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EFFECTS OF HALOTHANE ON CALCIUM RELEASE FROM SARCOPLASMIC RETICULUM OF RABBIT PSOAS AND SEMITENDINOSUS SKINNED MUSCLE FIBERS

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Abstract—Calcium release from sarcoplasmic reticulum was investigated using skinned fibers isolated from rabbit semitendinosus and psoas muscles, representative of slow and fast fibers, respectively. In both types of fibers, halothane at the concentration of 0.03% (v/v) enhanced the Ca^{2+}-induced calcium release. In the absence of cytoplasmic free Ca^{2+}, halothane induced calcium release in a dose-dependent manner, with a similar sensitivity for both semitendinosus and psoas fibers. These results are discussed in connection with muscular diseases such as malignant hyperthermia in which the crisis is triggered during anesthesia by halothane.

Halothane is an anesthetic known to trigger the malignant hyperthermia (MH)† crisis in genetically predisposed man and pigs [1]. MH seems to result from a defect in skeletal muscles associated to abnormal Ca^{2+} homeostasis [2].

Halothane, like Ca^{2+}, can induce calcium release from sarcoplasmic reticulum [3–5]. Furthermore, its potentiating effect on Ca^{2+}-induced calcium release has been observed in several studies [6–8]. Many investigations on the primary defect in MH have focused on Ca^{2+} transport in the sarcoplasmic reticulum (SR). In particular, a higher sensitivity to the calcium-releasing effects of Ca^{2+} and halothane has been reported in MH susceptible muscles [6].

Skeletal muscle fibers are defined as slow and fast with regard to their mechanical, metabolic and biochemical properties [9–11]. In the present work, slow and fast fibers were isolated from rabbit semitendinosus (ST) and psoas (PS) muscles. Using the skinned fibers technique, we investigated the halothane-induced calcium release from SR in both types of fibers.

To date, it is not known whether the MH defect is predominant in a particular type of fiber. Our results suggest that the MH defect is present in slow and fast fibers.

MATERIALS AND METHODS

Solutions. Experimental solutions were made with KMS, $MgMS_2$, $CaMS_2$, ATP, PIPES, and EGTA. Free Ca^{2+} concentrations were calculated according to the data used by Horiuti [12], at 10° and pH 7.0. Ionic strength was adjusted to 200 mM, PIPES to 20 mM, and MgATP and free Mg^{2+} to 3.5 and 1.5 mM, respectively. EGTA concentration was fixed between 0.1 and 10 mM according to the conditions desired (see Table 1). Halothane was purchased from Aldrich Chemical Co. (Strasbourg, France) and its concentration was expressed as per cent volume of the reaction mixture. It was introduced with an Hamilton syringe straight into the final solution.

Experimental procedure. Skinned fibers were prepared by a modification of the procedure previously described [13]: rabbit fiber bundles were removed from ST and PS muscles and immediately placed in a skinning solution containing 170 mM K-propionate, 10 mM imidazole, 5 mM EGTA, 2 mM Mg-acetate, and 2 mM ATP, pH 7.0. The fibers were incubated in this solution for 4 hr at 4°. The solution was changed every hour. The bundles of fibers were then transferred to another skinning solution of same composition made up in 50% glycerol (v/v), and stored at −20° until use.

A single fiber was isolated from the bundle in a solution containing 10 mM EGTA, and silk thread was attached to each end using a stereomicroscope. The apparent diameter of the skinned fiber was then measured (approximately 40 μm).

One fiber end was tied to a fixed hook while the other was connected to an U-gauge Shinkoh (Tokyo, Japan) or Cambridge Technology (Cambridge, U.S.A.) transducer. The fiber rested in an experimental trough in which solutions could be rapidly changed within 200 msec by a custom designed injection system. The sarcomere length was set to 3.0 μm using a diffraction pattern produced by a Spectra-Physics He–Ne laser. Isometric tension was monitored on a Gould 2200 recorder. All experiments were performed at 10°. We have chosen to perform experiments at 10° because at higher temperatures, deteriorations of skinned fibers are much more rapid. At 10°, it is therefore possible to perform many assays on the same fiber, without run down of the fiber.

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† Abbreviations used: EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetate; MH, malignant hyperthermia; MS, methanesulfonate $CH_3SO_3^-$; PIPES, piperazine-*N*,*N*′-bis ethylene sulfonate; PS, psoas; SR, sarcoplasmic reticulum; ST, semitendinosus.

Table 1. Composition of experimental solutions

	KMS	$MgMS_2$	$CaMS_2$	EGTA	PIPES	ATP	Free Ca^{2+}
Relaxing solutions	105	5.2		10	20	4.4	
	129	5.0		2	20	4.6	
	134	5.0		0.1	20	4.6	
Loading solution	98	5.1	3.3	10	20	4.4	0.25
Rigor solutions	129	1.7		2	20		
	153	1.5		10	20		
Releasing solutions	130		1.7	10	20		0.1
	126		3.9	10	20		0.32
	120		6.7	10	20		1
	116		8.6	10	20		3.16
	114		9.5	10	20		10

The abbreviation MS is for methanesulfonate. The concentrations are given as millimolar except free calcium which is given as micromolar, ionic strength 200 mM, pH 7.0 and temperature 10°. In relaxing and loading solutions, the Mg^{2+} and MgATP concentrations were kept at 1.5 and 3.5 mM, respectively. In rigor solutions, Mg^{2+} was 1.5 mM; and it was absent from the releasing solutions.

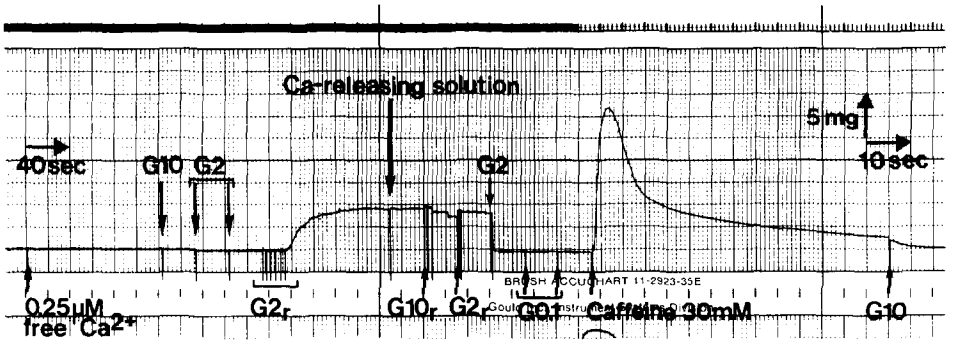

Fig. 1. Sequence for determinations of Ca-induced Ca release in a skinned fiber. Solutions were exchanged at the times indicated by deflections in tension records. The following solutions were applied: (1) calcium loading solution containing 0.25 µM free calcium for 2 min; (2) relaxing solutions containing first 10 then 2 mM EGTA (G10 and G2); (3) rigor solution (ATP-free) in 2 mM EGTA (G2r); (4) calcium-releasing solutions containing various free calcium concentrations (0.01–10 µM) in the absence or presence of 0.03% (v/v) halothane for 15, 30 or 60 sec; (5) rigor solutions containing first 10 then 2 mM EGTA (G10r and G2r); (6) relaxing solutions in 2 then 0.1 mM EGTA (G2 and G0.1); (7) 30 mM caffeine in relaxing solution G0.1. Controls (without application of the calcium-releasing solutions) were performed before and after each test. The ratio of the areas of caffeine signals (assay over control) gives the percentage of calcium remaining in the SR.

The total amount of calcium in the SR of a skinned fiber was determined from the integral of the tension signal under maximal release conditions: 30 mM caffeine in 0.1 mM EGTA [14].

Ca^{2+}-induced calcium release in the absence of ATP: effects of halothane. The rate of calcium release induced by Ca^{2+} was determined essentially as previously described [6]. A sequence of such assays is illustrated on Fig. 1. The SR was first loaded with Ca^{2+} by incubation in a loading solution containing 0.25 µM free Ca^{2+} for 2 min. ATP was removed by washing with an ATP-free solution until a steady rigor tension developed. Calcium-releasing solutions containing various concentrations of free Ca^{2+} without ATP in 10 mM EGTA, were then applied for various periods of time in the absence or presence of 0.03% halothane (v/v). The release of calcium was measured without ATP to prevent both calcium uptake and tension of the fiber. Calcium remaining in the SR was determined by applying a 30 mM caffeine solution. Controls (without application of the calcium-releasing solution) were performed before and after each test to correct deteriorations in tension responses. The average of two caffeine-induced control tensions corresponds to 100% of calcium remaining in the SR. The amount of calcium remaining in the SR after application of the calcium-releasing solutions was determined as a percentage of control.

Calcium release was determined from the difference between the amount of calcium remaining in the SR in the control and the test. We have measured

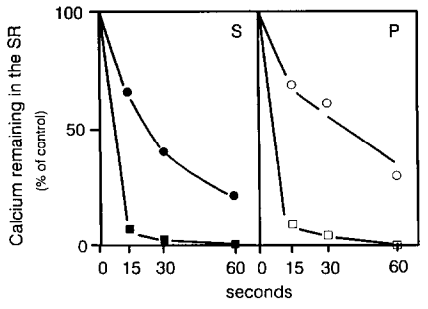

Fig. 2. Time course of calcium release from sarcoplasmic reticulum (SR) of a rabbit semitendinosus (S) and a psoas (P) skinned fiber. The SR was first loaded with 0.25 μM free Ca^{2+} for 2 min. A rigor solution was then applied to ensure complete depletion of ATP. Calcium-releasing solution containing various free Ca^{2+} concentration in the absence or presence of 0.03% halothane (v/v) was applied for various periods of time. Calcium remaining in the SR was then completely released by applying a 30 mM caffeine solution. It was expressed in percentage of control (without application of the calcium-releasing solution). Experiments were performed at 10°, pH 7.0, and in the absence of MgATP. Circles: calcium release induced by 1 μM free Ca^{2+}. Squares: calcium release induced by 1 μM free Ca^{2+} with 0.03% halothane (v/v).

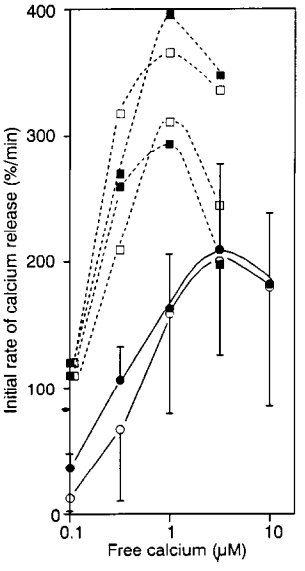

Fig. 3. Dependence of calcium release on free Ca^{2+} concentrations in absence or presence of halothane. Experiments were performed using rabbit skinned fibers isolated from psoas (open symbols) and semitendinosus (closed symbols) muscles, at 10° pH 7.0, and in the absence of MgATP. Circles: without halothane (N = 4 for psoas and N = 6 for semitendinosus). Results are expressed as mean + confidence interval (for Student's *t*-test, P = 0.20). Squares: with 0.03% halothane (v/v) (each curve corresponds to an experiment on one fiber). The rate of calcium release was obtained from the slope of the calcium release curve between the zero and the 15 sec points. It was expressed as percentage of calcium release/min.

calcium release for various Ca^{2+} concentrations and for various periods of time. The rate of calcium was then determined from the slope of the calcium release curve between the zero and the 15 sec points.

Halothane–induced calcium release in the presence of ATP. The SR was first loaded with calcium as described above. Solutions containing various concentrations of halothane in 0.1 mM EGTA were then applied, initiating transient tensions. The fiber was then exposed to the 30 mM caffeine solution to release the calcium remaining in the SR, initiating a test signal. Controls (without application of halothane treatment) were performed before and after each test and the average of two caffeine-induced control signals corresponds to 100% of calcium remaining in the SR. The percentage of calcium released by halothane was assessed from the difference between the amounts of calcium remaining in the SR in the control and the test.

RESULTS

Ca^{2+}-induced calcium release: effects of halothane in the absence of ATP.

The time course of calcium release induced by 1 μM free Ca^{2+} with or without 0.03% halothane (v/v) is shown in Fig. 2. Halothane increased the rate of calcium release induced by 1 μM free Ca^{2+} in both ST and PS fibers. The rate of calcium release was obtained from the slope of the calcium release curve between the zero and the 15 sec points. The rate of calcium release from the SR (measured as percentage of calcium release/min) increased with free Ca^{2+} concentration up to 3 μM in both ST and PS fibers (Fig. 3). Higher concentrations of Ca^{2+} inhibited calcium release. There was no significant difference between the two types of fibers. In the presence of 0.03% halothane (v/v), the rate of calcium release induced by Ca^{2+} was roughly doubled; further, the optimal concentration of free Ca^{2+} was shifted from 3 to 1 μM. There was no difference in the effect of halothane on the Ca^{2+}-induced calcium release in ST and PS fibers.

Calcium release induced by halothane in the presence of ATP.

In the last series of experiments, such as shown in Fig. 4, the fibers were incubated with MgATP, and the effect of halothane concentrations ranging between 0.0025 and 0.1% (v/v) on calcium release was assessed. The results are plotted in Fig. 5 for ST (S) and PS (P) fibers. Large variations in calcium release were observed in repeated experiments. Yet, in most cases, the amount of calcium released increased in proportion with the halothane concentration in both ST and PS fibers. Further, the threshold of calcium release was obtained with about 0.005% halothane (v/v) for the two categories of fibers.

DISCUSSION

The main conclusion emerging from the present work is that rabbit ST and PS fibers respond similarly to the calcium-releasing effect of halothane with or without Ca^{2+}. These findings in rabbit muscles are in agreement with two human studies which report no relationship between fiber type distribution and

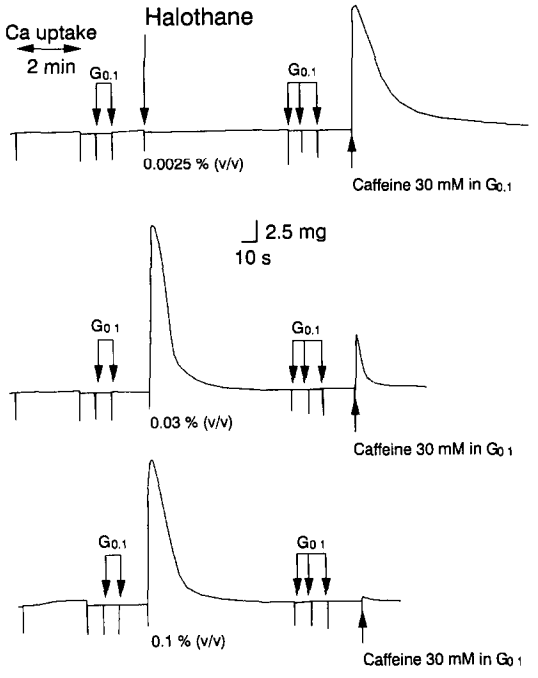

Fig. 4. Halothane induced calcium release in a relaxed skinned fiber. Traces shown are representative of assays performed on a single skinned fiber. The SR was first loaded with 0.25 μM free calcium for 2 min at 10°. After two washes with relaxing solution G0.1, various amounts of halothane in G0.1 was applied. Finally, 30 mM caffeine was injected to release the calcium remaining in the SR.

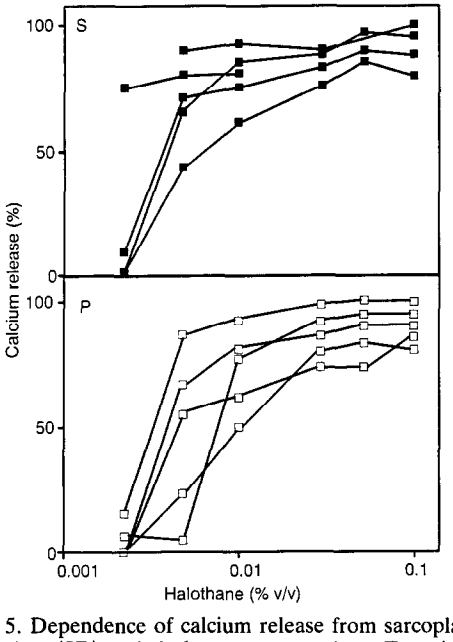

Fig. 5. Dependence of calcium release from sarcoplasmic reticulum (SR) on halothane concentrations. Experiments were performed at 10°, pH 7.0, in presence of 1.5 mM free Mg^{2+} and 3.5 mM MgATP on skinned fibers isolated from rabbit semitendinosus (S, closed symbols) and psoas (P, open symbols) muscles. Experimental procedure was as described in Materials and Methods. Each curve represents an experiment on one skinned fiber.

response to caffeine or halothane *in vitro* [15, 16]. Furthermore, these studies show no difference in fiber type distribution between MH susceptible and normal patients. Fiber type is thus not a determinant of MH contracture test results in human. It is reasonable to extrapolate our conclusion to slow and fast fibers from human and porcine normal muscles, and to infer that the defect of calcium release in MH susceptible muscles is shared by both slow and fast fibers.

Halothane 0.01% (v/v in solution) is approximately 1 mM [17]. Solubility coefficient (water–air) of halothane is 0.79 and 1.35 at 37° and 20°, respectively [18]. According to these values, and by extrapolation of the data of Price and Ohnishi [19], we have determined that 1 mM halothane in water (0.01% v/v) is in equilibrium with 1.1% (v/v in air) at 10°. The halothane concentrations used (e.g., around 0.01% v/v in solution) were in the range of those applied during anesthesia (around 1% v/v in gas), or in the contracture test of sensitivity to halothane routinely performed by the European MH group [20]. Nevertheless, we observed on single fibers a threshold of sensitivity to halothane lower than those measured on bundles of normal fibers under electrical stimulation. This difference could be due to better access of drug in the single fiber preparation.

As an important consequence of the results reported in the present work, the use of muscle biopsies in which slow and fast fibers are mixed is validated to assay the MH susceptibility.

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