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Short communication

9-Methyl-7-bromoeudistomin D induces Ca^{2+} release from cardiac sarcoplasmic reticulum

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Abstract

9-Methyl-7-bromoeudistomin D (MBED), the most powerful caffeine-like releaser of Ca^{2+} from skeletal muscle sarcoplasmic reticulum, induced Ca^{2+} release from the cardiac sarcoplasmic reticulum. MBED (5 μM) and caffeine (1 mM) caused rapid Ca^{2+} release from the fragmented cardiac sarcoplasmic reticulum in a Ca^{2+} electrode experiment. [^{3}H]MBED bound to a single class of high-affinity binding sites in cardiac sarcoplasmic reticulum membranes (K_d = 150 nM). These results suggest that MBED binds to a specific binding site on cardiac sarcoplasmic reticulum membranes to induce Ca^{2+} release from the cardiac sarcoplasmic reticulum. Thus, MBED is a useful probe for characterizing Ca^{2+} release the channels not only in skeletal sarcoplasmic reticulum but also in cardiac sarcoplasmic reticulum.

Keywords: MBED (9-methyl-7-bromoeudistomin D); Ca^{2+} release channel; Cardiac sarcoplasmic reticulum; Ca^{2+} releaser; Specific binding site

1. Introduction

The rapid intracellular release and uptake of Ca^{2+} by the sarcoplasmic reticulum is essential for the process of muscle contraction and relaxation (Ebashi, 1991). In cardiac muscle, the influx of Ca^{2+} via L-type Ca^{2+} channels triggers Ca^{2+} release by opening the sarcoplasmic reticulum Ca^{2+} channel. This process is known as Ca^{2+}-induced Ca^{2+} release (Nabauer et al., 1989; Wier, 1990) and is mediated by Ca^{2+} release channels known as ryanodine receptors (Coronado et al., 1994; Meissner, 1994).

We have shown that 9-methyl-7-bromoeudistomin D (MBED), a derivative of eudistomin D isolated from the marine tunicata *Eudistoma olivaceum* (Kobayashi et al., 1989), induces Ca^{2+}-release from skeletal muscle sarcoplasmic reticulum in a manner similar to caffeine (Seino et al., 1990; Ohizumi, 1997). Furthermore, it has been demonstrated that MBED shares the same binding site as caffeine in skeletal muscle sarcoplasmic reticulum (Fang et al., 1993) and vascular smooth muscle (Adachi et al., 1994). MBED induces Ca^{2+} release from the superficial Ca^{2+} stores that are needed for activation of Ca^{2+}-dependent K^+ channels in smooth muscles (Imaizumi et al., 1996). Here we describe the first direct evidence that MBED binds to the specific binding site to induce Ca^{2+} release from cardiac muscle sarcoplasmic reticulum. MBED may be a valuable tool for clarifying the molecular regulatory mechanism of Ca^{2+} release via cardiac Ca^{2+}-induced Ca^{2+} release channels.

2. Materials and methods

2.1. Materials

^{3}H-labeled MBED (Fig. 1) was synthesized as follows (Fang et al., 1993). Bromoeudistomin D (Kobayashi et al., 1989; Seino et al., 1990) was acetylated by treatment with acetic anhydride and methylated with [^{3}H]methyl iodide

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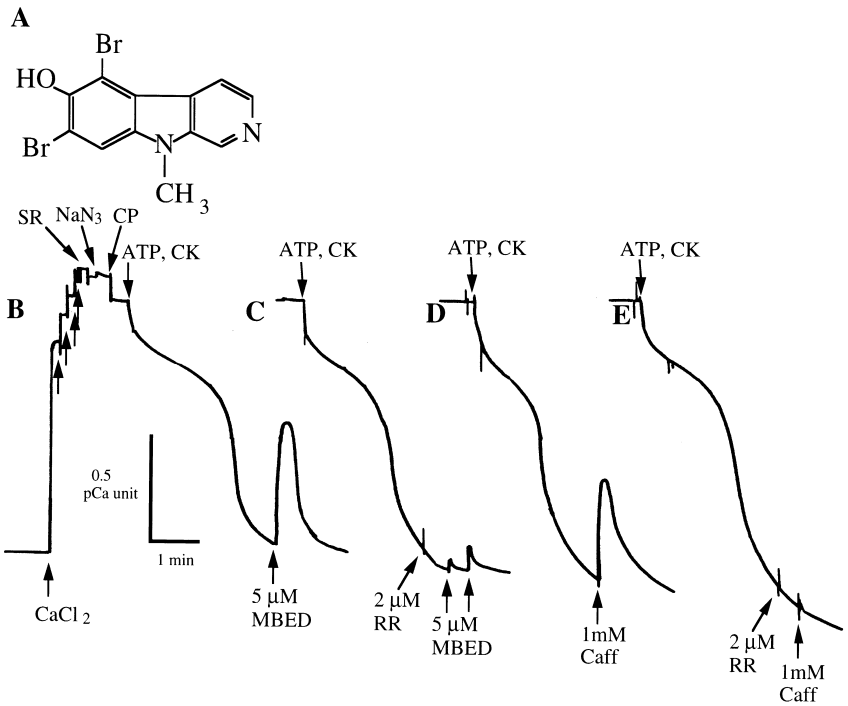

Fig. 1. Chemical structure of 9-methyl-7-bromoeudistomin D (MBED) (A) and typical recording traces of Ca^{2+} release induced by MBED or caffeine (Caff) from the cardiac sarcoplasmic reticulum vesicle in the presence or absence of ruthenium red (RR) in Ca^{2+} electrode experiments (B–E). Vertical calibration bars indicate responses for voltage change (10 mV) corresponding to 0.5 pCa unit. In C to E, the traces are those after addition of ATP and creatine kinase (CK). CP, creatine phosphate.

followed by saponification to give [^{3}H]MBED. [^{3}H]MBED was purified as reported previously. $^{45}CaCl_2$ (0.7 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Aprotinin, benzamidine and pepstatin A were from Sigma (St. Louis, MO, USA). Caffeine, ruthenium red, *p*-amidino-phenylmethanesulfonyl fluoride, dithiothreitol and iodoacetamide were from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Experimental animals

Experimental protocols were approved by the Animal Research Committee of Tohoku University. Male mongrel dogs (10–15 kg), male rabbits (Japanese white, 1.5–2.5 kg) and male guinea-pigs (Hartley, 300–400 g) were used. The animals were anesthetized by intravenous (dogs and rabbits, 30 mg/kg) or intraperitoneal (guinea pigs, 30 mg/kg) injection of pentobarbital sodium, and then the hearts were removed.

2.3. Preparation of cardiac sarcoplasmic reticulum

Cardiac sarcoplasmic reticulum vesicles were prepared from canine and rabbit cardiac muscle by the method of Harigaya and Schwartz (1969) with some modification. The homogenate of cardiac muscle in 3 volumes of 50 mM Tris–maleate (pH 7.0) was centrifuged at $2400 \times g$ for 20 min. The vesicles remaining in the supernatant were centrifuged at $9000 \times g$ for 20 min. The supernatant was further centrifuged at $35\,000 \times g$ for 40 min. The pellet fraction was suspended in a solution containing 50 mM KCl and 20 mM Tris–maleate (pH 7.0) and centrifuged at $35\,000 \times g$ for 40 min.

2.4. Ca^{2+} electrode experiments

The extravesicular Ca^{2+} concentration of the canine cardiac sarcoplasmic reticulum vesicle suspension was measured at 30°C with a Ca^{2+} electrode prepared as described previously by Nakamura et al. (1986). The Ca^{2+} electrode showed a Nernstian response (slope, 27–29 mV/pCa unit). The assay mixture contained 5 mM $MgCl_2$, 2 mM NaN_3, 0.05 mM $CaCl_2$, 90 mM KCl, 50 mM MOPS (3-(*N*-morpholino)-propanesulfonic acid)–KOH buffer (pH 7.0), 0.6 mg/ml cardiac sarcoplasmic reticulum, 5 mM creatine phosphate, 0.1 mg/ml creatine kinase and 1 mM ATP. The Ca^{2+} uptake reaction was started by simultaneous addition of ATP and creatine kinase.

2.5. Ca^{2+} efflux measurement

$^{45}Ca^{2+}$ efflux from rabbit cardiac sarcoplasmic reticulum vesicles passively preloaded with $^{45}Ca^{2+}$ was measured at 0°C as described previously (Seino et al., 1990). After a 12-h preincubation with 5 mM $^{45}CaCl^2$, the sarcoplasmic reticulum suspension was diluted with 100 volumes of an ice-cold reaction medium containing 0.4 mM $CaCl_2$, 1.9 mM EGTA, 90 mM KCl and 50 mM MOPS–KOH (pH 7.0). The aliquots (0.15 ml) of the diluted suspension were filtered through Millipore filters (HAWP type, 0.45 μm pore size) and washed three times with 0.5 ml of a solution containing 5 mM $LaCl_3$, 5 mM $MgCl_2$,

90 mM KCl and 50 mM MOPS–KOH (pH 7.0). The amount of $^{45}Ca^{2+}$ remaining in the sarcoplasmic reticulum vesicles was measured by counting the radioactivity of the washed filters.

2.6. Preparation of cardiac sarcoplasmic reticulum membranes

Cardiac sarcoplasmic reticulum membranes were prepared from guinea-pig cardiac muscle by the method of Tada et al. (1983) in the presence of protease inhibitors: 76.8 mM aprotinin, 0.1 mM *p*-amidino-phenylmethanesulfonyl fluoride, 0.8 mM benzamidine, 1 mM iodoacetamide and 0.7 μM pepstatin A. Nonspecific binding of [^{3}H]MBED to membranes from guinea-pigs was low compared with that to membranes from dogs or rabbits. The protein concentration was determined by using a protein assay kit (Bio-Rad) with bovine albumin as a standard.

2.7. [^{3}H]MBED binding assay

Cardiac sarcoplasmic reticulum membranes (200 mg/ml) were incubated with [^{3}H]MBED (50 nM) for 45 min at 0°C in a solution containing 0.3 M sucrose, 1 M NaCl, 2 mM dithiothreitol, 0.1 mM *p*-amidino-phenylmethanesulfonyl fluoride and 20 mM Tris–HCl (pH 7.3). The bound radioactivity was measured as described previously (Fang et al., 1993).

3. Results

3.1. Extravesicular Ca^{2+} concentration of the fragmented cardiac sarcoplasmic reticulum vesicle suspension

Upon the addition of 1 mM ATP, free Ca^{2+} concentrations decreased rapidly due to the formation of Ca–ATP complexes and further decreased gradually due to Ca^{2+} uptake by the sarcoplasmic reticulum (Fig. 1B). When the Ca^{2+} concentration was reduced to submicromolar levels, the apparent Ca^{2+} uptake slowed. At this point, the addition of 5 μM MBED or 1 mM caffeine to Ca^{2+}-filled sarcoplasmic reticulum induced immediate Ca^{2+} release followed by Ca^{2+} reuptake (Fig. 1B and D). The rate of Ca^{2+} reuptake was almost the same as that before the addition of MBED or caffeine. Pretreatment of sarcoplasmic reticulum with 2 μM ruthenium red (Fig. 1C and E) or 10 mM $MgCl_2$ (data not shown) blocked the effect of MBED and caffeine.

3.2. $^{45}Ca^{2+}$ efflux from the fragmented cardiac sarcoplasmic reticulum

The effects of MBED on $^{45}Ca^{2+}$-release from cardiac sarcoplasmic reticulum were measured at 0°C, a temperature at which the Ca^{2+} pump does not work. The amount of $^{45}Ca^{2+}$ remaining in the vesicle decreased after dilution of the suspension of sarcoplasmic reticulum preloaded passively with $^{45}CaCl_2$. The time course of the change in $^{45}Ca^{2+}$ content of the sarcoplasmic reticulum was plotted logarithmically as shown in Fig. 2A. The $^{45}Ca^{2+}$ efflux was accelerated markedly by MBED over the concentration range of 0.3–30 μM. Fig. 2B shows the concentration–response curve for MBED for $^{45}Ca^{2+}$ efflux. The EC_{50} value for $^{45}Ca^{2+}$ efflux was approximately 0.3 μM.

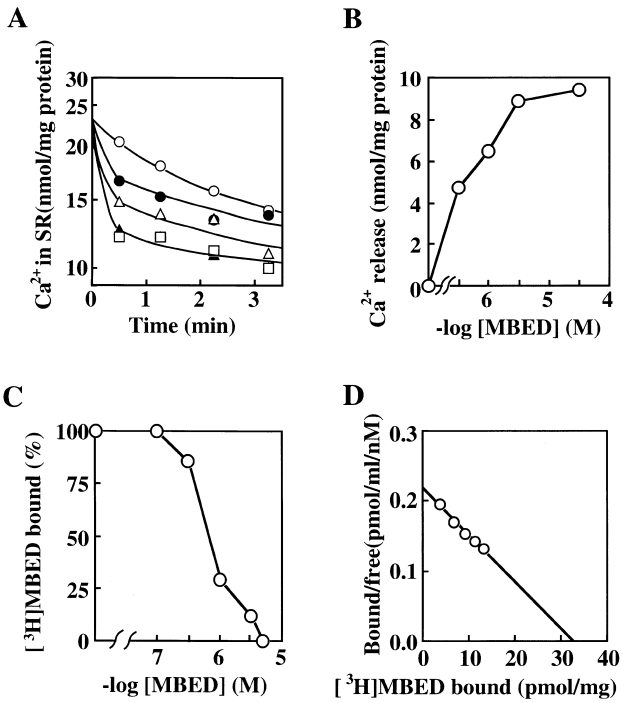

Fig. 2. Stimulatory effect of MBED on $^{45}Ca^{2+}$ efflux and [^{3}H]MBED binding in cardiac sarcoplasmic reticulum. (A) Time course of $^{45}Ca^{2+}$ release induced by different concentrations of MBED in the cardiac sarcoplasmic reticulum vesicle. (B) Concentration–response curve for MBED on $^{45}Ca^{2+}$ efflux from cardiac sarcoplasmic reticulum. (C) Effect of unlabelled MBED on [^{3}H]MBED binding to cardiac sarcoplasmic reticulum membranes. (D) Scatchard analysis of [^{3}H]MBED binding to cardiac sarcoplasmic reticulum membranes. In (A), the concentrations of MBED were 0 μM (○), 0.3 μM (●), 1 μM (△), 3 μM (▲) and 30 μM (□). Four preparations from different animals were used for each experiment (A–D).

3.3. [^{3}H]MBED binding to the cardiac sarcoplasmic reticulum membranes

[^{3}H]MBED binding to the cardiac membranes was inhibited by unlabeled MBED (Fig. 2C) with a IC_{50} value of 0.5 μM. The specific binding of [^{3}H]MBED to cardiac membranes was saturable and of high-affinity. Scatchard analysis showed that MBED bound to a high-affinity binding site with $K_d = 150$ nM and $B_{max} = 33$ pmol/mg (Fig. 2D).

4. Discussion

We have found that MBED induces marked Ca^{2+} release from the heavy fraction of fragmented skeletal muscle in a caffeine-like manner (Seino et al., 1990). Our previous studies also indicate that MBED shares a binding site with caffeine in skeletal muscle sarcoplasmic reticulum (Fang et al., 1993) or smooth muscle microsomes (Adachi et al., 1994). However, it remained to be determined whether the effect of MBED extends to cardiac or smooth muscle and brain tissue. In the present experiment, we have shown for the first time the Ca^{2+}-releasing action of MBED on the cardiac sarcoplasmic reticulum. In the Ca^{2+} electrode experiment, MBED, like caffeine (1 mM), induced Ca^{2+} release from the cardiac sarcoplasmic reticulum. This release was inhibited by typical inhibitors of Ca^{2+}-induced Ca^{2+} release channels such as ruthenium red or Mg^{2+}. The $^{45}Ca^{2+}$ efflux rate from cardiac sarcoplasmic reticulum was accelerated by MBED with an EC_{50} value of 0.3 μM. In the binding experiments reported here, [^{3}H]MBED bound to cardiac sarcoplasmic reticulum membranes in a replaceable and saturable manner, indicating the existence of a specific binding site. This site was of a single class with high affinity ($K_d = 150$ nM) and was comparable to that in skeletal muscle sarcoplasmic reticulum ($K_d = 40$ nM) (Fang et al., 1993) or in smooth muscle microsomes ($K_d = 50$ nM) (Adachi et al., 1994). A probable explanation for the results is that MBED binds to the specific binding site on cardiac sarcoplasmic reticulum membranes to cause Ca^{2+} release through cardiac Ca^{2+}-induced Ca^{2+} release channels.

It has been revealed that the ryanodine receptor is identical to the Ca^{2+}-induced Ca^{2+} release channels of sarcoplasmic reticulum (Ebashi, 1991). Genes encoding ryanodine receptors have been referred to as ryanodine receptor-1, ryanodine receptor-2 and ryanodine receptor-3. Ryanodine receptor-1 and ryanodine receptor-2 appear to be expressed predominantly in skeletal muscle and heart, respectively, while the ryanodine receptor-3 is expressed in brain, smooth muscle and epithelial cells (McPherson and Campbell, 1993). In this experiment, MBED and caffeine were demonstrated to activate cardiac Ca^{2+}-induced Ca^{2+} release channels, suggesting stimulation of ryanodine receptor-1 and ryanodine receptor-2 by both of the drugs. Ryanodine binds to cardiac sarcoplasmic reticulum preparations with a K_d value of 1–4 nM (Coronado et al., 1994; Meissner, 1994), which is much lower than that of MBED (150 nM). Furthermore, [^{3}H]ryanodine binding to cardiac sarcoplasmic reticulum membranes was not inhibited by MBED even at concentrations up to 10 μM (Ohizumi et al., unpublished data), suggesting that MBED binds to a different site from that of ryanodine. These observations suggest that the pharmacological action of MBED at the ryanodine receptor-2 is distinct from that of ryanodine. It has been reported that in smooth muscle Ca^{2+} release for activation of Ca^{2+}-activated K^+ channels is induced by MBED, whereas that for contraction is elicited by caffeine but not MBED, suggesting two distinct types of Ca^{2+}-induced Ca^{2+} release, only one of which is MBED sensitive (Imaizumi et al., 1996).

In conclusion, MBED has become an essential probe for clarifying the molecular properties of the caffeine-sensitive Ca^{2+}-release store of not only skeletal muscle but also cardiac or smooth muscle. A detailed investigation of the effects of modulators of Ca^{2+}-induced Ca^{2+} release channels on [^{3}H]MBED binding to cardiac sarcoplasmic reticulum is now in progress.

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