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Thyroid Hormones Selectively Modulate Human Alcohol Dehydrogenase Isozyme Catalyzed Ethanol Oxidation†

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ABSTRACT: Thyroid hormones are potent, instantaneous, and reversible inhibitors of ethanol oxidation catalyzed by isozymes of class I and II human alcohol dehydrogenase (ADH). None of the thyroid hormones inhibits class III ADH. At pH 7.40 the apparent K_i values vary between 55 and 110 μM for triiodothyronine, 35 and >200 μM for thyroxine, and 10 and 23 μM for triiodothyroacetic acid. The inhibition is of a mixed type toward both NAD^+ and ethanol. The binding of the thyroid hormone triiodothyronine to $\beta_1\gamma_1$ ADH is mutually exclusive with 1,10-phenanthroline, 4-methylpyrazole, and testosterone, identifying a binding site(s) for the thyroid hormones, which overlap(s) both the 1,10-phenanthroline site near the active site zinc atom and the testosterone binding site, the latter being a regulatory site on the γ-subunit-containing isozymes and distinct from their catalytic site. The inhibition by thyroid hormones may have implications for regulation of ADH catalysis of ethanol and alcohols in the intermediary metabolism of dopamine, norepinephrine, and serotonin and in steroid metabolism. In concert with other hormonal regulators, e.g., testosterone, the rate of ADH catalysis is capable of being fine tuned in accord with both substrate and modulator concentrations.

Metabolic pathways are regulated by a variety of mechanisms. The most immediate form of regulation is through specific interactions of substrates, products, and effectors with allosteric enzymes. Other levels of metabolic control are exerted by hormonal regulation and through the biosynthesis and degradation of constituent enzymes.

These considerations underlie the study of human alcohol dehydrogenase (ADH).[1] Three classes of structurally similar physicochemically distinct, dimeric isozymes are expressed by at least five genetic loci (Vallee, 1985). The catalytic properties of these isozymes differ markedly with respect to a number of alcohols and aldehydes in the intermediary metabolism of dopamine (Mårdh & Vallee, 1985), norepinephrine (Mårdh et al., 1985, 1986a), serotonin (Consalvi et al., 1986), and steroids (Frey & Vallee, 1980). Their number and distribution also vary both with the complexity of the organism

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[1] Abbreviations: ADH, alcohol dehydrogenase; K_i', apparent inhibition constant at fixed ethanol and NAD^+ concentrations; OP, 1,10-phenanthroline; 4-MePz, 4-methylpyrazole.

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Table I: Apparent K_i Values for Triiodothyroacetic Acid, Thyroxine, and Triiodothyronine Inhibition of ADH Isozymes[a]

isozyme class	isozyme	triiodothyroacetic acid	thyroxine	triiodothyronine
	$\alpha\beta_1$	10	>200	NI
	$\beta_1\beta_1$	20	>200	NI
	$\alpha\gamma_1$	13	40	80
I	$\alpha\gamma_2$	20	110	70
	$\beta_1\gamma_1$	13	60	70
	$\beta_1\gamma_2$	12	>200	110
	$\gamma_1\gamma_1$	14	100	60
II	π	23	35	55
III	χ	NI	NI	NI

[a] Determined as the concentration (μM) of inhibitor that yields 50% inhibition of initial reaction velocities in assays of 1.0 mM ethanol, 2.5 mM NAD^+, and 0.3 μM enzyme in 0.1 M sodium phosphate, pH 7.4 at 25 °C. NI = no inhibition noted up to 200 μM thyroid agent.

under study and with tissue specificity (Vallee, 1985). We have reported that testosterone and its physiologically active metabolite 5α-dihydrotestosterone are potent and selective allosteric inhibitors of ethanol oxidation catalyzed by class I γ ADH homo- and heterodimers (Mårdh et al., 1986b).

These studies are here extended to the effects of thyroid hormones on the catalytic potency of a variety of ADH isozymes. These hormones are, indeed, potent and isozyme-specific inhibitors of ADH-catalyzed ethanol oxidation. The results suggest a modulating role of thyroid hormones on ADH-mediated oxidoreduction reactions.

Materials and Methods

Human livers were obtained at post mortem examination, and the ADH isozymes were purified to homogeneity by described methods (Wagner et al., 1983; Ditlow et al., 1984; Wagner et al., 1984). The present study was performed with the class I isozymes $\alpha\alpha$, $\alpha\beta_1$, $\beta_1\beta_1$, $\alpha\gamma_1$, $\alpha\gamma_2$, $\beta_1\gamma_1$, $\beta_1\gamma_2$, and $\gamma_1\gamma_1$ and class II (π) and III (χ) ADH. Thyroxine, triiodothyronine, triiodothyroacetic acid, and testosterone were used as purchased from Sigma Chemical Co., St. Louis, MO.

The rate of ethanol oxidation in the presence or absence of hormones was monitored by the formation of NADH at 340 nm with a Varian Cary 219 spectrophotometer. All experiments were carried out with an enzyme-saturating NAD^+ concentration, 2.5 mM, in 0.1 M sodium phosphate, pH 7.4 at 25 °C, except class III ADH, which was assayed in 0.1 M glycine–NaOH at pH 10.0. Thyroxine and triiodothyronine solutions were freshly prepared in 0.1 M sodium hydroxide (Gilleland & Shore, 1969) and diluted 1:60 into the reaction mixture.

Results

Triiodothyroacetic acid and thyroxine inhibit ethanol oxidation catalyzed by all class I and II isozymes examined, while triiodothyronine inhibits class II ADH and the γ-subunit-containing isozymes of class I but not those composed only of α- and/or β-subunits (Table I). None of the thyroid agents inhibit the class III isozymes. The inhibition of class I and II isozymes is reversible instantaneously; e.g., a 10-fold dilution of 100 μM triiodothyronine and 0.8 μM $\beta_1\gamma_1$ ADH restores activity to 92% of that expected at this lower triiodothyronine concentration. For each member of the class I isozymes, triiodothyroacetic acid is the most potent inhibitor; at pH 7.4 the K_i values for triiodothyroacetic acid vary from 10 to 20 μM, while those for thyroxine and triiodothyronine vary from 40 μM to higher than their solubility limits, 200 μM (Table I).

For most of the isozymes the inhibition profiles for triiodothyronine and triiodothyroacetic acid extend over an inhibitor concentration range at least 2 orders of magnitude (Figure 1A,B) with Hill coefficients close to unity (Table II). The triiodothyronine inhibition of $\alpha\gamma_1$-, $\alpha\gamma_2$-, and $\beta_1\gamma_2$-catalyzed ethanol oxidation differs from this general pattern; the range of inhibition is much narrower with Hill coefficients of 1.8, 1.9, and 2.1, respectively, indicative of positive cooperativity (Figure 1A, Table II). In marked contrast, the concentration range of thyroxine inhibition is much wider, with Hill coefficients varying from 0.4 to 0.8 for the different isozymes (Figure 1C, Table II). For each isozyme the value is less than 1.0 as is observed when there is negative cooperativity.

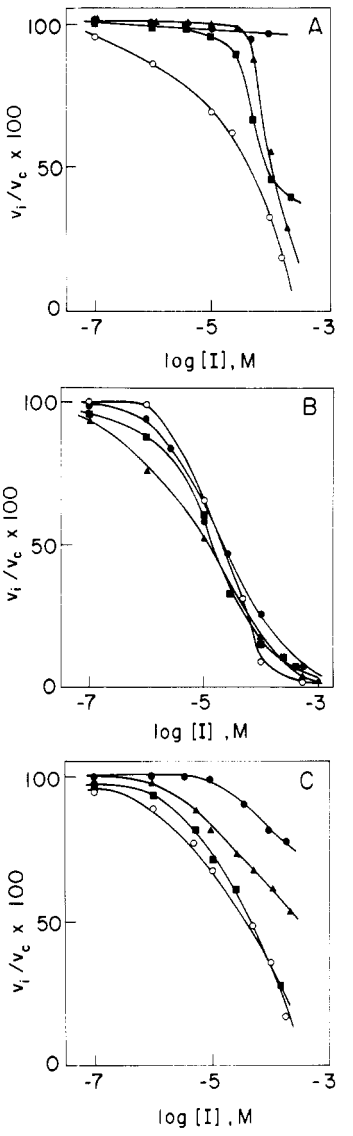


FIGURE 1: Inhibition of ADH-catalyzed ethanol oxidation by (A) triiodothyronine, (B) triiodothyroacetic acid, and (C) thyroxine as represented by $\beta_1\beta_1$ (●), $\alpha\gamma_1$ (■), $\beta_1\gamma_2$ (▲), and π (○) ADH. Initial velocities, measured in the presence, v_i, and absence, v_c, of a thyroid hormone, are plotted versus the logarithm of inhibitor concentration to the limits of their solubility. Assay conditions: 1.0 mM ethanol/2.5 mM NAD^+/0.3 μM enzyme in 0.1 M sodium phosphate (pH 7.40) at 25 °C. Reactions were initiated by the addition of ethanol.

All three thyroid agents inhibit class II ADH to a similar degree, with K_i values varying from 23 to 55 μM (Table I). The Hill coefficient for triiodothyroacetic acid is close to unity, 1.2, similar to the class I isozymes. In contrast, both triiodothyronine and thyroxine yield Hill coefficients less than unity, 0.7 and 0.6, respectively (Table II).

The mode of inhibition by thyroid hormones with respect to substrate and coenzyme has been determined over the

Table II: Hill Coefficients for Triiodothyroacetic Acid, Thyroxine, and Triiodothyronine Inhibition of Class I and II ADH Isozymes[a]

isozyme class	isozyme	triiodothyroacetic acid	thyroxine	triiodothyronine
	$\alpha\beta_1$	1.2	0.5	
	$\beta_1\beta_1$	0.9	0.5	
	$\alpha\gamma_1$	0.8	0.8	1.8
I	$\alpha\gamma_2$	0.9	0.7	1.9
	$\beta_1\gamma_1$	0.9	0.4	1.1
	$\beta_1\gamma_2$	0.8	0.8	2.1
	$\gamma_1\gamma_1$	1.0	0.8	0.7
II	π	1.2	0.6	0.7

[a] Calculated from plots of log $(v_c/v_i - 1)$ versus the log of inhibitor concentration, the slopes of which yield the Hill coefficients (Levitzki & Koshland, 1969). v_c and v_i refer to the velocity in the absence and presence of inhibitor, respectively.

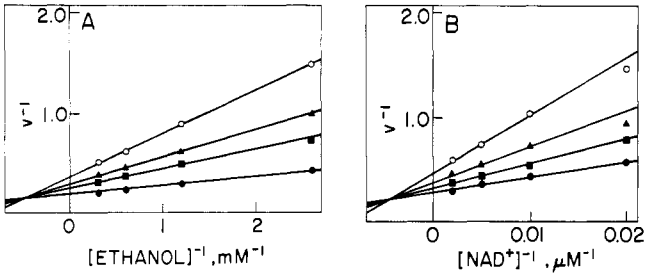


FIGURE 2: Lineweaver–Burk plots of triiodothyroacetic acid inhibition of $\beta_1\gamma_1$ ADH catalyzed ethanol oxidation for ethanol (A) and NAD^+ (B). Initial velocities, expressed as μM NAD^+ h^{-1} (mg of protein)$^{-1}$, were determined without (●) and with 10 (■), 20 (▲), and 40 (○) μM triiodothyroacetic acid. In (A) NAD^+ is at 2.5 mM, and in (B) the ethanol concentration is 5.0 mM. Other assay conditions are as described in Figure 1. The K_i values are 13 and 14 μM, respectively.

concentration range from 0.4 to 3.2 mM ethanol at 2.5 mM NAD^+ and from 25 to 500 μM NAD^+ at 5.0 mM ethanol for the $\beta_1\gamma_1$ isozyme. Triiodothyronine, triiodothyroacetic acid, and thyroxine inhibition is of mixed type versus both substrate and coenzyme, indicating the presence of an enzyme–coenzyme–substrate–inhibitor complex at steady state. The K_i values for the mixed inhibition of triiodothyroacetic acid toward ethanol and NAD^+ are 13 and 14 μM, respectively (Figure 2).

Previous studies of ethanol oxidation catalyzed by ADH isozymes have identified two sites of inhibitor action, one near the active site metal ion where 4-methylpyrazole and 1,10-phenanthroline bind (von Wartburg et al., 1964; Eklund et al., 1982) and another, allosteric site on the γ-subunit-containing isozyme where testosterone binds (Mårdh et al., 1986b). The kinetics of the triiodothyronine inhibition of $\beta_1\gamma_1$ isozyme were examined to determine whether or not the thyroid binding site(s) overlap(s) either or both of these sites. Dixon plots of 1/velocity, v, versus the concentration of the first inhibitor at a constant substrate concentration and at different concentrations of the thyroid hormone (Segel, 1975) are parallel. This demonstrates that the thyroid hormone strongly competes with each of the other three inhibitors for a site on the enzyme (Figure 3). The K_i values for triiodothyronine competition with 1,10-phenanthroline, 4-methylpyrazole, and testosterone are 153, 159, and 179 μM, respectively.

Discussion

Triiodothyronine (Ia), thyroxine (Ib), and triiodothyroacetic acid (Ic) selectively inhibit isozymes of human liver alcohol dehydrogenase (Chart I). Ia and Ib differ structurally by virtue of an iodine atom in the 3′-position; they inhibit the γ-containing class I isozymes with nearly the same average

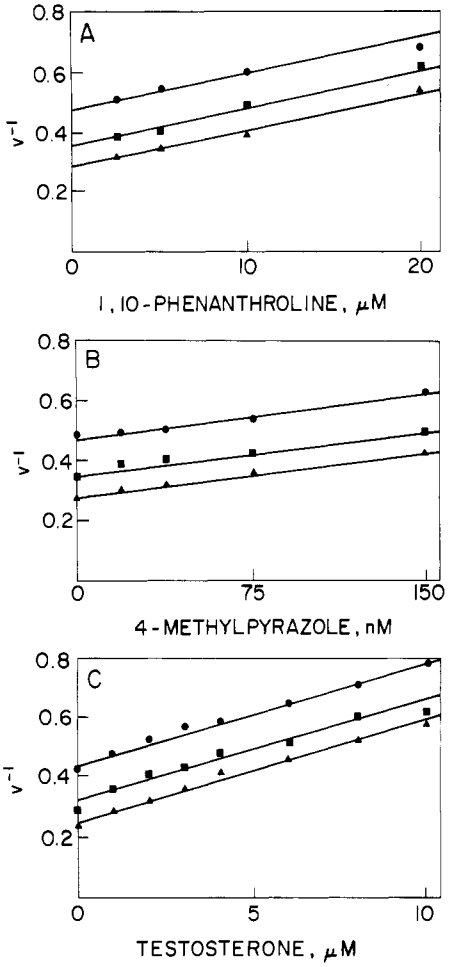
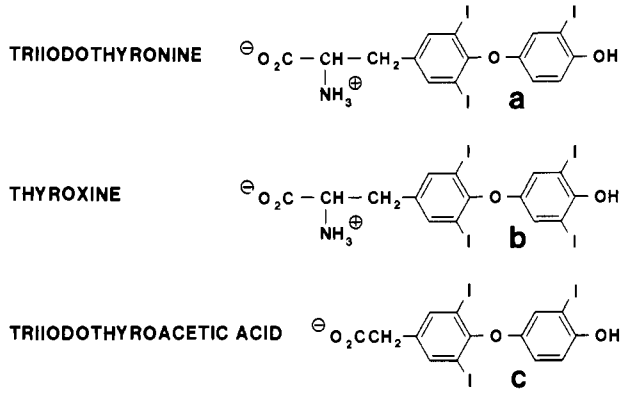


FIGURE 3: Dixon plots of the combined inhibition of $\beta_1\gamma_1$ ADH catalyzed ethanol oxidation by triiodothyronine (Ia) and 1,10-phenanthroline (A), by Ia and 4-methylpyrazole (B), and by Ia and testosterone (C). Initial velocities, expressed as μmol of NAD^+ h^{-1} (mg of protein)$^{-1}$, were determined at 5.0 mM ethanol without (▲) and with 75 (■) and 150 (●) μM Ia. Other assay conditions are as described in Figure 1. For OP, the instantaneous inhibition was monitored by adding the enzyme last, and for 4-MePyz and testosterone the inhibition was monitored by adding ethanol after a 5-min incubation of enzyme, NAD^+, and inhibitor.

Chart I



inhibition constants, 80 ± 22 and 102 ± 62 μM, respectively. These agents either do not or only weakly inhibit the $\beta_1\beta_1$ and $\alpha\beta_1$ isozymes. However, conversion of the alanyl portion of Ia into the acetate moiety of Ic results in much stronger inhibition toward all the class I isozymes, the average K_i value being 15 ± 4 μM (Table I). All three thyroid hormones inhibit class II to a similar degree, 23–55 μM, and completely fail to inhibit class III ADH.

Thyroid hormones are known to modulate the activities of malate, ornithine, α-glycerophosphate, aldehyde, and alcohol dehydrogenases (Wolff & Wolff, 1957; Wolff, 1962; von Wartburg et al., 1964; McCarthy et al., 1968; Gilleland & Shore, 1969). While the mechanism of this action is not fully understood, these hormones were first thought to inhibit by binding directly to the active site zinc atom of the enzyme. Different types of inhibition patterns have been observed. Thus thyroxine has been reported to be a competitive inhibitor both of the substrate and of the cofactor of malic dehydrogenase (Wolff & Wolff, 1957) but a noncompetitive inhibitor of glutamic dehydrogenase (Wolff, 1962).

In the present study, the inhibition by all three thyroid hormones is of the mixed type, both toward substrate and toward coenzyme, indicating that inhibitor, substrate, and coenzyme bind simultaneously to the enzyme (Figure 2). The closely similar K_i values for Ic inhibition of ethanol and NADH, 13 and 14 μM, respectively, suggest a single site of hormone interaction. Similarly, the inhibition constant of the thyroid hormone, Ia, is essentially the same regardless of whether 1,10-phenanthroline, 4-methylpyrazole, or testosterone is used to compete with the hormone in inhibiting the $\beta_1\gamma_1$ enzyme. However, in these cases thyroid hormone binding is essentially mutually exclusive with each of these inhibitors. Previous studies have shown that testosterone binds only to the γ-containing subunits at an allosteric site (Mårdh et al., 1986b), while 1,10-phenanthroline binds to the active site zinc atom (Eklund et al., 1982). Since testosterone and 1,10-phenanthroline bind at different sites on the enzyme (Mårdh et al., 1986b), the thyroid hormone must be assured to overlap both of these separate sites. The possible range of interaction of all these agents is quite large, ~15–17 Å for the thyroid hormones, ~11 Å for testosterone, and ~8 Å for 1,10-phenanthroline, making it reasonable that one binding site for the thyroid hormone could account for its inhibition patterns toward substrate, coenzyme, and other inhibitors of ADH. However, direct binding studies of the hormones to the enzyme will be needed to establish whether more than one thyroid hormone binding site exists.

In some cases the inhibitory profile indicates cooperativity by the thyroid hormones (Figure 1 and Table II). Triiodothyronine inhibits $\alpha\gamma_1$-, $\alpha\gamma$-, and $\beta_1\gamma_2$-catalyzed ethanol oxidation with positive cooperativity while that of thyroxine reveals negative cooperativity, quite similar to testosterone inhibition of γ-subunit-containing class I isozyme (Mårdh et al., 1986b). Assessment of the nature of this cooperativity requires determination of the affinities of the different ligands to the enzyme but indicates that there may be interactions between the subunits (Seydoux et al., 1974; Mårdh et al., 1986b). Such binding of a modulator to one or to both subunits of an enzyme is a potent means for the regulation of enzyme activity. Interestingly, class II ADH exhibits negative cooperativity both in the presence of triiodothyronine and in the presence of thyroxine (Table II), indicating similar but not necessarily identical subunit interactions during catalysis by class II ADH.

A putative regulator of human alcohol dehydrogenase such as the thyroid hormones would be important in the regulation of ethanol metabolism. Thyroxine was recognized early as an inhibitor of yeast ADH (Wolff & Wolff, 1957), and later thyroid hormones were shown to inhibit both the unresolved human liver enzyme and liver ADH of several mammalian species (von Wartburg et al., 1964; McCarthy et al., 1968; Gilleland & Shore, 1969; Truchot, 1973). It has proven difficult to correlate changes in thyroid status and rate of ethanol metabolism, in part possibly owing to the species chosen for the studies (Smith & Dawson, 1985). A few human in vivo studies have given conflicting results (Goldberg et al., 1960; Kalant et al., 1962). Further work will likely clarify whether the thyromimetic actions include modulation of enzyme activities in addition to those of calorigenesis, protein synthesis, glycogenolysis, and plasma cholesterol content.

ACKNOWLEDGMENTS

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Registry No. **a**, 6893-02-3; **b**, 51-48-9; **c**, 51-24-1; ADH, 9031-72-5; CH_3CH_2OH, 64-17-5; Zn, 7440-66-6.

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