

Effects of Dantrolene on Rat Diaphragm Muscle during Postnatal Maturation

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Background: **Dantrolene is the only known effective treatment for malignant hyperthermia. However, its effects on diaphragm muscle during postnatal maturation remain unknown.**

Methods: **The effects of dantrolene (10^{-8} to 10^{-4} M) were investigated *in vitro* on diaphragm muscle strips in adult rats and in postnatal rats aged 3, 10, and 17 days, and compared with those of ryanodine (10^{-8} to 10^{-6} M). The authors studied contraction and relaxation under isotonic and isometric conditions (29°C, Krebs-Henseleit solution, tetanic stimulation at 50 Hz). Data are mean ± SD.**

Results: **During postnatal maturation, the authors observed a progressive increase in active force developed per cross-sectional area (from 34 ± 25 to 69 ± 32 mN/mm^2; $P < 0.05$) and maximum shortening velocity (from 2.9 ± 0.5 to 4.9 ± 1.4 L_{max}/s; $P < 0.05$). Dantrolene induced a negative inotropic effect in diaphragm muscles in isotonic and isometric conditions in all groups, but this effect was significantly less marked in the 3-day-old rats compared with older rats. Dantrolene did not induce significant lusitropic effects during postnatal maturation. Developmental changes in the pharmacologic response to dantrolene were more rapid than those of ryanodine.**

Conclusion: **Dantrolene induced less pronounced negative inotropic effects on the diaphragm in neonatal rats as compared with adult rats. Our study suggests that developmental changes in the pharmacologic response to dantrolene are more rapid than those of ryanodine.**

IN skeletal and diaphragmatic muscles, postnatal maturation is associated with important ultrastructural changes, including changes in fiber type, distribution, and size[1]; biochemical differentiation, including elimination of embryologic and neonatal myosin isoforms[2,3]; changes in metabolic capacity[4,5]; and progressive development of the sarcoplasmic reticulum (SR).[5] These changes result in an improved diaphragm contractility.[6] However, the precise mechanisms by which maturation induces changes in the contractile performance of diaphragm muscle remain a matter of debate.

Dantrolene, the only known effective treatment for malignant hyperthermia,[6] is a postsynaptic skeletal muscle relaxant that inhibits calcium release during excitation–contraction coupling[7,8] and reduces the myoplasmic free calcium concentration in a dose-dependent manner.[9] The molecular basis of the action of dantrolene remains not completely understood but is generally presumed to involve either direct or indirect inhibitory effects on ryanodine receptor (RyR) Ca^{2+} channels of the SR.[10,11] The effects of dantrolene on muscle during postnatal maturation remain unknown. In adults, dantrolene induces a major negative inotropic effect on skeletal and diaphragmatic muscles.[12] During postnatal maturation, some quantitative and qualitative changes occur in the biochemical composition of the SR, especially changes regarding RyR3,[13] an isoform of RyR that is predominantly expressed in the diaphragm. Therefore, the extent of the inotropic effect of dantrolene on diaphragmatic muscle during postnatal development could be related to the state of maturation of different element in the contractile system, particularly that of RyR.

We therefore conducted an *in vitro* study on the effects of dantrolene on rat diaphragm muscle during postnatal maturation. We tested the hypothesis that maturation may modify the effects of dantrolene on diaphragm muscles.

Materials and 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 of the official edict of the French Ministry of Agriculture. After birth, rat pups were kept in cages with their mother. Adult rats received rat chow and water *ad libitum.* A 12-h light–dark cycle was provided. Experiments were performed on Wistar rats aged 3 days (n = 18), 10 days (n = 18), 17 days (n = 20), and 10–12 weeks (adult, n = 21).

After brief anesthesia with ether, a median laparotomy was performed, and a muscle strip from the ventral costal diaphragm was carefully dissected from the muscle *in situ* while the ribs and the central tendon were left intact, as previously reported.[14,15] With this procedure, diaphragmatic fibers were parallel and of approximately equal length.[15] This diaphragm strip was vertically sus-

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pended in a 200-ml jacketed reservoir with Krebs-Henseleit bicarbonate buffer solution that contained 118 mM sodium chloride, 4.7 mM potassium chloride, 1.2 mM magnesium sulfate, 1.1 mM dipotassium hydrogen phosphate, 25 mM sodium hydrogen carbonate, 2.5 mM calcium chloride, and 4.5 mM 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 per minute). After a 30-min stabilization period, at the initial muscle length at the apex of the length-active isometric tension curve (L_{max}), diaphragm muscle strips recovered their optimal mechanical performance.[14,15] At the end of the study, the cross-sectional area (millimeters squared) was calculated from the ratio of muscle weight to muscle length at L_{max}, assuming a muscle density of 1. Body weight was measured at the moment of killing.

All drugs were purchased from Sigma-Aldrich Chimie (L'Isle d'Abeau, Chesnes, France). Because dantrolene is poorly soluble in aqueous media, we used dimethylsulfoxide as a solvent, as previously reported.[12] Blood therapeutic concentrations of dantrolene range from 0.3 to 6 μg/ml (10^{-6}–10^{-5} M).[7] Therefore, five concentrations (from 10^{-8} to 10^{-4} M) were tested in a cumulative manner, with a 10-min period between each concentration. In a preliminary study (data not shown), we observed that the effects of the highest concentration of dantrolene remained stable between 15 and 60 min and that dimethylsulfoxide alone had no significant effect, as previously reported in hamster diaphragmatic muscle.[12]

Although expression of the RyR genes is modified during development, its pharmacologic consequences remain unknown. Therefore, in separated groups of diaphragmatic muscles, we also assessed the effect of ryanodine during postnatal maturation. Five concentrations of ryanodine (10^{-8}, 3.10^{-8}, 10^{-7}, 3.10^{-7}, and 10^{-6} M) were tested in a cumulative manner, with a 10-min period between each concentration. These concentrations are in the range of concentrations used in an other *in vitro* study, assessing the effect of ryanodine on neonatal and adult rat heart.[16]

Electromagnetic Lever System and Recording

The electromagnetic lever system has been previously described.[12,14,15] 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 L_{max}, 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.[12,14,15]

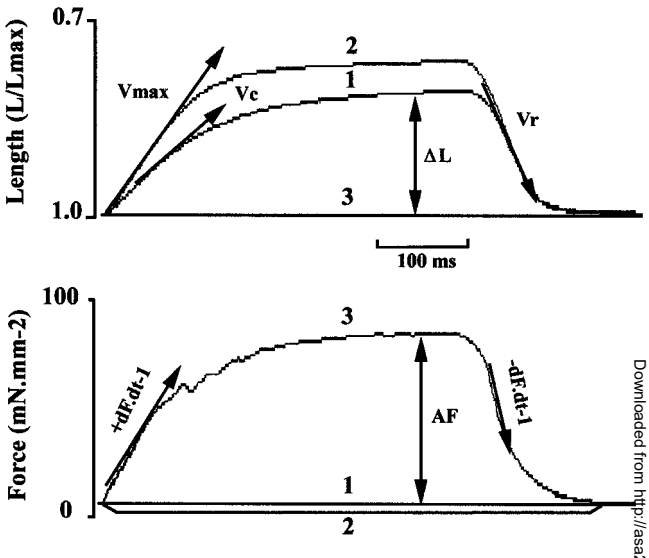

Fig. 1. Mechanical parameters of contraction and relaxation (*Top*) Muscle shortening length (L/L_{max}) plotted *versus* time (*Bottom*) Force (mN/mm^2) plotted *versus* time. Tetanus 1 was isotonic and loaded with preload only to determine the maximum extent of shortening (ΔL), maximum shortening velocity (Vc), and maximum lengthening velocity (Vr). Tetanus 2 was loaded with preload and was abruptly clamped to zero-load immediately after the electrical stimulus to determine the maximum unloaded shortening velocity (V_{max}). Tetanus 3 was fully isometric at L_{max} to determine the maximum active force (AF) and the peak of the positive (+dF/dt) and negative (−dF/dt) force derivatives.

Mechanical Parameters

Mechanical parameters were calculated from three consecutive tetanic contractions preloaded at L_{max}. The first contraction was isotonic and loaded with preload only, and 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.[12,15] The third contraction was fully isometric at L_{max}. The maximum extent of shortening (ΔL), maximum shortening velocity (Vc), and maximum lengthening velocity (Vr) were determined from the first contraction. The maximum unloaded shortening velocity (V_{max}), as calculated by means of the zero-load clamp technique, was determined from the second contraction. The maximum active isometric force (AF), and the peak of the positive (+dF/dt) and negative (−dF/dt) force derivatives were determined from the third contraction (fig. 1).

Maximum unloaded shortening velocity, Vc, AF, ΔL, and +dF/dt tested the contraction phase (inotropy). According to the most accepted theory of contraction,[17] cross bridges act as independent force generators, and thus AF depends on the elementary force produced per cross bridge and the total number of cross bridges formed. Vr and −dF/dt tested the relaxation phase. Nev-

Table 1. Physical Characteristics and Mechanical Parameters of Contraction, Relaxation, and Contraction–Relaxation Coupling in Adult and Postnatal Rats

	3-day-old (n = 18)	10-day-old (n = 18)	17-day-old (n = 20)	Adult (n = 21)
Physical characteristics				
Body weight (g)	7.6 ± 1.1*	17.0 ± 1.6*	28.6 ± 3.9*	306 ± 31
Diaphragm strip				
Weight (mg)	3.9 ± 2.1*	4.8 ± 2.0*	6.4 ± 2.1*	14.0 ± 3.4
Section (mm^2)	0.70 ± 0.3*	0.75 ± 0.2*	0.84 ± 0.2*	1.1 ± 0.4
L_{max} (mm)	5.7 ± 1.4*	6.1 ± 0.7*	7.7 ± 1.1*	11.3 ± 1.3
Contraction				
ΔL (%L_{max})	34 ± 10	29 ± 9	33 ± 9	32 ± 6
V_{max} (L_{max}/s)	2.9 ± 0.5*	3.3 ± 0.7*	3.9 ± 1.1*	4.9 ± 1.4
Vc (L_{max}/s)	1.8 ± 0.6*	2.0 ± 0.6*	2.9 ± 1.0	3.3 ± 1.0
AF (mN/mm^2)	34 ± 25*	43 ± 20*	64 ± 23	69 ± 32
+dF/dt ($mN \cdot mm^{-2} \cdot s^{-1}$)	241 ± 211*	360 ± 170*	530 ± 191*	1,997 ± 376
Relaxation				
Vr (L_{max}/s)	5.6 ± 1.9	6.5 ± 2.0	6.6 ± 1.9	7.0 ± 1.4
−dF/dt ($mN \cdot mm^{-2} \cdot s^{-1}$)	660 ± 497*	1,044 ± 626	1,769 ± 730*	1,320 ± 614
Contraction–relaxation coupling				
Vr/ΔL ($L_{max} \cdot s^{-1} \cdot \%L_{max}^{-1}$)	0.20 ± 0.09	0.24 ± 0.07	0.21 ± 0.08	0.24 ± 0.04
(−dF/dt)/AF^{-1}(s^{-1})	19.2 ± 4.2*	23.7 ± 3.9	26.2 ± 5.3	27.0 ± 4.0

Data are mean ± SD.

* $P < 0.05$ *versus* adult rats.

L_{max} = initial muscle length at the apex of the length-active isometric tension curve; ΔL = maximum extent of shortening; V_{max} = maximum unloaded shortening velocity; Vc = maximum shortening velocity; AF = maximum isometric active force normalized per cross-sectional area; +dF/dt = maximum positive peak force derivative; Vr = maximum lengthening velocity; −dF/dt = maximum negative peak force derivative.

ertheless, because changes in the contraction phase induce coordinated changes in the relaxation phase, relaxation parameters cannot assess lusitropy; therefore, variations in contraction and relaxation must be considered simultaneously to quantify drug-induced changes in lusitropy.[12,14,15] Thus, we calculated the ratios Vr/ΔL and (−dF/dt)/AF, which assessed lusitropy in isotonic and isometric conditions, respectively.

Statistical Analysis

Data are expressed as mean ± SD. Comparisons of control values between groups were performed using analysis of variance. Comparisons of several means were performed using repeated-measure analysis of variance and Newman-Keuls test. All *P* values were two-tailed, and a *P* value < 0.05 was required to reject the null hypothesis. Statistical analysis was performed using NCSS 6.0 software (Statistical Solutions Ltd, Cork, Ireland).

Results

Physical characteristics and mechanical parameters of contraction, relaxation, and contraction–relaxation coupling in adult and postnatal rats in control conditions are shown in table 1. During postnatal maturation, we observed significant increases in body weight, diaphragm strip weight, section, and L_{max} (table 1). We also observed significant increases in mechanical parameters testing inotropy in isotonic (V_{max}, Vc) and isometric (AF, +dF/dt) conditions (table 1). Vr was not significantly modified by postnatal maturation, whereas −dF/dt was significantly lower in 3- and 10-day-old rats compared with adult rats. The ratio Vr/ΔL, which assessed lusitropy in isotonic conditions, was not significantly modified during postnatal maturation. In contrast, the ratio (−dF/dt)/AF, which assessed lusitropy under isometric conditions, was significantly higher in 10- and 17-day-old rats as compared with 3-day-old and adult rats (table 1).

Dantrolene induced a significant and concentration-dependent negative inotropic effect in diaphragm muscles under low (V_{max}) and high (AF) loads in the four groups (fig. 2). However, this negative inotropic effect was significantly less marked in the 3-day-old rats as compared with older rats (fig. 2). Whatever the concentration, dantrolene did not induce any significant contracture. Dantrolene induced no significant changes in the Vr/ΔL ratio (fig. 3). Dantrolene also induced no significant changes in the ratio (−dF/dt)/AF except at the highest concentration (10^{-4} M) in the two groups of oldest rats (17-day-old and adults; fig. 3).

Ryanodine induced a significant and concentration-dependent negative inotropic effect in diaphragm muscles under low (V_{max}) and high (AF) loads in the four groups (fig. 4). This negative inotropic effect was significantly more marked in the adult group under low and high loads as compared with the three other groups and was significantly less marked in the 3-day-old group under low loads (fig. 4).

To compare the developmental changes in the pharmacologic responses to dantrolene and ryanodine, we compared these two drugs at equipotent concentrations

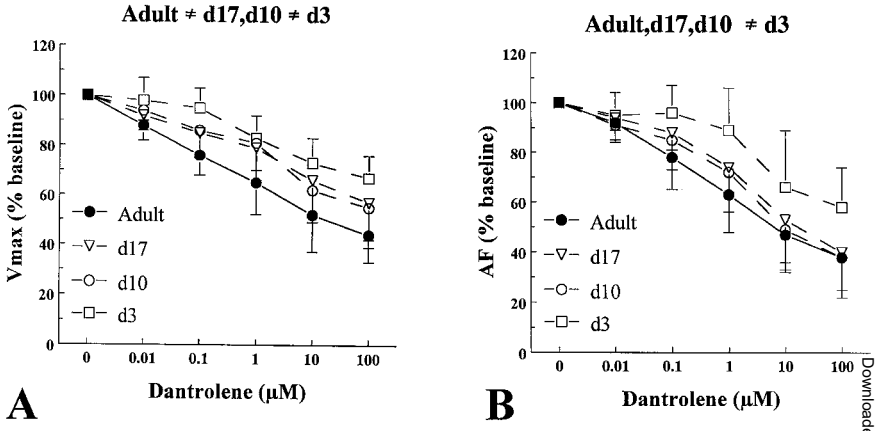

Fig. 2. Inotropic effects of dantrolene in isotonic (*A*) and isometric (*B*) conditions in diaphragm muscle during postnatal maturation. d3 = 3-day-old rats; d10 = 10-day-old rats; d17 = 17-day-old rats. V_{max} = maximum unloaded shortening velocity; AF = isometric active force normalized per cross-sectional area. Data are mean ± SD. Significant differences between groups refer to $P < 0.05$.

(100 μM dantrolene and 0.3 μM ryanodine), *i.e.*, at concentrations inducing comparable negative inotropic effect in the adult rat (AF: 38 ± 13 *vs.* 34 ± 8% of baseline, NS). Accordingly, although the global maturation of diaphragmatic muscle appeared to be a continuous process (table 1), the depressant action of dantrolene appeared to occur very rapidly during course of development, even more rapidly than that of ryanodine (fig. 5).

Discussion

We studied the effects of dantrolene on the intrinsic contractility and relaxation of isolated rat diaphragm muscle during postnatal maturation. The main result of our study is that the negative inotropic effect of dantrolene was significantly less pronounced in the 3-day-old rats as compared with older rats. Moreover, the developmental changes in the pharmacologic response to dantrolene (*i.e.*, a response comparable to that observed in the adult) appeared to occur more rapidly than those observed with ryanodine.

The effect of postnatal maturation on physical characteristics and mechanical parameters of contraction, relaxation, and contraction–relaxation coupling observed in our study (table 1) are similar to those previously described in other studies.[7,18,19] In all groups, dantrolene induced a significant and concentration-dependent negative inotropic effect in diaphragm muscle. The effects observed in adult rats were similar to those previously described in other species and in various skeletal muscles, including diaphragm.[12,20,21]

In diaphragm, as well as in other muscles, postnatal maturation is associated with important ultrastructural changes, including changes in fiber type, distribution, and size[1,18]; biochemical differentiation, including elimination of embryologic and neonatal myosin isoforms[2,3,22–24]; changes in metabolic capacity[2–4,25]; and progressive development of the SR[5] (table 2). These changes result in a progressive improvement in diaphragm contractility, leading to an increase in the tension developed and an enhanced rate of muscle shortening, and a decrease in the duration of contraction and half-relaxation time.[5,6,19] Some investigators have proposed that these changes could be mainly related to the postnatal transitions in myosin heavy chain (MHC) isoform expression.[22] Indeed, the progressive increase in velocity and force seems strongly associated with the progressive decrease in MHC–neonatal isoform expression and the progressive increase in MHC-2X and MHC-2B isoform expression.[22] In contrast, other studies suggest that there is only a low correlation between MHC isoform expression (especially MHC IIB) 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 diaphragmatic increase in veloci

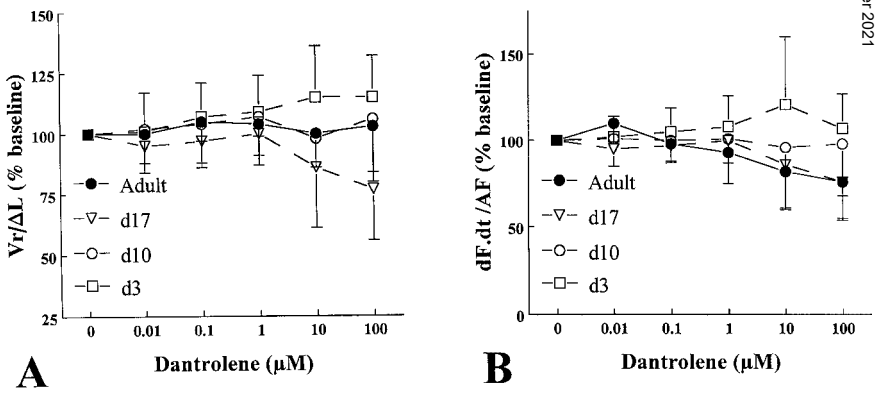

Fig. 3. Lusitropic effects of dantrolene in isotonic (*A*) and isometric (*B*) conditions in diaphragm muscle during postnatal maturation. d3 = 3-day-old rats; d10 = 10-day-old rats; d17 = 17-day-old rats; Vr/ΔL = ratio of maximum lengthening velocity to maximum shortening extent; (−dF/dt)/AF = ratio of maximum negative force derivative to maximum active force. At 10^{-4} M, (−dF/dt)/AF was significantly different from baseline values only in 17-day-old and adult rats ($P < 0.05$). There was otherwise no significant difference between groups. Data are mean ± SD.

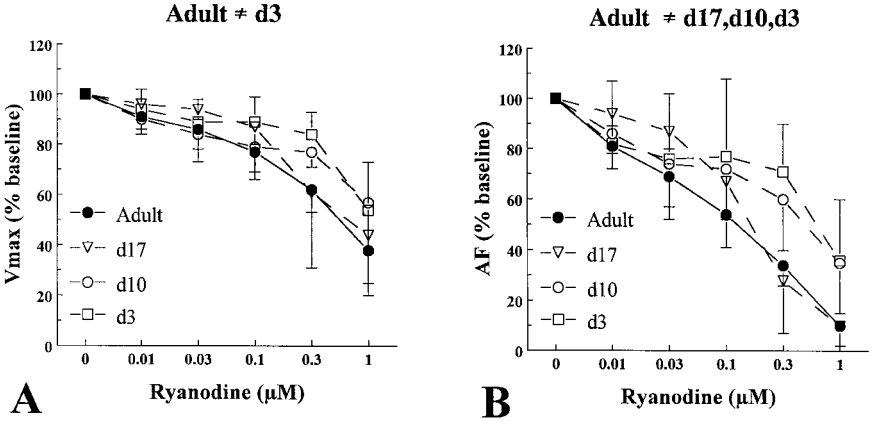

Fig. 4. Inotropic effects of ryanodine in isotonic (*A*) and isometric (*B*) conditions in diaphragm muscle during postnatal maturation. d3 = 3-day-old rats; d10 = 10-day-old rats; d17 = 17-day-old rats; V_{max} = maximum unloaded shortening velocity; AF = isometric active force normalized per cross-sectional area. Data are mean ± SD. Significant differences between groups refer to $P < 0.05$.

ty.[26] Other investigators have proposed that the increase in velocity and force could be related to an increase in total number of cross bridges and in peak total rate of energy release.[19]

The negative inotropic effect of dantrolene was significantly less marked in 3-day-old rats as compared with older rats. The effect of dantrolene is thought to be related to an inhibition of calcium release from the SR by either direct or indirect interaction with the RyR.[8,10,11] In adult skeletal and diaphragmatic muscles, the type 1 isoform of RyR is essential in triggering contraction. Expression of RyR1 requires approximately 3–4 weeks to reach the high levels that are maintained throughout adult life.[27] Another isoform, RyR3, is predominantly expressed during fetal and neonatal development and has been shown to play a physiologic role in excitation–contraction coupling of neonatal skeletal muscles.[13,28] RyR3 is already expressed during fetal development, but its expression is maximum during the neonatal phase (2–15 days) in the rat.[13] Moreover, RyR3 is more expressed in the diaphragm than in other skeletal muscles.[13] Therefore, our results may suggest that RyR3, which is predominantly expressed in the neonatal phase, is less susceptible to the action of dantrolene than RyR1. Because dantrolene may also act on multiple other sites such as triadin and FKBP12 proteins,[8] we cannot rule out the hypothesis that maturation of these proteins and changes in their binding to RyR could also be involved in the decreased susceptibility of neonatal rats to dantrolene. We therefore analyzed the pharmacologic response to ryanodine during diaphragmatic muscle maturation, because ryanodine is a highly specific inhibitor of RyR.[29] We observed that developmental changes of the response to dantrolene were more rapid than those of ryanodine (fig. 5). This is an indirect argument that suggests that dantrolene does not act only directly on the RyR. Further studies are required to elucidate this point.

Dantrolene did not modify isotonic relaxation (fig. 3), suggesting that it did not modify the calcium reuptake by the SR. This result agrees with those previously reported in hamster diaphragm muscle.[12] Dantrolene did not significantly modify isometric relaxation, except at the highest concentration (fig. 3). This result agrees with previous results in skeletal muscles.[12,30] Therefore, our results suggest that dantrolene up to 10^{-5} M, did not alter myofilament calcium sensitivity. At 10^{-4} M, dantrolene induced a significant decrease in the ratio $(-dF/dt)/AF$, indicating a negative lusitropic effect under high load and suggesting an increase in myofilament calcium sensitivity. It should be pointed out that this effect was not observed in 3-day-old rats (fig. 3B). In any case, our results indicate that dantrolene induced very weak lusitropic effects that are not markedly modified during development.

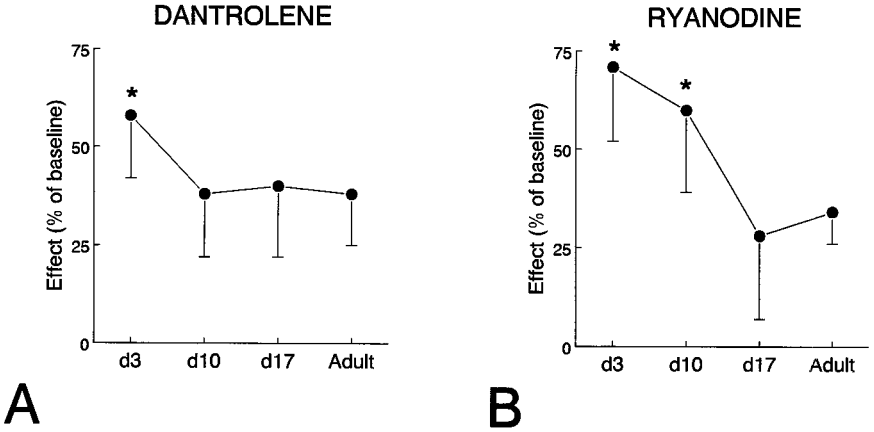

Fig. 5. Comparison of the response to dantrolene (100 μM; *A*) and ryanodine (0.3 μM; *B*) expressed as percent of baseline active force (AF) during postnatal maturation. d3 = 3-day-old rats; d10 = 10-day-old rats; d17 = 17-day-old rats. These concentrations induced comparable negative inotropic effect in the adult rat. Developmental changes in the pharmacologic response to dantrolene were more rapid than those of ryanodine. Data are mean ± SD. *$P < 0.05$ *versus* adult.

Table 2. Developmental Changes in Rat Diaphragm Muscle Myosin Heavy Chain (MHC) Isoforms Composition, Fiber Type Proportion (Expressed As a Relative Percent of the Total Complement Observed at a Given Age), and Various Enzyme Activities*

Variables	Day 0	Days 3–4†	Week 1	Week 2†	Week 3†	Week 4	Week 6	Adult†
MHC isoforms (%)								
Embryonic	68	55	51	26	8	0	0	0
Slow	14	16	13	25	22	29	26	27
2A	18	29	36	38	40	34	29	31
2X	0	0	0	11	23	33	38	31
2B	0	0	0	0	7	4	7	11
Fiber type (%)								
I			8	23	30	36	34	33
IIa			68	50	42	42	36	30
IIb			18	21	26	22	30	37
IIc			6	6	2	0	0	0
Enzyme activities								
SDH (OD 10^3)	86							36
AChE (nmol · mg^{-1} · h^{-1})	0.89		0.88		1.00	0.99		0.86
BuChE (nmol · mg^{-1} · h^{-1})	1.56		1.54		0.75	0.43		0.21
AChE/BuChE	1.76		1.75		0.75	0.43		0.31
LDH (μmol · mg^{-1} · h^{-1})	45		48		45	42	43	75
AK (μmol · mg^{-1} · h^{-1})	25		60		65	61	90	77
β-OAC (μmol · mg^{-1} · h^{-1})	4		14		16	13	17	12
PKK (μmol · mg^{-1} · h^{-1})	1		2.5		2.1	2	3	5
CK (μmol · mg^{-1} · h^{-1})	60		180		190	220	290	330

Data represent average values obtained by Brozanski *et al.*,[24] Johnson *et al.*,[22] Zhan *et al.*,[23] Fratacci *et al.*,[18] Brzin *et al.*,[25] and Kelly *et al.*[3]

* Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), total succinate-dehydrogenase activity (SDH) reflecting fiber oxidative capacity, β-hydroxyacyl-CoA dehydrogenase (β-OAC) reflecting fatty acid oxydation, malate dehydrogenase (MDH), reflecting the citric acid cycle, 1-phosphofructokinase (PFK) and lactate dehydrogenase (LDH), reflecting glycolysis capacity, and creatine kinase (CK) and adenylokinase (AK), reflecting high-energy phosphate metabolism. † A stage of development assessed in the current study.

OD = optical density.

The effects of dantrolene on diaphragm contraction may have potential clinical consequences. Dantrolene decreases tidal volume,[31] while minute ventilation is maintained by increasing respiratory rate.[32] Dantrolene-induced muscle weakness can contribute to prolonged postoperative tracheal intubation.[33,34] Our study demonstrates that dantrolene did not markedly alter diaphragmatic relaxation, whereas it induced significant negative inotropic effect in all age groups. The highly compliant infant rib cage places a greater functional demand on the diaphragm during ventilation than does the structurally stable rib cage in older children and adults, and a greater diaphragmatic contraction is needed to maintain a comparable tidal volume in an infant.[35] Thus, dantrolene could more markedly contribute to respiratory distress in infants as compared with adults. However, this effect might be counterbalanced by the fact that neonatal diaphragm is less susceptible to dantrolene than adult diaphragm (fig. 2). Further *in vivo* studies are mandatory to assess the ventilatory consequences of dantrolene during postnatal maturation.

The following points must be considered in the assessment of the relevance of our results. First, this study was conducted at 29°C. Nevertheless, the stability of the preparation is not sufficient at 37°C. Only very low temperature (18–20°C) can markedly modify the negative inotropic effect of dantrolene.[31] Second, our study did not enable us to detect a small increase in resting tension that has been recently reported at low concentrations of dantrolene.[36] Nevertheless, this phenomenon is thought to involve high-affinity binding sites and thus to occur at very low concentrations (10^{-9} M) of dantrolene, and is associated with a positive inotropic effect that was not observed in our study, even at the lowest concentration (10^{-8} M). Lastly, maturation in the rat may differ from that in humans. The literature has provided few data to enable reliable extrapolation between rat and human diaphragm maturation. A 3-day-old rat has a ratio of body weight to adult body weight of approximately 2–3%, a 10-day-old rat a ratio of 5%, and a 17-day-old rat has a ratio of 8%. Considering the body weight growth in humans, 3- and 10-day-old rats appear equivalent to premature infants, and a 17-day-old rat appears equivalent to a newborn infant. Considering lung function, there is a close match between rat and human.[3] Considering MHC isoform maturation, a 17-day-old rat appears equivalent to a human newborn.[22–24,38] Considering fiber type proportion, a 3-day-old rat appears equivalent to a premature infant, and a 17-day-old rat appears equivalent to a human newborn.[39]

In conclusion, in neonatal rats, dantrolene induced less pronounced negative inotropic effects on the diaphragm as compared with older rats, and did not induce significant lusitropic effects. Developmental changes in the pharmacologic response to dantrolene were more rapid than those of ryanodine. This is an indirect argument suggesting that dantrolene does not act only directly on the RyR.

References

1. Poggi PC, Marchetti R, Scelsi A, Calligaro A, Casasco A: An enzyme-histochemical, ultrastructural and morphometric study on fetal and neonatal rat diaphragms. J Submicrosc Cytol Pathol 1990; 22:515–21
2. LaFramboise WA, Daood MJ, Guthrie RD, Butler-Browne GS, Whalen RG, Ontell M: Myosin isoforms in neonatal rat extensor digitorum longis, diaphragm, and soleus muscles. Am J Physiol 1990; 259:L116–22
3. Kelly AM, Rosser BWC, Hofman R, Panettieri RA, Schiaffino S, Rubinstein NA, Nemeth PM: Metabolic and contractile protein expression in developing rat diaphragm muscle. J Neurosci 1991; 11:1231–42
4. Smith D, Green H, Thomson J, Sharatt M: Oxydative potential in developing rat diaphragm, EDL, and soleus muscle fibers. Am J Physiol 1988; 254:C661–8
5. Maxwell LC, McCarter RJM, Kuehl TJ, Robotham JL: Development of histochemical and functional properties of baboon respiratory muscles. J Appl Physiol 1983; 54:551–61
6. Moore BJ, Feldman HA, Reid MB: Developmental changes in diaphragm contractile properties. J Appl Physiol 1993; 75:522–6
7. Ward A, Chaffman MO, Sorkin EM: Dantrolene: A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in malignant hyperthermia, the neuroleptic malignant syndrome and an update of its use in muscle spasticity. Drugs 1986; 32:130–68
8. Pessah N, Lynch C III, Gronert GA: Complex pharmacology of malignant hyperthermia (editorial). ANESTHESIOLOGY 1996; 84:1275–9
9. Lopez JR, Gerardi A, Lopez MJ, Allen PD: Effects of dantrolene on myoplasmic free [Ca2+] measured in vivo in patients susceptible to malignant hyperthermia. ANESTHESIOLOGY 1992; 76:711–9
10. Parness J, Palnitkar SS: Identification of dantrolene binding sites in porcine skeletal muscle sarcoplasmic reticulum. J Biol Chem 1995; 270:18465–72
11. Fruen BR, Mickelson JR, Louis CF: Dantrolene inhibition of sarcoplasmic reticulum Ca2+ release by direct and specific action at skeletal muscle ryanodine receptors. J Biol Chem 1997; 272:26965–71
12. Langeron O, Coirault C, Fratéa S, Orliaguet G, Coriat P, Riou B: Effects of dantrolene on the diaphragm muscle of the normal and myopathic hamster. Br J Anaesth 1998; 81:553–5
13. Tarroni P, Rossi D, Conti A, Sorrentino V: Expression of the ryanodine receptor type 3 calcium release channel during development and differentiation of mammalian skeletal muscle cells. J Biol Chem 1997; 272:19808–13
14. Coirault C, Chemla D, Pery-Man N, Suard I, Salmeron S, Lecarpentier Y: Isometric relaxation of isolated diaphragm muscle: Influence of load, length, time and stimulation. J Appl Physiol 1994; 76:1468–75
15. Coirault C, Chemla D, Pery N, Suard I, Lecarpentier Y: Mechanical determinants of isotonic relaxation in isolated diaphragm muscle. J Appl Physiol 1993; 75:2265–72
16. Tanaka H, Shigenobu K: Effect of ryanodine on neonatal and adult rat heart: developmental increase in sarcoplasmic reticulum function. J Moll Cell Cardiol 1989; 21:1305–13
17. Huxley AF: Muscle structure and theories of contraction. Prog Biophys Chem 1957; 7:255–318
18. Fratacci MD, Levame M, Rauss A, Atlan G: Rat diaphragm during postnatal development. II. Resistance to low- and high-frequency fatigue. Reprod Fertil Dev 1996; 8:399–407
19. Coirault C, Lambert F, Joseph T, Blanc FX, Chemla D, Lecarpentier Y: Developmental changes in crossbrisges properties and myosin isoforms in hamster diaphragm. Am J Respir Crit Care Med 1997; 156:959–67
20. Meyler WJ, Wesseling H, Agoston S: The effects of dantrolene sodium on cardiac and skeletal muscle in rats. Eur J Pharmacol 1976; 39:127–31
21. Oba T, Hotta K: Similar inhibitory effects of dantrolene sodium on twitch tension and on silver ion-induced contracture in skeletal muscle. Eur J Pharmacol 1987; 143:275–8
22. Johnson BD, Wilson LE, Zhan WZ, Watchko JF, Daood MJ, Sieck GC: Contractile properties of the developing diaphragm correlate with myosin heavy chain phenotype. J Appl Physiol 1994; 77:481–7
23. Zhan WZ, Watchko JF, Prakash YS, Sieck G: Isotonic contractile and fatigue properties of developing rat diaphragm. J Appl Physiol 1998; 84:1260–8
24. Brozanski BS, Daood MJ, Watchko JF, LaFramboise WA, Guthrie RD: Postnatal expression of myosin isoforms in the genioglossus and diaphragm muscles. Pediatr Pulmonol 1993; 15:212–9
25. Brzin M, Sketelj J, Tennyson V, Kiauta T, Budininkas-Schoenebeck M: Activity, molecular forms, and cytochemistry of cholinesterases in developing rat diaphragm. Muscle Nerve 1981; 4:505–13
26. Powers SK, Farkas GA, Demirel H, Coombes J, Fletcher L, Hughes MG, Hodge K, Dodd SL, Schlenker EH: Effects of aging and obesity on respiratory muscle phenotype in Zucker rats. J Appl Physiol 1996; 81:1347–54
27. Kyselovic J, Leddy JJ, Ray A, Wigle J, Tuana BS: Temporal differences in the induction of dihydropyridine receptor subunits and ryanodine receptors during skeletal muscle development. J Biol Chem 1994; 269:21770–7
28. Bertocchini F, Ovitt CE, Conti A, Barone V, Schöler HR, Bottinelli R, Reggiani C, Sorrentino V: Requirement for the ryanodine receptor type 3 for efficient contraction in the neonatal skeletal muscles. EMBO J 1997; 16:6956–63
29. Sutko JL, Airey JA, Welch W, Ruest L: The pharmacology of ryanodine and related compounds. Pharmacol Rev 1997; 49:53–98
30. Ohta T, Ito S, Ogha A: Inhibitory action of dantrolene on Ca-induced Ca2+ release from sarcoplasmic reticulum in guinea pig skeletal muscle. Eur J Pharmacol 1990; 178:11–9
31. Oliven A, Chandler Deal E, Kelsen SG: Effect of dantrolene on ventilation and respiratory muscle activity in anaesthetized dogs. Br J Anaesth 1990; 64:207–13
32. Farquhar R, Leslie GC, Part NJ: How is ventilation maintained in the presence of the muscle relaxant, dantrolene sodium? A study in the anaesthetized rat. Br J Pharmacol 1986; 88:79–86
33. Watson CB, Reierson N, Norfleet EA: Clinically significant muscle weakness induced by oral dantrolene sodium prophylaxis for malignant hyperthermia. ANESTHESIOLOGY 1986; 65:312–4
34. Hara Y, Kato A, Horikawa H, Kato Y, Ichiyanagi K: [Postoperative respiratory depression thought to be due to oral dantrolene pretreatment in a malignant hyperthermia-susceptible patient]. Masui 1988; 37:483–7
35. Bryan AC, Mansell AL, Levison H: Development of the mechanical properties of the respiratory system, Development of the Lung. Edited by Hodson WA. New York, Marcel Dekker, 1977, pp 66–78
36. Nelson T, Lin M, Zapata-Sudo G, Sudo RT: Dantrolene sodium can increase or attenuate activity of skeletal muscle ryanodine receptor calcium release channel: Clinical implication. ANESTHESIOLOGY 1996; 84:1368–79
37. Zeltner TB, Caduff JH, Gehr P, Pfenninger J, Burri PH: The postnatal development and growth of the human lung. I. Morphometry. Respir Physiol 1987; 67:247–57
38. Lloyd JS, Brozanski BS, Daood M, Watchko JF: Developmental transitions in the myosin heavy chain phenotype of human respiratory muscle. Biol Neonate 1996; 69:67–75
39. Keens TG, Bryan AC, Levinson H, Ianuzzo CD: Developmental pattern of muscle fiber types in human ventilatory muscles. J Appl Physiol 1978; 44:909–13