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Interaction of Amiodarone and Desethylamiodarone With Solubilized Nuclear Thyroid Hormone Receptors

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The mechanisms of action of the potent antiarrhythmic drug amiodarone are unknown. However, amiodarone and its abundant metabolite, desethylamiodarone, bear a striking structural resemblance to thyroid hormones. In addition, certain cardiac electrophysiologic effects of amiodarone treatment are similar to those of hypothyroidism. These facts suggest that amiodarone or desethylamiodarone could be acting, in part, by blocking thyroid hormone action. Because thyroid hormones are known to act through nuclear receptor proteins, the binding of amiodarone and desethylamiodarone was measured to nuclear extracts derived from human lymphocytes, bovine atrium and ventricle and rat liver.

The capacity of increasing concentrations of amiodarone and desethylamiodarone nuclear extracts to block receptor binding of radiolabeled triiodothyronine (T_3) in a standard in vitro competition assay was tested. Nuclear extracts demonstrated only minimal binding to amiodarone. However, all receptor preparations had substantial affinities (K_D) for the desethyl analog: lymphocyte, 8.6 μM; atrium, 35.0 μM; ventricle, 26.9 μM and liver, 8.6 μM. Desethylamiodarone accumulates in very large quantities in parenchymatous organs during long-term amiodarone treatment. Taking its usual therapeutic serum level (about 4 μM or 2.7 $\mu g/ml$) as an estimate of intranuclear concentration, desethylamiodarone would partially saturate nuclear thyroid hormone receptors in several different tissues, including the heart.

Thus, amiodarone treatment may exert some of its electrophysiologic effects by metabolic conversion to desethylamiodarone. This metabolite may then exclude thyroid hormone from nuclear receptor sites within the myocardium.

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Amiodarone is an important antiarrhythmic agent whose mechanism of action is not yet fully understood. Current information suggests that amiodarone may act through its influence on nuclear receptors for thyroid hormone in target tissues (1). Amiodarone and its abundant metabolite, desethylamiodarone, are structurally similar to the physiologically active iodothyronines, thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) (Fig. 1). Furthermore, long-term amio-

darone therapy is associated with prolongation in duration of cardiac action potential and electrical refractoriness (2,3), changes that may figure significantly in its antiarrhythmic efficacy. These effects resemble electrophysiologic changes occurring with hypothyroidism (4); they are prevented by concomitant administration of thyroid hormone (2).

These considerations have led to the concept that amiodarone therapy may modulate the interaction of thyroid hormone and its receptors in myocardium and other tissues. However, no experimental data have been presented to evaluate a potential influence of amiodarone and its metabolites on binding of thyroid hormone to tissue receptors. Such data may be valuable in clarifying the mechanism behind the metabolic alterations associated with long-term amiodarone therapy. We therefore examined the capacity of amiodarone and desethylamiodarone to compete for the binding of T_3 to specific nuclear thyroid hormone receptors in rat liver, bovine heart and human lymphocytes. Rat liver was utilized because nuclear thyroid hormone receptors from this tissue have been the prototype in numerous studies of the binding properties of thyroid hormone analogs. Bovine heart provided an abundance of fresh tissue from each animal for studies of myocardial nuclear thyroid hormone receptors.

Amiodarone

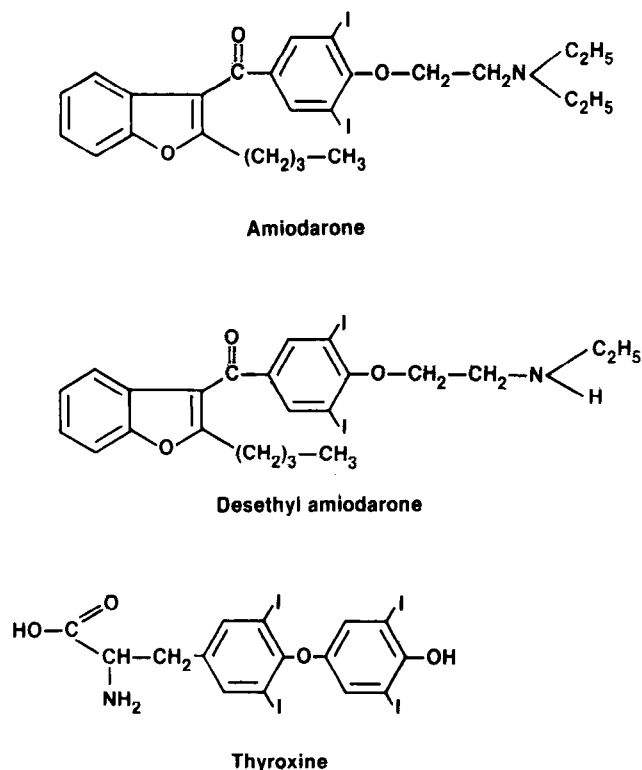**Desethyl amiodarone**

Thyroxine

Figure 1. Structural formulas for amiodarone, desethylamiodarone and thyroxine. Each molecule contains at least one diiodinated aromatic ring and an ether linkage. Steric considerations suggest that the optimal space-filling match occurs when the iodinated aromatic ring of amiodarone or desethylamiodarone corresponds with the phenolic iodinated (**rightward**) ring of thyroxine.

Lymphocytes furnished the only readily accessible source for nuclear thyroid hormone receptors in humans.

Methods

Reagents. High specific activity (^{125}I)3,5,3'-triiodo-L-thyronine (^{125}IT$_3$, 1,200 μCi/μg) was obtained from New England Nuclear. Unlabeled triiodothyronine (T$_3$) and thyroxine (T$_4$) were purchased from Sigma Chemical Company. Chromatographically pure reverse T$_3$ was purchased from Calbiochem-Behring. The unlabeled thyroid hormones (T$_4$, T$_3$, reverse T$_3$) were checked for purity by high performance liquid chromatography as described previously (5). T$_3$ and reverse T$_3$ contained less than 0.5% contaminants (measured as optical density at wavelength of 280 nm) and were used unpurified. Thyroxine intermittently contained trace amounts of 3,5-T$_2$ and T$_3$ and was purified when necessary (6).

Pure powdered amiodarone and desethylamiodarone were supplied by Sanofi Laboratories and were found to be homogeneous by high performance liquid chromatography (7). Amiodarone and desethylamiodarone contained undetectable amounts of the opposite analog at detector sensitivities

that could identify 0.1% contaminant. High performance liquid chromatography also confirmed the solubility and stability of amiodarone and desethylamiodarone in dilute ethanol solutions. Stock solutions of amiodarone or desethylamiodarone, 10 mM in pure ethanol, were diluted directly into assay buffer just before use. Assay preparations containing various concentrations of either drug remained clear and showed no evidence of precipitation during the course of the experiments.
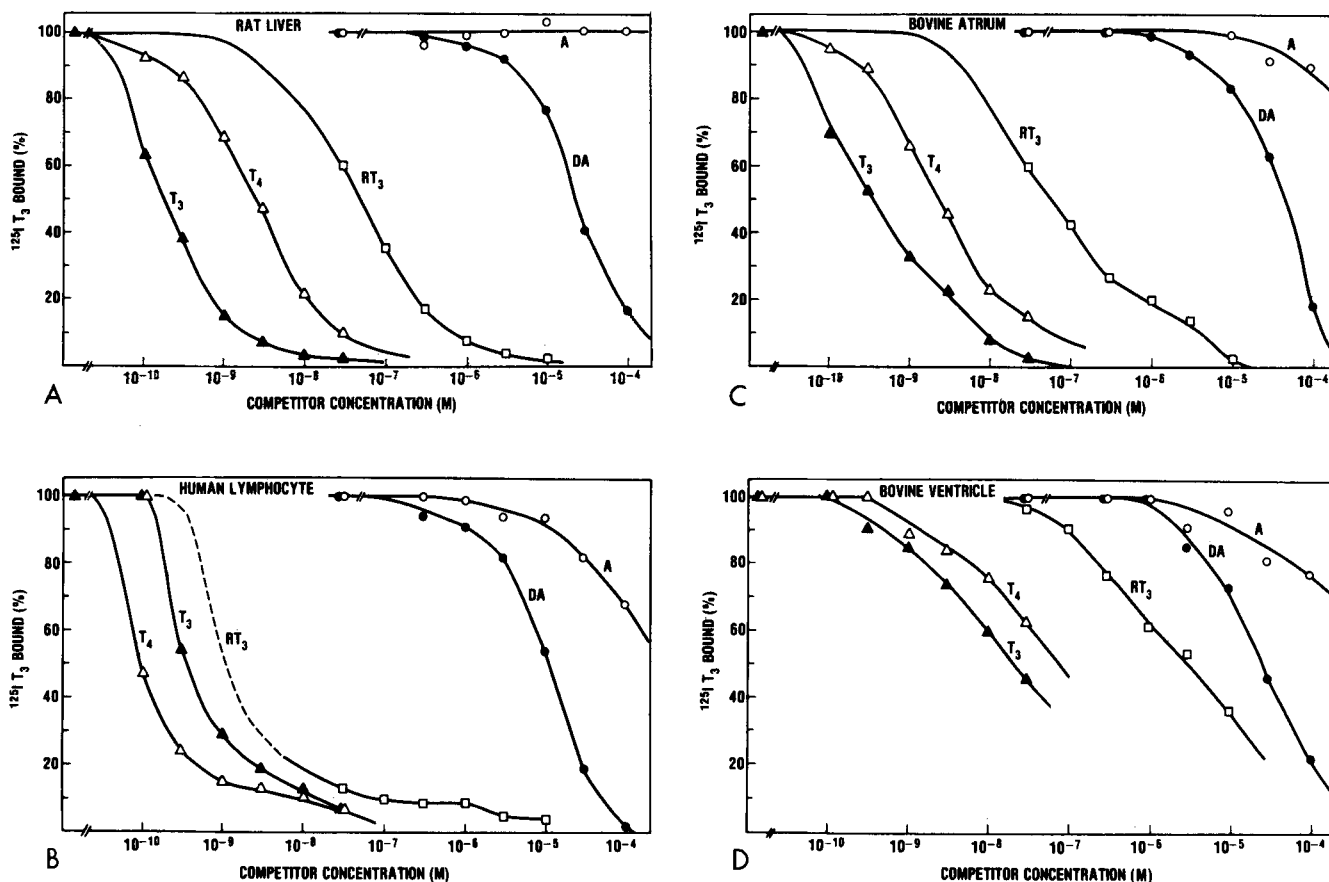
Receptor preparation. Solubilized nuclear thyroid hormone receptors from rat liver, bovine atrium and bovine ventricle were prepared as described previously (6). Briefly, 100 g of fresh tissue (rat liver, bovine ventricle or bovine atrial appendage) was homogenized in 300 ml of warm (37°C) 2.1 M sucrose solution containing 5.0 mM magnesium chloride and 0.1 mM phenylmethylsulfonylfluoride. Nuclei were isolated from filtered homogenate by pelleting through cold 2.1 M sucrose (25,000 $\times$ g for 2 h). Nuclei were washed in buffer (30 mM Tris, pH 7.6, 2 mM calcium chloride, 1.1 mM magnesium chloride) containing 0.5% Triton X-100, then extracted in either 40 ml (liver) or 20 ml (atrium and ventricle) of buffer containing 0.2 M (NH$_4$)$_2$SO$_4$. The insoluble fraction was removed by centrifugation (50,000 $\times$ g for 2 h) and the supernatant fraction, containing thyroid hormone binding activity, was stored in liquid nitrogen. Chromatin extracts were also prepared from lymphocytes derived from euthyroid human donors as described previously (8).

Hormone-binding studies. The relative receptor binding of each test compound was measured by binding site competition between ^{125}IT$_3$ and different concentrations of each test compound: reverse T$_3$ (30 nM to 10 $\mu$$M$), amiodarone and desethylamiodarone (0.3 $\mu$$M$ to 0.1 mM) and T$_3$ and T$_4$ (0.1 nM to 30 nM). Hormone binding reactions contained 0.1 nM ^{125}IT$_3$ in a total reaction volume of 0.5 ml. For saturation analysis, bound hormone was separated from free hormone on Sephadex G-25 columns (2.0 ml bed volume) as described previously (6). Binding site competition between ^{125}IT$_3$ and nonradioactive T$_3$ or T$_4$ was not influenced by the presence of maximal concentrations of the solvent for amiodarone and desethylamiodarone (ethanol, 1% in assay buffer).

Experimental conditions. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council DHEW Pub. No. (NIH) 74-23.

Results

Binding to nuclear thyroid hormone receptors from rat liver (Table 1, Fig. 2A). Liver nuclear extract bound triiodothyronine (T$_3$) best (equilibrium dissociation constant K$_D$ = 0.05 nM) with lower affinities for thyroxine (T$_4$) (K$_D$ = 0.89 nM) and reverse T$_3$ (K$_D$ = 16.9 nM). These results

Figure 2. Percent binding of a radiolabeled 3,5,3' triiodo-L-thyronine ($^{125}IT_3$) to solubilized nuclear thyroid hormone receptors plotted as a function of competitor concentration (**horizontal logarithmic axis**). Nuclear thyroid hormone receptors were obtained from rat liver (**A**), human lymphocytes (**B**), bovine atrium (**C**) and bovine ventricle (**D**). Potency of inhibition of radioligand binding indicates the relative affinity of each competitor for nuclear thyroid hormone receptors, with greater potency indicated by a more leftward displacement of the competition curve. The relative analog affinities were the same for receptors derived from liver, atrium and ventricle: $T_3 > T_4 >$ reverse T_3 (RT_3) > desethylamiodarone (DA) > amiodarone (A). Human lymphocyte receptors showed their characteristic higher affinity for T_4 relative to T_3. Amiodarone (**open circles**) showed only a minimal competition for $^{125}IT_3$ binding, indicating little or no affinity for nuclear thyroid hormone receptors isolated from these tissues. The **dashed line** in **B** shows a theoretical extrapolation of the reverse T_3 results.

are consistent with previous reports (6). Desethylamiodarone demonstrated active competition for T_3 binding to the receptor but with a lower affinity than T_3, T_4 or reverse T_3. Amiodarone did not show any competition in the concentration range tested. Thus, the desethylated analog of amiodarone bound to the rat liver nuclear receptor ($K_D = 8.6 \mu M$) whereas amiodarone did not bind.

Binding to human lymphocyte chromatin extracts (Table 1, Fig. 2B). Consistent with previous findings, we observed abundant high affinity T_3 ($K_D = 1.2$ nM) and T_4 binding ($K_D = 0.8$ nM). Reverse T_3 had a higher affinity in this mixed binding system than in rat liver (K_D estimated: 0.9 nM). Desethylamiodarone again showed active binding to this mixed receptor system ($K_D = 8.6 \mu M$. Amiodarone also showed modest displacement of $^{125}IT_3$, a result that was not consistent with the findings in rat liver. However, the apparent affinity of amiodarone for nuclear thyroid hormone receptor was much less than that of desethylamiodarone or any of the thyroid hormones. It is improbable that this observed amiodarone binding was due to contamination of the amiodarone with the desethylated analog because nuclear thyroid hormone receptors derived from rat liver did not show a similar amiodarone displacement and our liquid chromatographic studies measuring the purity of the crystalline compounds indicated no contamination of the amiodarone with desethylamiodarone.

Binding to nuclear thyroid hormone receptors from bovine atrium and ventricle (Table 1, Fig. 2C and 2D). Relative to the ventricle, atrium contained four to five times as much T_3 binding activity on a tissue wet-weight basis. For atrium, the order and affinities of hormone binding were very similar to values obtained from rat liver (T_3 $K_D = 0.3$ nM; T_4 $K_D = 1.7$ nM; reverse T_3 $K_D = 39.8$ nM). Desethylamiodarone again showed full $^{125}IT_3$ displacement ($K_D = 35.0 \mu M$) with only slight $^{125}IT_3$ displacement by amiodarone (estimated $K_D = 220 \mu M$).

Table 1. Receptor Affinity for T_3, T_4, Reverse T_3 and Desethylamiodarone

Receptor Source	Equilibrium Dissociation Constant (K_D)			
	T_3 (nM)	T_4 (nM)*	Reverse T_3 (nM)*	DA (μM)*
Rat liver	0.05	0.89	16.9	8.6
Human lymphocyte	0.36	0.08	0.9	8.6
				220†
Bovine atrium	0.30	1.7	39.8	35.0
Bovine ventricle	10.1	79	3,300	26.9

*$K_D = [(C_{50}) (K_D T_3)]/[(K_D T_3 + {}^{125}IT_3)]$, where C_{50} = molar concentration of competitor at 50% inhibition of T_3 binding $K_D T_3$ is the K_D of T_3 binding; ${}^{125}IT_3$ = the molar concentration of the radiolabeled T_3 (0.1 nM in these studies). †K_D for amiodarone binding to receptors from human lymphocytes. DA = desethylamiodarone.

Although material obtained from the ventricle showed less T_3 binding activity and lower affinity binding (T_3 K_D = 10.1 nM; T_4 K_D = 79 nM; reverse T_3 K_D = 3.3 μM) than the atrium, the order of hormone preference was the same in ventricle, atrium and rat liver. As in the atrium, desethylamiodarone showed active receptor binding (K_D = 26.9 μM); amiodarone exhibited a small amount of displacement.

Discussion

Desethylamiodarone competition for thyroid hormone receptors. Our results demonstrate that desethylamiodarone has a substantial capacity to compete for thyroid hormone binding to nuclear thyroid hormone receptors derived from three different tissues in three different species, including humans. In general, this capability is not shared by the parent compound, amiodarone, although some weak competitive binding is noted in human lymphocytes (Fig. 2B). This capacity suggests that desethylamiodarone may be able to influence metabolic processes governed by thyroid hormone in a variety of tissues. Desethylamiodarone has a much lower affinity for nuclear thyroid hormone receptors than triiodothyronine (T_3) or thyroxine (T_4) (Table 1). However, very large amounts of desethylamiodarone accumulate in parenchymatous organs of treated subjects (up to 6,500 mg/kg in human liver) (9). Steady state blood concentrations of desethylamiodarone in patients on long-term amiodarone therapy approximate 4 μM (2.7 μg/ml) (10). If similar concentrations appear in cell nuclei, our data suggest that partial saturation of nuclear thyroid hormone receptors by desethylamiodarone is a distinct possibility. Thus, desethylamiodarone competition for thyroid hormone receptors may be significant in the clinical use of this drug, both as a potential source of beneficial antiarrhythmic action, and as a factor in genesis of side effects in the many organ systems influenced by amiodarone treatment.

In addition to the binding properties of desethylamiodarone, our findings also demonstrate an even higher affinity of nuclear thyroid hormone receptors for reverse T_3. Be-

cause long-term amiodarone treatment results in a two- to fourfold rise in serum reverse T_3 levels (1), greater reverse T_3 occupancy of nuclear thyroid hormone receptor sites is a possible outcome of this therapy. The metabolic consequences of such a change are, at present, unknown. However, higher reverse T_3 levels could also act, possibly in concert with desethylamiodarone, to displace thyroid hormone from its nuclear receptor and thereby contribute to a hypothyroid state within the heart or other tissues.

Role of blockade of thyroid hormone receptors in antiarrhythmic effects of amiodarone. Thyroid hormone levels clearly modulate myocardial repolarization. Isolated myocardium from thyroidectomized rabbits exhibits prolongation in the plateau phase of the action potential (4). Singh and Vaughan Williams (2) demonstrated that long-term amiodarone therapy produces repolarization delay similar to that caused by thyroidectomy. The repolarization changes induced by amiodarone can be prevented by concomitant thyroid hormone treatment (2). More recently, Singh and Nademanee (1) suggested that amiodarone therapy may influence cardiac muscle, in part, by blocking the actions of T_3 on nuclear thyroid hormone receptors. Our findings provide explicit experimental support for this concept by demonstrating a potential for blockade of nuclear thyroid hormone receptors in heart and other tissues during amiodarone therapy and by identifying a specific molecular species most likely responsible for this blockade. Transformation of amiodarone to desethylamiodarone and subsequent blockade induced by this metabolite may explain the early electrophysiologic results.

This postulated action of desethylamiodarone is consonant with present knowledge of the pharmacokinetic and pharmacodynamic aspects of amiodarone therapy in man. Thus, the long delay in onset of maximal antiarrhythmic efficacy of oral therapy and the relatively modest effects on repolarization with intravenous dosing despite equivalent blood and tissue levels of amiodarone may be explained by a requirement to convert amiodarone to desethylamiodarone, accumulate large intracellular stores of desethylamiodarone and await the relatively slow changes that would be

expected with blockade of nuclear thyroid hormone receptors.

If desethylamiodarone competition for T_3 and T_4 binding to nuclear thyroid hormone receptors is responsible, in part, for certain cardiac effects of amiodarone therapy, then desethylamiodarone must occupy the receptor site without activation. Such a blocking effect would be unique among various substances known to interact with nuclear thyroid receptors. In the past, an extensive survey of thyroid hormone analogs failed to identify any compound with receptor antagonist properties (10).

If blockade of nuclear thyroid receptors is an important mediator of the cardiac actions of amiodarone therapy, this leaves unexplained the fact that effective amiodarone therapy is not uniformly accompanied by clinically overt generalized hypothyroidism. Although hypothyroidism develops in an appreciable number of patients receiving amiodarone, such patients generally have reduced levels of plasma T_3 and T_4, an effect usually ascribed to an unusual metabolic response to the abnormal iodide load released with metabolic breakdown of amiodarone and desethylamiodarone (1,11). Current results offer no suggestion of myocardial selectivity for desethylamiodarone, because this substance appears able to bind nuclear thyroid hormone receptor sites with a similar affinity in all tissues studied (Table 1). These results do suggest that receptor affinity for T_3 is distinctly lower in the ventricles. This could permit desethylamiodarone to compete with T_3 more effectively in the ventricles than in other tissues. Such an explanation would not apply to the atria, which exhibit a clinical sensitivity to antiarrhythmic actions of amiodarone therapy exceeding that of the ventricles.

Therapeutic implications of the study. Caution must be used in interpreting our results. Relative concentrations of desethylamiodarone, amiodarone, reverse T_3, T_3 and T_4 in the vicinity of nuclear thyroid hormone receptors during long-term amiodarone therapy is unknown. In addition, the relation between nuclear thyroid hormone receptors and electrical functioning of the myocardium is uncertain. Other thyroid hormone receptors (for example, those on plasma membrane) may also be important in mediating thyroid hormone action on electrical activity. Ultimate determination of the capacity of amiodarone and desethylamiodarone to influence thyroid hormone action in the heart and other tissues must rely on measurement of metabolic indexes. Certain cardiac electrophysiologic effects of amiodarone therapy have no apparent connection with blockade of nuclear thyroid hormone receptors, for example, blockade of inactivated sodium channels (12,13). Moreover, the relation of potential thyroid-mediated changes (delayed repolarization and prolonged refractoriness) and antiarrhythmic efficacy has not been fully delineated.

The electrophysiologic and clinical effects of long-term treatment with independently administered desethylamiodarone are unknown. Electrophysiologic studies to date have focused on results of short-term administration of desethylamiodarone (14), at time points before any likely influence resulting from inhibition of nuclear thyroid hormone receptors. Despite this multitude of uncertainties, our results suggest that further exploration of nuclear thyroid hormone receptor blockade may elucidate important aspects of antiarrhythmic efficacy and other consequences of amiodarone therapy.

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