Free oxygen radicals mediate the effect of arachidonic acid and prostaglandin G_2 on calcium uptake by sarcoplasmic reticulum of masseter muscle*

Eiichiro Okabe, Eiji Hiyama, Masakazu Oyama, Hideto Ogawa, and Haruo Ito

*Department of Pharmacology (Chief: Prof. Haruo Ito), Kanagawa Dental College,
82 Inaoka-cho, Yokosuka 238, Japan*

[Accepted for publication: April 20, 1982]

Key words: prostaglandins / free radicals / masseter muscle / ATPase / calcium

Introduction

The generation of oxygen-free radicals appear to be a likely candidate as intracellular mediator in the ischemic process. Tappel et al.¹⁾ postulated that ischemia reperfusion injury can be partially attributed to free radical production which enhances lipid peroxidation at the cell membrane. Lipid peroxidation, in turn, leads to membrane alterations from phospholipid depletion and also leads to inhibition of the membrane associated enzymes²⁾. Further, the generation of free radicals from the metabolic pathways of arachidonic acid can also result in the formation of lipid peroxides which may cause lysis of the red cells, destruction of the endothelial cells and peroxidation of the lysosomal and mitochondrial membranes. These radicals, being highly reactive forms of oxygen, may cause mechanical abnormalities of the excitation-contraction coupling system in the masseter muscle.

This study was designed to investigate the possibility that the effect of prostaglandins on the calcium transport of the sarcoplasmic reticulum of the canine masseter muscle may be mediated by production of free radicals.

Materials and Methods

Sarcoplasmic reticulum vesicles (SR) were prepared from a minced canine masseter

muscle by modification of the method of Harigaya and Schwartz³⁾. The oxalate-supported calcium uptake and calcium-stimulated, magnesium-dependent adenosine triphosphatase (Ca^{2+} -ATPase) activity of the SR were determined according to previous reports^{4,5)}.

The prostaglandin (PG) cyclic endoperoxides PGG_2 and PGH_2 were biosynthesized from radiolabeled arachidonic acid by using the cyclooxygenase from sheep seminal vesicle microsomes⁶⁾. The cyclooxygenase inhibitor indomethacin (Sigma Chemical, St. Louis, U.S.A.; 5 mg/kg, iv) was treated 30 minutes before isolation of the SR.

The SR isolated from the normal or indomethacin-treated animal was pre-incubated (10 ml; 27°C) at the desired pH for 2.0 or 2.5 minutes prior to addition of Mg, ATP and Ca (also pre-equilibrated to the temperature). Then, arachidonic acid (P-L Biochemicals, Milwaukee, U.S.A.; 200 μ g/ml), 11,14,17-eicosatrienoic acid (P-L Biochemicals, Milwaukee, U.S.A.; 200 μ g/ml), PGG_2 (10 μ g/ml), PGH_2 (10 μ g/ml), PGE_2 (Ono Chemical, Osaka, Japan; 10 μ g/ml), acetone or ethanol vehicle was added 1.0 minutes before superoxide dismutase (SOD, Sigma Chemical, St. Louis, U.S.A.; 10 μ g/ml final concentration), d-mannitol (20 mM final concentration) or substrates. SOD was used to scavenge the $\cdot O_2^-$ and d-mannitol to scavenge the $\cdot OH^{\cdot}$. SOD was added 0.5 minutes before d-mannitol or the substrates. d-Mannitol was added 0.5 minutes before the substrates. The pH of each individual

* General principles of this paper were presented at "The 29th annual meeting of the Japanese Association for Dental Research (Osaka, 1981)".

reaction was adjusted with an imidazole buffer following the method of Fabiato and Fabiato⁸⁾ and then re-checked prior to initiation of the reaction sequence.

Results and Discussion

Our observations in this experiment appear to be in agreement with the data of Arkhipenko et al.⁹⁾ who identified a specific defect in the sarcoplasmic reticulum (SR) from the ischemic skeletal muscle; i.e., uncoupling of the calcium transport from ATP hydrolysis.

The results at pH 7.0 (Fig. 1-A) show that arachidonic acid and PGG₂ produced a significant reduction in calcium uptake velocity, or in Ca²⁺-ATPase activity. In the presence of SOD, both the decrease in calcium uptake velocity and in Ca²⁺-ATPase activity caused by arachidonic acid or PGG₂

were restored to a normal level. The reduction in calcium uptake velocity and Ca²⁺-ATPase activity induced by arachidonic acid was inhibited by treatment with indomethacin. When calcium uptake velocity or Ca²⁺-ATPase activity was reduced by PGG₂, indomethacin had no effect on the reduction in calcium uptake velocity, or in Ca²⁺-ATPase activity. d-Mannitol was, by itself, incapable of affecting any reduction in calcium uptake velocity or Ca²⁺-ATPase activity induced by arachidonic acid or PGG₂. The addition of 11,14,17-eicosatrienoic acid, PGH₂ or PGE₂ caused no significant reduction in calcium uptake velocity, or in Ca²⁺-ATPase activity. Figure 1-B shows the effect of arachidonic acid and PGG₂ on calcium uptake velocity and on Ca²⁺-ATPase activity at pH 5.5. Arachidonic acid and PGG₂ produced a significant reduction in the calcium uptake velocity, but Ca²⁺-ATPase activity

Fig. 1 The effect of prostaglandins and fatty acids on calcium uptake velocity and on Ca²⁺-ATPase activity at pH 7.0 (A) and pH 5.5 (B). Where indicated, 11,14,17-ESA = 11,14,17-eicosatrienoic acid, AA = arachidonic acid, IDM = indomethacin, SOD = superoxide dismutase, M = mannitol. Calcium uptake velocity and ATPase activity measured under standard condition are indicated as C. Each bar represents the mean (n=8) and the error bar $\pm$ one SEM. ^aSignificantly (p<0.01) different from 11,14,17-ESA. ^bSignificantly (p<0.01) different from AA. ^cSignificantly (p<0.01) different from acetone vehicle. ^dSignificantly (p<0.01) different from PGG₂ alone.

was not affected. Indomethacin inhibited the reduction in calcium uptake velocity induced by arachidonic acid, but did not inhibit that by PGG₂. SOD had no effect on the reduction in calcium uptake velocity caused by arachidonic acid or PGG₂. d-Mannitol, together with SOD, was capable of inhibiting the reduction in calcium uptake velocity induced by arachidonic acid or PGG₂. The addition of 11,14,17-eicosatrienoic acid, PGH₂ or PGE₂ had no effect on calcium uptake velocity or on Ca²⁺-ATPase activity.

The ethanol or acetone vehicles, in the same concentration as in the various solutions, had no effect on calcium uptake velocity or on Ca²⁺-ATPase activity at either pH 7.0 or pH 5.5. Similarly, SOD, d-mannitol, SOD plus d-mannitol or indomethacin had little effect by themselves.

In conclusion, it can be suggested that prostaglandin synthesis induced by exogenous arachidonic acid causes damage to the SR function in the masseter muscle and that this damage is the result of free oxygen radicals generated by conversion of PGG₂ to PGH₂. In the process of acidosis, it appears that the superoxide anion radical ($\cdot\text{O}_2^-$) is generated first and subsequently, the more lethal hydroxyl radical ($\cdot\text{OH}$), and that protons directly depress SR calcium transport by uncoupling the calcium transport from ATP hydrolysis.

References

- 1) Tappel, A. L.: Lipid peroxidation damage to cell components. *Fed. Proc.* **32**: 1870, 1973.
- 2) Hochstein, P. and Jain, S. K.: Association of lipid peroxidation and polymerization of membrane proteins with erythrocyte aging. *Fed. Proc.* **40**: 183-188, 1981.
- 3) Harigaya, S. and Schwartz, A.: Rate of calcium binding and uptake in normal animal and failing human cardiac muscle. *Circ. Res.* **25**: 781-794, 1969.
- 4) Okabe, E. and Hess, M. L.: Effect of acidosis on the excitation-contraction coupling system of cardiac muscle. *Jpn. Circul. J.* **45**: 1291-1296, 1981.
- 5) Hess, M. L., Okabe, E. and Kontos, H. A.: Proton and free radical interaction with the calcium transport system of cardiac sarcoplasmic reticulum. *J. Mol. Cell. Cardiol.* **13**: 767-772, 1981.
- 6) Hamberg, M., Svensson, J., Wakabayashi, T. and Samuelsson, B.: Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proc. Natl. Acad. Sci. U.S.A.* **71**: 345-349, 1974.
- 7) Bawn, K. and Fridovich, I.: Superoxide radical and superoxide dismutase: Threat and defense. *Acta Physiol. Scand. (Suppl.)* **492**: 9-18, 1980.
- 8) Fabiato, A. and Fabiato, F.: Effects of pH on the myofilaments and sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J. Physiol. (London)* **276**: 233-255, 1978.
- 9) Arkhipenko, Y. V., Bilenko, M. V., Dobrina, S. K., Kagan, V. E., Kozlou, Y. P. and Shelenkoua, L. N.: Ischemic damage to the sarcoplasmic reticulum of skeletal muscles: the role of lipid peroxidation. *Bull. Exp. Biol. Med.* **83**: 683-686, 1977.