EFFECT OF FORCED RUNNING ON RAT SKELETAL MUSCLE WITH ACRYLAMIDE NEUROPATHY

Yasutomo Okajima and F. Patrick Maloney

From the Division of Rehabilitation Medicine, University of Arkansas for Medical Sciences, Little Rock, AR 72205 and John L. McClean Memorial VA Hospital, 4300 West 7th Street, Little Rock, AR 72203, USA

ABSTRACT: This study was performed to evaluate the effect of prolonged forced running on rat lower limb muscles with acrylamide neuropathy. Twenty-four 4-week-old rats were divided into three groups of eight rats. Acrylamide was given to two groups of sixteen rats to induce mild paralysis. Eight rats with acrylamide injections were forced to run 3200 m/day on a treadmill for five weeks. Running activities showed the rate of body weight gain and aggravated paralysis. Although the wet weight of tibialis anterior (TA) and extensor digitorum longus (EDL) muscles was reduced by running, that of soleus (SOL) muscles was unchanged. The ratio of their weight to their body weight (W/ww) remained constant regardless of exercise. Protein content (PC) of muscles was not altered by exercise, either. We postulated that exercise-induced worsening of paralysis in acrylamide neuropathy rats was not caused by muscle pathology. Deterioration of neuropathic condition due to exercise was suggested.

Key words: acrylamides, exercise, muscle, rat.

There were several experimental reports about exercise effects on skeletal muscle of healthy rats (4, 6, 11, 13), but few were available about those effects on that of neuropathy rats. His (10) showed that weight and twitch tension increase in exercised rat skeletal muscle during the process of reinervation, and suggested that dynamic exercise was effective in the treatment of patients recovering from peripheral nerve injuries. However, exercise sometimes cause muscle weakness in patients with lower motor neuron lesions (2, 3, 16, 23).

Reitman (30) and Vilhko (31) demonstrated histological evidence of muscle damage after running exercise of already weakened muscles. Herbstin (19, 21) observed the same kind of adverse effect of exercise (swimming and weight-lifting) in his biochemical studies of nerve-injured rat skeletal muscles.

The purpose of this study was to evaluate the exercise effect of prolonged forced running on the degree of paralysis, muscle weight, and protein content in partially paralyzed rats and to hypothesize the mechanism of exercise-induced muscle weakness which is sometimes seen in some patients with lower motor neuron disease.

METHODS

Experimental paralysis was produced by acrylamide injections, a well studied neuropathy (8, 27, 29). Chronic mild intoxication (10, 15) was induced while maintaining the rats running ability.

Twenty-four 4-week-old Wistar rats were equally divided into three groups: Group 1, sedentary without acrylamide; Group 2, sedentary with acrylamide; and Group 3, exercised with acrylamide. A dose of 50 mg/kg of a 2% acrylamide solution (Sigma Chemical Co.) in saline was administered intra-peritoneally via a Teveurin syringe three times a week (on Mon, Wed, and Fri) to Groups 2 and 3 for the first four weeks. This schedule was followed by a once weekly injection for the next six weeks. The same schedule of saline injections was applied to the controls in Group 1. Group 3 was exercised on a treadmill for the last five weeks before sacrifice. Standard Purina rodent diet and water were given ad libitum. Plastic animal housing was 10.5" x 16" x 8" deep. Animal wards were maintained at 72 ± 2°F. Lighting was controlled by automatic light timers set on a 12 hour on/off cycle.

An endless belt type treadmill was used for running activities on which rats were conditioned to run by an electrical shock of 120 V, 3-5 mA, 30-40 msec of duration. The interval of the electrical current was set at 3-5 sec. In order to avoid exhaustion and frequent electrical shocks, rats were removed immediately from the treadmill when they were shocked three times consecutively. Rats were placed to run 3200 m/day, 5 times a week on this 3° inclined treadmill. Running speed was set at 10-15 m/min.

Body weights and paralysis of each rat were evaluated weekly. Paralysis was rated by the appearance of four signs of weakness. One of them was the upgazing phenomenon (8, 10, 27) of hind limbs when rats were dropped from a few inches onto a flat surface. Another three signs, namely flip-over tendency of the hind feet (9), crossed or wide-based hind limbs, and waddling gait (9, 27), were observed on a 20° inclined treadmill. Each of these paralysis signs was rated as 1
Table 1. Muscle weight difference

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>BW (g)</th>
<th>±</th>
<th>TA (mg)</th>
<th>±</th>
<th>SOL (mg)</th>
<th>±</th>
<th>EDL (mg)</th>
<th>±</th>
<th>TA/EDL (mg/mg)</th>
<th>±</th>
<th>SOL/EDL (mg/mg)</th>
<th>±</th>
<th>EDL/TA/EDL (mg/mg)</th>
<th>±</th>
<th>MMO/MMO (mg/mg)</th>
<th>±</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>415±4</td>
<td>38±4</td>
<td>230±12</td>
<td>17±1</td>
<td>175±12</td>
<td>13±1</td>
<td>34±4</td>
<td>20±2</td>
<td>1.38±0.24</td>
<td>0.19±0.06</td>
<td>0.55±0.07</td>
<td>0.30±0.11</td>
<td>0.43±0.07</td>
<td>0.46±0.11</td>
<td>1.37±0.15</td>
<td>1.12±0.16</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>415±4</td>
<td>38±4</td>
<td>230±12</td>
<td>17±1</td>
<td>175±12</td>
<td>13±1</td>
<td>34±4</td>
<td>20±2</td>
<td>1.38±0.24</td>
<td>0.19±0.06</td>
<td>0.55±0.07</td>
<td>0.30±0.11</td>
<td>0.43±0.07</td>
<td>0.46±0.11</td>
<td>1.37±0.15</td>
<td>1.12±0.16</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>415±4</td>
<td>38±4</td>
<td>230±12</td>
<td>17±1</td>
<td>175±12</td>
<td>13±1</td>
<td>34±4</td>
<td>20±2</td>
<td>1.38±0.24</td>
<td>0.19±0.06</td>
<td>0.55±0.07</td>
<td>0.30±0.11</td>
<td>0.43±0.07</td>
<td>0.46±0.11</td>
<td>1.37±0.15</td>
<td>1.12±0.16</td>
</tr>
</tbody>
</table>

*Individual values converted to logarithms. Mean ± S.D. determined by Student's t-test.

**Significantly different from group 1 (p<0.05).

**Significantly different from group 2 (p<0.05).

RESULTS

Two rats in Group 3 did not run continuously without having frequent electrical shocks and were excluded from this experiment.

The body weight of all rats increased steadily as is depicted in Fig. 1. Acrylamide treatment and/or running activities slowed the rate of weight gain, but the acrylamide-treated Group 2 was not significantly lighter than Group 1. However, the rats exercised in Group 3 weighed significantly less (p<0.05) than Groups 1 and 2 (Table 1).

The ratio of the BW to TA EWL was constant for TA/BW which was significantly less (p<0.05) in acrylamide-treated groups compared with controls. Protein content was the same for all three groups.

**DISCUSSION**

Acrylamide causes dose-dependent muscle weakness secondary to peripheral neuropathy of the dying-back type (10, 19). Under the circumstances of this study mild prolonged muscle weakness occurred without rat weight loss. However, exercise aggravated the degree of paralysis and decreased the rate of BW gain (Fig. 1, Table 1). We believe the running distance of 3,200 m/day at 10-15 m/min was sufficient to show exercise effects on muscle when compared to other reports (22, 30). We thought deterioration of paralysis was caused by muscle atrophy. But the reduction of muscle weight was small as compared with the deterioration of paralysis.

Herborn (21) showed prolonged-exercise-induced weight reduction of synergystically tennostomized rat skeletal muscles although he found weight increased in less exercised muscles like other investigators (14, 26). Gordon (13) did not find weight increase in his exercised rat skeletal muscle. He thought that relative undernutrition from over-exercise was a causative factor to inhibit exercise hypertrophy of muscle. Carrow (4) found that the prolonged exercise of endurance-type induced reduction in muscle fiber diameter and muscle volume. Hatanu (17), Herborn (20), and KRNELL (24) showed that electrical stimulation induced hypertrophy of rat skeletal muscles. However, Domelaar (7), KRNELL (24) and Pette (28) demonstrated atrophy with more prolonged stimulation. They also showed that exercise-induced decrease of muscle fiber diameter was due to the fiber type conversion from Type II to Type I muscle fibers.

Our results did not show exercise-induced atrophy of muscles according to the values of WWM/BW (Table 1). But the absolute BW was significantly smaller in exercised neuropathy rats except for SOL, muscles. It is well known that SOL muscles are composed mostly of Type I fibers, while TA and EDL muscles contain Type 2 fibers. So the reduction of absolute weight of exercised TA and EDL muscles might be caused by fiber type conversion from Type II to Type I regardless of acrylamide neuropathy.

In muscle protein content, Crockett (5) and Hoender (18) found that restricted activity resulted in a decrease of muscle PC. However, Herborn (19, 21) found lower muscle PC in intensely exercised rats with partial denervation. We did not detect such an exercise-induced PC change in exercised muscles. Our method of protein assay may be too gross to detect a minute change in muscle under these experimental conditions.

The mechanism of overwork weakness in peripheral nerve diseases still remains unsolved, but deterioration of neuromorphology (1) rather than muscle pathology (11, 13, 19, 22) due to heavy exercise might be a causative factor.

**ACKNOWLEDGEMENTS**

We are grateful to Dr. Karl D. Stroud for his general support and to Dr Kenji Hattori and Dr Naosuke Chino for their academic assistance. This study was supported by Veterans Administration Research funds.

**REFERENCES**

17. Hatanu, E., Jura, K. & Buta, J.: Effect of electrical...
Table 1. Muscle weight difference

<table>
<thead>
<tr>
<th>Group</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>341.4 ± 41</td>
<td>341.4 ± 41</td>
<td>341.4 ± 41</td>
</tr>
<tr>
<td>TA (mg)</td>
<td>672 ± 33</td>
<td>672 ± 33</td>
<td>672 ± 33</td>
</tr>
<tr>
<td>SOL (mg)</td>
<td>207 ± 22</td>
<td>207 ± 22</td>
<td>207 ± 22</td>
</tr>
<tr>
<td>EDL (mg)</td>
<td>217 ± 14</td>
<td>217 ± 14</td>
<td>217 ± 14</td>
</tr>
<tr>
<td>TA/EDL (mg/mg)</td>
<td>2.83 ± 0.03</td>
<td>2.83 ± 0.03</td>
<td>2.83 ± 0.03</td>
</tr>
<tr>
<td>SOL/EDL (mg/mg)</td>
<td>0.55 ± 0.01</td>
<td>0.55 ± 0.01</td>
<td>0.55 ± 0.01</td>
</tr>
<tr>
<td>SOL/EDL (mg/mg)</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.01</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>PC/WWW (mg/mg)</td>
<td>137.3 ± 13</td>
<td>137.3 ± 13</td>
<td>137.3 ± 13</td>
</tr>
</tbody>
</table>

* Individual values converted to logarithms. Mean & S.D. recalculated by anlogies.

Significantly different from group 1 (p < 0.05).

Significantly different from group 2 (p < 0.05).

Fig. 1. Paralysis grade. Paralysis was graded by the appearance of hind limb flapping, hind foot splaying, waddling gait pattern, crossed or widen-based hind limbs. Paralysis was noted 3-2 weeks after the injection of acrylamide injection.

RESULTS

Two rats in Group 3 did not run continuously without having frequent electrical shocks and were excluded from this experiment.

The body weight of all rats increased steadily as is depicted in Fig. 1. Acrylamide treatment and/or running activities slowed the rate of weight gain, but the acrylamide-treated Group 2 was not significantly lighter than Group 1. However, the rats exercised in Group 3 weighed significantly less (p < 0.05) than Groups 1 and 2 (Table 1).

Paralysis was first observed 2-3 weeks after acrylamide administration was initiated. The acrylamide-treated groups showed their greatest paralysis at 9-10 weeks of age. The paralysis grade of the exercised rats in Group 3 was significantly worse (p < 0.05) with Mann-Whitney's U-test than the sedentary rats in Group 2 from the age of 12 to 14 weeks (Fig. 1).

The wet weight of TA, SOL, and EDL were lighter in Group 2 and lightest in Group 3 (Table 1). Significant weight differences (p < 0.05) were found in TA and SOL muscles between Groups 1 and 2 and in TA and EDL muscles between Groups 2 and 3. However, the ratio of the WWW to BW was constant except for TA/EDL which was significantly less (p < 0.05) in acrylamide-treated groups compared with controls. Protein content was the same for all three groups.

DISCUSSION

Acrylamide causes dose-dependent muscle weakness secondary to peripheral neuropathy of the dybing type. Under the circumstances of this study mild prolonged muscle weakness occurred without rat weight loss. However, exercise aggravated the degree of paralysis and decreased the rate of BW gain (Fig. 1).

REFERENCE


17. Hatano, E., Joda, K. & Buta, Y.: Effect of electrical
Y. Okajima and F. P. Maloney

cal stimulation of sciatic nerve of rats after partial denerva-
21. Herrington, G. J., Jawed, M. M., Gordon, F. E. & Dis-
24. Kortell, D., Eribeck, G., Viercy, B. A. & Donnerfurst, V.: Effects of physiological amounts of high and low-rate chronic stimulation on fast-switch muscle of the cut hind-
25. Kunin, C. L., Jawed, M. M., Herrington, G. J. & Dis-
tanso, J. F.: Overwork effect on partially denervated rat

27. Pettorini, D., Maffet, W., Leiner, E. & Viensa, G.: Time dependent effects on contractile properties, fiber popula-
tion, myosin light chains and enzymes of energy metabo-


Address for offprints: Yutatomo Okajima, M.D.
Dept. of Rehabilitation Med
Tokyo Sambai Hospital
1-4-3 Mita, Minato-ku
Tokyo 106
Japan


LONG-LASTING UNILATERAL MUSCLE WASTING AND WEAKNESS FOLLOWING INJURY AND IMMOBILISATION

O. M. Rutherford, D. A. Jones and J. M. Round

From the Department of Medicine, University College London, London, UK

ABSTRACT. Quadriceps strength and size was measured in a small group of subjects (n=7) 1 to 5 years after full mobilisation following some form of unilateral lower limb trauma. The mean maximum voluntary isometric force (MVC) was significantly lower for the injured (I) compared to the unjured (UI) leg (369 N: 139 vs. 535 N: 131, p < 0.01). Electrical stimulation superimposed on the voluntary contractions demonstrated that all subjects were able to maximally activate the quadriceps of both legs. Mean quadriceps (cross-sectional area (CSA) was significantly lower in the I (64 cm²: 12.8) compared to the UI leg (80 cm²: 12.8, p < 0.01). One subject with marked unilateral weakness and wasting took part in a 3-month strength training study for the injured leg. After training the I/UI ratio had been restored to nearly 100% (94% MVC; 88% CSA). These results would suggest that longer and more intensive physiotherapy is required in the immediate post-injury period to restore muscle strength and size to severely atrophied muscle.

Key words: muscle, immobilisation atrophy, strength, size, strength training.

Muscle weakness and atrophy are well-known side effects which occur with limb immobilisation. The values quoted for the loss of strength vary between 20 and 50% (1, 7, 13, 20) with 10 to 50% decreases in muscle volume, cross-sectional area and fibre area (2, 9, 13, 20, 21). Rehabilitation involves exercise to strengthen the affected muscles and once mobility has been regained it is generally assumed that the muscle has returned close to its full size and strength but there are suggestions that this may not be the case. After 14 weeks of intensive rehabilitation strength decrements of up to 35% have been reported between the injured and uninjured limb in army personnel and even six months after the resumption of military duties differences of 10–25% were still evident (6, 8). We know of no studies following this type of patient over the next few years to see whether full strength ever returns.

We report here results of quadriceps strength and size in a small group of subjects 1 to 5 years after full mobilisation following some form of unilateral lower limb trauma. A pilot study of the effect of the studies training was carried out in one subject who had marked unilateral wasting and weakness.

METHODS

Subjects. Seven subjects (6 male, 1 female) were studied. Details of the injury, time of immobilisation and time since full mobilisation are given in Table 1. All procedures were approved by the Committee on the Ethics of Clinical Investigation, University College Hospital.

Quadriiceps strength. Maximum voluntary isometric contrac-
tions (MVC) of the quadriceps were measured in a con-
ventional strength testing chair (1). The best of three MVCs was measured at least two separate days. A percutaneous twitch superimposition technique was used to test whether subjects were able to maximally activate the quadriceps during the isometric contraction (for full description of method see ref. 17). The quadriceps was stimulated at 1 Hz with a voltage sufficient to activate over 90% of the muscle and the height of the twitches before and during the voluntary contra-
tion were compared. During a truly maximum contrac-
tion no extra force is generated by the stimulation. When the contraction is submaximal the height of the superimposed twitch can be used to estimate the degree of inhibition from the known relationship between superimposed twitch height and the percentage of maximum force being generated (17).

Measurement of quadriceps size. Quadriceps cross-section-
al area (CSA) was measured from a Computed Tomogra-
phy (CT) X-ray scan taken at half femur height. Scans were taken on a Philips Tomoscan 350 with a scanning time of 4.8 s and a slice thickness of 9 mm. Images were analysed off-line on a locally designed interactive medical imaging package running on a PDP 11/3 (13). The area of the quadriceps and femur were measured semi-automatically using a contour following programme with manual editing where necessary.

Training regime. A preliminary training study was carried out on one subject (K. W.) who trained the injured leg for three months. The exercise consisted of one-legged knee exten-
sion training on a conventional isometric multi-gym appa-