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X - RAY MICROANALYSIS OF CARDIOCYTES IN THE SNELL DWARF MOUSE

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SUMMARY: X-ray microanalysis has been used to determine elemental content in the nucleus, myofibrillar cytoplasm and mitochondrially enriched cytoplasm of cardiocytes in Snell dwarf mice in comparison with phenotypically normal mice from the same strain. It was found that there was significantly lower chlorine concentration in all three subcellular locations and significantly lower sodium concentration in the nucleus of dwarf mouse cardiocytes. In both normal and dwarf mice, statistically significant subcellular compartmentalization was found for phosphorus, sulfur, and potassium.

INTRODUCTION. It has been well demonstrated that some pituitary tropins affect heart muscle structure and function of many animal species and of human subjects. In the case of GH (1,2) and PRL (3,4), this action is exerted directly; on the contrary, TSH influences heart muscle indirectly, through stimulation of secretion of thyroid hormones, which are known to have a selective effect on cardiocytes (5,6). Furthermore, it has been shown that thyroid hormones influence electrolyte distribution in some tissues, including the heart, by their action on the passive Na-K permeability of cell membranes (7) and/or by stimulation of the Na-K pump (8,9) as well as by the enhancement of tubular reabsorption of Na in kidneys (10,11). Also, PRL and GH exert an effect on the electrolyte and water content of the body, mainly by influence on renal function (12) and on ion transport across the wall of the intestine (13).

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Abbreviations: NUC - nucleus, CYT - myofibrillar cytoplasm, MEC - mitochondrially-enriched cytoplasm at poles of nuclei, GH - growth hormone, PRL - prolactin, TSH - thyrotropin. T3 - triiodothyronine, T4 - thyroxine.

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To date, there have been no reports on the elemental distribution in the tissues of Snell dwarf mice (14). It is now generally accepted that the primary endocrine lesion in mouse dwarfism is in the anterior pituitary, causing lack of GH and PRL (15) as well as TSH (16), followed by dwarfism (14), infertility (17), and severe secondary hypothyroidism (18). The study described in the present paper compares the elemental content in NUC, CYT and and MEC of the cardiocytes in Snell hypopituitary mice with that in phenotypically normal mice from the same strain. Moreover, we have evaluated elemental compartmentalization between the above mentioned three subcellular locations within the cardiocytes.

MATERIALS AND METHODS. Snell dwarf mice (dw/dw) were produced in a closed colony at the University of Texas Health Science Center at San Antonio by mating the heterozygous normal carriers of the dw gene. The dwarfs remained with their parents during two consecutive lactations and were subsequently housed with phenotypically normal (non-dwarf; ?/+) female littermates. the animals were maintained in a room with controlled illumination (14h light:10 h dark, lights on at 0600 h) and temperature (22 ± 2°C) and with constant access to Wayne Breeder Blox and tap water. The study was performed in adult animals (approximately 4 months of age). Five female dwarf mice and 5 phenotypically normal females from the same strain were used. Animals were sacrificed by cervical dislocation. Heart tissue was prepared for electron probe X-ray microanalysis as previously reported (19) except that thinner sections were cut at colder temperature. Briefly, small apex samples were frozen in liquid propane and ultrathin (0.2 μm) cryosections were cut at - 100°C with a glass knife on a LKBV ultramicrotome with modified cryokit. Freeze drying was performed at -100°C in the LKB cryochamber. Sections were supported on a Formvar film over a 1.5-mm slot in a 3-mm carbon grid. The section was covered by a carbon-coated Formvar film. The carbon grid was placed in a beryllium-carbon specimen holder and sections were examined at 25 kV in the STEM mode in a JEOL JSM-35C scanning electron microscope. Beam current was standardized by setting the specimen current to 2.0×10^{-10} amps on the Be specimen holder. Other parameters were 1.5 cm specimen-detector distance, 40° take off angle. X-ray spectra were acquired for 100 sec using a Tracor Northern detector and X-ray analysis system (NS-880). The Hall (20) peak-to-continuum method of quantitation was employed after correcting spectra for extraneous continuum. Salt-polyvinylpyrrolidone standards were used to convert elemental peak-to-continuum values to concentration units (mmoles/kg dry weight). Ten myocardial cells were analyzed from each animal. For each cell, spectra were collected from the nucleus (NUC), myofibrillar cytoplasm (CYT), and the mitochondrially enriched cytoplasm (MEC) near the nucleus. Data were statistically analyzed by computer using the BMDP2V repeated measures program of the BMDP statistical package (21). For each of the seven elements detected (Na, Mg, P, S, Cl, K, Ca), a (2 x 3) factorial (group by subcellular location) two-way analysis of variance (ANOVA) was performed, with repeated measures on subcellular location. Duncan's multiple range test was used to determine which means were significantly different.

RESULTS AND DISCUSSION. The intracellular elemental concentrations are shown in Table 1. The results of the present study indicate that the Cl concentration (in each examined subcellular location) and Na concentration (in

TABLE 1. Intracellular elemental concentrations (mmole/kg dry wt) in cardiocytes of Snell dwarf mice (dw/dw) and phenotypically normal (?/+) mice of same strain.*

	Normal			Dwarf		
	NUC	CYT	MEC	NUC	CYT	MEC
Na	287 ± 37[a,b]	272 ± 43[c]	212 ± 40[b,c]	208 ± 13[a]	228 ± 22	200 ± 39
K	287 ± 23[d]	263 ± 24[e]	217 ± 20[d,e]	273 ± 20[f]	252 ± 17[g]	212 ± 14[f,g]
Cl	216 ± 30[h,i]	200 ± 30[j,k]	176 ± 38[h,j,l]	149 ± 23[i]	144 ± 56[k]	139 ± 35[l]
Mg	48 ± 10	41 ± 6	32 ± 2	41 ± 7	40 ± 7	39 ± 4
Ca	27 ± 5	22 ± 3	26 ± 8	16 ± 3	17 ± 3	24 ± 4
P	362 ± 46[m,n]	256 ± 34[m,o]	470 ± 49[n,o]	394 ± 21[p,q]	300 ± 27[p,r]	465 ± 22[q,r]
S	165 ± 18[s]	172 ± 19[t]	225 ± 30[s,t]	155 ± 8[u]	163 ± 7[v]	215 ± 5[u,v]

*Data were obtained by electron probe X-ray microanalysis of freeze-dried sections, as described in Materials and Methods. Data are means ± SEM of 5 animals, 10 cells per animal. Means with common superscripts are significantly different ($p < 0.05$, Duncan's multiple range test).

nucleus only) are significantly lower in the cardiocytes of dwarf mice than in those of phenotypically normal mice. These findings are possibly connected with the absence of PRL and GH (15) and with the extremely low serum T3 and T4 levels (16) in Snell dwarfs. Since PRL (22), GH (23) and also the thyroid hormones (10, 11) are known to reduce substantially the renal excretion of Na, Cl and water, their deficiency presumably results in salt wasting and leads to extracellular hypochloremia and hyponatremia. Moreover, PRL and GH (13) have been found to stimulate the transport of fluid, Na, Cl and other ions across the wall of the intestine; therefore lack of these hormones can result in lower absorption of salt from the intestine. In turn, extracellular hypochloremia in hypopituitary mice can result in the loss of intracellular Cl, as has been shown for liver cells incubated in low-Cl solution (24), and is probably responsible for the significantly lower Cl concentration in the three probed subcellular compartments (NUC, CYT, MEC) of the cardiocytes of dwarf mice as compared to nondwarf mice. Though the Na-K pump activity should be reduced in the case of thyroid hormone deficiency (9), for dwarf mice this effect is probably balanced by enhancement of the pump current (or Na ions outflux) because of the extracellular hypochloremia-induced hyperpolarization of the cell membrane (24). Moreover, the assumption can be offered that the

passive Na and Cl ion fluxes into the cell are significantly decreased in Snell mice as a consequence of deficiency of thyroid hormones. Such a possibility is supported by the finding that T3 increases the passive permeability of the cell membrane for Na and K (7). Furthermore, the passive influx of Na ions has been assumed to accompany a movement of passively distributed Cl ions into the cell (24). Thus, hypothyroidism can impede the movement of Na and Cl ions into the cell interior. We have reported previously a positive correlation between the concentration of Na and Cl for different tumor cell types and their normal cell types of origin as well as for various slowly and rapidly dividing cell types (see 25 for review) and for developing mouse cardiocytes during postnatal maturation (26). Similar positive correlation between Na and Cl is also observed in the present study. However, it is not clear why the differences between dwarf and nondwarf animals were more distinct for both Na and Cl in NUC than in CYT (even insignificant for Na in the latter location).

Reports in the literature led us to speculate that the lack of GH and PRL and deficiency of thyroid hormones could affect the concentration of ions other than Na and Cl in the cardiocytes of dwarf mice. It has been shown that PRL stimulates the transport of K, Ca and Mg across the wall of the intestine (27). Excess of GH results in hyperphosphatemia and hypercalcemia in blood (28, 29). Hypercalcemia, hyperphosphatemia and hypomagnesemia are known consequences of hyperthyroidism (30). An excess of thyroid hormones increases total body K content (30). T3 increases intracellular K concentration and decreases intracellular Na concentration in the rat heart by direct stimulation of the Na-K pump (31), i.e. by the same mechanism which is thought to be involved in thyroid hormone-induced calorigenesis (8). However, in some recent papers the above mentioned theory of calorigenic action of thyroid hormones has been criticized (7, 32, 33). In the present study no significant differences have been found in the K, Mg, Ca, P, or S concentrations in any probed subcellular location between dwarf and normal mouse cardiocytes.

With regard to subcellular compartmentalization of elements, only P was found to be significantly different between NUC and CYT. It was in higher concentration in NUC than in CYT in both normal and dwarf mouse cardiocytes, which agrees with findings for hamster (19), rat and guinea pig (34) cardiocytes and is related to the presence of nucleic acids in the NUC. Furthermore, P was significantly higher in the MEC than in the NUC and CYT for both dwarf and normal mice. With regard to other compartmental differences, S was significantly higher and K was significantly lower in the MEC than in the NUC and CYT for both groups.

In conclusion, the present study has shown Na and Cl to be lower in cardiocytes of Snell dwarf mice than in normal mice, which may be related to the lack of GH and PRL and to thyroid hormone deficiency in these animals. It is of interest to us to determine if similar elemental differences exist in tissues other than heart muscle and also to determine what would be the effect of substitution of deficient hormones.

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