Effects of dantrolene on the diaphragm muscle of the normal and myopathic hamster

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Summary
Dantrolene is the only known effective treatment for malignant hyperthermia. However, its effects on the myopathic diaphragm remain unknown. The effects of dantrolene 10⁻⁸ to 10⁻⁴ mol litre⁻¹ on diaphragm muscle strips in normal (n=12) and myopathic hamsters (n=13) were investigated in vitro in response to tetanic stimulation. We studied contraction under isotonic and isometric conditions. Data are presented as mean (SD) per cent of baseline. Dantrolene induced a negative inotropic effect in normal and myopathic hamsters but no significant difference was observed between groups (active force at 10⁻⁴ mol litre⁻¹: 34 (7) vs 32 (11)%; ns). We conclude that dantrolene induced a comparable negative inotropic effect on diaphragm muscle in normal and myopathic hamsters. (Br. J. Anaesth. 1998; 81: 553–555).

Keywords: pharmacology, dantrolene; malignant hyperthermia; muscle, diaphragm; hamster

Dantrolene is a postsynaptic skeletal muscle relaxant that inhibits calcium release from the sarcoplasmic reticulum (SR), by either direct or indirect interaction with skeletal muscle Ca⁺⁺ release channel of the SR (i.e. ryanodine receptor).¹ Thus dantrolene is the only known effective treatment for malignant hyperthermia (MH).² However, dantrolene induces a negative inotropic effect on normal skeletal and diaphragm muscles, but the effects of dantrolene on myopathic muscles remain unknown. Genetically induced myopathy in the Syrian hamster offers a unique opportunity to compare the pharmacological effects of drugs in normal and myopathic muscles. Indeed, the myopathic Syrian hamster is used widely as an animal model as it develops both autosomal recessive cardiomyopathy and polymyopathy that have been well characterized.³,⁴ Therefore, we conducted an in vitro study on the effects of dantrolene on diaphragm muscle in normal and myopathic hamsters.

Materials and methods
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. Experiments were conducted using 13, 6-month-old myopathic Syrian hamsters (Strain Bio 14.6, Bio Breeders Inc., Fitchburg, MA, USA) and 12 normal Syrian hamsters (Strain F1B, Bio Breeders Inc.). Myopathic hamsters of both sexes develop generalized myopathy from the age of 6 weeks.¹⁴ After brief anaesthesia with ether, a muscle strip from the ventral costal diaphragm was dissected carefully from the muscle in situ.¹ This diaphragm strip was immediately suspended vertically in a 200-ml jacketed reservoir, maintained at 29°C with a thermostatic water circulator, with Krebs–Henseleit bicarbonate buffer solution (CaCl₂, 2.5 m mol litre⁻¹, pH 7.40). The bathing solution was bubbled with 95% oxygen–5% carbon dioxide. Preparations were field-stimulated by means of two platinum electrodes. Experiments were conducted after a 30-min stabilization period, at the initial muscle length at the apex of the length–active isometric tension curve (İmax), in tetanus mode at 50 Hz (10 trains min⁻¹ of 300 ms duration, 1 ms rectangular pulses). Cross-sectional area was calculated from the ratio of muscle weight to muscle length, assuming a muscle density of 1. Because dantrolene is poorly soluble in aqueous media, we used dimethylsulphoxide (DMSO) as a solvent. Therapeutic concentrations of dantrolene range from 0.3 to 10 mg ml⁻¹ (1–30 × 10⁻⁴ mol litre⁻¹).³ Therefore, five concentrations of dantrolene were tested in a cumulative manner: 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵ and 10⁻⁴ mol litre⁻¹, with a 15-min period between each concentration. In a preliminary study, we observed that the effects of the highest concentration of dantrolene remained stable between 15 and 60 min, and that DMSO alone had no significant effect. All drugs were purchased from Sigma-Aldrich Chimie (L’Isle d’Abeau Chesnes, France).

MECHANICAL PARAMETERS

The electromagnetic lever system has been described previously.⁴ Briefly, the load applied to the muscle was determined using a Servo mechanism-controlled current through the coil of an electromagnet. Muscular shortening induced displacement of the lever, which modulated the light intensity of a photo-
Parameters characterizing contraction were shortening velocity (vc), extent of shortening (Δl), peak of the positive force derivative normalized per cross-sectional area (+dFdr⁻¹) and active force normalized per cross-sectional area (AF). Mechanical variables were calculated from three consecutive tetanic contractions preloaded at lmax with increasing afterload from zero load to fully isometric contraction. The first contraction was clamped abruptly to zero load just after the electrical stimulus, enabling determination of the maximum unloaded shortening velocity (vcmax). The second contraction was isometric and loaded with preload only. The maximum extent of shortening (Δlmax) was determined from this contraction. The last contraction was fully isometric at lmax. The maximum active force (AFmax) and the peak of the positive (+dFdr⁻¹max) force derivative were determined from this fully isometric contraction.

STATISTICAL ANALYSIS

Data are expressed as mean (sd). Comparisons of control values between groups were performed using the Student’s t test. Comparison of several means were performed using repeated-measure analysis of variance and Newman–Keuls test. All P values were two-tailed, and P<0.05 was considered significant. Statistical analysis was performed using NCSS 6.0 software (Statistical Solutions Ltd, Cork, Ireland).

Results

Body weight was lower in myopathic hamsters than in controls (100 (4) vs 144 (5) g, P<0.05). There was no significant difference in lmax between groups (9.6 (1.7) vs 9.6 (2.1) mm; ns). Under control conditions, mechanical variables testing inotropy were significantly impaired in myopathic hamsters compared with normal hamsters: Δlmax: 20 (5) vs 34 (7) %lmax (P<0.05); vcmax: 1.4 (0.5) vs 4.5 (0.5) lmax s⁻¹ (P<0.05); AFmax: 66 (27) vs 101 (37) mN mm⁻² (P<0.05); +dFdr⁻¹max: 745 (350) vs 1314 (525) mN mm⁻² s⁻¹ (P<0.05).

In normal and myopathic hamsters, dantrolene induced a significant and concentration-dependent negative inotropic effect in diaphragmatic muscle under low (vcmax) and high (AFmax) loads. At a concentration of 10⁻⁴ mol litre⁻¹, dantrolene induced a maximum decrease in AFmax and vcmax in normal hamsters (34 (7) % of baseline (P<0.01) and 47 (7) % of baseline (P<0.05)) and in myopathic hamsters (32 (11) % of baseline (P<0.01) and 46 (10) % of baseline (P<0.05)). There were no significant differences in the inotropic effects of dantrolene between normal and myopathic hamsters (fig. 1). As baseline values of AF were markedly reduced in myopathic compared with normal hamsters, the absolute values of AF after dantrolene administration in myopathic hamsters were very low (fig. 1).

Discussion

We have studied the effects of dantrolene on the intrinsic contractility of isolated diaphragm muscle in normal and myopathic hamsters. The negative inotropic effect of dantrolene was comparable in normal and myopathic hamsters. Dantrolene induced a significant and concentration-dependent negative inotropic effect in the normal hamster with a plateau between 10⁻³ and 10⁻¹ mol litre⁻¹ (fig. 1). Moreover, the maximum decrease in AFmax was similar to the maximum decrease in adductor pollicis muscle force after administration of dantrolene in humans. These results suggest that our experimental model was clinically relevant. There were no significant differences in the negative inotropic effects of dantrolene between normal and myopathic hamsters (fig. 1). The negative inotropic effect of dantrolene on skeletal muscle is thought to be related to its effect on calcium release from the SR. Our results suggest that the effects of dantrolene on the ryanodine receptor complex are similar in normal and myopathic hamsters. Nevertheless, as contraction was markedly impaired in myopathic hamsters, it should be noted that the absolute values of AFmax after dantrolene in myopathic hamsters were very low. Therefore, whereas the pharmacological effects of dantrolene are not modified in myopathic muscle, its consequences on an
already impaired ventilatory function may be greater (fig. 1). Dantrolene may also affect breathing pattern in decreasing tidal volume while minute ventilation is maintained by increasing ventilatory frequency.\(^6\)

The following points must be considered in the assessment of the clinical relevance of our results. First, this study was conducted at 29°C. However, it should be pointed out that the inotropic effect of dantrolene observed in our study was comparable with that observed \textit{in vivo} in humans at 37°C.\(^5\)

Second, the cardiomyopathic Syrian hamster provides a useful and reproducible model of muscle disease, with early development of focal myolytic and necrosis lesions in skeletal and diaphragmatic muscles,\(^3\) that mimic those observed in human muscle dystrophies.\(^1\) Thus this animal model seems to be relevant for the assessment of the effects of dantrolene on myopathic diaphragm function.

In summary, in this study conducted on isolated diaphragm muscle in response to tetanic stimulation, dantrolene induced a comparable negative inotropic effect in normal and myopathic muscles and may be responsible for a diaphragmatic dysfunction.

References