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Effects of the thyroid hormone derivatives 3-iodothyronamine and thyronamine on rat liver oxidative capacity

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Abstract.

Thyronamines T₀AM and T₁AM are naturally occurring decarboxylated thyroid hormone derivatives. Their *in vivo* administration induces effects opposite to those induced by thyroid hormone, including lowering of body temperature. Since the mitochondrial energy-transduction apparatus is known to be a potential target of thyroid hormone and its derivatives, we investigated the *in vitro* effects of T₀AM and T₁AM on the rates of O₂ consumption and H₂O₂ release by rat liver mitochondria. Hypothyroid animals were used because of the low levels of endogenous thyronamines. We found that both compounds are able to reduce mitochondrial O₂ consumption and increase H₂O₂ release. The observed changes could be explained by a partial block, operated by thyronamines, at a site located near the site of action of antimycin A. This hypothesis was confirmed by the observation that thyronamines reduced the activity of Complex III where the site of antimycin action is located. Because thyronamines exerted their effects at concentrations comparable to those found in hepatic tissue, it is conceivable that they can affect *in vivo* mitochondrial O₂ consumption and H₂O₂ production acting as modulators of thyroid hormone action.

Key words. Thyroid hormone derivatives, O₂ consumption, ROS production, Mitochondria

1. Introduction

It has long been known that synthetic decarboxylated analogues of the thyroid hormone exhibit thyromimetic effects (Tomita and Lardy, 1956; Roth, 1957) and that thyroxine undergoes decarboxylation in rats (Lang and Klitgaard, 1959). Investigation on the biological function of decarboxylated thyroid hormone derivatives has received new impulse by the discovery of the endogenously produced 3-iodothyronamine (T₁AM) (Scanlan et al., 2004), which has been detected in several tissues of mouse (Scanlan et al., 2004) and rat (Saba et al., 2010), as well as in human blood (Saba et al., 2010). T₁AM is a potent *in vitro* agonist of the trace amine-associated receptor type 1 (TAAR1) a G_s-protein-coupled membrane receptor, and *in vivo* rapidly induces hypothermia and bradycardia (Scanlan et al., 2004). Exogenous T₁AM has also been found to produce negative inotropic and chronotropic effects in isolated working rat hearts (Chiellini et al., 2007) and endogenous T₁AM concentration exceeds T₃ concentration by about 10-fold in cardiac tissue (Saba et al., 2010). Effects on mouse body temperature, heart rate and cardiac output have also been obtained by administering thyronamine (T₀AM), another naturally occurring relative of thyroid hormone, which is less potent than T₁AM (Scanlan et al., 2004). Several recent studies have supported the concept that T₁AM and T₀AM are signaling molecules able to affect physiological manifestations of thyroid hormone acting through non-genomic effectors (Ianculescu and Scanlan, 2010).

It has previously been hypothesized that the mitochondrion is the main target for other thyroid hormone derivatives, such as the diiodothyronines. The inner membrane of rat liver mitochondria contains iodothyronine binding sites, showing the greatest affinity for 3,5-diiodothyronine (3,5-T₂) and 3,3'-diiodothyronine (3,3'-T₂) (Goglia et al., 1994) and the administration of such substances to hypothyroid rats stimulates liver mitochondrial respiration (Lanni et al., 1993). Thyroid thermogenesis has also been associated to mitochondrial effects (Cioffi et al., 2010, Videla et al., 2010), although alternative mechanisms, particularly interference with calcium homeostasis, have been proposed (Cannon and Nedergaard, 2010).

For these reasons, it seemed interesting to investigate whether addition of thyronamines to isolated mitochondria may affect both oxygen consumption and reactive oxygen species (ROS) release, whose rate is strongly dependent on the rate of the electron flow through the respiratory chain. In order to minimize potential interference by thyroid hormone and its derivatives, we chose to perform our experiments using liver mitochondria from hypothyroid rats.

2. Materials and Methods

2.1. Materials

All chemicals used (Sigma Chimica, Milano, Italy) were of the highest grades available. T₁AM and T₀AM were synthesized as described elsewhere (Hart et al., 2006).

2.2. Animals

The experiments were carried out on eight 70-day-old male Wistar rats, supplied by Nossan (Correzzana, Italy) at day 45 of age. From day 49 thyroid activity was chronically inhibited by i.p. administration of PTU (1 mg/100 g body weight, once per day for 3 weeks). All rats were kept under the same environmental conditions and were provided with water ad libitum and commercial rat chow diet (Nossan).

The treatment of animals in these experiments was in accordance with the guidelines set forth by the University's Animal Care Review Committee.

2.3. Preparation of homogenates and mitochondria

The animals were sacrificed by decapitation and livers were rapidly excised and placed into ice-cold homogenisation medium (HM) (220 mM mannitol, 70 mM sucrose, 1 mM EDTA, 0.1% fatty acid-free albumin, 10 mM Tris, pH 7.4). Livers, freed from connective tissue were weighed, finely minced, and washed with HM. Finally, tissues were gently homogenised (20% w/v) in HM using a glass Potter-Elvehjem homogeniser set at a standard velocity (500 rpm) for 1 min.

The homogenates, diluted 1:1 with HM, were freed of debris and nuclei by centrifugation at 500 g for 10 min at 4°C. The resulting supernatants were centrifuged at 10,000 g for 10 min. The mitochondrial pellets were resuspended in washing buffer (WB) (220 mM mannitol, 70 mM sucrose, 1 mM EGTA, 20 mM Tris, pH 7.4) and recentrifuged as described above. This procedure was repeated twice before final suspension in WB. Mitochondrial protein was measured by the biuret method (Gornall et al., 1949).

2.4. Analytical procedures

Mitochondrial respiration was monitored at 30° C by a Gilson respirometer in 1.0 ml of incubation medium (145 mM KCl, 30 mM Hepes, 5 mM KH₂PO₄, 3 mM MgCl₂, 0.1 mM EGTA, pH 7.4) with 0.2 mg of mitochondrial protein and succinate (10 mM) (plus rotenone 5 μM) or pyruvate/malate (10/2.5 mM) as substrates, in the absence and in the presence of 500 μM ADP.

The respiratory chain includes four catalytic complexes. Complex I (NADH-ubiquinone oxidoreductase) and Complex II (succinate-ubiquinone oxidoreductase) represent parallel pathways through which electrons are transferred to ubiquinone, while Complex III (ubiquinone-cytochrome *c* reductase) and Complex IV (cytochrome *c* oxidase) act sequentially as the final common pathway transferring electrons from ubiquinone to oxygen. Pyruvate/malate and succinate are used as selective Complex I or Complex II substrates, respectively.

The energy released as electrons flow through the respiratory chain is converted into a H⁺ gradient through the inner mitochondrial membrane. In the presence of ADP, this gradient dissipates through the ATP synthase complex (Complex V) promoting ATP synthesis. In the absence of ADP, the movement of H⁺ through ATP synthase ceases and the H⁺ gradient builds up causing electron flow and O₂ consumption to slow down. These conditions are conventionally referred as State 4 and State 3, respectively.

The rate of mitochondrial H₂O₂ release was measured at 30° C following the increase in fluorescence (excitation at 320 nm, emission at 400 nm) due to oxidation of *p*-hydroxyphenylacetate (PHPA) by H₂O₂ in the presence of horseradish peroxidase (HRP) (Hyslop and Sklar, 1984) in a computer-controlled Jasco fluorometer equipped with a thermostatically controlled cell-holder. The reaction mixture consisted of 0.1 mg/ml mitochondrial proteins, 6 U/ml HRP, 200 μg/ml PHPA, and either 10 mM succinate plus 5 μM rotenone or 10 mM pyruvate/2.5 mM malate, which were added at the end to start the reaction in a medium containing 145 mM KCl, 30 mM Hepes, 5 mM KH₂PO₄, 3 mM MgCl₂, 0.1 mM EGTA, pH 7.4. Measurements with the different substrates were also performed in the presence of 500 μM ADP. Various respiratory inhibitors and substrates are commonly used to obtain information on possible alterations at the sites of ROS generation. Therefore, the effects of two respiratory inhibitors were investigated: rotenone (Rot), which blocks the transfer of electrons from Complex I to ubiquinone (Palmer et al., 1968), and antimycin A (AA), which interrupts electron transfer within the ubiquinone-cytochrome *b* site of Complex III (Turrens

et al., 1985). Inhibitor concentrations (5 μM Rot, 10 μM AA) which do not interfere with the detection PHPA-HRP system were used (Venditti et al., 2003).

In each series of experiments, rates of O_2 consumption and H_2O_2 release were measured on mitochondrial suspensions incubated for 10 min with 10 μl of dimethyl sulphoxide (DMSO), or DMSO and T_1AM , or DMSO and T_0AM . The final concentration of DMSO in incubation media was 0.02% (v/v), whereas those of T_1AM and T_0AM ranged from 10^{-9} to 10^{-5} M.

Preliminary experiments showed that the rates of O_2 consumption and H_2O_2 release were not modified by 0.02% DMSO.

To determine monoamine oxidase (MAO) dependent H_2O_2 production, the rate of H_2O_2 release was measured on mitochondrial suspensions incubated as above described, in the absence of respiratory substrates and in the presence and in the absence of pargyline, a MAO A and MAO B inhibitor (Panova et al., 1997). Subsequently, we measured H_2O_2 release rates during State 4 and State 3 respiration in the presence and in the absence of pargyline. When pargyline was used, mitochondrial suspensions were pre-incubated for 1 h with 5 μM pargyline.

We also measured the activity of the four respiratory chain Complexes. The activities of the first three complexes were assayed in a Beckman (Fullerton, CA USA) model DU 640 spectrophotometer using the method of Ragan et al. (1987). Complex IV activity was determined polarographically at 30° C using a Gilson glass respirometer equipped with a Clark oxygen electrode (Yellow Springs Instruments, Ohio USA) by the procedure of Barré et al. (1987).

Respiratory complex activities were measured using mitochondrial suspensions containing 0.02% DMSO in the absence and in the presence of T_1AM or T_0AM added 10 min before the assay at final concentration of 10^{-5} M.

2.5. Data analysis

Experiments were carried out using eight mitochondrial preparations from eight different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of all the sample means. The results, expressed as means \pm standard error, were analyzed by one-way analysis of variance. When a significant F ratio was found, individual groups were compared by the Student-Newman-

Keuls multiple range test. Probability values (P) < 0.05 were considered as significant. Experiments concerning MAO-dependent H_2O_2 production were carried out using three mitochondrial preparations from three different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of all the sample means and the results were expressed as means \pm SD.

3. Results

3.1. Oxygen consumption

As shown in Fig. 1, using succinate as substrate, the rates of State 4 and State 3 oxygen consumption were reduced by addition of either T_1AM or T_0AM . With the former compound, significant reduction of State 4 and State 3 respiration rates were observed at concentrations $\geq 10^{-6}$ M and 10^{-8} M, respectively. With the latter compound, the effect was greater and occurred at lower concentration, since significant reduction of State 4 and State 3 respiration was observed at concentrations $\geq 10^{-7}$ M and 10^{-9} M, respectively.

Using pyruvate/malate as substrates no significant effect was produced by either T_1AM or T_0AM on State 4 respiration rate. State 3 respiration rate was not significantly affected by T_0AM , whereas it was slightly reduced by T_1AM at concentrations $\geq 10^{-8}$ M.

3.2. H_2O_2 release

The rates of succinate-supported H_2O_2 release are shown in Fig. 2. H_2O_2 release was increased by T_1AM and to an even greater extent by T_0AM . Both compounds were effective at concentrations $\geq 10^{-8}$ M. After addition of rotenone to succinate-supplemented mitochondria H_2O_2 release was reduced (data not shown) but the response to T_1AM and T_0AM was still observed in the same concentration range. Further addition of antimycin A increased H_2O_2 release (data not shown), but the response to T_1AM and T_0AM was lost. In the presence of ADP, i.e. during the State 3 respiration, the rates of H_2O_2 release were increased by T_1AM , at concentrations $\geq 10^{-8}$ M, and by T_0AM , at concentrations $\geq 10^{-7}$ M. Under these conditions the maximum effect of T_0AM was greater than the maximum effect of T_1AM .

The rates of pyruvate/malate supported H_2O_2 release are shown in Fig. 3. H_2O_2 release was increased by T_1AM and to a smaller extent by T_0AM . Both compounds were effective at concentrations $\geq 10^{-7}$ M. The

addition of antimycin A to the mitochondria supplemented with pyruvate/malate determined an increase in H_2O_2 release (data not shown) and under these conditions no significant change was produced by either T_1AM or T_0AM . In the presence of rotenone, H_2O_2 release by pyruvate/malate supplemented mitochondria was increased (data not shown), and no further change was produced by T_1AM or T_0AM .

In the presence of ADP, i.e. during the State 3 respiration, H_2O_2 release was increased by T_1AM at concentrations $\geq 10^{-6}$ M and by T_0AM at concentrations $\geq 10^{-8}$ M.

The rates of H_2O_2 production in the absence of respiratory substrates are shown in Fig. 4. Because H_2O_2 was not produced in the presence of the MAO inhibitor pargyline (unreported data), our results suggest that both thyronamines undergo oxidative deamination. H_2O_2 production was higher in the presence of T_0AM suggesting that the latter may be a better substrate than T_1AM for MAO. Notably, calculations based on these results suggest that oxidative deamination by MAO would produce only minor decrease in thyronamine concentration (the maximum predicted effect is 6% decrease in T_0AM concentration).

3.3. Effect of MAO inhibitor on H_2O_2 release by respiring mitochondria

The rates of succinate-supported H_2O_2 release in the presence and in the absence of the MAO inhibitor pargyline are shown in Fig. 5. In accordance with the previous experiments, H_2O_2 release was significantly increased in the presence of $\geq 10^{-8}$ M T_1AM or T_0AM , during both State 4 and State 3. Significant increase in H_2O_2 release was also observed after exposure to pargyline, even though the effect of T_0AM during State 3 only became significant at concentrations $\geq 10^{-7}$ M. However, in the presence of pargyline the stimulation of H_2O_2 release produced by T_0AM was significantly lower during both State 4 and State 3, whereas that produced by T_1AM was significantly lower during State 4.

The rates of pyruvate/malate supported H_2O_2 release in the presence and in the absence of the MAO inhibitor are shown in Fig. 6. In the absence of pargyline, the rates were significantly increased by T_1AM at concentrations $\geq 10^{-7}$ M during both State 4 and State 3. After pargyline addition, H_2O_2 release rates were still increased by T_1AM at concentrations $\geq 10^{-6}$ M, but the effect of T_1AM was significantly lower at concentrations $\geq 10^{-6}$ M and 10^{-8} M during State 4 and State 3, respectively. The H_2O_2 release rates were also significantly increased by $\geq 10^{-6}$ M T_0AM both in State 3 and State 4, although the latter effect was minimal. The response to T_0AM was not significantly modified after pargyline addition.

3.4. Activity of respiratory chain complexes

As shown in Fig. 7, the activities of Complexes I, II, and IV of the respiratory chain were not affected by 10^{-5} M T₁AM and T₀AM. Conversely, the activity of Complex III was decreased to a similar extent by both compounds.

4. Discussion

It is well established that many actions of thyroid hormones are mediated by modulation of gene expression, triggered by hormone binding to nuclear receptors (TRs) (Samuels, et al., 1988). TRs have much higher affinity for 3,5,3'-triiodothyronine (T₃) than for thyroxine (T₄). So, although T₄ is the main product of thyroid secretion, it is regarded as a prohormone, which must be activated by deiodination to T₃ in order to initiate thyroid hormone action. The genomic effects mediated by TRs, like all transcriptional regulations, occurs on a relatively slow time scale. However, there are many rapid effects associated with thyroid hormones, that occur on a time scale precluding a T₃-TRs transcriptional mechanism (Yen, 2001).

Since Horst et al. (1989) showed a rapid stimulation of hepatic oxygen consumption by 3,5-diiodo-L-thyronine (3,5-T₂) it has been suggested that iodothyronines other than T₃ and T₄ can play a physiological role. Among these iodothyronines, 3,5-T₂ appears to influence energy metabolism. In vitro it stimulates rat liver cytochrome oxidase (Lanni et al., 1994) and when injected into hypothyroid rats, it is able to enhance resting metabolic rate through a pathway which does not depend on protein-synthesis and is more rapid than that of T₃ (Lanni et al., 1996).

More recently, there has been renewed interest for deiodinated and decarboxylated derivatives of thyroid hormones, such as T₁AM and T₀AM, which rapidly induce hypothermia and bradycardia in mouse through a mechanism independent of gene transcription (Scanlan et al., 2004).

Although T₁AM and T₀AM can dose-dependently couple TAAR1 to cAMP production (Scanlan et al., 2004), it is not yet clear whether TAAR1 is the only endogenous receptor for these molecules. In particular, decreases in body temperature and cardiac function are not consistent with increased cAMP production at the cellular level. Therefore, it has been suggested that either TAAR1 activation is not coupled to Gs proteins in some tissues, or thyronamines may interact with other TAAR subtypes (Zucchi et al., 2006). An alternative

possibility is that thyronamine effects may be mediated by their interaction with receptors different from TAARs. Notably, T₁AM has recently been reported to be accumulated within cells, suggesting the existence of intracellular targets (Saba et al., 2010).

The results reported in this paper appear to support such a hypothesis providing strong evidence that both thyronamines are able to influence the mitochondrial function in a subcellular preparation. Unlike 3,5-T₂ T₁AM and T₀AM do not appear to require cytoplasmic factors to produce mitochondrial effects, and they produce opposite functional consequences. Whereas treatment of hypothyroid rats with 3,5-T₂ stimulates the respiratory activity of rat liver mitochondria (Lanni et al., 1993), *in vitro* addition of T₁AM or T₀AM produces an inhibitory effect which may be consistent with their ability to induce hypothermia *in vivo* (Scanlan et al., 2004).

Interestingly, in some cases T₀AM appears to affect O₂ consumption to a greater extent than T₁AM, whereas it is less effective in inducing hypothermia *in vivo* (Scanlan et al., 2004). This could be explained by a greater intracellular accumulation of T₁AM *in vivo*. This hypothesis is supported by the observation that, in various cellular lines, unlabelled T₀AM decreases labelled T₁AM uptake to lesser extent than T₁AM itself (Ianculescu et al., 2009).

Electron transport within the inner mitochondrial membrane is a major biological process leading to ROS generation. Superoxide radical anion ($O_2^{\bullet-}$), the product of univalent oxygen reduction, is converted by superoxide dismutase into hydrogen peroxide, whose rate of release by intact mitochondria is often used to establish the capacity of such organelles to produce ROS. The rate of H₂O₂ generation by mitochondrial respiratory chain is related to the concentration and degree of reduction of the autoxidizable electron carriers (Boveris and Chance, 1973), which increases when the rate of electron flow decreases (Tzagaloff, 1982). Reduction in O₂ consumption rate indicates that, in the presence of thyronamines, electron flow rate along the respiratory chain decreases which is consistent with increased H₂O₂ production. Another process potentially contributing to mitochondrial ROS production is thyronamine oxidation by monoamine oxidases, which are located in the outer mitochondrial membrane and catalyze oxidation of biogenic amines by molecular oxygen which is reduced to H₂O₂. In fact, we obtained evidence that a reaction different from electron carrier autoxidation contributed to the increased H₂O₂ observed after exposure to T₁AM or T₀AM, since significant effect was obtained using pyruvate/malate in spite of minor changes in respiration rate, and H₂O₂ release rate was significantly reduced in the presence of pargyline.

Analysis of H_2O_2 production in the absence of the respiratory substrates showed that the rates of H_2O_2 generation due to autoxidation of electron carriers and to oxidative deamination of thyronamines were not additive. This result is not surprising since respiring mitochondria are able to largely remove ROS produced by both respiratory chain and other sources (Zoccarato et al., 2004). In accordance with this hypothesis, it has been reported that respiratory substrates lead to removal of H_2O_2 generated by MAO, favouring reduced glutathione regeneration (Sandri et al., 1990).

Further information on the site of action of thyronamine derives from the different effects of respiratory chain inhibitors on H_2O_2 release. Following thyronamine addition, mitochondrial preparations did not display any significant change in the rates of H_2O_2 release in the presence of antimycin A with both substrates (i.e. succinate and pyruvate/malate). No change was also observed in the presence of rotenone with pyruvate/malate as substrates. This result is consistent with the expected lack of changes in autoxidizable carrier concentration and suggest that thyronamines lead to a partial block of electron flow acting at a site which is located close to the site of antimycin action. Therefore, we determined the activities of the Complexes which constitute the respiratory chain and found that incubation with thyronamines caused reduced activity of Complex III, which is the target of antimycin. An action occurring at this level might also explain the different effects evoked by thyronamines on mitochondrial respiration sustained by Complex I and Complex II linked substrates. Indeed, since succinate-supported electron flow is higher than pyruvate/malate-supported flow, it is possible that an incomplete block of the electron flow through Complex III may reduce the former while producing minor effects on the latter.

The physiological implications of our findings require further investigations. Changes in State 3 succinate-supported O_2 consumption and H_2O_2 release rates were found in presence of 10 nM T_1AM . This substance has been found in rodent tissues including liver (Scanlan et al., 2004). Although the subcellular distribution of T_1AM is not known, recent research has showed that its concentration in liver is higher than in other tissues, including heart, and averages 92 pmol/g tissue, i.e. is in the order of 10^{-7} M (Saba et al., 2010). These results agree with the observation that, in rat, T_3 and T_4 concentrations (Saba et al., 2010; Escobar-Morreale et al., 1996) as well as aromatic amino acid decarboxylase activity (Inagaki and Tanaka, 1974) are higher in liver than in heart. Moreover, on the light of the our results, they support the idea that endogenous T_1AM is able to produce relevant effects on O_2 consumption and ROS production by liver mitochondria, at least in hypothyroid rats.

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Figure legend

Fig. 1. Effects of in vitro addition of T₁AM (▲) and T₀AM (○) on rates of State 4 and State 3 oxygen consumption by liver mitochondria from hypothyroid rats. Final T₁AM and T₀AM concentrations ranged from 1 to 10⁴ nM. The data, reported as percentage of the control, are expressed as means ± SEM. Experiments were carried out using eight mitochondrial preparations from eight different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of the eight sample means. The control values obtained in the presence of succinate (10 mM) + rotenone (5 μM) were 46.8±2.0 and 151.7±6.3 μmol O/min/mg mitochondrial protein during State 4 and State 3 respiration, respectively. The control values obtained in the presence of pyruvate/malate (10/2.5 mM), were 16.3±0.8 and 37.2±1.7 μmol O/min/mg mitochondrial protein during State 4 and State 3 respiration, respectively. ^a significant T₁AM vs. control; ^b significant T₀AM vs. control. The level of significance was chosen as *P* < 0.05. The horizontal axes do not start at zero in order to make the effects more clearly visible.

Fig. 2. Effects of in vitro addition of T₁AM (▲) and T₀AM (○) on rates of succinate supported H₂O₂ release by liver mitochondria from hypothyroid rats. Final T₁AM and T₀AM concentrations ranged from 1 to 10⁴ nM. The data, reported as percentage of the control, are expressed as means ± SEM. Experiments were carried out using eight mitochondrial preparations from eight different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of the eight sample means. The control values obtained in the presence of Succ, Succ + Rot, Succ + Rot + AA, and Succ + Rot + ADP were 139.4±1.2, 98.4±2.1, 857.9±4.4, and 63.9±0.3 pmol H₂O₂/min/mg mitochondrial protein, respectively. ^a significant T₁AM vs. control; ^b significant T₀AM vs. control. The level of significance was chosen as *P* < 0.05. The horizontal axes do not start at zero in order to make the effects more clearly visible.

Fig. 3. Effects of in vitro addition of T₁AM (▲) and T₀AM (○) on rates of pyruvate/malate supported H₂O₂ release by liver mitochondria from hypothyroid rats. Final T₁AM and T₀AM concentrations ranged from 1 to 10⁴ nM. The data, reported as percentage of the control, are expressed as means ± SEM. Experiments were carried out using eight mitochondrial preparations from eight different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of the eight sample means.

The control values obtained in the presence of Pyr/mal, Pyr/mal + Rot, Pyr/mal + AA, Pyr/mal + ADP were 183.5 ± 3.2 , 204.0 ± 7.1 , 937.0 ± 12.4 , and 101.1 ± 2.1 pmol H_2O_2 /min/mg mitochondrial protein, respectively.^a significant T_1AM vs. control; ^b significant T_0AM vs. control. The level of significance was chosen as $P < 0.05$.

Fig. 4. Rate of H_2O_2 generation by MAO catalyzed oxidative deamination of T_0AM and T_1AM . The data, are expressed as means \pm SEM. Final T_1AM and T_0AM concentrations ranged from 1 to 10^4 nM. Experiments were carried out using eight mitochondrial preparations from three different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of the three sample means.

Fig. 5. Effects of in vitro addition of T_1AM and T_0AM in the presence (■) and in the absence (□) of 5 μM pargyline on rates of succinate supported H_2O_2 release by rat liver mitochondria from hypothyroid rats. Final T_1AM and T_0AM concentrations ranged from 1 to 10^4 nM. The data, reported as percentage of the respective controls, are expressed as means \pm SEM. Experiments were carried out using eight mitochondrial preparations from eight different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of the eight sample means. In the presence of the inhibitor, the control values were 93.9 ± 0.9 and 57.5 ± 1.5 pmol H_2O_2 /min/mg mitochondrial protein, during State 4 and State 3, respectively. In the absence of the inhibitor, the control values were 101.1 ± 2.1 and 64.0 ± 0.2 pmol H_2O_2 /min/mg mitochondrial protein, during State 4 and State 3, respectively. ^a significant vs. control; ^b significant vs. value obtained in the absence of inhibitor. The level of significance was chosen as $P < 0.05$.

Fig. 6. Effects of in vitro addition of T_1AM and T_0AM in the presence (■) and in the absence (□) of 5 μM pargyline on rates of pyruvate/malate supported H_2O_2 release by rat liver mitochondria from hypothyroid rats. Final T_1AM and T_0AM concentrations ranged from 1 to 10^4 nM. The data, reported as percentage of the control, are expressed as means \pm SEM. Experiments were carried out using eight mitochondrial preparations from eight different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of the three sample means. In the presence of the inhibitor, the control

values were 163.2 ± 4.5 and 90.3 ± 1.7 pmol H_2O_2 /min/mg mitochondrial protein, during State 4 and State 3, respectively. In the absence of the inhibitor, the control values were 183.8 ± 1.0 and 99.5 ± 0.4 pmol H_2O_2 /min/mg mitochondrial protein, during State 4 and State 3, respectively. ^a significant vs. control; ^b significant vs. value obtained in the absence of inhibitor. The level of significance was chosen as $P < 0.05$.

Fig. 7. Effects of in vitro addition of T_1AM (\blacktriangle) and T_0AM (\circ) on activities of respiratory complexes from liver mitochondria from hypothyroid rats. Final T_1AM and T_0AM concentrations are 10^4 nM. The data, reported as percentage of the control, are expressed as means \pm SEM. Experiments were carried out using eight mitochondrial preparations from eight different rats, each run in duplicate or triplicate from which means were calculated for each sample. Then an average was taken of the three sample means. The control values of Complex I, Complex II, Complex III, and Complex IV, referred to mg of mitochondrial protein, were 34.5 ± 1.1 nmol NADH oxidized/min, 12.5 ± 0.7 nmol 2,6-dichloro-phenolindophenol reduced/min, 101.4 ± 2.8 nmol cytochrome c reduced/min, and 0.94 ± 0.05 μ mol O reduced/min, respectively. ^a significant T_1AM vs. control; ^b significant T_0AM vs. control. The level of significance was chosen as $P < 0.05$.

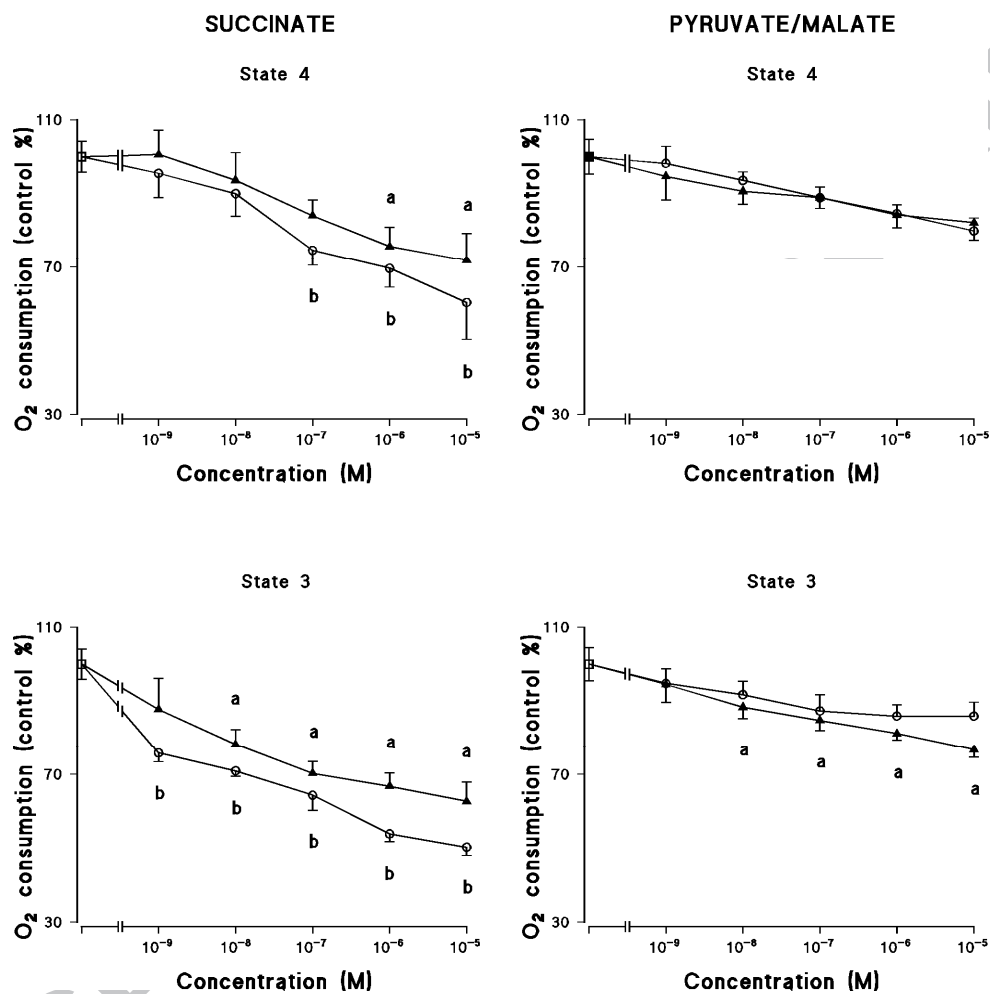


Fig.1

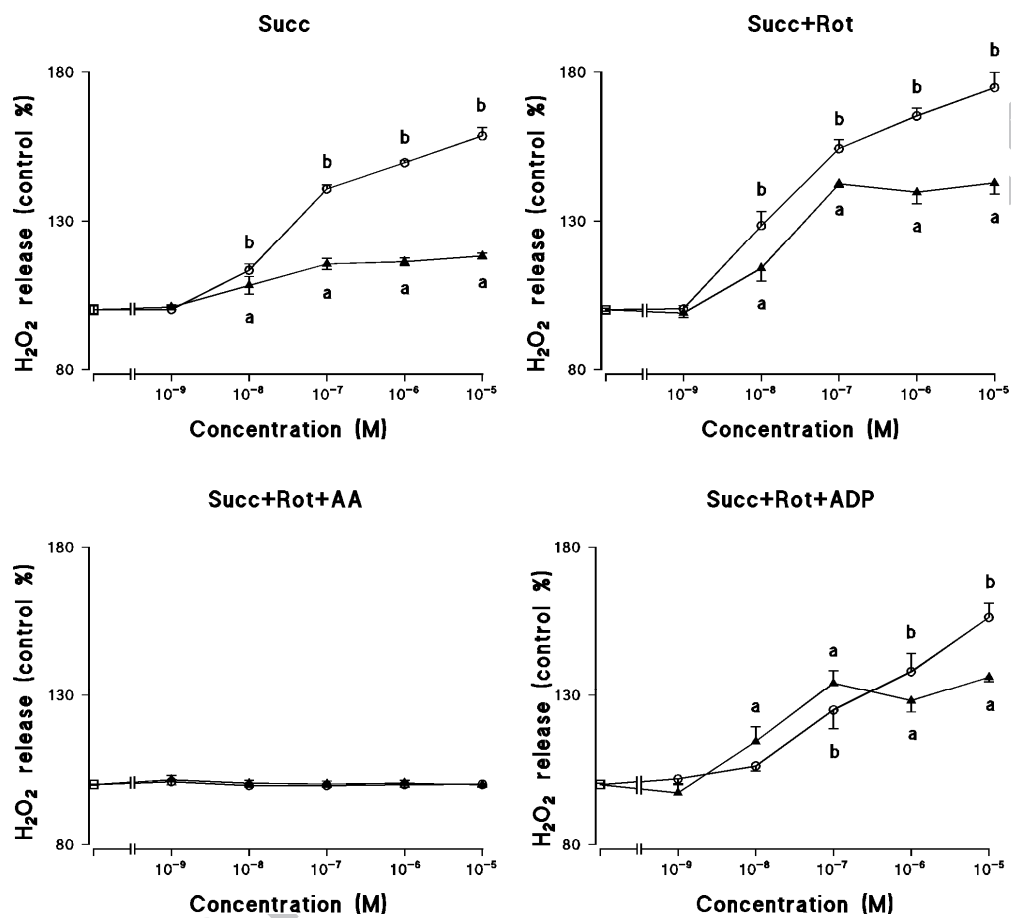


Fig. 2

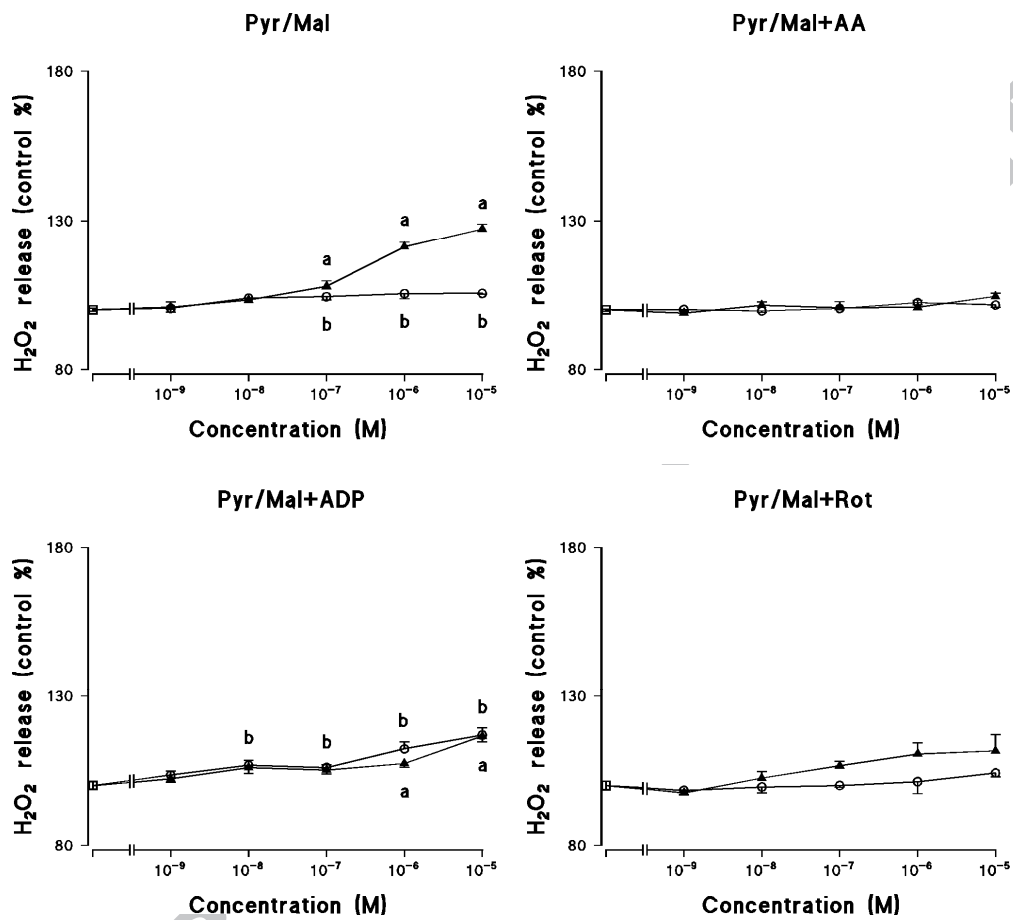


Fig. 3

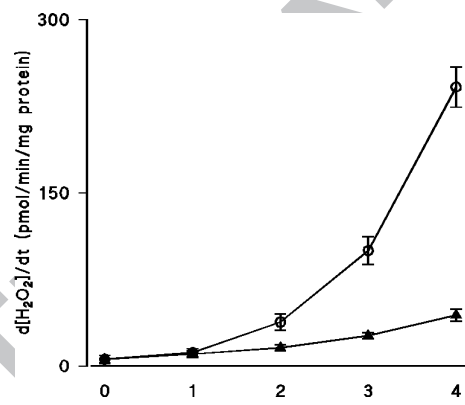
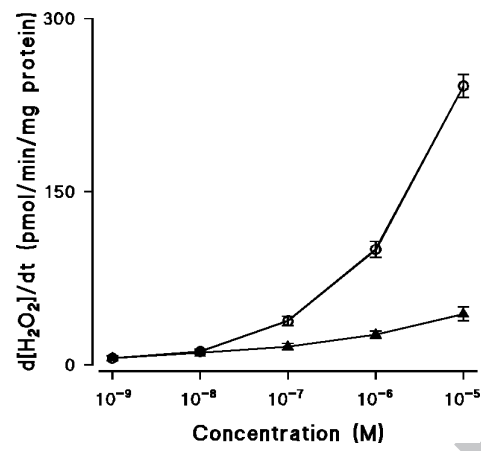


Fig. 4

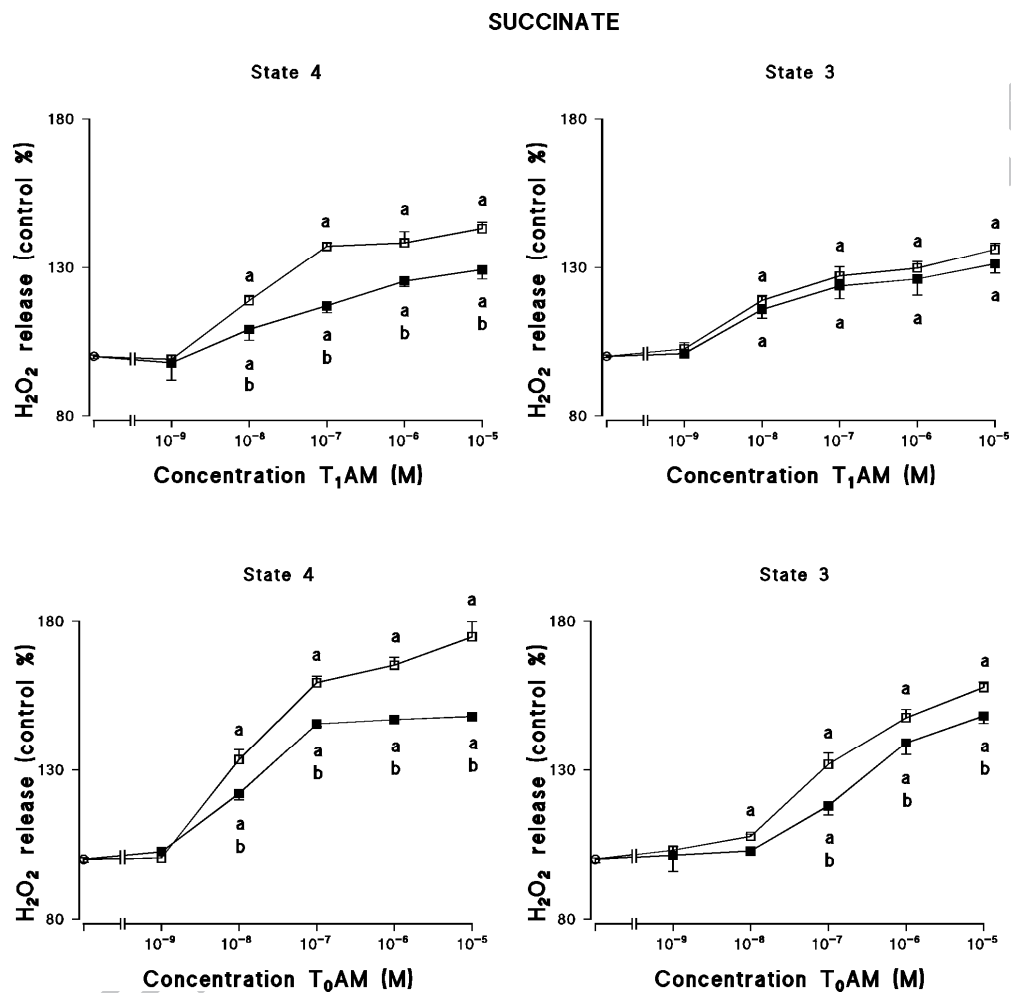


Fig. 5

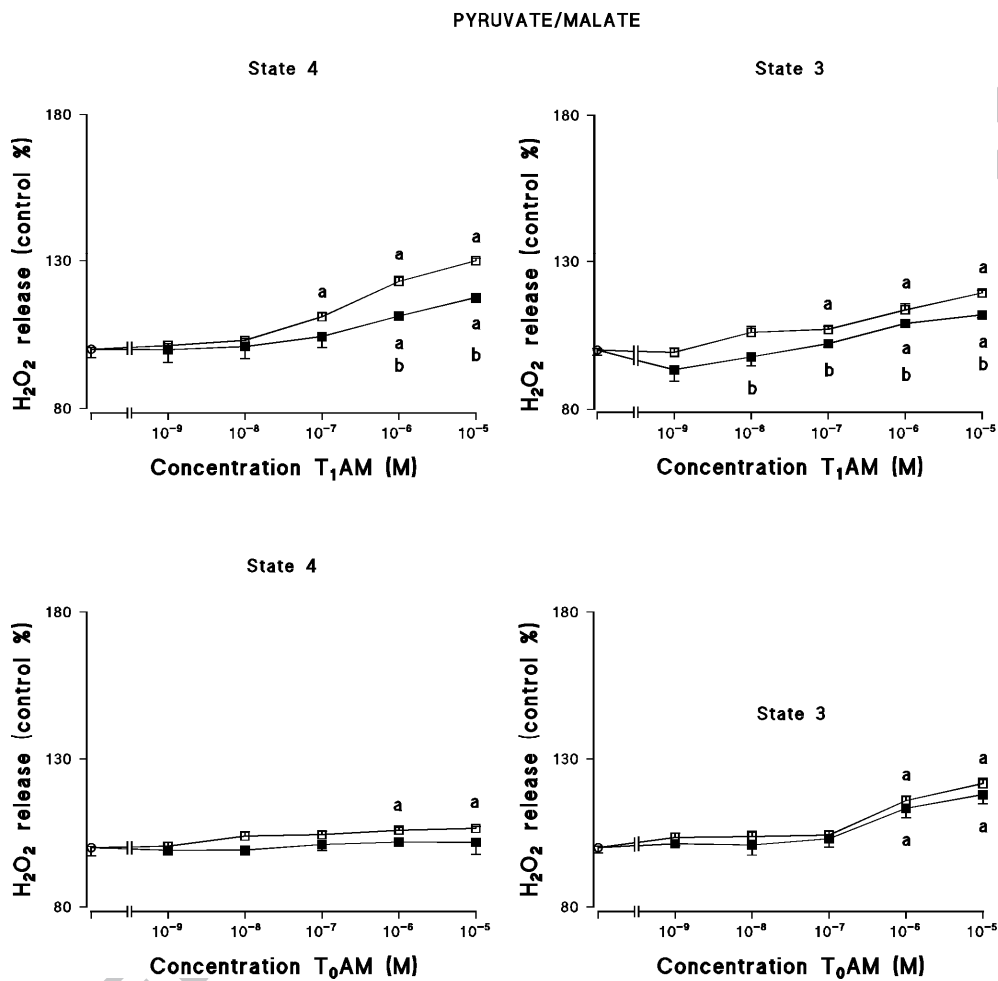


Fig. 6

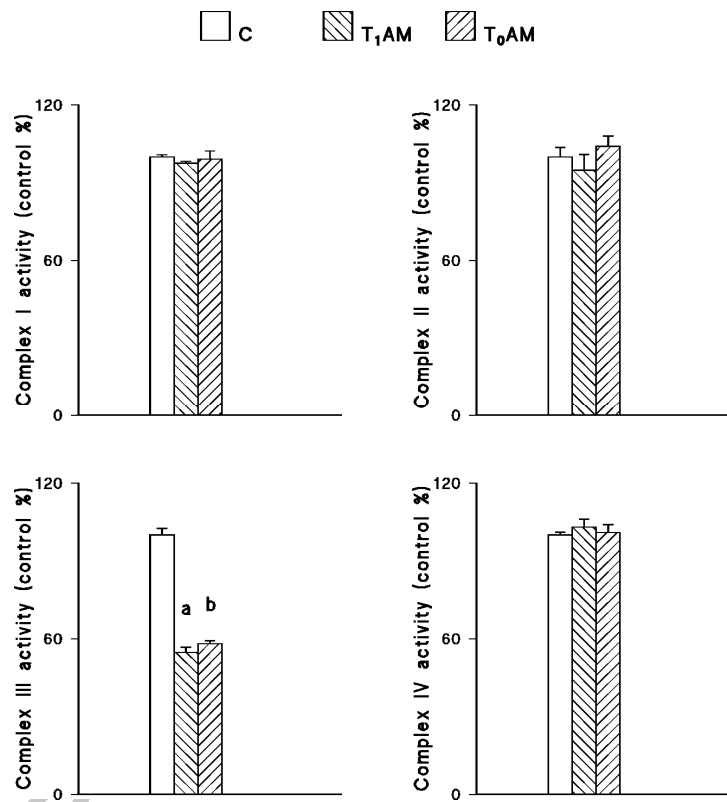


Fig. 7

Highlights

Thyronamines reduce the in vitro mitochondrial oxygen consumption. Thyronamines increase the in vitro mitochondrial hydrogen peroxide oxygen release. Thyronamine are oxidized by mitochondrial monoamine oxydase. Inhibition of monoamine oxydase reduces thyronamine induced increase in H₂O₂ release. Thyronamines reduce the activity of complex III of the respiratory chain.