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Effects of anti-triadin antibody on Ca^{2+} release from sarcoplasmic reticulum

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The monoclonal antibody, mAb GE 4.90, raised against triadin, a 95 kDa protein of sarcoplasmic reticulum (SR), inhibits the slow phase of Ca^{2+} release from SR following depolarization of the T-tubule moiety of the triad. The antibody has virtually no effect on the fast phase of depolarization-induced Ca^{2+} release nor on caffeine-induced Ca^{2+} release. Since the slow phase of depolarization-induced Ca^{2+} release is also inhibited by dihydropyridines (DHP), these results suggest that triadin may be involved in the functional coupling between the DHP receptor and the SR Ca^{2+} channel.

Triadin; Excitation-contraction coupling; Ca^{2+} release; Sarcoplasmic reticulum

1. INTRODUCTION

The dihydropyridine (DHP) receptor is recognized now to be the major protein that senses the change in the membrane potential of the transverse (T) tubule and transmits that signal to the junctional foot protein (JFP) of the sarcoplasmic reticulum (SR) [1–3], initiating the release of Ca^{2+} from the SR [4–6]. The apparent close proximity between the DHP receptor and the JFP [7] and their close association during myogenesis [8] might suggest direct communication between the T-tubule and SR proteins by which a conformational change induced in the DHP receptor by depolarization of the T-tubule would directly alter the Ca^{2+} release protein of the SR [9,10]. However, the fact that is has not yet been possible to recombine these two proteins, although they have both been isolated for several years, may suggest the involvement of a third protein in their interaction [9].

Recently some of us have presented evidence that triadin is a major constituent of the triad junction which binds both to the DHP receptor and the JFP [11,12]. This protein is an intrinsic protein of the terminal cisternae (TC) with a distribution and content approximately the same as those of the JFP [13]. The role of this protein as a physical mediator between the DHP receptor and the JFP could readily explain both the apparent close association between the latter two proteins as well as the failure to demonstrate direct physical coupling. If triadin forms the physical link between the voltage sensor and Ca^{2+} release, it is also possible that it may play a role in excitation–contraction coupling. We have developed a specific monoclonal antibody (GE 4.90) directed against this protein [13]. In this communication we report that the antibody produces appreciable effects on T-tubule-mediated Ca^{2+} release from isolated triadic vesicles.

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Abbrevations: DHP, dihydropyridine; DIR, depolarization-induced Ca^{2+} release; e-c coupling, excitation-contraction coupling; HEPES, N-2 hydroxyethyl piperzine-N-2 ethanesulfuric acid; JFP, junctional foot protein; mAb, monoclonal antibody; MES, 2-(N-morpholino) ethanesulfonic acid; PMSF, phenylmethyl sulfonyl fluoride; SR, sarcoplasmic reticulum; TC, terminal cisternae; T-tubule, transverse tubule.

2. EXPERIMENTAL

2.1. *Preparation of microsomal fraction enriched in the triad vesicles*

The triad-containing microsomal fraction was prepared from rabbit leg and back muscles by differential centrifugation as described previously [14]. After the final centrifugation, the sedimented fraction was homogenized in a solution containing 0.3 M sucrose, 0.15 M potassium gluconate, proteolytic inhibitors (PEI: 0.1 mM PMSF, 10 μg/ml aprotinin, 0.8 μg/ml antipain, 2 μg/ml trypsin inhibitor) and 20 mM MES (pH 6.8) at a final protein concentration of 20–30 mg/ml. The preparation was quickly frozen in liquid nitrogen and stored at −70°C.

2.2. *Antibody preparation*

Monoclonal antibody GE 4.90 against triadin was produced in mouse ascites and purified by Bakerbond ABX column chromatography as described in detail in [13]. The purified antibody was concentrated on an Amicon ultrafiltration apparatus, and stored at −20°C in the presence of 35% glycerol. For Ca^{2+} release experiments, the antibody was dialyzed against a solution containing 20 mM MES, pH

6.8, to remove glycerol, since glycerol was found to activate both depolarization-induced Ca^{2+} release and caffeine-induced Ca^{2+} release even at low concentrations (e.g. 0.5%).

2.3. *Induction and assay of depolarization-induced Ca^{2+} release*

These were done as described previously [15]. The vesicular fraction (3.2 mg/ml) was incubated in 0.15 M potassium gluconate, 20 mM MES (pH 6.8) with or without mAb GE 4.90 for 30 min at 22°C. The vesicles (1.6 mg/ml) were then incubated in a solution containing 0.15 M potassium gluconate, 200 μM $CaCl_2$, 0.5 mM $MgCl_2$, 0.75 mM Na_2-ATP, 5.0 mM phosphoenolpyruvate, 10 U/ml pyruvate kinase, 9 μM arsenazo III, and 20 mM MES, pH 6.8 (Solution A_1) at 22°C. Solution A_1 was found to contain 2–7 mM endogenous Na^+, as determined by atomic absorption spectrophotometry. At various times after the addition of ATP, Solution A_1 was mixed with an equal volume of Solution B_1 containing 0.15 M choline chloride, 9 μM arsenazo III, 20 mM MES (pH 6.8). The amount of [^{14}C] SCN^- taken up by purified T-tubule vesicles increased upon incubation of the vesicles in Solution A_1, while it decreased upon mixing Solution A_1 with Solution B_1 ([16]; details to be published elsewhere). This suggests that incubation of triad vesicles in Solution A_1 led to polarization of the T-tubule moiety making the lumenal side more positive, while mixing Solution A_1 with Solution B_1 led to depolarization. The time-course of depolarization-induced Ca^{2+} release was recorded using a stopped-flow spectrophotometer system [15]. The time-course of Ca^{2+} release was so complex in the presence of antibody that the data could not be fitted by a single model. Satisfactory data fitting could be achieved in the following way. The initial rate of Ca^{2+} release in the fast phase ($A_f \cdot k_f$, where A_f is the amount of Ca^{2+} released in the fast phase, and k_f is the rate constant of Ca^{2+} release in the fast phase) was calculated by fitting of an exponential model, $y = A_f\{1-\exp(-k_f t)\}$, to an early portion of the release time-course ($0 \le t \le 0.1$ s). On the other hand, the initial rate of Ca^{2+} release in the slow phase ($A_s \cdot k_s$) and the lag period that preceded Ca^{2+} release in the slow phase (t_l) were calculated by fitting an exponential model, $y = y' + A_s[1-\exp\{-k_s(t-t_l)\}]$, to a 0.05 s $\le t \le$ 4.0 s portion of the time-course of Ca^{2+} release.

2.4. *Induction and assay of caffeine-induced Ca^{2+} release*

The vesicles (3.2 mg/ml) were incubated in 0.15 M KCl with or without addition of mAb for 30 min at 22°C. For active Ca^{2+} loading of SR, the vesicles (1.6 mg/ml) were incubated in a solution of 0.15 M KCl, 0.5 mM Mg ATP, 5.0 mM phosphenolpyruvate, 10 U/ml pyruvate kinase, 9 μM arsenazo III, and 20 mM MES, pH 6.8 (Solution A_2). After incubation for 4–8 min, Ca^{2+} release was induced by mixing one part of Solution A_2 with one part of Solution B_2 containing 0.15 M KCl, 4 mM caffeine, 9 μM arsenazo III, 20 mM MES (pH 6.8). The time-course of caffeine-induced Ca^{2+} release was recorded using the stopped-flow spectrophotometer system, and the data were analyzed by computer fitting of a single exponential function.

3. RESULTS

Isolated triad vesicles were incubated with various concentrations of anti-triadin mAb GE 4.90, and Ca^{2+} release from SR was induced by depolarization of the T-tubule moiety of the triads. Incubation of the vesicles with increasing concentrations of mAb GE 4.90 ($\le$ 40 μg/mg protein) resulted in a partial inhibition of Ca^{2+} release in the slow phase of depolarization-induced release (Figs. 1 and 2), while the fast phase, which is completed within 0.1 s, was virtually unaffected by mAb (Figs. 1 and 2). Consequently, the biphasic characteristics of the Ca^{2+} release time-course became more pronounced upon increase of the mAb concentration (e.g. Fig. 1, curves c,d,e). The most remarkable effect of

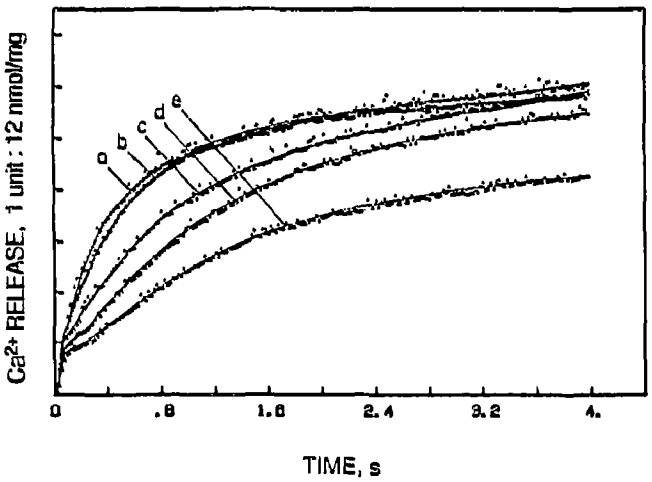

Fig. 1. Stopped-flow spectrophotometric traces showing the time-course of depolarization-induced Ca^{2+} release from isolated triad vesicles after incubation with various amounts of anti-triadin mAb, GE 4.90. The concentration of mAb added (μm/mg membrane protein): curve a, 0; b, 2.0; c, 4.5; d, 10.0; e, 40.0. Each trace was obtained by signal averaging about 25 traces.

the mAb GE 4.90 was the increase of the lag phase that preceded the slow phase of Ca^{2+} release (t_l) as shown in Fig. 3.

In the same concentration range of antibody as above ($\le$ 40 μg/mg protein), mAb GE 4.90 has no appreciable effect on caffeine-induced Ca^{2+} releae (data, not shown), which is induced by direct stimulation of SR [15,17]. A number of mAbs raised against the T-tubule and SR proteins affected neither depolarization-induced Ca^{2+} release nor caffeine-induced release [18], indicating that the inhibition of depolarization-induced Ca^{2+} release by mAb GE 4.90 represents a specific effect characteristic

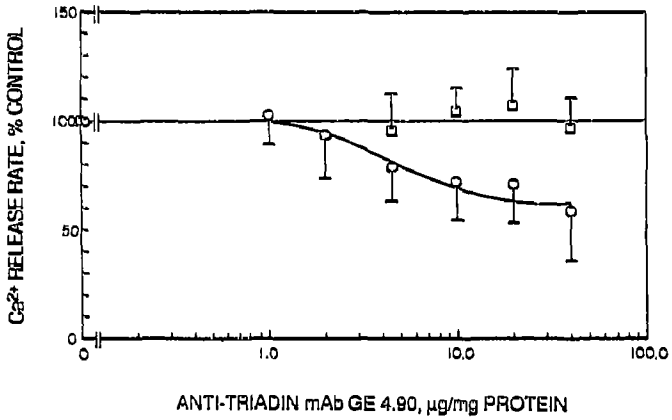

Fig. 2. Relative rates of Ca^{2+} release in the fast phase (□) and in the slow phase (○) of depolarization-induced Ca^{2+} release from isolated triad vesicles as a function of the amount of added mAb GE 4.90. Ca^{2+} release was induced by depolarization after incubation of the vesicles with various concentrations of mAb GE 4.90, the time-course of release was monitored in the stopped-flow spectrophotometer system, and the rates were calculated as described in Experimental. Each datum point is the average of a total of 96–220 traces from 4–9 experiments ±S.D.

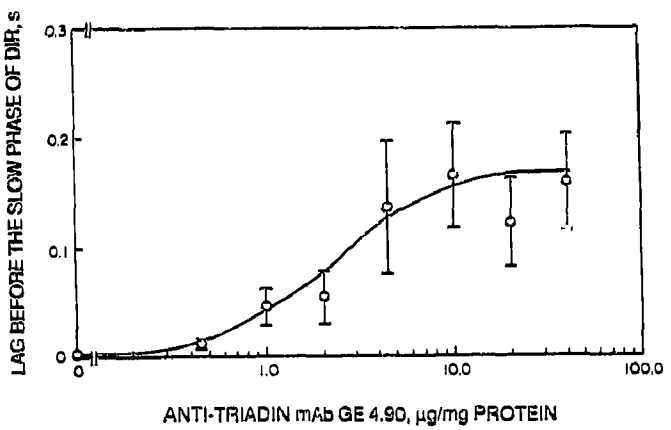

Fig. 3. Duration of the lag period that precedes Ca^{2+} release in the slow phase of depolarization-induced Ca^{2+} release as a function of the concentration of mAb GE 4.90 added during pre-incubation. Ca^{2+} release was induced from the triad vesicles, as described in the legend to Fig. 2. The lag period that preceded the slow phase (t_l) was calculated by computer fitting (see Experimental). Note that the t_l value sharply increases with the increase of the mAb concentration. Each datum point represents the average ± S.D.

for this particular mAb. mAb GE 4.90, up to 30 μg/mg protein, had no appreciable effect on Ca^{2+} uptake; the amount of the Ca^{2+} taken up at the steady-state of Ca^{2+} uptake after treatment of the vesicles with 30 μg mAb GE 4.90/mg protein was 100.4 ± 8.7% (average ± S.D., n=4) of the sample without treatment. This excludes the possibility that the observed inhibition of Ca^{2+} release might be caused by indirect effects such as reduced Ca^{2+} accumulation in the SR vesicle.

4. DISCUSSION

Depolarization-induced Ca^{2+} release from SR is controlled by the attached T-tubule as demonstrated previously [15]. Furthermore, the slow phase of depolarization-induced release is mediated by the DHP receptor of the T-tubule, as evidenced by the fact that it is inhibited by Ca^{2+} release blockers of the dehydropyridine class [19] and by an antibody against the α_1 subunit of the DHP receptor [20]. Therefore, the present finding that the anti-triadin antibody mAb GE 4.90 inhibits depolarization-induced Ca^{2+} release provides direct support for a role of triadin in the DHP receptor-mediated T-tubule-to-SR communication.

The fast phase of depolarization-induced Ca^{2+} release, which is also mediated by the T-tubule [15], is not influenced by the anti-triadin antibody. As shown in recent studies [20,21], several other antibodies and DHP also have differential effects on the two phases of depolarization-induced Ca^{2+} release. Thus, the two phases might be controlled by different types of transmission mechanisms. The role of these two phases in physiological excitation–contraction coupling remains to be investigated.

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REFERENCES

[1] Rios, E. and Brum, G. (1987) Nature 325, 717–720.
[2] Lamb, G.D. and Walsh, T. (1987) J. Physiol. 393, 595–617.
[3] Tanabe, T., Beam, K.G., Powell, J.A. and Numa, S. (1988) Nature 33, 134–139.
[4] Imagawa, T., Smith, J.S., Coronado, R. and Campbell, K.P. (1987) J. Biol. Chem. 262, 11636–11643.
[5] Hymel, L., Inui, M., Fleischer, S. and Schindler, H.G. (1987) Proc. Natl. Acad. Sci. USA 85, 411–415.
[6] Lai, F.A., Erickson, H.P., Rousseau, E., Liu, Q.-Y. and Meissner, G. (1987) Nature 231, 315–319.
[7] Block, B.A., Imagawa, T., Campbell, K.P., Franz, M. and Franzini-Armstrong, C. (1988) J. Cell Biol. 107, 2587–2600.
[8] Yuan, S., Arnold, W. and Jorgensen, A.O. (1991) J. Cell Biol. 112, 289–301.
[9] Caswell, A.H. and Brandt, N.R. (1989) Trends Biochem. Sci. 14, 161–165.
[10] Rios, E., Ma, J. and Gonzales, A. (1991) J. Musc. Res. Cell Motil. 12, 127–135.
[11] Brandt, N.R., Caswell, A.H., Wen, S.-R. and Talvenheimo, J.A. (1990) J. Memb. Biol. 113, 237–251.
[12] Kim, K.C., Caswell, A.H., Talvenheimo, J.A. and Brandt, N.R. (1990) Biochemistry 29, 9281–9289.
[13] Caswell, A.H., Brandt, N.R., Brunschwig, J.-P. and Purkerson, S. (1991) Biochemistry 30, 7507–7513.
[14] Kim, D.H., Ohnishi, S.T. and Ikemoto, N. (1983) J. Biol. Chem. 258, 9662–9668.
[15] Ikemoto, N., Antoniu, B. and Kim, D.H. (1984) J. Biol. Chem. 259, 13151–13158.
[16] Ikemoto, N., Antoniu, B. and Kang, J.-J. (1992) Biophys. J. (abstract).
[17] Donaldson, S.K.B. (1985) J. Gen. Physiol. 86, 501–525.
[18] Rosemblatt, M.S., Perez, G., Antoniu, B., Reilley, E. and Ikemoto, N. (1990) Transductions in Biological Systems (C. Hidalgo, J. Bacigalupo, E. Jaimovich and J. Vergara eds.) pp. 371–382, Plenum Publishing Corp., NY.
[19] Ohkusa, T., Carlos, A.D., Kang, J.-J., Smilowitz, H. and Ikemoto, N. (1991) Biochem. Biophys. Res. Commun. 175, 271–276.
[20] Ohkusa, T., Smilowitz, H.M. and Ikemoto, N. (1990) Biophys. J. 57, 498a.
[21] Ohkusa, T., Kang, J.-J., Antoniu, B., Rosemblatt, M.S., Jay, S.D., Campbell, K.P. and Ikemoto, N. (1991) Biophys. J. 59, 44a.