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Comparisons between the inorganic content of healthy and hypertensive rat tissues by inductively coupled plasma-mass spectrometry

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The inorganic contents of bone, brain, erythrocyte, heart, kidney cortex, kidney medulla, liver, lung, muscle and plasma from spontaneously hypertensive rats were compared with those of the same tissues from healthy Sprague-Dawley rats. A general inductively coupled plasma-mass spectrometry method developed for multi-element determinations of most of the elements present in biological tissues was used. Variations were found not only for major elements, as expected, but also for many trace elements in several tissues.

Keywords: hypertension, inductively coupled plasma-mass spectrometry, inorganic content, rat tissues

Introduction

Research on essential hypertension has not yet revealed its intimate origin. If its increased prevalence among blacks and the development by inbreeding of strains of spontaneously hypertensive rats seem to point to a polygenetic predisposition, it appears that behavior patterns, stress, obesity, oral contraceptives, but more importantly, the intake of dietary sodium are all external factors that also can lead to essential hypertension among those with a genetic predisposition. It is now strongly believed that essential hypertension, which accounts for 90–95% of hypertension, is a result of the combined action of genetic and environmental factors (Kaplan 1988).

To identity the heredity factors involved, three major paths of studies are currently being pursued (Cotran *et al.* 1989):

(i) The existence of a primary non-structural genetic defect in the kidney's ability to excrete sodium and water in response to the inevitable periodic increases in blood pressure, thus resulting in an inability to correct and reverse those periodic variations.

(ii) The existence of a defect in cell membrane sodium or calcium transport, heightening vascular reactivity to vasoconstrictive agents thus increasing blood pressure. Such defects have already been reported in red blood cells for sodium, and in platelet and fat cells for calcium in subjects affected by hypertension.

(iii) The existence of a primary heightened sympathetic response to stress or other neurogenic influences resulting in rises in blood pressure.

The three hypotheses are not mutually exclusive and two of them directly involve inorganics. Variations in the homeostasis of sodium and, to a lesser extent, calcium are then undoubtedly related to the prevalence of essential hypertension. Moreover, one of the three mechanisms suspected to be disturbed in renal hypertension (5–10% of hypertension) is sodium homeostasis (Gayton *et al.* 1986), and a second one, the renin–angiotensin system, also alters blood volume via the action of angiotensin II on aldosterone, the latter enhancing sodium retention (Skeggs 1986).

Knowing that the concentration of most elements does not vary independently in biological tissues but is closely linked to the behavior of many others—modifications in the concentration of sodium, for instance, can exert an influence on as many as 13 elements (Rae 1981), we can expect changes in the concentration of many trace inorganics. Up to now, more than 54 inter-relations between two distinct

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elements have been identified and these kinds of interactions are often at the origin of synergistic effects that can enhance the evolution of many pathological disorders. It is already well known that once hypertension has established, structural changes occur in the small muscular arteries and arterioles, contributing to increased vascular resistance and thus heightening blood pressure. Moreover, essential hypertension produces small lesions at the kidney level known as benign nephrosclerosis (Cotran *et al.* 1989). Those obvious changes induced by the elevated blood pressure endured by organs or tissues could mask more subtle variations caused by changes in salt concentrations, which in turn could accentuate tissue degradation. Consequently, a comparison of the total inorganic contents of the tissues of healthy normotensive rats to those of spontaneously hypertensive rats could generate useful information about the possible implications of trace elements in the stress and cell necrosis affecting organs sensible to long-term hypertension.

In the past few years, inductively coupled plasma-mass spectrometry (ICP-MS) has rendered possible precise multi-elemental determinations of trace elements at very low concentrations. Measurements at the low $ng\,g^{-1}$ level of more than 30 elements can now be realized routinely in a complex biological matrix in less than 30 min using the standard additions methodology. We have determined the precision and detection limits of this ICP-MS routine method recently (Schmit *et al.* 1991) and have applied it successfully on similar types of tissues (Gélinas *et al.* 1992).

This work aims to compare the inorganic contents (33 elements) of 10 tissues (bone, brain, erythrocyte, heart, kidney cortex, kidney medulla, liver, lung, muscle and plasma) of healthy normotensive Sprague-Dawley rats with those of spontaneously hypertensive rats, to pin-point their significant differences (90% confidence limit) via a multivariate analysis.

Materials and methods

Instrumentation

The measurements were done on an Elan ICP/MS Model 250 from SCIEX/Perkin-Elmer (Rexdale, Canada) under the following conditions: plasma argon flow, $16.0\,l\,min^{-1}$; nebulizer argon flow, $0.5\,l\,min^{-1}$; auxiliary argon flow, $1.2\,l\,min^{-1}$; r.f. power, 1.1 kW; reflected power, < 2 W. The standard torch was used throughout. Sample uptake for the Meinhard concentric nebulizer was precisely controlled by a Gilson Minipuls 2 (Mandel Scientific Co., Guelph, Canada) peristalic pump working at a flow rate of $0.4\,ml\,min^{-1}$. The ion sampling depth, defined as the distance between the aperture of the sample cone and the downstream turn of the r.f. coils, was fixed at 15 mm. The sampler and skimmer nickel cone apertures were 1.1 and 0.9 mm, respectively.

Reagents and solutions

The standard reference solutions used in this work were prepared from SCP-Science (Montreal, Canada) $1000\,\mu g\,ml^{-1}$ ICP standards diluted with ultrapure water obtained with a Milli-Q system (Millipore, Mississauga, Canada). Merck Suprapur (SCP-Science) 65% nitric acid was used for tissue digestion in a microwave bomb. NBS SMR #1577a Bovine Liver standard reference material (NIST, Gaitheburg, MD) was used throughout for calibration and reference purposes.

Tissue preparation and digestion

Male Sprague-Dawley rats and Spontaneously Hypertensive rats (~ 300 g) of the same lines were killed by decapitation and the studied organs immediately removed, perfused with a 300 mM mannitol solution (buffered at pH 7.5), and frozen at -22°C. A 500 mg amount of crude tissue and 1.25 ml of ultrapure 8 M nitric acid were mixed into the internal PTFE cup (30 ml) of a Parr (Moline, USA) microwave acid digestion bomb and allowed to digest for 2 min at medium power inside a standard microwave oven. After cooling for 30 min at room temperature, followed by 30 min in dry ice, the digest was poured into a 25 ml calibrated flask and the internal PTFE cup rinsed three times with ultrapure Millipore water. All the fractions were collected in the flask and an internal standard of $100\,mg\,ml^{-1}$ of rhodium was added. The resulting volume was adjusted to 25 ml with ultrapure (Milli-Q) water. Aliquots of solution were pipetted into polypropylene flasks and appropriate solutions of standards were added for element determination by the standard addition method.

All sample manipulations were done inside a class 100 clean room. Sample introduction was done via an automatic sampler introduction system working on a class 100 clean surface.

Element determination

The standard addition subroutine from the instrument software was used. Linear regression on a minimum of three additions (four measurements, $r \geq 0.999$) was used throughout for element determination. Each measurement represents the average of four replicates. An NBS SMR #1577a Bovine Liver digest solution was inserted every 10 measurements. Each measurement was done after 2 min of rinsing with ultrapure (Milli-Q) water and an additional 2 min of sample introduction to allow instrument response stabilization. Rinsing and stabilization times have been determined in preliminary experiments as appropriate to avoid memory effects and sample introduction instabilities, respectively.

Spectral interferences being much more frequent in the low mass region, the analyzed mass range (m/z = 5–238) was divided into two different zones: the low mass range (m/z = 5–90) and the high mass range (m/z = 91–238). The elements of the low mass zone were determined in the high resolution mode (resolution of 0.6 atomic mass unit at 10% peak height), while the elements of the high mass zone were determined in the low resolution mode (resolution of 1.0 atomic mass unit at 10% peak height). The division of the full range in two distinct and contiguous zones also allows a better optimization of the ion lens system of the instrument. This helps to compensate for the mass effect during ion transmission and thus improves the detection limits. The optimized voltages applied to the lens system in high resolution mode and low resolution mode were: +2.99 and +3.98 V (barrel lens), −7.44 and −5.56 V (plate lens), −17.75 and −19.63 V (Einzel lens unit), and, finally, −5.28 and −6.05 V (stop lens), respectively.

Precision

Confidence limits are given by: mean value $\pm ts/\sqrt{n}$ where s is the standard deviation and t is the t-table value at 90% confidence level. The estimated uncertainties are based on the combined effects of the digestion method, the imprecision and instabilities of the instrument, and the biological material variability.

Statistical multivariate analysis

Statistical multivariate analysis was used to identify the significant differences between both series of tissues (90% confidence limit). In a first step, SAS software was used to compute a discriminant analysis that tests tissue differences by comparing variances within and between each kind of tissue and extracts the most significant elements. Second, principal component analysis (SPAD software) applied on the previously calculated correlation matrices was used to explain the main discriminant sources of the different tissues.

Results and discussion

Exhaustive screening of the inorganic content of tissues allows detection of a pool of about 45 different elements. Among them, carbon, nitrogen and oxygen are not currently measurable by ICP-MS, and phosphorus, sulfur, chlorine and bromine determinations require particular precautions that are not compatible with multi-elemental analysis (Date & Stuart 1988, Jiang & Houk, 1988).

Detection limits

Precision and quantitative detection limits of the methodology have been established in a recent study (Schmit *et al.* 1991). They were evaluated via repeated spike recoveries in an organic matrix similar to the one generated by the digestion of the rat tissues. Those limits lay between 0.125 and 1.0 ng ml^{-1} for most of the elements (Table 1). Only bismuth, iron, scandium, silicon and iodine could not be measured precisely below 10 ng ml^{-1}.

Table 2 presents the inorganic contents of 10 tissues of spontaneously hypertensive rats. The general elemental distribution among the 10 tissues appears similar to the one found in normotensive Sprague-Dawley rat tissues and their concentrations lie in the same range (Gélinas *et al.* 1992). However, several significant divergences (90% confidence limit) can be found for some of the most important trace elements for tissue integrity (Table 3).

Table 1. Evaluation of elemental detection limits of the method spike recoveries in a complex organic matrix

Element	Spike (ng ml^{-1})	Found[a] (ng ml^{-1})	Confidence limit (90%)
Ag	0.125	0.130	0.018
Co	0.125	0.121	0.005
Cr	0.125	0.122	0.004
Li	0.125	0.129	0.006
Mn	0.125	0.127	0.006
Mo	0.125	0.124	0.008
Sb	0.125	0.122	0.015
Sr	0.125	0.123	0.005
Tl	0.125	0.117	0.004
V	0.125	0.128	0.004
Y	0.125	0.125	0.002
As	0.250	0.233	0.037
Ba	0.250	0.253	0.024
Cd	0.250	0.249	0.022
Hg	0.250	0.244	0.028
Pb	0.250	0.235	0.036
Sn	0.250	0.254	0.025
Zr	0.250	0.227	0.019
U	0.250	0.235	0.008
Al	0.50	0.44	0.03
B	0.50	0.49	0.06
Cu	0.50	0.47	0.03
Ni	0.50	0.46	0.05
Rb	0.50	0.50	0.05
Se	0.50	0.51	0.07
Ti	0.50	0.48	0.03
W	0.50	0.49	0.03
Zn	1.00	1.02	0.09
Bi	10.00	7.92	0.24
Fe	10.00	10.36	0.37
Sc	10.00	8.34	0.34
Si	10.00	14.16	1.73
I	10.00	6.86	0.48

[a]Mean of five independant determinations.

Table 2. Inorganic contents of individual male spontaneously hypertensive rat tissues

Element	Brain		Heart		Erythrocyte		Liver		Muscle		Bone		Plasma		Lung		Kidney Cortex		Kidney medulla	
	mean[a] ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)	mean ($\mu g\,g^{-1}$)	confidence limit (90%)
Ag	0.006	0.001	0.004	0.001	0.006	0.001	0.010	0.001	0.008	0.001	0.265	0.025	0.003	0.001	0.002	0.001	0.006	0.001	0.007	0.001
Al	0.473	0.061	0.562	0.059	0.570	0.061	1.095	0.052	1.173	0.088	1.585	0.093	0.589	0.045	0.945	0.057	0.443	0.044	0.311	0.028
As	0.114	0.013	0.108	0.016	11.787	0.600	0.194	0.011	0.138	0.007	0.637	0.040	0.035	0.009	0.768	0.060	0.079	0.006	0.117	0.011
B	0.078	0.007	0.108	0.010	0.104	0.008	0.310	0.015	0.105	0.009	1.849	0.090	0.001	0.001	ND		0.139	0.010	0.163	0.009
Ba	0.036	0.006	0.010	0.001	0.056	0.005	0.013	0.001	0.028	0.011	5.967	0.329	ND		0.007	0.001	0.006	0.001	0.007	0.001
Bi	ND[b]		ND		ND		0.002	0.002	0.003	0.002	0.001	0.001	ND		ND		ND		0.003	0.001
Ca	20.099	1.451	8.956	0.251	32.462	2.298	11.918	0.246	12.958	0.209	117744.5	7011.0	52.471	5.084	14.216	1.885	21.923	1.933	10.476	1.191
Cd	0.001	0.000	0.004	0.002	0.002	0.001	0.003	0.002	0.005	0.001	0.062	0.006	0.017	0.004	0.001	0.000	0.041	0.009	0.109	0.011
Co	0.003	0.001	0.010	0.001	0.011	0.001	0.013	0.001	0.002	0.001	2.257	0.190	0.002	0.001	0.004	0.001	0.048	0.010	0.045	0.004
Cr	0.247	0.016	0.250	0.011	0.212	0.017	0.280	0.020	0.153	0.007	0.224	0.008	0.118	0.009	0.352	0.018	0.218	0.010	0.277	0.013
Cu	2.489	0.129	4.846	0.155	1.051	0.092	3.545	0.072	0.886	0.058	1.061	0.054	1.146	0.040	1.215	0.048	9.146	0.410	5.682	0.195
Fe	34.959	1.333	102.392	3.606	853.763	24.406	153.297	6.082	21.319	2.437	54.034	3.321	2.764	0.184	150.793	0.761	90.074	5.549	84.213	4.816
Hg	0.001	0.000	0.001	0.001	0.0004	0.000	0.002	0.001	0.001	0.001	0.225	0.017	ND		ND		0.004	0.000	0.010	0.001
K	5026.425	179.47	5691.52	53.08	5343.13	218.34	4617.48	102.25	7699.06	93.96	1763.67	92.91	475.77	19.19	4174.54	139.55	3166.47	64.73	4397.17	128.35
Mg	150.68	3.12	250.55	6.83	96.78	2.97	213.78	5.85	277.55	5.66	5435.87	146.99	34.49	2.17	202.22	8.25	275.96	7.77	261.43	17.19
Mn	0.301	0.019	0.390	0.011	0.092	0.005	2.170	0.101	0.116	0.006	0.527	0.026	0.003	0.001	0.153	0.007	1.367	0.044	1.814	0.062
Mo	0.047	0.008	0.057	0.003	0.003	0.000	0.584	0.027	0.017	0.001	0.194	0.013	ND		0.053	0.004	0.392	0.019	0.383	0.023
Na	1708.38	41.38	1696.01	50.84	2243.52	108.39	1394.15	45.61	972.53	11.51	12278.18	954.26	8041.03	228.15	2424.10	103.07	1533.65	38.19	2810.76	99.37
Ni	0.003	0.001	0.002	0.001	0.023	0.002	0.051	0.005	0.028	0.003	1.006	0.068	0.001	0.001	0.013	0.002	0.100	0.010	0.217	0.015
Pb	0.001	0.000	0.010	0.001	0.014	0.001	0.192	0.013	0.042	0.006	0.208	0.017	ND		0.011	0.001	0.092	0.011	0.109	0.006
Rb	1.988	0.059	3.143	0.099	2.589	0.109	5.460	0.116	4.319	0.103	1.075	0.057	0.185	0.011	2.598	0.090	5.182	0.166	7.166	0.236
Sb	0.002	0.001	0.008	0.001	0.001	0.001	0.029	0.003	0.011	0.001	0.055	0.004	0.001	0.001	0.044	0.007	ND		0.004	0.001
Sc	0.002	0.001	ND		0.003	0.002	0.002	0.001	0.001	0.001	0.437	0.035	ND		ND		0.016	0.002	0.022	0.011
Se	0.089	0.007	0.222	0.028	0.788	0.058	0.438	0.026	0.211	0.016	0.219	0.027	0.439	0.043	0.358	0.033	1.350	0.061	1.477	0.091
Sn	0.001	0.001	0.011	0.001	0.087	0.117	0.104	0.012	0.081	0.007	0.212	0.018	0.001	0.001	0.001	0.001	0.020	0.002	0.029	0.003
Sr	0.058	0.006	0.025	0.003	0.036	0.007	0.081	0.008	0.052	0.004	44.738	1.429	0.035	0.007	0.050	0.006	0.030	0.002	0.051	0.004
Ti	1.134	0.058	1.317	0.085	0.682	0.111	0.527	0.027	0.254	0.015	1.110	0.085	0.486	0.037	1.793	0.133	1.220	0.082	1.752	0.075
Tl	0.001	0.000	0.011	0.001	0.091	0.012	0.001	0.001	0.020	0.002	0.002	0.001	0.002	0.001	0.001	0.001	0.028	0.004	0.156	0.008
V	0.038	0.006	0.025	0.002	0.059	0.012	0.056	0.004	0.045	0.007	0.054	0.005	0.065	0.010	0.020	0.002	0.037	0.005	0.055	0.009
W	0.001	0.001	ND		0.004	0.005	0.001	0.001	0.002	0.001	0.009	0.001	ND		ND		ND		0.003	0.001
Y	0.001	0.001	ND		ND		0.001	0.001	ND		0.004	0.001	ND		ND		ND		ND	
Zn	12.127	0.670	20.686	0.958	8.025	1.258	23.304	0.796	12.599	0.687	175.041	4.193	1.526	0.082	14.710	0.522	29.294	1.350	30.552	1.009
Zr	0.028	0.006	0.073	0.009	0.032	1.389	0.193	0.016	0.098	0.013	0.150	0.009	0.054	0.010	0.046	0.008	0.071	0.007	0.125	0.010

[a]Each value is the average of 10 independent determinations on each of the 10 different tissues. Each determination is the average of four successive measurements.
[b]Not determined or below quantitative detection limit.

Table 3. Significant differences (90% confidence limit) between the inorganic concentrations of tissues from spontaneously hypertensive and healthy Sprague-Dawley rats (%)

Element	Brain	Heart	Erythrocyte	Liver	Muscle	Bone	Plasma	Lung	Kidney cortex	Kidney medulla
Na	+18.8[a]	+25.0	+7.9	+53.7	+77.8	+26.8	+38.6	+33.2	+25.9	+20.4
Ca		−33.7	−17.2			+11.7	−18.5		−10.5	−28.7
K		+29.2		+13.4	+16.2	+75.9			+15.0	+10.1
Mg						+12.2	−24.5	+13.5		
Al					+24.4		+16.6			
As	+22.8							+23.3		
Ba						+11.0				
Co						+65.2				
Cr	+15.4					−62.1	−2.5[b]	+32.3		
Cu									+10.5	+20.1
Ni						+15.0				
Rb	+99.4				+21.6					
Se		−10.0	−13.7				−13.9			
Sr			−55.6							
Ti			−29.3					−20.8		
Zn	+24.7	+21.2								

[a]Percentages are obtained by substracting the concentrations of the Sprague-Dawley rat from those of the spontaneously hypertensive rat, divided by the latter, multiplied by 100.
[b]Statistically non-significant at 90% confidence level.

The first and most noticeable differences concern the major elements (i.e. calcium, sodium, potassium and magnesium).

Sodium and calcium. The role of sodium retention by the kidneys in the etiology of hypertension is well understood today (Blaustein & Hamlyn 1984). In renal tubular cells, sodium transport is controlled by the cytosolic calcium concentration. A diminution of cytosolic calcium induces a decline in global sodium exchanges across the membrane (Chase 1984, Windhager *et al.* 1988). An altered regulation of the calcium transport systems in the tubular cells, via an activation of a Ca^{2+} ATPase, would consequently produce secondary disorders in sodium reabsorption and would result in an elevated expulsion rate of calcium from the cell, a lower cytosolic calcium concentration, a higher permeability of brush border membranes for sodium and, ultimately, an increased sodium reabsorption. The response from plasmatic volume regulatory mechanisms to the increase in sodium concentration would then generate hypertension (Gmaj *et al.* 1988). This hypothesis seems in accordance with the 25.9 and 20.4% rise of sodium, and the 10.5 and 28.7% decreases of calcium in kidney cortex and kidney medullas, respectively, in hypertensive rats.

On the other hand, many anomalies in the structure and function of erythrocytes have been described and suggest a more generalized problem at the membrane level (Gmaj *et al.* 1988). Reductions of Ca^{2+} concentration in erythrocyte membranes, platelet cells and synapsis membranes of neuronal cells have been recorded (Imaoka 1987). Decreases of 33.7, 17.2 and 18.5% of calcium, associated with rises of 25.0, 7.9 and 38.6% of sodium in hearts, erythrocytes and plasmas of spontaneously hypertensive rats also agree with those recent results. As expected, intracellular increases of sodium are common to every tissue exposed to hypertension (between 7.9 and 77.9%) and are probably a consequence of the higher sodium retention by kidneys.

As well, the increase of 11.7% of calcium in bones of hypertensive rats is accompanied by increases in elements that show marked and specific accumulations in bones: barium (11.0%), nickel (15.0%) and cobalt (65.2%).

Potassium. Surprisingly, despite simultaneous increases in sodium, potassium levels measured in hypertensive tissues are generally higher than those in normotensive tissues. Sodium and potassium are usually regulated by an antagonism mechanism in the excretion function of the kidney, as shown by their negative correlation coefficient of −0.76 obtained consecutively to the multivariate analysis.

Thus, the Na–K ATPase pump within membranes cannot account for either increase.

Magnesium. Magnesium has often been described as a protecting element against hypertension and a deficiency can lead to a gradual augmentation of arterial pressure (Karppanen 1985). Even if hypertensive rats show higher levels of magnesium in lungs and bones than normotensive rats (13.5 and 12.2% more, respectively), their magnesium concentration in plasmas shows a marked decrease (−24.5%). More studies will be necessary to explain the importance of magnesium in the etiology of hypertension but a low concentration in blood must definitively be considered as an additional risk factor.

Even if the rise in blood pressure caused by a severe intoxication by certain trace elements is well documented, the name of the differences existing in the normal trace inorganic content between normotensive and hypertensive animals exposed to the same environment is still unknown. Trace element variations found in this study are probably not all directly implicated in the etiology of hypertension. However, for many of them, a relation with hypertension or other cardiovascular diseases has been reported by different authors.

Aluminum. The 24.4% increase of aluminum in hypertensive rat muscles could well be a consequence of the 16.6% increase observed in plasmas. Increases in the concentration of aluminum in plasma have already been reported for other diseases (Allen 1981).

Arsenic. The 22.8 and 23.3% increases of arsenic in brains and lungs from hypertensive rats must be attributed to an incomplete perfusion of the hypertensive tissues since erythrocytes, the main cumulative tissue for arsenic, show no significant variations. Lungs and brains are particularly difficult to perfuse efficiently.

Chromium. In contrast to the carcinogen Cr(VI) that penetrates into red blood cells, Cr(III) is an essential trace element that is bound to plasmatic globulins when transported towards tissues. Since its absorption by the gastro-intestinal tract is very limited (0.4%), a decrease in its concentration could generate physiological problems for the entire organism. Moreover, hypertension has been negatively correlated with Cr(III) concentration in plasma (Punsar 1975). The slight reduction of Cr(III) in plasmas from hypertensive rats may not be significant when taken alone (−2.5%), but it becomes more threatening when the very significant drop of 62.1% in bones is considered [bones contain the only bioavailable stock of Cr(III) of the body (Stoecker & Li 1988)]. The low dietary absorption rate combined with those two decreases could be a consequence of, or constitute, a supplementary risk factor for hypertension.

On the other hand, the 32.3% rise of chromium in lungs from hypertensive rats must be attributed to Cr(VI) (Teraoka 1981) that would not play a role in hypertension. Besides the fact that the lung is the only organ known to accumulate chromium with aging (Langard & Hensten-Petterson 1981), no explanation was found for this higher concentration, nor for the similarly high levels found in brains (+15.4%).

Copper. The similarity between degenerative changes of the cardiovascular system associated with cardiac diseases and cupric dietary deficiencies led to the hypothesis that copper plays a role in the etiology of certain human cardiovascular diseases. Recently, elevated plasma concentration of copper have been measured in subjects suffering from hypertension or cerebal atherosclerosis (Watson 1987). While copper concentration is slightly higher in every hypertensive rat tissue, only kidney cortex and medullas show significant changes (10.5 and 20.1% higher, respectively).

Rubidium. Increases of rubidium in hypertensive tissues are correlated with those of potassium but they are less pronounced. Only brain tissues behave differently with a 99.4% rise (while potassium is only 7.3% higher). Since rubidium levels are usually uniform within members of a given species and are somewhat independent of exposure, the origin of this difference could be genetic.

Selenium. The influence of selenium on cardiovascular functions is well admitted today. A long-term selenium deficiency leads to Keshan disease, a severe cardiomyopathy. A negative correlation was also shown between its abundance and degenerative coronary and cardiovascular diseases in certain countries where soil concentrations of selenium are low (Finland, China, New Zealand, etc.) (Oster *et al.* 1986). It is even believed that selenium could be beneficial to patients suffering from ischemic diseases and hypertension since its protective action against hypoxy and free radicals could minimise cardiovascular tissue damage (Watson 1987). Plasmas, erythrocytes and hearts from hypertensive rats show significant reductions of selenium (13.9, 13.7

and 10.0%, respectively), while brains, bones and lungs have non-significant lower concentrations. Hypertension could play a role in the generation of those differences.

Strontium. Strontium distribution within tissues is usually similar to that of calcium. Thus, it is not surprising to measure a lower concentration in almost every tissue from the spontaneously hypertensive rats (−55.6% for erythrocytes and non-significant decreases for hearts, bones, plasmas, lungs and kidneys). The difference encountered for erythrocytes may seem elevated but it could be related to the structural and functional changes in their membranes associated with hypertension.

Titanium. The higher levels of titanium in lungs and erythrocytes from normotensive rats remain unexplained. The correlation between the two (20.8 and 29.3%, respectively) could be attributed to residual erythrocytes trapped in lungs following an incomplete blood perfusion. Moreover, membrane changes in erythrocytes from hypertensive rats could reduce titanium penetration within their cytoplasm. As well, titanium is more concentrated in lungs than in any other organ and thus they could be the first organs affected by a variation of its concentration.

Zinc. Even if zinc is implicated in numerous biochemical reactions and was proved to be an antagonist of copper, no relation could be found between increases of 24.7% in brains and 21.2% in hearts, and hypertension. Since every tissue from normotensive rats contains less zinc than the ones from hypertensive rats, the differences could be attributed to genetic factors specific to each strain of rats.

Biological variations within the 10 samples of each tissue from either strain of rats being very low, hypertension seemingly causes profound modifications in the inorganic content of many tissues. Curiously, soft tissues with a high inorganic load (liver and kidneys) exhibit no significant differences in their trace inorganic content (copper excepted) when exposed to high blood pressure. This situation probably results from the fact that their exposure to inorganics via food, air and water, which is mostly responsible for their high inorganic content, was carefully controlled and presumed identical for both strains of rats.

Conclusion

The main objective of this study was to evaluate the differences in the inorganic content of tissues from spontaneously hypertensive and healthy Sprague-Dawley rats, taking advantage of the capability of multi-element analysis by ICP-MS. It is presumptuous to assert, without additional research, that the differences found between the trace element contents of the two strains of rats are all directly related to hypertension. However, it is clear from this work that ICP-MS offers great possibilities for synergistic studies and identification of inorganic tracers in biomedical research.

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