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ORIGINAL ARTICLE

Calcium channel blockers are inadequate for malignant hyperthermia crisis

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Abstract

Purpose Malignant hyperthermia (MH) results from disordered calcium (Ca^{2+}) homeostasis in skeletal muscle during general anesthesia. Although Ca^{2+} channel blockers may be given to treat the tachycardia and circulatory instability, coadministration of Ca^{2+} channel blockers and dantrolene is contraindicated during MH crisis. We evaluated the effect of Ca^{2+} channel blockers on Ca^{2+} homeostasis and their interactions with dantrolene in human skeletal muscle.

Methods Human skeletal muscle samples were obtained by biopsy and divided into two groups according to the results of the Ca^{2+}-induced Ca^{2+} release rate test. Differentiated myotubes were labeled with Fura-2, and changes in the 340/380-nm ratio were used to calculate changes in Ca^{2+} concentration following nifedipine treatment in the absence or presence of dantrolene.

Results Nifedipine induced a transient increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in a dose-dependent manner. The half-maximal concentration (EC_{50}) for nifedipine was 0.718 ± 0.329 μM in the accelerated group and 1.389 ± 0.482 μM in the nonaccelerated group ($P = 0.009$). The addition of 50 μM dantrolene attenuated by 15.4% the increase in $[Ca^{2+}]_i$ caused by the 0.5 μM nifedipine.

Conclusion Ca^{2+} channel blockers led to increased $[Ca^{2+}]_i$ in human skeletal muscle cells. The increase is thus scarcely affected by dantrolene treatment. Data provide a greater physiologic basis for avoiding the use of Ca^{2+} channel blockers during MH crisis.

Keywords Malignant hyperthermia · Calcium channel blocker · Dantrolene

Introduction

Malignant hyperthermia (MH) is a potentially fatal complication caused by depolarizing muscle relaxants and/or volatile agents during general anesthesia. A disorder of calcium (Ca^{2+}) regulation by the sarcoplasmic reticulum (SR) of skeletal muscle is thought to be responsible [1–4]. Ca^{2+} channel blockers are often used during anesthesia and may be given to control the tachycardia and circulatory instability present before MH is recognized. However, Ca^{2+} channel blockers should be avoided during MH crisis and are contraindicated in the presence of dantrolene, the specific remedy for MH. Shortly after introduction of dantrolene, cardiac collapse was seen after a combination of verapamil and dantrolene was given to swine and dogs [5, 6]. Whereas dantrolene alone produced a mild increase in arterial pressure, in verapamil-pretreated animals, it profoundly depressed cardiac function and markedly increased serum potassium (K^+). Hence, the combination of Ca^{2+} channel blockers and dantrolene is contraindicated. However, an amlodipine–dantrolene combination did not increase serum K^+ or cause severe cardiac collapse in pigs [7]. Verapamil, classified as belong to the

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phenylalkylamine group, induced hyperkalemia by reducing cardiac output and organ perfusion, further aggravating depression of contractility. Amlodipine, a member of the dihydropyridine group, has only minor effects. Use of Ca^{2+} channel blockers to treat arrhythmia in MH crisis should be avoided [4]; the European Malignant Hyperthermia Group (EMHG) guideline recommends administration of amiodarone or β-blockers [8]. It is unclear what effect Ca^{2+} channel blockers have on skeletal muscle cells, although their depressive effect on cardiac muscle is well documented [5–7]. Here, we evaluated the effects of Ca^{2+} channel blockers and their interactions with dantrolene on Ca^{2+} homeostasis in human skeletal muscle.

Methods

Preparation of myotubes

This study was reviewed and approved by the review board of our institutional ethical committee. Written informed consent was obtained from patients and their families.

Human skeletal muscle samples were obtained by quadriceps or biceps brachii muscle biopsy. Thirteen patients susceptible for the development of MH were identified (Table 1) and classified into an accelerated group ($n = 7$) and a nonaccelerated group ($n = 6$) based on the Ca^{2+}-induced Ca^{2+} release (CICR) rate test according to the Endo protocol [10, 11]. In brief, chemically stripped muscle fibers were made from biopsied muscle tissue using saponin, and fibers were treated with varying concentrations of Ca^{2+} (0, 0.3, 1.0, 3.0, and 10.0 μM). The tension of each sample was measured using a force transducer, and CICR rates were calculated. Mean normal CICR values were determined using samples from 12 individuals with negative in vitro contracture tests (IVCT; EMHG protocol) and caffeine-halothane contracture tests (CHCT; North American MH Group protocol) [12]. CICR values 2 standard deviations (SD) above the mean of normal individuals were defined as being accelerated. Susceptibility to MH is traditionally diagnosed by the CICR rate test [13–15]; IVCT was not clinically used in Japan. An accelerated CICR rate indicates predisposition for the development of MH, and the accelerated group comprised two individuals who developed rank 5–6 MH crises according to the Clinical Grading Scale (CGS) [9].

Table 1 Patient characteristics and results from the calcium-induced calcium release rate (CICR) rate test

No.	Age (years)	Gender	MH history	Result
1	64	F	MH family	Accelerated
2	45	M	MH family	Accelerated
3	18	M	MH (CGS 38 rank 5)	Accelerated
4	1	F	MH (CGS 20 rank 4)	Accelerated
5	7	F	No	Accelerated
6	89	M	MH family	Accelerated
7	35	F	MH (CGS 50 rank 6)	Accelerated
8	4	M	No	Nonaccelerated
9	28	F	No	Nonaccelerated
10	37	F	MH family	Nonaccelerated
11	15	M	No	Nonaccelerated
12	53	M	No	Nonaccelerated
13	75	M	No	Nonaccelerated

Accelerated CICR rate test denotes predisposition to MH. The brother of patient 1 died with a high fever during general anesthesia. The brother of patient 2 had an episode of MH (CGS 58, rank 6) in halothane anesthesia with succinylcholine. Patient 5 did not have an MH episode but had congenital scoliosis with torticollis. The daughter of patient 6 died with a high fever after general anesthesia

MH malignant hyperthermia, *CGS* Clinical Grading Scale [9]

Cell culture

Biopsied skeletal muscle cells were maintained in Dulbecco's modified Eagle medium (DMEM/F12; Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated bovine calf serum (FBS; Sigma-Aldrich, St. Louis, MO, USA) containing 1% ampicillin, kanamycin sulfate (Sigma-Aldrich), and amphotericin B (Invitrogen) in 25 cm^2 cell-culture flasks (Corning, New York, NY, USA) and a 5% carbon dioxide (CO_2) atmosphere at 37°C. The medium was changed every 3 days. After 2–3 weeks in culture, the cells were plated on 35-mm glass-bottom culture dishes with 10-mm microwells (MatTek, Ashland, MA, USA) and allowed to grow for 10–14 days [16] in DMEM/F12 with 2% FBS until the myoblasts fused to form myotubes. We used myotubes formed by fusion of myoblasts with a fiber-like shape and multiple nuclei.

Ca^{2+} imaging of myotubes

Myotubes were washed in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered salt solution (HBSS) containing 130.0 mM sodium chloride (NaCl), 5.4 mM potassium chloride (KCl), 20.0 mM HEPES, 2.5 mM calcium chloride ($CaCl_2$), 1 mM magnesium chloride ($MgCl_2$), and 5.5 mM glucose at pH 7.4. The myotubes were loaded with 5.0 μM Fura-2 (Dojindo, Tokyo, Japan) in HBSS for 1 h at 37°C and subsequently washed with HBSS to remove excess extracellular Fura-2. Microwell plates containing Fura-2-loaded myotubes were mounted on a fluorescence microscope (Nikon, Tokyo, Japan) and alternately stimulated at 340 and 380 nm. Fluorescence emissions at 510 nm were acquired every 619 ms for 40 min using a cooled high-speed digital video camera (ORCA-AG; Hamamatsu Photonics, Hamamatsu, Japan). Regions of

interest (ROIs) were determined within the cytoplasm of cells. Paired 340/380 fluorescence ratios for each ROI were calculated every 5 s to measure intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) using a calibration curve (Fura-2 Calcium Imaging Calibration Kit; Invitrogen, Carlsbad, CA, USA).

Nifedipine

Only myotubes that demonstrated increased $[Ca^{2+}]_i$ in response to 10.0 mM caffeine (Wako, Osaka, Japan) were used for experiments, and alpha-actinin in immunofluorescence staining was used to confirm the cellular composition of caffeine-reacting cells. A 50-mM nifedipine stock solution in dimethylsulfoxide (DMSO) was diluted with HBSS to the following working concentrations: 0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 15.0, and 50.0 μM. These solutions were perfused into the microwells at a rate of 1.2 ml/min at 37°C. Myotubes were treated with each concentration of nifedipine for 2 min and washed with HBSS. Fura-2 fluorescence was measured, and the $[Ca^{2+}]_i$ was derived from 340/380-nm ratios calculated using a Ca^{2+} imaging system. The changes in ratios were calculated from the difference between the maximal response following nifedipine treatment and the preceding baseline. Caffeine-induced changes in Fura-2 fluorescence were used as a positive control.

Three Ca^{2+} channel blockers

Stock solutions of 50 mM nifedipine, verapamil, or diltiazem in DMSO were diluted with HBSS to make a 50.0 μM working solution. Ca^{2+} channel blockers were perfused into the sample microwells at a rate of 1.2 ml/min at 37°C. Myotubes were treated in random order with each concentration of drug for 2 min and washed with HBSS. Nifedipine-, verapamil-, or diltiazem-induced changes in Fura-2 fluorescence were measured. Data denote the ratio of verapamil or diltiazem to nifedipine.

Dantrolene

Myotubes were treated with 0.5 μM nifedipine alone for 2 min as a control and washed with HBSS. After pretreatment with 50.0 μM dantrolene, 0.5 μM nifedipine was added for 2 min. Fura-2 fluorescence was measured, and the 340/380-nm ratios were calculated. The same procedure was performed with 1.5 and 5.0 μM nifedipine. Data represent the percentage of control dantrolene-free condition response.

Data analysis

Changes in ratios were calculated from the difference between the maximal response and the preceding baseline. Data analysis was performed using PRISM software (GraphPad Software, San Diego, CA, USA) with Excel-based templates (Microsoft, Redmond, WA, USA). Values shown are the mean ± standard error of mean (SEM). Unpaired *t* tests were used for statistical comparisons between accelerated and nonaccelerated groups. Paired *t* tests were used to for comparisons within groups. *P* values <0.05 were considered to be statistically significant.

Results

Effect of nifedipine on $[Ca^{2+}]_i$ in skeletal muscle

Treatment of human skeletal myotubes with nifedipine induced a transient increase in $[Ca^{2+}]_i$ (Fig. 1a). When the nifedipine dose–response curves were normalized to the maximal response observed after treatment with 50 μM nifedipine, the curve of the accelerated group was shifted to the left compared with the nonaccelerated group (Fig. 1b). The nifedipine-induced half-maximal concentration (EC_{50}) for activation was 0.718 ± 0.329 μM ($n = 7$) in the accelerated group, and 1.389 ± 0.482 μM ($n = 6$) in the nonaccelerated group ($P = 0.009$) (Table 2), and DMSO had no effect on the 340/380 ratio. The EC_{50} values for caffeine were 2.553 ± 0.162 mM ($n = 7$) and 4.918 ± 0.231 mM ($n = 6$) for the two groups, respectively ($P = 0.003$).

Effect of different L-type Ca^{2+} channel blockers on $[Ca^{2+}]_i$

We next examined the effects of three representative L-type Ca^{2+} channel blockers, nifedipine, verapamil, and diltiazem, on $[Ca^{2+}]_i$ in myotubes. Whereas similar increases in $[Ca^{2+}]_i$ were observed in myotube treated with verapamil or diltiazem, nifedipine induced greater changes.

Effect of dantrolene on nifedipine-induced changes in $[Ca^{2+}]_i$

Myotubes were treated with dantrolene or untreated as a control before application of nifedipine (Fig. 2a). In the presence of 50.0 μM dantrolene, the increased $[Ca^{2+}]_i$ induced by 0.5, 1.5, and 5.0 μM nifedipine was attenuated by 15.4%, 27.9%, and 28.1% in the accelerated group compared with control response (Fig. 2b). Treatment with 1.5 and 5.0 μM nifedipine significantly attenuated the increased $[Ca^{2+}]_i$ compared with control response ($P = 0.0066, 0.0007$).

Discussion

We isolated skeletal myotubes from patients predisposed to MH and showed that nifedipine increased $[Ca^{2+}]_i$. Depolarizing stimuli were not used, although pretreatment with

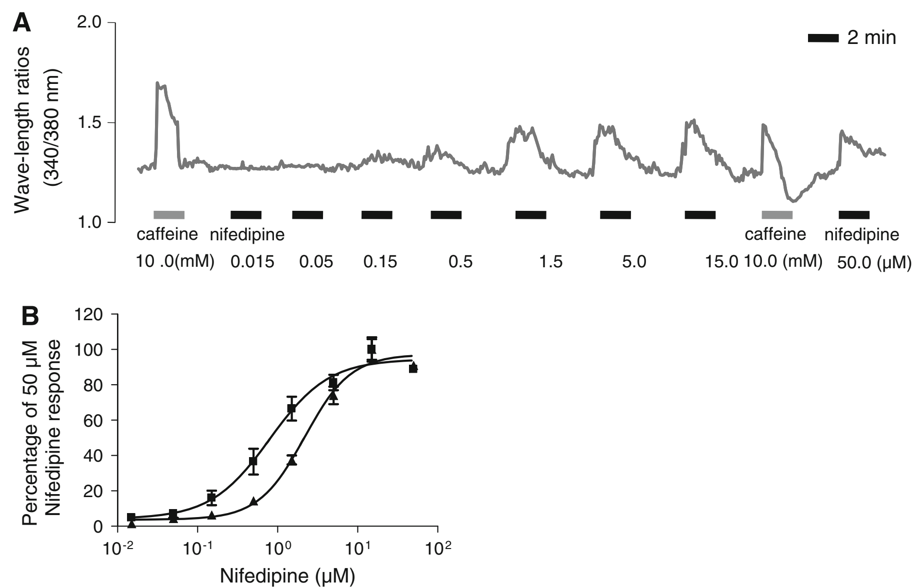

Fig. 1 The effect of nifedipine on intracellular calcium $[Ca^{2+}]_i$ of myotubes. **a** Representative data demonstrating the transient Ca^{2+} response following nifedipine treatment. Nifedipine increased $[Ca^{2+}]_i$ in primary human skeletal myotubes. **b** Representative nifedipine dose–response curve for the percentage increase in Ca^{2+} response. *Squares*, accelerated; *triangles*, nonaccelerated. These curves were normalized to the maximal response observed with 50 μM nifedipine. Data are the mean ± standard error of mean

Table 2 Half-maximal concentration (EC_{50}) values for nifedipine

Group	EC_{50} for nifedipine (μM)	No. mycotubes	EC_{50} for caffeine (mM)	No. mycotubes
Accelerated	0.718 ± 0.329*	7	2.553 ± 0.162*	7
Nonaccelerated	1.389 ± 0.482	6	4.918 ± 0.231	6

Values are mean ± standard error of mean

* $P < 0.05$ versus nonaccelerated group

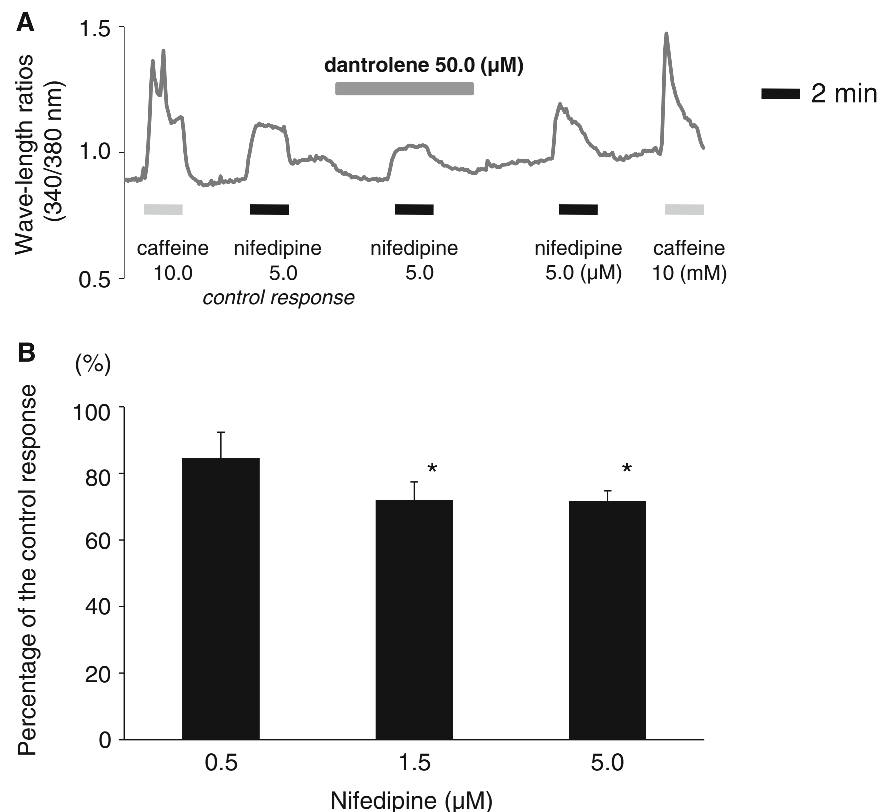

Fig. 2 The effect of dantrolene on nifedipine-induced intracellular calcium $[Ca^{2+}]_i$ changes. **a** Representative data demonstrating the transient Ca^{2+} response following nifedipine treatment in the absence and presence of dantrolene. **b** Data represent the percentage of control response, which was taken in a dantrolene-free condition. Data are mean ± standard error of mean. *$P < 0.05$ versus control response

nifedipine has been used in vitro studies to reduce $[Ca^{2+}]_i$ release after cell stimulation with depolarizing agents such as KCl [17, 18]. Additionally, the increase in $[Ca^{2+}]_i$ by nifedipine was only slightly modified by dantrolene.

Ca^{2+} channel blockers are used to treat hypertension, coronary artery disease, and heart failure at 10-nM concentrations. There are no reports of MH induced by Ca^{2+} channel blockers taken as normal medication. The EC_{50} for nifedipine on skeletal muscle is tenfold higher than the commonly used therapeutic doses. Intravenous use may cause a rapid and greater increase in $[Ca^{2+}]_i$ and chain reaction, as Ca^{2+} concentrations are already high during the MH crisis. Calcium homeostasis during the MH crisis differs from the normal state, and the use of Ca^{2+} channel blockers involving elevated myoplasmic $[Ca^{2+}]_i$ may not be safe.

Whereas nicardipine is clinically popular, nifedipine is often used in in vitro studies. As both are classified as belonging to the dihydropyridine group, other Ca^{2+} channel blockers were investigated. All three Ca^{2+} channel blockers tested in this study, nifedipine, verapamil, and diltiazem, increased $[Ca^{2+}]_i$. L-type Ca^{2+} channel blockers are classified into dihydropyridine (DHP), phenylalkylamine (PAA), and benzothiazepine (BTZ) groups based upon their dihydropyridine-receptor (*DHPR*) binding sites; they vary in the number of binding segments [19–24]. Interestingly, in this study, drug potency correlated with the number of binding sites on *DHPR*, i.e., nifedipine > verapamil > diltiazem. In a previous study, nifedipine increased $[Ca^{2+}]_i$ in human skeletal muscle cells obtained from volunteers. However, it did not affect $[Ca^{2+}]_i$ in PC12 cells, which lack both *DHPR* and *RYR1*. An increase of $[Ca^{2+}]_i$ caused by nifedipine could be explained by Ca^{2+} release from ryanodine-sensitive stores through a membrane-potential-dependent mechanism [25]. Skeletal muscle shows specific interaction; *DHPR* and *RYR1* directly interact without using Ca^{2+} as a second messenger. Based on the specific mechanism of the skeletal muscle, Ca^{2+} blockers increase $[Ca^{2+}]_i$ by release of Ca^{2+} through the ryanodine receptor, mediated by facilitation of DHPR gating. We previously reported that nifedipine increased $[Ca^{2+}]_i$ in myotubes with both *DHPR and RYR1*; however, it did not affect $[Ca^{2+}]_i$ in human embryonic kidney (HEK)-293 cells expressing *RYR1* but lacking *DHPR* [26]. Data in the study reported here are thus consistent with the previous study: Ca^{2+} channel blockers release Ca^{2+} from the sarcoplasmic reticulum (SR) without directly affecting the ryanodine receptor, but they require *DHPRs* [25].

We compared the effect of predisposition on the development of MH, classified using the CICR rate test. The EC_{50} for nifedipine was 0.718 μM in the accelerated group with MH predisposition and 1.389 μM in the nonaccelerated group. The CICR rate test decides the predisposition for MH according to the acceleration of Ca^{2+} release from SR, using skinned fibers that hold SR membrane function. The acceleration of CICR rate indicates a disorder of *RYR1* function. That nifedipine binds on *DHPR* causes Ca^{2+} release. As with *RYR1* stimulators such as caffeine and halothane [15], the EC_{50} for nifedipine in the accelerated group was half that in the nonaccelerated group. The accelerated group is thus more sensitive to nifedipine. In other words, that nifedipine binds on *DHPR* may cause the similar effect on *RYR1* with *RYR1* stimulators. Dantrolene sodium, the only effective treatment for MH, reduces the uncontrolled rise in $[Ca^{2+}]_i$ by binding the N-terminus of *RYR1* [27], the region responsible for *RYR1* activation. It thus stabilizes the closed state of the *RYR1* channels and inhibits Ca^{2+} efflux from the SR [28]. In the study reported here, the concentration of dantrolene used, 50 μM, is consistent with clinical use. Indeed, EMHG recommendations for treating MH result in 24-h plasma dantrolene concentrations of 14–18 mg/l (45–57 μM) [29]. Using skeletal myotubes, we showed that clinical doses of dantrolene attenuate the nifedipine-induced $[Ca^{2+}]_i$ increase by only about 20% compared with dantrolene-free conditions. *DHP*- bound Ca^{2+} channel blockers also bind to the N-terminal of *RYR1*; thus, dantrolene could inhibit the binding *RYR1* conformational-sensitive site. Whereas we did not observe a greater interaction of clinical concentrations of nifedipine and dantrolene in skeletal muscle, the effect of dantrolene was insufficient to prevent nifedipine-induced $[Ca^{2+}]_i$ increase. Thus, even in the presence of dantrolene, the use of Ca^{2+} channel blockers is not advisable. Recent data suggest that dantrolene may act by suppressing entry of Ca^{2+} from extracellular fluid [29–32]. In this study, the nifedipine-induced increase in $[Ca^{2+}]_i$ was approximately 80% of that seen in the absence of dantrolene; therefore, $[Ca^{2+}]_i$ probably derived from Ca^{2+} stores. This is consistent with a previous report [25] showing that nifedipine is able to promote Ca^{2+} release in the absence of extracellular Ca^{2+}. Taken together, these data indicate that increased $[Ca^{2+}]_i$ seen following nifedipine treatment is derived from the SR.

In conclusion, we used myotubes isolated from skeletal muscle of individuals predisposed to MH to show that Ca^{2+} channel blocker treatment led to increased $[Ca^{2+}]_i$. Importantly, the increases in $[Ca^{2+}]_i$ were seen even in the presence of dantrolene. These data provide a greater physiologic basis for the current practice of avoiding Ca^{2+} channel blocker in an MH crisis.

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References

1. Hopkins PM. Malignant hyperthermia: advances in clinical management and diagnosis. Br J Anaesth. 2000;85:118–28.
2. Stowell KM. Malignant hyperthermia: a pharmacogenetic disorder. Pharmacogenomics. 2008;9:1657–72.
3. Larach MG, Gronert GA, Allen GC, Brandom BW, Lehman EB. Clinical presentation, treatment, and complications of malignant hyperthermia in North America from 1987 to 2006. Anesth Analg. 2010;110:498–507.
4. Rosenberg H, Davis M, James D, Pollock N, Stowell K. Malignant hyperthermia. Orphanet J Rares Dis. 2007;2:21–34.
5. Saltzman LS, Kates RA, Corke BC, Norfleet EA, Heath KR. Hyperkalemia and cardiovascular collapse after verapamil and dantrolene administration in swine. Anesth Analg. 1984;63:473–8.
6. Lynch C 3rd, Durbin CG Jr, Fisher NA, Veselis RA, Althaus JS. Effects of dantrolene and verapamil on atrioventricular condition and cardiovascular performance in dogs. Anesth Analg. 1986;65: 252–8.
7. Freysz M, Timour Q, Bemaud C, Bertrix L, Faucon G. Cardiac implications of amlodipine-dantrolene combinations. Can J Anaesth. 1996;43:50–5.
8. Glahn KP, Ellis FR, Halsall PJ, Muller CR, Snoeck MMJ, Urwyler A, Wappler F; European Malignant Hyperthermia Group. Recognizing and managing a malignant hyperthermia crisis: guidelines from the European Malignant Hyperthermia Group. Br J Anasth. 2010;105:417–20.
9. Larach MG, Localio AR, Allen GC, Denborough MA, Ellis FR, Gronert, GA, Kaplan RF, Muldoon SM, Nelson T E, Ording H, Rosenberg H, Waud BE, Wedel DJ. A clinical grading scale to predict malignant hyperthermia susceptibility. Anesthesiology 1994;80:771–9.
10. Endo M, Iino M. Measurement of Ca release in skinned fibers from skeletal muscle. Methods Enzymol. 1988;157:12–26.
11. Ohta T, Endo M, Nakano T, Morohoshi Y, Wanikawa K, Ohga A. Ca-induced Ca release in malignant hyperthermia-susceptible pig skeletal muscle. Am J Physiol. 1989;256:358–67.
12. Oku S, Mukaida K, Nosaka S, Sai Y, Maehara Y, Yuge O. Comparison of the in vitro caffeine-halothane contracture test with the Ca-induced Ca release rate test in patients suspected of having malignant hyperthermia susceptibility. J Anesth. 2000;14:6–13.
13. Ibarra MCA, Wu S, Murayama K, Minami M, Ichihara Y, Kikuchi H, et al. Malignant hyperthermia in Japan. Anesthesiology. 2006;104:1146–56.
14. Migita T, Mukaida K, Kawamoto M, Kobayashi M, Yuge O. Fulminant-type malignant hyperthermia in Japan: cumulative analysis of 383 cases. J Anesth. 2007;21:285–8.
15. Migita T, Mukaida K, Hamada H, Kobayashi M, Nishino I, Yuge O, Kawamoto M. Effects of propofol on calcium homeostasis in human skeletal muscle. Anaesth Intensive Care. 2009;37:415–25.
16. Liberona JL, Caviedes P, Tascon S, Hidalgo J, Giglio JR, Sampaio SV, Caviedes R, Jaimovich E. Expression of ion channels during differentiation of a human skeletal muscle cell line. J Muscle Res Cell Motil. 1997;18:587–98.
17. Squecco R, Bencini C, Piperio C, Francini F. L-type Ca^{2+} channel and ryanodine receptor cross-talk in frog skeletal muscle. J Physiol. 2004;555:137–52.
18. Yang T, Allen PD, Pessah IN, Lopez JR. Enhanced excitation-coupled calcium entry in myotubes is associated with expression of RyR1 malignant hyperthermia mutations. J Biol Chem. 2007;282:37471–8.
19. Hofmann F, Biel M, Flockerzi V. Molecular basis for Ca^{2+} channel diversity. Annu Rev Neurosci. 1994;17:399–418.
20. Opie LH. Pharmacological differences between calcium antagonists. Eur Heart J. 1997;18:71–9.
21. Hockerman GH, Peterson BZ, Johnson BD, Catterall WA. Molecular determinants of drug binding and action on L-type calcium channels. Annu Rev Pharmacol Texicol. 1997;37:361–96.
22. Yamaguchi S, Okamura Y, Nagao T, Adachi-Akahane S. Serine residue in the IIIS5-S6 linker of the L-type Ca^{2+} channel alpha 1C subunit is the critical determinant of the action of dihydropyridine Ca^{2+} channel agonists. J Biol Chem. 2000;275:41504–11.
23. Hirota K, Hashiba E, Yoshioka H, Kabara S, Matsuki A. Effects of three different L-type Ca^{2+} entry blockers on airway constriction induced by muscarinic receptor stimulation. Br J Anaesth. 2003;90:671–5.
24. Striessnig J, Grabner M, Mitterdorfer J, Hering S, Sinnegger MJ, Glossmann H. Structural basis of drug binding to L Ca^{2+} channels. Trends Pharmacol Sci. 1998;19:108–15.
25. Weigl LG, Hohenegger M, Kress HG. J. Dihydropyridine-induced Ca^{2+} release from ryanodine-sensitive Ca^{2+} pools in human skeletal muscle cells. Physiol. 2000;525 2:461–9.
26. Migita T, Mukaida K, Hamada H, Kawamoto M. Do Ca^{2+} channel blockers improve malignant hyperthermia crisis? Eur J Anaesth. 2009;26:124.
27. Paul-Pletzer K, Yamamoto T, Bhat MB, MA J, Ikemoto N, Jimenez LS, Morimoto H, Williams PG, Parness J. Identification of a dantrolene-binding sequence on the skeletal muscle ryanodine receptor. J Biol Chem. 2002;277:34918–23.
28. Fruen BR, Mickelson JR, Louis CF. Dantrolene inhibition of sarcoplasmic reticulum Ca^{2+} release by direct and specific action at skeletal muscle ryanodine receptors. J Biol Chem. 1997;272: 26965–71.
29. Cherednichenko G, Ward CW, Feng W, Cabrales E, Michaelson L, Samso M, Lopez JR, Allen PD, Pessah IN. Enhanced excitation-coupled calcium entry in myotubes expressing malignant hyperthermia mutation R163C is attenuated by dantrolene. Mol Pharmacol. 2008;73:1203–12.
30. Cherednichenko G, Hurne AM, Fessenden JD, Lee EH, Allen PD, Beam KG, Pessah IN. Conformational activation of Ca^{2+} entry by depolarization of skeletal myotubes. Proc Natl Acad Sci USA. 2004;101(44):15793–8.
31. Dirksen RT. Checking your DOCCs and feet: molecular mechanisms of Ca^{2+} entry in skeletal muscle. J Physiol. 2009;587:3139–47.
32. Bannister RA, Pessah IN, Beam KG. The skeletal L-type Ca^{2+} current is a major contributor to excitation-coupled Ca^{2+} entry. J Gen Physiol. 2009;133:79–91.