Diaphragm strength and cross-bridge properties during chronic growth hormone hypersecretion

Y. Lecarpentier*, C. Coirault*, B. Riou*, D. Chemla*, J.J. Mercadier*


ABSTRACT: The aim of the study was to determine diaphragm performance and cross-bridge properties in rats bearing a growth hormone (GH)-secreting tumour.

The tumour was induced by subcutaneous injection of GH-hypersecreting cells (GC cells) into the flank. Eighteen weeks after GC cell injection, rats developed a GH-secreting tumour (45.4±5.1 g) and the GH plasma level reached 5,408±648 µg L⁻¹ in GH rats versus 7.2±2.2 µg L⁻¹ in control rats (p<0.001). Diaphragm mechanics and cross-bridge properties were studied by applying the equations of A. Huxley to isolated diaphragm strips (control rats: n=12; GH rats n=20).

In comparison to control rats, the total tension and total number of cross-bridges mm⁻² in GH rats were lower in both twitch and tetanus (p<0.001). A linear relationship was observed between total tension and total cross-bridge number (r=0.94; p<0.001). Conversely, the cross-bridge single force and peak mechanical efficiency did not differ between control and GH rats, in either twitch or tetanus modes.

In conclusion, the diaphragm strength was significantly lower in rats bearing growth hormone secreting tumours, and this was essentially determined by the lower cross-bridge number mm⁻² without change in cross-bridge single force. Eur Respir J 1999; 13: 1070–1077.

Chronic hypersecretion of growth hormone (GH) induces numerous morphological, functional, and metabolic consequences [1, 2]. Respiratory disorders have been documented in patients with GH hypersecretion syndrome [3–7]. Pneumomegaly was first described by Cushing and Davidooff [3]. Lung growth has been attributed to an increase in the size [4, 6] or number [8] of alveoli. The assessment of the respiratory contractile function during GH hypersecretion syndrome appears to be of interest for several reasons: 1) death due to respiratory disorders is three times the expected rate [9]; 2) GH hypersecretion results in generalized muscle weakness and wasting [10]; and 3) respiratory muscle strength is an important determinant of pulmonary volumes [11]. In human acromegaly, respiratory muscle strength investigated by measuring maximal inspiratory and expiratory pressures has been shown to be normal [6, 8]. In another study, inspiratory or expiratory muscle force has been shown to be below normal limits in seven out of 10 patients [12].

A decline in respiratory muscle strength might be due to a dysfunction of the diaphragm. The first aim of this study was to determine whether diaphragm muscle strength was decreased in rats bearing a GH-secreting tumour [13]. In striated muscles, myosin cross-bridges represent molecular motors generating force. Diaphragm performance is, to a large extent, determined both by the number and the single force of cross-bridges. The hypothesis that potential changes in diaphragm performance were due to alterations in the total number, single force and kinetics of the cross-bridges was also tested. On the basis of the theory of Huxley [14], three main steps of the cross-bridge cycle were investigated, in particular the attachment step, the power stroke and the detachment step [15–17].

Materials and methods

Model of rat bearing a growth hormone-secreting tumour

Care of the animals and performance of all experiments were in accordance with the Helsinki recommendations. GH-hypersecreting cells (GC cells) [18] were cultured in Ham’s F10 medium supplemented with 15% horse serum and 2.5% foetal calf serum (Gibco-BRL, Cergy-Pontoise, France). Animals were anaesthetized with sodium methohexitone anaesthesia (40 mg·kg⁻¹ i.p.) before GC cell injection. A suspension of 10–15×10⁶ GC cells in Hank’s medium (0.3 mL) was subcutaneously injected into the flank of 10–12-week-old female Wistar Furth rats (Ifa Credo, L’Arbresle, France). Tumour growth occurred in 90% of the injected rats [13]. Rats were maintained on a regular 12-h light–dark cycle, fed ad libitum, and weighed weekly. Diaphragm function was studied in isolated muscle strips from tumour-bearing rats, 18 weeks after GC cell injection (n=20). Diaphragm strips were also studied from control animals of the same age (n=12).

Hormonal immunoassays

After brief anaesthesia, blood samples were immediately collected into heparinized, chilled tubes, and the plasma stored at -20°C until assay. Plasma GH was measured by means of radioimmunoassays [18].
Mounting procedure

After blood sample collection, the animals were laparotomized. A muscle strip from the ventral part of the costal diaphragm was carefully dissected from the muscle in situ and attached to an electromagnetic force transducer in a tissue chamber containing Krebs–Henseleit solution (in mM): 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.1 H2PO4, 25 NaHCO3, 2.5 CaCl2, and 4.5 glucose. The solution was bubbled with 95% O2/5% CO2 and maintained at 22°C and pH 7.40. The strip was electrically stimulated in twich mode by means of two platinum electrodes delivering rectangular pulses of 1 ms duration at 0.17 Hz. Diaphragm muscles were also stimulated in tetanus conditions as follows: electrical stimulus 1 ms duration; stimulation frequency 33 Hz; train duration 250 ms; and train frequency 0.17 Hz. Experiments were carried out at the resting length (L0) which corresponds to the apex of the resting length–active tension curve.

The electromagnetic lever system

The electromagnetic device consisted of an aluminum lever which was cemented to a coil suspended in the field of a permanent magnet [15]. A force couple developed when an electric current passed through the coil. The lever displacement was measured by means of a photodiode transducer composed of an incandescent lamp, a miniature photodiode and a preamplifier acting as a current-to-voltage converter. The light emitted by the lamp was modulated by the lever displacement and current alterations in the photodiode were converted into voltage alterations. The linearity of the system ranged 0–5 mm of muscle shortening.

Mechanical analysis

Mechanical parameters were calculated from two contractions at L0, recorded in both twitch and tetanus modes. The first contraction was abruptly clamped to zero-load just after the electrical stimulus. The second contraction was fully isometric. The following mechanical indices were used: maximum unloaded shortening velocity of contraction 1 (Vmax); and total force was the peak isometric force of contraction 2. Force was normalized per muscle cross-sectional area (mm2) of the diaphragm strip which was calculated from the ratio of fresh muscle weight to muscle length at L0, assuming a muscle density of 1. Velocity was expressed in L0·s−1, tension in mN·mm−2 and time in s.

The hyperbolic tension–velocity (P–V) relationship was derived from the peak velocity (V) of 7–10 isotonic afterloaded contractions, plotted against the isotonic load level normalized per cross-sectional area (P), by successive load increments, from zero-load up to the isometric tension. Experimental data from the isotonic (P–V) relationship were fitted according to the equation of H ILL [19] (P=a(V+b)=(cPmax+a)b, where -a and -b are the asymptotes of the hyperbola as determined by multilinear regression, and cPmax, is the calculated peak isometric tension for V=0, from the equation.

Characteristics of cross-bridges and energetics

The equations of HUXLEY [14] were used to calculate the rate of total energy release (E; in mW·mm−2), the isotonic tension (Pisoc; in mN·mm−2) and the rate of mechanical energy (Wm; in mW·mm−2) as a function of velocity (V). E is given as:

\[
E = \frac{msar/2}{2V} \left( \frac{h}{f_1 + g_1} \right) \times \left\{ g_1 + f_1 \left( \frac{V}{\Phi} \right) \left( 1 - e^{-\Phi} \right) \right\} \quad (1)
\]

where msar/2 is the cross-bridge number per mm2 at peak isometric tension [14]; sar is the resting sarcomere length at L0; f1 is the maximum value of the rate constant for cross-bridge attachment; and g1 and g2 (which appear in equation 2) are the peak values of the rate constants for cross-bridge detachment [14]. The instantaneous movement x of the myosin head relative to actin varies from 0 to h, the step size of the cross-bridge which is defined by the translocation distance of the actin filament per adenosine triphosphate (ATP) hydrolysis and produced by the swing of the myosin head [20]; f1 and g1 correspond to x=h, and g2 corresponds to x=0 [14]; e is the free energy required to split one ATP molecule [20–22]; l is the distance between two actin sites; Φ=(f1+g1)/2-b. For reasons of dimensions of equations, Φ was multiplied by sar/2 as compared to the initial hypothesis [14]. Consequently, calculations of f1, g1, and g2 were divided by sar/2 as compared to those described previously [15–17] and were given by the following equations:

\[
f_1 = -\frac{g_1 + \sqrt{g_1^2 + 4g_2g_1}}{2} (2)
\]

\[
g_1 = \frac{2wb}{ehG} (3)
\]

\[
g_2 = \frac{2V_{\text{max}}}{h} (4)
\]

The maximum value of total energy release (Emax) occurs at Vmax. The minimum value of rate of total energy release (E0; in mW·mm−2) occurs under isometric conditions; E0 is equal to the product of a × b [14, 23] and is also given by the equation:

\[
E_0 = \frac{(msar/2)^2}{2V} \left( \frac{h}{f_1 + g_1} \right) \quad (5)
\]

The maximum turnover rate of myosin adenosine triphosphatase (ATPase) per site under isometric conditions (kcat; in s−1) is E0/msar/2:

\[
k_{\text{cat}} = \frac{h}{2V} \times \frac{f_1 g_1}{f_1 + g_1} (6)
\]

The isotonic tension Pisoc is given by the equation [14]:

\[
P_{\text{isoc}} = \frac{msar w}{2V} \times \frac{f_1}{f_1 + g_1} \times \left[ 1 - \frac{V}{\Phi} \left( 1 - e^{-\Phi} \right) \right] \left( 1 + \frac{1}{2} \left( \frac{f_1 + g_1}{g_2} \right)^2 \frac{w}{\Phi} \right) \quad (7)
\]

where w is the mechanical work of a single cross-bridge. The elementary force per single cross-bridge in
isometric conditions (\( \Pi \); in pN) is \( \Pi = \frac{P_{\text{Hux, max}}}{(\text{msar}/2)} \) or

\[
\Pi = \frac{w}{I} \times \frac{f_1}{f_1 + g_1}
\]  

(8)

The cross-bridge number per mm\(^2\) at peak isometric tension is \( \text{msar}/2 = P_{\text{Hux, max}} \). The rate of mechanical work is \( W_M = P_{\text{Hux}} \times V \). The normalized peak rate of mechanical work (n\( W \)) is defined as follows \([23]\):

\[
n_{W} = \frac{W_{\text{M,max}}}{(cP_{\text{max}})}
\]

where \( W_{\text{M,max}} \) is the peak value of \( W_M \) and \( cP_{\text{max}} \) is the maximum calculated velocity for \( P=0 \) in the equation of HILL \([19]\). At any given load level, the mechanical efficiency (Meff) of the muscle is defined as the ratio of \( W_M \) to \( E \) \([14]\):

\[
\text{Meff} = \frac{W_M}{E}
\]

Meff,max is the maximum value of Meff.

Values of A. Huxley’s equation constants

A stroke size of 11 nm has been determined by means of optical tweezers \([24]\) and is supported by the tridimensional structure of the myosin head \([25]\). The distance \( f_1 \) is equal to 36 nm. The free energy required to split one ATP molecule per contraction site is \( e = 5.1 \times 10^{-20} \) J. The mechanical work (\( w \)) of a single cross-bridge is equal to 0.75 \( e \) \([14]\), so that \( w = 3.8 \times 10^{-20} \) J.

Data analysis

Data are expressed as mean\( \pm \)SEM. Control rats were compared to GH rats using the unpaired Student’s t-test after ANOVA; p-values <0.05 were required to rule out the null hypothesis. Linear regression was based on the least squares method. The asymptotes -a and -b of the hyperbola of A. Hill were calculated by multilinear regression and the least squares method.

Results

Effects of tumour growth on body weight and plasma hormone levels

At the time of sacrifice, the mean weight of the tumour was 45.4\( \pm \)5.1 g. Body weight was higher in GH tumour-bearing rats than in control rats (495\( \pm \)38 g versus 228\( \pm \)9 g; \( p<0.001 \)). GH plasma levels were markedly higher in tumour-bearing rats than in control rats (5,408\( \pm \)648 mg L\(^{-1} \) in GH rats versus 7.2\( \pm \)2.2 mg L\(^{-1} \) in control rats, \( p<0.001 \)).

Mechanics

In both twitch and tetanus modes, \( V_{\text{max}} \), total tension, total number of cross-bridges per mm\(^2\), maximum value of...
total energy release ($E_{\text{max}}$) and $W_{\text{M,max}}$ were significantly lower in GH than in control rats (figs. 1 and 2). The percentage of total tension at which $W_{\text{M,max}}$ occurred did not differ between GH and control rats either in twitch (35±1% versus 31±2%; NS) or in tetanus (28±1% versus 27±2%; NS) modes. The single force of cross-bridges ($P$) and the $nW_{\text{M}}$ did not differ between the two groups (figs. 1 and 2). The absolute value of the asymptote -a of the $P$--$V$ hyperbola was significantly lower in GH than in control rats in twitch (10.5±1.3 versus 15.1±1.9 mN·mm$^{-2}$; $p<0.01$) and in tetanus (7.6±0.9 versus 11.9±1.7 mN·mm$^{-2}$; $p<0.01$) modes. The absolute value of the asymptote -b of the $P$--$V$ hyperbola did not differ between GH and control rats in twitch (0.53±0.05 versus 0.64±0.07 L0·s$^{-1}$; NS) and tetanus (0.27±0.03 versus 0.32±0.04 L0·s$^{-1}$; NS).

Cross-bridge kinetics and energetics

In both twitch and tetanus modes, rate constants for attachment ($f_1$) and detachment ($g_1$) and the maximum turnover rate of the myosin ATPase ($k_{\text{cat}}$) did not differ significantly between control and GH rats (fig. 3). Conversely, the rate constant for detachment ($g_2$) was significantly lower in GH than in control rats (fig. 3). Peak mechanical efficiency did not differ significantly between control and GH rats, either in twitch or in tetanus modes (fig. 2). The percentage of total tension at which $M_{\text{eff,max}}$ occurred did not differ between GH and control rats in either twitch (61±2% versus 61±2%; NS) or tetanus (65±2% versus 70±2%; NS) modes.

Relationships between parameters

In the study groups as a whole, there was a direct linear relationship between total tension and total cross-bridge number, but no relationship between $V_{\text{max}}$ and $k_{\text{cat}}$ (fig. 4). There was a negative linear relationship between $nW_{\text{M}}$ and both $M_{\text{eff,max}}$ and cross-bridge single force (fig. 5). There was a direct linear relationship between $M_{\text{eff,max}}$ and cross-bridge single force and between $E_{\text{max}}$ and $W_{\text{M,max}}$ (fig. 6).

Discussion

It has been demonstrated that there was an intrinsic alteration in diaphragm performance in rats bearing a GH-secreting tumour. In this model, the marked fall in total tension was attributed, at least partly, to the decline in the total number of cross-bridges. Conversely, no changes in
the single force of myosin molecular motors or in peak mechanical efficiency were observed.

Respiratory muscle strength in growth hormone hypersecretion syndrome

In this study, intrinsic diaphragmatic performance was markedly impaired, as attested by the lower tension in rats bearing a GH-secreting tumour as compared to control rats. GH hypersecretion results in generalized muscle weakness and wasting [10]. Experimental muscular hypertrophy induced by GH hypersecretion has been shown to be functionally inefficient, the contractile strength being reduced. In human acromegaly, maximal inspiratory pressure and maximal expiratory pressure have been shown to be normal [6, 8]. Conversely, in 10 patients with acromegaly, LANDELLI et al. [12] have shown that maximal respiratory pressures, either inspiratory (3/10 patients) or expiratory (3/10 patients) or both (1/10 patient) were decreased. However, maximal respiratory pressures do not assess the intrinsic function of the diaphragm muscle. Moreover, maximal respiratory pressures are not specific to diaphragm muscle, and accessory respiratory muscles may play a compensatory role and minimize the decrease in maximal inspiratory pressure in human acromegaly. Assuming that the GH plasma level is a determinant of muscle weakness [10], the marked fall in diaphragm contractile strength in rats with a GH-secreting tumour might be due to higher GH plasma levels compared with those observed in human acromegaly. Moreover, values of GH plasma levels observed in rats bearing a GH-secreting tumour were similar to those obtained in mouse transgenic model [26].

Number and force of cross-bridges

The total number and the single force of cross-bridges represent the main determinants of muscle tension. In the present study, the single force, number and kinetics of cross-bridges were calculated on the basis of the classic equations of HUXLEY [14]. Cross-bridge single force (P) was not altered in GH tumour-bearing rats and only the total cross-bridge number-mm⁻² decreased significantly (fig. 1). The drop in total cross-bridge number accounted, at least in part, for the diaphragm weakness. Peak isometric tension was linearly related to the total number of cross-bridges-mm⁻² (fig. 4), but not to the single force of the myosin head. Diaphragm peak tension has previously been shown to be related to the decline in the total cross-bridge number with no changes occurring in cross-bridge single force, in experimental models such as cardiomypathic Syrian hamsters [15], and during developmental changes [16]. Conversely, in a rabbit submitted
to a chronic volume and pressure overload, the fall in both the total cross-bridge number and cross-bridge single force accounted for the decrease in diaphragm strength [17]. Values of cross-bridge single force in the present study were of the same order of magnitude as those previously measured by means of optical tweezers and glass needle techniques and ranged 3–7 pN [24, 27, 28].

No relationship between maximum unloaded shortening velocity and maximum turnover rate of myosin adenosine triphosphatase

There was no relationship between $V_{max}$ and $k_{cat}$ (fig. 4), as previously observed in the diaphragm of cardiomyopathic Syrian hamsters and during development [15, 16] and in the diaphragm of rabbits suffering from congestive heart failure [17]. This finding contrasts with the classic linear relationship between $V_{max}$ and maximum actin-activated myosin ATPase activity in skeletal muscles [29] and during compensatory cardiac hypertrophy. In the theory of A. Huxley, $V_{max}$ is related to the detachment rate constant ($g_2$) which has been predicted to limit $V_{max}$ [14, 22]. The limiting step of the overall detachment process is thought to correspond to the adenosine diphosphate (ADP) release and consequently the maximum velocity of movement [30]. Conversely, $k_{cat}$, the inverse of the overall duration of the cross-bridge cycle, varies with the rate constant for attachment ($f_1$), which is considered to be the limiting step of the cross-bridge cycle [22, 30]. Loop 2, at the actin-binding site of the myosin head, may "tune" the rate-limiting step of the myosin ATPase cycle and consequently $k_{cat}$ [30]. As suggested by SPUDICH [30], these two loops might exert their regulatory function partly independently of each other. This may account in part for the absence of any relationship between $V_{max}$ and $k_{cat}$ (fig. 4). This is corroborated by the fact that alterations in loop 2 can markedly modify the myosin ATPase activity, without inducing corresponding changes in velocity [31].
A reduction in motor-unit action potential duration, even in the absence of clinical symptoms or muscle weakness, has been reported [33]. There is a cellular patchy myopathic disturbance in the proximal muscles of acromegalic patients. Myopathy in GH hypersecretion consists of segmental fibre degeneration, foci of small cell infiltration, thickening of capillary basement membranes and variable areas of hypertrophy and atrophy of both type I and type II fibers. In proximal limb muscles, hypertrophy of both type I and type II has been reported [33]. Hypertrophy of type I and atrophy of type II muscle fibres have also been found in acromegalic patients [34]; however, muscle changes are not significantly correlated with GH levels.

Clinical relevance

It is important to consider diaphragm function during GH hypersecretion in humans for several reasons. Firstly, the presence of pulmonary disease in patients with GH hypersecretion has been shown to increase morbidity and mortality. Indeed, death due to respiratory disease has been found to be three-times more likely in acromegaly [9]. Secondly, GH hypersecretion increases airway resistance. In humans, narrowing of the upper airways with the development of airflow obstruction has many aetiological factors in acromegaly [35, 36]. Acromegaly induces an increase in the size of organs and tissues, including the tissues of the mouth, pharynx and larynx. Narrowing of the small airways might be due to an increase in the wall thickness of the intrapulmonary conducting airways, which leads to a reduction in the size of the lumen [5]. An increase in upper and small airway resistances induces an increase in breathing work which may lead to diaphragm fatigue and/or weakness. In association with intrinsic
alterations of muscular cells due to GH hypersecretion, this may partly account for the impairment of diaphragm strength [12].

In conclusion, in growth hormone tumour-bearing rats, there was intrinsic impairment of diaphragm strength. This was associated with a significant fall in the number of cross-bridges without changes occurring in cross-bridge single force. The maximal efficiency did not change and, in terms of cross-bridge kinetics, only the detachment rate constant (gs) was altered.

References