD groove formed by contributions

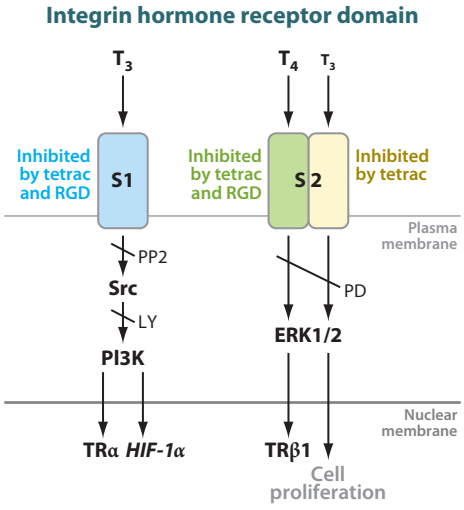


Figure 2

Schematic rendering of the thyroid hormone plasma membrane receptor domain on integrin αvβ3. The domain consists of two discrete binding sites with distinct downstream transduction pathways. Site 1 (S1) binds only 3,3′,5-triiodo-L-thyronine (L-T_3) and results in activation of the Src-PI3K pathway, in nuclear translocation of TRα resident in cytoplasm, and in induction of expression of the *hypoxia-inducible factor 1α* (*HIF-1α*) gene. S2 binds L-thyroxine (T_4) and, to a lesser extent, T_3, and it causes activation of the ERK1/2 cascade. This results in cancer cell proliferation, blood vessel cell proliferation, and nuclear translocation of cytoplasmic TRβ1. The two receptor sites for T_3 can be distinguished by the fact that the RGD peptide, which binds to integrin αvβ3, inhibits the actions of T_3 at S1 but does not inhibit the actions of T_3 at S2. These distinctions are thought to arise from allosteric differences in the binding characteristics of the two hormones. Abbreviations: ERK, extracellular regulated kinase; LY, LY-294002, a PI3K inhibitor; PD, PD-98059, an ERK1/2 activation inhibitor; PI3K, phosphatidylinositol 3-kinase; PP2, a Src kinase inhibitor; RGD, arginine-glycine-aspartate; TRβ1, thyroid hormone receptor β1. Reproduced from Reference 6 (HY Lin et al., *Am. J. Physiol. Cell. Physiol.* 296:C980–91, 2009), with permission of the publisher.

from the αv and β3 monomers (29) was carried out to estimate the fit of hormone analogs into the proposed site. Analysis of pharmacodynamic properties of the receptor site for thyroid hormone indicates that this site and the RGD recognition site are not identical (6). Furthermore, studies comparing the activities of unmodified tetrac and nanoparticulate tetrac at the receptor reveal increased potency of the nanoparticle and a pattern of gene transcription for these two molecules that is only 80% identical (30). The unmodified tetrac and nanoparticulate formulation of tetrac must therefore fit into the receptor pocket differently.

GENE TRANSCRIPTION MEDIATED BY THE INTEGRIN RECEPTOR IN CANCER CELLS: COMPARISON OF ACTIONS OF UNMODIFIED TETRAC AND NANOPARTICULATE TETRAC

Multiple cancer cell survival pathway genes are affected coherently by tetrac in an aggressive human breast cancer cell line (**Tables 1** and **2**). That is, proapoptotic genes are transcribed in response to tetrac, and expression of antiapoptotic genes is suppressed. In addition, the *thrombospondin 1* (*THBS1*) gene is transcribed in tetrac-treated cells, thus inhibiting angiogenesis. This gene is almost invariably suppressed in cancer cells. Whereas nanoparticulate tetrac has effects on gene transcription that are similar to those of unmodified tetrac, the nanoparticle formulation turns off

Table 1 Genes regulated by tetrac and nanoparticulate tetrac in MDA-MB-231 breast cancer cells

Gene	Function of gene product	Increase or decrease
THBS1	Thrombospondin 1, inhibits angiogenesis	↑
CASP2	Caspase 2, promotes apoptosis	↑[a]
BCL2L14	B cell lymphoma-2, promotes apoptosis	↑[a]
CXCL10	Anti–endothelial cell chemokine	↑
EDN1	Endothelin-1	↑
CBY1	Catenin inhibitor	↑
SOCS4	Suppressor of cytokine signaling	↑
CTNNA1	Catenin (Wnt oncogene pathway)	↓
CTNNA2	Catenin (Wnt oncogene pathway)	↓
Cyclins	Cell cycle regulators	↓
XIAP	X-linked inhibitor of apoptosis protein	↓
MCL1	Myeloid cell leukemia-1 factor	↓[a]
CTSL1	Cathepsin 1, recruits endothelial cells	↓
Interleukins	Inflammatory cytokines	↓
NR1D1	Nuclear receptor Rev-erba, (orphan nuclear receptor, regulator of circadian rhythm)	↓
EGFR	EGF[b] receptor	↓[a]

[a]Effect seen only with nanoparticulate tetrac (30).
[b]EGF, epidermal growth factor.

expression of the *EGF receptor* (*EGFR*) gene and differentially affects expression of several other genes (30). The EGF protein is commonly a growth factor for cancer cells, hence the development of the anti-EGF receptor antibody cetuximab (Erbitux®) as an anticancer agent (31, 32).

The results described above were obtained in cells deprived of T_4 and T_3 for several days before the exposure to unmodified and nanoparticulate formulations of tetrac. Thus at the integrin receptor, not only do the formulations inhibit certain actions of T_4 and T_3, but they also have distinctive actions of their own at the receptor that are transduced into patterns of gene transcription inimical to cancer cell survival.

Table 2 Genes regulated by tetrac and nanoparticulate tetrac in medullary thyroid carcinoma cells

Gene	Function of gene product	Increase or decrease
DFFA	Promotes apoptosis	↑
CASP2	Promotes apoptosis	↑
CASP8AP2	Promotes apoptosis	↑
CDKN2C	Cyclin-dependent kinase inhibitor	↑
THBS1	Thrombospondin 1, inhibits angiogenesis	↑
αE-Catenin	Supports metastatic potential of cells; resists induction of apoptosis	↓
VEGFA	Vascular endothelial growth factor	↓

From Reference 34.

MODULATION OF GROWTH OF HUMAN TUMOR XENOGRAFTS VIA THE INTEGRIN RECEPTOR FOR THYROID HORMONE

The genotypic findings obtained with tetrac led to a series of studies of xenografts of human solid tumors in the nude mouse. Tetrac and nanoparticulate tetrac (the latter at one-tenth the dose of unmodified tetrac) strongly inhibited the growth of human renal carcinoma cells (33), medullary carcinoma of the thyroid (34), follicular thyroid cancer cells (35), chemoresistant breast cancer cells (30, 36), and pancreatic cancer cells (37).

Importantly, treatment with tetrac (**Figure 3**) and nanoparticulate tetrac reduces the tumor cell component of the xenografts and also reduces the hemoglobin content, which is an index of vascularity of the tumors (**Figure 4**). The molecular basis for the reduction in tumor-associated neovascularization has several components. First, thyroid hormone analogs that are agonists at the integrin receptor are proangiogenic (1–3, 19), at least in part via the release by endothelial cells of basic fibroblast growth factor (bFGF, or FGF2) (19). Tetrac and nanoparticulate tetrac block this action of agonist thyroid hormone analogs such as T_4 and T_3 that are present in the intact, xenografted animal. Epidermal growth factor (EGF) is also proangiogenic (38), and thyroid hormone can modulate activity of EGF at the latter's receptor (39). We propose that tetrac inhibits the supportive effect of endogenous thyroid hormone analogs on neovascularization induced by EGF. Second, in the absence of thyroid hormone, tetrac blocks induction of neovascularization by vascular endothelial growth factor (VEGF) and bFGF (20). This action of tetrac was demonstrated in the chick chorioallantoic membrane (CAM) model of angiogenesis. Third, acting via the integrin receptor, tetrac modulates transcription of genes relevant to angiogenesis. These genes include

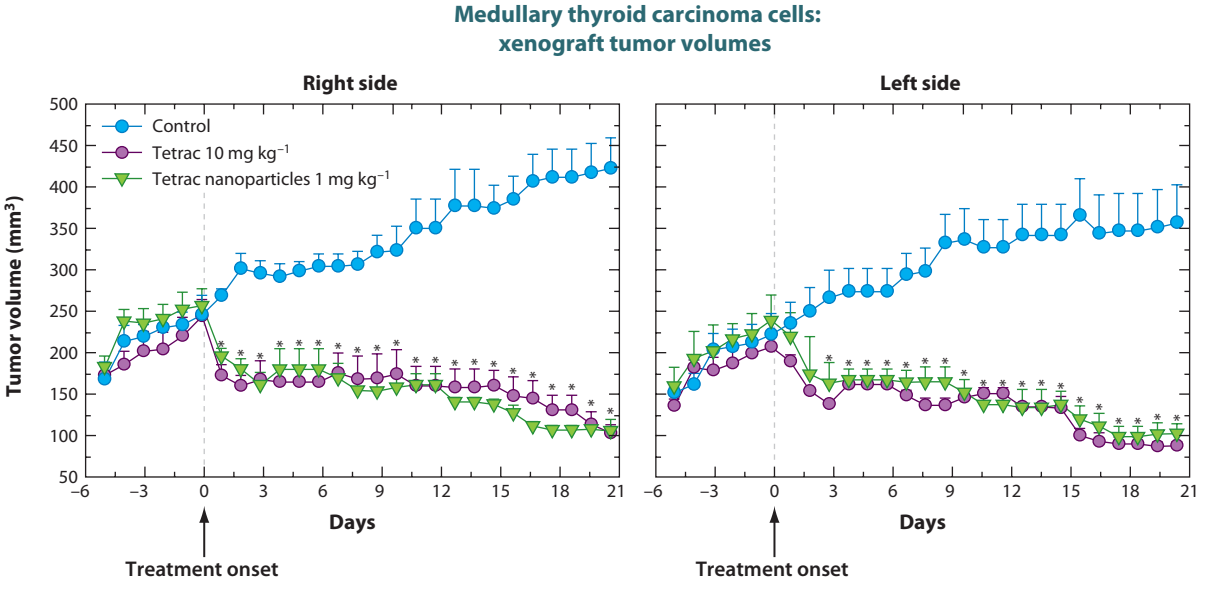


Figure 3

Effect of tetraiodothyroacetic acid (tetrac) and nanoparticulate tetrac on the growth of medullary thyroid carcinoma cell xenografts in the nude mouse. Treatment with tetrac (10 mg per kg daily, intraperitoneally) or tetrac nanoparticles (1 mg per kg daily, intraperitoneally) was begun after measurable growth was established in both flanks of each animal, and tumor volume was measured daily for 21 days. From days 1–3 onward, significant reductions in tumor volume occurred (*$P < 0.01$ for each treatment group when compared with the control group). Reproduced from Reference 34 (M Yalcin et al., *J. Clin. Endocrinol. Metab.* 95(4):1972–80, 2010), with permission of the publisher.

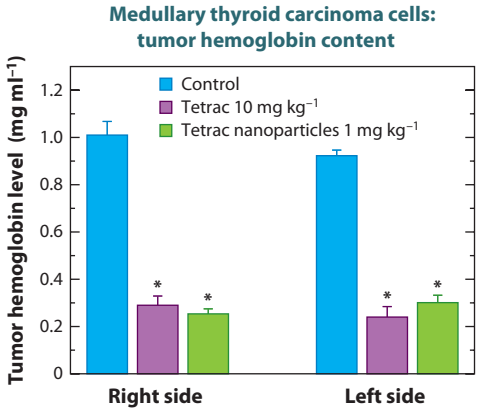


Figure 4

Decrease in xenograft hemoglobin content in medullary thyroid carcinoma cell murine xenografts in the presence of tetraiodothyroacetic acid (tetrac) or nanoparticulate tetrac. Hemoglobin content, an indication of vascularity in the xenografts, was significantly reduced in animals treated with tetrac or nanoparticulate tetrac ($P < 0.01$), when compared with the control animal group. Reproduced from Reference 34 (M Yalcin et al., *J. Clin. Endocrinol. Metab.* 95(4):1972–80, 2010), with permission of the publisher.

THBS1 (30), as mentioned above, and *VEGF-A* (30) (**Tables 1** and **2**). VEGF-A protein is an important inducer of tumor-related blood vessel support. *THBS1* expression is stimulated by tetrac, and *VEGF-A* expression is inhibited by tetrac formulations.

In the xenograft models studied to date, systemic administration of tetrac formulations has not induced intratumor hemorrhage. Thus the antiangiogenic action of the agents is, so far, not one that appears to disrupt the existing blood supply.

The routes by which tetrac formulations can be delivered to tumors are several. Systemic administration has been shown to be efficacious against xenografts (33–35). One can conjecture that stents coated with tetrac might be inserted into supply vessels for the treatment of primary tumors or multiple metastases. The blood-brain barrier is currently considered impermeable to the 200-nm tetrac formulations designed to preclude entry into cells by unmodified tetrac. Convection-enhanced delivery (40) can be used for delivery of nanoparticulate tetrac to brain tumors, and direct intratumoral administration of the product in brain is currently under study in an animal model of glioma (J. Palomo & M. Vogelbaum, unpublished observations).

We can conclude that the cell surface receptor for thyroid hormone mediates proliferative effects of circulating T_4 and T_3 on tumor cells and on tumor-associated blood vessels and that tetrac and nanoparticulate tetrac block these proliferative effects of the hormone. However, tetrac formulations have chemotherapeutic and antiangiogenic actions that transcend simple blockade of thyroid hormone effects. As described above, these chemotherapeutic actions are complex. They do, however, produce desirable effects on multiple cell survival pathways in tumor cells.

RETENTION OF DOXORUBICIN BY DOXORUBICIN-RESISTANT CANCER CELLS EXPOSED TO TETRAC

The basis for doxorubicin resistance in cancer cells is activity of the plasma membrane multidrug resistance [MDR, mediated by P-glycoprotein (P-gp)] pumps that export chemotherapeutic agents when such agents are taken up from extracellular fluid (41). Treatment of doxorubicin-resistant human breast cancer (MCF-7) cells with tetrac increases the intracellular retention time of

doxorubicin and renders the cells doxorubicin sensitive (36). The retention times of other chemotherapeutic agents exported by the P-gp may be similarly increased by tetrac formulations. The mechanism of this action of tetrac is not established but may relate to the action of the drug on the Na^+/H^+ antiporter and intracellular pH. That is, tetrac inhibits thyroid hormone–mediated support of Na^+/H^+ antiporter activity (24), thus promoting intracellular acidosis. The decrease in intracellular pH decreases activity of P-gp and may also inhibit transcription of the *P-glycoprotein* gene (42).

RADIOSENSITIZATION OF TUMOR CELLS BY TETRAC

Integrin $\alpha v\beta 3$ has been reported to modulate cancer cell responses to radiation (43). Hercbergs et al. (44) have recently shown that mouse glioma cells exposed briefly to micromolar concentrations of tetrac in vitro exhibit up to a threefold increase in radiosensitivity. There appears to be retention of cell "memory" of the transient exposure to the hormone analog for at least a week; that is, radiosensitivity is demonstrable seven days following a 1-h exposure, followed by washout of the agent. Nanoparticulate tetrac is also an effective radiosensitizing agent, as demonstrated in a human lung cancer cell line (A. Hercbergs, F.B. Davis, P.J. Davis, and J. Leith, unpublished observations). Such studies need to be extended to xenografts. The mechanism of this action of tetrac is not known.

INTEGRIN RECEPTOR–MEDIATED TISSUE ACTIONS OF THYROID HORMONE ANALOGS THAT ARE UNRELATED TO CANCER CELLS AND TO TUMOR-RELATED BLOOD VESSEL CELLS

Retinopathy

The integrin receptor for thyroid hormone is under consideration as a target in states of nonmalignant neovascularization, such as proliferative retinopathy. Tetrac has been tested in the hyperoxia model of induction of retinal vessel proliferation in the newborn mouse. This model is considered relevant to proliferative retinopathy; intraocular and systemic tetrac (S.A. Mousa, unpublished observations; D. Duh, unpublished observations) along with other integrin $\alpha v\beta 3$ antagonists (45) have been found to reduce blood vessel proliferation in the model. Interestingly, nanoparticulate tetrac administered intraperitoneally has been found to be effective in this system.

Stimulation of Angiogenesis

Prior sections of this review describe the antiangiogenic properties of the thyroid hormone analog tetrac in the context of tumor-related vasculature or certain retinopathies. However, the potent proangiogenic qualities of T_4 and T_3 may be desirable in chronically ischemic tissues. El Eter et al. have described, in a model of hind-limb ischemia in the rabbit, arterial budding and increased capillary/muscle fiber ratios in response to infused thyroid hormone (46). T_4 was used in these studies, although, as pointed out in a subsequent section (see Platelet Function, below), T_3 would be more desirable because it does not promote platelet aggregation.

An action of T_3 on wound healing has been described by Safer and colleagues (47) and attributed to effects of the hormone on keratinocytes (48). Although it seems likely that actions of the hormone on angiogenesis may also have contributed to the observed effects, this possibility has not been addressed in experimental models. Pharmacologically, observations such as these may encourage further studies of local or systemic administration of agonist thyroid hormone analogs to salvage damaged or hypoxic tissues.

Bone Cell Function

Integrin αvβ3 is highly expressed by osteoclasts (49). This suggests that the bone demineralization of hyperthyroidism may be attributable to the presence of this thyroid hormone integrin receptor on the cell surface of osteoclasts. Consistent with this suggestion is the ability of RGD peptides to oppose induction of osteoporosis by thyroid hormone in an animal model (9), despite the contention that suppression of endogenous thyrotropin in thyrotoxicosis underlies the associated osteoporosis (50). Interestingly, integrin αvβ3 has recently been described on human osteoblast-like cells and is proposed to mediate the ERK-dependent proliferative effect of thyroid hormone on such cells (51). If the predominant effect of normal circulating levels of thyroid hormone on bone is on osteoclasts, then combination therapy in the osteoporotic patient with tetrac, to oppose T_3- and T_4-induced osteoclastic activity, and parathyroid hormone (PTH) or recombinant PTH 1–34, to promote osteoblastic activity, seems to merit further study.

Platelet Function

The integrin is also represented on human platelets (52). Thus it is not surprising to observe that thyroid hormone stimulates platelet aggregation and platelet adenosine triphosphate (ATP) release (22). The receptor on platelets distinguishes between thyroid hormone analogs: T_4 is an agonist, whereas T_3 has no effect on platelet aggregation. If one is to consider testing the use of thyroid hormone as a proangiogenic agent in narrowed vessels, then T_3 is the agent of choice, whereas in the setting of wound healing, the proangiogenic and platelet aggregatory features of T_4 may be desirable. Recent epidemiologic data indicate that there is an increased risk of ischemic stroke in young adults with hyperthyroidism (53). It is conceivable that the action of T_4 on platelets is relevant to these clinical findings.

Integrin-Mediated Actions of Thyroid Hormone on Neurons

Yonkers & Ribera (54, 55) have described participation of the integrin receptor in the increase by T_4 in voltage-gated neuronal Na^+ currents; T_3 was inactive. The model for these studies was the sensory neuron of the zebrafish. Interestingly, the p38 isoform of mitogen-activated protein kinase—rather than ERK1/2—was implicated in transduction of the hormonal signal. In the intact organism, tetrac produced behavioral (touch-response) effects consistent with actions of thyroid hormone observed on these sensory neurons. In unpublished studies, M. Zhou and H.J. Cao at the Ordway Research Institute have extended these observations in sensory neurons to mouse brain slices and have shown tetrac-inhibitable shortening of latency of onset of Na^+ current and increased current amplitude in response to application of 10^{-7} M T_4. These data suggest that nongenomic effects of the hormone on Na^+ currents could contribute to the cognitive effects of the hormone (14) and raise the possibility that unmodified tetrac, crossing the blood-brain barrier, may affect cognition.

Other nongenomic effects of thyroid hormone on neurons, including neuroprotection, involve PI3K activation (56) and thus are consistent with an integrin-initiated mechanism (6). However, the possibility of involvement of the αvβ3 receptor has not been considered. Neuronal migration in brain also has been shown to be nongenomically directed by T_4 and not T_3, and to be linked to control of the state of actin in the nerve cell (57), but a role for the integrin receptor in this action has not been sought.

Immune Function and Thyroid Hormone

Potentiation by thyroid hormone of the antiviral action of interferon-γ (IFN-γ) (58) and of IFN's effect on HLA-DR expression (23) was shown to be tetrac- and triac-inhibitable before the integrin receptor for thyroid hormone was identified (1). However, these IFN-based observations—and the fact that IFN action could also be potentiated by T_4 that is covalently bound to agarose (23) and thus excluded from the cell interior—make it likely that such actions of the hormone are initiated at the cell surface receptor.

INTEGRIN RECEPTOR–MEDIATED CELLULAR ACTIONS OF THYROID HORMONE ANALOGS

Actions of Thyroid Hormone on Plasma Membrane Ion Transporters

That iodothyronines contribute to the set point of Na^+/H^+ antiporter (transporter) activity is mentioned above. Originally described in rat myoblasts by Incerpi and coworkers (59), this effect of T_3 was subsequently shown to originate at the cell surface (24). Triac inhibits stimulation of the transporter by T_3, implicating the integrin receptor in this action of the hormone. One function of this effect of thyroid hormone was shown to be enhancement of the ability of muscle cells to recover from an acid load (59).

Activity of the sodium pump Na,K-ATPase can be stimulated nongenomically by T_3. In studies conducted in alveolar cells, Lei and coworkers (13) described insertion of pump units into plasma membrane and increased ATPase activity with exposure of cells to T_3 in vitro. Activation of both ERK1/2 and PI3K was shown to be required for this effect (13). As noted above, the thyroid hormone integrin receptor that is activated by T_3 can lead to stimulation of these two kinases (6), whereas T_4 activates only ERK1/2. This is consistent with, but does not prove, the concept that the αvβ3 receptor initiates the action of T_3 on the sodium pump. The action of the hormone on Na,K-ATPase infers a contribution of circulating T_3 to a basal level of activity of the ATPase.

Intracellular Protein Trafficking

Activated from the cell surface by T_4 or T_3, cytoplasmic ERK1/2 translocates to the cell nucleus (60, 61), where it serine-phosphorylates a diverse group of nucleoproteins that include TRβ1 (16, 60), ERα (estrogen receptor α) (4), STAT1α (signal transducer and activator of transcription-1α) (23, 60), and the oncogene suppressor protein, p53 (61). In the case of each of these proteins, we have identified the specific function to which the phosphorylation is relevant. The transcriptional activities of TR (62) and ER (4) are increased by T_4-directed phosphorylation. STAT1α is involved in transducing signals of interferons, and this effect is modulated by thyroid hormone. Other intracellular proteins whose compartmental distributions are affected by thyroid hormone, in addition to the nuclear hormone receptor TRβ1, include TRα1 and Trp230. Yen and coworkers (63) and Cheng and colleagues (64) observed a decade ago that T_3 stimulated the transfer of TRβ1 from cytoplasm to the nucleus; the molecular mechanism for this was not shown. We have recently reported that this trafficking of TRβ1 in response to thyroid hormone originates at the integrin receptor (16) and appears to involve ERK1/2 that is also resident in cytoplasm and that forms complexes with TR when activated. It is also clear that T_3 can act at the integrin to cause TRα1 to move to the nucleus from cytoplasm (6). Trp230 is a coactivator protein important to thyroid hormone action in the nucleus. T_3 promotes the movement of Trp230 from the Golgi apparatus to the nucleus (65). It is not known whether the integrin receptor is involved in this action of

thyroid hormone. Because the levels of thyroid hormone available to cells are relatively stable, the effects of thyroid hormone on processes such as protein trafficking within cells are presumed to contribute to setting the rate of protein transfer.

Intracellular Disposition of Integrin αvβ3 in Thyroid Hormone–Treated Cells

The cellular uptake of integrin αvβ3 by endocytosis has been described to lead to perinuclear accumulation of the protein (66), and a process of return of the integrin to the plasma membrane (recycling) has also been reported (67). In thyroid hormone–treated, permeabilized cells containing fluorescently labeled antibodies to specific monomers, it was surprising to find on confocal microscopy and on cell fractionation that the αv monomer undergoes nuclear uptake, whereas the β3 monomer is apparently restricted to the cytoplasm (68). Furthermore, the αv monomer was associated in the nucleus with several coactivator proteins, including p300 (68). Tetrac blocks this action of agonist thyroid hormones. It has not been determined whether the nuclear uptake of the αv monomer modifies gene transcription rates. Certain infectious agents, e.g., West Nile virus (69), may bind to the heterodimeric integrin on the cell surface, and it will be important to determine whether the αv monomer is a vehicle for nuclear importation of such agents. Among the pharmacologic implications of such a possibility is transient interference with cell uptake of infectious agents—for example, after acute exposure—via the hormone receptor on αvβ3.

CONCLUSIONS

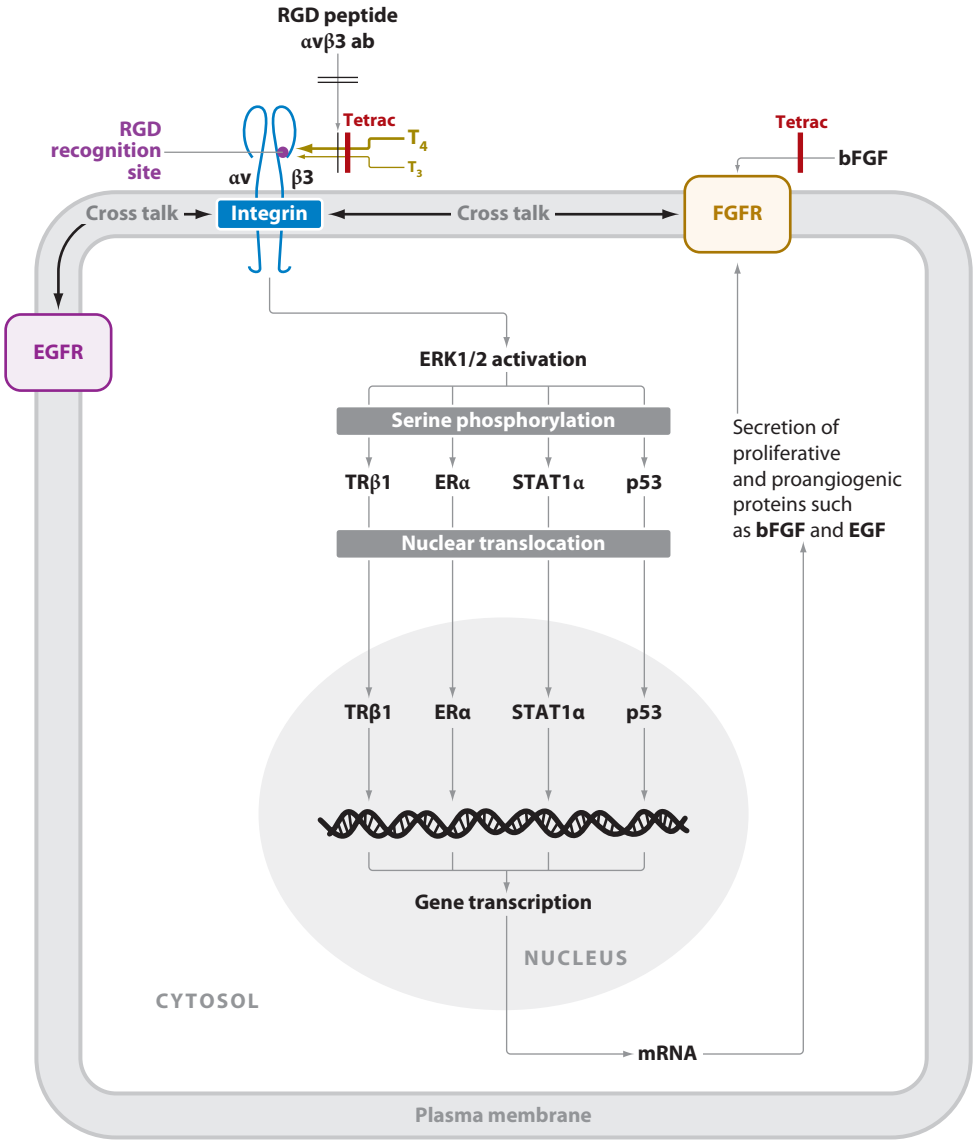
The discovery that a thyroid hormone integrin receptor exists on the plasma membrane of tumor cells and of dividing endothelial cells and vascular smooth muscle cells has permitted new insights into the physiologic actions of the hormone. Some of these actions are summarized in **Figure 5**. The receptor is on an integrin known to transduce a number of specific extracellular

Figure 5

Schematic depiction of the signaling pathways initiated by thyroid hormones and inhibited by tetraiodothyroacetic acid (tetrac) in cells bearing the integrin receptor for thyroid hormone analogs. These analogs also modulate the activities of certain growth factors via ("executive") cross talk of the thyroid hormone integrin receptor with plasma membrane receptors for growth factors such as vascular endothelial growth factor (VEGF) (20), basic fibroblast growth factor (bFGF) (20), and epidermal growth factor (EGF) (39) that are clustered with the integrin. Both L-thyroxine (T_4) and 3,3′,5-triiodo-L-thyronine (T_3) bind to integrin αvβ3. The downstream effects of such binding are universally inhibited by tetrac or nanoparticulate tetrac and only selectively inhibited by arginine-glycine-aspartate (RGD) peptide. Monoclonal antibody to αvβ3 also blocks agonist actions of T_4 and T_3 at the integrin receptor. When the integrin receptor transduces hormonal signals via the extracellular regulated kinase (ERK) 1/2 pathway, complex cellular events such as angiogenesis and tumor cell proliferation result. In addition, ERK activation via the thyroid hormone integrin receptors causes specific serine phosphorylation of certain cytosolic proteins such as TRβ1 (thyroid hormone receptor β1) (60), ERα (estrogen receptor α) (4), STAT1α (signal transducer and activator of transcription-1α) (23) and p53 (61). These phosphoproteins then translocate to the cell nucleus, where they promote transcription of specific genes whose gene products have diverse functions. Tetrac binds to the thyroid hormone integrin receptor and displaces agonist thyroid hormone analogs such as T_4 and T_3; in addition to inhibiting actions of T_4 and T_3 at the complex integrin receptor site depicted in **Figure 2**, tetrac is also capable via the receptor of inhibiting proangiogenic actions of vascular growth factors (20) at their specific receptors in either the absence or presence of thyroid hormone. This presumptively occurs via receptor cross talk mentioned above. The existence of cooperative relationships between integrin αvβ3 and the VEGF receptor (70) and the EGF receptor (71) has been described by others. Abbreviations: ab, antibody; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; mRNA, messenger ribonucleic acid.

matrix protein signals, to engage in cross talk with growth factor receptors clustered with the integrin, and to influence specific gene expression. Not surprisingly, the location of the receptor on the integrin and the complexity of the receptor domain allow thyroid hormone analogs to modulate a number of cellular activities. These activities include proliferation of cancer cells and cancer-relevant angiogenesis, differential regulation of genes related to cancer cell survival pathways, plasma membrane transporter activities, and cell radiosensitivity.

Pharmacologically, the thyroid hormone analog receptor on integrin αvβ3 offers potentially useful pathways for clinical management of cancer cell proliferation, as xenograft studies have recently suggested, and also of nonmalignant syndromes that are vascular growth factor–dependent, such as certain skin disorders or proliferation of eye blood vessels. The cancer-relevant assets of a particular antagonist thyroid hormone analog, tetrac, at the receptor include induction of



apoptosis, opposition of intrinsic antiapoptotic mechanisms, and inhibition of the angiogenic activities of several vascular growth factors, including VEGF and bFGF.

We have reformulated tetrac—and certain other hormone analogs—as nanoparticles. Tetrac is a low-grade thyromimetic within the cell but acts exclusively as an antagonist at the "integrin receptor." One of the distinctive features of the formulation is that tetrac is covalently bound to the exterior of the biodegradable particle in a design that permits insertion of the hormone analog into the integrin receptor for thyroid hormone and allows specific modulation of the behavior of the receptor. Thus nanoparticulate tetrac does not gain access to the cell interior; rather, it acts exclusively at the integrin and hence avoids promoting a number of traditional thyromimetic actions. Furthermore, the formulation results in increased potency of the compounds and in effects on specific gene expression that sometimes differ—and desirably so—from those of unmodified tetrac. Nanoparticulate thyroid hormone analogs are a new family of agents that, in the case of tetrac, show promise in tumor management. In the case of T_4 and T_3, the nanoparticulate compounds may be useful in non-oncologic settings, such as the promotion of angiogenesis in settings of ischemia.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Annual Review of Pharmacology and Toxicology

Volume 51, 2011

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