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Introduction

Ca^{2+} release from the sarcoplasmic reticulum (SR) plays a key role in excitation-contraction coupling (EC-coupling) in skeletal muscle[1,2]. It is well known that ryanodine, a plant alkaloid, promotes Ca^{2+} release from skeletal and cardiac SR and interferes with the inactivation of Ca^{2+}-induced Ca^{2+} release (CICR) from SR[3]. The alkaloid binds with high affinity to a receptor localized in the heavy fraction of SR (HSR)[4]. The purified ryanodine receptor[5,6] is identical in morphology with the "feet" structures to span the transverse tubule-SR junction and form caffeine-sensitive Ca^{2+} channels. It has been reported that ryanodine locks the Ca^{2+} release channels of SR in an open state and that its high affinity binding site is localized in terminal cisternae of SR[4]. These studies revealed that the ryanodine receptor is identical with CICR channels of SR[7]. One of the useful approaches to achieve a better understanding of the molecular mechanism of Ca^{2+} release is the application of specific drugs that affect the releasing mechanisms.

It has been reported that caffeine increases the Ca^{2+} sensitivity of CICR channels and the open probability of the channels at saturating Ca^{2+} concentrations. Numerous studies using skinned skeletal muscle fibres and isolated SR membrane preparations have revealed the presence of a caffeine-sensitive Ca^{2+} release pathway through CICR channels. However, the characterization of the caffeine receptor site in Ca^{2+} release channels has not been possible because of its low affinity and the detailed molecular mechanism of Ca^{2+} release from SR remains unresolved.

We have reported that bromoeudistomin D (BED), a derivative of eudistomin D isolated from the Caribbean tunicate Eudistoma olivaceum, induces Ca^{2+} release from HSR. Our pharmacological studies indicate that BED is approximately 500 times more potent than caffeine in Ca^{2+} releasing activity. For the purpose of finding the inhibitor in order to investigate the function of CICR channels, numerous analogues of BED were synthesized.

Materials and Methods

HSR was prepared from skeletal muscle of male rabbits as reported previously[8]. In order to estimate the Ca^{2+} releasing activity, the concentration of extravesicular Ca^{2+} in the HSR suspension was measured at 30°C with a Ca^{2+} electrode as described previously[9]. $^{45}Ca^{2+}$ release from HSR passively preloaded with $^{45}Ca^{2+}$ was measured at 0°C according to the method of Nakamura et al[10]. [^{3}H]MBED and [^{3}H]ryanodine binding experiments were performed by the method of Seino et al[9].

Results and Discussion

In the course of our survey of inhibitors of Ca^{2+}-induced Ca^{2+} release (CICR) in natural products and their derivatives, we have been succeeded in finding 4,6-Dibromo-3-hydroxycarbazole (DBHC) as a CICR inhibitors. The pharmacological properties of DBHC were examined. In Ca^{2+} electrode experiments, DBHC (10^{-4} M) markedly inhibited Ca^{2+} release from the heavy fraction of sarcoplasmic reticulum (HSR) induced by caffeine (1 mM) and BED (10^{-5} M). DBHC (10^{-4} M) abolished $^{45}Ca^{2+}$ release induced by caffeine (1 mM) and BED (10^{-5} M) in HSR. These results indicate DBHC to be a CICR inhibitors. As shown in Fig. 1b-1d, inhibitory effects of CICR blockers such as procaine, ruthenium red and Mg^{2+} on $^{45}Ca^{2+}$ release were clearly observed at Ca^{2+} concentrations from pCa 7 to pCa 5.5, and were decreased at Ca^{2+} concentrations higher than pCa 5.5 or lower than pCa 7. However, DBHC decreased $^{45}Ca^{2+}$ release induced by Ca^{2+} over the wide range of extravesicular Ca^{2+} concentrations (Fig. 1a). These results indicate that the inhibitory effects of procaine, ruthenium red and Mg^{2+} but not DBHC are suppressed at high Ca^{2+} concentrations and that DBHC is a novel type of CICR inhibitors having unique pharmacological properties. [^{3}H]Ryanodine binding to HSR was suppressed by ruthenium red, Mg^{2+} and procaine, but was not affected by DBHC up to 10^{-4} M. [^{3}H]Ryanodine binding to HSR was enhanced by caffeine and BED. DBHC antagonized the enhancement in a concentration-dependent manner. 9-[^{3}H]Methyl-7-bromo-eudistomin D, a [^{3}H]-labeled analogue of BED, specifically bound to HSR. Both DBHC and caffeine increased the Kd value without affecting the Bmax value, indicating a competitive mode of inhibition (Fig. 2). These results suggest that DBHC binds to the caffeine binding site to block Ca^{2+} release from HSR. This drug is a novel type of inhibitor for the CICR channels in SR and may provide a useful tool for clarifying the Ca^{2+} releasing mechanisms in SR.

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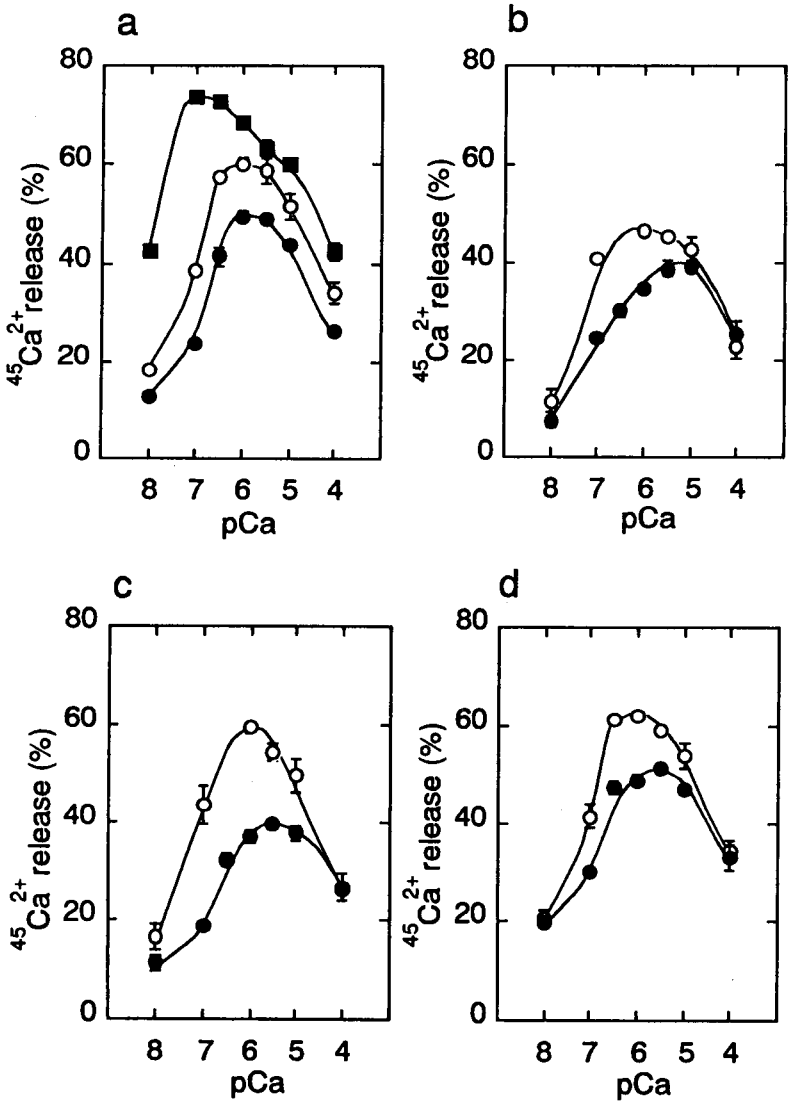

Fig. 1. Inhibitory effects of CICR inhibitors on $^{45}Ca^{2+}$ release at various Ca^{2+} concentrations. $^{45}Ca^{2+}$ release at various concentrations of free Ca^{2+} was measured during 1 min after dilution. Each value was normalized against the amount of $^{45}Ca^{2+}$ in HSR at zero time. (a) Control (○), 10^{-4} M DBHC (●), 10^{-5}M BED (■). (b) Control (○), 3 mM procaine (●). (c) Control (○), 30 nM ruthenium red (●). (d) Control (○), 3×10^{-5} M Mg^{2+} (●). Data are mean ± s.e.mean (n = 4).

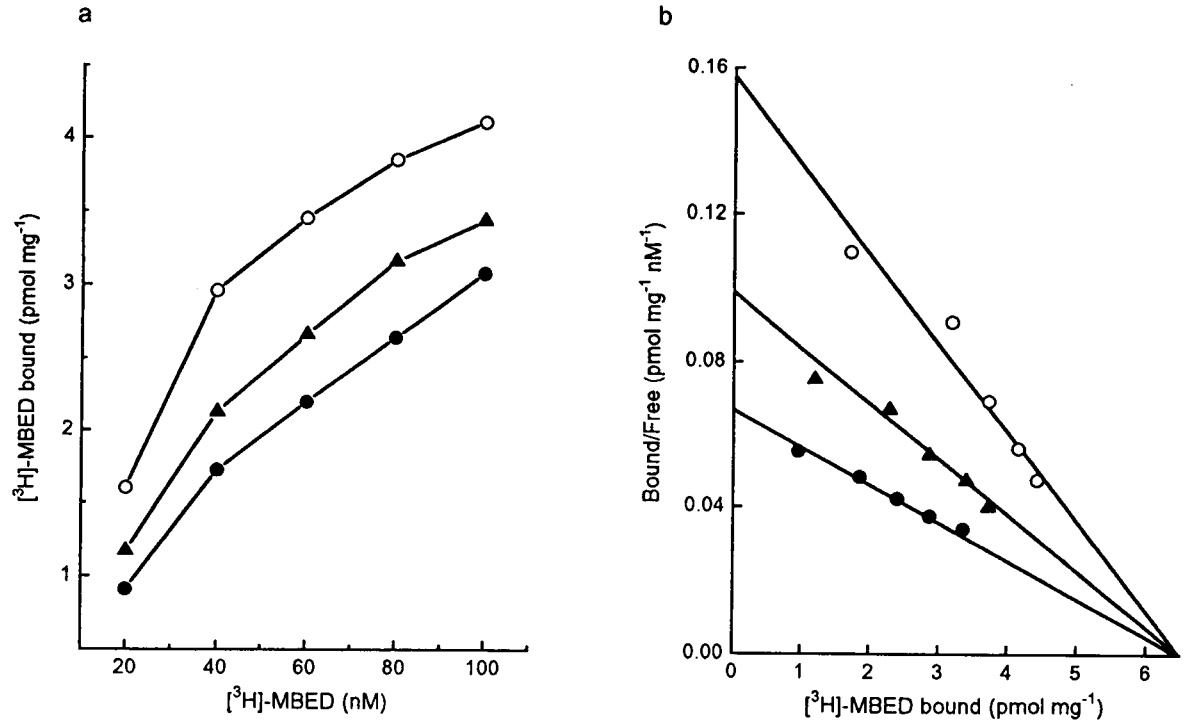

Fig. 2. Effects of DBHC and caffeine on [^{3}H]MBED binding. HSR (0.3 mg ml^{-1}) was incubated with increasing concentration of [^{3}H]-MBED from 20 to 100 nM for 45 min at 0°C. (a) [^{3}H]-MBED binding was measured in the presence or absence (○) of 0.05 mM DBHC (●) or 0.5 mM caffeine (▲) and is plotted. (b) [^{3}H]-MBED binding in (a) is presented as a Scatchard plot.