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# Effects of Arg-Gly-Asp-Ser on $Ca^{2+}$ transport of myocardial sarcoplasmic reticulum in rat septic shock[1]

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**KEY WORDS** peptide RGDS; sarcoplasmic reticulum; calcium; myocardium; septic shock

**AIM**: To study the effects of Arg-Gly-Asp-Ser (RGDS), a synthetic short peptide of fibrinogen degradation, on the $Ca^{2+}$ transport function of cardiac sarcoplasmic reticulum in rat septic shock. **METHODS**: RGDS 5 $\mu mol \cdot kg^{-1}$ was injected iv at 4 h and 14 h after cecal ligation and puncture (CLP) operation on rats. Highly purified membrane of sarcoplasmic reticulum (SR) was prepared from rat hearts. Assays were made of ATP-dependent $Ca^{2+}$ uptake by cardiac SR and [$^3$H] ryanodine binding to SR. **RESULTS**: The initial rate and the capacity of SR $Ca^{2+}$ uptake were increased by 104 % ($P < 0.01$) and 12 % ($P < 0.05$), respectively, paralleled by an increase in $Ca^{2+}$-ATPase activity and a decrease in calcium accumulation of myo- cardium of septic rats, whereas the $B_{max}$ and $K_d$ values of $Ca^{2+}$ activated [$^3$H]ryanodine binding to SR were unaffected after RGDS administration. **CONCLUTIONS**: The results indicated that RGDS have cardioprotective effects of maintaining $Ca^{2+}$ homeostasis of cardiac myocytes by enhancing SR $Ca^{2+}$ uptake in rat septic shock.

Arg-Gly-Asp-Ser (RGDS) is a fragment of peptide degradated from fibrinogen Aα chain, RGDS prevents platelet-dependent thrombus formation in experimental artery thrombosis[1,2]. Our previous work showed that RGDS prevented the calcium overload and attenuated enzyme release from ischemia-reperfused rat heart[3]. It is considered that $Ca^{2+}$ overload of myocardium occurs during septic shock and consequently contributes to the development of cardiac dysfunction[4,5]. Cardiac SR plays an important role in the regulation of cytosolic $Ca^{2+}$ concentration and thereby is a main regulator of cardiac contraction and relexation. The purpose of this study was to investigate the roles of RGDS on $Ca^{2+}$ transport of cardiac SR in septic shock.

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## MATERIALS AND METHODS

Rat heart experiments were performed on 24 ♂ Wistar rats weighing 270 ± 30 g. Sepsis was induced by cecal ligation and puncture (CLP)[6]. Rats were randomly divided into 3 groups: (1) Control group: rats were sham-operated and were injected with normal saline (NS); (2) Shock group: rats were injected with NS 5 $mL \cdot kg^{-1}$ via tail vein 4 h and 14 h following CLP operation; (3) RGDS group: rats were injected via tail vein with RGDS 5 $\mu mol \cdot kg^{-1}$ (dissolved in NS 5 $mL \cdot kg^{-1}$) 4 h and 14 h after CLP. Hearts were excised at 18 h postoperation into ice cold NS for preparation of SR vesicles. The SR vesicles purified by discontinuous sucrose gradient centrifugation were divided into longitudinal SR (0.8 - 1.0 $mol \cdot L^{-1}$ sucrose interface) and junctional SR (1.0 - 1.2 $mol \cdot L^{-1}$ sucrose interface)[7].

Protein content of the SR vesicles was determined[8].

ATP-dependent $Ca^{2+}$ uptake by cardiac SR was assayed[9] with modification. Ten μg longitudinal SR vesicles were preincubated at 37 ℃ for 1 min in 0.2 mL of reaction mixture containing: KCl 120, HEPES 20 (pH 7.2), $MgCl_2$ 3, $NaN_3$ 5, sodium oxalate 5 ($mol \cdot L^{-1}$), Ruthenium Red 5 $\mu mol \cdot L^{-1}$, and egtazic acid 500 $\mu mol \cdot L^{-1}$ to control the free [$^{45}Ca^{2+}$] in the range of 0.1 - 20 $\mu mol \cdot L^{-1}$ (185 GBq $\cdot mol^{-1}$ $Ca^{2+}$) as determined by the SPECS computer program[10]. The reaction was initiated by the addition of ATP 3 $mmol \cdot L^{-1}$. The ATP-dependent $Ca^{2+}$ uptake was calculated as the difference in the activities between the presence and absence of ATP.

SR $Ca^{2+}$-ATPase activity was measured[11] in the presence or absence of free [$Ca^{2+}$] 5 $\mu mol \cdot L^{-1}$.

[$^3$H] Ryanodine binding assay was carried out[12] with modification. The standard assay mixture in a final volume of 0.2 mL contained KCl 120, HEPES 20 (pH 7.2), NaCl 300 ($mmol \cdot L^{-1}$), egtazic acid buffered free [$Ca^{2+}$] 50 μmol

$\cdot L^{-1}$, [$^3$H]ryanodine 2.5 – 50 nmol$\cdot L^{-1}$ with a radioactivity of $12 \times 10^{16}$ dpm$\cdot$mol$^{-1}$, and in the absence/presence of unlabelled ryanodine 50 μmol$\cdot L^{-1}$. The binding reaction was initiated by the addition of junctional SR vesicles 40 μg and proceeded for 30 min at 37 ℃. The specific binding was defined as the bound radioactivity displaceable by unlabelled ryanodine 50 μmol$\cdot L^{-1}$. The nonspecific binding ranged from 5 % to 18 % depending on the concentration of ligand.

Total calcium content of myocardium was measured with atomic absorbance photometer (GGX-1).

RGDS was synthesized at Sino-Germany Cooperative Laborotary of Biogenic Drugs in Beijing Medical University. The purities (98 % – 99 %) of intermediates and the products were confirmed by TLC and HPLC, the amino acid sequence was determined by FAB-MS with VG-ZAB-MS high resolution GS/MS/DS[13]. $^{45}CaCl_2$ (590 TBq$\cdot$kg$^{-1}$) and [$^3$H] ryanodine were purchased from New England Nuclear (DuPont Co). Other chemicals and reagents were of AR.

Results were expressed as $\bar{x} \pm s$. Statistical analyses were performed using one-way ANOVA, the Student-Newman-Keuls $q$ test was used for multiple comparisons.

## RESULTS

**Effect of RGDS on the $Ca^{2+}$ uptake and $Ca^{2+}$-ATPase activity of SR in septic shock** The ATP-dependent $Ca^{2+}$ uptake by cardiac SR was increased with incubation time. The initial rate (described as the capacity of uptake within the first minute) and the capacity of $Ca^{2+}$ uptake by rat SR were decreased by 56 % (48 ± 11 mmol$\cdot$kg$^{-1}$ for control *vs* 21 ± 9 mmol$\cdot$kg$^{-1}$ for shock, $P<0.01$) and 25 % (126 ± 12 mmol$\cdot$kg$^{-1}$ for control *vs* 95 ± 6 mmol$\cdot$kg$^{-1}$ for shock, $P<0.01$) compared with control group, respectively (Fig 1).

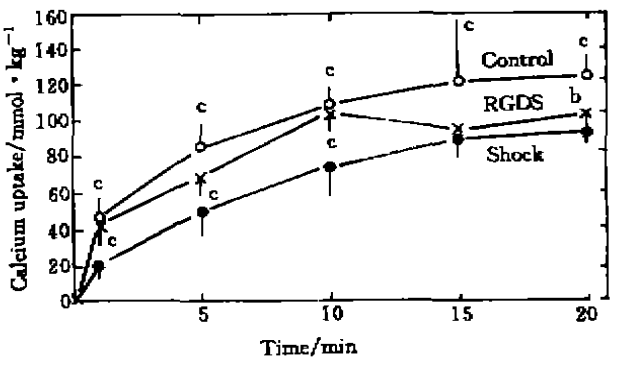


**Fig 1. Effect of RGDS on ATP-dependent $Ca^{2+}$ uptake by cardiac longitudinal SR in septic shock rats.** $n=7$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* shock group.

Compared with control group, the SR $Ca^{2+}$-ATPase activity of shock rats was also decreased (Tab 1).

**Tab 1. Effects of RGDS on cardiac SR $Ca^{2+}$-ATPase activity and myocardial calcium content in rat septic shock.** $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* shock group. $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* control group.

| Group | $Ca^{2+}$-ATPase activity, mmol$\cdot$Pi$\cdot$kg$^{-1}\cdot$h$^{-1}$ $n=8$ | Calcium content, mmol/kg dry wt $n=6$ |
|---|---|---|
| Control | 41 ± 6$^c$ | 17 ± 4$^c$ |
| Shock | 24 ± 7$^f$ | 33 ± 8$^f$ |
| RGDS | 33 ± 7$^{be}$ | 15 ± 4$^{cd}$ |

In the group of RGDS administration, the initial rate and the capacity of SR $Ca^{2+}$ uptake was 43 ± 4 mmol$\cdot$kg$^{-1}$ and 105 ± 6 mmol$\cdot$kg$^{-1}$, increased by 104 % ($P<0.01$) and 12 % ($P<0.05$) *vs* shock group (Fig 1). There was also an increase in SR $Ca^{2+}$-ATPase activity in comparison to shock rats (Tab 1).

There was a relationship between $Ca^{2+}$ uptake activities and $Ca^{2+}$ concentrations in 3 groups (Fig 2).

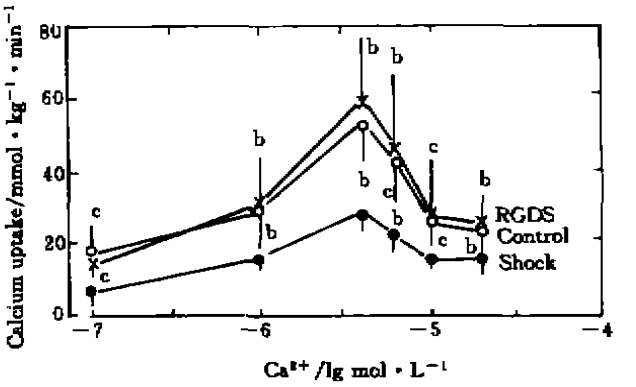


**Fig 2. Effect of RGDS on $Ca^{2+}$ uptake in rat cardiac longitudinal SR.** $n=7$, $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ *vs* shock group.

Data analysis on the substrate-velocity relationship (Eadie-Hofstee plot, $v$ against $v/[S]$) indicated that the $V_{max}$ for $Ca^{2+}$ was decreased by 85 % (53 ± 24 mmol$\cdot$kg$^{-1}\cdot$min$^{-1}$ *vs* 29 ± 5 mmol$\cdot$kg$^{-1}\cdot$min$^{-1}$ for control and shock, $P<0.05$) while for RGDS administrated rats, the $V_{max}$ for $Ca^{2+}$ was increased (60 ± 17 mmol$\cdot$kg$^{-1}\cdot$min$^{-1}$, $P<0.05$)

in comparison to shock rats. The $K_m$ values were calculated to be $3.1 \pm 1.3$ $\mu mol \cdot L^{-1}$, $2.0 \pm 0.8$ $\mu mol \cdot L^{-1}$ and $4.9 \pm 1.4$ $\mu mol \cdot L^{-1}$ for control, septic shock, and RGDS group, respectively ($P > 0.05$), which indicated that the affinity of $Ca^{2+}$ to SR $Ca^{2+}$-ATPase were not affected either for septic or for RGDS group.

**Effect of RGDS on SR [$^3$H]ryanodine binding**

In sepsis, the amount of [$^3$H]ryanodine binding to rat SR was decreased (Fig 3).

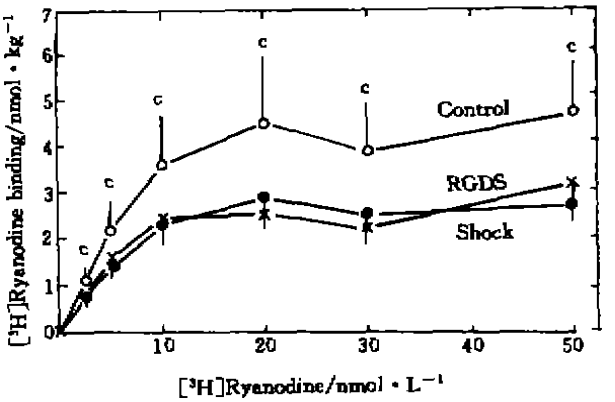


**Fig 3. Effect of RGDS on [$^3$H]ryanodine binding to rat cardiac junctional SR in septic shock. $n = 8$, $\bar{x} \pm s$. $^cP < 0.01$ *vs* shock group.**

Scatchard plot analysis indicated that the maximal binding ($B_{max}$) was reduced by 41 % ($6.6 \pm 2.0$ nmol $\cdot kg^{-1}$ for control versus $3.9 \pm 0.3$ nmol $\cdot kg^{-1}$ for shock, $P < 0.01$), while the $K_d$ value remained unchanged ($12.4 \pm 1.9$ nmol $\cdot L^{-1}$ for control and $10.4 \pm 2.8$ nmol $\cdot L^{-1}$ for shock, $P > 0.05$). After RGDS, there were no obvious differences in SR [$^3$H] ryanodine binding between the group of shock and RGDS, the $B_{max}$ ($4.1 \pm 0.7$ nmol $\cdot kg^{-1}$) and $K_d$ ($10.4 \pm 2.4$ nmol $\cdot L^{-1}$) values were not differ from those of shock rats (Fig 3, 4).

**Effect of RGDS on myocardial calcium content**

The calcium content of rat myocardium was elevated in septic shock, In RGDS group, it was reduced and had no difference from control group (Tab 1).

## DISCUSSION

Administration of RGDS 5 $\mu mol \cdot kg^{-1}$ ameliorated the impairment in SR $Ca^{2+}$ uptake and calcium accumulation in myocardium of septic rats. Since

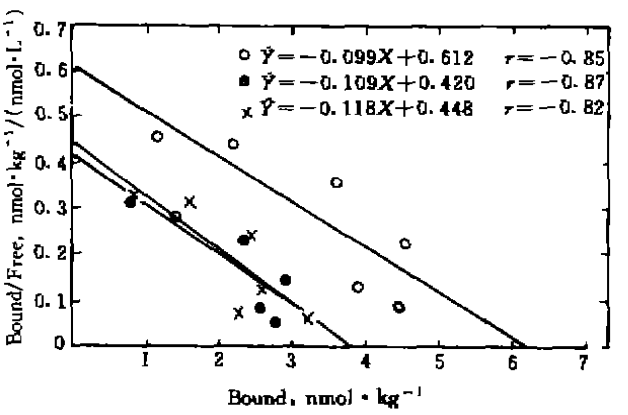


**Fig 4. Scatchard plot of [$^3$H]ryanodine binding to rat cardiac junctional SR. Control (○), shock (●), RGDS (×), $n = 8$.**

calcium overload has been assumed to be a causative factor for myocardial depression occured in septic shock[4,5], the effects of RGDS may be protective against calcium accumulation of cardiac myocytes and be benificial to cardiac function of septic rats.

The cellular mechanisms by which RGDS influence $Ca^{2+}$ regulation of cardiac SR was unclear. The observed alterations in SR $Ca^{2+}$ uptake either in septic shock or after RGDS administration could not be attributed to changes in the intracellular $Ca^{2+}$ concentration, for the affinity ($K_m$ value) for $Ca^{2+}$ were not affected.

Studies have proved that $Ca^{2+}$ release from cardiac SR takes place through a ryanodine-sensitive $Ca^{2+}$ release channel[12,14]. The data presented here demonstrated that the affinity for [$^3$H] ryanodine binding ($K_d$ value) was unaffected while the number of ryanodine receptor ($B_{max}$ value) which implies the number of $Ca^{2+}$ release channel of cardiac SR was reduced in septic shock. The results that RGDS administration caused no changes in $B_{max}$ and $K_d$ values of SR [$^3$H] ryanodine binding indicated that the effects of RGDS were distinct from actions on SR $Ca^{2+}$ release channel.

This work revealed that RGDS had cardioprotective effect on septic rats, the enhancement of SR $Ca^{2+}$ uptake activity may be one of the mechanisms by which RGDS exerts its function.

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## 精-甘-天冬-丝氨酸对脓毒性休克大鼠心肌肌浆网钙转运的影响[1]

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**关键词** 肽 RGDS；肌浆网；钙；心肌；脓毒性休克

**目的**：探讨一种人工合成的纤维蛋白原降解肽片段 RGDS 对脓毒性休克大鼠心肌肌浆网钙转运功能的影响. **方法**：大鼠盲肠结扎穿孔术后 4 h 和 14 h 分两次尾静脉注射 RGDS 5 $\mu mol \cdot kg^{-1}$. 制备大鼠心肌肌浆网(SR)膜；测定 SR $Ca^{2+}$ 摄取和 [$^3$H]ryanodine 受体结合功能. **结果**：RGDS 组大鼠心肌 SR 摄 $Ca^{2+}$ 率及摄 $Ca^{2+}$ 量分别较休克组提高 104 % ($P<0.01$)和 12 %($P<0.05$)，而心肌 SR 钙释放通道-[$^3$H]ryanodine 受体结合 $B_{max}$ 和 $K_d$ 值没有明显变化. 同时 RGDS 还可以减轻休克大鼠心肌组织钙聚积. **结论**：RGDS 提高休克大鼠心肌 SR $Ca^{2+}$ 摄取功能，维持心肌细胞钙稳态，具有心肌保护作用.