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Abnormal Levels of Urinary Catecholamines in Dystrophic Mice and Hamsters (39167)

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Progressive muscular dystrophy (PMD) in man and in other animals is generally classified as a primary myopathy, although evidence is being presented that other nonmyogenic factors might be involved. For example, present results implicate several hypotheses in muscular dystrophy: (a) a general lesion in all organs, perhaps at the site of the cell membrane (1); (b) a systemic or circulatory problem (2); or (c) a neurotrophic dysfunction (3).

Previous work in our laboratory was supportive to the idea that a generalized metabolic defect in dystrophic mice was present. Clues from studies on sterol metabolism and Kreb cycle intermediates suggested to us that humoral and/or central nervous system effects were implicated in the mouse disease process (4). This idea was not new since speculation of the involvement of the sympathetic system and endocrine glands was first made by Bramwell (5). Bramwell admitted, however, that the evidence at that time (1925) was suggestive rather than conclusive.

Substantive data is now being accumulated hinting to a relationship between PMD and catecholamine metabolism. In human patients, Stern, Herkovic, and Misirlija (6) measured a marked increase in the urinary excretion of adrenaline and noradrenaline. Also, a considerable alteration of the ratio of adrenaline (E) to noradrenaline (NE) in the blood has been described by Stern *et al.* (7). Similar results were obtained by Gordon and Dowben (8) in dystrophic mice (Jackson 129/Re). In the latter study, increased levels of catecholamine were found in mouse muscle, adrenal glands, and urine. Also, 24 hr urine samples from affected animals contained high levels of dopamine (DA). Negative correlation in urinary catecholamine excretion rates in humans was found by Mendell *et al.* (9).

Since it has been established that urinary catecholamines are useful for diagnostic purposes for other disease states (10, 11), it was of interest to verify or disprove previous findings for the dystrophic animal using a new analytical method that did not depend on the conversion of catecholamines to fluorescent or volatile derivatives.

The present method is based on the combination of liquid–solid extraction, cation exchange chromatography, and controlled potential electrochemistry. The high sample throughput and few critical reagents make this approach very suitable for routine measurement of catecholamines in tissue and urine. A previous paper introduced the principles involved in our new method and reviewed the earlier literature (12). A detailed procedure and evaluation of its application to urinary catecholamines will be described elsewhere (13). One important advantage of the present method over previous procedures is the detection of 1 ng/ml or less of DA, DOPA, NE, and E in a single step. The precision of the methodology for normal levels is ± 5% relative standard deviation for a sample assayed eight times over a 4-day period (13).

Materials and methods. Dystrophic animals. Twelve dystrophic Syrian hamsters (Bio 14.6) and a like number of control littermates were used for this study. The two groups were divided into three subgroups and housed four to a cage. Ran-

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domly, two animals from each subgroup were placed in metabolic cages for urine collection.

In addition to the above animal model, 12 dystrophic (C57B1/6w) and 12 control mice were studied.

Animals were housed in plastic cages in a room equipped with a 12-hr light, 12-hr dark cycle. Food and water were available *ad lib.*

Standard urine pool. A random urine pool from normal animals was acidified to pH 2, 6 *M* HCl, and standard solutions containing four catecholamines were added to make the final concentrations approximately 100 ng/ml NE and *ℓ*-DOPA, 40 ng/ml EPI, and 160 ng/ml DA. The exact concentration of each of the compounds was then determined by running the urine pool and the urine pool standard through the calibration procedure described elsewhere (13).

Animal urine collection. All urine collections covered a 24-hr period. As a matter of routine, three or four mice were placed in a metabolism cage 16–18 hr prior to 24-hr collections. Urinary samples were collected into cylinders containing 0.5 ml of 6 *M* HCl starting at 9:00 AM each morning. Total volumes were recorded, the urine poured into glass counting vials with *plastic* liners and tops, and the samples immediately frozen. Because of their size, only two hamsters were needed for obtaining 24-hr collections. The data are reported in terms of ng/24-hr specimen/animal.

Assay for catecholamines. The details of the procedure have been previously described (13). The method is capable of simultaneous assay of 3,4-dihydroxyphenylalanine (DOPA), dopamine (DA), norepinephrine (NE), and epinephrine (E) in urine. All four compounds can be detected to a sensitivity of 1.0 ng/ml and with a relative standard deviation of ± 5.0%. The very selective procedure is based on the combination of liquid–solid extraction, bonded phase cation-exchange chromatography and controlled-potential hydrodynamic electrochemistry.

Results. In the first part of our study, 24-hr urine samples from dystrophic hamsters (Bio 14.6) were assayed. Two animals were placed in each metabolic cage. Six samples were collected over a 2-week period and another four samples were collected over a single-week period. The hamster data is presented in Fig. 1. Dystrophic hamsters excreted about twice as much NE as did normal hamsters ($P = 0.01$). The increase for E was approximately three times control values ($P < 0.001$). DOPA and DA levels were similar in the two groups. Because our hamster data on DA was at variance with previously published results for the mouse (8), we returned to the mouse, an alternative animal model used in our previous studies (4 and references therein).

Twelve dystrophic (C57B1/6w) and twelve control littermate mice were randomly divided into two groups and three subgroups. Urine samples were collected five times the first week (Expt I), six times during the next 3 weeks (Expt II), and five times during the last 3 weeks (Expt III). A total of 40–44 individual specimens were obtained during this period. Because of the small volumes of urine excreted by mice in 24 hr, even as a group of four, the standard deviation based on a single mouse was

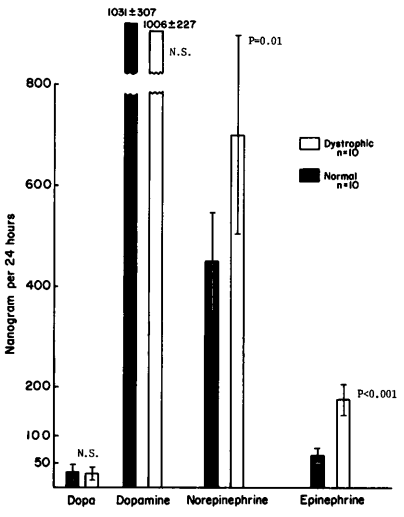

FIGURE 1

FIG. 1. Urinary samples from hamsters were collected over a 24-hr period. Mean and standard deviations of measured catecholamines from both control (black bar) and dystrophic (open bar) hamsters are compared. The difference between normal and dystrophic excretion of NE ($P = 0.01$) and E ($P < 0.001$) were highly significant.

quite large. The combined data produced a relative standard deviation of less than ± 10%. Individual subgroups did not show any differences during the 2 months of sample measurements, and the data for each subgroup and experimental period were combined for Fig. 2.

In Fig. 2 a slight decrease in DOPA levels was measured for dystrophic mice. This difference was due to some very high control values obtained in Experiment II and Subgroup 2. The DOPA values measured for the combined groups, however, were not considered to be very significant (P = 0.20). DA levels in both dystrophic and control groups were the same and represented quantitatively the greatest concentration of catecholamines. As previously measured in hamsters, dystrophic mouse values for NE and E were significantly elevated over control values ($P < 0.001$). NE dystrophic values were twice as great as normal NE values, while E values (8 ± 1 ng/24 hr) were substantially (4-fold) increased for dystrophic mice (32 ± 4 ng/24 hr).

Discussion. Investigations of the urinary catecholamines have contributed greatly to our knowledge of their role in the physiologic and psychologic economy of the body (11). Since the unaltered amines in the urine represent only a small fraction of the quantities released into the blood, it is apparent that they need not always serve as a reliable indicator of production. It is to be expected that these substances would be affected by the rate of release, the relative blood flow to the liver and other organs, and to alterations in renal clearance.

Despite these real problems, urinary catecholamine data accumulated in the present study and by previous workers are of interest in muscular disease. While our data support the conclusion of increased excretion of NE and E in dystrophic mice (8), no alteration of DA excretion was found in either mouse or hamster studies by our more specific analytical method (12, 13).

The literature indicates that an increase in content of active catecholamines in muscle would affect skeletal muscle function. It has been observed that catecholamines delay and partially reverse the onset of muscle fatigue under a variety of circumstances and also affect the capacity of resting muscle to perform work (14). We conjecture, therefore, that the observation of Sandow and Brust (15), that dystrophic mouse muscle was more resistant to fatigue than control muscles following maximal strength or tentanizing stimuli, may be referable to the muscles' catecholamine content (16).

Propositionally larger adrenal and pituitary glands were demonstrated in dystrophic mice as compared to normal animals (8). The enlarged adrenal glands reported previously in these animals may be ascribed to the stress of the disease.

A more intimate relationship between sex-linked pseudohypertrophic muscular dystrophy (DMD) and biogenic amine, abnormal metabolism in human subjects has been suggested recently by several investigators. These reports indicated increased levels of serotonin in cerebrospinal fluid (17), decreased rate of [5-^{14}C]serotonin (5-hydroxytryptamine) uptake of platelets (18), elevation of urinary epinephrine and norepinephrine (6), and an increase in catecholamine fluorescence of muscle fibers (19).

Of particular interest are the attempts to

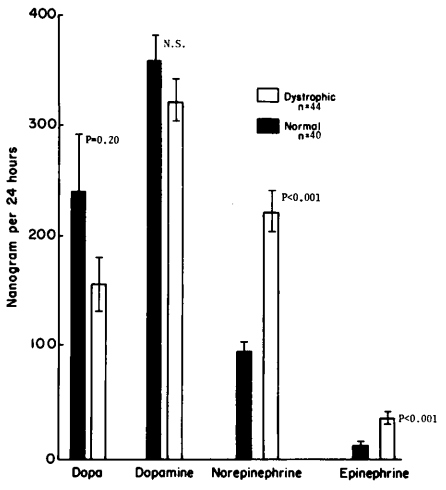

FIGURE 2

FIG. 2. Twenty-four-hour samples from control (black bar) and dystrophic (open bar) mice were collected. Except for DOPA levels, catecholamine excretion in mice was less than hamster urinary values. Although dystrophic mice showed a decrease in DOPA values as compared to control values, the decrease was not significant (P = 0.20). Increases in levels of NE ($P < 0.001$) and E ($P < 0.001$) in dystrophic urine were highly significant.

reproduce the histopathological characteristics of the disease in animals. By a combination of aortic ligature and small doses of vasoactive agents such as serotonin (5HT) or noradrenaline, the characteristic histopathological lesions of DMD (20) and increases in plasma enzyme levels were observed (21). A variation of this theme was supplied by Yu *et al.* (22) and supported the relationship between the DMD and biogenic amine. In Yu's experiments, a monoamine oxidase inhibitor, pargyline, produced a characteristic myopathy of DMD, although the lesions are limited to the soleus. Another experimental myopathy has been reported by using 5HT in combination with imipramine, an agent known to block the uptake of biogenic amines (23).

The one negative finding in regard to catecholamine metabolism and muscular dystrophy is the paper by Mendell *et al.* (9). This study weighs heavily against the possibility that abnormal quantities of the circulating biogenic amines alone were directly involved in the pathogenesis of DMD. Despite this lone negative conclusion, the weight of evidence from previous literature, as well as the present studies in animal models, suggests an abnormality in catecholamine metabolism in muscular dystrophy.

Regardless of the cause–effect relationship between increased levels of NE and E in urine of dystrophic animals as compared to control values, we feel that the monitoring of urinary catecholamines by this simple, fast, and efficient analytical method will serve as a useful barometer in following the course of the disease under dietary and/or drug manipulation.

Summary. Twenty-four-hour urine was collected from normal and dystrophic mice and hamsters for catecholamine determinations. A new method of analysis was used whereby 3,4-dihydroxyphenylalanine (DOPA), dopamine (DA), norepinephrine (NE), and epinephrine (E) were measured simultaneously. The procedure is based on a combination of liquid–solid extraction, cation exchange chromatography, and controlled potential electrochemistry.

The results of these experiments indicated that while DA levels were similar in both normal and pathological animal urine, DOPA levels decreased slightly in the dystrophic mouse but not the hamster, and NE and E levels in dystrophic groups were two and four times greater than normal in both species.

The data supports the concept of biochemical alterations in tissue other than muscle. While not necessarily supportive to catecholamine abnormality as the primary cause of muscular dystrophy, the present data cast doubt that this disease is a primary muscle disease.

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