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Effects of Iodine upon the Structure and Function of Mitochondria

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Abstract

The influence of iodine in its positive and negative monovalent form upon the oxygen consumption in euthyroid and thyroidectomized rats and the oxidative phosphorylation in liver mitochondria isolated from both groups of animals, as well as the spontaneous swelling and total ATPase activity of mitochondria have been studied.

It was established that the administration of ICl increased the oxygen consumption of normal and thyroidectomized rats while under the same conditions no effect was found with NaI . IBr stimulated the oxygen consumption *in vitro* in liver mitochondria isolated both from normal and thyroidectomized rats and decreased the P/O ratio while NaI had no effect. I_2 and IBr increased the swelling and inhibited the ATPase activity of isolated rat liver mitochondria, while these effects were not observed when KI was used. The thyroidstatic 1-methyl-2-mercaptoimidazol decreased the stimulating effect of iodine upon the swelling of mitochondria and to a certain extent lowered its inhibiting effect upon the ATPase activity.

It is concluded that iodine in its positive monovalent form has a thyroxine-like effect upon the structure and function of isolated rat liver mitochondria, as well as *in vivo* upon the respiration of euthyroid and thyroidectomized rats.

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The interrelations between the mitochondria and the thyroid hormones have drawn the attention of the scientists for a long time. High doses of thyroid hormones both *in vivo* and *in vitro* uncouple the processes of oxidative phosphorylation [1-6]. Low physiological doses stimulate to an equal degree the oxygen consumption as well as the esterification of inorganic phosphate which is not accompanied by a change of the coefficient P/O [5]. An increase in the oxygen consumption and a decrease in the P/O ratio was observed *in vitro* under the influence of I_2 and ICN [6]. Iodine enhances the uncoupling effect of the thyroid hormones [5].

One of the sensitive reactions of mitochondria to the action of the thyroid hormones is their swelling. Similar effect was obtained using I_2 and ICN [6, 10, 11]. Iodine inhibits the ATPase activity of mitochondria [12].

It is supposed that only iodine in its positive monovalent form is biologically active [13]. According to Mohnach [13] the iodine atoms in positions 3, 3′ and 5′ in the thyroxine molecule are negatively charged, while the iodine atom in position 5 is positively charged. This iodine atom has the ability to change its redox state ($I^{1+} \rightleftharpoons I^{1-}$) which is probably connected with the hormonal effect of thyroxine. It was suggested that thyroxine associates with the lipids of the cell membrane, whereupon the thyroxine is degraded by a free radical mechanism, resulting in the release of some or all iodine in the forms of $I^{\cdot}$ or I^{+} [14].

The aim of this work was to study the effect of iodine in its positive and negative monovalent form upon the oxygen consumption both in normal and thyroidectomized animals, the reactions of oxidative phosphorylation in mitochondria isolated from both groups of animals, the spontaneous swelling and the total ATPase activity of the mitochondria.

Methods

Thyroidectomized and sham-operated Wistar rats were used in both *in vivo* and *in vitro* studies, but isolated liver mitochondria were used for *in vitro* experiments.

The oxygen consumption of the rats was determined according to a modified method of Kalabuhov in a closed system [15]. The animals were injected intraperitoneally with water solutions of ICI or NaI (25 μg/150 g body weight). The oxygen consumption was measured before the injecting and followed daily up to the fourth day after the injection.

Mitochondria were isolated by differential centrifugation in 0.25 M

sucrose, 0.02 M Tris-HCl buffer and 0.001 M EDTA, pH 7.4 [16]. The oxygen consumption of mitochondria (3 mg mitochondrial protein/ml) was determined according to Warburg's method by mitochondria incubation for 20 min at 28° C in a medium containing 40 μmoles phosphate buffer, pH 7.4, 10 μmoles $MgCl_2$, 3.5 μmoles ADP, 2.5 μmoles NaF, 35 μmoles glucose, 30 μmoles substrate and 0.15 mg hexokinase. The final volume was 2 ml. The phosphorylation was followed by the decrease of the inorganic phosphate in the incubation medium. Glutamate was used as a substrate of oxidation. The degree of swelling of mitochondria, suspended in 0.125 M KCl and 0.02 M Tris-HCl buffer was measured spectrophotometrically by the change of the optical density at 520 nm [17].

The activity of the mitochondrial ATPase (3 mg mitochondrial protein/ml) was determined following the increase of the inorganic phosphate after a 15 min incubation at 37° C in a medium containing 200 μmoles NaCl, 10 μmoles KCl, 10 μmoles $MgCl_2$, 6 μmoles ATP and 40 μmoles Tris-HCl buffer, pH 7.4 [18]. The final volume was 2 ml.

In this work water solutions of iodine in various forms were used: negative monovalent form (NaI and KI), positive monovalent form (ICl and IBr) and I_2.

Results

The data shown in Fig. 1 illustrate the effect of compounds containing positively and negatively charged iodine on the oxygen consumption of rats. The results show that intraperitoneal injection of ICl (iodine in positive form) increases the oxygen consumption of normal animals and a maximum of about 30% was reached on the fourth day after the injection. Similar effect was observed in animals with thyroidectomy, but the maximum was reached on the first day. On the fourth day after ICl injecting the oxygen consumption of the thyroidectomized animals fell down to the initial level. No significant changes in the oxygen consumption were observed both in normal and thyroidectomized animals when NaI was administered (iodine in negative form).

Figure 2 shows the effect of iodine on the oxygen consumption and the oxidative phosphorylation of isolated rat liver mitochondria from normal and thyroidectomized animals. About a 40% increase of the oxygen consumption, accompanied by a decrease of the P/O ratio was found in mitochondria isolated from both groups of animals when 5×10^{-5} M IBr (final concentration) was added to the incubation medium. If NaI was used instead of IBr, this effect was not observed.

The effect of different forms of iodine on the spontaneous swelling of mitochondria is shown in Fig. 3. KI did not change significantly the swelling of mitochondria while it was increased by I_2 and IBr. In this

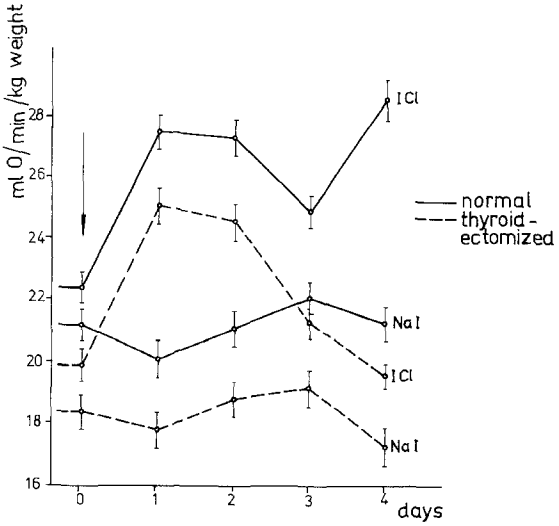

Figure 1. Effect of iodine on the oxygen consumption of rats. The arrow shows the administration of iodine containing compounds.

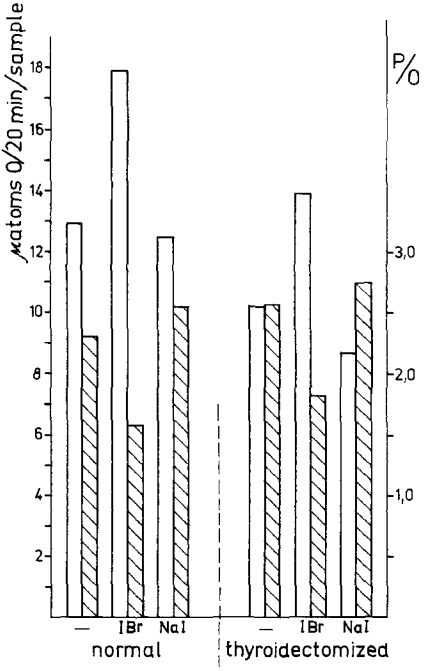

Figure 2. Influence of iodine upon the respiration and oxidative phosphorylation in isolated rat liver mitochondria. □ oxygen consumption; ▨ coefficient P/O.

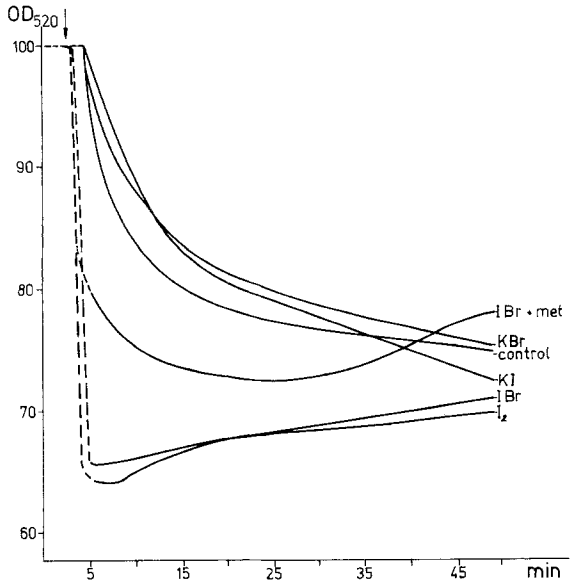

Figure 3. Effect of iodine upon the swelling of isolated rat liver mitochondria. Ordinate — percentage of the optical density compared to the control value (100%). The administration of iodine containing compounds or water is shown by an arrow. Met = 1-methyl-2-mercaptoimidazol.

case a decrease in the optical density of about 35% was observed. The concentration of iodine in these compounds was 5.10^{-4} M. The studies with KBr in the same concentration showed that the anion of bromine did not influence the swelling of mitochondria. It has to be noted that at a 5.10^{-4} M concentration of iodine the initial rapid swelling was followed by a period of gradual shrinking and the optical density reached a level which was quite close to that of the control. The effect of IBr upon the swelling of mitochondria was less evident when the well known thyroidstatic drug 1-methyl-2-mercaptoimidazol was added in equimolar concentration to the medium.

Figure 4 illustrates the effect of iodine upon the activity of mitochondrial ATPase. The activity of the ATPase decreased parallelly with the increasing of the concentrations of I_2 and IBr. The control study with KBr at the same concentrations showed that the bromine anion was inactive. 1-methyl-2-mercaptoimidazol lowered to a certain extent the inhibiting effect of iodine upon the ATPase activity of mitochondria. Under the conditions described KI did not influence the activity of mitochondrial ATPase.

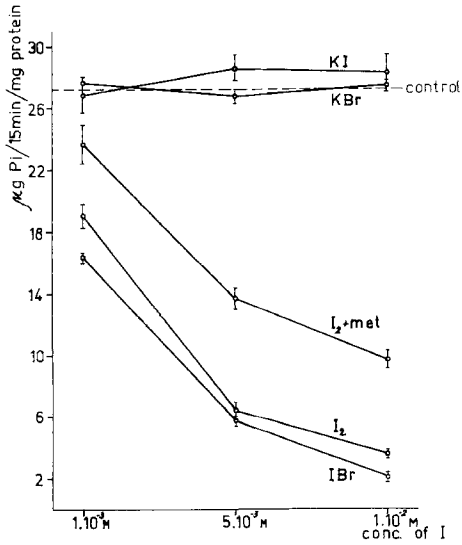

Figure 4. Effect of iodine upon the ATPase activity of isolated rat liver mitochondria. Met = 1-methyl-2-mercaptoimidazol.

Discussion

The experimental data point out that iodine in positive monovalent form (I^{1+}) shows both *in vivo* and *in vitro* biological effects similar to those of the thyroid hormones. The administration of low doses of ICl unlike NaI increased the oxygen consumption in euthyroid and thyroidectomized rats in the next few days. The metabolic effect of ICl on thyroidectomized rats disappeared faster in comparison with the same effect upon unoperated animals and was reduced to the initial level after the second day. It seems the mechanism of ICl action is different in both groups of animals. It is probable that ICl acts directly upon the cell respiratory system in thyroidectomized rats, while in euthyroid animals iodine accumulates in the thyroid gland and stimulates the synthesis and secretion of thyroid hormones, which are responsible for the increased oxygen consumption.

The presence of IBr in the incubation medium uncoupled the processes of oxidative phosphorylation in liver mitochondria isolated from normal and thyroidectomized rats increasing oxygen consumption and decreasing the P/O ratio. These results are in accordance with the experiments of Rall *et al.* [6] which indicated that I_2 and ICN uncouple

the processes of respiration and oxidative phosphorylation in mitochondria.

I_2 and IBr caused a fast swelling of liver mitochondria. This phenomenon is probably connected with the physico-chemical changes in the membrane phospholipids of the mitochondria which interact with iodine. The molecular iodine has a specific effect upon model phospholipid membranes lowering their electric resistance and increasing their permeability [19-21].

I_2 and IBr, similarly to thyroxine inhibited the total ATPase activity of mitochondria while KI had no effect. It seems this inhibitory effect is also due to the positively charged iodine.

The data of the identical effects of I_2 and IBr upon the swelling and the ATPase activity of mitochondria and on the other hand the lack of any effect of KI make it possible to suggest that the positively charged iodine is the active component of the iodine molecule too.

1-methyl-2-mercaptoimidazol lowered the stimulatory effect of iodine upon the swelling of mitochondria and decreased to a certain extent its inhibitory effect upon ATPase activity. The action of this thyroidstatic drug is probably connected with an acceptance of iodine in the imidazol nucleus.

The molecular mechanism by which the iodine compounds affect the structure and function of the mitochondria is unclear.

The interaction between the iodine ions and the lipoprotein components of the mitochondrial membrane results in physico-chemical alterations of the latter, which are related to phenomena as swelling of mitochondria, increase of the oxygen consumption, uncoupling of the oxidative phosphorylation and decrease of the ATPase activity. These changes occur most probably as a result of a decrease in the resistance and the biopotentials of the mitochondrial membrane accompanied with an increase of its permeability and conductivity.

Using lecithin bimolecular membranes it has been established that iodine and the charged groups of lecithine form donor - acceptor complexes with charge transfer and an increase in conductivity [19, 21, 22, 24]. In these complexes the lecithin molecules act as donors, and the iodine atoms as acceptors of electrons [24-27]. The resistance of the phospholipid membrane decreases in the presence of I_2, which dissociates into labile I^- and such bound in a complex lecithine. I^+ [28]. The conductivity of the phospholipid membrane increases with the increase of I_2 concentration [29, 29a]. At a concentration of 1.10^{-5} M a decrease of the membrane resistance by three orders of magnitude is observed, while I^- in the same concentration has a negligible effect [29].

It has been shown that when lecithin or cholesterol-palmitin membranes are placed in contact with vapours of I_2, the energy of activation of the membrane decreases and its conductivity increases [23].

According to the Chemiosmotic hypothesis the decrease of the membrane potential leads to uncoupling of the oxidative phosphorylation [30]. The uncoupling agents increase the proton conductivity of both the mitochondrial membrane [31, 32] and the artificial bimolecular phospholipid membranes [33, 34]. These compounds increase the permeability of the phospholipid micelle to H^+ or OH^- [35] and decrease the resistance of some artificial membranes [36].

The uncoupling of the oxidative phosphorylation may be caused either by a decrease of the membrane potential, by protonation of the nucleophylic groups participating in the reactions of coupling or by transfer of charges across the membrane, which interfere with the coupling mechanisms [37].

In the mitochondrial membrane (like in other cellular membranes) the fixed negative charges predominate, due to which the membrane components interact more readily with I^+ than with I^-. In this way the difference between the action of I^+ and I^- may be explained. I^+ is a strong oxidizer and should interact rapidly with the negatively charged groups of the membrane.

Neutralization of the charges on the mitochondrial membrane facilitates the transfer also of ions, including I^-.

The similarity between the effects of iodine compounds and the thyroxine observed *in vivo* and *in vitro* gives us reason to suggest that:

1. De-iodination of the thyroid hormone constitutes a necessary step in the realization of its effect upon the structure and function of mitochondria.
2. The active components in the molecule of the thyroid hormone, as regards its biochemical effect, are the iodine atoms.
3. The possible places of interaction between the membrane and the iodine ions (I^+) are the negative charges fixed in the membrane.
4. Neutralization of the charges of the mitochondrial membrane is the major event leading to changes in its resistance, conductivity, permeability and coupling mechanisms of the oxidative phosphorylation.

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