Mechanism of N-Ethylmaleimide-Induced Contraction of the Frog Sartorius Muscle

S. N. Grishin, A. V. Shakirzyanova, A. Yu. Teplov*, I. M. Fatkhutdinov, V. V. Valiullin, and A. L. Zefirov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 3, pp. 248-250, March, 2006
Original article submitted June 24, 2005

We studied parameters of the frog sartorius muscle contraction initiated by ryanodine receptor agonists in the presence of ROS donors. We hypothesized that sodium nitroprusside and hydrogen peroxide inhibit initiation of contractions by N-ethylmaleimide and that this effect of ROS donors on parameters of N-ethylmaleimide-induced contractions is due to a direct effects of sodium nitroprusside and hydrogen peroxide on N-ethylmaleimide, but not to inactivation of ryanodine receptors in the sarcoplasmatic reticulum of frog skeletal muscle.

Key Words: *muscle contraction; N-ethylmaleimide; reactive oxygen species; ryanodine receptors*

Cisterns of the sarcoplasmatic reticulum (SPR) in skeletal muscle fibers (MF) are the main sites of intracellular Ca^{2+} storage and participate in the regulation of its content determining the strength of MF contraction. Ca^{2+} is released with participation of ryanodine receptors (RyR) located in SPR membrane near transverse tubules contacting with potential-dependent L-type Ca channels (dihydropyridine receptors) via special feet protruding into the cytosol towards dihydropyridine receptors. The action potential induces conformational changes in these receptors, which leads to activation of RyR, Ca^{2+} release from SPR, and its binding to troponin C, which triggers muscle contraction [5]. Skeletal MF SPR has type I ryanodine receptors (RyR_1) [1]. The regulatory sites for Ca^{2+}, Mg^{2+}, ATP, and calmodulin are located on the RyR foot [5]. In addition, RyR has functionally different sites for oxidation and S-nitrosylation; oxidation of these sites modifies channel activity [3]. Sulfhydryl reagent analysis of RyR molecule revealed the presence of thiols involved in the regulation of MF contractile function; failure of muscle work in some pathologies can be due to disorders in this regulation [3,8]. We studied RyR_1-agonist-induced contractions of the frog sartorius muscle MF in response to application of N-ethylmaleimide (NEM) in the presence of ROS.

MATERIALS AND METHODS

Experiments were carried out on lake frogs in autumn-winter. Sartorius muscle preparation was placed into a cuvette (20-21°C) and during constant perfusion by Ringer's solution (115.0 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, and 11.0 mM $NaHCO_3$, pH 7.3) was stretched at a force of 500 mg (isometric conditions) over 40 min. Muscle contraction was induced with RyR agonist added to the cuvette with a microdosing device when perfusion was stopped. Muscle contractions were recorded using a photoelectric transformer, tension changes were recorded by an autorecorder [2]. The contractile function of MF was evaluated by the force of contraction in mg and rate of contractions in mg/sec (determined by the ratio of contraction force to duration of maximum tension development in seconds).

Department of Physiology, *Department of Pathophysiology, Kazan State Medical University. ***Address for correspondence:*** alikteplov@mail.ru. A. Yu. Teplov

0007-4888/06/1413-0278

Carbacholine (200 μM), caffeine (1 mM), NEM, sodium nitroprusside, H_2O_2, and dandrolene (100 μM; Sigma) were used in the study. Agonists (carbacholine, caffeine, NEM) initiating contractions were added to the cuvette at 20-min intervals with perfusion stopped. For evaluating the effects of substances the solution in the cuvette was replaced with a fresh portion before adding the agonist. Effects of the agents were evaluated by comparing contractile characteristics of MF before and after addition of the test substances.

The interaction between nitrogen monoxide and MF was provided by 15-min perfusion with solution containing sodium nitroprusside. The effects of dandrolene and H_2O_2 on MF were studied after 15-min perfusion of MF with solution containing these agents in above concentrations. The results were processed using SigmaStat 3.0 software.

RESULTS

For evaluating the parameters of intact muscle contractions induced by carbacholine we selected a sparing regimen of contractions (20-min intervals between contractions), when the muscle developed the constant amplitude in response to carbacholine in the working concentration during the entire experiment and the initial level of stretching was restored during relaxation (Table 1). The latent period of contraction in response to carbacholine, in contrast to the latent period of contractions with other agonists, was very short, and it was impossible to measure its length by our methods.

Caffeine sensitized RyR_1 molecule to Ca^{2+} and caused muscle contraction because of RyR activation [1]. Addition of caffeine into incubation medium initiated MF contractions with characteristics not differing from those of carbacholine-induced contractions (Table 1).

Characteristics of MF contractions induced by addition of NEM constituted 150% of time of the maximum tension development and 157% of contraction force *vs.* carbacholine-induced contraction (Table 1), with a latent period of 3±1 sec (n=6). After preliminary 20-min washout repeated addition of NEM into the cuvette caused muscle contraction different from the first one: prolongation of latent period to 250%, time of maximum tension development to 267%, decrease of contraction force to 46%, and rate of contractions to 17% in comparison with the first contraction of the muscle in response to NEM, which seemed to indicate a decrease in the muscle sensitivity to this agent.

NEM alkylates three thiol classes in rabbit skeletal muscle RyR, causing three phases of the channel modification, depending on the duration of exposure and concentration [6]. High concentrations of NEM first decrease, then increase, and then again (now irreversibly) decrease the probability of the channel opening. Low concentrations caused the release of Ca^{2+} from SPR, this effect of NEM being completely reversible [3]. These data suggest that frog muscle contractions under these conditions are associated with activation of RyR. Therefore, addition of RyR blocker dandrolene caused no contraction of sartorius muscle in response to agonists (carbacholine, caffeine, NEM), which confirms that the effects of these agents are mediated through RyR activation.

ROS can dose-dependently induce RyR activation and stimulate their inhibition [4,9]. Low concentrations of nitrogen monoxide virtually do not modify channel activity, while its high level causes activation [7,10]. H_2O_2 in submillimolar concen-

TABLE 1. Parameters of Frog Sartorius Muscle Contractions Caused by Carbacholine (Cc), N-Ethylmaleimide (NEM), and Caffeine (Cf) Alone and in the Presence of ROS Donors Sodium Nitroprusside (SNP) and H_2O_2

Applied substances	Force of contraction, mg	Rate of contraction, mg/sec	Time of maximum tension, sec	Number of experiments
Cc	508.0±56.7	91.5±36.1	6.00±0.58	6
Cf	489.5±64.2	93.2±29.8	5.50±0.66	5
NEM	796.7±98.1	83.7±24.8	9.00±0.73	6
SNP+Cc	501.5±59.0	92.1±33.4	5.80±0.63	5
SNP+Cf	497.2±66.7	94.5±32.2	5.40±0.71	5
SNP+NEM	248.4±85.2	109.8±42.9	7.50±0.92	5
H_2O_2+Cc	511.3±64.3	89.0±41.9	6.10±0.69	5
H_2O_2+Cf	498.4±70.7	90.4±32.5	5.70±0.68	5
H_2O_2+NEM	269.4±60.9	105.9±44.3	7.00±1.01	5

trations induces activation of Ca^{2+} release from SPR and inhibits the channel in millimolar concentrations [5]. In our experiments sodium nitroprusside and H_2O_2 caused no contractions of the frog sartorius muscle. However, the force and rate of muscle contractions induced by NEM decreased significantly in the presence of nitrogen monoxide and H_2O_2 (Table 1). NEM in the presence of sodium nitroprusside caused contractions with the following characteristics: time of maximum tension development 100%, force of contraction 46.4% of carbacholine-induced contraction (Table 1).

NEM in the presence of H_2O_2 caused contractions with the following characteristics: time of maximum tension development 168%, force of contraction 58%, rate of contraction 26% of carbacholine-induced contraction (Table 1). The parameters of contractions initiated by carbacholine and caffeine did not change in the presence of ROS in the same concentrations.

Hence, ROS (nitrogen monoxide and H_2O_2) in concentrations negligible for RyR suppressed NEM-induced muscle contraction. Presumably, this effect is due to the effects of these compounds directly on NEM, but not to RyR inactivation. This is confirmed by the results of studies demonstrating the absence of nitrogen monoxide and H_2O_2 effects on muscle contractions induced by carbacholine and caffeine (Table 1) and by the data of authorsdemonstrating in a study of RyR incorporated in bilipid layer that nitrogen monoxide donors in concentrations causing no RyR activation inhibited NEM-induced release of Ca^{2+} from SPR [6]. These authors hypothesized that NEM in low concentrations was not a selective thiol reagent and activated RyR without alkylation of critical thiols.

Hence, NEM can be used as an effective initiator of muscle contractions for model experiments in neuromuscular physiology.

The study was supported by grant NSh-1383. 2003.4.

REFERENCES

1. O. P. Balezina, *Uspekhi Fiziol. Nauk*, **33**, No. 3, 31 (2002).
2. R. Blattner, H. I. Classen, H. Denert, and H. Dering, *Experiments on Isolated Smooth Muscle Preparations* [in Russian], Moscow (1983).
3. B. Aghdasi, J. Z. Zhang, Y. Wu, *et al.*, *J. Biol. Chem.*, **272**, No. 6, 3739-3748 (1997).
4. T. G. Favero, A. C. Zable, and J. J. Abramson, *Ibid.*, **270**, No. 43, 25 557-25 563 (1995).
5. C. Franzini-Armstrong and F. Protasi, *Physiol. Rev.*, **77**, 699-729 (1997).
6. E. V. Menshikova, E. Cheong, and G. Salama, *J. Biol. Chem.*, **275**, No. 47, 6775-6780 (2000).
7. L. G. Meszaros, I. Minarovic, and A. Zahradnikova, *FEBS Lett.*, **380**, Nos. 1-2, 49-52 (1996).
8. T. Naganuma, T. Murayama, and Y. Nomura, *Arch. Biochem. Biophys.*, **364**, No. 2, 133-142 (1999).
9. G. Salama, E. V. Menshikova, and J. J. Abramson, *Antioxid. Redox. Signal*, **2**, No. 1, 5-16 (2000).
10. A. Zahradnikova, I. Minarovic, R. C. Venema, and L. G. Meszaros, *Cell Calcium*, **22**, No. 6, 447-454 (1997).