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Expression of NIS in the thyroid and pituitary of female rats after a single dose of potassium iodide

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Objective. The aim of this work was to study the expression of NIS in the thyroid and anterior pituitary in rats after a single dose of iodide appropriate to the content of iodide in iodine-positive points in the thyroid and pituitary.

Methods. A total of 41 inbred rat females of local laboratory strain weighing 250-300 g at the stage of diestrus and/or metestrus were used. Pituitaries and thyroids were dissected from 15 control rats at the same time as these from four groups of 6-8 rats each which were given various doses of potassium iodide dissolved in 0.5 ml distilled water (6 rats - 1 μg/100 g body weight; 8 rats - 4 μg/100 g ; 6 rats - 8 μg/100 g ; 6 rats - 25 μg/100 g.) by gavage at 48 h before sacrifice. In 6 rats of control group the concentration of iodine in thyroids and pituitaries was estimated in terms of percent by weight in dry tissue (wt% I^{-2} dry tissue) using the wavelength dispersive spectrometry (WDS) quantitative analysis. The expression of NIS in thyroids and pituitaries in terms of the percentage of positive immunostained area (% PA) was measured by streptavidin-biotin method using specific polyclonal antibodies.

Results. In thyroids, the concentration of iodine in iodine-positive points ranged from 2.5 to 59.3 (mean of 16.7±3.0) in terms of wt% I^{-2} dry tissue (100 % iodine-positive points), while in pituitaries it ranged from 0.17 to 6.3 (mean of 1.4±0.3) in all points and 2.2±0.4 in iodine-positive points. Histochemical reaction for NIS in the pituitaries at 48 hours after iodide administration showed a dose related increase beginning from 4 μg/100 g (from 1.8±0.7 to 12.9±1.0 % PA, respectively to the dose of iodide), while such increase in the thyroids started from 8 μg/100g (from 3.7±1.2 to 9.1±2.0 % PA). It remained still increased in pituitaries after the dose of 8 μg/100g (11.4±1.0 % PA) and 25 μg/100g (13.9±1.5 % PA), while such increase in thyroids was found only after the dose of 25 μg/100g (11.9±2.8 % PA).

Conclusion. It was found that in the pituitaries of rat females the expression of NIS started after the dose of 4 μg iodide/100g, while that in the thyroids started after 8 μg iodide/100g. Thus, it may be suggested that the pituitary appears more susceptible to the level of iodide in blood.

Key words: WDS, NIS, iodide, pituitary, thyroid, rats

It is well known that after a single intraperitoneal administration of potassium iodide with a radioactive tracer to healthy rats in a dose of 5 times higher than the total amount of iodine in the thyroid, the organically bound iodine in the thyroid of these rats becomes not detectable for as long as the level of inorganic iodine in serum is higher than 19 g % (Wolff-Chaikoff effect; Wolff and Chaikoff 1948).

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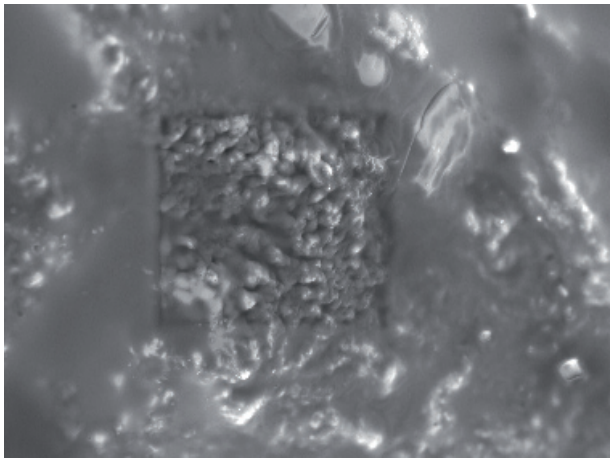

Fig 1A. Representative scanning electron micrograph of the sample of human thyroid dehydrated by drying at 100 °C for 45 min. This scan was taken *after* the square of the tissue (50 μm²) in the middle of this picture has been exposed to 20 kV x-ray flux and the estimation of iodine-positive points has been taken

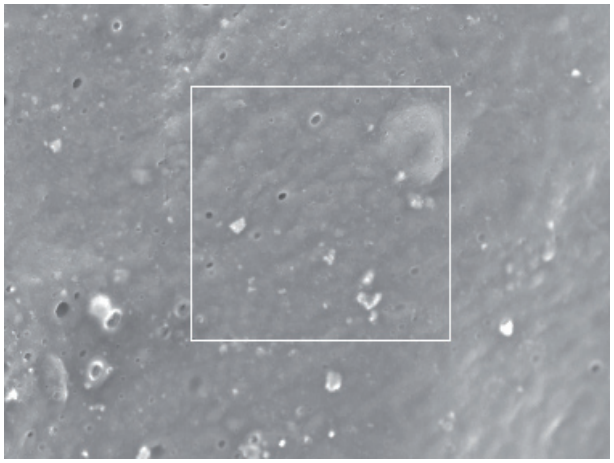

Fig 1B. Representative scanning electron micrograph of the sample of rat thyroid dehydrated by drying at 100 °C for 45 min. This picture has been taken *before* the square of the tissue (50 μm²) in the middle was exposed to 20 kV x-ray flux to estimate iodine-positive points

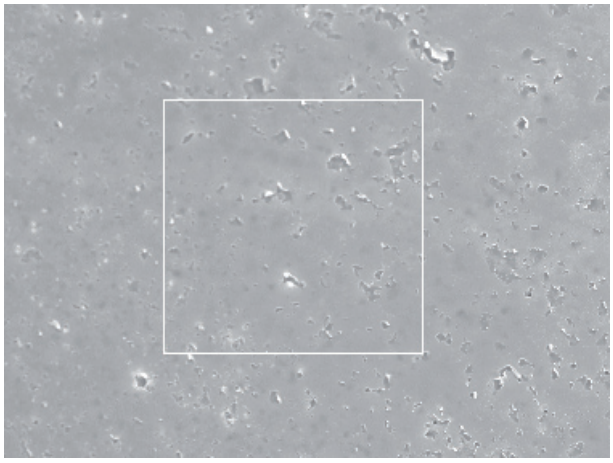

Fig 1C. Representative scanning electron micrograph of the sample of rat pituitary dehydrated by drying at 100 °C for 45 min. This picture has been taken *before* the square of the tissue (50 μm²) in the middle was exposed to 20 kV x-ray flux to estimate iodine-positive points

The concentration of iodine in the thyroid has been estimated by various methods (Tadros et al. 1981; Hansson et al. 2008). However, the significance of iodine concentration in various organs is not yet well understood, since a majority of methods based on catalytic reactions such as gas chromatography of iodine in the form of iodo-acetone or iodo-butanol, iodide ion selective electrodes, X-ray fluorescent spectrometry, isotope exchange and neutron activation analysis do not appear sensitive enough for the estimation of minute amounts of iodine (such as 0.1 μg/g). Thus, a majority of them does not seem useful and reliable (Andrasi et al. 2007).

In contrast, because of its high resolving power the wavelength dispersive spectrometry (WDS) allows the estimation of very low concentration of this microelement without any destruction of tissue matrix, but requires the dehydration of samples. A new method of biological sample preparation for microelements definition using WDS has been recently developed in the Department of Physical Chemistry, South Ural State University (Basalaeva et al. 2009) as shown for human thyroid (Fig. 1A), rat thyroid (Fig. 1B) and rat pituitary (Fig. 1C).

Quantitative estimation of iodine in the tissues with the aid of this method not only will permit to obtain fundamental data on the level of iodine in various tissues, but also on the mechanism of iodine action in those tissues and organs including the interrelations between the effective iodine dose and resulting iodine concentration in the appropriate tissue which, finally, will also correspond and contribute to the mechanism of the original approach by Wolff and Chaikoff (1948). Though recently the quantitative estimation of iodine in various brain structures has been conducted with the aid of epithermal and radiochemical neutron activation analysis (Andrasi et al. 2007), the pituitary has not yet been subjected to investigation.

Natrium iodide symporter (NIS) is a key plasma membrane protein that mediates active iodine uptake

by the thyroid, lactating breast, and several other tissues (see the Discussion). Nevertheless, some report also appeared on the presence of NIS in the pituitary (Mitsuma et al. 1997). In this investigation, NIS has been selected as a marker of the presence or absence of iodine action on the appropriate organ.

Materials and Methods

Animals. A total of 41 inbred female rats of local laboratory strain weighing 250-300 g at the stage of diestrus and/or metestrus were used. They were kept in light (12:12 hr light:dark cycle; lights on at 7.00 a.m. local time) and temperature (24±1 °C) controlled animal room and fed standard commercial laboratory diet and tap water *ad libitum*. The experiment was conducted by observing humane principles as presented by European Community Directive 86/609/EC.

A total of 15 rats served as control and 26 rats were given various doses of potassium iodide per 100 g body weight in 0.5 ml distilled water by gavage (e.g. 6 rats - 1 μg; 8 rats - 4 μg; 6 rats - 8 μg; 6 rats - 25 μg). Immediately before sacrifice the blood sample was taken from jugular vein in ether anesthesia and then the animals were sacrificed under continuing ether anesthesia.

Wavelength dispersive spectrometry (WDS) quantitative analysis. From the thyroids and pituitaries of 6 control rats the tissue fragments of no more than 2 mm thickness were obtained and subjected to the procedure of sample preparation. First, they were dried at 100 °C for 45 min (Basalaeva et al. 2009). After that they were coated with platinum by JFC-1600 Auto Fine Coater (JEOL, Tokyo, Japan) and examined in a JEOL JSM-6460 LV raster electron microscope (REM) (JEOL, Tokyo Japan) equipped with wave length dispersive X-ray analyzer Inca 500 (Oxford Instruments, High Wycombe, UK) at 20 kV and 2.5 nA . Silver iodide (AgI) and 20 % gelatine solution with known concentrations of thyroxine were used as standards. WDS counting times were 20 sec per analyzed point. Each sample was counted 5 times (see Figs. 1A, 1B and 1C). The analyses were carried out by elemental imaging using the multispectral analysis program Oxford Inca Energy Wave Crystal EWC453 (Oxford Instruments, High Wycombe, UK).

The results were expressed as percent by weight in dry tissue (wt% I^{-2} g/100 g) in each class and the averages were compared by one-way ANOVA followed by the Duncan multiple range test at $p<0.05$.

Morphological evaluation. The samples of the pituitary with osseous tissues and the thyroids with the fragments of trachea were fixed by 10 % neutral formol and embedded in paraffin. Osseous and cartilage tissues were decalcified. Serial thin parallel slices were made, stained by hematoxyline-eosine and examined by light microscope.

Immunohistochemistry. The expression of natrium-iodide symporter (NIS) in the thyroid and pituitary was determined by streptavidin-biotin method with the use of polyclonal antibodies to NIS (1:100 v/v), purchased from Abbiotec (San Diego, CA). Paraffin-embedded tissue sections were deparafinized with xylene, rehydrated in sequential ethanol/water baths of descending ethanol concentrations, and subjected to microwave fixation for 15 min in 10 mmol citrate buffer. Endogenous peroxidase was quenched with 0.3 % hydrogen peroxide in methanol for 10 min. Nonspecific binding was inhibited by 1 % (weight/volume) mouse serum albumin (MSA) for 30 min at room temperature. Primary antibodies were added to the slides in blocking 1 % (w/v) MSA solution and incubated for 30 min at room temperature. Tissue sections were incubated for 30 min at room temperature with a peroxidase-conjugated secondary antibody in blocking buffer. After washing, tissue sections were incubated for 5 min at room temperature with biotinyl tyramide (1:50 v/v) in 50 mmol Tris HCl (0.01 % H_2O_2, pH 8.0) followed by 30 min incubation with alkaline phosphatase–conjugated streptavidin (1:100 v/v) in blocking buffer.

All sections were investigated by light microscope Axiostar plus (Carl Zeiss, Jena, Germany) equipped by the scanner with a 35 mm digital-camera (Cannon Power Shot A520), and afterwards saved and analyzed with the BioVision Professional 3.0 (West Medica Handels GmbH, Vienna, Austria) for computerized image analysis and quantification of NIS.

Statistical evaluation. For statistical evaluation Statistica for Windows 6.0 was used. The results were expressed as mean ± SEM for each group. The differences between groups were analyzed by one-way ANOVA followed by Duncan's multiple range test, the values of $p<0.05$ being considered as significant.

Results

As found by electron-probe microanalysis (WDS), the concentration of iodine in the thyroid (iodine-positive points) ranged from 2.5 to 59.3 (mean of 16.7±3.0) wt % I^{-2} dry tissue (Fig. 1B) and that in the pituitary from 0.0 to 6.0 (mean of 1.4±0.3) wt % I^{-2} dry tissue (Fig. 1C). By such a way, the ratio of iodine concentration in the

thyroid versus the pituitary has been 12:1 in terms of all estimated points and 7.1:1 in terms of iodine positive points (Table 1, Fig. 2).

Table 1

Organ	Parameter	
Thyroid	Number of measurement	18
	iodine -positive points (%)	100
Pituitary	Number of measurement	32
	iodine -positive points (%)	65.6

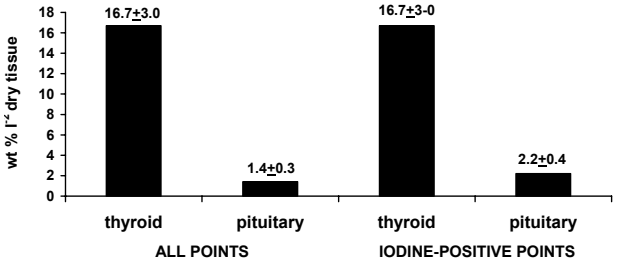

Fig. 2. Wavelength dispersive spectrometry (WDS) quantitative analysis of iodine concentration levels in thyroids and pituitaries of female rats (wt% I-2 dry tissue). * = p<0.05 vs. all points per organ

In this experiment the Wolff-Chaikoff phenomenon started from the iodine dose of 25 μg/100 g which has been about 5 times higher than the amount of iodine in the thyroid. From this follows that such dose of iodine which will correspond to the amount of iodine in the thyroid should be about 5 μg/100 g (Wolff and Chaikoff 1948). Thus, as based on the same scheme, the dose of iodide which will be about five times higher than that corresponding to iodine concentration in iodine positive points in the pituitary should be 3.3 μg/100 g, while that which corresponds to the true pituitary iodine concentration in such points should be 0.6 μg/100 g.

As based on the above findings, following doses of potassium iodide were chosen for the use in our experiments. Thus, the dose of 1 μg KI/100 g (after being slightly rounded) has been found out as that corresponding to iodine concentration in iodine positive points in the pituitary, while that of 4 μg KI/100 g appeared, in this respect, about 5 times higher. In addition, however, the dose of 8 μg KI/100 g appeared about 1.5 times higher than the concentration of iodine in the thyroid, while that of 25 μg KI/100 g has been about 5 times higher than the concentration of iodine in the thyroid.

Histochemical reaction for NIS in the pituitaries at 48 hours after iodide administration showed a dose related

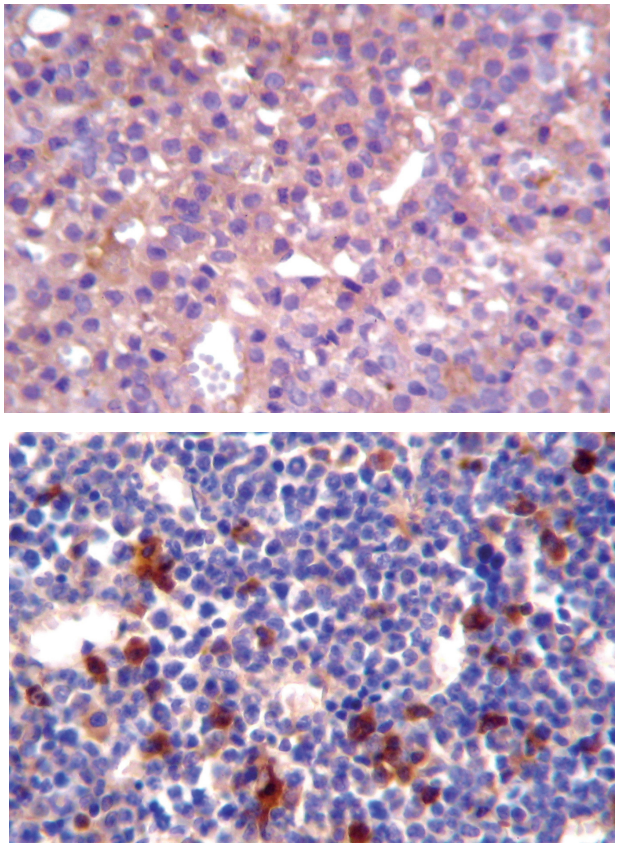

Fig. 3. Immunohistochemical staining with specific antibodies to NIS in the pituitary of female rats (original magnification: x 400: significant increase of NIS at 48 hrs after KI administration in a dose of 4 μg/100 g (lower panel) compared to control (upper panel)

increase beginning from the dose of 4 μg/100 g (e.g. from 1.8±0.7 % PA in controls to 12.9±1.0 % PA). In the thyroid, however, such increase started from the dose of 8 μg/100g (e.g. from 3.7±1.2 % PA in controls to 9.1±2.0 %

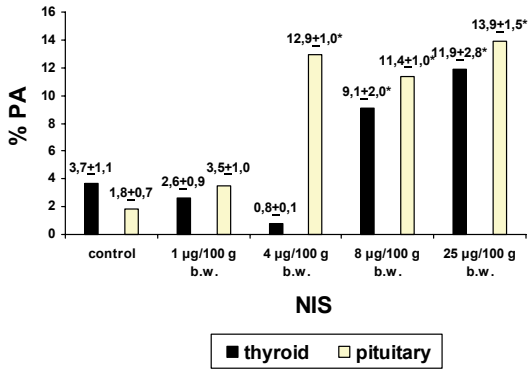

Fig. 4. Significant increase of NIS in thyroids and pituitaries of female rats at 48 hours after KI administration * = p<0.05 vs. control group

PA). As shown in Fig. 4, increased expression of NIS in the pituitary persisted after the dose of 8 μg/100g (11.4±1.0 % PA) and 25 μg/100g (13.9±1.5 % PA), while in the thyroid after 25 μg/100g only (11.9±2.8 % PA).

Discussion

Recently, the role of iodide in extrathyroidal tissues has been extensively discussed repeatedly (Cann et al. 1999; Cann 2006). Though, in general, iodide cannot be organified in those tissues, there are some exceptions to this rule. Actually, the expression of NIS has been found in several extrathyroidal tissues such as salivary gland, parotid gland, submandibular gland, pituitary, pancreas, testis, mammary gland, gastric mucosa, prostate, ovary, adrenals, heart, thymus and lung (Spitzweg et al. 1998).

The uptake of iodide as mediated by the sodium iodide symporter (NIS) and followed by iodide organification in several extrathyroidal tissues may serve, indeed, to numerous physiological functions including antimicrobial defense of tissue surfaces at the environment/body interface such as gastrointestinal tract, cornea or skin (Majerus and Courtois 1992), supply of iodine to the fetus (mammary gland) (Rillema and Rowady 1997) and fertility (uterus, oviduct) (Brown-Grant and Rogers 1972). In addition, the formation of certain iodoproteins (e.g. iodolactones, iodoaldehydes) (Dugrillon 1996) both in the thyroid gland and in extrathyroidal tissues may provide important antiproliferative and antioxidative effects, thereby controlling cellular integrity, cell proliferation, and tumorigenesis (Eskin 1970; Kato et al. 1994).

The results of our experiment showed that the response of pituitary and thyroid to a single relatively low inorganic iodine dose appears organ specific. Thus, the increase of NIS expression

In these experiments, the increase of NIS in the pituitary of rat females after the administration of inorganic iodide has been found to start from the dose of 4 μg/100g, while that in the thyroid started from 8 μg/100g. From this it may be suggested that the pituitary appeared more sensitive to the effect of iodide than the thyroid itself.

In addition, it has been also observed that a single dose of iodide (4 μg/100g) results in a slight and short-term (less than 120 hr) hyperfunction of pituitary-thyroid system in female rats (Basalaeva et al. 2010b). Possibly, it may be assumed that transient thyroid hyperfunction resulting from the dose of 4 μg/100g iodide could be secondary being induced by the pituitary.

It should be also noted, that the dose of 4 μg/100g has been targeted to reach about 5 times higher level of iodine in the pituitary iodine-positive points as respected the original investigations by Wolff and Chaikoff (1948), while the dose of 8 μg/100g has been targeted to reach about 5 times higher concentration of such points in the thyroid. It may be supposed that the response of the pituitary as well as that of the thyroid is being started after the certain proportion of iodine concentration has been reached between the iodide level in blood and the level of iodine in iodine-positive points in these organs. Actually, our previous investigation (Basalaeva et al. 2010a) showed the increase of apoptotic enzymes activity (e.g. effectory caspase 32 and initiatory caspase 8) in the pituitary and, moreover, this investigation possibly permitted to suggest that such increase of caspases activity in the pituitary may be related to the increase of iodide in blood after the administration of iodide in a dose of 4 μg/100g. This view may be further supported by the findings that, after the iodide dose of 4 μg/100g, neither any changes in the activity of caspases nor in the level of NIS were found in the thyroid. Such synchronous and unidirectional changes of caspases activity and NIS possibly permit the suggestion on certain role of iodine in the regulation of apoptosis in the above mentioned endocrine organs. However, further research should possibly contribute to elucidate these still not well understood and elucidated functions.

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