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SHORT COMMUNICATION

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Iodine concentration in different human brain parts

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Abstract Iodine is one of the most important essential elements as demonstrated by the fact that its deficiency can cause goitre. Nevertheless, quantitative data on its concentration in biological materials, especially in the human brain, are scarce. There is therefore a demand for accurate and reliable information on iodine in these types of samples. The purpose of the present work was to determine the concentration of total iodine in some control human brain parts by rapid radiochemical neutron activation analysis. Our second goal was to determine I distribution between lipid fraction and in brain tissue without lipid by applying two types of solvent extraction methods. The results were checked by the analysis of biological standard reference materials with certified or literature values for iodine and good agreement was found.

Keywords Iodine · Human brain · Radiochemical neutron activation

Introduction

The role of iodine, especially in the synthesis of thyroid hormones which regulate a wide variety of physiological processes in living organisms, has been known for a long time[1, 2]. Essentiality of the element has long been recognized, since iodine deficiency, which leads to goitre, is highly prevalent worldwide [3, 4]. Newborns are particularly sensitive to the effects of both iodine deficiency and iodine excess because of the risk of thyroid impairment [5].

The major part of this element enters living organisms via the food chain. Intake of iodine is also affected by the iodine content of soil, fertilizers, drinking and irrigation water, by the use of human and veterinary drugs and iodine feed supplements, and further by the use of disinfectants and sanitizers in the dairy industry. Iodine often occurs as a pollutant in terrestrial ecosystems. Another strong source of iodine is the sea. It is emitted both as part of the sea spray and in the form of halogenated organic gases. Reactor accidents may also release ^{131}I to the biosphere, while ^{129}I emissions are associated with nuclear fuel reprocessing.

Given the importance of this element for different scientific fields it is surprising that reliable data on its concentration levels in various body fluids and organs (other than thyroid), food articles, environmental samples and pharmaceutical products are scarce. This shortfall of information is mainly due to the lack of adequate analytical techniques to determine iodine in such complex matrices.

Because of the low concentration levels (μg and ng) of iodine typical of most animal and plant tissues, its quantitative determination requires sensitive analytical methods. These include methods based on catalytic reactions [6, 7], gas chromatography of iodine in the form of iodoacetone and iodobutanone [8], X-ray fluorescence spectrometry [4, 9], the use of iodide-ion-selective electrodes [10, 11] and most lately inductively coupled plasma mass spectrometry, especially with the isotope dilution method [12, 13]. Two very sensitive methods for determination of traces of iodine, with the advantage of freedom from reagent blanks, are thermal neutron activation in either its destructive or radiochemical form [14, 15], and epithermal neutron activation analysis for non-destructive determination of this element when its concentration is somewhat higher [16, 17]. A rapid radiochemical activation analysis of different food articles and diets was previously developed and opti-

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mised for the determination of low concentration of iodine [18, 19].

The present work focuses on iodine concentrations in different parts of the human brain using the accurate and reliable radioanalytical method described below. Iodine has been determined in human brain samples by burning the activated samples in oxygen atmosphere and separating ^{128}I using oxidation–reduction and extraction cycles [20, 21, 22, 23]. Another objective of this work was to extend the method for the determination of iodine concentration not only in whole brain samples (high fat content) but – by applying two types of solvent extraction – in lipid fraction and in brain tissue without lipid. Major emphasis is on five brain parts of five German control patients, but three Hungarian control patients are also included. To prove the accuracy of the applied technique National Institute of Standard and Technology standard reference materials (NIST SRMs) were analysed.

Experimental

Sample collection

Brain samples were obtained from the Institute of Neuropathology, University of Munich, where samples were pathologically classified and stored at –70°C until required. Tissue samples were dissected from the brains about 24 h after death. Upon reception samples were lyophilized in a freeze-dryer (–8°C, 10^{-3} bar) for 7 days until constant weight. The dried samples were weighted accurately and used for experiments. Instruments used for collecting tissue, storing and transporting were of the same materials in all cases and care was taken to avoid contamination of samples during collection and treatment (ceramic-, Ti-tools, pre-cleaned polyethylene vessels, high-purity laboratory). Tools and plastic containers were thoroughly cleaned in analytical-grade nitric acid (soaking for 24 h) and doubly distilled water before use.

The samples were collected from five German control patients of 51–73 years of age (mean age 58 years). All subjects taken into consideration were diseased for reasons not involving the nervous system. For solvent extraction experiments samples of three Hungarian control patients were also used (mean age 70 years). The samples were taken from both hemispheres in the case of Hungarian patients. The brain's different regions are known to be devoted to different functions; therefore bulk analysis is not adequate. Biological functions of brain regions sampled with respective dry mass contents are summarized in Table 1.

Radiochemical separation of ^{128}I radionuclide

Neutron activation analysis (NAA), in general, has a high sensitivity for iodine. However, for measuring trace amounts of iodine it is necessary to employ either a preconcentration NAA (PNAA) or a radiochemical NAA (RNAA) procedure to separate iodine from elements such as Br, Cl, Na and Mn, which induce high activities in the samples during neutron activation [9].

The first step was irradiation: 30–50 mg of lyophilised human brain samples and an appropriate aliquot (10 µL) of KI standard solution (10 µg g^{-1} for KI) were sealed in polyethylene tubes and irradiated simultaneously for 5 min in the pneumatic transfer system of the research reactor of Berlin (BER II) at a neutron flux of 8×10^{13} n cm^{-2} s^{-1}.

Irradiation was followed by a Schöniger combustion method to extract ^{128}I from brain samples. About 4 g of pure cellulose packed into ashless filter paper (the relatively long burning time of cellulose ensures the total oxidation of the sample) was placed in the Pt basket of a 4-L Schöniger flask; then approximately 2 mL of concentrated KI solution (50 mg g^{-1} KI) was added by weight to the filter paper as a carrier. A balloon was attached to the side tube of the flask to act as a safety valve during ignition. The flask was well flushed with oxygen. A good seal was ensured by wetting the ground joint with 10% (m/m) Na_2SO_3 solution. The irradiated brain sample was weighted into the Pt basket, the filter paper was ignited, and the magnetic stirrer was switched on for 20 min to splash the walls and basket with the 35 mL of absorbing solution (30 mL 0.05 M H_2SO_4+5 mL 10% (m/m) Na_2SO_3). The iodine in samples (independent of the original bound) was reduced to iodide by Na_2SO_3.

Iodine was isolated from the absorbing solution by oxidation–reduction and extraction cycles. Iodine was first extracted in a separating funnel into 2×5 mL carbon tetrachloride (oxidization of iodide was ensured with the addition of 1 mL 10% (m/m) $NaNO_2$ to the absorbing solution prior to the first extraction). The aqueous phase was discharged. In the second extraction step iodine was again reduced to iodide soluble in the aqueous phase with the addition and extraction into 1 mL 10% (m/m) Na_2SO_3. The organic phase was discharged. The third and final extraction was performed into carbon tetrachloride (2 then 3 mL) by adding 2 mL 10% (m/m) $NaNO_2$ (which again served as oxidant of iodide) and 2 mL 2.5 M H_2SO_4 solutions to the aqueous phase. The final iodine extract was filtered through Whatman phase filter paper, collected in a glass vial, fitted with a ground glass stopper to prevent evaporation of the solvent, and measured in a well-type detector. Isolation procedure took less then 30 min.

Table 1 Functions and dry mass content of the investigated brain parts

Brain region	Function	Dry mass content (%)
Area occipitalis (AOC)	vision	20.9
Caput nuclei caudati (CNC)	movement (inhibition)	17.8
Putamen (PUT)	movement (inhibition)	19.4
Globus pallidus (GLP)	movement (facilitation)	21.1
Thalamus (THA)	limbic system, emotion	19.1
Parietal lobe (PAR)	knowledge	17.0
Cortex entorhinalis (CER)	autonom system	16.4
Ammon's horn (AMH)	emotion, learning, memory	18.5
Cortex frontalis parasaggitalis (CFP)	cognitive function	16.9
Cortex frontalis basalis (CFB)	cognitive function	16.7
Pulvinar thalami (PTH)	sensory system	20.0
Vermis cerebelli (VEC)	equilibrium	18.0
Substantia nigra (SUN)	movement (facilitation)	25.7
Gyrus hippocampus (GHC)	limbic system	16.8

Relative standard deviation is ±3%

Calculation of iodine content

The γ-activity of the isolated ^{128}I nuclide (^{127}I (n,γ) ^{128}I; $t_{1/2}$=25 min; $E_{\hat{\gamma}}$ = 433) was measured by a well-type HPGe detector and was compared to the activity of the irradiated KI standard solution (since no matrix was present in the standard solution, it was measured directly, without extraction). Cooling period lasted for approximately 45 min. Counting time was set to 20 min for brain samples and 5 min for standard, which resulted in a statistical error of <5%. Iodine concentrations in brain samples calculated from activity measurement were calculated using the relative method.

After γ-measurement, sample solutions were diluted with carbon tetrachloride to determine the chemical yield by spectrophotometry for each sample individually. Absorption of elemental iodine carrier in every sample solution was measured at 516 nm. For quantification, two independent (inactive) standard iodine solutions of 0.1333 mg g^{-1} were prepared. Absorbance results for the two standard solutions were averaged. Chemical yield and subsequently iodine content of brain samples was calculated.

For the localization of trace elements two extraction methods were used. First, apolar lipids were extracted with diethyl ether (2×2h, 2×200 μL). In further experiments both apolar and polar lipids were removed from the brain samples using an *n*-hexane/isopropanol (3:2) solvent mixture (2 h, 800 μL) and iodine content was measured in every fraction.

Results and discussion

As an advantageous technique for precise and reliable determination of μg and ng quantities of iodine, thermal INAA is sensitive enough, but separation of short-lived iodine is imperative due to the high interfering activities of chlorine, bromine and sodium. These and some other interferences can be reduced but not completely eliminated by epithermal NAA and thus often radiochemical separation of induced ^{128}I is required.

In Ljubljana several accurate and reliable techniques have been developed for the determination of ^{127}I in different biospheric samples (water, food articles, urine, biological and environmental samples, standard reference materials etc.) using a short irradiation (1–30 min, Triga Mark II Reactor) to induce ^{128}I. These were applied in NAAL-Berlin (BER II Reactor at flux of 8×10^{13} n cm^{-2} s^{-1}) [19, 20, 21]. One of them, a combustion in oxygen atmosphere method (Schöniger), is very convenient for the determination of iodine in dry human brain samples. Since cellulose powder was used to ensure the total oxidation of samples, its blank contents were to be taken into account. Analysis of cellulose showed that iodine content was about 1 ng g^{-1}. Thus blanks are negligible in practice. The isolation procedure takes less than 30 min, so counting can be started after approximately one half-life of ^{128}I; thus iodine can be assessed in a wide concentration range (from ng g^{-1} to mg g^{-1} levels) and in different matrix. Because the half-life of ^{128}I is only 25 min, irradiation and measuring times can be kept short. This is a prerequisite for dealing with many samples per day and for keeping the unit cost low.

Most important, however, is the fact that the procedure, as worked out, provides a pure ^{128}I spectrum even at very unfavourable chlorine to iodine or bromine to iodine ratios and thus extremely high accumulation factors are achieved. Initially, small peaks due to ^{24}Na and ^{82}Br were sometimes present, but after introducing filtration of the final CCl_4 phase through Whatman phase separating paper, the spectra were radiochemically highly pure. Yields, which are determined by spectrophotometry after irradiation by measuring the absorbance of an added amount of iodine carrier in carbon tetrachloride are 80–95%. Chemical yields for iodine were determined for each sample aliquot individually.

Table 2 Results of iodine content in biological standard reference materials (SRMs) (ng g^{-1} dry weight; mean±s.d., *N*=3)

SRM	Certified[a] or literature values[b]	Our values
NIST 1577b Bovine liver[b]	218±46 [21]	188±41
NIST 1577 Bovine liver[b]	270±30 [16] 222±15 [29] 180±12 [14] 187±12 [30]	209±21
NIST 1566 Oyster tissue[b]	2,720±200 [21]	2,800±140
NIST 1566a Oyster tissue[a]	4,460±420 [25]	4,380±150
NIST 1571 Orchard leaves[b]	160±20 [16] 173±4 [29] 177±8 [30]	175±6

The limit of detection for iodine using the above irradiation conditions and well-type HPGe detector is about 5–10 ng g^{-1} dry weight, obtained from the background under the 433 keV peak in real sample spectra. Detection limits are calculated according to the equation given by Currie [24]. Accuracy was determined by the analysis of NIST standard reference materials [25]. Table 2 gives the obtained results. The differences to certified or literature values are within ±10%. It should be noted that our choice of reference materials was very restricted by the need to find those that were certified or at least had information values for iodine. From the good agreement of results obtained it can be concluded that iodine radioisotopes are fully released on ignition and that the combustion method described here represents a useful and sensitive technique for the determination of iodine in various samples of biological origin including human brain samples. The precision of the analytical technique was tested by repeated analysis of these reference materials. The precision, in terms of relative standard deviation, is 5% at about 50–100 ng g^{-1} levels of iodine.

About 15–20% and about 30–40% of the mass of brain parts were separated (number of determinations: 27) in the first (removing apolar lipids with diethyl ether) and second extractions (extraction with solvent mixture), respectively. Data of solvent extraction showed that about 70% of iodine content remained in brain tissue (number of determinations: 10). Knowledge of the localization and concentration of trace elements would be an aid to understanding the molecular biology of the brain. The nature and site of I binding is still unknown in the human brain, but it must be much stronger than that of its chemical homologue Cl, which is highly mobile. According to our earlier results, more than 90% of trace metals (e.g. Fe, Zn)

Table 3 Iodine results from both hemispheres of three (A,B,C) Hungarian control patients (ng g^{-1} dry weight)

Brain region	Hemisphere	Iodine content			Mean±s.d.
		A	B	C	
Pulvinar thalami (M)	left side	478	900	630	669±213
	right side	865	1,012	780	886±117
Gyrus hippocampus (G)	left side	419	487	276	394±108
	right side	314	653	270	412±210
Substantia nigra (M)	left side	704	n.a.	1,090	897±273
	right side	1,014	2,898	806	910±147
Vermis cerebelli (G)	left side	240	243	360	281±68
	right side	235	112	420	256±155

G and M indicates that the region was classified as grey matter (G) or as a structure of mixed composition (M)
Instrumental statistical error of individual values is 5%
n.a. not analysed

Table 4 Iodine concentrations in brain regions of five German control patients (A–E, left hemisphere, ng g^{-1} dry weight)

Brain part	A	B	C	D	E	Mean±s.d.
CFP	66	124	2,250	123	5,340	104±33
CFB	64	138	2,920	99	n.a.	100±37
AOC	47	77	5,810	96	7,100	74±25
PAR	122	108	4,140	94	4,950	108±14
THA	103	88	9,300	59	4,090	83±23
CNC	n.a.	n.a.	5,930	86	n.a.	86
PUT	256	n.a.	7,330	62	12,400	159±137
GLP	42	n.a.	4,430	65	4,950	54±16
AMH	52	n.a.	9,000	n.a.	3,000	52
CER	n.a.	94	4,120	n.a.	3,900	52

n.a. not analysed
Instrumental statistical error of individual values is 5%

remained in brain tissue [26]. The present finding indicates the high lipid solubility of iodine and its complexes.

By using factors calculated from the water content of the samples (Table 1) it is possible to compare the results obtained by different analytical techniques and to convert values from the literature expressed on a wet matter basis.

From our data (Table 3) it can be seen that corresponding regions from the two hemispheres of three human brains revealed similar concentrations in general, which is in accordance with other essential element studies [26, 27]. Interestingly, in certain cases (e.g. pulvinar thalami of person A) significant differences could be seen in concentrations between the two hemispheres. Instrumental statistical errors (1σ) for individual measurements were about 5%. Enrichment of I was observed in basal ganglia (pulvinar thalami) and in brainstem (substantia nigra). It is an interesting finding that substantia nigra, which is one of the most intensely pigmented structures in the human brain, was found to have the highest I content. The lowest I concentration can be seen in vermis cerebelli. It can be concluded that generally higher I concentration was observed in regions of mixed composition than in grey matter. The concentration range for I turned out to be similar to those reported in the literature [28].

Data on I concentrations in brain parts of German control patients are summarized in Table 4. Very high I quantities (μg g^{-1}) were found in brain regions of two control subjects and therefore these patients were excluded from mean calculations. The reason for this anomaly is unknown. Another difficulty was that iodine content of certain brain samples could not be measured, since insufficient quantities remained after determining other trace elements [26]. It seems from standard deviation that there is a wide range for individual values. Satisfactory agreement was found between our results by the RNAA method and those by epithermal NAA in the literature [28].

It is known that thyroid necessitates some essential elements other than iodine in the thyroid hormone (T_4) (e.g. Zn and Se). By using our previous data on Zn distribution in normal patients we studied the possible correlation of I and Zn. Analysis of mean data for brain regions did not reveal correlation between the two elements. When every sample data was taken into account, correlation appeared to be somewhat stronger (R=0,467 with p=7.9%).

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