

EFFECT OF LONG-TERM ADMINISTRATION OF ARSENIC(III) AND BROMINE WITH AND WITHOUT SELENIUM AND IODINE SUPPLEMENTATION ON THE ELEMENT LEVEL IN THE THYROID OF RAT

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Received: June 10, 2005; Accepted: September 25, 2005

Key words: Iodine/Thyroid gland/Selenium/Bromine/Arsenic

The aim of this study was to evaluate the influence of arsenic and bromine exposure with or without iodine and selenium supplementation on the element level in the thyroid of rats. Four major groups of Wistar female rats were fed with respective diets: group A - standard diet, group B - iodine rich diet (10 mg I/kg food), group C - selenium rich diet (1 mg Se/kg) and group D - iodine and selenium rich diet (as in group B and C). Each group was divided into four subgroups per 7 animals each receiving either NaAsO₂ ip (6.5 mg.kg⁻¹ twice a week for two weeks and 3.25 mg.kg⁻¹ for six weeks) or KBr in drinking water (58.8 mg.l⁻¹) for 8 weeks or combined administration of both substances. Remaining subgroup served as controls. After 8 weeks thyroid glands were analyzed by ICP-MS for As, Br, Se, and I content. The exposition of rat to arsenic or bromine causes the accumulation of these elements in the thyroid gland (~18 ppm of As, ~90 ppm of Br) and significantly affects iodine and selenium concentration in the thyroid. In iodine and/or selenium supplemented rats the bromine intake into the thyroid was lowered to ~50% of the level in unsupplemented animals. Also selenium thyroid level elevated due to KBr administration was lowered by iodine supplementation in the diet. The accumulation of arsenic in the thyroid was not influenced by selenium or iodine supplementation; however, As(III) administration increased iodine thyroid level and suppressed selenium thyroid level in selenium or iodine supplemented group of animals.

INTRODUCTION

An increased incidence of thyroid cancer has been reported especially in the female population of the United States^{1,2}, in most other affluent countries (Scandinavia, Israel)³ and as well in the Czech Republic⁴. According to epidemiological, clinical and experimental studies, both the dietary deficiency and excess of iodine have been associated with increased risk of thyroid carcinomas⁵⁻⁸. In this connection, the effect of salt water fish and shellfish consumption as a measure of iodine exposure has been discussed⁹⁻¹¹.

Apart from iodine, which is the main constituent of thyroid hormones, a second essential trace element - selenium - is required for appropriate thyroid hormone synthesis, activation and metabolism. Adequate supply with these essential elements needs to be provided to the organism either by a balanced diet or supplementation¹²⁻¹⁴.

However, other dietary substances may be present in seawater, and subsequently taken up by fish and seafood, that interact with iodine metabolism or may be carcinogenic to the thyroid without any relation to iodine metabolism¹⁵. One of such dietary factors of seafood origin is bromine, the goitrogenic effect of which cannot be

excluded due to its chemical relationship with iodine. Its increasing use in both, industry and agriculture introduces bromine into the alimentary chain in ever-growing quantities. Another sea-water bound element is carcinogenic metalloid arsenic; its interaction with selenium has been known for more than half a century¹⁶.

This study was undertaken to estimate the influence of arsenic and bromine exposure on selenium and iodine thyroid content in experimental animals fed standard and iodine- or selenium-rich diet.

MATERIALS AND METHODS

Animals and diets

Female Wistar rats (Charles River, FRG) weighing 90-100 g were used for the experiments. After adaptation period of 5 days, the animals were assigned to four experimental groups being fed with different experimental diets.

Control group (A) received a standard diet (C 1042, Altromin, Lage, FRG), containing 17% protein, 57% carbohydrates, 5% fat, 3% fiber (cellulose) 8% of a mixture of vitamins and salts and 10% of water. Selenium content

in the diet was 0.252 mg/kg food and iodine content was 0.151 mg/kg food; iodine supplemented group (B) received the standard diet with the addition of 10 mg iodine/kg food; selenium supplemented group (C) received the standard diet with the addition of 1 mg selenium/kg food; selenium and iodine supplemented group (D) received the standard diet with the additions as in group B and C. Animals were fed their respective diets for 8 weeks.

For each of four separate groups in which one of the four diets was supplemented, 28 animals were used and divided into four subgroups ($n=7$ in each subgroup) according to following scheme: subgroup 1 received no additional treatment; subgroup 2 received NaAsO_2 (Sigma Chemical Co.), *ip*, 6.5 mg/kg body weight twice per week for two weeks and 3.25 mg/kg bw for six weeks; subgroup 3 received KBr (Sigma Chemical Co.) 58 mg/l in drinking water; subgroup 4 - received NaAsO_2 as in subgroup 2 and KBr as in subgroup 3.

The animals were housed under conditions of constant humidity, temperature and light/dark cycles 12h/12h. They had free access to drinking water and food. Diets were replaced daily, body weights were determined weekly.

Sample preparation and analytical procedures

At the end of 8-week experimental period the animals were sacrificed under ether anesthesia by cutting jugular veins. The thyroids were excised, weighed immediately, sealed in aluminum foil and stored frozen at -20°C until analysis.

Digestion of thyroid glands was carried out in 30 ml Teflon vials (Savillex) using 2 ml of concentrated nitric acid (purris p.a., Fluka Chemie, Switzerland), capped and left overnight on a heating desk at 150°C . The content in vials was almost evaporated, and then diluted with 2% HNO_3 . As an internal standard, 10 ppb of In (Astasol, Analytica Ltd., Czech Rep.) was added.

Element analysis was performed by ICP-MS spectrometer (PQ3, VG Elemental); sample solutions were brought into nebulizer by means of peristaltic pump. Following isotopes were measured: ^{75}As , ^{79}Br , ^{127}I , ^{82}Se and ^{115}In .

Digestion and analysis were checked by the measurement of the reference material (8414 Bovine Muscle Powder, NIST, Washington DC).

The element contents are expressed in micrograms of the element measured per gram of wet tissue weight [$\mu\text{g}\cdot\text{g}^{-1}$ wtwt].

Statistical analysis

All results are presented as the arithmetical mean \pm standard deviation (SD). Student's *t*-tests were performed to obtain levels of significance between the groups. Number of animals (*n*) used per group are stated in the tables.

RESULTS

The growth curves were similar in all groups of animals supplemented by respective diets. The administration of arsenic (III) and/or potassium bromide did not exhibit any influence on the body weight increments within the groups. The average body weight gains in animals after eight-week supplementation with iodine- and/or selenium-enriched diet are given in Table 1.

Significant decrease in the average body weight gain was observed in rats supplemented with selenium enriched diet.

The results of arsenic, bromine selenium and iodine determination in the rat thyroid gland are summarized in Table 2. Since the standard diet lacked arsenic and bromine, these elements were not detectable in non-treated animals.

Arsenic. In As (III) and As (III) + KBr treated animals of control group A, the concentration of arsenic reached the level of 18.0 and 18.9 $\mu\text{g/g}$ wet tissue, respectively. The level remained unaffected by iodine supplementation. In selenium supplemented animals the concentration of arsenic was enhanced in group C2 compared to groups C4 and D2. In the other groups the additional administration of bromine together with arsenic did not cause any difference in arsenic concentration in the thyroid.

Bromine. In KBr and KBr + As (III) treated rats the bromine content in thyroid gland was elevated to ~ 90 ppm. Both, selenium and iodine enriched diet sig-

Table 1. Effect of the diet supplementation on the average weight gain of rats

Subgroup	Diet	weight gain [% of starting weight]
A1	Standard diet	174.7 \pm 12.7
B1	Iodine rich diet	150.6 \pm 18.4
C1	Selenium rich diet	131.0 \pm 23.1 *
D1	Iodine + Selenium rich diet	153.9 \pm 14.1

Groups are defined in the text; $n = 7$ animals per group. Values represent mean \pm SD* $P < 0.01$ vs. control subgroup A1

Table 2. Concentration of arsenic, bromine, selenium and iodine in the thyroid gland of rats fed for eight weeks standard, iodine and/or selenium enriched diet combined with arsenic(III) and/or potassium bromide treatment.

Treatment	n	As [$\mu\text{g} \cdot \text{g}^{-1}$ wtw]	Br [$\mu\text{g} \cdot \text{g}^{-1}$ wtw]	Se [$\mu\text{g} \cdot \text{g}^{-1}$ wtw]	I [$\mu\text{g} \cdot \text{g}^{-1}$ wtw]
A. Standard diet					
1. Control	7	n.d.	n.d.	0.20 ± 0.02	82.5 ± 42.1
2. As ^{III}	7	18.0 ± 5.2*	n.d.	0.28 ± 0.12	83.5 ± 44.6
3. KBr	7	n.d.	91.1 ± 32.2*	0.53 ± 0.11*	73.7 ± 28.9
4. KBr + As ^{III}	6	18.9 ± 6.4*	90.2 ± 22.9*	0.55 ± 0.10*	91.6 ± 16.9
B. Iodine enriched diet					
1. Control	7	n.d.	n.d.	0.22 ± 0.08	54.1 ± 33.6
2. As ^{III}	7	18.9 ± 7.4*	n.d.	0.26 ± 0.04	120.0 ± 16.7**
3. KBr	7	n.d.	48.7 ± 6.0*#	0.38 ± 0.06*#	57.7 ± 48.2
4. KBr + As ^{III}	6	18.9 ± 7.6*	48.5 ± 11.0*#	0.37 ± 0.09*#	82.1 ± 42.7
C. Selenium enriched diet					
1. Control	7	n.d.	n.d.	0.34 ± 0.09#	148.7 ± 40.5#
2. As ^{III}	6	20.7 ± 4.0*	n.d.	0.27 ± 0.05	171.7 ± 27.5#
3. KBr	7	n.d.	58.3 ± 8.6*#	0.50 ± 0.13#	144.7 ± 10.9#
4. KBr + As ^{III}	7	15.2 ± 2.7*	51.0 ± 10.3*#	0.40 ± 0.06#	134.6 ± 24.8#
D. Iodine + Selenium enriched diet					
1. Control	7	n.d.	n.d.	0.29 ± 0.07#	126.4 ± 35.2#
2. As ^{III}	7	15.9 ± 4.4*	n.d.	0.22 ± 0.03#	127.1 ± 63.3
3. KBr	7	n.d.	34.4 ± 11.4*#	0.33 ± 0.06#	86.2 ± 50.1
4. KBr + As ^{III}	7	15.9 ± 3.6*	35.9 ± 7.3*#	0.32 ± 0.04#	109.6 ± 32.0

Data represent mean ± SD, n.d. = not detectable
 Significant differences p<0.05 * versus control of the same group;
 # versus corresponding subgroup of control group A

nificantly diminished bromine uptake into the thyroid of all animals compared to control group A. Iodine supplementation (group B) resulted in the decrease of bromine content to 54% of A3 and A4 control groups. Selenium supplementation (group C) decreased the bromine level to ~60%, and the combined iodine and selenium supplementation (group D) to ~40% of A3, A4 control groups. Additional treatment with As(III) did not affect the levels of bromine in the thyroid.

Selenium. Selenium supplementation enhanced Se content in the thyroid from 0.20 ± 0.02 (A1) to 0.34 ± 0.09 $\mu\text{g}/\text{g}$ wet tissue (C1), and in combination with iodine supplementation to 0.29 ± 0.07 (D1). No effect of iodine supplementation on selenium thyroid level was found.

Selenium concentration was enhanced in all KBr and KBr + As(III) treated rats compared with respective control subgroup - to ~270% in group A, to 170% in group B and to 150% in group C. In animals supplemented with combined iodine and selenium enriched diet (group D) no such effect was observed. In selenium supplemented rats the As(III) treatment caused a significant decrease of selenium thyroid level to 80% of respective control subgroups C1, D1 level.

Iodine. In control animals fed standard diet the iodine content in the thyroid remained unaffected by either KBr or As(III) treatment. Iodine supplementation exerted no effect on thyroid iodine level (group B) compared to group A. Selenium supplementation caused significant elevation

of iodine content in all subgroups compared to group A, the highest level being in As(III) treated rats (C2). In selenium and iodine supplemented rats (group D) the increase was significant in control rats only (D1).

DISCUSSION

In the present study the eight-week administration of sodium arsenite to female rats (twice a week, 6.5 mg/kg bw, ip, for 2 week and 3.25 mg/kg bw, ip, for six week) and/or potassium bromide (58 mg/l in drinking water) did not lead to any obvious signs of toxicity; no significant differences in the weight gain evolution were found between treated and untreated animals within each group fed respective diet.

The administration of selenium-rich diet (1.25 mg/kg food) for 8 weeks resulted in a decrease of weight gain compared to animals fed standard diet (Table 1) and could reflect some slight toxic effect of selenium supplementation at this level. However, this does not correspond to the results of long term (8–10 weeks) selenium supplementation experiments in which the selenium content in animal diet up to 2 mg Se/kg food was referred in Wistar rats to be still a physiological value¹⁷. The dietary excess of iodine (10 mg/kg food) had no effect on the weight gain.

The progressive increase in dietary content of iodine from 0.15 mg to 10 mg/kg food did not result in an increase in thyroidal iodine concentration in control animals (group B1). These findings are in accordance with the recently published data assuming the high dietary iodine supply (>0.8 mg/kg food) to overcharge compensatory capacity of iodine homeostasis with the consequence of iodine accumulation in extrathyroidal tissues (esp. skin) (ref.¹⁸). On the other hand, the increase in dietary selenium from 0.25 mg to 1.25 mg/kg of food (group C1) resulted in enhanced selenium concentration in the thyroid as well as in elevated iodine thyroid level.

In 1974, the thyroid-toxic properties of bromide ion were observed by Van Logten et al., pointing to impairment of thyroid function¹⁹. Later the inhibition of iodine uptake was claimed to be one of possible mechanism of action on the thyroid gland²⁰. Our data demonstrate that bromine accumulates easily in the thyroid. In agreement with recently published data^{21,22} the uptake of bromine into the thyroid was found to be dependent on the stage of iodine supply. In animals fed iodine enriched diet the bromine intake into the thyroid was lowered to ~50% of bromine thyroid level of animals fed standard diet. Combined iodine and selenium supplementation resulted in an additive protective effect. The concentration of bromide ions administered in our experiment has not reached the critical concentration to contribute to lowering iodine uptake into thyroid gland; however a strong elevating effect on selenium thyroid level was seen, moderated by iodine supplementation.

Arsenic and selenium are metalloids with similar chemical properties and metabolic fates. Inorganic arsenic has been shown to modify metabolism of selenium and

influence the anti-carcinogenic activity of inorganic and organic selenium compounds^{23,24}. Some data also demonstrate that pre- or co-exposure to inorganic selenium exerts protective effect against toxicity of arsenic^{25–27}. In our study the level of arsenic cumulation in the thyroid was not significantly influenced by dietary selenium supplementation. However, the administration of arsenite suppressed the access of Se into the thyroid of selenium supplemented animals (group C2, D2).

In view of the results presented, it can be concluded that arsenic and bromine have been shown to influence significantly the thyroid level of iodine and selenium, both closely associated with thyroid metabolism. The potential occurrence of simultaneous exposure to these elements in human population deserves further studies designed to clarify the nature and effect of these interactions.

ACKNOWLEDGEMENT

This study was supported by the Cancer Registry of Norway, Oslo, Norway and by the Grant Agency of the Czech Republic (No. 305/05/0344).

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