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Ca-release Channel of the Sarcoplasmic Reticulum of the Snake (Reptile) Skeletal Muscle

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ABSTRACT

To investigate properties of Ca-release channel in the reptile skeletal muscle, electrophoretical analysis, purification of RyR, [^{3}H]ryanodine binding study, and ^{45}Ca-release were carried out in the SR vesicles prepared from the snake skeletal muscle.

The snake SR vesicle has the single high molecular weight protein band on SDS-PAGE, and its mobility was similar with that of rat skeletal SR vesicles. The high molecular weight band on SDS-PAGE was found in the [^{3}H]ryanodine peak fractions (Fr_{5-7}) obtained from the purification step of the RyR. Maximal binding site and Kd of the snake SR RyR were 6.36 pmole/mg protein and 17.62 nM, respectively. Specific binding of [^{3}H]ryanodine was significantly increased by calcium and AMP ($P<0.05$), but not or slightly inhibited by tetracaine, ruthenium red (5.4%), or $MgCl_2$ (21%).

^{45}Ca-release from the SR vesicles loaded passively was significantly increased by the low concentration of calcium (1~10 μM) and AMP (5 mM)($P<0.05$), but significantly decreased by the high concentration (300 μM) of calcium, tetracaine (1 mM), ruthenium red (10 μM), and $MgCl_2$ (2 mM)($P<0.05$).

From the above results, it is suggested that snake SR vesicles also have the RyR showing the similar properties to those of mammalian skeletal RyR with the exceptions of no or slight inhibition of [^{3}H]ryanodine-binding by tetracaine, ruthenium red, or $MgCl_2$.

Key Words: Ca-release channel, Ryanodine receptor, Sarcoplasmic reticulum, Snake

Abbreviations: AMP, Adenosine-5'-monophosphate; DIFP, Diisopropyl fluorophosph-ate; EGTA, Ethylene glycol-bis (β-aminoethyl ether) N,N,N',N'-tetraacetic acid; PMSF, Phenylmethylsulfonyl fluoride; Chaps, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate; RyR, Ryanodine receptor; EDTA, Ethylenediamine tetraacetic acid; PAGE, Polyacrylamide gel; SDS, Sodium dodecyl sulfate

INTRODUCTION

Skeletal muscle contraction is essential for the movement and for the maintenance of specific pose of animals. One of the important regulatory factors that control it is the fluctuation of intracelluar calcium concentration. The resting concentration of free Ca^{2+} in the cytosol of any cell is extremely low ($\leqq 10^{-7}$~10^{-8} mol/L), compared with extracellular fluid (~10^{-3} M), but when the depolarization of SR in response to neuronal stimulus drives Ca^{2+} from extracellular fluid or intracellular repository into cytosol, arriving on a certain level, it is known that muscle contraction is triggered. Particularly as skeletal muscle contraction is maintained for long time, even when Ca^{2+} outside of skeletal muscle cell membrane is removed, it is

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admitted that the Ca^{2+} release from intracellular storing place is a more crucial contributor to the intracellular Ca^{2+} required for contraction (Ebashi 1976; Endo 1977), however, how this signal from skeletal muscle membrane to SR is transduced is not elucidated exactly. Many studies of SR Ca^{2+} release just indicate that there exist Ca^{2+}-release channels (ryanodine receptor=RyR) (Cadwell and Caswell 1982; Campbell *et al.*, 1987; Lai *et al.*, 1987; Lai *et al.*, 1988; Smith *et al.*, 1985).

Ca^{2+}-release channels are placed in SR membrane adjacent to T-tubules formed from invagination of plasma membrane and consist of four 565 kD polypeptides in the forms of homotetramer or two homooligomer (Fleischer and Inui 1989; Lai *et al.*, 1982; Lai *et al.*, 1989), which however exhibit a little difference in electrophoretic mobility. According to the recent studies, RyRs have several kinds of isoform, its distribution is different from each tissues (skeletal muscle, cardiac muscle, brain) (Lai *et al.*, 1988; Smith *et al.*, 1985; Meissner and Henderson 1987; McPherson *et al.*, 1991), it is assumed that they have isoforms varying with species functionally and immunologically (Airey *et al.*, 1993; Seok *et al.*, 1992, 1994), and found out that there exist three genes (ryr-1, ryr-2, and ryr-3) for them (McPherson and Campbell 1993).

In functional aspect, SR Ca^{2+} release of mammalian skeletal muscle is activated by intracellular Ca^{2+} and adenine nucleotides and inhibited by Mg^{2+}. Among these, $[Ca^{2+}]_i$ stimulates the Ca^{2+} release in $\sim\mu M$ concentration level, but depresses it in increased $[Ca^{2+}]_i$ level (Meissner 1984; Meissner *et al.*, 1986). RyR of cardiac muscle has similar characteristics with that of skeletal muscle, but it is activated primarily by Ca^{2+} introduced from extracellular fluid and releases Ca^{2+} more rapidly than that of skeletal muscle (Meissner and Henderson 1987). However, it was reported that RyR of invertebrate lobster skeletal muscle is stimulated by Ca^{2+} much in higher concentration and immunologically it does not react with that of vertebrate skeletal muscle (Seok *et al.*, 1992). Besides both in fishes and in amphibian, the properties of RyR had been already reported (Seok *et al.*, 1995; Lamb and Stephenson 1990), however, in reptiles there is no report on the existence of the calcium release channel and its functional properties.

Therefore to investigate the properties of Ca-release channel in the SR of the reptile skeletal muscle, we carried out electrophoretical analysis, purification of RyR, [^{3}H]ryanodine binding study, and Ca^{2+} induced ^{45}Ca-release in the SR vesicles of the snake skeletal muscle.

MATERIALS AND METHODS

Isolation of SR vesicles

Isolation of SR vesicles was followed as described by Seok *et al.*, (1995). After killing snake, and removing the snake skin, about 40 g of the snake skeletal muscle was excised, minced, and homogenized with a Polytron (kinematica) of for 90 sec (3×30 sec) at the middle setting in 7.5 volumes of the medium containing 0.1 M NaCl, 20 mM K/Pipes, pH 6.8, 0.1 mM EGTA, 1 mM EDTA, 0.2 mM PMSF, 10 mM aprotinin, 1 μM leupeptin, 1 μM pepstatin, 1 mM benzamidine and 1 mM iodoacetate. The homogenates were centrifuged at 2500 ×g for 30 min (Sorvall; GSA rotor) and the resulting supernatant was passed through three layers of cheesecloth and then centrifuged for 30 min at 33,500×g (Sorvall ultracentrifuge; T-865 rotor). Pellets were resuspended in a 0.6 M KCl solution (containing 0.6 M KCl, 20 mM K/Pipes, pH 7.0, 100 μM EGTA, 75 μM $CaCl_2$, 0.2 mM PMSF and 1 μM leupeptin) incubated for 30 min in ice and sedimented by centrifugation for 30 min at 126,000×g (T865 rotor). After resuspension of the pellets in 20 ml of 0.6 M KCl solution, membranes were loaded onto 40%, 30% and 20% discontinuous sucrose gradients in medium of 0.6 M KCl solution and centrifuged for 16 hr at 121,500×g (Sorvall; AH 629 rotor). Membrane sedimenting between the 40% and 30 % sucrose region of the gradients were collected, slowly diluted with 2 volumes of cold water, pelleted by centrifugation for 30 min at 126,000×g (T865 rotor) and then resuspended in 0.3 M sucrose, 5 mM K/Pipes, pH 7.0 and stored in ~0.25 ml aliquots at −70℃.

SDS-polyacrylamide gel electrophoresis and isolation of RyR

SR vesicles were analysed in SDS-polyacrylamide gel electrophoresis which was performed in the Laemmli buffer system (Laemmli 1970) using 3~12% linear polyacrylamide running gels and 3% stacking gels. About 50~100 mg of SR of skeletal muscle of rat and snake and of canine cardiac muscle were treated by sample buffer, boiled for 3 min, and then electrophoresed for 16 hrs. After electrophoresis the gel was fixed in the solution of 12% trichloroacetic acid and were stained by colloidal Commassie Blue staining method (Neuhoff *et al.*, 1988).

On the other hand, to confirm which band binds to [^{3}H]ryanodine, they were purified by rate density gradient centrifugation as described by Lai *et al.*, (1988). Snake SR vesicles (1.2 mg/ml) were solubilized in the solution of 1.5% Chaps (1 M NaCl, 20 mM Na/Pipes, pH 7.4, 100 μM EGTA, 500 μM $CaCl_2$, 5 mM AMP, 5 mg/ml phosphatidylcholine, 100 μM dithiothreitol, and 1 μM leupeptin with or without ~3 nM [^{3}H]ryanodine) for 1 hr at room temperature and for 1hr at 4℃, loaded on 7~20% linear sucrose gradient, and centrifuged for 16 hrs at 121,500× g (Sorvall, AH 629 rotor). After centrifugation they were separated into 16 fractions, 2 ml of each, from the bottom. To find the peak [^{3}H] ryanodine radioactivity among them, 50 μl of each fraction was removed into plastic vial containing scintillation cocktail, and counted with liquid scintillation counter (Packard). Unlabeled solubilized solution of snake SR vesicles were also fractionated by the same way as in labeled solubilized solution and electrophoresed.

[^{3}H]ryanodine binding

[^{3}H]ryanodine binding was followed essentially as described by Seok *et al.*, (1994). After SR vesicles (30~50 μg) were incubated for 2 hr at 20℃ in high concentration of KCl solution (1 M KCl, 20 mM K/Pipes, pH 7.4, 0.1 mM $CaCl_2$ 5 mM AMP, 0.2 mM PMSF, 2 μM leupeptin, 2 mM dithiothreitol and 2~64 nM [^{3}H]ryanodine diluted with 40 volumes of cold water, filtered through Whatman filter (GF/B, pore size 1 μm) soaked with 2% polyethylenimine, and washed with cold water (5 ml×3). The washed filter papers were put into plastic vial containing scintillation cocktail, and their radioactivity were determined by liquid scintillation counter (Packard). Nonspecific binding was estimated using a 1,000-fold excess of unlabeled ryanodine. The specific binding (B) in each concentration was the value measured by subtracting nonspecific binding from the total binding and the value of Kd and Bmax of ryanodine binding were determined by applying to the equation, $B = Kd \times B/F + Bmax$ (F=[^{3}H]ryanodine free concentration). Another binding experiments were carried out in the same way as described above after adding 20 nM [^{3}H]ryanodine in which the effects of Ca^{2+} (1~300 μM), AMP (5 mM), tetracaine (1 mM), ruthenium red (10 μM) and $MgCl_2$ (1 mM) on [^{3}H]ryanodine binding of Ca^{2+}-release channel were examined.

$^{45}Ca^{2+}$-release in Snake SR vesicles

The SR vesicles of snake skeletal muscle (~3 mg) were preincubated for 30 min in the ice in the solution of 0.1 M KCl [0.1 M KCl, 20 mM K/Pipes, pH 7.0, 1 μM leupeptin, 0.2 mM PMSF, 0.1 mM EGTA and 0.1 mM $CaCl_2$] and centrifuged for 30 min at 121,500×g (AH 629 rotor). The pellet was resuspended in a small volume of 0.1 M KCl solution. 5 μl of the vesicle suspension was incubated with 5 μl of 2 mM ^{45}Ca, which was made in the solution of 0.1 M KCl, for 2 hrs at 20℃. After incubation, ^{45}Ca-release was initiated by diluting vesicles 100-fold into isoosmolar efflux or rinse media and was stopped at 25 sec and 60 sec by filtrating 0.4 ml aliquot on a filter paper (Gelman GA/6; pore size=0.45 μm). Vesicles on filters were washed manually three times with 1 ml of rinse media. Filters washed were put into plastic vials with scintillation cocktail and the radioactivity remaining in the vesicles on filters was determined by liquid scintillation counter. Extravesicular calcium of efflux media was adjusted to various concentrations by Fabiato's computer programs (1988) and Ca-release stimulants (AMP) or inhibitors ($MgCl_2$, ruthenium red, or tetracaine) were added to efflux media. Rinse media contained 0.1 M KCl, 20 mM K/Pipes, pH 7.0, 2 mM EGTA, 10 μM ruthenium red and 2 mM

$MgCl_2$.

Materials

$^{45}CaCl_2$ (10.5 mCi/ml) and [^{3}H]ryanodine were purchased from Dupont-New England Nuclear, AMP, Protease inhibitors and SDS-molecular weight markers from Sigma. All other chemicals were of analytical grade. The significance of the results were examined using student *t*-test.

RESULTS

Identification of RyR

SDS gel analysis of Chaps-solubilized, purified fractions of SR vesicles of snake skeletal muscle was shown in figure 1. It was found that snake SR vesicles had a high molecular weight band (lane 3) which had a similar mobility with the band (lane 2) of RyR of mammalian skeletal muscle, and a little slower mobility than SR vesicles of canine cardiac muscle. When radioactivity of each of 16 fractions obtained in purification procedures with [^{3}H] ryanodine was measured, peak was appeared in fraction numbers from 5 to 7 (Fr_{5-7})(Fig. 2 Panel A), and high molecular weight protein band was found in the same fractions obtained in purification steps without [^{3}H]ryanodine (Fig. 2 Panel B).

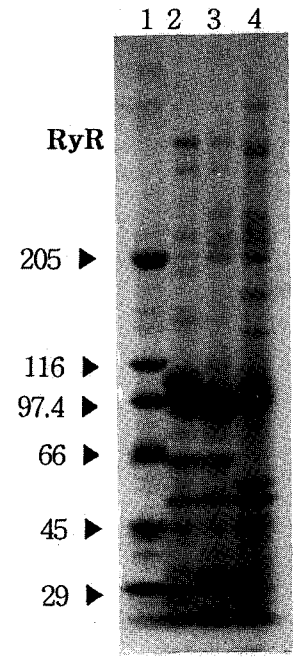

Fig. 1. SDS-gel of SR vesicles of rat, snake skeletal and canine cardiac muscle. SDS-polyacrylamide gel (3~12%) electrophoresis of molecular weight standard (lane 1), rat (lane 2), snake (lane 3), and canine (lane 4). Molecular weight values ($\times 10^{-3}$) of standard protein s are indicated on the left. RyR=ryanodine receptor.

[^{3}H]ryanodine binding to SR vesicles

[^{3}H]ryanodine binding of snake SR vesicles was increased by the increase of [^{3}H]ryanodine concentration, that is nearly saturated binding in concentration of 100 nM, which was confirmed by Scatchard analysis. This analysis data was summarized in Fig. 3 and Table 1 indicating that the maximum value of specific high affinity [^{3}H]ryanodine binding of snake SR vesicles was 6 pmole per mg protein of SR vesicles and the value of Kd was 18 nM. These results indicate that ryanodine receptors actually exist in the SR of the snake, reptile, skeletal muscle. Non specific [^{3}H]ryanodine binding to the SR vesicles was low to the level of 3~6%.

Elevation of free Ca^{2+} concentration 1 μM to 10 μM in the 20 nM [^{3}H]ryanodine binding to SR vesicles increases the amount of specific binding significantly from 0.423±0.042 pmole/mg protein to 0.697±0.052 pmole/mg protein. In the case of elevation of free Ca^{2+} concentration to 300 μM, the specific binding was increased to 0.717±0.064 pmole/mg protein significantly compared to that in 1 μM Ca^{2+} concentration ($P < 0.005$). When 5 mM AMP was added to reaction solution, the amount of binding was increased by 50~73%, compared to that in the presence of calcium only (Table 2). When the effects of tetracaine, ruthenium red or $MgCl_2$ on the specific binding amount (1.237±0.106 pmole/mg protein) in the presence of 300 μM Ca^{2+} and 5 mM AMP were observed, tetracaine didn't affect on that, and ruthenium red and $MgCl_2$ inhibited by the degree of 5.4% and 21% respectively, but it was not significant statisti-

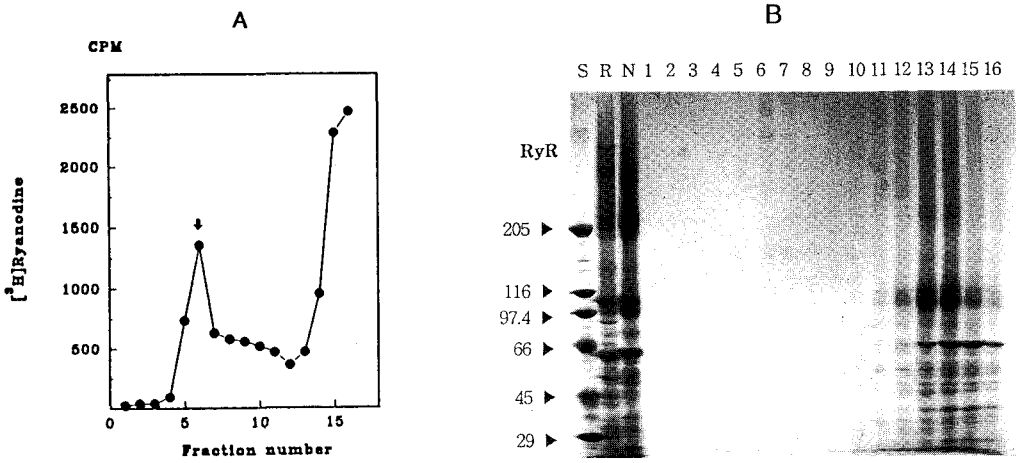

Fig. 2. Sedimentation profile and SDS analysis of Chaps-solubilized, purified snake RyR. A, snake SR vesicles (1.2 mg of protein/ml) were solubilized with Chaps (1.5%) in a medium containing 1.0 M NaCl, 20 mM Na/Pipes, pH 7.4, 100 μM EGTA, 500 μM $CaCl_2$, 5 mM AMP, 5 mg/ml phosphatidylcholine, 100 μM dithiothreitol, 1 μM leupeptin, 200 μM PMSF, and 3 nM [^{3}H]ryanodine. The solubilized proteins were loaded onto a linear 7~20% sucrose gradient in the above medium containing 1% Chaps and centrifuged at 4℃ in a Sorvall AH629 rotor for 16h at 26,000 rpm. 16 fractions of 2 ml were collected and analyzed for [^{3}H]ryanodine radioactivity. The arrow indicates the position of [^{3}H]ryanodine peak fraction. B, SDS-polyacrylamide gel electrophoresis of molecular weight standard (lane S), rat skeletal (lane R), snake skeletal (lane N) vesicles (30 g of protein each), and purified fractions (Fr1~16= lane 1~16) without [^{3}H]ryanodine. Molecular weight values ($\times 10^{-3}$) of standard proteins are indicated on the left.

Table 1. Ryanodine binding sites and K_D values of SR vesicles of the snake skeletal mucle

Bmax (pmole/mg protein)	K_D (nM)
6.36±0.19	17.62±0.03

Data(mean±SE) were obtained from Scatchard analysis of 8 binding experiments. [^{3}H]ryanodine binding to the SR vesicles of the snake skeletal muscle was done for 2 h at 20 oC in the solution containing 1 M KCl, 20 mM K/Pipes, pH 7.4, 100 μM $CaCl_2$, 5 mM AMP, 0.2 mM PMSF, 100 μM dithiothreitol, 1 μM leupeptin, and 2~64 nM [^{3}H]ryanodine. Nonspecific binding was assessed using a 1,000-fold excess of unlabeled ryanodine.

Table 2. [^{3}H]Ryanodine binding to SR vesicles of snake skeletal muscle

Specific binding of [^{3}H]ryanodine in the presence of Ca^{2+}		
1	100	300 μM
0.423±0.042	0.697±0.052*a	0.0717±0.064*a
+5 mM AMP		
0.633±0.091	1.227±0.081*b,c	1.237±0.106*b,c
		pmole/mg protein

[^{3}H]Ryanodine binding was carried out for 1 hr at 20℃ in the medium containing 1 M KCl, 20 mM K/Pipes, pH 7.0, 200 μM PMSF, 100 μM dithiothreitol, 1 μM leupeptin, and 20 nM [^{3}H]ryanodine in the various concentrations of $CaCl_2$ with or without 5 mM AMP. Nonspecific binding was ass-essed using a 1,000-fold excess of unlabeled ryanodine. *a, c: Significantly different from the corresponding value of 1 M Ca^{2+} ($P<0.005$). *b: Significantly different from the corresponding value of each concentration of Ca^{2+} ($P<0.005$).

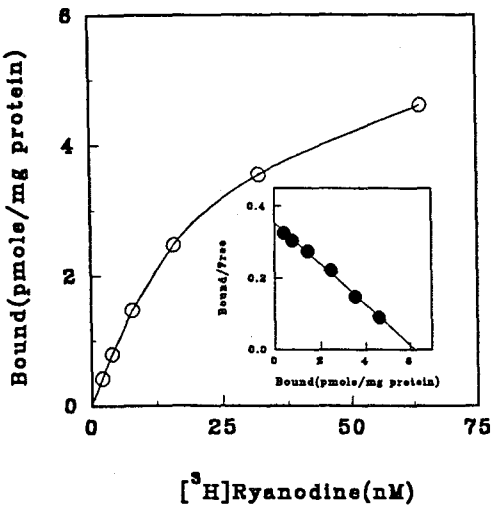

Fig. 3. Specific binding of [³H]ryanodine to snake SR vesicles.
[³H]Ryanodine binding was carried out in the medium containing 1 M KCl, 20 mM K/Pipes, pH 7.4, 100 μM $CaCl_2$, 5 mM AMP, 200 μM PMSF, 100 μM dithiothreitol, 1 M leupeptin, and 2~64 nM [³H]ryanodine. Nonspecific binding was assessed using a 1000-fold excess of unlabeled ryanodine. Inset; Scatchard plot of the saturation data.

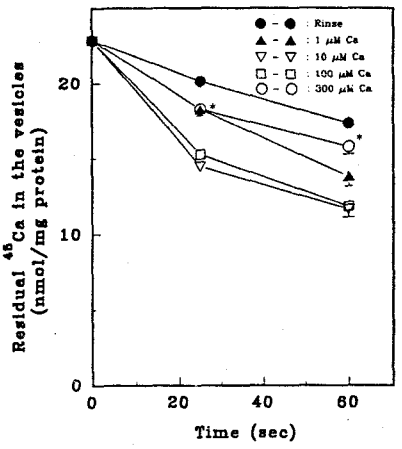

Fig. 4. Ca-induced ^{45}Ca release from the snake SR vesicles. ^{45}Ca was loaded into SR vesicles by passive diffusion for 2 hrs at 20℃ in the medium containing 0.1 M KCl, 20 mM K/Pipes, pH 7.0, and 1 mM ^{45}Ca. Release was started with the addition of rinse or release solution, and stopped with filtration through GA/6 filter at 25 and 60 sec. Rinse contianed 0.1 M KCl, 20 mM K/Pipes, pH 7.0, 2 mM EGTA, 10 μM ruthenium red, and 2 mM $MgCl_2$. Free calcium concentration in the release solution was changed by Fabiato's computer program (1988). *: Significantly different from the corresponding value of 10 μM Ca ($P<0.001$).

cally (Table 3).

Ca^{2+} release from SR vesicles

To investigate the regulation of snake skeletal SR Ca^{2+}-release channel, ^{45}Ca efflux behavior of passively loaded SR vesicles was studied. Fig 4 shows the residual ^{45}Ca in the vesicles at 25 and 60 sec after being diluted into media that contained varying concentrations of free Ca^{2+} (1 to 300 μM). When they are released in free Ca^{2+} rinse solution, the residual ^{45}Ca was 20.10 $\pm$0.03 and 17.35$\pm$0.37 nmole/mg protein at 25 and 60 sec respectively, but the addition of 10 μM Ca^{2+} in efflux media increased ^{45}Ca-release to 14.48$\pm$0.14 and 11.67$\pm$0.28 nmole/mg protein, significantly ($P<0.005$). On the other hand, the elevation of Ca^{2+} concentration in efflux media to 300 μM decreased the amount of release to 18.25$\pm$0.41 and 13.83$\pm$0.63 nmole/mg protein significantly, comparing to that in 10 μM of Ca^{2+}($P<0.05$). When 5 mM AMP was added to the ef-

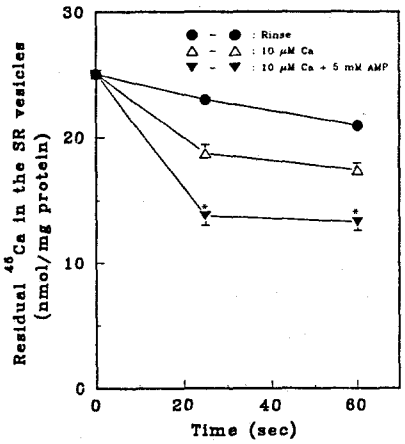

Fig. 5. Effect of AMP on the Ca-induced ^{45}Ca-release from the snake SR vesicles. *: Significantly different from the corresponding value of 10 μM Ca ($P<0.005$).

Table 3. Effect of inhibitors for calcium release channel on [^{3}H]ryanodine binding to the SR of snake skeletal muscle

Specific binding of [^{3}H]ryanodine in the presence of 300 μM Ca+5 mM AMP			
Cantrol	1 mM Tetracaine	10 μM Ruthenium red	1 mM $MgCl_2$
1.237±0.106	1.247±0.058	1.170±0.055	0.977±0.064
			pmole/mg protein

[^{3}H]Ryanodine binding was carried out for 1 hr at 20℃ in the medium containing 1 M KCl, 20 mM K/Pipes, pH 7.0, 300 μM $CaCl_2$, 5 mM AMP, 200 μM PMSF, 100 μM dithiothreitol, 1 μM leupeptin, and 20 nM [^{3}H]ryanodine with or without SR Ca-channel inhibitors (tetracaine, ruthenium red or $MgCl_2$).

Table 4. Effect of some inhibitors on the ^{45}Ca-release form passively loaded SR vesicles of the snake skeletal muscle

		Residual ^{45}Ca in the SR vesicles after releasing at	
		25	60 sec
	Rinse	22.05±0.50	19.96±0.39
A	10 μM Ca	17.70±0.44	16.13±0.36
	10 μM Ca+2 mM $MgCl_2$	20.27±0.45*	18.59±0.42*
	Rinse	22.82±0.51	20.65±0.40
B	10 μM Ca	17.35±0.45	16.73±0.37
	10 μM+10 μM Ruthenium Red	22.5±0.42*	21.49±0.58*
	Rinse	23.58±0.91	20.67±1.29
C	10 μM Ca	18.06±0.76	15.09±0.81
	10 μM Ca+1 mM Tetracaine	22.40±0.83*	18.22±0.75*
			nmole/mg protein

^{45}Ca was loaded into SR vesicles by passive diffusion for 2 hrs at 20℃ in the medium containing 0.1 M KCl, 20 mM K/Pipes, pH 7.0, and 1 mM ^{45}Ca. Release was started with the addition of rinse or efflux media, and stopped with filtration through GA/6 filter at 25 and 60 sec. Rinse contained 0.1 M KCl, 20 mM K/Pipes, pH 7.0, 2 mM EGTA, 10 μM ruthenium red, and 2 mM $MgCl_2$. Tetracaine was added into ^{45}Ca loading solution in 30 min after starting incubation and efflux media, and $MgCl_2$ and ruthenium red was only added into efflux media. Data (mean±SE) were obtained from 4 (A and B) ~6 (C) experiments. *: Significantly different from the corresponding value of 10 μM Ca ($P< 0.05$).

flux media containing 10 μM of Ca^{2+}, Ca^{2+}-induced ^{45}Ca release was increased apparently (Fig. 5)($P<0.005$).

To observe the effect of some Ca-release channel blocking agents ($MgCl_2$, ruthenium red, or tetracaine) on the ^{45}Ca-release from SR vesicles, they were added to efflux media containing 10 μM Ca^{2+}, respectively. 2 mM $MgCl_2$ inhibited ^{45}Ca release by 10 μM Ca^{2+}, significantly (Table 4)($P<0.05$), 10 μM ruthenium red also inhibited the ^{45}Ca-release by 10 μM Ca^{2+} efflux media, significantly (Table 4)($P<0.05$), and when 1 mM tetracaine was added to 10 μM Ca^{2+} efflux media, 10 μM Ca^{2+}-induced ^{45}Ca-release was significantly inhibited (Table 4)($P<0.05$).

DISCUSSION

This study describes the presence and functional properties of Ca-release channel of the snake skeletal muscle SR. Snake SR Ca-release channel showed similar properties with the

mammalian Ca-release channel.

Snake SR has a single high molecular weight band like a mammalian skeletal RyR band's mobility on SDS polyacrylamide gel (PAGE) (Fig. 1). Its sedimentation coefficient was 30 S and peak fraction of [^{3}H]ryanodine binding in the RyR purification steps was coincident with the single high molecular weight band on SDS PAGE of nonlabeled fractions (Fig. 2 panel A and B). Maximum [^{3}H]ryanodine binding site (Bmax) and Kd value (Table 1) of snake SR were smilar with those of rabbit skeletal SR (Pessah *et al.*, 1986). [^{3}H]ryanodine binding was increased by Ca^{2+} (~300 μM) and AMP, but it was not inhibited by tetracaine or slightly inhibited by ruthenium red (10 μM) and $MgCl_2$ (1 mM)(Table 3). Effect of Mg^{2+} on [^{3}H]ryanodine binding was similar with that suggested by Pessah *et al.* (1985) who reported that millimolar Mg^{2+} effectively inhibits the ryanodine binding to skeletal receptors but has little effect on the binding to cardiac receptors.

$^{45}Ca^{2+}$ release from the snake skeletal SR was activated by micromolar Ca^{2+} and millimolar AMP (Fig. 4 and 5), and inhibited by high concentration of Ca^{2+} (300 μM)(Fig. 4), millimolar Mg^{2+}, micromolar ruthenium red (10 μM), and millimolar tetracaine (Table 4). There is no Ca^{2+} induced ^{45}Ca-release by high concentration of Ca^{2+} (millimolar) which was found in lobster's skeletal SR (Seok *et al.*, 1992). These functional properties of snake SR Ca-release channel were similar with those of mammalian skeletal SR Ca-release channel (Meissner 1984; Meissner *et al.*, 1986; Fleischer and Inui 1989). However we did not try to investigate the functional properties of the snake SR in the level of the single channel. So, to confirm the exact functional properties of snake Ca-release channel, single channel study using purified RyR will be considered.

In the above binding and flux studies, there is one question. "Why is [^{3}H]ryanodine binding not or slightly inhibited by Ca-release channel blocker (tetracaine, ruthenium red, or $MgCl_2$) or high Ca^{2+} ?" We think this study is not enough to answer about that. Therefore many other structural and functional studies must be needed to define better the process of excitation-contraction coupling in muscle, and the mechanism of Ca^{2+} release from sarcoplasmic reticulum

In conclusion, the present study suggest that snake, reptile, skeletal muscular SR also has Ca-release channels, their some functional properties are similar with those of mammalian channels

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=국문초록=

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파충류 골격근의 근소포체에서 칼슘유리 채널의 존재를 밝히고저 뱀 골격근에서 근소포체를 분리하여 SDS-PAGE 전기영동, RyR의 정제, [^{3}H]ryanodine 결합실험 및 ^{45}Ca 유리 실험으로 아래와 같은 결과를 얻었다.

1) 뱀골격근 소포체도 단일 band의 high molecular weight 단백을 가지고 있고, 그 mobility는 포유류 골격근의 것과 유사했다.

2) RyR의 정제과정에서 얻어진 [^{3}H]ryanodine의 peak 결합 분획에서 high molecular weight의 단백분획이 발견되었다.

3) 뱀 골격근 SR vesicles에 대한 [^{3}H]ryanodine의 maximum binding site와 Kd값은 각각 6.36 pmole/mg protein과 17.62 nM이었으며, [^{3}H]ryanodine의 특이성 결합은 칼슘과 AMP에의해 유의성있게 증가되었고 ($P<0.005$), tetracaine에 의해 억제되지 않았으나 ruthenium red와 $MgCl_2$에 의해 일부만 억제되었다.

4) 근 소포체로부터 ^{45}Ca 유리는 낮은 농도의 칼슘 (1~10 μM)과 AMP에 의해 증가되었고 ($P<0.05$), 고농도의 칼슘 (300 μM), tetracaine, ruthenium red 또는 $MgCl_2$에 의해 억제되었다 ($P<0.05$).

이상의 실험성적으로 파충류 (뱀)의 골격근에도 칼슘유리 채널이 있어 근 수축시 세포내 칼슘농도 조절에 관여할 수 있을 것으로 여겨지며, 채널의 기능적 특징 일부가 포유류의 것과 유사한 것으로 사료된다.