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Gender-Related Differences in a Process of the Age-Dependent Alterations of the Elements in Monkey Sino-Atrial Node

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Gender differences in the trace elements of monkey sino-atrial (SA) node were investigated in a process of age-dependent alterations. Sixty hearts from Japanese and rhesus monkeys (30 male and 30 female) used were aged ranging from 1-day- to 30-year-old. The elements were analyzed using an inductively coupled plasma-atomic emission spectrometer (ICP-AES). Advancing age decreased all the trace elements. Ca, P, S and Mg significantly decreased. The correlation coefficients of Ca and P were −0.178±0.081 ($p<0.05$) and −0.088±0.022 ($p<0.05$) in male (n=30), and −0.095±0.026 ($p<0.05$) and −0.069±0.017 ($p<0.05$) in female (n=30), respectively. The age-dependent coefficients for Fe/Ca, Zn/Ca, Fe/P, Fe/S, Zn/S, Fe/Mg and Zn/Mg were exhibited markedly in male, but all was less in female. In gender-related differences, only a ratio of P/Ca ($p<0.05$) was significantly observed with ageing. The trace elements such as Cu, Se and Sn were less detected in the SA nodes. These results indicate that the age-dependent changes in the ratios of elements are appeared more rapidly in male monkey SA node, and the gender difference is observed in ratio of P/Ca. The different attenuations may be involved with the age- and gender-related SA nodal functions.

Key Words: Gender difference, Trace elements, Age-dependent alteration, Cardiac pacemaker, Monkey sino-atrial node

INTRODUCTION

Mineral concentrations in animal tissues play an important role for the effects of contamination on animal health and safety of animal origin products in human nutrition. In our recent studies, the trace elements in monkey sino-atrial (SA) nodes were attenuated along with ageing [1,2]. This phenomenon was similar to the changes in heart tissues of other animals such as rat and porcine. In human SA node, however, we did not find the age-dependent alterations of trace elements, presumably due to a limitation of only elder persons (65 to 102 year-old) [3]. In monkey hearts with a large rang of ages, the almost elements decreased in an age-related manner [4]. In our laboratory, the age-dependent changes in the vascular tissues are quite different from those of the heart. The elements in the vascular tissues were accumulated along with ageing.

Recently, a gender medicine has been focused worldwide. Although most of gender differences is a lifetime, gender disagreements have been found in the occurrences of diseases and the effectiveness of drugs. With an advance in years, the differences result from sex hormones, and may be due to the fundamental differences of genomes. As a result, the gender-related alterations of physiological and pharmacological functions are produced [5,6]. In human cardiovascular responses to adrenaline, α-adrenergic effects are predominant in female, whereas β-adrenergic effects are predominant in male [7]. Cardiac functions are declined with advancing ageing [8,9]. The decline might be in partial due to age-dependent alterations of the element contents, accompanied with gender differences. Recently out of balances of the element ratios (Ca/P, Mg/Ca and Cu/Zn etc.) as well as the decline of elements have been shown to be closely related with the occurrence of diseases [10-12]. However, little is yet known concerning the age-dependent and gender-related differences for the trace elements in SA node (cardiac pacemaker), closely involved with cardiac functions. In the present experiments, therefore, the alterations in the elements and the ratios of pair elements induced by sex differences along with ageing were investigated using monkey SA node tissues with a large range of ages. An age of the monkey is considered to be equivalent to one third of human age.

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ABBREVIATIONS: SA node, sino-atrial node.

METHODS

All experiments were carried out according to the guidelines laid down by the Nara Medical University Animal Welfare Committee, and also under the terms of the Declaration of Helsinki.

Sampling

Sixty Japanese monkeys (30 male and 30 female) weighing 2～15 kg were used in a range between 1-day- and 30-year-old. The SA nodal tissues were removed from anatomical position, and were confirmed by means of histological observation we have experienced. After removing the right atrium, strips of the SA node tissue (5～7 mm in width) were obtained by dissecting the tissue perpendicular to the crista terminalis, as described previously [1,13,14]. The preparation was fixed with 10 % formalin.

Determination of elements

Samples were washed throughly with distilled water and were dried at 80°C for 16 h, as described in our previous reports [1-4]. After 1 ml nitric acid was added, they were heated at 100°C for 2 h in a dry-block bath (EB-303; Iuchi, Tokyo, Japan). After the addition of 0.5 ml perchloric acid, they were heated at 100°C for an additional 2 h. The samples were adjusted to a volume of 10 ml by adding ultrapure water (Milli-Q Jr.; Millipore, Tokyo, Japan) and were filtered through the filter paper with a pore size of 4 μm (No. 7; Toyo Roshi Co., Osaka, Japan). The resulting filtrates were analyzed with an inductively coupled plasma-atomic emission spectrometer (ICP-AES) (ICPS-1000III; Shimadzu Co., Kyoto, Japan). The conditions were 1.2 kW from the radio-frequency generator, a plasma argon flow rate of 1.2 l/min, a cooling gas flow of 14 l/min, a carrier gas flow of 1.0 l/min, a 20-μm entrance slit, a 30-μm exit slit, a height

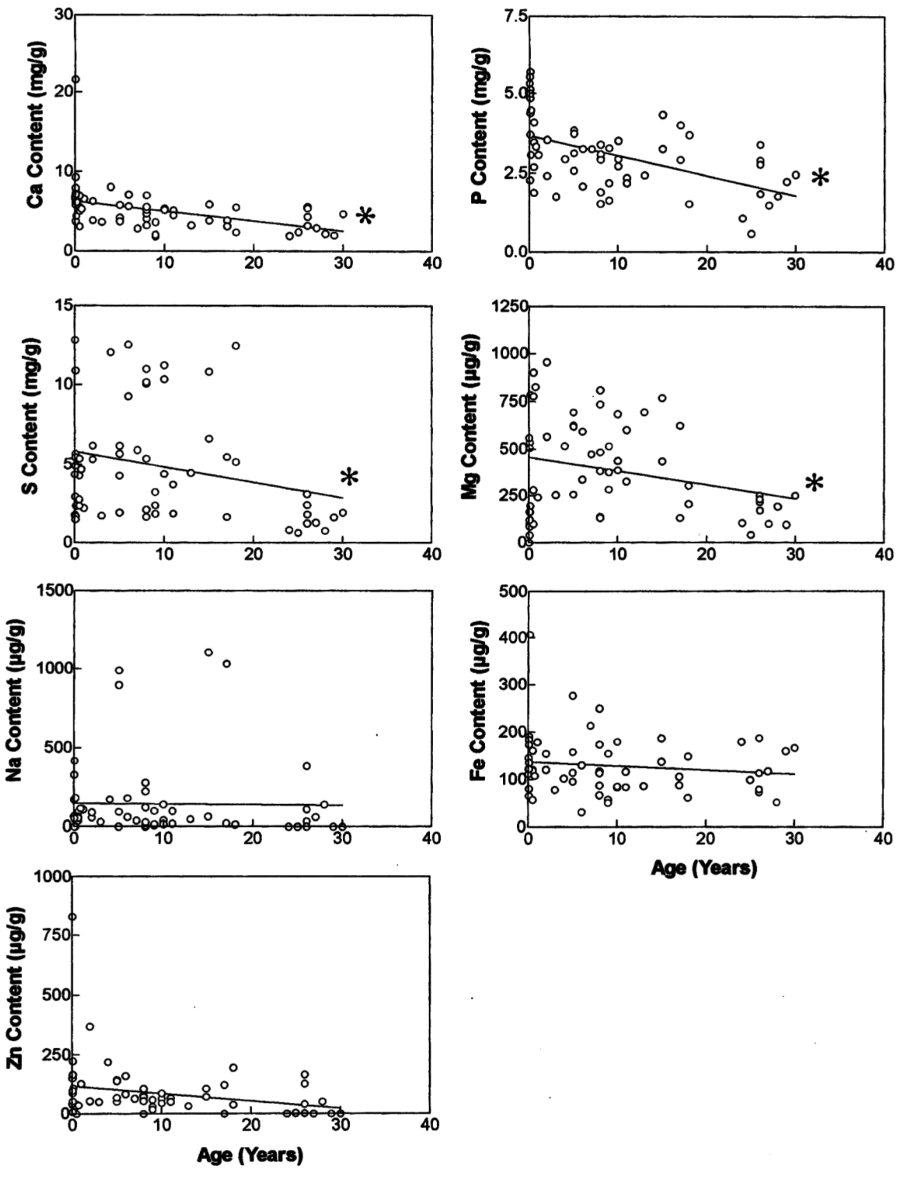

Fig. 1. Age-related slopes for the elements in monkey SA node. Values are represented as mean±SEM. *$p < 0.05$, with respect to coefficient slope.

of observation of 15 mm, and an integration time lapse of 5 s. The amount of element was expressed on a dry-weight basis.

Statistical analysis

Statistical analyses were performed using the GraphPad Prism Version 2.0 (GraphPad Software Inc., San Diego, CA, USA), as according to our previous reports [1,2]. The values were compared by paired *t*-test with analysis of variance (ANOVA). A p-value less than 0.05 was considered to be statistically significant. Data were expressed as the mean± SEM.

RESULTS

Age-dependency of the elements

Using ICP-AES analysis, the element contents such as Mg, Na, P, S, Fe, Zn, Se, Cu, Sn and Co were estimated. The element contents of all the SA node (n=60) analyzed in this study were 5.149±0.355 mg/g in Ca, 3.093±0.147 mg/g in P, 4.981±0.460 mg/g in S, 0.387±0.033 mg/g in Mg, 0.088±0.0015 mg/g in Zn, 0.143±0.0032 mg/g in Na, and 0.128±0.008 mg/g in Fe. Other trace elements such as Se, Co, Sn and Cu were less detected in the SA node. Fig. 1 shows the age-related changes in each element in the SA nodes of both male and female monkeys. Advancing ages reduced most of element contents age-dependently. The significant slopes (n=60) were -0.123 ± 0.035 ($p<0.001$) in Ca, -0.062 ± 0.014 ($p<0.001$) in P, -0.099 ± 0.048 ($p<0.05$) in S, and -0.744 ± 0.348 ($p<0.05$) in Mg.

For each sex, the correlation coefficients for the age-dependent attenuations of Ca and P were -0.178 ± 0.081 ($p<0.05$) and -0.088 ± 0.022 ($p<0.05$) in male (n=30), and -0.095 ± 0.026 ($p<0.05$) and -0.069 ± 0.017 ($p<0.05$) in female (n=30), respectively (Fig. 2). The attenuation slopes of Ca and P were relatively shaper in male monkeys. Mg

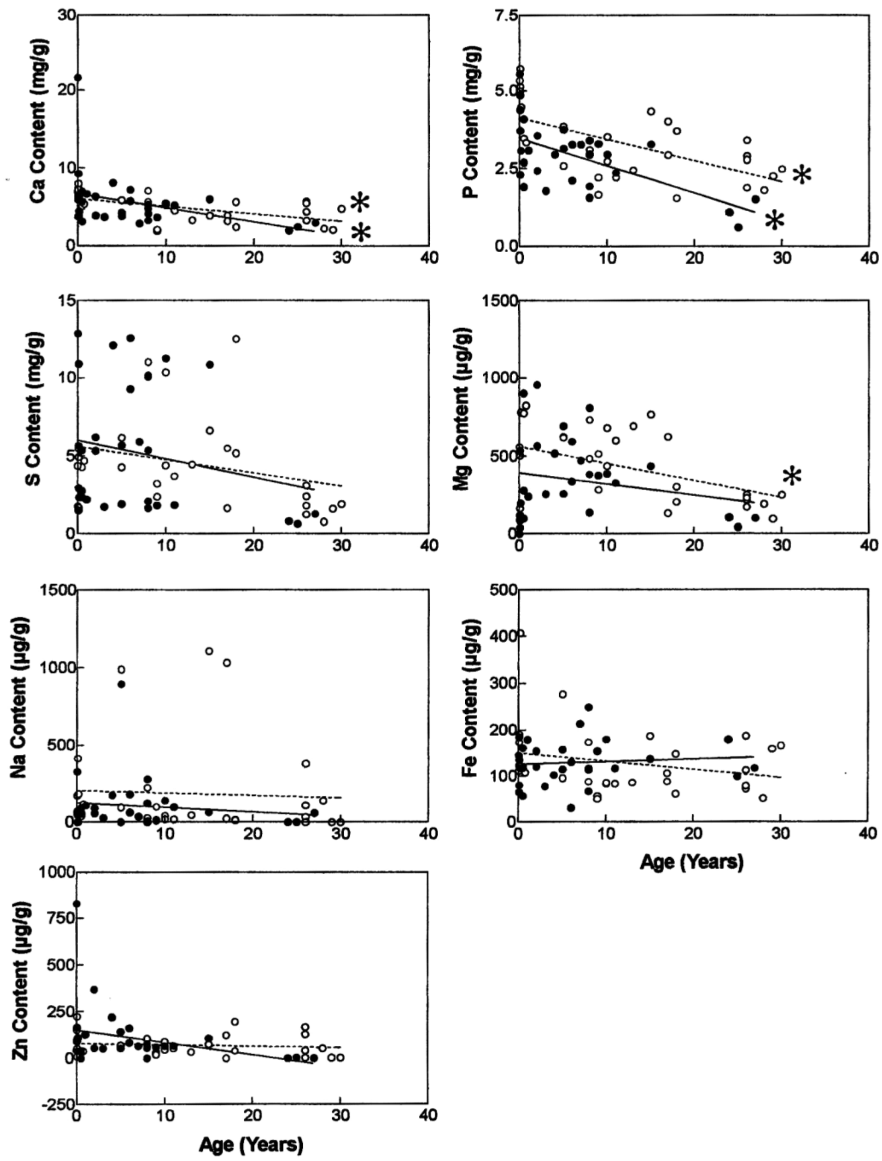

Fig. 2. Age-related alterations with aging for the elements in monkey SA node. Open circles and solid line indicate the male monkey SA node (n=30), and filled circles and dashed line the female monkeys (n=30). Values are represented as mean± SEM. *$p<0.05$, with respect to coefficient slope.

in the female also decreased significantly with ageing (−0.109±0.042, $p<0.05$). Other elements were not caused the changes to significant extent. With ageing, Zn of both sexes tended to decrease, and the male monkeys had a shaper slope. Fe of the male was failed to alter, but the slope of female was sharper.

Gender-related differences of the correlation among elements along with ageing

The correlations for pair of elements were compared between male and female monkeys. The correlation with P

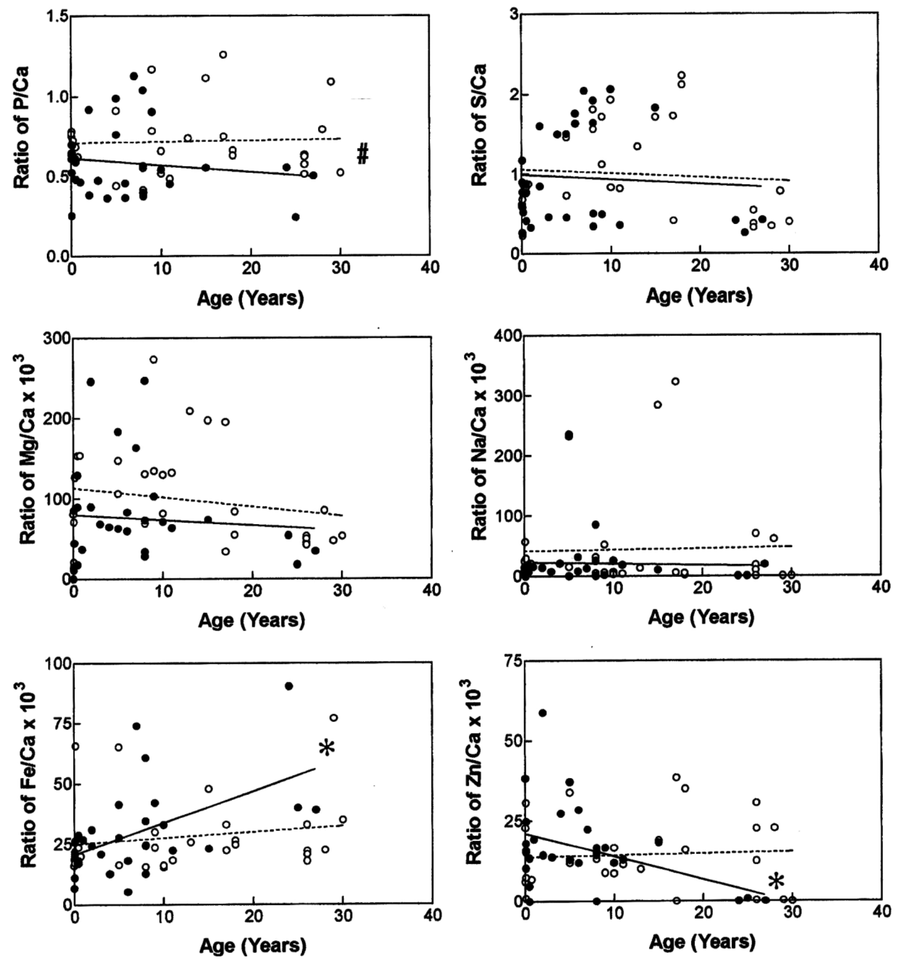

Fig. 3. Gender-related differences in each element as a base of Ca content in the SA node along with ageing. Open circles and solid line indicate the male monkeys (n=30), and filled circles and dashed line the female monkeys (n=30). Values are represented as mean±SEM. *$p<0.05$, with respect to coefficient slope. #$p<0.05$, with respect to the gender difference.

Table 1. Correlation coefficients among the elements in monkey SA node

		P	S	Mg	Na	Fe	Zn
Ca	Male	−0.144 ⌉#	−0.070	−0.080	−0.044	0.532*	−0.423*
	Female	0.036 ⌋	−0.081	−0.186	0.026	0.162	0.049
P	Male		−0.044	−0.040	−0.072	0.662***	0.068
	Female		−0.112	−0.250	0.010	0.184	0.080
S	Male			0.085	−0.016	0.643***	−0.448*
	Female			0.091	0.048	0.265	−0.005
Mg	Male				−0.112	0.382*	−0.417*
	Female				−0.083	0.163	−0.047
Na	Male					0.023	−0.089
	female					0.306	0.081
Fe	Male						−0.296
	female						0.016

Significance (n=30) is represented as *,#$p<0.05$, ***$p<0.001$.

as a base of Ca content (P/Ca) was not significant; -0.144 ± 0.020 in male and -0.036 ± 0.001 in female monkeys (Fig. 3). The gender difference in age-related ratio of P/Ca was significant ($p<0.05$). The correlation coefficients were -0.423 ± 0.178 ($p<0.05$) in Zn/Ca and 0.532 ± 0.114 ($p<0.05$) in Fe/Ca in the male, and the others were not significant. These correlation coefficients among all the elements are summarized in Table 1.

As a base of P content (Fig. 4), the correlation coefficient of Fe/P was significant in male monkeys; 0.662 ± 0.053 ($p<0.001$). Furthermore, the close relations were observed in only male monkeys; 0.643 ± 0.021 ($p<0.001$) in Fe/S and

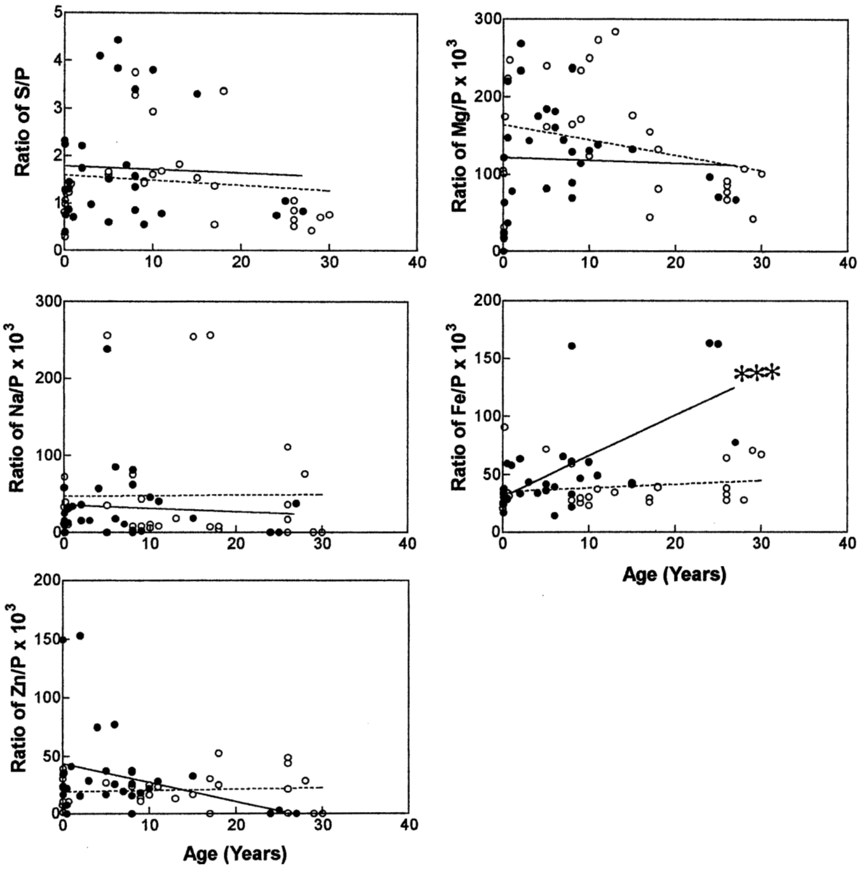

Fig. 4. Gender- and age-related differences in each element as a base of P content. Correlation coefficients among the elements in monkey SA node are present along with ageing. Open circles and solid line indicate the male monkeys (n=30), and filled circles and dashed line the female monkeys (n=30). Values are represented as mean±SEM. ***$p<0.001$, with respect to coefficient slope.

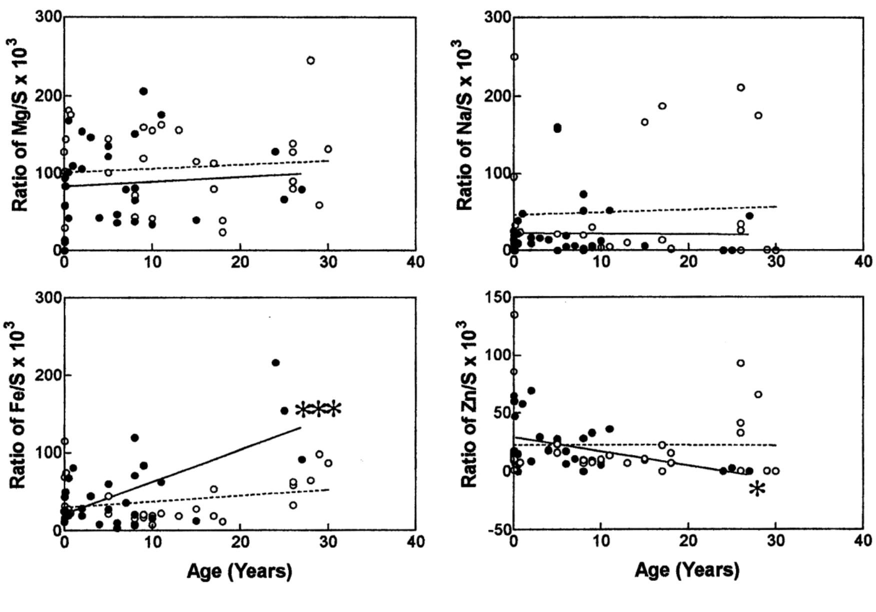

Fig. 5. Gender-related differences in each element as a base of S content in the SA node along with ageing. Open circles and solid line indicate the male monkeys (n=30), and filled circles and dashed line the female monkeys (n=30). Values are represented as mean±SEM. *$p<0.05$, ***$p<0.001$, with respect to coefficient slope.

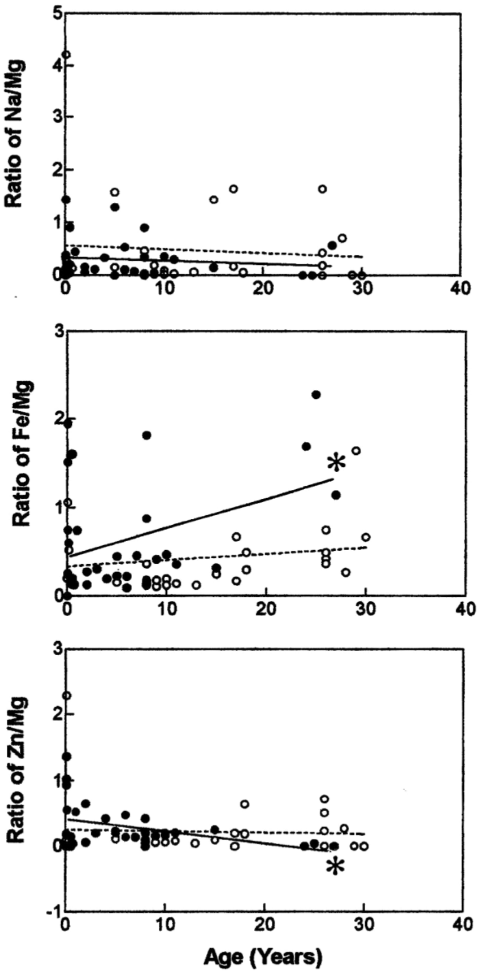

Fig. 6. Gender-related differences in each element as a base of Mg content in the SA node along with ageing. Open circles and solid line indicate the male monkeys (n=30), and filled circles and dashed line the female monkeys (n=30). Values are represented as mean± SEM. *$p<0.05$, with respect to coefficient slope.

-0.448 ± 0.013 ($p<0.05$) in Zn/S (Fig. 5), and also 0.382 ± 0.017 ($p<0.05$) in Fe/Mg and -0.417 ± 0.015 ($p<0.05$) in Zn/Mg (Fig. 6).

DISCUSSION

This study for monkey SA node revealed as follows: (a) the elements (Ca, P, S and Mg) decreased with ageing in both male and female monkeys, but the slopes were not similar, (b) the elements of each sex were more or less attenuated with ageing, and Ca and P deceased in both sexes and Mg in female significantly, (c) the age-related alteration ratios for the pair elements (*i.e.*, Zn/Ca, Fe/P and Fe/S) were remarkable in male but not at all in female monkeys, and (d) the significant gender-related difference with aging exhibited in only a ratio of P/Ca.

Sino-atrial (SA) node plays a key role for cardiac functions as a pacemaker. With an increase in age, vagal predominance occurs, which suppresses the cardiac functions [8,9]. In histological respects, an ageing process has been shown that the density of nodal cell per volume of rat SA node decreases [15]. In the monkeys under 1 year-old or below, the contents of Ca and P in SA node were higher as compared with the adult and elder monkeys [1]. Neonatal SA node beats at faster rate due to higher density of Ca^{2+} channel and the hyperpolarization-activated inward current (a pacemaker current) [16-19]. So, the trace elements are needed to regulate and maintain the cardiac functions and the development. We have already reported for the age-dependent alterations of the trace elements not only in the SA node, but also in the vascular and cardiac valvar tissues in monkey [1] and human [3,20]. Advancing age greatly increases the contents of Ca and P in the arteries and cardiac valves but inversely decreases them in SA node and cardiac muscles [2,21-24]. Mineralization causes pathological alterations, and the resultant calcification (or atherosclerosis) gradually advances along with ageing. The calcification is elicited as a result of a degenerative process within the cardiovascular fibrous tissues [25-28].

Recently, anti-ageing and gender medicines have been intensely focused. The gender-related differences might reflect to cardiac functions, responsible for the differences of sex hormones along with ageing. α-adrenergic effects are predominant in female, and β-adrenergic effects are predominant in male [7], suggesting that blood pressure may be higher sensitive for female, whereas heart rate may be higher sensitive for male. The difference might be partly resulted from the gender-related differences of trace elements. In this study, all the elements in monkey cardiac tissues were attenuated along with ageing, as our previous reports [1,2,4]. The age-dependent coefficients for Zn/Ca, Fe/Ca, Fe/P, Fe/S, Zn/S, Fe/Mg and Zn/Mg were exhibited markedly in male, but all was less in female. The correlations of pair elements are dependent on the change in either element. When either element has a large alteration with aging, the ratio of pair elements is largely changed. We are now unable to explain clearly, but the ageing phenomena in SA node (heart) advance more rapidly in male than female monkeys. The phenomena would be mainly caused by the alterations of Ca, P, Fe, S, Zn and Mg. The gender difference was significant in the relation between Ca and P. The role of Ca/P has already been shown in bone [29,30], auditory ossicles [31], and teeth [32,33]. Furthermore, the Ca/P ratio plays a role for vascular calcification of aorta and coronary arteries [12,34]. Thus, unbalances of the element ratios cause some diseases. The elements in monkey SA node are attenuated by advancing ages, leading to the age-related decline of SA nodal functions. The role of element ratios for the SA node functions is unclear yet.

Physiological functions including heart deteriorate along with ageing. Ageing increases vagal predominance, and suppresses cardiac functions, *i.e.*, a prolongation of R-R interval [35-37]. The SA node also decreases the functions with ageing, and simultaneously might compensate for the age-related deterioration [38]. The age-dependent changes are the decreases in the number of the SA nodal cells, the pharmacological receptors and the ionic channels, and in the intracellular signal transductions. The changes are produced by the expression of genes encoding proteins and alter the receptor sensitivities for autonomic nervous transmitters, resulting in the marked age-related alterations of cardiac functions (such as heart rate, conduction system and cardiac output).

The elements and the trace elements play an important role for the functions [39]. In the present experiments, Ca, P, S and Zn elements in the SA node decreased markedly, consistent with our previous reports [1,2]. The attenuations of major elements such as Ca and P may be closely involved with the age-related cardiac functions (the contraction system). Major functions of Na are (a) osmotic control, (b) electrolytic balances and current, (c) stability of polyelectrolytes, DNA, and membranes, and (d) connections of chemical uptake of organ metabolites. In this study, Mg decreased in male but increased in female. Lower Mg is related with autistic spectrum disorders [37]. Mg plays an important role as Mg-ATP for energetic metabolism. Lack of Mg may lead to ischemic heart diseases. Zn in male was markedly reduced with aging. Zn is (a) the most common catalytic metal ion in cytoplasm, (b) involving with a variety of enzyme reactions, (c) an organization of chromosomes, and (d) DNA and RNA regulatory functions. Zn is considered to be one of most important ageing factors. Fe and S act as enzymes for reactions of dihydrogen and dinitrogen.

It has recently found that abnormal ratios of elements caused several diseases; Mg/Ca, Cu/Zn and Cu/Fe in uterine cancer and myoma [11,40] and breast and cervical cancers [10], and Cu/Zn and Cu/Fe in diabetes [41]. In this study, the age-dependent ratios of Fe and the elements (such as Fe/Ca, Fe/P, Fe/S and Fe/Mg) were altered markedly (see Fig. 3), since the alteration of Fe was small. Fe was not altered in male, but was reduced in female. The relationship between Zn and Fe was also remarkable. These differences may be related to gender differences of an occurrence of the diseases such as cardiac ischemia and acrohypothermy [38]. As a result, the gender differences produce finally a difference of lifetime. Anyway the close age-dependent relations with the alterations of each element, the element ratios and the correlation coefficients are responsible for diminution of the SA node. In the present analyses, the trace elements such as Se, Cu, Co and Sn were less detected in the SA node, but might be involved with the age-dependent diminution of cardiac functions.

Thus, the alterations of element contents in the SA node are modulated by age-related changes in sex hormones. In cardiac conduction system, the fibrosis and fatty influtratin increase with aging [42]. Also, the collagen in human SA node increases in accord with ages [16,43]. The connective tissue increases but the muscle tissue diminishes [44]. Therefore, the age-related attenuations of these elements and the alterations of element ratios might be related with the diminution of the muscle tissues and the decrease in the number of SA nodal cells. Extensive experiments are needed to elucidate it more in detail.

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