Molecular and Cellular Endocrinology 317 (2010) 141–147

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Inhibition of goiter growth and of cyclic AMP formation in rat thyroid by 2-iodohexadecanal

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ARTICLE INFO

Article history:
Received 13 October 2009
Received in revised form
11 December 2009
Accepted 18 December 2009

Keywords:
Thyroid
Iodolipids
Antigoitrogenics

ABSTRACT

Introduction: Thyroid autoregulation has been related to intraglandular content of an unknown putative iodocompound. The thyroid is capable of producing different iodolipids such as 6-iodo-deltalactone (ILδ) and 2-iodohexadecanal (2-IHDA). Data from different laboratories have shown that these iodolipids inhibit several thyroid parameters. ILδ has an antigoitrogenic action but no data about the action of 2-IHDA on this parameter has been published.

Objectives: to study the action of 2-IHDA on methimazole (MMI)-induced goiter and analyze if this compound can cause the involution of preformed goiter.

Results: Administration of MMI to rats during 10 days increased thyroid weight by 112%. This effect was significantly inhibited by the simultaneous injection of 20 μg/day of 2-IHDA (51% vs. MMI) while iodine or non iodinated hexadecanal were without action. Thyroidal proliferating cell nuclear antigen (PCNA) content was increased by MMI while 2-IHDA decreased this value (control: 100%; MMI: 190 ± 11; MMI + 2-IHDA: 134 ± 10). Serum TSH was increased after MMI administration and 2-IHDA did not modify this parameter (control: 1.89 ± 0.10; MMI: 8.19 ± 0.93 ng/ml; MMI + 2-IHDA: 7.38 ± 0.72). Treatment with MMI increased thyroidal cAMP content (control: 16.1 ± 0.82, MMI: 42.4 ± 4.6 fmol/mg protein) while injection of 2-IHDA significantly decreased this value (22.3 ± 2.0). Goiter prevention by 2-IHDA was also observed at 30 days of treatment reducing total number of cells (51% inhibition) and epithelial height (81% inhibition).

Goiter involution was induced after withdrawal of MMI and injection with 2-IHDA, KI or saline. 2-IHDA led to a reduction of 74.5% in thyroid weight after 3 days while spontaneous involution (saline) was only of 32%. KI failed to alter this value. This significant involution was accompanied by a reduction in the number of cells (66%).

Administration of the iodolipids did not produce significant changes in several serum parameters such as total T_3 and T_4, cholesterol, transaminases, urea and creatinine.

Conclusion: 2-Iodohexadecanal, as 6-iodo-deltalactone, prevents goiter growth in rats and opens a potential therapeutic application of iodolipids.

1. Introduction

Although thyroid gland function is mainly under the control of pituitary TSH, other factors may also play a role in this process (Dumont et al., 1992). Iodine is not used only by the thyroid to synthesize thyroid hormones but also directly influences a number of thyroid parameters such as thyroid proliferation and function (Pisarev and Gartner, 2000; Panneels et al., 2009). Bray (1968) showed that thyroids from rats fed with iodine-deficient diet were more sensitive to TSH. Goiter produced experimentally by thyouracils or low iodine diet may develop before an increase in serum TSH is detected (Berthier and Lemarchand-Beraud, 1978; Matsuzaki et al., 1978) explaining why in some areas goiter coexists with normal levels of serum TSH and T_4 (Pisarev et al., 1970). Moreover the administration of KI reduced the goitrogenic action and the stimulation of thyroid protein synthesis induced by TSH, cAMP and cGMP in mice (Pisarev and Itoiz, 1972). Thyroid autoregulation may be defined as the capability of the intracellular content of iodide to modulate the gland function and its response to other regulatory factors. Iodine must be organified in order to exert its

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doi:10.1016/j.mce.2009.12.026

effect (Van Sande et al., 1975). The nature of this/these iodocompound/s is still unknown. Several candidates have been suggested as intermediates such as an iodoprotein (Lissitzky et al., 1961) or T_3 (Juvenal and Pisarev, 1981), but their possible role remains doubtful. It was shown that, besides thyroid hormones, other iodinated compounds, iodinated lipids, are biosynthesized by the thyroid gland. Among these, 6-iodo-5-hydroxy-8,11,14-eicosatrienoic acid δ-lactone (ILδ) and 2-iodohexadecanal (2-IHDA) have shown to inhibit several thyroid parameters and its participation in thyroid autoregulation has been suggested (Pisarev and Gartner, 2000; Panneels et al., 2009).

The occurrence *in vivo* was demonstrated for ILδ in thyroid tissue from a patient with Graves' disease treated with iodide (Dugrillon et al., 1994), although the formation of ILδ in other species could not be detected unless exogenous arachidonic acid was added (Boeynaems and Hubbard, 1980; Pereira et al., 1990). The synthesis of 2-IHDA derived from plasmalogens, the major iodolipid formed in horse thyroid, was demonstrated by Pereira et al. (1990). This iodolipid was also detected in the thyroid of rats and dogs (Panneels et al., 1996) and in beef thyroid (our unpublished results).

ILδ, omega lactone (ILω) and the free acid 14-iodo-15-hydroxy-5,8,11-eicosatrienoic acid (I-OH-A) inhibit several thyroid parameters such as cell proliferation (Pisarev et al., 1988; Dugrillon et al., 1990), iodide uptake (Chazenbalk et al., 1988), H_2O_2 production (Krawiec et al., 1988), goiter growth (Pisarev et al., 1988) and inositol-1,4,5-triphosphate (IP3) formation (Dugrillon and Gartner, 1995). On the other hand, 2-IHDA inhibits NADPH oxidase (Ohayon et al., 1994), H_2O_2 production (Panneels et al., 1994a) and adenylate cyclase (Panneels et al., 1994b).

No effects of 2-IHDA in *in vivo* systems have been studied. Therefore the objective of this work, was to analyze the possible antigoitrogenic effect of 2-IHDA, to analyze wether this compound can cause the involution of preformed goiter and to determine its possible side effects.

2. Materials and methods

2.1. Animals

Female Wistar rats (90–120 bw) were employed in these studies. The animals were kept on regular diet and water *ad libitum*. They were cared under the NIH guidelines for experimental animals.

2.2. Goiter induction

Goiter was induced by the administration of MMI, 5 mg/day/100 g bw in 0.2 ml of saline for variable periods of time, as will be indicated. The test substances were injected ip at the same time. At the end of each experiment the animals were sacrificed. Blood was obtained and serum was separated. Thyroid gland weight was expressed per 100 g of bw and the percent inhibition of goiter was calculated as follows:

$$\%\ \text{variation} = \frac{1 - (\text{experimental} - \text{control})}{(\text{MMI} - \text{control})} \times 100$$

2.3. Involution of performed goiter

The rats were distributed into two groups: (a) solvent-treated and (b) injected with MMI, 5 mg/day for 10 days, i.p., to induce goiter. The treatment with the goitrogen was then discontinued and the rats were distributed into the following subgroups:

(b1) without further treatment (saline); (b2) 20 μg/day of 2-IHDA, (b3) 20 μg/day of KI both i.p. The animals were killed 3, 7 and 10 days after this last treatment. The ratio thyroid weight mg/body weight (g) × 100 was determined.

2.4. Morphological analysis

Thyroid glands were fixed with 10% neutral-buffered formalin, embedded in paraffin and cut in 5 μm sections for histochemical studies. Another group was frozen at −80 °C for western blot analysis. Sections were stainedwith hematoxylin–eosin.

Morphological changes induced by the different treatments were analyzed by light microscopy and pictures were taken using a digital camera (Sony U-LH100HGAPO). The sections were analyzed using Image J Software (1.40 g Wayne Rasband, National Institute of Health, USA).

The morphometric measurements: numbers of epithelial cells, epithelial height and lumen area were quantified in one section crossing the centre of the lobe, considered to be representative of the entire gland. All measurements were done at a magnification of 100×.

2.5. Western blot analysis

Thyroid glands were washed with cold PBS and lysed in buffer RIPA (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% Nonidet P40, 0.1% SDS, 0.5% deoxycholate) plus PMSF 0.5 mM, benzamidine 2.5 mM, aprotinin 10 μg/ml, leupeptin 1 μg/ml, pepstatin 1 μg/ml.

Proteins (50 μg) were electrophoresed on 10% polyacrylamide gels and transferred to nitrocellulose membranes. Immunoblotting was carried out with polyclonal anti PCNA antibodies (dilution 1:500, Santa Cruz Biotechnology, USA) in phosphate buffer saline solution (PBS) with 0.2% Tween 20 (Sigma–Aldrich, St. Louis, MO) and 5% BSA. Immunochemical detection was performed using a specific antibody and anti rabbit IgG antibody conjugated with peroxidase. Specific proteins were visualized using the enhanced chemiluminescence method (Amersham Pharmacia Biotech, USA). Densitometric analysis was performed using the NIH ImageJ analysis Software (1.40 g Wayne Rasband, National Institute of Health, USA) and results were corrected for protein loading by normalization for β-actin expression.

2.6. Cyclic AMP determination

Thyroid glands were homogenized immediately after sacrifice in 1 ml of ethanol with an Ultra-Turrax tissue homogenizer, in ice. The suspension was centrifuged and cyclic AMP was determined in the supernatant using the radioimmune assay system (RIA) by the method described by Steiner et al. (1969) with some modifications (Del Punta et al., 1996). The antibody used was provided by the NIH (National Hormone and peptide program, Dr. A.F. Parlow).

2.7. Serum determinations

T_3 and T_4 were measured by RIA. Thyrotropin (TSH) was determined in duplicate by RIA using the kits provided by the NIDDKD (NIH, Bethesda, Md, USA). Results are expressed in terms of rTSH-RP-3 (rat TSH reference Preparation 3). Intra and interassay coefficients of variation for TSH were 8.7% and 13.4%, respectively.

The serum was separated and used to determine cholesterol, alanine transaminases (ALT), aspartate transaminases (AST), urea and creatinine using standard methods.

2.8. Materials

All reagents were purchased from Sigma Chemical Co (St. Louis, MO). ILδ was synthesized and purified as already described (Pisarev et al., 1988) and 2-IHDA was prepared according to the procedures described by Ohayon et al. (1994).

2.9. Statistical analysis

Statistical analysis was performed using ANOVA followed by Student–Newman–Keuls test for multiple comparisons. Data are expressed as mean ± S.E.M. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Prevention of goiter growth

Body weight of the rats did not show significant changes under the different treatments (results not shown). As shown in Table 1 rats treated with KI, ILδ or 2-IHDA did not show any change in the weight of their thyroids compared to controls. Administration of MMI, 5 mg during 10 days caused a significant increase in thyroid weight by 112%. Daily injections of 2-IHDA, 1 or 5 μg, failed to alter significantly thyroid weight, but at a dose of 10 and 20 μg/day it reduced the goitrogenic action of MMI by 40% and 51%, respectively. ILδ at 20 μg/day caused an inhibition of 48% while KI and hexadecanal (HD), the precursors in the *in vitro* synthesis of 2-IHDA, were without effect. For the following studies a concentration of 20 μg/day of both compounds (2-IHDA and ILδ) was employed.

Table 1
Prevention of MMI-induced goiter in the rat: 10 days of treatment.

Treatment	Thyroid wt (mg)	Body wt (g)	Thyroid wt (mg)/body wt (g) × 100	% Inhibition (vs. MMI)
Control	11.8 ± 0.5	138.1 ± 4.5	8.6 ± 0.3	
MMI	26.1 ± 0.6	142.4 ± 6.7	18.3 ± 0.5^{ooo}	
2-IHDA 20 μg	10.0 ± 0.5	130.2 ± 6.2	7.7 ± 0.3	
ILδ 20 μg	10.1 ± 0.4	127.9 ± 3.3	7.9 ± 0.3	
MMI + 2-IHDA 1 μg	25.2 ± 0.6	139.4 ± 5.6	18.1 ± 0.3	–
MMI + 2-IHDA 5 μg	22.9 ± 0.7	131.1 ± 5.5	17.5 ± 0.9	–
MMI + 2-IHDA 10 μg	20.0 ± 0.4	139.1 ± 4.2	14.4 ± 0.3*	40
MMI + 2-IHDA 20 μg	18.4 ± 0.4	138.7 ± 5.6	13.3 ± 0.5**	51
MMI + ILδ 20 μg	19.1 ± 0.4	140.2 ± 3.1	13.6 ± 0.3**	48
MMI + HAD 20 μg	20.9 ± 0.5	139.1 ± 4.4	15.0 ± 0.3	–
MMI + KI 20 μg	20.7 ± 0.7	141.8 ± 5.1	14.6 ± 0.9	–

The rats were treated simultaneously with MMI and/or the iodolipids for 10 days. Each value is the average of four experimental determinations by quintuplicate. Results are expressed as the mean ± S.E.M. of each group. % of inhibition was calculated as described in Section 2.
* $P < 0.05$ compared with MMI.
** $P < 0.01$ compared with MMI.
^{ooo} $P < 0.001$ compared with control.

In order to study whether the antigoitrogenic effect of 2-IHDA is due to an action at the pituitary, plasma TSH levels were measured. Table 2 shows TSH, T_3 and T_4 serum levels of rats submitted to the different treatments. Injection of MMI during 10 days caused a significant increase in TSH (416%). No significant changes were observed in rats treated with ILδ and 2-IHDA alone vs. control, or MMI plus ILδ and MMI plus 2-IHDA vs. MMI treated rats.

As cAMP has been shown to mimic the effect of TSH on thyroid growth its level in the glands of rats was measured. As can be seen in Table 3 administration of MMI for 10 days, caused a 162% increase in cAMP content. Simultaneous administration of 2-IHDA decreased MMI stimulation by 57% at 10 μg/day, 76% at 20 μg/day; while the injection of ILδ at 20 μg/day caused an inhibition of 70%

As a marker of cell proliferation, PCNA was examined by Western blots in the thyroids of rats injected with MMI, MMI plus 2-IHDA or its non iodinated precursor, HD. MMI treatment led to strong increase in PCNA levels (90%); this expression was significantly reduced by 2-IHDA injection (62%) while the injection of HD failed to alter this value (Fig. 1).

Table 2
Serum TSH, T_3 and T_4 after administration of iodolipids.

Treatment	TSH (ng/ml)	T_3 (nmol)	T_4 (nmol)
Control	1.89 ± 0.16	1.75 ± 0.15	75.0 ± 6.4
ILδ 20 μg	1.75 ± 0.40	1.78 ± 0.30	77.0 ± 5.2
2-IHDA 20 μg	1.66 ± 0.28	1.40 ± 0.20	65.0 ± 3.3
MMI	9.75 ± 1.03	0.78 ± 0.15**	40.0 ± 4.0**
MMI + 2-IHDA 20 μg	7.38 ± 0.67	1.01 ± 0.03**	43.0 ± 7.1**
MMI + ILδ 20 μg	7.45 ± 0.82	0.85 ± 0.15**	49.0 ± 7.2**

The rats were treated simultaneously with MMI and/or the iodolipids for 10 days. Each value is the average of four experimental determinations by quintuplicate. Results are expressed as the mean ± S.E.M. of each group.
** $P < 0.01$ compared with control.

Table 3
Effect of increasing doses of 2-IHDA on intrathyroid cAMP levels.

Treatment	cAMP (fmol/mg protein)	% Inhibition
Control	16.1 ± 1.4	
MMI	42.4 ± 4.6^{oo}	
MMI + 2-IHDA 5 μg	30.2 ± 2.5	
MMI + 2-IHDA 10 μg	27.4 ± 1.6**	40
MMI + 2-IHDA 20 μg	22.3 ± 2.0**	51
MMI + ILδ 20 μg	24.0 ± 2.5**	48

The rats were treated simultaneously with MMI or MMI plus iodolipids for 10 days. cAMP was measured as indicated in Section 2. Each value is the average of four experimental determinations by quintuplicate. Results are expressed as the mean ± S.E.M. of each group. % of inhibition was calculated as described in Section 2.
** $P < 0.01$ compared with MMI.
^{oo} $P < 0.01$ compared with control.

In order to exclude a transient effect of the iodolipid a more prolonged time of treatment was performed. Rats were treated during 30 days with the different compounds. The injection of MMI raised the thyroid weight by 318% respect to the control value. This stimulation was inhibited by 2-IHDA (36%) and ILδ (32%) (Table 4). Total cell number and the epithelial height were increased in the MMI group (95% and 120%, respectively) while the lumen area was smaller (53%). Simultaneous treatment with 2-IHDA reduced cell number and epithelial height (83% and 51% inhibition, respectively), while the lumen area value, was close to the control value. Similar results were obtained with the simultaneous injection of ILδ (Table 4 and Fig. 2).

3.2. Involution of preformed goiter

In order to explore whether 2-IHDA can cause the involution of preformed goiter, the following design was applied: rats were

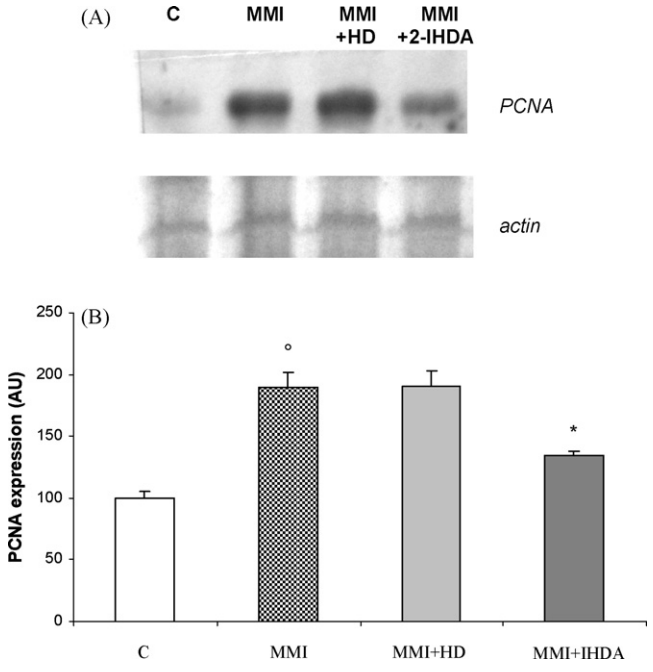

Fig. 1. PCNA levels after 10 days of treatment. PCNA was measured and quantified as described. (A) Representative autoradiography of a Western blot. (B) Densitometric analysis of the western blot. Data are expressed as the level of PCNA relative to actin. Each value represents the mean ± S.E.M. of three independent experiments (5 animals each). Control value was taken as 100. $^{o}P < 0.05$ vs. control; $^{*}P < 0.05$ MMI vs. MMI + 2-IHDA (20 μg).

Table 4
Prevention of MMI-induced goiter in the rat: 30 days of treatment.

Treatment	Thyroid wt (mg)/body wt (g) × 100	Cells number ($\times 10^{-3}$)/μm^2	Epithelial height (μm)	Lumen area (μm^2)
Control	6.2 ± 0.2	6.4 ± 0.4	5.4 ± 0.3	1334 ± 58
MMI	26.0 ± 1.1^{ooo}	12.5 ± 0.4^{ooo}	11.9 ± 0.5^{ooo}	709 ± 26^{oo}
MMI + 2-IHDA 20 μg	18.8 ± 0.7**	9.4 ± 0.3***	6.5 ± 0.2***	1189 ± 32*
MMI + ILδ 20 μg	19.7 ± 0.8**	10.0 ± 0.4***	6.7 ± 0.3***	1135 ± 45*

The rats were treated simultaneously with MMI or MMI plus iodolipids for 30 days. Each value is the average of four experimental determinations by quintuplicate. Results are expressed as the mean ± S.E.M. of each group.
* $P < 0.05$ compared with MMI.
** $P < 0.01$ compared with MMI.
*** $P < 0.001$ compared with MMI.
^{oo} $P < 0.01$ compared with control.
^{ooo} $P < 0.001$ compared with control.

injected with MMI for 10 days. The antithyroid drug caused a 151% increase in thyroid weight when compared to that of the control rats. At this point MMI was discontinued and the rats were distributed into the following groups: (a) pretreated with MMI, without further treatment (spontaneous involution); pretreated with the goitrogen and then treated with (b) 2-IHDA, 20 μg/day, or (c) KI, 20 μg/day. The animals were killed after 3, 7 and 10 days of these last treatments. Administration of 2-IHDA led to a reduction of 74.5% in goiter weight after 3 days. Spontaneous involution was only of 32% and KI failed to alter this value. After 7 and 10 days the reduction caused by 2-IHDA administration was the same of that obtained with KI (7 days: spontaneous involution, 56%; 2-IHDA, 87.5%; KI, 82%; 10 days: spontaneous involution, 58%; 2-IHD, 98%; KI; 95%) (Fig. 3). This was a direct inhibitory effect of 2-IHDA on the thyroid since TSH level, at 3 days, was the same whatever the treatment was as it was expected (MMI: 33.62 ng/ml; spontaneous involution: 1.8 ± 0.3 ng/ml; 2-IHDA: 2.6 ± 0.8 ng/ml; KI: 2.0 ± 0.5 ng/ml).

After 10 days of MMI treatment the follicular cell number (Fig. 4) and the epithelial height were increased (74% and 114%, respectively). After 3 days of the withdrawal of MMI treatment, only the injection with 2-IHDA led to a significant reduction of these parameters; follicular cell number: 66% inhibition, epithelial height: 25% reduction (c: 5.44 ± 0.16 μm; MMI: 11.6 ± 0.24 μm; sp. invol.: 11.5 ± 0.21 μm; KI: 10.85 ± 0.17 μm; 2-IHDA: 10.0 ± 0.22 μm ($P < 0.05$ vs. sp. invol)). After 7 days of

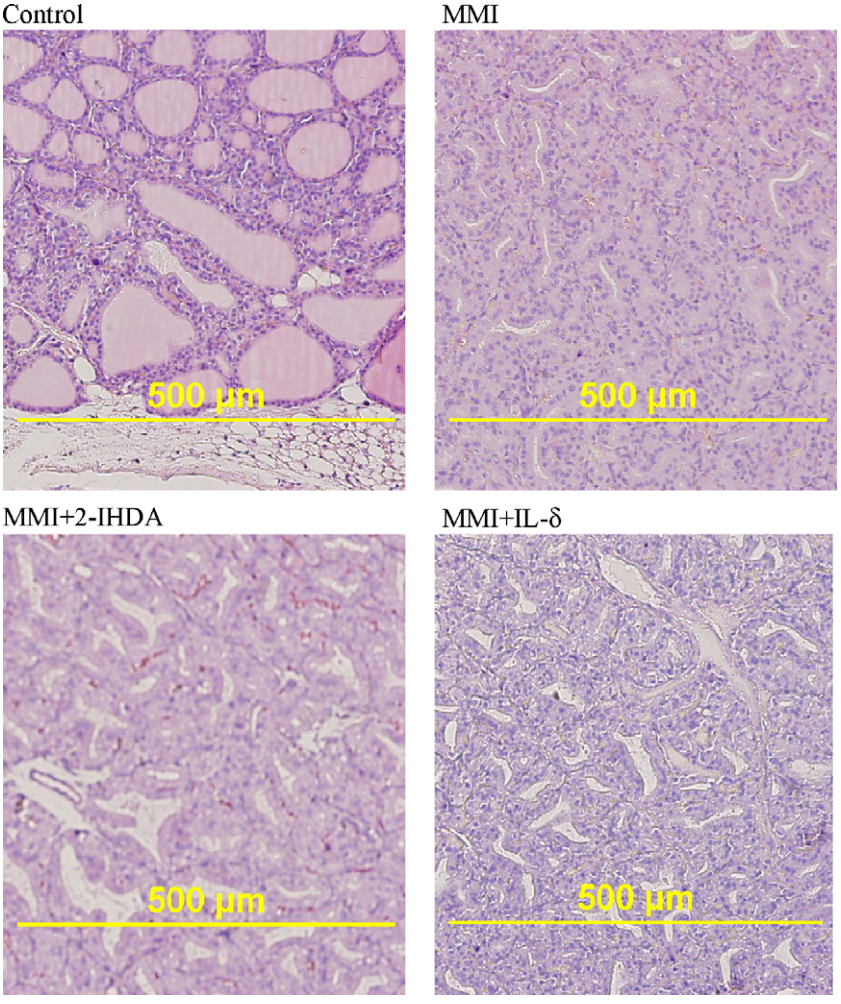

Fig. 2. Thyroid morphology of control, goitrous and treated rats. The rats were treated simultaneously with MMI or MMI plus iodolipids for 30 days.

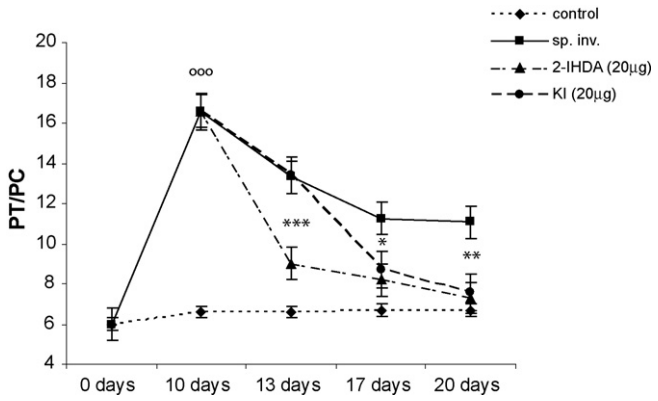

Fig. 3. Involution of preformed goiter. The rats were treated with MMI (5 mg/day) for 10 days and were then treated as indicated. Data are expressed as the ratio of thyroid weight/body weight × 100. Each value represents the mean ± S.E.M. of three independent experiments (5 animals each). $^{ooo}P < 0.001$ (MMI vs. control); $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (2-IHDA vs. spontaneous involution).

treatments, although a more pronounced effect with 2-IHDA was obtained there was no statistically significance difference with KI treatment (results not shown). Regarding lumen area value, the treatment with MMI led to a reduction of 42%. After the interruption of MMI treatment, there were no significant differences between the values obtained with the different treatments and those of spontaneous involution at any time of the assay (c: $1451 \pm 53\ \mu m^2$; MMI: $847 \pm 42\ \mu m^2$; sp. invol.: $1110 \pm 59\ \mu m^2$; KI: $1172 \pm 71\ \mu m^2$; 2-IHDA: $1135 \pm 68\ \mu m^2$).

3.3. Collateral effects

The effects of the injection of 2-IHDA for 10 days on several serum parameters were examined. None of the parameters examined, such as T_3 and T_4 (Table 2), cholesterol, AST and ALT transaminases, urea and creatinine were significantly different from those of control rats (results not shown).

4. Discussion

The actions of iodide are relieved by PTU and MMI, compounds which inhibit iodide organification. Therefore an organic iodocompound, called XI, was proposed to be the intermediate of its action (Van Sande et al., 1975). First attempts to identify this putative intermediate have been unsuccessful but iodinated lipids have been found in glands from different species and they reproduce some of the effects of KI.

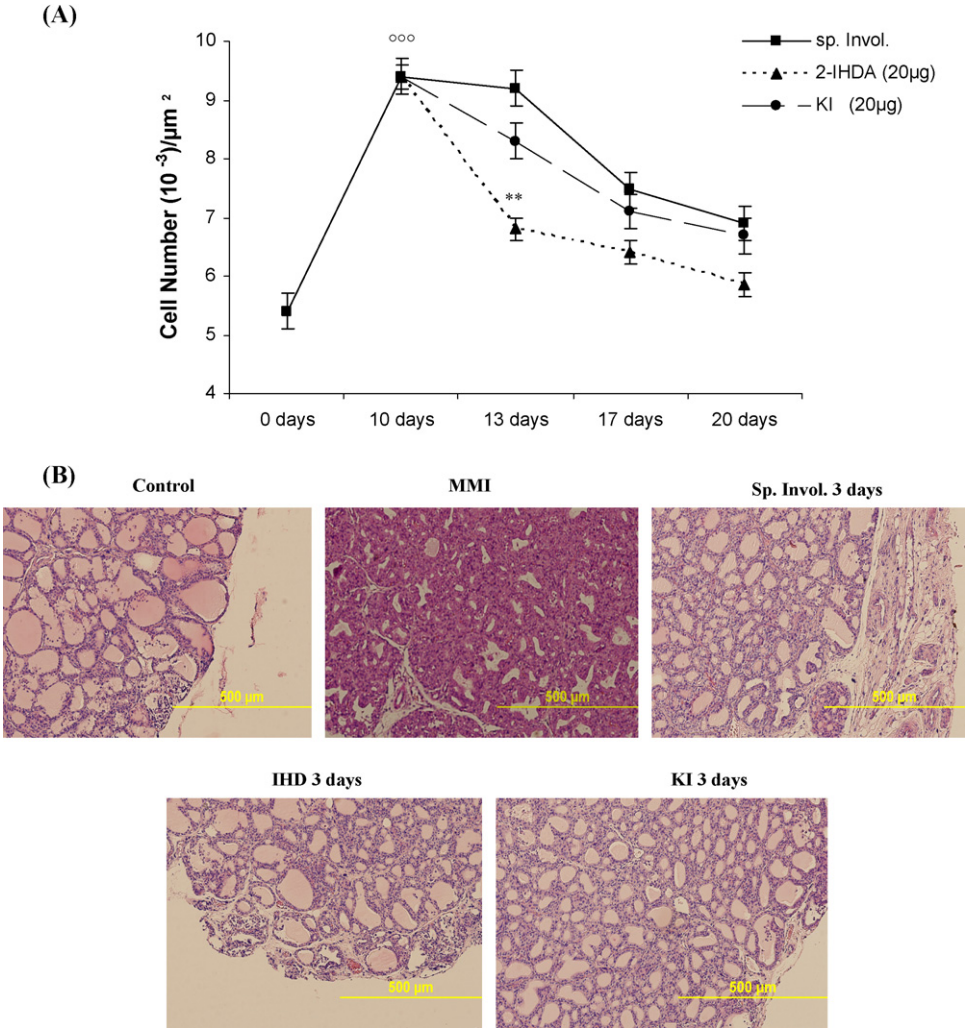

Fig. 4. The rats were treated with MMI (5 mg/day) for 10 days and were then treated as indicated. Thyroid glands were fixed with 10% neutral-buffered formalin, embedded in paraffin and sectioned in 5 µm sections for histochemical studies. (A) The number of epithelial cells was quantified as described. Each value represents the mean ± S.E.M. of three independent experiments (5 animals each). $^{ooo}P < 0.001$ (MMI 10 days vs. control); $^{**}P < 0.01$ (2-IHDA vs. spontaneous involution). (B) Involuting thyroids after 3 days with the different treatments

Boeynaems and Hubbard (1980) have reported the conversion of exogenous free arachidonic acid into 5-hydroxy-6-iodo-8,11,14-eicosatrienoic delta lactone (ILδ) in rat thyroid and Dugrillon et al. (1994) demonstrated that this compound is synthesized by a human gland. Pereira et al. (1990) found α-iodohexadecanal (2-IHDA) as the major iodolipid in horse thyroid while Panneels et al. (1996), have shown the synthesis of this iodocompound in cultured dog thyroid cells. There is still no data about the presence of 2-IHDA in human thyroid.

2-IHDA inhibits NADPH oxidase (Ohayon et al., 1994), H_2O_2 production (Panneels et al., 1994a) and cAMP (Panneels et al., 1994b), but the effects on cell growth has not been studied. The aim of this work was to further explore the possible role of 2-IHDA in thyroid autoregulation studying its antigoitrogenic action.

In order to explore the possible mechanism of action of 2-IHDA on thyroid growth, serum TSH, T_3 and T_4 were measured. In rats treated with MMI, TSH showed a 5-fold increase, while T_3 and T_4 were significantly decreased as it was expected. Simultaneous injection of 2-IHDA and MMI did not change serum TSH, T_3 or T_4. These results show that the antigoitrogenic action of 2-IHDA is not due to an inhibition of pituitary TSH synthesis or secretion in rats, since serum TSH, which was increased by MMI injection, remained the same in rats treated with MMI plus 2-IHDA. Moreover intrathyroidal cAMP levels, which are involved in the goitrogenic action of TSH (Dumont et al., 1992), were reduced by the injection of 2-IHDA, demonstrating a direct effect on the thyroid. It was shown that KI decreased the goitrogenic effect of TSH and of cAMP in mice (Pisarev and Itoiz, 1972). I-OH-A inhibited MMI-induced goiter and this inhibition was associated with a decrease in the thyroid cAMP content (Pisarev et al., 1988). In cultured dog thyroid cells, 2-IHDA reproduced the inhibitory effect of iodide on cAMP accumulation (Panneels et al., 1994b). The same authors demonstrated also, a direct inhibition on the adenylyl cyclase activity in human thyroid membranes. On the other hand a direct inhibition of thyroid growth caused by 2-IHDA has now been observed in *in vitro* cell culture studies with FRTL-5 cells (to be published). As it was demonstrated that thyrocytes-specific Gαq/Gα11-deficient mice lacked the normal proliferative thyroid response to TSH or goitrogenic diet, an effect on this cascade cannot be ruled out (Kero et al., 2007). Moreover, 2-IHDA inhibits H_2O_2 production and this decrease resulted from an inhibition of this cascade (Panneels et al., 1994a).

It is known that TSH regulates the synthesis of several angiogenic factors (Eggo, 2003; Ramsden et al., 2005) and early events during goitrogenesis involve the expansion of thyroid microvasculature (Many et al., 1984; Gérard et al., 2008). A regulation of thyroid blood flow and angiogenesis factors by iodide was demonstrated (Michalkiewicz et al., 1989; Yamada et al., 2006; Gérard et al., 2009). 2-IHDA could have also an inhibitory effect on these parameters. Further studies are necessary to clarify this point.

The action of the iodolipid cannot be ascribed to the iodide that would originate from their possible dehalogenation since injection of KI failed to cause a change in thyroid growth as a consequence of the MMI treatment.

The action of 2-IHDA is not a transient effect since the inhibition was observed after more prolonged times of treatment (30 days). The increase in thyroid weight caused by MMI is directly related to a raise in cell number and epithelium height, lumen area reduction and follicular cell hypertrophy. Treatment with 2-IHDA inhibited cell number and epithelial height suggesting a control not only on cell proliferation, but also on cellular hypertrophy.

2-IHDA was able not only to prevent the growth of MMI-induced goiter but also caused the involution of preformed goiter. 2-IHDA caused a significant reduction in goiter weight after 3 days of treatment compared to the spontaneous involution. KI alone failed to alter this value. Once more, the possibility that the results obtained with 2-IHDA may be due to a release of iodide originated by deiodination of the iodolipid may be ruled out since KI failed to alter the spontaneous involution after 3 days.

These results contrast with those published by Mutaku et al. (2002) who have found that after 3 days of iodide administration, glandular weight decreased significantly. The discrepancy may be attributed to the different models employed. Mutaku et al. administered 200 μg/day of KI while we have injected only 20 μg/day. On the other hand these results confirm those published by Many et al. (1985) who have found the same pattern of involution in animals fed either with a moderate or a high iodine diet. Patel et al. (2000) observed a spontaneous involution at day 4 after withdrawal of the goitrogenic treatment (iodide deficient diet). Necrosis and apoptosis were observed during involution (Rognoni et al., 1987) and this later process is stimulated by iodine (Mutaku et al., 2002) as well as an increase in the oxidative stress (Poncin et al., 2008). This seems to be the process involved in 2-IHDA involution induction, since TSH levels are the same among the different groups.

There was a strong correlation between the decrease in thyroid weight, the number of glandular epithelial cells and height of epithelium during goiter involution. The decline of these parameters was also accelerated by the injection of 2-IHDA during the first 3 days of involution. The significant reduction of the follicular cell number but only a slight decrease in epithelium height after 3 days of 2-IHDA injection and preliminary results of our laboratory measuring caspase 3 and tunnel assay support the hypothesis that 2-IHDA induces an apoptotic cell death during involution (to be published).

Another of the objectives of the present studies was to determine whether the administration of iodolipids causes side effects. As a first step we measured some serum parameters as an index of liver impairment and a reflection of kidney function. Under the present experimental conditions no significant changes were observed in the serum parameters. It was demonstrated in previous studies that ILδ had no toxic side effects (Pisarev et al., 1994). It should be remarked that since 2-IHDA has been already demonstrated to be a naturally occurring compound (Pereira et al., 1990; Panneels et al., 1996) it is not surprising that it does not show dramatic toxic side effects on other organs. On the other hand, possible toxic effects would not be expected physiologically since XI is not supposed to diffuse out.

In conclusion, these results show for the first time that 2-IHDA has an antigoitrogenic action without side effects in the time tested. It may be on account that as 2-IHDA is formed inside the follicles, exogenous administration may not fully mimic the *in vivo* effects. The presents results open the possibility that 2-IHDA could be a potential useful therapeutic agent.

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgments

We thank Valerie Panneels (Heidelberg University) for critical reading of the manuscript. This work was supported by grants from the Argentine National Research Council (CONICET), the National Agency for the Promotion of Science and Technology (ANPCYT) and the University of Buenos Aires. MAD, GJ, OP, MAP, and GJJ are Established Researchers from CONICET, LT is a doctoral fellow from CNEA and RO is a doctoral fellow from the ANPCYT.

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