Effects of postnatal maturation on energetics and cross-bridge properties in rat diaphragm

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The diaphragm muscle is the principal inspiratory muscle, chronically active from birth and generating adequate force to sustain ventilation. Thus it is the only skeletal muscle that may be strictly regarded as being essential. During postnatal maturation, in diaphragm muscle as well as in other skeletal muscles, major ultrastructural, biochemical, and metabolic changes occur, resulting in an improved contractility (9, 20, 30, 31, 38, 41, 46). However, the precise mechanisms by which maturation induces changes in the contractile performance of diaphragm muscle remain incompletely understood (8, 12, 19, 31, 47).

In diaphragm muscle, as well as in other striated muscles, mechanical processes result from the cyclic interaction between two contractile proteins, actin and myosin. The mechanical and energetic properties of the muscle depend on actomyosin cross-bridge (CB) cycling because CBs produce a power stroke that drives the myosin molecules along the actin filaments (17, 18).

According to the most widely accepted theory of muscle contraction (17, 18), CBs act as independent force generators, and muscle force depends on both the elementary force produced per single CB and the total number of CBs formed (17, 18). In a recent study performed in the hamster, Coirault et al. (8) have suggested that developmental changes in diaphragm muscle force were associated with changes in CB number and kinetics but not with changes in the elementary force produced per single CB or in mechanical efficiency. However, species differences regarding changes in CB properties during postnatal maturation might exist, although neonatal rats have become widely used as experimental laboratory animals, especially in cardiorespiratory physiological and pharmacological fields (3, 37, 38, 43, 46). Moreover, Coirault et al. (8) have studied only 1-day- and 1-wk-old hamsters, whereas it may be important to study additional intermediate stages during postnatal maturation. This study may be especially relevant in the rat, whose transition from a fetal to an adult pattern of contractile protein isoforms has been reported to be more complex and delayed compared with other mammal species (22, 27). In fact, at term, the rat diaphragm muscle myosin heavy chain (MHC) phenotype is a composite of neonatal MHC (MHCneo 66%), MHCslow (12%) and MHC2A (22%). In contrast, at birth, the human diaphragm is...
more mature, being composed of more fast adult MHC than the rat diaphragm, with 15% MHC.neo, 32% MHC.slow, 47% MHC.2A, and 6% MHC.2B (27).

We, therefore, conducted an in vitro study on the effects of postnatal maturation on diaphragm muscle CB properties of 3-day-old, 10-day-old, 17-day-old, and adults rats. We hypothesized that, despite marked changes in the contractile protein isoform composition of the rat diaphragm muscle during postnatal development, there are no changes in unitary force production per CB but that the increase in the total number of CBs per cross-sectional area with aging contributes to the developmental changes in contractility.

METHODS

Animals and study design. Care of the animals conformed to the recommendations of the Helsinki Declaration, and the study was performed in accordance with the regulations laid down by the French Ministry of Agriculture. After birth, rat pups were kept in cages with their mothers. Adult rats received rat chow and water ad libitum. A 12-h light-dark cycle was provided. Experiments were performed on Wistar rats – 12 wk (adult, n = 20), 17 days (n = 20), and 10–12 wk (adult, n = 20).

After a brief anesthesia with ether, a median laparotomy was performed and a muscle strip from the ventral diaphragm was carefully dissected from the muscle in situ, as previously reported (7). With this procedure, the diaphragmatic fibers were parallel and of approximately equal length. This diaphragm strip was vertically suspended in a 200-ml jacketed reservoir with Krebs-Henseleit bicarbonate buffer solution that contained (in mM) 118 sodium chloride, 4.7 potassium chloride, 1.2 magnesium sulfate, 1.1 dipotassium hydrogen phosphate, 25 sodium hydrogen carbonate, 2.5 calcium chloride, and 4.5 glucose. The jacketed reservoir was maintained at 29°C with continuous monitoring of the solution temperature. The bathing solution was bubbled with 95% oxygen-5% carbon dioxide, resulting in a pH of 7.40. Preparations were field stimulated with 1-ms rectangular pulses, at a rate of 50 Hz for 300 ms, to induce a tetanic contraction (10 contractions/min). A frequency of stimulation of 50 Hz was used because it assures a maximal tetanus across all age groups without increasing the risk of inducing high-frequency fatigue (29). After a 30-min stabilization period, at the initial muscle length at the apex of the length-active isometric tension curve (Lmax), diaphragm muscle strips recovered their optimal mechanical performance (7). At the end of the study, the cross-sectional area (in mm²) was calculated from the ratio of fresh muscle weight to muscle length at Lmax, assuming a muscle density of 1.06. Body weight was measured at the moment the animal was killed.

Electromagnetic lever system. The electromagnetic lever system has been previously described (7, 23, 33). Briefly, the load applied to the muscle was determined by means of a servomechanism–controlled current through the coil of an electromagnet. Muscular shortening induced a displacement of the lever, which modulated the light intensity of a photoelectric transducer. The initial preload (resting force), which determined Lmax, was automatically maintained constant throughout the experiment. All analyses were made from digital records of force and length obtained with a computer, as previously described (7, 23).

Mechanical parameters. The main mechanical parameters were calculated from three consecutive tetanic contractions preloaded at Lmax. The first contraction was isometric and loaded with preload only. The second contraction was loaded with preload and was abruptly clamped to zero load immediately after the electrical stimulus, according to the zero-load clamp technique (4). The third contraction was fully isometric at Lmax. The maximum extent of shortening (ΔL) and maximum lengthening velocity (Vr) were determined from the first contraction. The maximum unloaded shortening velocity (Vmax), as calculated by means of the zero-load clamp technique, was determined from the second contraction. The maximum active isometric force normalized per cross-sectional area (AF), and the peak of the positive (+) and negative (−) maximum contraction rate (dF/dt) force derivatives normalized per cross-sectional area were determined from the third contraction (Fig. 1).

Energetic parameters and CB properties. Calculations of muscle energetics and characteristics of CBs were determined from Huxley’s equations (17), as previously described (5, 8, 24–26). The force-velocity (F-V) relationship was derived from the peak velocity (V) of various afterloaded contractions, plotted against the isotonic load level normalized...
Fig. 2. A typical 10-day-old diaphragm muscle contracting at 10 different afterload levels from zero load to isometric load and stimulated in tetanus (50 Hz, train duration = 350 ms). $L_{\text{max}}$ is the initial muscle length corresponding to the apex of the length-force curve. Top: $LL_{\text{max}}$ plotted vs. time. Bottom: force (mN/mm$^2$) plotted vs. time.

The maximum turnover rate of myosin ATPase in isometric conditions ($k_{\text{cat}}$, s$^{-1}$) is given by the following equation

$$k_{\text{cat}} = E_0/(em) = h/(2l) \cdot (f_1g_1)/(f_1 + g_1)$$

Assuming that one molecule of ATP is split in each CB cycle, the total duration of the time cycle ($t_s$), the total CB cycle duration ($t_c = 1/k_{\text{cat}}$), the duration of the power stroke [time stroke ($t_p$)], the duty ratio ($t_p/t_c$), and the mean velocity of each CB ($v_0$) were calculated as

$$t_s = L_{\text{max}}/(mnhE_0)$$

$$v_0 = h/t_s$$

$P_{\text{Hex}}$ is given by

$$P_{\text{Hex}} = m \cdot w \cdot l^{-1} \cdot f_1 \cdot (f_1 + g_1)^{-1} \cdot [1 - V \cdot f_1^{-1} \cdot (1 - e^{-V/2\varphi})]$$

$$[1 + (f_1 + g_1)^2 \cdot g_1^{-2} \cdot V/(2\varphi)]$$

where $w$ is the Wm of a unitary CB. The elementary force per unitary CB in isometric conditions ($\pi$, pN) is given by the following equation

$$\pi = P_{\text{Hex max}}/m = (wl) \cdot f_1/(f_1 + g_1)$$

$Wm$ is given by

$$Wm = P_{\text{Hex}}V$$

At any given load, the mechanical efficiency (Eff) of the muscle is defined as the ratio of $Wm$ to $E$, and $Eff_{\text{max}}$ is the maximum value of Eff.

A stroke size ($h$) of 11 nm has been determined by means of optical tweezers (11) and is supported by the three-dimensional structure of crystallized myosin head (10, 35). The distance $l$ is equal to 36 nm (36). The free energy required to split one ATP molecule is $5.1 \times 10^{-20}$ J. Because $w$ is 0.75e, the value of $w$ is $3.8 \times 10^{-20}$ J (48).

Statistical analysis. Data are expressed as means ± SD. Comparisons of several means were performed by using one-way ANOVA and Newman-Keuls test. F-V relationship was fitted to a hyperbola by using multilinear regression and the least-squares method. Correlation between two variables was performed by using the least-squares method. All $P$ values were two-tailed, and a $P$ value of <0.05 was required to reject the null hypothesis. Statistical analysis was performed with the use of NCSS 6.0 software (Statistical Solutions, Cork, Ireland).

RESULTS

As shown in Table 1, we observed significant differences in body weight, diaphragm strip weight, section, and $L_{\text{max}}$.

### Table 1. Physical characteristics changes with development

<table>
<thead>
<tr>
<th></th>
<th>3-Day-Old Rat</th>
<th>10-Day-Old Rat</th>
<th>17-Day-Old Rat</th>
<th>Adult Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>7.6 ± 1.0*</td>
<td>17.0 ± 1.6*</td>
<td>28.6 ± 3.9*</td>
<td>289.1 ± 3.3*</td>
</tr>
<tr>
<td>Diaphragm strip</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Weight, mg</td>
<td>3.9 ± 0.6*</td>
<td>5.4 ± 2.2*</td>
<td>6.4 ± 2.2*</td>
<td>11.0 ± 0.4*</td>
</tr>
<tr>
<td>Section, mm$^2$</td>
<td>0.69 ± 0.3*†</td>
<td>0.81 ± 0.2*</td>
<td>0.85 ± 0.2*</td>
<td>1.19 ± 0.3*</td>
</tr>
<tr>
<td>$L_{\text{max}}$, mm</td>
<td>5.7 ± 1.4*‡</td>
<td>6.1 ± 1.1*</td>
<td>7.7 ± 1.1*</td>
<td>9.1 ± 1.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 20 rats for each group). $L_{\text{max}}$, initial muscle length corresponding to the apex of the length-force curve.

* $P < 0.05$ vs. adult rats. † $P < 0.05$ vs. 17-day-old rats.
Table 2. Effects of postnatal maturation on main mechanical parameters

<table>
<thead>
<tr>
<th></th>
<th>3-Day-Old Rats</th>
<th>10-Day-Old Rats</th>
<th>17-Day-Old Rats</th>
<th>Adult Rats</th>
</tr>
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<tbody>
<tr>
<td><strong>Contraction</strong></td>
<td></td>
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<tr>
<td>Initial resting force, mN</td>
<td>6 ± 1*</td>
<td>8 ± 1*</td>
<td>10 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>ΔL, %Lmax</td>
<td>33 ± 9</td>
<td>29 ± 9</td>
<td>33 ± 9</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>Vmax, mN/mm²/s</td>
<td>2.9 ± 0.5†‡</td>
<td>3.2 ± 0.8†‡</td>
<td>4.0 ± 1.1*</td>
<td>5.1 ± 1.2</td>
</tr>
<tr>
<td>AF, mN/mm²</td>
<td>34 ± 25†‡</td>
<td>43 ± 20†‡</td>
<td>64 ± 24*</td>
<td>77 ± 19</td>
</tr>
<tr>
<td>+dF/dt, mN-mm⁻²s⁻¹</td>
<td>241 ± 21†‡</td>
<td>360 ± 169†‡</td>
<td>530 ± 191*</td>
<td>990 ± 276</td>
</tr>
<tr>
<td><strong>Relaxation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vr, Lmax/s</td>
<td>5.6 ± 2.0*</td>
<td>6.5 ± 2.0*</td>
<td>7.4 ± 2.8</td>
<td>8.3 ± 1.5</td>
</tr>
<tr>
<td>−dF/dt, mN-mm⁻²s⁻¹</td>
<td>660 ± 498†</td>
<td>1,045 ± 627†</td>
<td>1,768 ± 730</td>
<td>2,090 ± 510</td>
</tr>
<tr>
<td>(−dF/dt)/AF, s⁻¹</td>
<td>19.3 ± 4.2†‡</td>
<td>23.7 ± 4.0*</td>
<td>25.6 ± 6.7</td>
<td>27.3 ± 2.9</td>
</tr>
</tbody>
</table>

Data are means ± SD (n = 20 rats). ΔL, maximum extent of shortening; Vmax, maximum unloaded shortening velocity; AF, maximum isometric active force normalized per cross-sectional area; +dF/dt, maximum positive peak force derivative normalized per cross-sectional area; Vr, maximum lengthening velocity; −dF/dt, maximum negative peak force derivative normalized per cross-sectional area; F, force. *P < 0.05 vs. adult rats. †P < 0.05 vs. 17-day-old rats. ‡P < 0.05 vs. 10-day-old rats.

Main mechanical parameters. During postnatal maturation, we observed significant increases in mechanical parameters testing inotropy in isotonic (Vmax) and isometric (AF, +dF/dt) conditions (Table 2). Vr and −dF/dt, which tested relaxation in isotonic (Vr) and isometric conditions (−dF/dt) were also significantly modified during postnatal maturation (Table 2). The ratio Vr/ΔL was only significantly different between 3-day-old and adult rats (Table 2). The ratio (−dF/dt)/AF, was significantly lower in 3- and 10-day-old rats than in other groups (Table 2).

Energetic characteristics. Postnatal maturation did not significantly change the curvature G of the F-V relationship (Table 3). There was a significant increase in the asymptote −a and −b of the hyperbola with postnatal maturation (Table 3). The peak of mechanical efficiency (Effmax) was not modified by postnatal maturation, although there was a significant increase in the rate of total energy release (Table 3).

CB properties. During postnatal maturation, there was a significant increase in the total number of CBs per cross-sectional area but not in the force developed by unitary CB (Table 4). The kcat was only significantly modified by maturation in adult rats (Table 4). Moreover, the maximum values of the rate constant for CB attachment and detachment significantly increased during postnatal maturation (Table 4). The maximum value of the rate constant for CB detachment was only significantly modified by maturation in adult rats (Table 4). Postnatal maturation was associated with a decrease in the total duration of the CB cycle and tss, associated with a decrease in the duty ratio (Table 4). An increase in the v0 of CBs was also observed during maturation (Table 4).

Relationships between the mechanical and energetic parameters. There was a strong linear relationship (r = 0.969, P < 0.001) between maximum total isometric force and m (Fig. 3), with the number of CBs increasing proportionally with force. There was also a significant correlation (r = 0.728, P < 0.001) between Vmax and m (Fig. 3). Conversely, there was neither significant correlation between AF and π (r = 0.252, P value not significant (NS)) nor between Vmax and π (r = 0.161, NS). A weak correlation (r = 0.541, P < 0.001) was noted between Vmax and kcat (Fig. 4). With the use of multiple correlation, it appeared that Vmax was more strongly correlated with age (r = 0.780, P < 0.001) than with kcat (r = 0.541, P = 0.006).

DISCUSSION

In our study, CB properties were calculated from mechanical data obtained in isolated diaphragm strips by using Huxley’s equations (17). Huxley’s theory (17)
remains the most commonly accepted theory of muscle contraction, and his equations have been applied in studies on the effects of different pathophysiological conditions (congestive heart failure, fatigue) or treatment administration (angiotensin-converting enzyme inhibition, nandrolone) on diaphragm muscle CB kinetics of various animal species (mouse, rabbit, rat) (5, 24–26, 40). The effects of postnatal maturation on physical characteristics and mechanical parameters of contraction, relaxation, and contraction-relaxation coupling of rat diaphragm observed in our study (Tables 1 and 2) are in agreement with those previously described in other rat diaphragm studies (12, 19, 46, 49).

Shortening velocity. $V_{\text{max}}$ increased significantly during postnatal maturation (Table 2), as previously reported (19). Some authors have proposed that these changes could be largely related to postnatal transitions in MHC isoform expression, especially the progressive decrease in MHCneo isoform expression and the progressive increase in MHC2X and MHC2B expression (19). In fact, MHC isoforms differ in their ATPase activity (47), and it has been suggested that these differences in actomyosin ATPase activity contribute to the relationship between MHC phenotype transitions and velocities of the diaphragm during early postnatal development (1, 19, 47). A lower actomyosin ATPase activity has been reported in newborn rats, compared with adult rats, with actomyosin ATPase activity depending on MHC isoform expression (47). The lowest actomyosin ATPase activity was observed in those fibers that expressed MHCneo and MHCslow, and it increased in rank order in IIa > IIX < IIb fibers (47). In contrast, other studies have suggested that there is only a weak correlation between MHC isoform expression and changes in diaphragmatic velocity during maturation, supporting the hypothesis that factors in addition to the postnatal transitions in MHC isoform expression are involved in regulating a diaphragmatic increase in velocity of shortening (34).

In the hamster diaphragm, Coirault et al. (8) did not observe any significant correlation between $k_{\text{cat}}$ and

<table>
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<tr>
<th>Table 4. Cross-bridge properties in adult and postnatal rats</th>
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<tr>
<td>3-Day-Old Rats</td>
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<tr>
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<tr>
<td>$f_{\text{1}}$, s$^{-1}$</td>
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<tr>
<td>$g_{\text{1}}$, s$^{-1}$</td>
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<tr>
<td>$g_{\text{2}}$, s$^{-1}$</td>
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<tr>
<td>$\pi$, pN</td>
</tr>
<tr>
<td>$m$, 10$^9$/mm$^2$</td>
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<tr>
<td>$k_{\text{cat}}$, s$^{-1}$</td>
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<tr>
<td>$t_{\text{c}}$, s</td>
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<tr>
<td>$t_{\text{n}}$, s</td>
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<tr>
<td>Duty ratio</td>
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<td>$v_{\text{o}}$, μm/s$^{-1}$</td>
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Data are means ± SD (n = 20 rats). $f_{\text{1}}$, Maximum value of the rate constant for cross-bridge attachment; $g_{\text{1}}$ and $g_{\text{2}}$, peak values of rate constants for cross-bridge detachment; $\pi$, elementary force per single cross bridge; $m$, total number of cross bridges per cross-sectional area; $k_{\text{cat}}$, turnover rate of myosin ATPase per myosin site in isometric conditions; $t_{\text{c}}$, total CB cycle duration; $t_{\text{n}}$, duration of the power stroke; duty ratio, $t_{\text{n}}$/t; $v_{\text{o}}$, mean velocity of a single cross bridge during the power stroke. *P < 0.05 vs. adult rats. †P < 0.05 vs. 17-day-old rats.

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In the hamster diaphragm, Coirault et al. (8) did not observe any significant correlation between $k_{\text{cat}}$ and

Fig. 3. Significant correlations between maximum isometric total force (TF) and the total number of cross bridges (m) (A), and $V_{\text{max}}$ and m (B).

Fig. 4. Significant but weak correlation between $V_{\text{max}}$ and turnover rate of myosin ATPase per myosin site ($k_{\text{cat}}$).
$V_{\text{max}}$ during postnatal maturation. In contrast, in rat diaphragm, we observed a weak but significant correlation between $k_{\text{cat}}$ and $V_{\text{max}}$. It should be pointed out that the number of animals studied ($n = 80$) is likely to have provided us a sufficient statistical power to detect this weak correlation (8). Our results suggest that the mechanisms underlying postnatal changes in $V_{\text{max}}$ are only partly dependent on actomyosin ATPase activity (1). In addition, according to Huxley’s theory, $k_{\text{cat}}$ is principally governed by $f_1$ and $g_1$, whereas $V_{\text{max}}$ is proportional to $g_2$. Therefore, complex changes in the rate constants of CB attachment and detachment during postnatal maturation (Table 4) may partly explain the weak correlation between $V_{\text{max}}$ and $k_{\text{cat}}$. We have observed that $k_{\text{cat}}$ did not vary significantly during the first 2 wk postpartum, whereas $V_{\text{max}}$ increased significantly from day 3 to day 17 and adulthood (Table 4). Accordingly, changes in the $t_c$ assuming that one ATP molecule is hydrolyzed per CB cycle, were inversely related to developmental changes in $k_{\text{cat}}$. These results agree with those by Coirault et al. (8) and, as suggested by these authors, may be related to the progressive disappearance of MHC neo isoforms and the expression of adult fast myosin isoforms. This hypothesis is consistent with biochemical studies (2, 16) and indicates that the overall cycle of ATP splitting takes place more slowly in immature than in adult diaphragms. Indeed, $t_c$ decreased significantly only in adult rats (Table 4). During the first 2 wk postpartum, there is an important increase in MHC slow and a concomitant reduction in the proportion of MHC neo (5, 9, 19, 47, 49). However, both MHC neo and MHC slow have a low actomyosin ATPase activity (47), suggesting that the increase in MHC slow counterbalances the decrease in MHC neo in circumstances in which myosin ATPase activity is concerned, and explains why $k_{\text{cat}}$ values remained unchanged between days 3 and 17 postpartum but increased thereafter. This is consistent with the results obtained for both $f_1$ and $g_1$ (Table 4). According to Huxley’s equations, $k_{\text{cat}}$ is principally governed by the two rate constants, $f_1$ and $g_1$ (17).

Active force and $\pi$. The $\pi$ values calculated in our study (Table 4) agree with those previously calculated from Huxley’s equations for adult hamster (8, 25) and rat diaphragm (26). In addition, $\pi$ values calculated in our study are also in the range of the average force between an actin filament and a single molecule of myosin as measured by pulling the filament with optical tweezers (32). During postnatal maturation, we observed a significant increase in diaphragmatic force and in the total number of CBs, as well as a strong linear relationship between maximum total isometric force and the total number of CBs (Fig. 5), whereas no change in $\pi$ was observed. Because the maximum total isometric force is the product of the number of CBs and $\pi$ (17), these results suggest that the increase in force of the developing diaphragm muscle are mainly related to the increase in the number of CBs. This hypothesis is in agreement with the results of Coirault et al. (8), obtained in hamster diaphragm. The total number of CBs per square millimeter reflects the cross-sectional density of CBs of a given muscle. During diaphragm postnatal maturation, different mechanisms may participate in the increase in the total number of CBs, in parallel with an increase in myofilament protein density (30), a reduction in the percentage of interstitial space relative to total muscle cross-sectional area (14), and an increase in fiber cross-sectional area (41). The lack of change in force per single CB observed in our study suggests that developmental increase in maximum total isometric force is not related to reorientation of oblique fibers into the longitudinal axis of the diaphragm muscle, as previously suggested by Coirault et al. (8). It should be pointed out that our methodology enables us only to calculate the number of active CBs but not the total number of CBs present. Indeed, we cannot make the difference between a decreased total number of CBs, leading to a decreased force, and a decrease in calcium available for contraction, also leading to a decreased force through a decrease in the number of active CBs. Because diaphragmatic contraction highly depends on intracellular calcium, mainly from the sarcoplasmic reticulum, rather than extracellular calcium, the maturation of the sarcoplasmic reticulum may have played an important role in the postnatal increase in force and number of active CBs observed in our study. Ryanodine receptors (RyR) are intracellular homotetrameric Ca$^{2+}$-release channels, whose subunits are encoded by three different genes indicated as RyR1, RyR2, and RyR3. In adult skeletal and diaphragmatic muscles, RyR1 is essential in triggering contraction. Expression of RyR1 requires about 3–4 wk to reach the high levels that are maintained throughout adult life (21). Another isoform, RyR3, more expressed in the diaphragm than in other skeletal muscles (44), is predominantly expressed during fetal and neonatal development and has been shown to play a physiological role in excitation-contraction coupling of neonatal skeletal muscles (3, 44). RyR3 is already expressed during fetal development, but its expression is maximum during the neonatal phase (2–15 days) in the rat (44). Therefore, the changes in CB cycling observed following the 2 wk postpartum may be in relation with the progressive disappearance of RyR3. Alternatively, the changes in force generation during postnatal maturation may be related to the MHC content per half sarcomere (13). In a recent study, maximum force values of rat diaphragm muscle bundles and single fibers were normalized for MHC content per half sarcomere to determine the effect of CB number on maximum specific force during maturation (13). MHC content per half sarcromere progressively increased during early postnatal maturation, but no change in force per half sarcromere MHC content was noted between days 0 and 14, except for fibers predominantly expressing MHC slow, which represent about 12% of total MHC content at day 14 and 0% between days 0 and 7 (47, 49). These results indicate that the difference in specific force mainly reflects differences in MHC content per half sarcomere. Our results are in agreement with this assumption because we observed that, during postna-
tal development, there was no increase in the unitary force per CB but that there was an increase in the total number of CB, which may reflect the increase in MHC content per half sarcomere.

**Mechanical efficiency.** We observed no significant changes in peak mechanical efficiency during postnatal maturation (Table 3). However, changes in myofibrillar ATPase activity are usually thought to be responsible for changes in the economy of the muscle force generation and/or in peak mechanical efficiency (48). It has been shown that slow-contracting muscles such as soleus, which has a low ATPase activity, have a greater economy of force generation than fast-contracting muscles with high ATPase activity (48). Accordingly, higher ATPase activity in adult diaphragm would be expected to decrease both maximum efficiency and the G curvature of the F-V relationship. In contrast, we observed no such changes during maturation in the rat (Table 3), as previously reported in hamster diaphragm muscle (8), despite a significant increase in $k_{cat}$. These results indicate that changes in myosin ATPase activity are not always associated to changes in contractile efficiency, as previously observed in cardiac muscle (42).

**Relaxation.** We also observed a progressive change in diaphragm relaxation during postnatal maturation (Table 2). However, these changes were mainly significant after the second week postpartum, as previously reported (45). The precise mechanisms by which postnatal maturation modified diaphragm relaxation remain unclear. Relaxation is controlled by a complex interplay between inactivation and loading conditions. The rate of inactivation is limited mainly by active $Ca^{2+}$ pumping by the sarcoplasmic reticulum, $Ca^{2+}$ removal from troponin C, and the instantaneous number of working CBs (6). Thus the increase in the total number of CBs noted in our study (Table 4) may have played a role in the improvement in relaxation observed during postnatal maturation by increasing the instantaneous number of working CBs. In addition, there is some evidence suggesting that active calcium movements by the sarcoplasmic reticulum could be altered in the neonatal diaphragm. Maxwell et al. (30) showed evidence of an immature sarcoplasmic reticulum in fibers from premature baboon diaphragms. Three factors could limit capacities for $Ca^{2+}$ release and reuptake in the diaphragm muscle. On the other hand, it has been suggested that CB kinetics have a limited influence on the overall time course of diaphragm relaxation (6).

**CB kinetics.** We observed marked differences in CB kinetics between the adult rat and the adult hamster (8). Although the value of a single CB was similar in the two species, in agreement with Huxley’s theory, the number of CBs, and the constants of attachment ($f_1$) and detachment ($g_1$, $g_2$) were lower in the rat than in the hamster (8). Despite these marked differences, we observed no important species difference in the postnatal maturation process concerning CB kinetics. This result suggests that postnatal maturation involves common mechanisms that do not markedly differ from one species to another. Nevertheless, further investigations on other mammal species should be performed to confirm this hypothesis.

**Limitations of the study.** The design and methodology of our study do not allow us to analyze CB kinetics at the single-fiber level or in relation to the differences in MHC expression. In muscle strips, series compliance and muscle fiber heterogeneity may affect mechanical properties and CB cycling kinetics. It is important to note that experiments designed to apply the principles of Huxley’s theory were performed on isolated frog sartorius muscles and not on isolated fibers (17). Huxley’s theoretical data (17) were fitted by means of Hill’s data (15) obtained from muscle strips and not from isolated fibers. The equations can, therefore, be applied to multicellular preparations such as diaphragm muscle strips. Importantly, four MHC isoforms have been identified in the hindlimb muscle of frogs (28). Therefore, the model accurately fits the mechanical properties of a muscle whose fiber composition includes different MHCs (17). In heterogeneous muscle, the F-V characteristics are thought to reflect the relative contribution of each fiber type (48). Likewise, according to the Huxley equations (17), CB characteristics are thought to reflect the average value of the myosin molecular motors. Therefore, in our study, the CB characteristics of the rat diaphragm strip probably reflected the mean CB behavior of the different MHCs, mainly MHCneo and MHCslow in day 3; MHCneo, MHCslow, and MHCslow in day 10; MHCslow, MHCslow, and MHCslow in day 17; and MHCslow, MHCslow, MHCslow, and MHCslow in adult rats (47, 49). Moreover, coexpression of MHC isoforms is not entirely restricted to the early postnatal period, and, in adult rat diaphragm muscle, ~14% of all fibers coexpress MHC isoforms (40). Thus, even when studies are performed at the single-fiber level, it does not totally resolve the problem related to the coexpression of multiple isoforms of MHC. In addition, when the specific force of diaphragm muscle fibers is corrected for the estimated MHC content per half sarcomere, specific force of fibers expressing different MHC isoforms is comparable (39). These results indicate that the force per CB is similar across MHC isoforms and that the difference in specific force reflects differences in MHC content per half sarcomere. Our results are in agreement with this assumption because we observed that, during postnatal development, there was no increase in the unitary force per CB but that there was an increase in the total number of CBs, which may reflect the increase in MHC content per half sarcomere.

In conclusion, in isolated rat diaphragm muscle, we have found that postnatal maturation was associated with an improved diaphragm contractility and relaxation. The increase in the number of CBs paralleled the postnatal improvement in diaphragm force generation. There were also important changes in CB kinetics during postnatal maturation, but the average force produced by a single CB and the peak mechanical efficiency remained unchanged. By comparing the rat and the hamster, it appears that there are few species
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