The Properties of Specific Binding Site of 125I-Radioiodinated Myotoxin α, a Novel Ca++ Releasing, agent in Skeletal Muscle Sarcoplasmic Reticulum

III. 10. The Properties of Specific Binding Site of ^{125}I-Radioiodinated Myotoxin *a*, a Novel Ca^{++} Releasing, agent in Skeletal Muscle Sarcoplasmic Reticulum

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Introduction

Myotoxin *a* (MYTX) isolated from prairie rattlesnakes (*Crotalus viridis viridis*) is a muscle-damaging polypeptide that is composed of 42 amino-acid residues including 3 disulfide bonds[1]. The electron-microscopic study has revealed that MYTX causes muscle degeneration and disturbance of the sarcoplasmic reticulum (SR) functions and muscle filaments2). Recently, we found that MYTX strongly induced Ca^{++} release from skeletal muscle SR with the EC_{50} value of 0.5 μM. The properties of MYTX-induced Ca^{++} release were different from those induced by the other Ca^{++}-releasing agents such as caffeine, MBED, a powerful caffeine-like Ca^{++} releaser, and adenosine 5'-(β,γ-methylene)triphosphate (AMP-PCP)[3].

In order to characterize the molecular mechanism of action of MYTX, we prepared ^{125}I-labeled MYTX (^{125}I-MYTX), and studied its binding to the heavy fraction of fragmented SR (HSR), in which the ryanodine receptor is enriched abundantly. Our results indicated that MYTX induced Ca^{++} release by binding to a specific site which is distinct from the ryanodine receptor. MYTX may be useful as a biochemical probe not only for clarifying the regulatory mechanism of physiological Ca^{++} release but also for purifying a novel type of Ca^{++} release channels or their regulatory proteins.

Materials and Methods

MATERIALS

HSR was prepared from rabbit skeletal muscle by the method of Kim et al.[4]. The ryanodine receptor was purified from skeletal muscle by the method of Shoshan-Barmatz and Zarka[5]. MBED was synthesized by the method described previously[6,7]. Chemicals used were obtained from following sources; crude venom of prairie rattlesnakes (*Crotalus viridis viridis*) from Sigma; ryanodine from S.B. Penick Company; $^{45}CaCl_2$ (25.9 Bq/pmol), ^{3}H-

ryanodine (2.22 kBq/pmol) and Na^{125}I (95.3 kBq/pmol) from Du-Pont New England Nuclear; All other chemicals were of reagent grade.

METHODS

MYTX (Fig. 1) was purified as described previously[2]. ^{125}I-Labeling of MYTX was performed by the chloramine T method. ^{125}I-MYTX binding was measured by the method of Penefsky[8] with a slight modification. ^{3}H-Ryanodine binding was measured as described previously[7]. The extravesicular Ca^{++} concentration was monitored with a Ca^{++} electrode prepared by the method of Nakamura et al.[9]. $^{45}Ca^{++}$ release from HSR passively preloaded with 45Ca++ was measured at 0°C as described previously[9] with a slight modification.

Results and Discussion

It was found that MYTX is a powerful Ca^{++} releaser in the HSR. ^{125}I-MYTX with high Ca^{++}-releasing ability was successfully prepared. It specifically bound to a single class of binding sites in HSR with a K_D of 0.4 μM and B_{max} of 6 nmol/mg protein (Fig.1). ^{125}I-MYTX binding was markedly inhibited by Na^{+} and K^{+}, whereas that was little affected by Ca^{++} and Mg^{++}. The binding activity was markedly decreased by spermine, a blocker of Ca^{++} release channels, and was not affected by the other modulators of Ca^{++} release such as caffeine, procaine or ruthenium red (Table 1). Spermine decreased the binding in a concentration-dependent manner with the IC_{50} value of 20 μM. Scatchard analysis of ^{125}I-MYTX binding indicated that the Bmax value was decreased by spermine, while the K_D value was not changed, indicating a noncompetitive mode of inhibition. ^{125}I-MYTX did not bind to the purified ryanodine receptor. Ca^{++} electrode experiments indicated that MYTX induced Ca^{++} release from HSR at 0.1 μM or more, which was abolished by spermine. The maximal response to MYTX (10 μM) was further increased by caffeine (10 μM) in $^{45}Ca^{++}$ release, probably indicating that the effects of MYTX and caffeine are synergistic in Ca^{++}-releasing action (Fig. 2). These results suggest that MYTX binds to an important regulatory protein of Ca^{++} release which is not the ryanodine receptor.

References

1) Fox J. W. et al., Biochemistry **18** (1979) 678.
2) Cameron D. L. and Tu A. T., Biochemistry **224** (1977) 89.
3) Funayama K. et al., Jpn. J. Pharmacol. **58** (1992) suppl. I, 208P.
4) Kim D. H. et al., J. Biol. Chem. **258** (1983) 9662.
5) Shoshan-Barmatz V. and Zarka A., Biochem. J. **285** (1992) 61.
6) Kobayashi J. et al., J. Pharm. Pharmacol. **40** (1988) 62.
7) Seino A. et al., J. Pharmacol. Exp. Ther. **256** (1991) 861.
8) Penefsky H. S., Methods Enzymol. **56** (1979) 527.
9) Nakamura Y. et al., J. Biol. Chem. **261** (1986) 4139.

Table 1. Effects of various modulators of Ca^{++} release on ^{125}I-MYTX binding to HSR. HSR vesicles (50 μg/ml) were incubated with ^{125}I-MYTX (0.3 μM) at 0° C for 60 min in the presence or absence (control) of various modulators. Values, expressed as percents of control, are means ± S.E.M. (n = 3). Significantly different from control: ** ($p < .01$).

Modulators	^{125}I-MYTX Bound
	% of Control
Control	100.0 ± 2.63
Caffeine (2 mM)	93.5 ± 10.10
MBED (10 μM)	106.5 ± 1.36
AMP-PCP (100 μM)	100.1 ± 6.22
Inositol (1,4,5)trisphosphate (10 μM)	104.1 ± 2.80
Procaine (10 mM)	113.4 ± 5.93
Ruthenium red (1 μM)	96.2 ± 2.66
Spermine (100 μM)	31.0 ± 3.78*
Dantrolene (10 μM)	98.8 ± 3.22

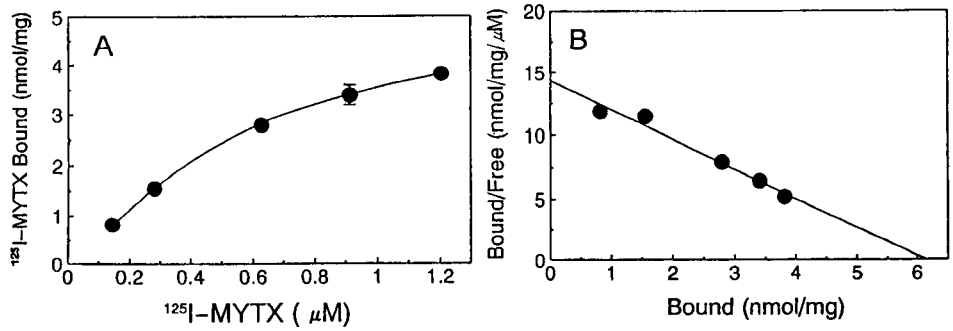

Fig. 1. Saturation (A) and Scatchard (B) plots of ^{125}I-MYTX binding to HSR. HSR (50 μg/ml) was incubated with ^{125}I-MYTX at various concentrations at 0°C for 60 min. Values are means with S.E.M. (n=3).

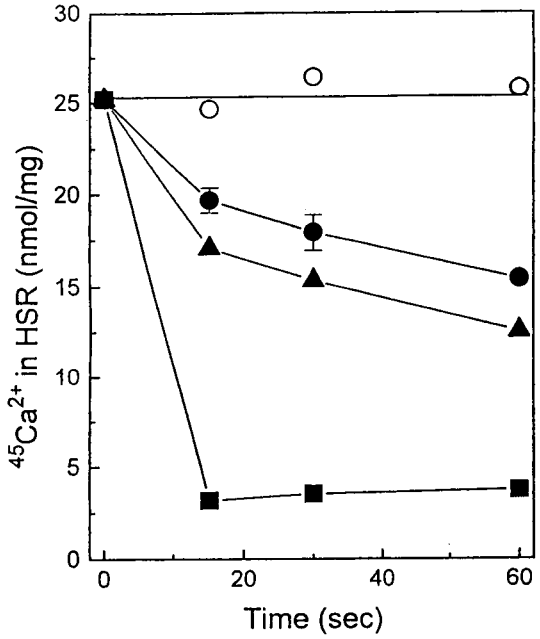

Fig. 2. Interrelations between the Ca^{++}-releasing activities of MYTX and caffeine in $^{45}Ca^{++}$ release experiments on HSR. The $^{45}Ca^{++}$ content of HSR was measured at 0° C by the filtration method. Values are means with S.E.M. (n = 3). Control (○); MYTX (10 μM) (●) ; caffeine (10 mM) (▲); MYTX (10 μM) plus caffeine (10 mM) (■).