Contractile properties of rat single muscle fibers and myosin and troponin isoform expression after hypergravity

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Stevens, Laurence, Cyril Bozzo, Tatiana Nemirovskaya, Valerie Montel, Maurice Falempin, and Yvonne Mounier. Contractile properties of rat single muscle fibers and myosin and troponin isoform expression after hypergravity. J Appl Physiol 94: 2398–2405, 2003. First published February 7, 2003; 10.1152/japplphysiol.00808.2002.—The effects of 19 days of hypergravity (HG) were investigated on the biochemical and physiological properties of the slow soleus muscle and its fast agonist, the plantaris. HG was induced by rotational centrifugation that led to a 2-G gravity level. The HG rats were characterized by a slower body growth than control, whereas the soleus muscle mass was increased by 15%. Using electrophoretic techniques, we showed that the distribution of myosin heavy chain and troponin T isoforms was not modified after HG in both soleus and plantaris. In contrast, the isoform expression pattern of two troponin subunits, troponin I and troponin C, was changed in a slow-to-fast manner only in the soleus. From tension-pCa relationships, changes in Ca2+ activation threshold by 0.18 pCa unit indicated a decrease in Ca2+ sensitivity of the myofilaments. In parallel, the changes in the contractile mechanism specifically linked to HG remained unknown. So a question needs to be raised: what kinds of modifications in contractile properties appear in HG conditions, and are they accompanied by changes in phenotypic properties? Consequently, are the changes in muscle properties at 2 G opposite those induced by 0 G; otherwise, is there a continuum in muscle characteristics following the gravity level from 0 G to 1 G to 2 G?

The aim of this paper was to analyze the effect of a 2-G environment on two muscles: the slow soleus and its fast agonist, the plantaris. We investigated the contractile properties of these muscles by using single skinned fibers, which permitted us to determine the Ca2+ sensitivity of the myofilaments. In parallel, the MHC and the three Tn subunit expression patterns were examined. The myosin molecule was chosen as a fine marker of muscle plasticity due to its abundance in striated muscles and its highly extended range of isoforms capable of being modified, as clearly demonstrated in microgravity. TnT, TnC, and TnI, which contribute to the regulation of muscle contraction and which have been previously studied in unloading conditions, were concomitant with a rise in the expression of the fast isoforms of these proteins and even with the appearance of MHC isoforms not expressed at the protein level in the normal soleus (MHC IIId/x, MHC IIb). Few changes appeared in fast muscles, such as extensor digitorum longus, plantaris, or tibialis anterior (41).

Therefore, it is now evident that the gravity factor has to be integrated as a parameter that modulates muscular properties and that its changes induce adaptive processes. However, until now, the effects of hypergravity (HG) have been less studied, at least for muscle contractile properties. Some data reporting HG effects after chronic centrifugation on muscle morphology (48) and biochemical characteristics have demonstrated for the slow soleus either a transition toward a slower muscle in young developing rats (24, 25) or no change in slow MHC content in adults (33). However, the changes in the contractile mechanism specifically linked to HG remained unknown. So a question needs to be raised: what kinds of modifications in contractile properties appear in HG conditions, and are they accompanied by changes in phenotypic properties? Consequently, are the changes in muscle properties at 2 G opposite those induced by 0 G; otherwise, is there a continuum in muscle characteristics following the gravity level from 0 G to 1 G to 2 G?

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ditions (2, 39), were also examined. The relation bet
between TnC and TnT subunit expressions and Ca2+
activation characteristics has already been well estab
lished. Indeed, TnC, the Ca2+-binding subunit, exists
in skeletal muscle as fast (TnCf) and slow (TnCs)
isoforms, with the modulation of the contractile re
sponse and the apparent Ca2+ affinity of the contrac
tile system being directly dependent on the TnC iso
form (21, 23, 28). The TnT subunit interacts with
tropomyosin and plays an important role in Ca2+ acti
vation and in the cooperativity process along the myo
fibrillar lattice (36). In the rat muscle, three different
slow isoforms (TnTs) and four fast isoforms (TnTf)
have already been described (2).

Our results indicated that HG conditions induced
changes in the Ca2+-activated properties of slow soleus
fibers. The expression pattern of MHC and TnT iso
forms was not altered in the soleus after HG, whereas
slow-to-fast transitions occurred for the TnC and TnI
molecules. The fast plantaris fibers were not modified,
either for their contractile properties or for their MHC
and Tn isoform expressions.

MATERIALS AND METHODS

Animals and Muscle Preparation

The experiments were carried out on adult male Wistar
rats (initial body weight ~200 g). The animals were ran
domly divided into a control (Cont, n = 9) group and a group
submitted to a 2-G centrifugation (HG, n = 9). The experi
ments received authorizations from both the Ministry of
Agriculture and the Ministry of Education (veterinary ser
vice of health and animal protection, authorization A59–682)
in France and the Animal Care Committee in the Institute of
Biomedical Problems in Moscow. After 19 days of HG, both
groups of rats were anesthetized with an intraperitoneal
injection of pentobarbital sodium (30 mg/kg), and muscles
were immediately dissected. The soleus and plantaris mus
cles were removed bilaterally and weighed. There was no
statistical difference in muscle mass between the right and
left sides. Then, for each animal, one muscle was frozen in
liquid nitrogen and stored at −80°C until SDS-PAGE anal
ysis, and the other muscle was chemically skinned for the
contractile experiments on single fibers. The skinnin
procedure was based on Ca2+ chelation by EGTA, which perme
abilized the sarcolemmal and transverse tubular mem
branes. The EGTA skinnning solution (see Solutions) was
applied for 24 h at 4°C. The skinned biopsies were stored at
−20°C in 50:50 glycerol-skinnning solution (storage solution).
Protease inhibitor leupeptin was added to the storage solu
tion (10 μg/ml) to prevent protein degradation. All samples
were brought in preserved temperature conditions (~80 and
−20°C) to the laboratory in Lille for the experiments.

Centrifugation Apparatus

The centrifugation was conducted in Moscow at the Insti
tute of Biomedical Problems. The apparatus consisted of a
velocity-controlled direct-current motor located in the verti
cal axis of the apparatus and driving two horizontal cross
arms (total length 4 m) at constant rotation speed. Four
free-swinging gondolas were jointed at the four extremities of
the horizontal arms. Each gondola contained five rats. Dur
ing centrifugation, the gondolas were tilted at a constant 60°
angle from vertical, depending on the chosen speed. Rotat
ions were done at a constant velocity of 21 rad/min. Given
the mass and the inertia of the gondolas, including the cages
and rats, this angular velocity led to 2-G resultant force. The
gondolas were equipped with a ventilation system and a light
system that reproduced a 12:12-h light-dark cycle. For ani
mal care (cleaning and feeding), the centrifugation was
stopped daily for 15 min every morning at 11 AM. Food and
water were available ad libitum. During the experiment, a
video camera control indicated that the rats remained inac
tive and kept a tight grip on the floor of the gondola during
first 2–3 days of centrifugation. Then they began to move
and to get food and water until the end of the experiment.
However, they walked slowly, with short steps. These quali
tative observations confirmed more specific reports on loco
motion and caged activity levels (12, 46). For the whole
duration of the study, Cont animals were kept in the cen
trifuge room in cages similar to the gondolas on the centrifuge
apparatus, so that all of the animals were exposed to the same
level of noise, lighting, and temperature (20 ± 1°C).

Experimental Procedures

For each experiment, a 2- to 2.5-mm single-fiber segment
was isolated from the skinned biopsy. A silk thread was tied
at each extremity, allowing the mounting of the fiber in an
experimental chamber with constant stirring, initially filled
with relaxing (R) solution. The fiber was held at one end by
small fixed forceps and at the other end by a clamp connected
to a strain gauge (Force transducer Fort 10, World Precision
Instruments; sensitivity 10 V/gg). The mounted fiber was
viewed through a high-magnifying binocular (×80) with a
micrometer, allowing fiber diameter measurements. Fibers
comparable to strips with a high degree of ellipticity were
discarded (~5%). The resting sarcomere length was mea
sured by means of a helium-neon laser (Spectra Physics)
directed perpendicular to the long axis of the fiber. Then the
fiber was stretched to ~120% of resting length to allow
maximal isometric tension development on ionic activation.
The resulting sarcomere length (2.6 ± 0.04 μm) was subse
quently regularly controlled and readjusted if necessary.
The output of the force transducer was amplified and recorded on
a graph recorder (Gould, model Windograph no. 40–8474–02) and simultaneously analyzed by computer software.

Solutions

All reagents were provided by Sigma Chemical (St. Louis,
MO). The composition of all solutions was calculated by the
Fabiato computer program (9), with final ionic strength at
200 mM. The pH was adjusted to 7.0, and ATP (2.5 mM) was
added in each solution. The skinnning solution was made up
of (in mM) 10 MOPS, 170 potassium propionate, 2.5 magnesi
um acetate, and 5 K2EGTA. The following solutions were
used for the experimental procedure: a washing (W) solution
composed of (in mM) 10 MOPS, 185 potassium propionate,
and 2.5 magnesium acetate; a R solution identical to the
storage solution; and pCa- or pSr-activating solutions con
sisting of W solution plus various concentrations of free Ca2+
or Sr2+ from CaCO3 or SrCl2, respectively, buffered with
EGTA and added in proportions to obtain the different pCa
values (7.0–4.2) or pSr values (5.0 and 3.4). To eliminate a
hypothetical influence of the sarcoplasmic reticulum (SR) on
the tension developed by the myofilaments, each fiber was bathed
for 20 min at the beginning of an experiment in a Brij
solution made up of R solution with 2% Brij 58 (polyoxyeth
ylene 20 cetyl ether). The nonionic Brij 58 detergent irreversibly
eliminated the ability of the SR of skinned muscles to
sequester and release Ca\(^{2+}\), without altering the actomyosin system.

**Tension-pCa Relationships**

All experiments were performed in a thermostatically controlled room (19 ± 1°C). At the beginning of each experiment, a maximal tension (P\(_0\)) was induced by applying a pCa 4.2 solution that contained enough calcium to saturate all TnC sites. An experimental sequence was defined as follows. The fiber was bathed in W solution, which eliminated EGTA traces from the previously applied R solution. Then the fiber was activated at a level of tension (P) in a given pCa solution, immediately followed by a maximal contraction P\(_0\). This procedure allowed the calculation of the relative tension (P/P\(_0\)). Finally, the fiber was relaxed in R solution. Fibers were rejected if force declined during a sustained contraction, or decreased by >20% during the whole experiment, and if tension-pCa series were not completely achieved. Data from four or five fibers, at least, were kept from each muscle biopsy. The tensions developed in submaximally activating solutions were expressed as fractions of P\(_0\) related to the Ca\(^{2+}\) concentration (in pCa), tension-pCa relationships. The tension-pCa experimental data were fitted to the Hill equation:

\[
P/P_0 = \frac{([Ca^{2+}]/K)^n_h}{1 + ([Ca^{2+}]/K)^n_h}
\]

where P/P\(_0\) is the normalized tension, n\(_h\) is the Hill coefficient, K is the apparent dissociation constant (pK = \(-\log K = p\text{Ca}_{50}\), where pCa\(_{50}\) is the pCa necessary to develop 50% of the P\(_0\)), and brackets denote concentration.

Different parameters can be deduced from the tension-pCa curves: the pCa threshold (pCa\(_{th}\)), defined as the lowest Ca\(^{2+}\) concentration required to obtain the development of tension; the pCa\(_{50}\) value; and n\(_h\), related to the steepness of the curve. Two n\(_h\) values were also calculated when the curve was asymmetric. We used the Hill plot linearization of the raw data, i.e., \(\log ([P/P_0] - 1)/([P/P_0])\) (28). Thus the data were best fitted by two straight lines, corresponding to n\(_1\), slope for P/P\(_0\) >50%, and n\(_2\), slope for P/P\(_0\) <50%.

**Functional Identification of Fiber Type**

The criterion for functional fiber identification was based on the difference in Ca\(^{2+}\) and Sr\(^{2+}\) activation characteristics between slow and fast fibers. Indeed, it has been demonstrated that fast muscle fibers are less sensitive to Sr\(^{2+}\) than are slow fibers. To minimize the number of tensions developed by the fiber, only two Sr\(^{2+}\) solutions, pSr 3.4 and 5.0, were applied. The application of pSr 3.4 solution elicited the maximal Sr\(^{2+}\) tension. The pSr 5.0 solution produced tensions close to 95% P\(_0\) in slow fibers and tensions ranging from 0 to 10% in fast fibers (23, 38, 44). Thus slow and fast fibers were clearly identified.

**Electrophoresis**

Frozen muscle tissue was pulverized under liquid N\(_2\) in a small steel mortar and used for the analyses of MHC and Tn subunits. Muscle powder was dissolved in an extraction buffer, as previously described (2).

**MHC isoforms.** As already described (43), the MHC composition was determined by SDS-PAGE on a 4.5% stacking gel and on a 7.5% separating gel. Electrophoresis was run for 18 h at 12°C (180-V constant, 13 mA per gel). After the gel run, the gel slabs were silver stained. The relative proportion of each MHC isoform in each muscle type was determined by integrating densitometry (see below). At least two independent measurements were performed on each sample. They were quite similar, and the mean value was reported.

**Immunoblotting**

Electrotransfer was carried out on a 0.2-μm nitrocellulose sheet (Advantec MFS, Pleasanton, CA). The membranes were blocked with a PBS solution (pH 7.4) containing 5% nonfat dry milk and 0.2% sodium azide. All of the membranes were incubated overnight with each primary antibody. A monoclonal antibody (5C5 from Sigma Chemical, specific to α-sarcomeric actin) allowed actin signal recognition. For TnI, the fast isoforms were identified with the JLT-12 monoclonal antibody from Sigma Chemical; the slow TnT isoforms were detected by using a polyclonal antibody previously characterized and provided by Härter et al. (17). For TnC, both slow and fast isoforms (50/50% recognition) were identified by another polyclonal antibody provided by Härter and Pette (18). For TnI, slow and fast isoforms were identified by two polyclonal antibodies also provided by Härter and Pette.

The first identification was performed for actin. The bound antibodies were then removed by an incubation at 50°C for 40 min with occasional stirring in a stripping buffer (100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.7). The membrane was washed twice in PBS at room temperature, and the success of the stripping was tested by incubating the membrane with the secondary antibodies corresponding to the previously tested antibodies and enhanced chemiluminescence (ECL) detection. The immunodetection of the other proteins was then performed as described above.

The primary antigen-antibody complexes were detected by a peroxidase staining kit (Sigma Chemical), consisting of extravadin peroxidase and biotinated goat conjugate antibodies against mouse or guinea pig IgG (Sigma Chemical). The signals were visualized by an ECL kit (Amer sham Pharmacia Biotech, Piscataway, NJ). Signal intensities were evaluated by an integrating densitometry software (GS-700 Imaging Densitometer, Biorad, Ivry Sur Seine, France). At least two independent measurements were performed on each sample (averaged value reported). To ensure that there was no muscle protein loss during the 19-day HG period. Thus the actin signal intensities expressed in percentage of control corresponded to 103.3 ± 2.2% (n = 9) for HG soleus and 109 ± 4.1% (n = 9) for HG plantaris muscles. Therefore, the actin signal could serve as internal control. The method of analysis for each Tn subunit has been described at the beginning of each respective result section.

**Statistical Analysis**

The data are presented as means ± SE. Student’s t-test was used to estimate differences among means, with the acceptable level of significance being set at P < 0.05.

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Table 1. BM and MWM for soleus and plantaris and ratio of MWM to BM after 19 days of centrifugation at 2 G

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<thead>
<tr>
<th></th>
<th>BM, g</th>
<th>MWM, mg</th>
<th>MWM/BM, mg/g</th>
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<tbody>
<tr>
<td>Cont</td>
<td>Initial</td>
<td>207 ± 2(9)</td>
<td>120.7 ± 3.6(18)</td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>208 ± 2(9)</td>
<td>138.8 ± 3.1(18)</td>
</tr>
<tr>
<td>HG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Final</td>
<td>254 ± 5(9)</td>
<td>215 ± 9(18)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nos. of rats (for body mass [BM]) and muscles (for muscle wet mass [MWM]) are shown in parentheses. Cont, control; HG, hypergravity. *Significantly different from Cont within a same muscle, P < 0.05.

RESULTS

Body Mass and Muscle Wet Mass

The initial body masses (BM) of Cont and HG rats were similar (Table 1). At the end of the 19-day experiment, rats of Cont and HG groups (n = 9) gained in weight an average of 83 and 46 g, respectively. The rats submitted to centrifugation yielded final BMs lower than those of Cont by 12%. The mean absolute wet mass of the soleus muscles was significantly increased by 15%. In contrast, after HG, the plantaris muscle mass remained unchanged. The ratio of muscle wet mass to BM was increased for soleus, whereas it was not significantly modified for plantaris. Fiber diameter measurements did not reveal any change in either muscles (see Table 3).

Myofibrillar Protein Expression in Whole Muscles

MHC protein isoforms in soleus and plantaris muscles. HG conditions during 19 days did not induce any change in the pattern of electrophoretically separated MHC isoforms in soleus and plantaris whole muscles (Fig. 1 and Table 2). MHC I and MHC IIA were expressed at similar levels in Cont and HG soleus. Similar proportions of MHC I, MHC IIA, MHC IId/x, and MHC IIB isoforms were found in Cont and HG plantaris.

Tn subunit isoforms. See Table 2 and Fig. 2.

TNT SUBUNIT. Polyclonal antibodies directed to TnTs (slow or fast) antibodies were different, it was not possible to determine accurate relative proportions into TnTs, TnT2s, and TnT3s and four bands representing the TnTf isoforms, TnT1f, TnT2f, TnT3f, and TnT4f. The proportions of the different isoforms within a TnT type (slow or fast) were related to the actin signal, as previously described (39). Because the two anti-TnT (slow or fast) antibodies were different, it was not possible to determine accurate relative proportions

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Table 2. Isoform expression patterns of MHC and troponin (TnT, TnI, and TnC) subunits in soleus and plantaris muscles from control rats and rats submitted to 2-G centrifugation

<table>
<thead>
<tr>
<th></th>
<th>Soleus</th>
<th>Plantaris</th>
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<tbody>
<tr>
<td>n</td>
<td>Cont</td>
<td>HG</td>
</tr>
<tr>
<td>MHC I</td>
<td>83.1 ± 2.8</td>
<td>82.1 ± 1.7</td>
</tr>
<tr>
<td>MHC IIA</td>
<td>16.9 ± 2.7</td>
<td>17.9 ± 1.7</td>
</tr>
<tr>
<td>MHC IId/x</td>
<td>3.0 ± 0.5</td>
<td>41.2 ± 2.1</td>
</tr>
<tr>
<td>MHC IIB</td>
<td>3.9 ± 0.2</td>
<td>39.1 ± 4.3</td>
</tr>
<tr>
<td>TnT1s</td>
<td>42.5 ± 2.0</td>
<td>41.7 ± 7.8</td>
</tr>
<tr>
<td>TnT2s</td>
<td>41.0 ± 2.0</td>
<td>43.1 ± 2.4</td>
</tr>
<tr>
<td>TnT3s</td>
<td>6.5 ± 1.2</td>
<td>15.2 ± 4.5</td>
</tr>
<tr>
<td>TnT1f</td>
<td>24.7 ± 2.0</td>
<td>27.2 ± 2.3</td>
</tr>
<tr>
<td>TnT2f</td>
<td>41.7 ± 0.5</td>
<td>38.3 ± 0.6</td>
</tr>
<tr>
<td>TnT3f</td>
<td>31.5 ± 0.4</td>
<td>30.9 ± 1.1</td>
</tr>
<tr>
<td>TnT4f</td>
<td>2.1 ± 0.6</td>
<td>3.6 ± 0.8</td>
</tr>
<tr>
<td>TnCs</td>
<td>75.5 ± 3.4</td>
<td>62.3 ± 4.2</td>
</tr>
<tr>
<td>TnCf</td>
<td>78.8 ± 3.9</td>
<td>59.1 ± 3.0</td>
</tr>
</tbody>
</table>

Values are means ± SE, in %; n, no. of muscles. MHC, myosin heavy chain; Tn, troponin; T, I, and C: Tn isoforms; S, slow isoform; F, fast isoform. *Significantly different from Cont within a same muscle, P < 0.05.
of total slow and total fast isoforms in a given muscle. Nevertheless, within each population (slow or fast), we were able to estimate the relative proportion of each isoform. Thus the proportions of TnT1s, TnT2s, and TnT3s found in Cont soleus were not significantly modified by HG. The four TnTf isoforms were expressed in Cont soleus, with TnT2f and TnT3f being predominant, whereas TnT1f was slightly lower and TnT4f was present at a very low level. The same distribution was found in HG soleus muscles. For plantaris, the relative expression of slow and fast TnT isoforms remained unchanged after HG.

**TnI subunit.** TnI only exists as two well-separated isoforms. Changes were estimated after successive application of the slow and the fast antibodies, and relative concentrations of slow (TnIs) and fast (TnIf) isoforms were evaluated with reference to the same actin signal. TnIs was predominantly expressed in Cont soleus, with TnT2f and TnT3f being expressed in Cont soleus, whereas TnIf increased. In plantaris, the respective expressions of TnIs and TnIf remained similar to those of Cont after HG.

**TnC subunit.** The expressions of TnCs and TnCf isoforms were compared on the same gel because the same polyclonal antibody was able to recognize both isoforms. Thus the proportion of each isoform could be measured as a percentage of total TnC. TnCs, the predominant isoform in Cont soleus muscles, represented ~79% of total TnC and was decreased by 25% after HG. The proportions of TnCf and TnCs in Cont plantaris were not modified by exposure to HG.

**Maximal Forces and Tension-pCa Relationships**

The P0 was recorded in the saturating pCa 4.2 solution (Table 3). After HG, slow soleus fibers as well as fast plantaris fibers did not show any change in absolute and normalized P0 compared with Cont. The tension-pCa relationships of the soleus and plantaris fibers are illustrated in Fig. 3. For the two muscles in Cont conditions, the curves showed classic distinct

Table 3. Contractile characteristics of slow-twitch fibers from soleus and fast-twitch fibers from plantaris in control and 2-G centrifuged rats

<table>
<thead>
<tr>
<th></th>
<th>Soleus</th>
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<th>Plantaris</th>
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<tr>
<td></td>
<td>Cont</td>
<td>HG</td>
<td>Cont</td>
<td>HG</td>
</tr>
<tr>
<td>n</td>
<td>41</td>
<td>52</td>
<td>42</td>
<td>40</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>74.7 ± 2.2</td>
<td>71.5 ± 1.9</td>
<td>69.3 ± 1.8</td>
<td>67.6 ± 2.0</td>
</tr>
<tr>
<td>P0, kN/m^2</td>
<td>3.54 ± 0.32</td>
<td>3.63 ± 0.35</td>
<td>3.90 ± 0.34</td>
<td>3.54 ± 0.31</td>
</tr>
<tr>
<td>nH</td>
<td>5.85 ± 0.02</td>
<td>5.80 ± 0.02</td>
<td>5.76 ± 0.02</td>
<td>5.76 ± 0.03</td>
</tr>
<tr>
<td>nF</td>
<td>6.63 ± 0.03</td>
<td>6.45 ± 0.02</td>
<td>6.31 ± 0.03</td>
<td>6.33 ± 0.04</td>
</tr>
<tr>
<td>pCa50</td>
<td>3.22 ± 0.11</td>
<td>3.73 ± 0.17</td>
<td>4.60 ± 0.43</td>
<td>4.25 ± 0.49</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of fibers; P0, maximal tension; pCa50, threshold for Ca2+ activation; pCa4.2, pCa at which tension is half-maximal; nH, slope of fitted line for tension-pCa curve; n1 and n2, Hill coefficients for relative tensions >50% and <50%, respectively. *Significantly different from Cont within a same muscle, P < 0.05.

Fig. 3. Tension-pCa relationships of single-skinned fibers in soleus (A) and plantaris (B) from C (●) and HG (○) rats. Values are means ± SE. Curves were fitted according to the Hill equation. P0, maximal tension.

**DISCUSSION**

This paper reports for the first time the effects of a 2-G centrifugation on the contractile properties of slow and fast rat muscle fibers in relation to their composition in MHC and Tn isoforms.

**Body and Muscle Masses After HG**

In our experiments, during the 19 days of HG, the animal growth was slowed down, and this led to a 12% lower mean BM for rats submitted to centrifugation, compared with Cont. This decline is in agreement with that described by other studies in animals exposed to HG for 14 days (35, 48, 49). These authors explained that the BM decreased because the rats reduced their food intake during the first days of HG, although food and water were provided ad libitum. The same observation was made by our operator. Moreover,
a commonly described response to centrifugation consists in a loss in fat mass due to a preferential fat degradation (3, 48). Centrifugation also evokes a stress response during the first 5 days that results in a transient elevation in circulating catecholamines and corticosterone, which contributes to the increased lipolysis (28).

Centrifugation used to produce an artificially induced gravity should theoretically result in an increased load on muscles in response to an additional imposed force. Therefore, a hypertrophic response may be expected. Indeed, we observed a 15% increase in muscle mass and a significantly higher muscle wet mass-to-BM ratio for the soleus muscle. No change was obtained for the plantaris muscle. A slight increase (7) or stable absolute masses (35, 48) have already been reported for slow extensor muscles, whereas decreases in the range of 5–15% (3, 7, 48) occurred in fast extensor or flexor muscles. Thus HG conditions in our study target preferentially the slow muscle. A surprising result was the unchanged diameters in 2-G soleus muscle fibers, also observed by Roy et al. (35). Therefore, the increase in muscle mass might be more likely related to an increase in noncontractile muscle components such as connective tissue (30) or complexes of the extracellular matrix (14, 37). The possibility of edema in the muscle should be considered; however, it has been reported that many indexes of the state of hydration of animals (water balance and total body water) were unaffected after 2 wk of centrifugation at 2 G (26, 31).

Contractile Protein Expression and Ca\(^{2+}\) Activation Properties

After HG, our results demonstrated that soleus and plantaris muscles expressed, respectively, the same MHC patterns as Cont. This is in agreement with the results obtained by other groups in soleus muscle (35) and in fast muscles such as plantaris (25) and medial gastrocnemius (35). Thus data on soleus underline the fact that HG does not induce change in MHC isoform composition, unlike microgravity conditions, which result in slow-to-fast transitions from MHC I to MHC IIa, II\(d/x\), and IIb. Nevertheless, a similarity between micro- and hypergravity situations might be found in the increased number of hybrid muscle fibers (32, 35, 40, 46).

This paper reports the first data relative to the expression pattern of Tn subunit isoforms at 2 G. In fast plantaris, no change in Tn subunit isoform composition was observed. In soleus, the proportions in TnT isoforms were similar to the Cont ones, for the slow as well as for the fast isoforms. On the contrary, the analysis of TnI and TnC expressions indicated transitions in the slow-to-fast direction, i.e., in the same direction as that observed in unloading conditions after hindlimb suspension (39). However, the level in TnI\(f\) was increased more after 14 days of unloading (×4) than after HG (×1.5), whereas the level in TnC\(f\) was increased more after HG (×1.9) than after microgravity (×1.09).

The functional significance of these changes in Tn subunit isoform expression could be discussed in terms of Ca\(^{2+}\)-activated properties. A large elevation in the steepness of the tension-pCa curve indicated an increased cooperativity among the different proteins of the thin filament. This could be related to the increased expression of the TnC\(f\) isoform (28). Moreover, the straightening of the HG curve might contribute to masking the amplitude of the decrease in Ca\(^{2+}\) affinity, which is more evident at pCa\(_{\text{thr}}\) than at pCa\(_{\text{50}}\). Nevertheless, the slightest direction of the rightward shift would be in agreement with the higher proportion of TnC\((f) (15). Surprisingly, the increase in the steepness of the curve was not accompanied by changes in the TnT molecule. This suggested that a protein such as tropomyosin strongly implied in the cooperative mechanisms within the thin filament (36) might have been transformed during HG. Further studies are needed to elucidate this point.

The changes in the tension-pCa relationship of the slow soleus fibers appeared comparable to those widely reported for different slow muscles from rat, monkey, and humans after simulated or real microgravity (11, 13, 22, 41, 50). No change was found in the tension-pCa relationship of fast plantaris fibers at 0 or 2 G. Thus microgravity (or HG) provoked a preferential adaptation to unloading (or increased load) for the Ca\(^{2+}\) activation properties of slow muscles.

Is There a Continuum for the Adaptation of Muscle Properties From 0 to 2 G?

Supporting this hypothesis already proposed by National Aeronautics and Space Administration-Ames group (48), we have described changes in absolute and relative muscle masses, which were lower at 0 G and higher at 2 G compared, respectively, with 1-G data. Moreover, in both situations, the adaptation concerned more selectively the slow soleus extensor, whereas no change appeared in the fast plantaris.

The other changes reported in this paper did not follow a continuum from 0 to 2 G. Indeed, the tension-pCa relationships were shifted in a similar way at 0 or 2 G compared with 1 G, and TnC and TnI isoforms exhibited slow-to-fast transitions at 0 and 2 G. The most surprising result was the absence of change in MHC and TnT isoform compositions after 2 G, because these molecules are precisely the most extensively and rapidly transformed in microgravity (39, 43). Thus muscle mass should be regulated in a continual way, whereas the functional contractile properties and the phenotypical changes would escape this principle.

Muscle Properties After 2 G Compared With Other Mechanical Overload Situations

Our results after 2-G centrifugation can be compared with others obtained in overload situations, which also induce muscle hypertrophy. Compensatory hypertrophy related to chronic functional overload occurs after
ablation, tenotomy, or denervation of synergistic muscles. In these different conditions, studies have reported changes in the MHC composition corresponding, in the soleus, to an increase in MHC I expression and a decrease in MHC IIa (27, 29) and, in the plantaris, to increases in native slow myosin Sm (47) or MHC I (1, 5) associated with a repression of MHC IIb (5, 27). The transitions toward slower contractile characteristics were also attested by slower twitch contraction, decreases in maximal shortening velocity in whole soleus or plantaris muscles (1, 5, 33, 34), and decreases in SR Ca^{2+} uptake (1, 19, 45). Moreover, increases in P_0 were described in soleus and plantaris at whole muscle (5, 33) or single-fiber (20) levels. The tension-pCa relationship was shifted in the leftward direction, compared with Cont, for both muscles, with the effect being larger for the slow soleus fibers (20).

Taken together, all of these results on mechanical overload are opposite to those found in microgravity and participate lightly in the continuum principle mentioned above. Proposing an interpretation of the discrepancies between 2-G centrifugation and other overload situations is somewhat tempting. Our hypothesis is based on the fact that compensatory growth of mechanically overloaded muscles is largely due to passive chronic stretch (16). During our experiments at 2-G centrifugation, the animals kept a tight grip on the floor (see MATERIALS AND METHODS), with the soleus thus being passively stretched (ankle in a dorsiflexed position). It can, therefore, be supposed that this stretch may be less and/or may be elicited more temporarily than during other overloading situations, because it occurred preferentially during the first days at 2 G. Although this might appear speculative, there are no data available at present that provide a possible explanation for the differences between 2-G centrifugation and other overload conditions.

To conclude, 2-G centrifugation proposed as a potential countermeasure to prevent the effects of microgravity could be considered to limit the atrophic process, as previously described (8). However, what appears most disturbing from our present data is the same orientation of HG effects and microgravity effects during spaceflights that result in a reduction in the Ca^{2+} affinity of the contractile system. Finally, all of these data underline the importance of the gravity factor in muscle physiology.

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