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Iodolactones and iodoaldehydes – mediators of iodine in thyroid autoregulation

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Summary: Within the last decades multiple iodolipid-classes have been identified in thyroid tissue. For a long time they have been supposed to be involved in thyroid autoregulation, but for the time being no specific compounds could be isolated. A new approach was stimulated by the finding that thyroid cells were able to iodinate polyunsaturated fatty acids to form iodolactones and by the identification of α-iodohexadecanal (α-IHDA) as the major compound of an iodolipid fraction. α-IHDA exerts multiple inhibitory effects on adenylate cyclase, NADPH-oxidase and thyroid peroxidase. Therefore, it is speculated as a mediator of the Wolff-Chaikoff-effekt and to be involved in the autoregulation of specific thyroid functions mediated by the cyclic adenosine-3′,5′-monophosphate (cAMP)-pathway. Meanwhile 6-iodo-5-hydroxy-8,11,14-eicosatrienoic acid δ-lactone (δ-iodolactone) has been identified in human thyroid tissue and it could be demonstrated that this iodoeicosanoid specifically inhibits signal transduction pathways induced by local growth factors such as epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Therefore, δ-iodolactones seem to act as mediators of iodine, especially in the autoregulation of cAMP-independent thyroid cell proliferation. We will summarize these important new findings and discuss the role of these iodolipids on thyroid cell growth regulation.

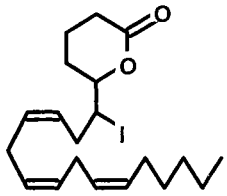
Zusammenfassung: In den vergangenen Jahrzehnten wurden verschiedene Iodlipidklassen in Schilddrüsengewebe nachgewiesen. Sehr bald wurde vermutet, daß sie an der Autoregulation der Schilddrüse beteiligt sind, jedoch konnten zunächst keine spezifischen Substanzen isoliert werden. Erst die Beobachtung, daß Schilddrüsenzellen mehrfach ungesättigte Fettsäuren unter der Bildung von Iodlaktonen umsetzen, sowie die Identifizierung des Iodaldehyds α-Iodhexadecanal (α-IHDA) als größte Fraktion innerhalb einer Iodlipidfraktion, erbrachte neue Anhaltspunkte für die weitere Erforschung. Es konnte gezeigt werden, daß α-IHDA mehrere inhibitorische Wirkungen auf die Adenylatzyclase, die NADPH-Oxidase und die schilddrüsenspezifische Peroxidase, besitzt. Deshalb wird es als Mediator des Wolff-Chaikoff-Effekts, sowie der Inhibition spezifischer Schilddrüsenzellfunktionen die durch das zyklische Adenosin-3′,5′-Monophosphat (cAMP) vermittelt werden, diskutiert. Als Hauptprodukt der Arachidonsäure-Iodierung wurde das δ-Lakton der 6-Iod-5-Hydroxy-8,11,14-Eicosatriensäure (δ-Iodlakton) in Schilddrüsengewebe nachgewiesen und es konnte gezeigt werden, daß dieses Iodeicosanoid spezifisch die durch lokale Wachstumsfaktoren, wie EGF und bFGF, induzierte Signaltransduktionmechanismen inhibiert. Deshalb scheinen δ-Iodlaktone als Mediatoren von Iod spezifisch an der Regulation der cAMP-unabhängigen Schilddrüsenzellproliferation beteiligt zu sein. Wir fassen diese neuen Ergebnisse zusammen und diskutieren die Beteiligung dieser Iodlipide an der Regulation des Schilddrüsenwachstums.

The regulation of thyroid metabolism by iodide involves numerous inhibitory effects. One of which is a decrease in cylic adenosine-3′,5′-monophosphate (cAMP) formation, this in turn results in an inhibition of all cAMP-mediated effects on the gland. In addition to the cAMP pathway there are other signal transduction mechanisms summarized as cAMP-independent mechanisms. One of them is the phosphatidylinositol (PI) cascade, which is also under the negative control of iodide. Because they are abolished by methimazole or propylthiouracil, most of the inhibitory effects induced by iodide depend on the organification step. For this reason organic iodocompounds have been postulated as mediators (for review see Wolff, 1989). Since thyroid cell proliferation as well as thyroid cell differentiation are reciprocally regulated by the cAMP and the PI cascades, at least two different mediators have been postulated to act on these two signal transduction pathways (Gärtner et al., 1985). The PI cascade enhances thyroid cell proliferation and leads to dedifferentiation, while the cAMP pathway upregulates specific thyroid functions and directly decreases thyroid cell proliferation, although in some thyroid in vitro-systems cAMP clearly stimulates proliferation. The main stimulus for thyroid cell proliferation seems to be mediated by local growth factors such as epidermal growth factor

(EGF) and basic fibroblast growth factor (bFGF) which act via tyrosin kinase receptors and the PI cascade (Gärtner et al., 1988). Therefore, the organic iodocompound that mediates the inhibitory effect of iodide on the PI cascade seem to be especially involved in thyroid growth regulation.

Thyroid iodolipids

Iodocompounds other than thyroid hormones were detected in thyroid homogenates more than 40 years ago. The physiological role was unknown but suggested to be involved in thyroid autoregulation (Taurog et al., 1956; Shah et al., 1973). Radio-iodine incorporation studies could resolve the nature of these iodocompounds by the use of chromatographic methods and they were classified as polar non-phosphatide lipids, neutral lipids and iodinated fatty acids (Shah et al., 1973; Chazenbalk et al., 1985). Specific compounds were identified when highly sensitive methods such as gas chromatography-mass spectrometry and nuclear magnetic resonance were used. Gas chromatography coupled with tandem mass spectrometry allowed the identification of 6-iodo-5-hydroxy-8,11,14-eicosatrienoic acid δ-lactone (δ-iodolactone, formula see fig. 1) in human thyroid tissue that has been treated in vivo with high concentrations of iodine before thyroidectomy (Dugrillon et al., 1994). This iodoeicosanoid had been identified as the major product of in vitro iodination of arachidonic acid by thyroid tissue (Boeynaems and Hubbard, 1980) and follicles (Dugrillon et al., 1990). The major product of non-polar lipid iodination in vitro has been identified as α-iodohexadecanal (α-IHDA, formula see fig. 1) by Pereira et. al, 1990. This compound is probably formed by the addition of iodine to the vinyl ether group of plasmalogens, although, its occurence in vivo has yet to be proven. An overview of thyroid iodolipid identification is given in table 1.



6-iodo-5-hydroxy-8,11,14-eicosatrienoic acid δ-lactone (δ-iodolactone)

α-iodohexadecanal (α-IHDA)

Fig. 1 Structural formulae of the major iodolactone and iodoaldehyde

Iodolipid effects

The participation of iodolipids in the regulation of thyroid autoregulation has been suggested since their discovery (Taurog et al., 1956; Shah et al., 1973). Since the formation of iodolipids has been related to the arachidonic acid metabolism and some iodolipids have been identified as free fatty acids (Chazenbalk et al., 1985), a crude fraction of iodinated derivatives of arachidonic acid has been tested on the iodine metabolism of calf thyroid tissue. These investigations show that iodolipids mimic the effects of iodide on thyroid iodine uptake and organification (Krawiec et al., 1988; Chazenbalk et al., 1988). An overview of iodolipid testing is given in table 2.

From investigations on thyroid cell proliferation it is known that iodide inhibits proliferation and that arachidonic acid derivatives participate in the regulation of thyroid cell proliferation (Gärtner et al., 1984). Therefore, the main product of arachidonic acid iodination by thyroid tissue, the δ-iodolactone, has been further investigated. Its formation from exogenous arachidonate in isolated porcine thyroid follicles was confirmed. It could be demonstrated that δ-iodolactone inhibited EGF-induced proliferation of isolated porcine thyroid follicles without requirement of further organification in a concentration range 50-fold lower compared to iodide. δ-Iodolactone had no effect on cAMP formation and seemed to be exclusively involved in cAMP-independent growth control. This has been confirmed by the findings that δ-iodolactone inhibits inositol-1,4,5-trisphosphate (IP_3) formation induced by EGF and its specificity has been proved as another iodolactone, 5-iodo-4-hydroxy-7,10,13,16,19-docosapentaenoic acid γ-lactone (γ-iodolactone), had no effect on thyroid cell proliferation and on IP3 formation. These results demonstrate an action of iodide and of δ-iodolactone at the calcium-dependent signal transduction mechanism modulating thyroid cell proliferation induced by EGF. δ-Iodolactone acts at or proximal to the generation of IP_3, whereas the TSH-dependent signal transduction seems to be unaltered. Therefore it has been speculated as a specific inhibitory mediator of iodide on growth factor-induced thyroid cell proliferation (Dugrillon et al., 1990; Dugrillon and Gärtner, 1995).

In order to further characterize the inhibitory effects of iodolactones in vivo, rats were treated with either δ-iodolactone or 14-iodo-15-hydroxy-5,8,11-eicosatrienoic acid ω-lactone (ω-iodolactone) simultaneously with a goitrogen or after goitrogen-inducet thyroid enlargement. The iodocompounds were either i.p. or p.o. Both compounds cause significant dose-related decrease of thyroid weight, with oral administration being less effective. Several serum parameters such as total T_3 and T_4 demonstrate no change during acute administration (Pisarev et al., 1994). These results give further support to the role of δ-iodolactone in growth regulation.

α-IHDA, which has been identified as major iodolipid in horse thyroid in vitro (Pereira et al., 1990), has been shown to inhibit NADPH-dependent H_2O_2 generation in porcine thyroid plasma membranes and in canine thyroid cells (Ohayon et al., 1994; Panneels et al., 1994a; Panneels et al., 1994b). It seems clear that this compound is responsible for the well-known Wolff-Chaikoff-effect by inhibition of NADPH oxidase as well as thyroid peroxidase. Further inhibitory effects at higher concentrations of α-IHDA were observed on TSH-induced cAMP and carbachol-induced IP_3 formation. No growth suppressing effect could be demonstrated by measuring the thymidine incorporation into canine thyroid cells.

Table 1 Reported iodolipid biosynthesis and identification in thyroid tissue and cells

Authors	System	Substance identified	Methods and remarks
Taurog et al., 1954 Taurog et al., 1956	sheep thyroid homogenates	iodolipid "Unknown I"	by radioiodine incorporation and TLC
Shah et al., 1973	human thyroid tissue	polar non-phosphatide lipid fraction	by radioiodine incorporation and TLC
a) Boeynaems and Hubbard, 1980 b) Boeynaems et al., 1981	rat thyroid lobes	a) 6-iodo-8,11,14-eicosatrienoic acid δ-lactone (δ-iodolactone) b) 5-iodo-7,10,13,16,19-docosapentaenoic acid γ-lactone (γ-iodolactone)	by radioiodine incorporation and HPLC as well as by GCMS; exogenous supplementation of a) arachidonic acid and b) docosahexaenoic acid
Chazenbalk et al., 1985	calf thyroid slices	iodinated fatty acids and neutral lipids	by radioiodine incorporation and TLC
Pereira et al., 1990	horse thyroid	α-iodohexadecanal (α-IHDA)	by radioiodine incorporation and HPLC as well as by GCMS and 1H-NMR
a) Dugrillon et al., 1990 b) Dugrillon et al,. 1994	a) porcine thyroid follicles b) human thyroid tissue	6-iodo-8,11,14-eicosatrienoic acid δ-lactone (δ-iodolactone)	a) by GCMS; exogenous supplementation of arachidonic acid b) by GCMSMS; treated with iodine in vivo before thyroidectomy

Table 2 Reported effects of iodolipids on thyroid growth and function

Authors	System	Substances tested	Main effects
Chazenbalk et al., 1984 Krawiec et al., 1988 Chazenbalk et al., 1988	calf thyroid slices	semi-purified preparation of iodoarachidonates	• decreased I-uptake • decreased PBI-formation (due to decreased H_2O_2 availability)
Pisarev et al., 1988	rats	14-iodo-15-hydroxy-5,8,11-eicosatrienoic acid (I-OH-A); 14-iodo-15-hydroxy-5,8,11-eicosatrienoic acid ω-lactone (ω-iodolactone); 6-iodo-8,11,14-eicosatrienoic acid δ-lactone (δ-iodolactone)	• prevented MMI-induced goiter
Dugrillon et al., 1990	porcine thyroid follicles	δ-iodolactone	• inhibited cell proliferation • no inhibition of cAMP formation
Krawiec et al., 1991	calf thyroid slices	I-OH-A; ω-iodolactone	• action at the plasma membrane level (decreased DOG- and AIB-uptake)
Pisarev et al., 1992	FRTL-5	ω-iodolactone; δ-iodolactone	• inhibited cell proliferation
Ohayon et al., 1994 Panneels et al., 1994a Panneels et al., 1994b	human and porcine thyroid membranes; dog thyroid cells	α-iodohexadecanal (α-IHDA)	• decreased NADPH-oxidase and TPO activity • decreased cAMP formation
Pisarev et al., 1994	rats	ω-iodolactone; δ-iodolactone	• involution of methimazole-induced goiter
Dugrillon et al., 1994 Dugrillon and Gärtner, 1995	human and porcine thyroid follicles	δ-iodolactone	• inhibited cell proliferation (no effects with a γ-iodolactone) • no inhibition of cAMP formation • inhibited EGF-induced IP_3 formation

Discussion

Today, two iodolipid families are discussed as mediators of iodine in thyroid autoregulation, iodinated derivatives of polyunsaturated fatty acids (iodolactones) and iodoaldehydes. Both groups of substances seem to differ in the amounts occuring in thyroid tissue as well as in their specific functions.

α-IHDA (α-iodohexadecanal) is the major iodolipid formed after iodide administration and together with other iodoaldehydes it seems to be involved in the regulation of specific thyroid functions as well as the Wolff-Chaikoff-effect. The decrease of adenylate cyclase acticity by α-IHDA might also suggest its participation in the regulation of cell proliferation, but it failed to decrease TSH-induced DNA formation in canine thyroid cells (Panneels et al., 1994b). Furthermore it has yet to be identified in vivo. For these reasons the possible role of α-IHDA in thyroid growth autoregulation remains debatable.

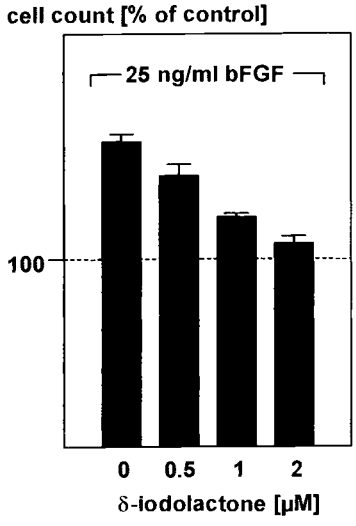


Fig. 2 Inhibitory effect of d-iodolactone on quiescent FRTL-5 cells stimulated with 25 ng/ml of bFGF. The cells were plated in the presence of 1.0 U/l TSH. After a 48 h resting phase without TSH, the cells were stimulated with bFGF and δ-iodolactone as indicated. The cell counts were determined after a stimulation period of 72h using a hemocytometer an expressed as means ± SD of triplicates (unpublished data, explanations of abbreviations see text).

δ-Iodolactone (6-iodo-5-hydroxy-8,11,14-eicosatrienoic acid δ-lactone) is the major compound of arachidonic acid iodination in thyroid tissue and has been identified in human tissue. The structural specificity, the selectivity for the inhibition of the EGF-induced signalling pathway, and the potency of δ-iodolactone are features which suggest an important role in the regulation of thyroid cell proliferation. In Fisher Rat Thyroid cell-Line (FRTL)-5 δ-iodolactone failed to exert these effects with such clearity (Pisarev et al., 1992). These experiments were performed under continuous stimulation of TSH and the cAMP pathway. We recently demonstrated that the δ-iodolactone already exerts all the previously described features on quiescent FRTL-5 cells stimulated by bFGF (unpublished data, fig. 2). These results again indicate that δ-iodolactones specifically inhibit cAMP-independent proliferation and make the FRTL-5 cells a suitable model to explore iodolipid effects.

Despite these convincing data, it is still uncertain, whether the formation of iodolipids in the basolateral membrane is dependent on specific peroxidases or if it is a more or less unspecific reaction. The thyroid specific peroxidase is located at the apical membrane, whereas the phospholipase C as well as the adenylate cyclase, which probably are the sites of action, are located at the apical membrane. Since the inhibitory effects of iodide are abolished in the presence of thionamides, one have to postulate that the iodocompounds responsible for these inhibitory effects are dependent on peroxidation. Whether this applies to the iodolactones as well as to the iodoaldehydes has yet to be confirmed. Further studies are necessary to clarify this important point.

Clinical importance may result from decreased iodolipid synthesis activity and from the essential nature of fatty acid precursors of iodolactones. Goitrogenesis and even tumorigenesis may be related to a relative δ-iodolactone deficiency and may be treated with iodide together with essential fatty acids or with δ-iodolactone by itself. From epidemiological point of view the nutritional variation in essential fatty acid ingestion may influence the iodolactone pattern synthesizend within the thyroid gland. Whether this is an explanation of variations in goiter prevalence is to be clarified.

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