Relationship between an Endogenous Calcium Binding Protein (S-100ab) and Ca^{2+} Transport in Frog Skeletal Muscle

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Abstract

Sarcoplasmic reticulum vesicles prepared from frog hind limbs were used to test the effect of a 21 Kd calcium-binding protein S-100ab on the mechanism of Ca^{2+} transport (release and uptake). The results show that the protein increases a Ca^{2+}-release by acting on sarcoplasmic Ca^{2+} channels. This event was also demonstrated by a modification of the ryanodine bond on its receptors. On contrast, the T-tubule receptors of voltage-gated Ca^{2+} channels, do not seem to be involved in the S-100 action. In fact, using single-fibre preparations, the mechanical parameters measured in twitch and tetanus were unchanged when μM concentrations of S-100ab were present in the perfusion solution.

Since the S-100ab effect appears to be modulated by GTP, it is possible that the protein could act on sarcoplasmic reticulum Ca^{2+} channels through an involvement of the GTP-binding protein system.

Key words: S-100, sarcoplasmic reticulum, tension development, Ca^{2+} transport, ryanodine binding.

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In the last years, the study of excitation-contraction (EC) coupling in skeletal muscle has been concerned with the mechanism by which the depolarization of sarcolemma determines the rise of Ca^{2+} conductance in Ca^{2+} channels present in a specific area of terminal cisternae [2, 17] identified as being part of the bridging structures (feet) between T-tubules and sarcoplasmic reticulum (SR) [6, 10]. This channel, formed by a tetrameric polypeptide (450 Kd per monomer) called ryanodine receptor (RR) or junctional foot protein, has been characterized for sequence and biochemical/physiological parameters [12, 14]. Another Ca^{2+}-channel (voltage-gated) extists in T-tubules, in conjunction with RR. It is a hetero-pentamer which also contains the DHP receptors in one subunit (a1) [1, 20]. Notice that the same channel type is present in cardiac muscle (L-type Ca^{2+}-channel) where it is the fundamental step linking the sarcolemmal depolarization with the contraction. Even if the Ca^{2+} fluxes via voltage-gated channels are not a prerequisite to myofibrillar contraction in the skeletal muscle, they must be present to trigger EC coupling. This process is accompanied by a movement of positive charges (gating charges) [18] across the T-tubule membranes which seems to initiate, by an unknown step, the opening of SR Ca^{2+}-channels. Several mechanisms have been proposed to explain the problem but to date the only possible conclusion is that "protein-protein interactions between the voltage-gated calcium channels in the trasverse tubule membrane and the calcium-release channels in the sarcoplasmic reticulum membrane are responsible for excitation-contraction coupling in skeletal muscle (W.A. Catteral)" [2].

About five years ago, Haimoto and Kato proposed that the E-F hand-type proteins, present in the muscle, might be enriched with another calcium binding protein named S-100 [9]. This protein, a dimer, was first purified by Moore [16]. It has a molecular weight of 21 Kd and has two high affinity and several low affinity binding sites to Ca^{2+}. It is present in the tissues of different origin in 3 different isoforms: S-100a_0 (αα), S-100a (αß), S-100b (ßß) or in mixture of them (S-100ab) [3]. Recently we showed that S-100 could have a physiological role in skeletal muscle function. In fact, two different S-100 isoforms, S-100a_0 and S-100b, not only activate the adenylate cyclase present in SR membrane [4] but also increase Ca^{2+} release in the same preparations which suggests that S-100 plays an important role in the control of Ca^{2+} release [5, 15].

The attempt to verify this hypothesis was carried out

using two different approaches: mechanical, by means of skeletal muscle single fibres, and biochemical using microsomal vesicles derived from fragmentation of SR.

Materials and Methods

a) Mechanical procedures: single fibres were dissected from the lateral head of the tibialis anterior muscle of frog (for mounting and cross-section area determination see ref. 13). The maximum velocity of shortening during the early phases of the contraction in frog single muscle fibres was determined as previously described [13]. An anodized aluminium experimental chamber (1.5 ml capacity) and a force trasducer (resonance frequency of 30 Khz and 2 mV/mg sensitivity) already described [13] were used. Stimuli (0.5 msec duration, 1.5 times the threshold strength) were applied by a pair of platinum plate electrodes (10 mm long, 4 mm apart) at 5 min intervals to evoke cycles of isometric twitch and tetanus (volley of alternating polarity stimuli for 0.35 sec duration). The experimental solutions (pH 7.1) were: Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 3.0 mM phosphate buffer) or Ringer plus 1 μM S-100ab, kept at constant temperature (18°C) by means of a thermoelectric module. The outputs from the force transducer were recorded by a 386 MS-DOS computer via an A/D converter (R-C Electronics Inc., Ca, USA) and analyzed by specific software.

b) S-100 purification: S-100ab protein was isolated from bovine brain and checked for contaminants as already described [7]. Intramolecularly cross-linked S-100 (S-100cl) was prepared according to Fulle et al. [7].

c) SR vesicle preparation: the SR fragmentation was carried out by starting with 50 g of skeletal muscle dissected from frog hind limbs [15]. After homogenization and two different centrifugation steps, the material was layered onto a discontinuous sucrose gradient (1.6-0.8 M), centrifuged at 70,000 x g x 16 hours; the fractions collected from first two steps were stored at -80°C until used.

d) Ca^{2+} transport: active uptake and passive release of Ca^{2+} was determined by the method previously reported [15] which utilizes ^{45}Ca as radiolabeled tracer and a filtration apparatus to determine the Ca^{2+} fluxes across the membranes at preselected time intervals. Ca^{2+} uptake was measured using 5 mM ATP to actively load the ion onto the vesicles, while the release was determined in presence of 10^{-6} M Ca^{2+}-free and in absence of ATP and adenine. The activity of Ca^{2+} pump system was also measured by a previously described method [15].

e) Ca^{2+}-ATP-ase activity: The Ca^{+2} ATP-ase assay was carried out in a final volume of 1 ml of incubation medium (5 mM ATP, 1 mM $CaCl_2$, 50 mM Tris-HCl) at pH 7.5 in presence of 25-30 μg of proteins and other agents as previously described (Fano' et al, 1989).

f) Binding experiments: the modification of ryanodine binding was determined in order to study the direct effect of S-100 on Ca^{2+} release channels. Aliquots of 0.2 ml (3-5 mg proteins) were incubated (0-120 min) at 25°C in the presence of different radiolabeled ryanodine concentrations. Nonspecific binding was determined for all experimental points by the inclusion of a 10^4-fold excess of cold ryanodine. The displacement effect induced by S-100 was carried out in samples of 50-200 μg of proteins preincubated for 30 min with 1-50 nM tritiated-ryanodine or 1.0 μM S-100ab.

Results

a) Mechanical determinations

Fig. 1 shows the traces of tetanic (panel a) and twitch (panel b) isometric tensions vs time, obtained from one muscle fibre in the absence (lower trace) and in the presence (upper trace) of 1 μM S-100ab. As can be seen, the protein addition did not modify the time courses of twitch and tetanic tension. The mean values of maximum tetanic tension (Tab. 1) relative to the two experimental conditions, as well as those of twitch peak tension (Tab. 1) and peak time do not differ significantly.

b) Ca^{2+} transport

In Fig. 2 the effect induced by S-100ab on Ca^{2+} uptake and release in vesicles derived from SR is reported. All the S-100 concentrations used (0.05-5.0 μM) did not significantly change the Ca^{2+} accumulation in the vesicles (Fig. 2 left). This fact is also confirmed by the data in which no modification of Ca^{2+}-dependent ATP-ase exists when 0.05-1.0 μM S-100 is present in the medium (Tab. 2). In contrast, if the same S-100 concentrations were checked

Table 1. Means (+ SEMs) for peak twitch and plateau tetanic tensions

DATE	- S-100	+ S-100
Twitch		
02/21/91	185.79 ± 3.17	184.08 ± 6.23
12/05/91	258.86 ± 5.15	257.84 ± 5.45
Tetanus		
02/21/91	328.22 ± 1.50	326.05 ± 9.90
12/05/91	315.83 ± 4.92	323.29 ± 2.18

Data referred to two muscle fibres in normal and plus 1 μM S-100 Ringer solutions. Tension values are expressed as KN/m^2 of cross-sectional area.

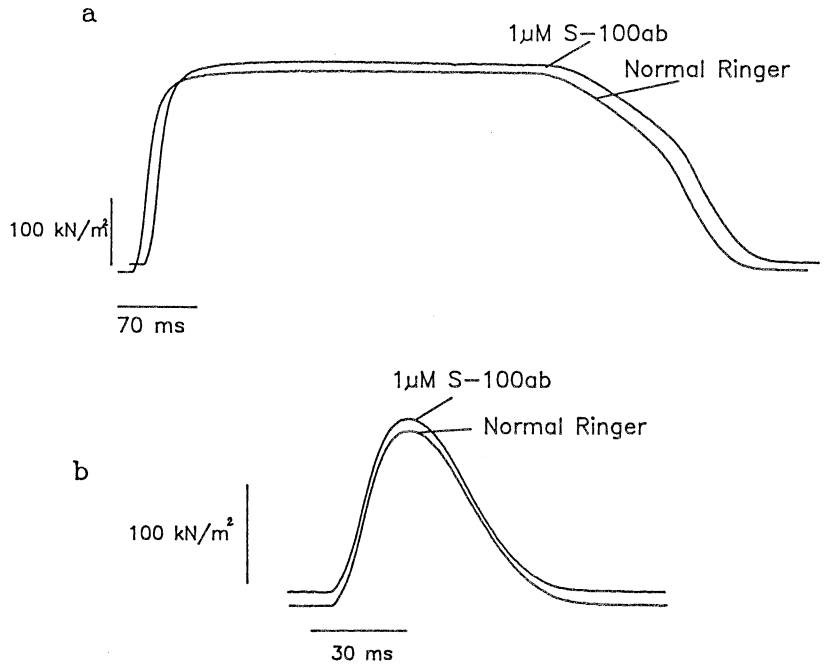

Figure 1. Plots of tension vs time developed by a single frog muscle fibre during isometric tetanus [panel (a)] and twitch [panel (b)].
In each panel the lower trace is obtained in the normal Ringer solution and the upper Trace is obtained in Ringer + 1 μM S-100ab. Traces of panel (a) are out of phase to better show their time course. Experimental temperature is 18°C.

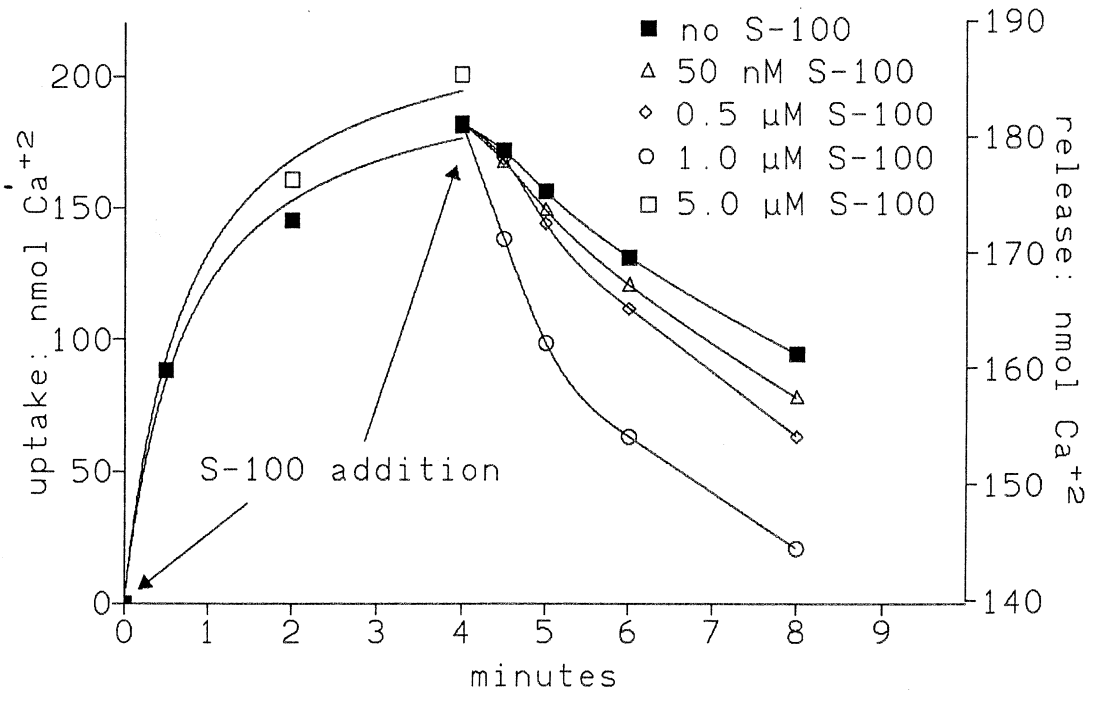

Figure 2: Effect of S-100ab on Ca^{2+} uptake and release in vesicles derived from SR fragmentation. The uptake was induced by 5 mM ATP in the presence of 5.0 μM S-100. Ca^{2+} release was measured on actively preloaded vesicles (in the absence of the protein) in the presence of 0.05-1 μM S-100, 10^{-6} M Ca^{2+}-free and in the absence of ATP or adenine. All the data reported are referred to one or three experiments carried out in triplicate (maximum SD=10.5%).

Table 2. Ca/dependent ATP-ase activity in presence of S-100ab.

S-100	0.00 μM	2.81 ± 0.10
S-100	0.05 μM	2.75 ± 0.35
S-100	0.50 μM	2.66 ± 0.23
S-100	1.00 μM	2.89 ± 0.12

The assay was carried out in presence of 25-50 μg prot. at pH 7.2. The released orthophosphate was estimated on 1 ml of clear supernatant and the specific activity was expressed as mol of Pi released per min per mg of proteins (M+SD, n=5).

on low-activated Ca^{2+} release (in the presence of μM Ca^{2+} concentrations but in the absence of adenine), a dose-dependent increase of this process was observed (Fig. 2 right). In the absence of adenine, the poor Ca^{2+} release induced by μM Ca^{2+} could have been increased when 10^{-11}-10^{-4} M GTP-s, an analogous non-hydrolyzed GTP, was present in the experimental solution (Fig. 3). This nucleotide doubled the Ca^{2+} release and changed the Ca^{2+}-dependent ATP-ase activity, with the same parameters (Tab. 3). In this condition the effect of 1 μM S-100 was not related to nucleotide concentration, and it reaches the maximum value when 10^{-5} M GTP-S was present in the medium. Other GTP derivatives were tested but the Ca^{2+} release stimulating capacity was very little and not dose-dependent (GDP) or was completely ineffective (GTP-ß-s) also on Ca^{2+}-ATPase (data not shown).

c) Ryanodine binding

The modification of ryanodine binding as a consequence of S-100 action is shown in Fig. 4. In fact, after an incubation of 120 min to saturate all the sites in presence of 0-50 nM Ryanodine, the specific binding was radically modified if 1 μM S-100 was present. The inset in Fig. 5 shows that the presence of the protein induced a decrease of Kd value (from 6.843 to 4.06). Vice versa Bmax carried out from Scatchard plot analysis rises when the binding was measured in presence of 1 μM S-100 (from 0.56 to 1.01 nmol/min/mg).

A particular aspect of the ryanodine/S-100 interaction is shown in Fig. 4; it is evident that the alteration of ryanodine binding induced by S-100 was completely abolished if the alkaloid and the protein were added to the vesicle-containing incubation solution together. On the contrary, if one of the two was preincubated separately for 20-30 minutes before adding the other, the binding effect was again present.

Discussion

The goal of this work was to determine if the increased Ca^{2+} release, induced by μM S-100 concentrations, was derived from a direct or indirect interaction between the protein and some components of Ca^{2+} channels present in the skeletal muscle.

The data presented here seem to exclude an effective

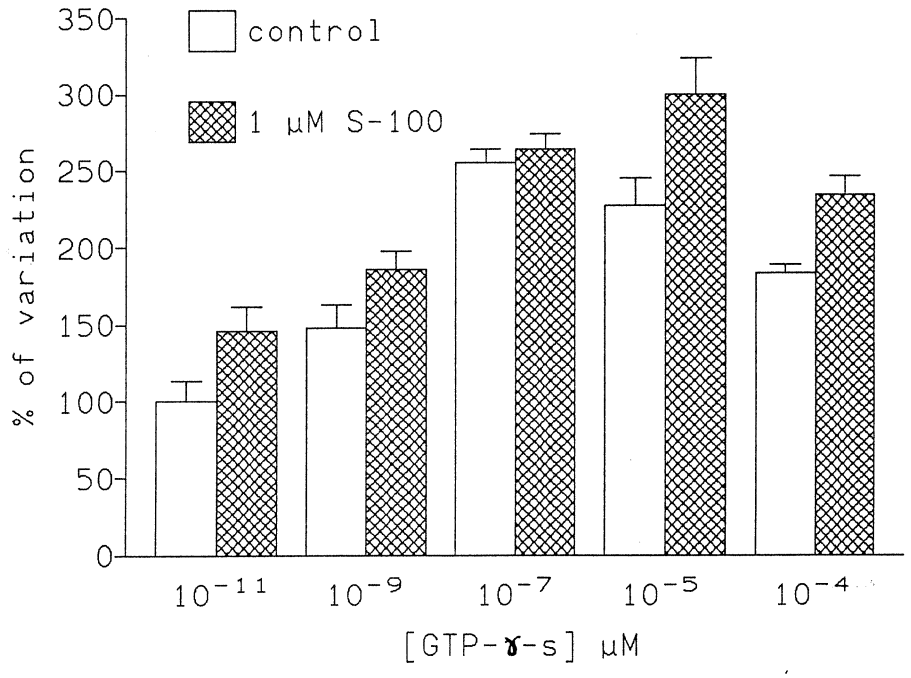

Figure 3: Effect of 1 M S-100ab on Ca^{2+} release (bars SD, n=5) measured for 4 min on vesicles actively preloaded with 5 mM ATP. The release was started adding to vesicles 10^{-6} M Ca^{2+}-free and S-100 in the presence of different (10^{-11}-10^{-4}) GTP-s concentrations. The data reported are plotted as the percentage of variations versus controls (182+24 pmol of Ca^{2+} released after 4 min in the absence of GTP-s and S-100).

S-100 action on DHP receptors localized in T-tubules because, when 1 μM S-100ab was added in the perfusion solution, none of the mechanical parameters measured were modified by the presence of the protein. Therefore even if some interactions occur, they are not physiologically significant. In contrast, the Ca^{2+}-channel capacity was strongly modified by S-100; it increased Ca^{2+} release and positively modified ryanodine- binding to its receptors. This is probably due to the existence of one or more regulatory sites on the Ca^{2+} channel (sarcoplasmic face) that have the characteristics required for promoting interaction with low molecular weight acid proteins which may be present in the medium [19].

However, the influence of S-100 on ligand-receptor complex between ryanodine and Ca^{2+} channel has many characteristic aspects that are only partially evident from our experiments. One of these is the S-100 positive effect on ryanodine binding that was evident only when the protein was added to vesicles before or after the ryanodine. One hypothesis is that S-100 is unable to increase

Table 3. Specific ATP-ase activity variations induced by GTP-s.

[GTP-s]	- S-100	+ S-100
0.01 nM	2.05 ± 0.41	2.11 ± 0.14
1.00 nM	2.37 ± 0.26	2.26 ± 0.33
0.10 uM	2.48 ± 0.31	2.32 ± 0.21
10.0 uM	2.41 ± 0.25	2.31 ± 0.26
100 uM	1.75 ± 0.36	1.96 ± 0.19

The assay was carried out in presence of 25-50 g prot. at pH 7.2. The released orthophosphate was estimated on 1 ml of clear supernatant and the specific activity was expressed as mol of Pi released per min per mg of proteins (M+SD, n=5).

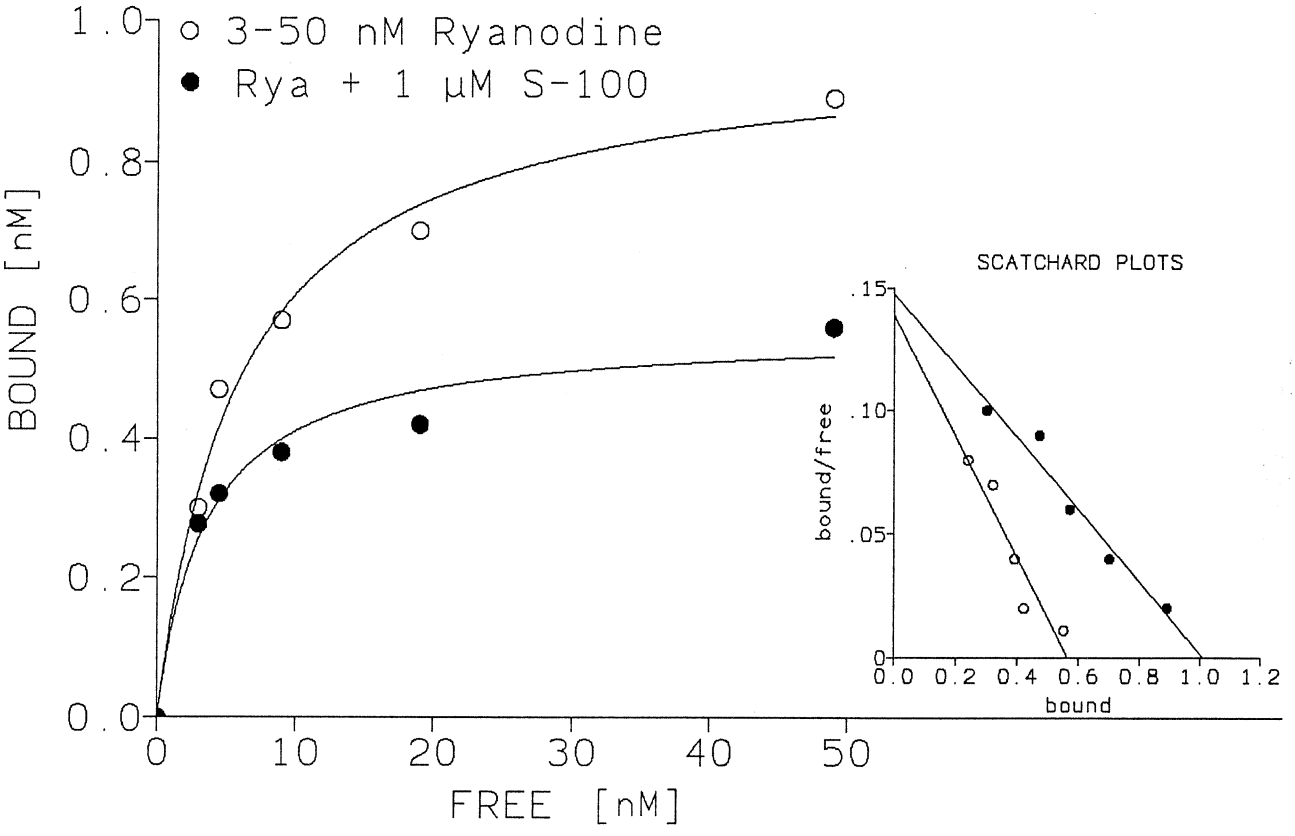

Figure 4: Specific binding of different (3-50 nM) [^{3}H]-ryanodine concentrations in absence (unfilled) or presence (filled) of 1 μM S-100ab. The protein was added after 120 min of vesicle preincubation with ryanodine to site saturation. The included graph shows the Scatchard plot analysis by which the Kd and Bmax values were calculated by In-plot Software.
All data plotted are the mean of four determinations.

	- S-100	+ S-100
Kd	*6.843+0.15*	*4.061+0.17*
Bmax	*0.563+0.02*	*1.01+0.06*

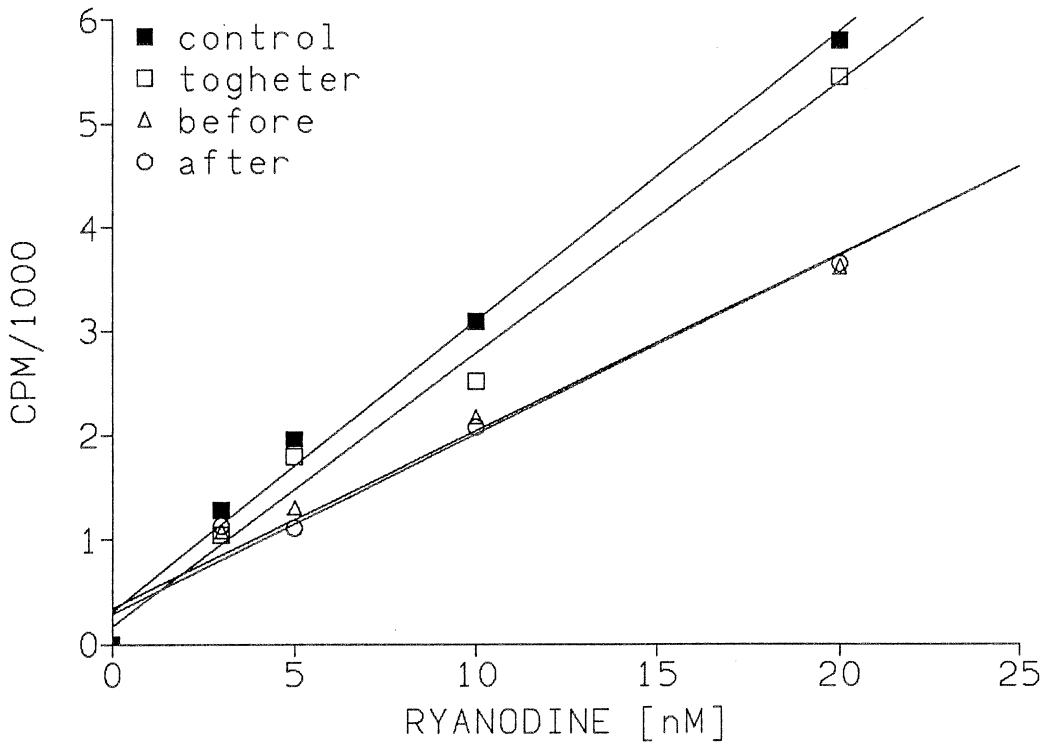

Figure 5: [^{3}H]-ryanodine binding on sarcoplasmic vesicles in the presence of 1 μM S-100ab. The protein was added to experimental solution together with the alkaloid or 30 min before/after the starting of vesicle incubation. Each point represents the mean of four measures (maximum SD=7.8%).

ryanodine affinity to the receptor when the bond is forming but this is possible only if the system is in its stationary (activated or not) phase. It should be noted that a direct link between S-100 and ryanodine can be excluded based on experiments in which 1 μM S-100ab were separated by gel filtration after incubation with 50 nM labeled ryanodine. Under these conditions, the S-100 fractions collected were not radioactive (not shown).

The possible interaction or, rather, a "functional interference" between S-100 and the ryanodine receptors can also be derived from experiments carried out in the presence of efficient GTP-s concentrations in which the same G-protein system involved in charge movement during EC cycle [8] may be interested in S-100 action. This hypothesis is also substantiated by the following considerations. The molecular structure of sarcoplasmic Ca^{2+} channel has a strong sequence analogy with the adenylate cyclase system [11]; for this reason, it is possible that agents, capable of effecting one, could also affect the others. Moreover, in the inner membrane network, there is a remarkable adenylate cyclase activity that is positively influenced by μM S-100 concentrations [4]. Since it is known that, in brain membranes, S-100 stimulates adenylate cyclase via a PTX-sensitive G-protein [7], it is possible that a similar situation exsists in skeletal muscle.

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