BIOCHEMICAL STUDY ON SPONTANEOUS THYMOMA RATS WITH MOTOR DYSFUNCTION

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Spontaneous thymoma rats, Buffalo/Mna (B/Mna), in which nephrotic syndrom (NS) has recently been observed, have notable features in connection with muscle diseases; they exhibit muscle fatigability and weakness. Some biochemical measurements used for diagnosis of muscle diseases and NS were performed in these rats. ACI strain served as a reference strain. Urinary creatinine level and serum enzyme activities such as CPK, aldolase, GOT and GPT in the B/Mna rats did not differ from those in the ACI rats. On the other hand, urinary creatine level, the ratio of urinary creatine to creatinine and serum total cholesterol level in the B/Mna rats were significantly greater than those in the ACI rats. B/Mna rats also showed proteinuria and hypoalbuminemia. These results indicate the possibility of some pathological change of skeletal muscles which may result at least partially from abnormal lipid metabolism and hypoproteinaemia as a consequence of NS, differing from the typical muscular dystrophy.

Keywords — creatinuria; hypercholesterolemia; motor dysfunction; nephrotic syndrome; thymoma

Much attention has been paid to the relationship between thymic abnormalities and muscle diseases. Complication such as thymoma in myasthenia gravis (MG), thymic abnormality in murine dystrophy, and presence of cells resembling striated muscle cells in human thymus suggest the relationship. Buffalo/Mna (B/Mna) strain was established as a rat strain in which thymoma develops spontaneously. These rats exhibited an exacerbated muscle fatigability and a muscle weakness and ultrastructural changes of the muscle cells.

There are several kinds of useful animal models for the investigation of muscle diseases. For instance, rats of experimental autoimmune myasthenia gravis and dystrophic mice have helped much to advance the etiologies of MG and muscular dystrophy, respectively. However, these animals, not ultimate models of many types of the diseases, can never give complete explanations for the etiologies. Though much attention has been paid to the relationship between thymic abnormality and muscular diseases, it still remains unclarified. It is expected that B/Mna rats would give a clue for the elucidation of the relationship.

On the other hand, in these rats, nephrotic syndrom (NS) characterized by albuminuria, hypoalbuminemia, hypercholesterolemia as well as focal and segmental glomerular sclerosis has recently been observed, and they begin to excrete albumin from 10th week of age when no sign of thymoma can be recognized yet. Though spontaneous NS is known in aged rats, it is not known in such young rats. It is expected again that the rats can be used as a model of NS which are useful for immunological studies of the syndrome and also for development of effective drugs for the syndrome which are seriously awaited.

Numerous biochemical investigations for muscle diseases have been performed in man and other animals. The present study was carried out in order to examine whether biochemical
changes in urine and serum which are relevant to muscular diseases and NS occurred in B/Mna rats.

MATERIALS AND METHODS

Urine Creatine and Creatinine — Six male rats of B/Mna strain (360–440 g in body weight, 65–67 weeks of age) were used and six male rats of ACI strain (285–335 g in body weight, 56–71 weeks of age) served as reference. Animals were placed in individual metabolic cages for urine collection for 24 h, and food (CE-2 pellet, CLEA Japan Inc.) and water were given ad libitum. Urine creatine and creatinine were determined by the Jaffe reaction.11) Creatine was converted to creatinine in a picric acid medium (pH 2) by heating in a boiling water bath for 2 h.

Enzyme Activities and Total Cholesterol Level in Serum, and Total Proteins in Urine and Serum — Six male rats of B/Mna strain (330–405 g in body weight, 72–75 weeks of age) were used and six male rats of ACI strain (265–320 g in body weight, 65–78 weeks of age) served as reference rats. Their thymus weight per Kg body weight were 35.8±4.0 and 0.4±0.0 g, respectively. Animals were anaesthetized by intraperitoneal injection of urethane (0.75 g/kg) and alpha-chloralose (20 mg/kg) after 12–18 h fasting. The blood was collected from right common carotid artery by means of an endoarterial cannula with care to avoid hemolysis, and after clotting, the serum was separated (4°C, 3000 rpm, 10 min). Serum glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities were determined by the method in ref.12. Serum creatine phosphokinase (CPK) and aldolase activities were measured by the methods of Oliver13) and Beisenherz et al.,14) respectively. Serum cholesterol level was measured by the method of Röschlau et al.15) Total proteins in urine and serum were measured by Lowry method.16)

SDS-gel Electrophoresis of Structural Proteins of Skeletal Muscles — The structural proteins were extracted from the extensor digitorum longus (EDL) and soleus muscles (Fig. 1). The preparations of sodium dodecyl sulfate-polyacrylamide (SDS) gels and the electrophoresis were performed in a manner similar to the procedure of Weber and Osborn.17) The 10% polyacrylamide gel buffer solution contained 0.15% N,N,N′,N′′-tetramethylethylenediamine and 0.075% ammonium persulfate. The samples of 0.06 ml in a solution of 0.02 M sodium phosphate (pH 7.2), 2% SDS and 50% glycerol were layered on the gels. The electrophoresis was conducted for 3 h at a constant current of 6 mA per gel. Proteins were stained with Coomassie brilliant blue.

Statistical Analysis — Statistical evaluations were performed by Student’s t-test and, when necessary, by Aspin-Welch method, a modified Student’s t-test.

RESULTS AND DISCUSSION

Creatinuria has a basic significance for muscu-
lar function, and it has been demonstrated in muscular dystrophy\textsuperscript{18,19} and a number of other diseases\textsuperscript{19,20} which are directly or indirectly related to muscle failure. As shown in Table I, the B/Mna rats showed a 2.63 times greater creatine excretion than the ACI rats. It seems to be interesting that the value (15.5 ±0.5 mg/d/kg body weight) is quite close to those in muscular dystrophy and other neuromuscular diseases in humans.\textsuperscript{18,19} Also the ratio of urinary creatine to creatinine in the B/Mna rats was 2.80 times greater than that in the ACI rats. When compared with dystrophic mice,\textsuperscript{21,22} the B/Mna rats seem to excrete more constantly greater amount of urinary creatine.

An elevation of urinary creatine level can occur only when serum creatine level is excessive or when renal tubular reabsorption of creatine is reduced. Possible causes of an elevation of serum creatine level are: (1) a degradation or a decrease of muscle fibers; (2) a decreased ability of creatine uptake by muscle cells; (3) an increased permeability of muscle cell membrane; (4) an abnormality of creatine synthesis system. The elevation of urinary creatine level in the B/Mna rats seems to be derived at least partially from the decrease of muscle weight\textsuperscript{6} and probably partially from the degradation and decrease of muscle fibers as observed in the femoral and extraocular muscle cells.\textsuperscript{7,8} Conversely, the excretion of a large amount of creatine excreted in urine may affect muscle activity because creatine play an important role in supplying energy for muscle contraction. It might give a possible explanation for the weakness and myogenic fatigability in the fast twitch muscles.\textsuperscript{6,8}

On the other hand, urinary creatinine in the B/Mna rats did not differ from that in the ACI rats (Table I). This result was inconsistent with the decrease of urinary creatinine observed in patients of Duchenne muscular dystrophy (DMD).\textsuperscript{18,19} Also no statistical difference in serum enzyme activities (CPK, aldolase, GOT, GPT) between the B/Mna and ACI rats was observed (Table II), but in serum CPK and aldolase activities there seemed to be some tendency towards a decrease. These results were in contradiction to the increases of these enzymes observed in patients of DMD.\textsuperscript{23,24} The SDS-gel electrophoresis patterns of the EDL and the soleus muscles of B/Mna rats did not indicate any significant abnormality in the structural proteins as the most fundamental materials for muscle contraction. The separation and decrease of myosin

\begin{table}[h]
\centering
\caption{Urinary Creatine and Creatinine}
\begin{tabular}{lccc}
\hline
 & Creatine & Creatinine & Creatine/creatinine \\
 & (mg/kg/d) & (mg/kg/d) & \\
\hline
B/Mna (n=6) & 15.5±0.5\textsuperscript{a)} & 27.2±1.6 & 0.56±0.03\textsuperscript{a)} \\
ACI (n=6) & 5.9±1.3 & 30.5±2.3 & 0.20±0.04 \\
B/A ratio & 2.63 & 0.91 & 2.80 \\
\hline
\end{tabular}
\textit{Each value represents the mean ± S.E.; a) indicates significant difference from ACI rats at p<0.001.}
\end{table}

\begin{table}[h]
\centering
\caption{Enzyme Activities (U/I) and Total Cholesterol (mg/ 100 ml) in Serum}
\begin{tabular}{lccccc}
\hline
 & CPK & Aldolase & GOT & GPT & Cholesterol \\
\hline
B/Mna (n=6) & 126.4±23.2 & 55.8±5.3 & 71.6±6.2 & 63.8±5.2 & 135.1±4.8\textsuperscript{a)} \\
ACI (n=6) & 327.8±92.6 & 69.5±6.7 & 75.3±06.3 & 63.9±4.7 & 63.9±4.7 \\
\hline
\end{tabular}
\textit{For other details see Table I.}
\end{table}
band has been observed in patients of DMD. Therefore it seems that myopathy in the B/Mna rats is obviously different from a typical muscular dystrophy in humans.

While the B/Mna rats showed an elevation of total cholesterol level in the serum (Table II). This result may suggest the possibility of some abnormality of lipid metabolism, and seems to have some relation to lipid droplets observed in the femoral and extraocular muscle cells. These findings are noteworthy in connection with myopathy associated with abnormal lipid metabolisms. As described in “introduction”, NS has recently been observed in B/Mna rats. Also in the present study proteinuria (383 ± 56 mg/kg/d) and hypoproteinemia (7.5 ± 0.2 g/100 ml) were observed in these rats, as compared with ACI rats (180 ± 28 mg/kg/d, 8.4 ± 0.1 g/100 ml, respectively). Therefore the elevation of serum cholesterol level may be relevant to NS, and hypoproteinemia could be a possible cause of muscle weakness and fatigability and variable changes in muscle fibers. Further studies of the myopathy will be required to clarify the relation to thymoma as well as NS.

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REFERENCES


