EXPERIMENTAL CALCIUM-MAGNESIUM DEFICIENCY IN RATS: CHEMICAL AND HISTOCHEMICAL STUDY

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Chemical and histochemical profiles of central nervous system tissues, skeletal and cardiac muscles and visceral organs of experimental calcium-magnesium deficient rats and control animals were studied. Six to eight weeks after a feeding schedule of a calcium-magnesium deficient diet, the calcium concentration in the kidney increased twenty-five fold over that of the control and there was a moderate increase in the spinal cord and skeletal muscle. Fluctuation of the magnesium concentration was insignificant.

A decrease in succinic dehydrogenase activity demonstrated in the cerebral cortex, cerebellum, spinal cord, kidney and skeletal muscle corresponds to tissue morphology, for example, atrophy and a decreased SDH type II muscle fiber was noted in gastrocnemius tissue specimens.

Acetylcholinesterase activity in the motor end-plate of the gastrocnemius muscle showed a slight decrease in enzyme activity and a swelling of the motor end-plate.

The severe histochemical changes of SDH and acid phosphatase activities in renal tubules and hepatic parenchymal cells suggest a progression of cellular catabolism related to lysosomal enzyme activity.

The relationship of cellular calcium and magnesium to metabolic regulation, such as enzyme activation, endocrine gland secretion and muscle function, has been well documented. A magnesium deficiency in rats producing neuropathological changes in cerebellar Purkinje cells has been reported (2). A recent electron microscopic study of parathyroid gland metabolism demonstrated that a low calcium concentration induced secretory stimulation and increased the convolutions of the intercellular membrane and a high concentration of calcium inhibited secretion and increased the number of phagolysosomes (4).

In this paper, the effects of a calcium and magnesium dietary deficiency on serum and tissue concentrations of these elements in the rat are described and an attempt is made to correlate the effect of the deficiency with the histochemical alterations.

MATERIALS AND METHODS

Healthy one month old Wistar strain rats (closed colony, random bred) of both sexes and weighing 110–190 grams were used. The experimental animals were...
fed a calcium-magnesium deficient diet purchased from the Oriental Yeast Co., Ltd. in Tokyo (Table 1) and deionised water offered ad lib. was measured and recorded. Control animals were fed MF regular rat chow purchased from the same company and were offered tap water. The rats were maintained on these diets for six to ten weeks.

A. Chemical analysis of serum and tissue calcium and magnesium

Using ether, the experimental and control animals were sacrificed and a sample of blood and tissue samples taken from the cerebrum, cerebellum, spinal cord, gastrocnemius, cardiac muscle, liver, kidney and duodenum were obtained in the shortest possible time.

The serum protein, calcium and magnesium concentrations were analysed with a Sequential Multiple Autoanalyser 12/60 (Technicon, Swiss) under standard methods.

The respective tissue specimens, weighing 200–1000 mg, were wet ashed with a mixture of 60% nitric acid and perchloric acid, heated until dissolved and diluted with double distilled water until a total amount of 10 ml was obtained. Determination of calcium and magnesium in these samples was then carried out using an atomic absorption spectrophotometer (Model AA-1 Nihon Jarrel-Ash Co., Tokyo) according to the recommended procedure.

The t-test was applied for statistical analysis of the data.

B. Histochemical demonstration of enzyme activity

Using ether, specimens of tissue from the cerebral cortex, cerebellum, cervical segment of spinal cord, gastrocnemius, liver and kidney were obtained from 17 experimental and 7 control animals. Specimens were prepared by fixing in cold 4% buffered paraformaldehyde for 8 hours at pH 7.4 or frozen in dry ice and cut 10 μ thick.

Tissue sections were incubated with or without substrate and buffer solution
TABLE 2. Protein, Calcium and Magnesium in the Sera of the Control Healthy and Ca-Mg Deficient Rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (g/dl)</th>
<th>Calcium (mg/dl)</th>
<th>Magnesium (meq/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control healthy rats (7)</td>
<td>7.33±0.28</td>
<td>8.33±0.21</td>
<td>2.72±0.12</td>
</tr>
<tr>
<td>Calcium and magnesium deficient rats (8)</td>
<td>6.45±0.24</td>
<td>7.24±0.45</td>
<td>1.85±0.21</td>
</tr>
<tr>
<td></td>
<td>0.01&lt;P&lt;0.02</td>
<td>0.02&lt;P&lt;0.05</td>
<td>0.01&lt;P&lt;0.02</td>
</tr>
</tbody>
</table>

± Standard error
The numbers in parentheses are the numbers of animals used.

RESULTS

The experimental animals fed a calcium-magnesium deficient diet became extremely weak with gross behavioral abnormalities and a pseudoedema of the face and trunk.

A. Quantitative analysis of calcium and magnesium

Totals of serum protein, calcium and magnesium concentration in both experimental and control animals appear in Table 2. Analysis of the data shows a significant reduction (P<0.05) of these concentrations in the experimental animals in comparison with the controls. Results of quantitative analysis of calcium and magnesium in the various tissues of the control and Ca-Mg deficient rats appear in Table 3.

TABLE 3. Calcium and Magnesium in the Various Tissues of the Control and Ca-Mg Deficient Rats. (μg/g wet weight)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Calcium</th>
<th>Magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control rat (7)</td>
<td>Ca-Mg deficient rat (7)</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>127.7±28.7</td>
<td>141.4±69.0</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>81.5±10.1</td>
<td>52.9±7.5‡</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>86.5±15.6</td>
<td>400.8±175.9</td>
</tr>
<tr>
<td>Cardiac muscle</td>
<td>36.1±3.0</td>
<td>42.4±5.0</td>
</tr>
<tr>
<td>Gastrocnemius muscle</td>
<td>51.4±3.8</td>
<td>68.8±7.0§</td>
</tr>
<tr>
<td>Liver</td>
<td>32.1±2.0</td>
<td>22.7±2.2‡</td>
</tr>
<tr>
<td>Kidney</td>
<td>68.2±3.3</td>
<td>1761.7±261.0++</td>
</tr>
<tr>
<td>Duodenum</td>
<td>70.9±4.6</td>
<td>45.7±3.7*</td>
</tr>
</tbody>
</table>

± Standard error
The numbers in parentheses are the numbers of animals studied.
FIG. 1. SDH activity of the anterior grey column of the spinal cord of the control rat. Showing a moderate to strong positive reaction of the spinal motor neurons (arrows) and moderate positive reaction of the neuropil. ×128

FIG. 2. SDH activity of the anterior grey column of the spinal cord of the Ca-Mg deficient rat. Showing a reduced enzyme activity of the neuronal perikaryon and a slight swelling of the motor neurons (arrows). ×128

FIG. 3. SDH activity of the cerebellar cortex of the control rat. Showing abundant positive cells in the granular cell layer (G), moderate positive reaction of the Purkinje cell and neuropil of the molecular layer (right side). ×320

FIG. 4. SDH activity of the cerebellar cortex of the Ca-Mg deficient rat. Showing a decrease in the number of SDH positive cells and reduced enzyme activity in the granular cell layer (G). ×320

FIG. 5. SDH activity of the cerebral cortex of the control rat. Showing a moderate to strong positive reaction of the pyramidal cell (arrows). ×320

FIG. 6. SDH activity of the cerebral cortex of the Ca-Mg deficient rat. Showing a slight decrease of SDH in the pyramidal cell perikaryon (arrows). ×320
Fig. 7. SDH activity of the gastrocnemius of the control rat. Showing an intensely strong positive reaction of type I muscle fiber (I), weak positive reaction of type II (II) and intermediate fiber. Strong SDH activity of the subsarcolemmal sarcoplasm indicated by arrows. ×320

Fig. 8. SDH activity of the gastrocnemius of the Ca-Mg deficient rat. Showing a decrease or disappearance of enzyme activity of type II muscle fibers (II). Type I muscle fiber showed slight hypertrophy and no enzyme activity in the subsarcolemmal sarcoplasm (arrows). Both types of muscle fibers are less angular. ×800

Fig. 9. Acetylcholinesterase of the motor end-plate (arrows) of the gastrocnemius of the control rat. ×320

Fig. 10. Acetylcholinesterase of the motor end-plate of the gastrocnemius of the Ca-Mg deficient rat. Showing a slightly swollen and irregular outline of the motor end-plate (arrow). ×320

Fig. 11. SDH activity of the hepatic parenchymal cells of the control rat. Showing enzyme activity evenly distributed throughout the cytoplasm. ×320

Fig. 12. SDH activity of the hepatic parenchymal cells of the Ca-Mg deficient rat. Showing clumping of SDH, dilatation of sinusoid (long arrows) and reduction in size (short arrows). ×320
magnesium concentrations in the various tissues can be found in Table 3. In all specimens, there is no statistically significant difference in the concentration of magnesium. The most interesting results are found in the calcium concentrations in the experimental animals. Results of Table 3 are summarized as 1) there is a prominent increase in calcium concentration in the kidney of the calcium-magnesium deficient rat, with a statistically significant difference \((P<0.001)\), 2) there is a slight and irregular increase in calcium concentration in tissue taken from the cerebral cortex, gastrocnemius and spinal cord, and 3) there was a significant decrease in calcium concentration in the small intestine \((P<0.01)\) and liver \((P<0.05)\).

B. Histochemical changes in enzyme activity

Cerebral cortex, cerebellum and cervical segment of spinal cord:

SDH activity in the perikaryon of the control spinal motor neuron showed a moderate to strong positive reaction. The neuropil of the anterior gray column of the control spinal cord exhibited a moderately positive reaction (Fig. 1). In experimental animals, the neuronal perikaryon of spinal motor neuron showed a prominent reduction of SDH activity with the perikaryon appearing slightly swollen (Fig. 2). Additionally, there was a slight decrease noted in the granular cells of the cerebellar cortex (Figs. 3, 4), pyramidal cells and neuropil of the 5th layer of the cerebral cortex (Figs. 5, 6).

Gastrocnemius:

In the control specimens, a strong positive reaction for SDH was seen in the small-sized muscle fibers and a weak positive reaction in the large-sized muscle fibers, i.e. type I and II muscle fibers. A small number of intermediate fibers gave a moderate to weak positive reaction. Type I fiber in cross section showed a strong positive reaction in the subsarcolemmal sarcoplasm and in the sarcoplasmic reticulum (Fig. 7). Type II fibers gave only a moderate to slight positive reaction; both types appeared angular in contour.

In the experimental specimens, a decrease in SDH activity was clearly recognized as atrophy which was seen in type II fibers. The contour of these fibers was less angular. Type I fibers demonstrated a slight hypertrophy or were seen without atrophy (Fig. 8).

Acetylcholinesterase activity at the motor end-plate of calcium-magnesium deficient rat tissue samples showed a slight decrease with slight swelling of the outline of the motor end-plate (Figs. 9, 10).

Liver and kidney:

In the controls, SDH was evenly distributed throughout the cytoplasm of the hepatic parenchymal cells and gave a slight to moderate positive reaction (Fig. 11).

In the experimental animals, the hepatic parenchymal cells were slightly reduced in size and the sinusoid appeared dilated. SDH was unevenly distributed throughout the cell with numerous clumps seen in the cytoplasm (Fig. 12).

Histochemically, the reaction for acid phosphatase indicated its role as a lysosomal “marker”. In the parenchymal cells of controls, acid phosphatase positive granules appeared localized along the bile canaliculi and also in the Kupffer cells in the sinusoid (Fig. 13).
FIG. 13. ACPase activity of the hepatic cell of the control rat. Showing ACPase positive granules along the bile canaliculi (arrows) and in the Kupffer cell (K). ×800

Fig. 14. ACPase activity of the hepatic cell of the Ca-Mg deficient rat. Showing an increase in positive granules (arrows) and hypertrophic Kupffer cells (K). ×800

FIG. 15. SDH activity of the renal convoluted tubules of the control rat. Showing a moderate to strong positive reaction of the proximal and distal convoluted tubules in the cortex of the kidney. ×320

FIG. 16. SDH activity of the renal convoluted tubules of the Ca-Mg deficient rat. Showing a decrease in enzyme activity of the proximal tubule and accumulation of SDH positive mass in the lumen of the tubule (arrow). ×320

FIG. 17. ACPase activity of the renal convoluted tubules of the control rat. Showing localization of small and moderate sized granules of acid phosphatase positive reaction in the basal portion of the tubular cell. ×800

FIG. 18. ACPase activity of the renal convoluted tubules of the Ca-Mg deficient rat. Showing increased enzyme activity and localization in the apical portion. ×800
In the experimental animals, acid phosphatase positive granules in increased numbers and of large irregular form were found along the bile canaliculi and throughout the cytoplasm of parenchymal cells (Fig. 14).

In the control kidney SDH activity showed a stronger positive reaction in the cortex than in the medulla. A strong positive reaction was also seen in cells at the basal area of both the proximal and distal convoluted tubules (Fig. 15).

In experimental animals, a low SDH activity was demonstrated in a small number of tubules in the outer layer of the renal cortex, presumably a part of the proximal convoluted tubule (Fig. 16).

Acid phosphatase positive granules were present in all cells of the nephron in the experimental animals. In the controls, these granules were abundant in the basal portion of the proximal convoluted tubule. Distal and collecting tubules, however, showed less enzyme activity than the proximal convoluted tubules. It is interesting that the acid phosphatase positive granules in the proximal and distal tubules showed an increase in enzyme activity throughout the cytoplasm, developing a granular size, especially in the brush border in the experimental animals (Fig. 17).

A reduction in SDH activity in skeletal muscle, motor neurons and renal tubules and atrophy of type II muscle fibers in tissue samples from calcium-magnesium deficient rats have been histochemically demonstrated. In kidney and liver tissue of experimental animals an increase in acid phosphatase activity was demonstrated. From these histochemical observations in the calcium-magnesium deficient rat, possible the relationship of decrease in SDH activity to induction of a process of soft tissue calcification and the relationship of the increase in acid phosphatase to increased cell catabolism will be discussed.

DISCUSSION

An objective of this study was to attempt to correlate in the rat, the state of a calcium-magnesium deficiency with histochemical changes occurring in visceral organs, nervous and muscle tissue. Chemical analysis of serum and tissue concentrations of calcium and magnesium in experimental and control animals were conducted.

In animals fed a calcium-magnesium deficient diet, concentration of calcium was prominently increased spinal cord, kidney and gastrocnemius muscle tissues and decreased in blood serum and cerebellum, liver and duodenum tissues. These observations, together with results of investigations on osteomalacia (unpublished) may help to confirm the report by Chertow et al. (4) concerning mechanisms indirectly affecting parathyroid hormone secretion.

Secondary hyperparathyroidism is known to occur after magnesium deficiency (6) and after uremia (1). Arieff and Massry (1) stated that a marked rise in the level of calcium in the brain was dependent on the presence of excess of parathyroid hormone. Chutkow (5) pointed out that the distribution of calcium in nervous tissue, including the interstitial fluid component, was more than 80% of brain calcium concentration in the intercellular compartment. Recent advances in the study of calcium metabolism, now associate extra-pyramidal motor disorders with calcification of the basal ganglia. And in cases of basal ganglia calcification, insufficient functioning of the parathyroid glands has been noted (3, 9, 13). At
this time, however, it is very difficult to suggest the mechanism producing an increase in the concentration of calcium in nervous and skeletal muscles and can only speculate that this is a response to an abnormality of parathyroid gland function, such as hypo- or hyperparathyroidism. In this study, the histochemical demonstration of reduced SDH activity seen in motor neuron perikaryon is a response to the lower oxidative function of the mitochondria which are affected by the high calcium concentration within the motor neuron. This has also been suggested by reports of chromatolysis and cell degeneration following magnesium deficiency (2) and mitochondrial swelling induced by calcium (8).

The histochemical changes of skeletal muscle, in particular the type II muscle fiber atrophy and reduced SDH activity, may be produced by the decrease in acetylcholinesterase and swelling of the motor end-plate. Mallette et al. (11) report that these changes are the basis of the motor neuron abnormality.

Low levels of dietary magnesium are reported to produce renal damage such as degeneration of the tubular epithelium in rabbits and rats (2). Maggi and Oddy (10) reported uniformly brown-black acid phosphatase positive granules appeared to fill the cytoplasm of proximal tubular cells following a short-time period of starvation in animals.

In this study, acid phosphatase activity appeared in the apical region or brush border and a reduced SDH activity was demonstrated in the proximal tubules of the calcium-magnesium deficient rat kidney. These results suggest that absorbed protein and non-protein substances excrete telolysosomes, previously reported by Maunsbach in 1969 (12).

The liver is of great importance in degradation as well as synthesis of proteins and amino acids. The changes in SDH localization and increase in acid phosphatase activity demonstrated in the hepatic cell and Kupffer cell may indicate a progression of cell catabolism. A fine structural study on enzyme changes in the liver of calcium-magnesium deficient rats will be reported at a later data.

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REFERENCES


