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2,2,4-Trimethylpentane induces Ca^{2+} release from the sarcoplasmic reticulum terminal cisterns

Vladimir B. Ritov and Elizaveta V. Men'shikova

School of Biology, M.V. Lomonosov Moscow State University, Moscow (U.S.S.R.)

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Using quin2, the effects of aliphatic hydrocarbons on the system of Ca^{2+}-induced Ca^{2+} release in isolated membranes of rabbit skeletal muscle terminal cisterns have been studied. The hydrocarbons were inserted into the membranes by means of hydrocarbon-containing liposomes. 2,2,4-Trimethylpentane (isooctane) caused a rapid release of 70–75% of Ca^{2+} taken up by the terminal cistern vesicles during the Ca^{2+}-pump operation. This effect was inhibited by the caffeine-induced Ca^{2+} release blockers – Mg^{2+}, ruthenium red and tetracaine. The same was observed with a decrease in the concentration of ATP that is known to activate the terminal cistern Ca^{2+} channels. The effect of 2,2,4-trimethylpentane on the longitudinal cistern fractions practically devoid of Ca^{2+}-channels was insignificant. Heptane, hexane and octane caused a slow release of 5–10% of the accumulated Ca^{2+} from the terminal cistern vesicles; no such effect was induced by decane.

1. Introduction

The Ca^{2+} release from the sarcoplasmic reticulum (SR) of skeletal muscles is an intermediate link between the electrical excitation of plasma membranes and the contractile response of muscle fibers [1]. The molecular mechanism of Ca^{2+} release from SR is still unknown. Therefore, a study of the Ca^{2+}-induced Ca^{2+} release system of the terminal cisterns of skeletal muscle SR is of special interest [2–6]. The central components of such a system are, in all probability, adenine nucleotide-dependent Ca^{2+}-channels [7–9]. Studies on the biochemical mechanisms underlying malignant hyperthermia induced by halothane anaesthesia demonstrated that the volatile anaesthetic halothane (1,1,1-trifluoro-2-chloro-2-bromoethane) can induce Ca^{2+} release from the terminal cisterns of SR [10–12]. It seems likely that the halothane-induced Ca^{2+} release is due to the increased affinity of the Ca^{2+} release system for Ca^{2+} [12]. Taking into account the specific effect of halothane, an investigation of the effects of alkanes on the system of Ca^{2+} release of skeletal muscle SR was initiated.

Correspondence: V.B. Ritov, School of Biology, M.V. Lomonosov Moscow State University, Moscow 119899, U.S.S.R.

2. Materials and Methods

2.1. Chemicals. Caffeine, imidazole, Hepes, ruthenium red, benzocaine, tetracaine and glycerol were purchased from Serva; phosphocreatine was from Sigma; creatine kinase and ATP were obtained from Reanal (Hungary); sucrose, KCl and potassium oxalate were from Merck; potassium gluconate was purchased from Fluka; benzoylcholine iodide was from Chemapol (Czechoslovakia); hexane, heptane, octane, decane, 2,2,4-trimethylpentane and halothane were Soviet products; alamethicin was isolated from the fungus *Trichoderma viride*. ATP and caffeine were separated from Ca^{2+}, using Dowex 50X8 (Serva) and Chelex 100 (Bio-Rad), respectively.

2.2. Methods. The experiments were carried out on two SR fractions, i.e., on the terminal cistern (TC) and longitudinal tubule (LC) fractions isolated from rabbit hind limb white skeletal muscle homogenates [13]. Protein concentration was determined by the biuret reaction. The uptake and release of Ca^{2+} by the SR vesicles were measured by quin2 fluorescence [14] in a medium (2 ml) containing 100 mM potassium gluconate, 1.5 mM $MgCl_2$, 2 mM ATP, 0.1 mM KH_2PO_4, 7 mM phosphocreatine, 2–3 I.U. creatine kinase, 20 μM Ca^{2+} (including contaminant Ca^{2+}), 25 μM quin2, 50–90 μg/ml of SR membrane protein and 10 mM Hepes (pH 6.8) (28 °C). The measurements were p.

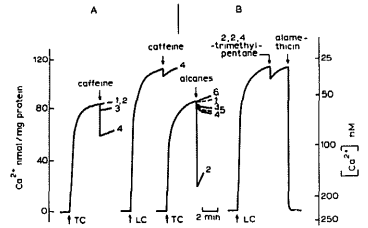

Fig. 1. Ca^{2+} transport by the terminal cistern (TC) and longitudinal cistern (LC) fractions as measured by quin2 fluorescence. The incubation medium contained: (A) 2 mM ATP + SR protein + 2 mM $MgCl_2$ (4) or 4 mM $MgCl_2$ (3) or 2 mM $MgCl_2$ + 3 μM ruthenium red (1) or 2 mM $MgCl_2$ + 0.2 mM tetracaine (2); (B) 2 mM ATP + SR protein + 1.5 mM $MgCl_2$. Additions: (A) 5 mM caffeine; (B) DPL (1) or DPL + decane (6) or DPL + hexane (3) or DPL + heptane (4) or DPL + octane (5) or DPL + 2,2,4-trimethylpentane (2). Concentrations: DPL, 5 μg/ml; alkanes, 3.5 μg/ml. Other conditions as described in 2.2. Methods.

formed on a Hitachi 850 spectrofluorimeter in a thermostated cell supplied with a vibrator. ATP hydrolysis was measured pH-metrically in a medium (4 ml) containing 100 mM NaCl, 4 mM $MgCl_2$, 2 mM ATP, 5 mM sodium oxalate, 20–25 μg/ml of SR protein and 2.5 mM imidazole (pH 7.0) (37 °C). The reaction was initiated by an addition of 25–35 μM $CaCl_2$.

3. Results and Discussion

Fig. 1 shows the kinetics of Ca^{2+} transport in the TC and LC fractions as measured by quin2 fluorescence. An addition of SR membranes to the incubation medium containing Mg-ATP, Ca^{2+} and quin2 results in a time-dependent decrease in fluorescence as a consequence of the ATP-dependent Ca^{2+} uptake by the SR vesicles. After the fluorescence response has reached a plateau, the fluorescence is maintained at a constant level for at least 10 min, which points to the lack of permeability of SR membranes for quin2. After addition of the channel-forming polypeptide alamethicin, the fluorescence returns to a level above the initial one due to the presence of contaminant Ca^{2+} in SR membrane preparations. The basal level of fluorescence of quin2 was shifted in all fluorescence plots presented herein, depending on the concentration of Ca^{2+} added together with SR membranes. In order to determine the amount of the accumulated Ca^{2+}, quin2 fluorescence was calibrated by an addition of 2–4 nmol $CaCl_2$ before the addition of SR membranes and after the addition of alamethicin. The free Ca^{2+} concentration in the incubation medium was determined as described by Rink [14]. Under the conditions used, the TC and LC fractions took up to about 80–100 nmol Ca^{2+}/mg protein during ATP hydrolysis, as a result of which Ca^{2+} concentration in the medium decreased to 0.05–0.1 μM. An addition of 5 mM caffeine after the saturation of SR vesicles with Ca^{2+} resulted in the liberation of about 30 nmol Ca^{2+}/mg protein from the TC fraction and of about 5 nmol Ca^{2+}/mg protein from the LC fraction (Fig. 1). The caffeine-induced Ca^{2+} release was inhibited by 3 μM ruthenium red, 0.2 mM tetracaine or by further addition of 2.5 mM $MgCl_2$, which is in good agreement with the literature data [15,16]. Halothane also induced Ca^{2+} release from the TC fraction; however, this reaction proceeded at rather a slow rate. The hydrocarbons were inserted into SR membranes within the composition of hydrocarbon-containing liposomes. The latter were obtained by the method of Batzri and Korn [17] by injecting dipalmitoyllecithin (DPL) (5 mg/ml) and a hydrocarbon (5 μl/ml) solution in ethanol with a Hamilton microsyringe. As can be seen from Fig. 1, an addition of DPL or of a DPL + decane solution in ethanol (2 μl) to the incubation medium after Ca^{2+} uptake does not change the Ca^{2+} concentration inside the SR vesicles. DPL in combination with hexane, heptane or octane causes a slow release of a small amount of Ca^{2+} (5–10%). When 2,2,4,-trimethylpentane (isooctane)-containing liposomes are present in the incubation medium, 70% of the accumulated Ca^{2+} are rapidly released from the SR vesicles (Fig. 1). Isooctane was found to liberate up to about 10% of the accumulated Ca^{2+} from the LC fraction which, as can be judged from the magnitude of the caffeine-induced effect (Fig. 1), contains a small amount of TC vesicles. The isooctane-induced Ca^{2+} release is inhibited by Mg^{2+}, ruthenium red and tetracaine. A decrease in the ATP concentration from 2 mM to 10 μM at a constant concentration of Mg^{2+} (0.5 mM) causes a significant decrease in the rate of Ca^{2+} release induced by isooctane (Fig. 2). At low concentrations of free Mg^{2+} (1.5 mM $MgCl_2$, 2 mM ATP), 3.5 μg/ml isooctane causes a maximal release of Ca^{2+}, because further additions of caffeine produce no effect. The half-maximum concentration of isooctane under these conditions is 1 μg/ml. At high Mg^{2+} concentrations causing the inhibition of the isooctane effect, the isooctane enhances the caffeine-induced Ca^{2+} release. An interesting finding is that hexane and heptane, which normally do not cause any significant release of Ca^{2+} even at low Mg^{2+} concentrations, have a stimulating effect on the caffeine-induced Ca^{2+} release. Octane has no effect at all, whereas decane has an inhibiting effect (Fig. 2).

Isooctane-containing liposomes were found to influence the kinetics of ATP hydrolysis by the TC fraction. When the rate of ATP hydrolysis in SR membranes was measured by the pH method, the $CaCl_2$ added to a medium containing Mg-ATP, oxalate and SR membranes increased the rate of ATP hydrolysis. After a

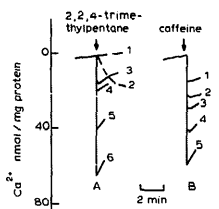

Fig. 2. Ca^{2+} release from the TC vesicles induced by 2,2,4-trimethylpentane (A) or caffeine (B) as measured by quin2 fluorescence. TC membranes were preincubated for 3–4 minutes in a medium containing: (A) 2 mM ATP + 1.5 mM $MgCl_2$ (6) or 1.5 mM $MgCl_2$ + 3 μM ruthenium red (1) or 1.5 mM $MgCl_2$ + 0.2 mM tetracaine (3) or 2.5 mM $MgCl_2$ (5) or 4 mM $MgCl_2$ (4) or 10 μM ATP + 0.5 mM $MgCl_2$ (2); (B) 2 mM ATP + 1.5 mM $MgCl_2$. Two minutes prior to caffeine addition, DPL (2) or DPL + decane (1) or DPL + octane (3) or DPL + heptane (4) or DPL + hexane (5) were added to the incubation medium. Concentrations: DPL, 5 μg/ml; alkanes, 3.5 μg/ml. The amount of Ca^{2+} in the SR vesicles before the addition of 2,2,4-trimethyl pentane or caffeine (5 mM) was taken for 100%. Other conditions as described in 2.2. Methods.

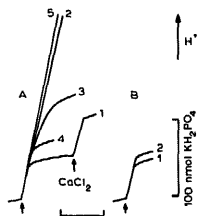

Fig. 4. Effects of benzoylcholine and caffeine on ATP hydrolysis in the TC vesicles as measured by the pH-method. The TC vesicles were preincubated for 2 min with 2 mM ATP + 4 mM $MgCl_2$ + contaminant Ca^{2+} (approx. 10 μM) (1) or 2.5 mM benzoylcholine (2) or 5 mM caffeine (4) or 2.5 mM benzoylcholine + 3 μM ruthenium red (3) or 5 mM caffeine + 10 mM benzocaine (5). ATP hydrolysis was induced by an addition of 100 nmol $CaCl_2$. Other conditions as described in 2.2. Methods.

definite time interval, the rate of ATP hydrolysis dropped to the initial level. This decrease reflects the accumulation of added Ca^{2+}, since a second addition of Ca^{2+} results in the activation of ATP hydrolysis (Fig. 3). Isooctane-containing liposomes added to the incubation medium cause a several-fold increase in the amount of ATP hydrolyzed during Ca^{2+} transport, i.e., a decrease of the Ca^{2+}/ATP ratio; a similar effect is induced by caffeine. The decrease of the Ca^{2+}/ATP ratio by isooctane and caffeine is observed only in the TC fraction and it can be prevented by ruthenium red and tetracaine (Fig. 3). Other *n*-alkanes tested had no effect on the Ca^{2+}/ATP ratio.

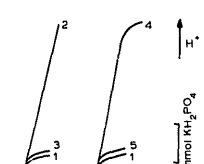

Fig. 3. ATP hydrolysis in the TC (A) and LC (B) vesicles as measured by the pH-method. The SR membranes were preincubated with 2 mM ATP + 4 mM $MgCl_2$ + contaminant Ca^{2+} (approx. 10 μM)[(1)] + 5 mM caffeine (5) or 5 μg/ml of DPL + 3.5 μg/ml of 2,2,4-trimethylpentane (2) or DPL + 2,2,4-trimethylpentane + 3 μM ruthenium red (4) or DPL + 2,2,4-trimethylpentane + 0.2 mM tetracaine (3). ATP hydrolysis was induced by an addition of 100 nmol $CaCl_2$. Other conditions as described in 2.2. Methods.

It can thus be assumed that isooctane stimulates the Ca^{2+}-channels of the Ca^{2+}-induced Ca^{2+} release system. Evidence for this assumption can be derived from the following facts. First, the TC fraction, which is rich in Ca^{2+} channels [9], is more sensitive to isooctane than the LC fraction. Second, the rate of Ca^{2+} release by isooctane diminishes with a decrease in the concentration of ATP which activates the Ca^{2+}-channels of TC [9]. Third, the blockers of the caffeine-induced Ca^{2+} release suppress the effect of isooctane. One cannot also exclude the possibility that isooctane, like caffeine and halothane, induces the transition of the Ca^{2+} release system to a state characterized by a high affinity for Ca^{2+} [4,12]. The isooctane-induced decrease of the Ca^{2+}/ATP ratio can also be explained in terms of the Ca^{2+}-channel activation.

The high specificity of isooctane in comparison with *n*-alkanes suggests that the Ca^{2+} release system comprises a hydrophobic cavity which is adapted to the trimethyl group. The fill-up of this cavity with structurally appropriate radicals results in the activation of the Ca^{2+}-channels. Fig. 4 shows that addition of benzoylcholine (2.5 mM), a benzoic acid choline ester, causes a decrease of the Ca^{2+}/ATP ratio in the TC fraction; this effect is blocked by ruthenium red. Benzocaine, a *p*-aminobenzoic acid ethyl ester, which is devoid of the trimethylammonium group, does not only decrease the Ca^{2+}/ATP ratio, but, contrariwise, inhibits the caffeine-induced decrease of Ca^{2+}/ATP.

One important finding of the present work, i.e., Ca^{2+} release from the TC vesicles under effects of 2,2,4-trimethylpentane, may be essential for a search into ways and means of pharmacological influence on

the SR Ca^{2+}-channel function. It is probable that the Ca^{2+} release from SR under the influence of structurally complex compounds [18] is, in some cases, due to hydrophobic interactions described above.

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