Low Oxygen Tension Induces Positive Inotropy and Decreases a^i_{Na} in Isolated Guinea-Pig Cardiac Ventricular Papillary Muscles

Ming-Jy Jao and Jung-Mou Yang

Department of Physiology and Biophysics
National Defense Medical Center
Taipei 100, Taiwan, ROC

Abstract

Effects of low oxygen on contractile force, intracellular Na^+ activity (a^i_{Na}), and action potential were simultaneously measured in isolated guinea-pig ventricular papillary muscles. Reduction of oxygen from control 488 to 150 mmHg biphasically increased and decreased the twitch tension, and decreased a^i_{Na} in muscles driven at 60 beats/min. The action potential duration (APD) was decreased but the maximum rate of upstroke ($\dot{V}_{max}$) was increased. In control, 1 μM epinephrine significantly increased the the action potential amplitude and twitch tension with decreases in the time to twitch peak (TTP), time for 50% relaxation (RT_{50}), and a^i_{Na}. After exposure to low oxygen for 10 min, with twitch tension elevated and TTP and RT_{90} increased, 1 μM epinephrine significantly increased the twitch tension and $\dot{V}_{max}$, and decreased the APD and a^i_{Na}. Pretreatment with reserpine inhibited the twitch tension, both at control and in the presence of epinephrine. But changes of action potential and a^i_{Na} in response to low oxygen and epinephrine were similar to those in control. Our results indicate that the isolated guinea-pig ventricular muscle needs a high oxygen tension to maintain a normal contractile function. Reduction of oxygen deteriorates the electrical and mechanical activities, most likely, by a coaxial graded hypoxia. The decreased a^i_{Na}, not associated with endogenous catecholamines, suggests that the activity of the Na^+-K^+ pump can be maintained in the superficial muscle cells despite of core-central hypoxia.

Key Words: guinea-pig cardiac ventricular papillary muscle, hypoxia, intracellular Na^+ Activity, Na^+-K^+ pump, reserpine

Introduction

In cardiac muscles, there is a general consensus that mechanisms responsible for enhancing contraction are associated with increases in intracellular Na^+, Ca^{2+}, pH, or the sensitivity of contractile protein to Ca^{2+}. Hypoxia can result in decreases in action potential duration and contractile force (4, 8, 27). However, an initially transient increase in the contractile force has been observed during early decrease of oxygen content both from *in vivo* and *in vitro* studies (8, 22, 25, 26). These findings suggest that graded decrease of oxygen content may enhance mechanical performance of cardiac muscles before contraction weakens and then fails. An endogenous release of catecholamines or cardioactive peptides may contribute to the positive inotropy (6, 10, 14). In another aspect, rigor contraction observed in the anoxic state has been attributed to an accumulation of intracellular Na^+ and Ca^{2+} (1, 2, 11). Reports of changes of a^i_{Na} in ventricular muscles during hypoxia are diverse (9, 16, 18, 20, 29). The systolic and diastolic $[Ca^{2+}]_i$ are also found to rise despite of a decline in tension during early hypoxia (2). In ferret papillary muscles, oxygen deficit may produce no change in the intracellular Ca^{2+} activity (a^i_{Ca}) and a transient increase in the intracellular pH before acidification occurs (7, 9). Less is known about mechanisms underlying the observed contractile enhancement during early hypoxia.

In the present study, we simultaneously

Corresponding author: Dr. Jung-Mou Yang, Department of Physiology and Biophysics, National Defense Medieal Center, Box 90048-503, Taipei, Taiwan, ROC. Tel: 886-2-23650267, E-mail: jmyang@ndmc1.ndmctsgh.edu.tw

monitored changes of contractile force, action potential, and a^i_{Na} in response to lowering of oxygen tension of the perfusate from high partial pressure to a physiological range in isolated guinea pig ventricular papillary muscles. Possible mechanisms involving the positive inotropy in association with the effects of oxygen change were explored. In some experiments, reserpine was given to eliminate effects of endogenous cardioactive transmitters from nerve terminals.

Materials and Methods Male

guinea-pigs (200-450 g) were sacrificed by cervical dislocation. A strand of fine papillary muscle, about 0.5 mm in diameter and 1-3 mm in length from the right ventricle, was carefully dissected and then mounted in a narrow perfusing chamber. One end of the muscle fiber was fixed and driven with a Grass stimulator. The other end was tied to a force displacement transducer (Cambridge, 403A) to measure tension. The sensitivity of the transducer was adjusted at 30 mV/mg. Two L-shaped insect-pins were used to fix the muscle fiber near the driven end, leaving a segment to be impaled without vigorous twitch displacement. The muscle fiber was driven at a rate of 60 beats/min and was stretched until the tension was 60-80% of maximum. The time from the start of stimulation to twitch peak (TTP) and the times for 50% and 90% relaxation (RT_{50} and RT_{90}) were measured and analyzed. The superfusing Tyrode solution contained (in mM) NaCl 135; KCl 5.4; $CaCl_2$ 1.8; $NaHCO_3$ 12; $MgCl_2$ 1.1; NaH_2PO_4 0.5; glucose 5.0; was bubbled with a gas mixture of 97% O_2 and 3% CO_2, and was maintained at 37 °C. The PO_2, PCO_2, and pH in perfusion chamber were measured by Acid-Base Lab 3 (Radiometer Copenhagen). In the reservoir, the parameters of PO_2, PCO_2, and pH were 825±10 mmHg, 37.5±3.0 mmHg, and 7.39±0.02, respectively. In the perfusion system, the pH slightly increased to 7.48±0.01, PO_2 and PCO_2 decreased to 488±26 and 33.1±3.0 mmHg when the perfusate flowed from the reservoir into the tissue chamber. The specimen was equilibrated for at least two hours to achieve a stable condition. In low oxygen studies the oxygen was withdrawn and replaced with equal amount of N_2. Changes of PO_2 and PCO_2 in the tissue chamber were monitored for 90 min (Fig. 1). In studies of endogenous catecholamine depletion, reserpine (5 mg/kg) was administered to the animal intraperitoneally 12 hr prior to the experiment.

The conventional microelectrode, made from borosilicate micropipettes, had a tip resistance in the range 10-40 MΩ when backfilled with 3 M KCl solution. The action potential was displayed on an oscilloscope. The maximal rate of upstroke of the action potential ($\dot{V}_{max}$) was recorded after the signal

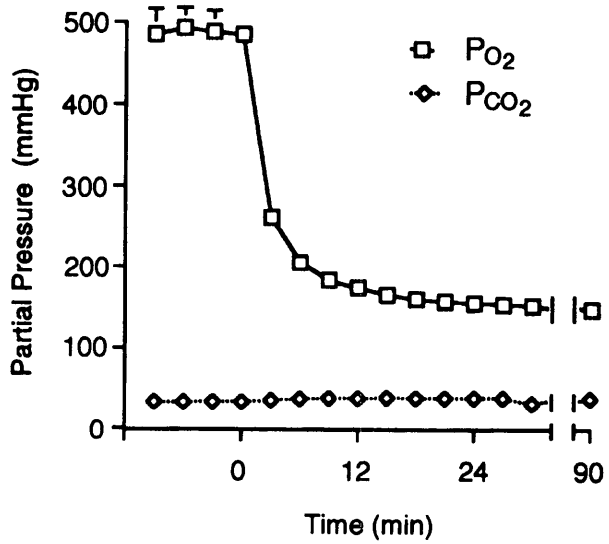

Fig.1. Changes of PO_2 (❑) and PCO_2 (◊) in perfusing chamber. In the open circulating system, the parameters of PO_2 and PCO_2 in the reservoir averaged 825 and 37.5 mmHg, respectively. For the purpose of electrical isolation, a polyethylene tube carrying the perfusion fluid from the reservoir to the perfusing chamber was submerged in the water bath and the tube was somewhat permeable to oxygen. Thus, when the reservoir was bubbled with nitrogen gas (at time 0), the oxygen in the perfusate of the tissue chamber was abruptly decreased but was then maintained at a rather stable level during the 90 min observation period.

was passed through a differential amplifier. The Na^+-selective microelectrode made from thick-wall pipettes was beveled and backfilled with 100 mM NaCl after silanization with a tiny amount of n-tributylchlorosilane (31). A 100-300 μm column of Na^+-selective liquid sensor (Fluka) was drawn into the tip. After each experiment, the sodium electrode was calibrated using a method of single-electrolyte solutions (17). The latter contains 100 mM, 10 mM, 1 mM NaCl, or 100 mM KCl. The potential response of Na^+-electrodes was 58.6±0.5 mV (n=42) per tenfold sodium activity change at 37 °C. The selectivity coefficient for K (k_{NaK}) was 0.01±0.001 (n=42). Signals from the conventional and Na^+-selective microelectrodes were passed through two identical low-pass filters with a fixed frequency of 0.2 Hz. The electrical potentials of the sodium and conventional electrodes (E^i_{Na} and $\overline{V}_m$) and their difference (a^i_{Na}) were recorded on a chart recorder. The intracellular Na^+ activity was calculated according to a modified Nicolsky equation (17):

$$E^i_{Na} - \overline{V}_m = E_o + S \log[a^i_{Na} + k_{Nak} a^i_K],$$

where E^i_{Na} and $\overline{V}_m$ are the respective potentials of sodium and conventional electrodes in cells. E_o is the constant potential of the electrometric system. S is the slope of potential response in calibration solutions

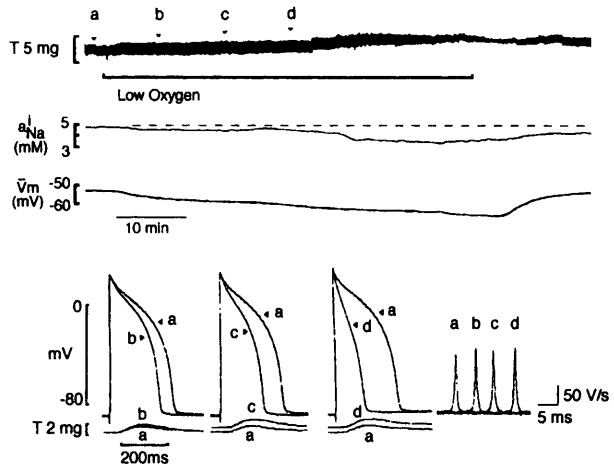

Fig. 2. Effects of oxygen reduction on the guinea-pig ventricular papillary muscle. Slow recordings of twitch tension (T), intracellular Na^+ activity (a^i_{Na}), and filtered membrane potential ($\bar{V}_m$) were shown in upper traces; Fast recordings of action potential, maximal rate of upstroke of action potential ($\dot{V}_{max}$), twitch tension taken at points a, b, c, and d in upper traces were shown below. Note that the twitch tension was biphasically changed, the APD and a^i_{Na} were continually decreased in low oxygen. The resting tension was slightly elevated during low oxygen.

for each electrode. a^i_K is the intracellular K^+ activity, and the reported value of 116 mM was used for calculation (4). It was noted that a significant loss of intracellular a^i_K was found only in muscle fibers exposed to hypoxia for more than 2 hr with marked depolarization (4). Changes of a^i_K and a^i_{Ca} during the experiment were neglected in this study because the exposure of the muscle fiber to low oxygen was studied within 20 min (2, 4,5, 11).

Epinephrine hydrochloride was purchased from Sigma Chemicals and freshly prepared during experiments. The experimental data were pooled for analysis, using only experiments which attained at least a level of 80% recovery. The twitch tension was analyzed as the relative change from control. The Student's *t*-test or Newman-Kruels test was employed to analyze the experimental data. The difference was considered significant when the p value was less than 0.05.

Results

Effects of Low Oxygen on a^i_{Na}

When the oxygen tension of the perfusate was reduced from a high level to a physiological range, twitch tension of the muscle fiber always biphasically increased for twenty to thirty minutes and then decreased below the control level. Ectopic beats usually occurred and easily dislodged the electrodes. In one muscle fiber, as shown in Figure 2, ectopic beat did not develop after exposure to low oxygen tension for 55 min. In this fiber the contractile force during low oxygen first increased slightly for 30 min, and then gradually decreased to below control. The a^i_{Na} and durations of the action potential at 30% and 90% repolarization (APD_{30} and APD_{90}) were decreased, but the $\dot{V}_{max}$ was increased during 60 min observation period. In another five papillary muscles, except the occurrence of ectopic beats, change in the twitch tension was similar in the low oxygen perfusate.

Effects of Epinephrine

Since a long exposure to low oxygen was usually associated with the development of ectopic rhythm from which a reliable recording was difficult to obtain, the effects of low oxygen and epinephrine were undertaken within 20 min. In control, 1 μM epinephrine increased the twitch tension and the action potential amplitude (APA) with decreases in a^i_{Na} and APD (Fig. 3). In eight muscle fibers tested, the twitch

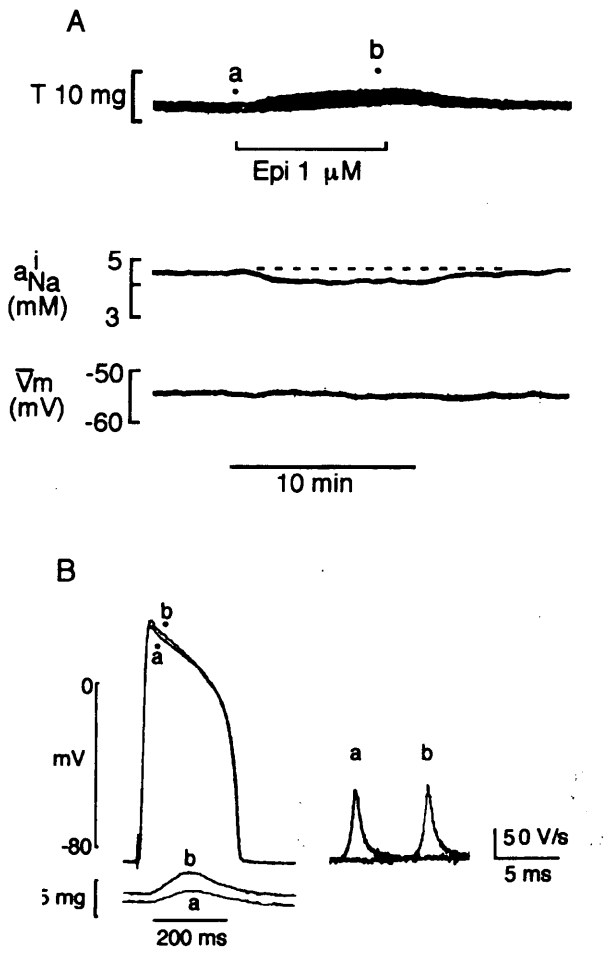

Fig. 3. Effects of epinephrine (Epi) on the guinea-pig ventricular papillary muscle. Abbreviations as in Fig. 2. After 1 μM epinephrine, the plateau was elevated and the action potential slightly decreased.

Table 1. Effects of Low Oxygen and Epinephrine on Action Potential, a^{i}_{Na}, an Twitch Tension in Normal and Reserpinized Guinea-pig Ventricular Papillary Muscles

	APA(mV)	APD_{30}(ms)	APD_{90}(ms)	$\dot{V}_{max}$(V/s)	a^{i}_{Na}(mM)	TT(%)
Normal muscle fibers (n=10)						
control	119±2	138±4	214±3	122±10	4.6±0.1	100
low oxygen	118±2	82±9*	153±10*	126±10	4.3±0.1*	256±17*
low oxygen + 1 μM Epi	117±2	47±5*†	134±9*†	131±12*†	3.9±0.1*†	588±86*†
Reserpinized muscle fibers (n=8)						
control	122±1	128±4	182±5	150±2	4.3±0.1	
low oxygen	123±1	96±7*	140±9*	154±3	3.8±0.1*	
low oxygen + 1 μM Epi	123±1	85±7*†	126±8*†	157±2*†	3.4±0.1*†	

Values are mean±SE *p<0.05 vs. control; †p<0.05 vs. low oxygen Abbreviations: Epi, epinephrine; APA, action potential amplitude; APD_{30} and $APD9_{90}$, duration of action potential at 30% and 90% repolarization; $\dot{V}_{max}$, maximal rate of depolarization; a^{i}_{Na}, intracellular Na^{+} activity; TT, twitch tension. The exposure to epinephrine was 6-8 min after oxygen reduction for 10 min.

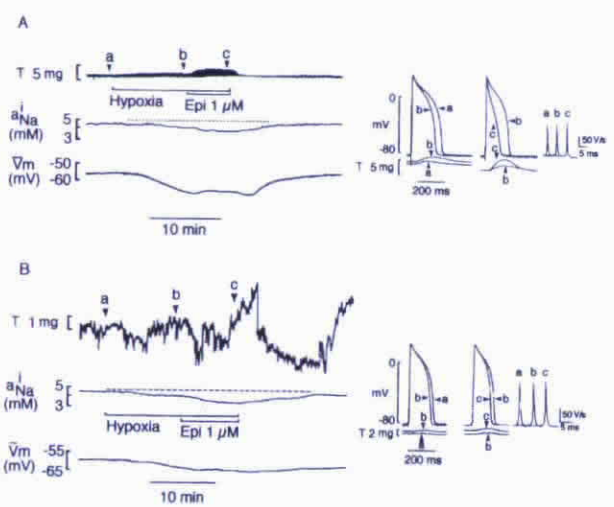

Fig. 4. Effects of oxygen reduction and oxygen reduction plus epinephrine (Epi) on the normal and reserpine-pretreated guinea-pig ventricular papillary muscles. A, normal muscle fiber; B, reserpinized muscle fiber. Abbreviations are the same as in Fig. 2. Note that the reserpinized fiber was too weak to keep a steady recording, but changes in a^{i}_{Na} and membrane potential were similar to those in non-reserpinized fibers.

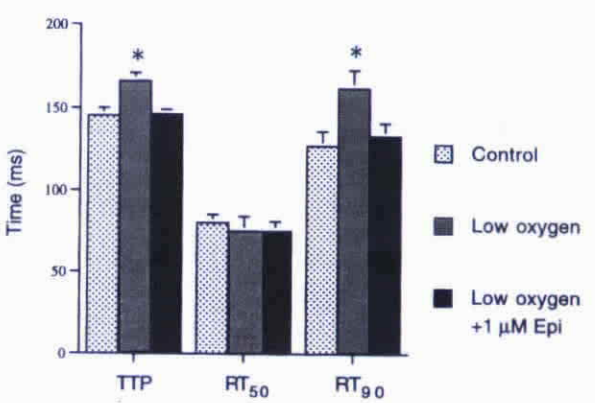

Fig. 5. Changes in the time to twitch peak (TTP) and the times for 50% and 90% relaxation (RT_{50} and RT_{90}) of the twitch tension by reduction of oxygen tension and subsequent addition of epinephrine in ten ventricular papillary muscles. Epinephrine was added after exposure of muscle fibers to low oxygen for ten minutes. In control, averages of the TTP, RT_{50}, and RT_{90} were 145±5, 82±5, and 127±9 ms, respectively. *: p<0.01, Newman-Kruels test.

tension was increased by 90% of the control. The time to twitch peak (TTP) and time for 50% relaxation (RT_{50}) were significantly decreased from 157±8 and 82±4 ms to 139±8 and 69±5 ms (p<0.005), respectively. But the time for 90% relaxation (RT_{90}) was insignificantly changed from 148±10 to 143±10 ms. The APA significantly increased from 122±2 to 127±2 mV (p<0.05). Both the APD_{30} and APD_{90} were insignificantly changed from 166±12 and 235±12 ms to 165±14 and 230±15 ms, respectively, but the $\dot{V}_{max}$ was insignificantly increased from 144±12 to 154±14 V/s. The a^{i}_{Na}, however, was significantly decreased from 4.5±0.1 to 3.9±0.2 mM, by nearly 12% of the control (p<0.05).

The effects of low oxygen and epinephrine are shown in Figure 4A. In this experiment, reduction of oxygen tension for 10 min slightly increased the twitch tension and $\dot{V}_{max}$, but decreased a^{i}_{Na}, APD_{30} and APD_{90}. The addition of 1 μM epinephrine increased further the twitch tension and $\dot{V}_{max}$, and further decreased a^{i}_{Na}, APD_{30} and APD_{90}. Similar changes were found in nine additional fibers and the results are summarized in Table 1. In addition, changes in TTP, RT_{50} and RT_{90} of the twitch tension were analyzed and shown in Figure 5. From this figure,

low oxygen tension increased not only TTP but also RT_{90} that was ameliorated by 1 μM epinephrine.

Reserpine was administered to deplete the endogenous catecholamine from the nerve terminal. The reserpinized cardiac muscle fiber responded erratically to electrical stimulation and was unable to maintain a steady resting tension. As shown in Figure 4B, the twitch tension was very weak and did not respond well to low oxygen and epinephrine. Except the twitch tension, changes in the a^i_{Na} and action potential were similar to those in non-reserpine-treated fibers (Fig. 4A) during low oxygen and the subsequent application of 1 μM epinephrine. Data of eight muscle fibers are summarized in Table 1.

Discussion

In the isolated guinea-pig ventricular papillary muscle, low oxygen in the perfusate can cause core-central hypoxia (3). During hypoxia, an inefficient anaerobic metabolism from glycogen and glucose utilization becomes a major alternative source of cellular ATP, and the production of lactate and pyruvate should be increased (12, 13, 19, 24). Therefore, a normal function of excitation-contraction coupling maintained after oxygen reduction depends on the magnitude of coaxial graded hypoxia in the papillary muscle (3). In the present study, the increased twitch tension after low oxygen can not attribute to an increase in Ca^{2+} currents because the plateau phase of the action potential is decreased and does not improve with epinephrine. Alternatively, the elevated resting tension and increased relaxation time are observed after reduction of oxygen, suggesting that the muscle is partially contractured by anoxic core (1, 8). The increased intrinsic tension can explain the increased twitch tension from the length-tension relationship. On the other hand, a progressive increase in cytosolic H^+ was observed when oxygen was low (our unpublished results). After a long period of low oxygen tension, a severe acidosis in the core of the papillary muscle can decrease Ca^{2+} inward currents and deteriorate the function of sarcoplasmic reticulums to uptake and release of Ca^{2+}, resulting in the decreased twitch tension (11, 15, 21). Therefore, the result in the mechanical activity depends on the extent of core-central hypoxic effect.

In the present study, the most prominent electrical event after reducing oxygen tension is a shortening of the APD with an increased $\dot{V}_{max}$. The membrane potentials measured by the microelectrodes are only in the superficial layer where oxygen supply is still adequate. In the similar maneuver, pH_i measured by H^+-selective microelectrodes was progressively decreased (unpublished observations). This suggests that, during low oxygen tension, cells in the core of the papillary muscle suffer from hypoxia. The production and release of H^+ from the core can decrease cellular pH in the superficial layer. Therefore, the decreased APD can be explained by a core central hypoxia, which can increase an outward current, probably the inward rectifier, I_{K1} and decrease the open probability of the Ca^{2+} channel (15, 21, 23, 28). The increased $\dot{V}_{max}$, possibly by an effect of electrical coupling between cells, indicates that the fast Na^+ inward current is not inhibited in the multicellular preparation.

The reported changes in a^i_{Na} during hypoxia are diverse, including no change in the dog and ferret, an increase in the sheep or guinea-pig, or a decrease in guinea-pig cardiac muscles (9, 16, 18, 20, 29). The different results are possibly due to varied experimental conditions and spatial inhomogeneous effect of prolonged hypoxia. In the present study, the impalements of the two microelectrodes within 200 mm in the superficial muscle cells are stable and the membrane potentials are quite similar in control and can completely recover after experiment. The inhomogeneous effects is unlikely in the early state unless a long exposure to low oxygen condition (3, 4). The decreased a^i_{Na} in the superficial cells indicates that a net flux of Na^+ is outwardly increased during reduction of oxygen. The explanation can be that (a) the activity of the Na^+-K^+ pump is maintained in adequate oxygen tension. That is why stimulation of the adrenoceptor, known to activate the Na^+-K^+ pump, further decreases a^i_{Na} (28, 30). (b) The intercellular accumulation of H^+, released from the core, enhances Na^+-H^+ exchange and then decreases a^i_{Na}. This can explain why there are decreases in a^i_{Na} and pH_i. One might argue that the decrease in a^i_{Na} is induced by endogenous release of catecholamines during hypoxia. In the present study, pretreatment with reserpine which can deplete the endogenous catecholamines from the nerve terminal directly inhibits the mechanical activity. But changes in a^i_{Na} and the membrane potential in response to oxygen reduction are similar to those without reserpine. These results suggest that changes in the electrical activity and a^i_{Na} in the superficial layer are mainly affected by anoxic core during reduction of oxygen, not associated with endogenous catecholamines.

In conclusion, the isolated papillary muscle needs high oxygen tension to maintain a normal excitation-contraction coupling. Once the oxygen tension is low, a varied extent of coaxial hypoxia from peripheral to core-center can produce paradoxical changes in a^i_{Na} and the mechanical and electrical activities. In the superficial muscle cells, the activity of Na^+-K^+ pump is maintained to keep a^i_{Na} low that is not due to endogenous catecholamines in low oxygen.

The initial increase in the twitch tension can be due to an enhancement of intrinsic resting tension by hypoxic core.

Acknowledgments

We thank Dr. H.-H. Wang of Columbia University and Dr. C.-Y. Chai of Institute of Biomedical Sciences, Academia Sinica, for their advice and encouragement. This study was supported by grants from National Science Council (NSC 81-0420-B-016-591 and NSC 84-2331-B-016-067 M04) and Shih-Chun Wang Memorial Fund.

References

1. Allen, D.G., and C.H. Orchard. Myocardial contractile function during ischemia and hypoxia. *Circ. Res.* 60: 153-168, 1987.
2. Allen, D.G., J.A. Lee, and G.L. Smith. The consequences of simulated ischemia on intracellular Ca^{2+} and tension in isolated ferret ventricular muscle. *J. Physiol.* 410: 297-323, 1989.
3. Aomine, M., M. Arita, S. Imanishi, and T. Kiyosue. Surface layer ATP-related contraction in isolated, superfused canine ventricular papillary muscle: an isotachophoretic analysis. *Jpn. J. Physiol.* 32: 895-910, 1982.
4. Baumgarten, C.M., C.J. Cohen, and T.F. McDonald. Heterogeneity of intracellular potassium activity and membrane potential on hypoxic guinea pig ventricles. *Circ. Res.* 49: 1181-1189, 1981.
5. Chapman, R.A., A. Coray, and J.A.S. McGuigan. Sodium-calcium exchange in mammalian ventricular muscle: A study with sodium-sensitive micro-electrodes. *J. Physiol.* 343: 253-76, 1983.
6. Dart, A.M., and R.A. Riemersma. Effects of acidosis on anoxic and exocytotic noradrenaline release from the heart. *J. Mol. Cell. Cardiol.* 21: 75-83, 1989.
7. Ellis, D., and J. Noireaud. Intracellular pH in sheep Purkinje fibres and ferret papillary muscles during hypoxia and recovery. *J. Physiol.* 383: 125-141, 1987.
8. Goldhaber, J.I., J.M. Parker, and J.N. Weiss. Mechanisms of excitation-contraction coupling failure during metabolic inhibition in guinea-pig ventricular myocytes. *J. Physiol.* 443: 371-386, 1991.
9. Guarnieri, T. Intracellular sodium-calcium dissociation in early contractile failure in hypoxic ferret papillary muscles. *J. Physiol.* 388: 449-465, 1987.
10. Haass, M., G. Richardt, and A. Schomig. Potentiation of potassium-evoked noradrenaline and neuropeptide Y co-release by cardiac energy depletion: role of calcium channels and sodium-proton exchange. *Naunyn-Schmiedebergs Archives Pharmacol.* 346: 410-418, 1992.
11. Haigney, M.C.P., H. Miyata, E.G. Lakatta, M.D. Stern, and H.S. Silverman. Dependence of hypoxia cellular calcium loading on Na^+-Ca^{2+} exchange. *Circ. Res.* 71: 547-557, 1992.
12. Hohl, C.M., and R.A. Altschuld. Response of isolated adult canine cardiac myocytes to prolonged hypoxia and reoxygenation. *Am. J. Physiol.* 260: C383-C391, 1991.
13. Jarmakani, J.M., T. Nagatomo, M. Nakazawa, and G.A. Langer. Effect of hypoxia on myocardial high-energy phosphates in the neonatal mammalian heart. *Am. J. Physiol.* 235 (Heart Circ. Physiol. 4): H475-H481, 1978.
14. Kagamu, H., T. Suzuki, M. Arakawa, and Y. Mitsui. Low oxygen enhances endothelin-1 (ET-1) production and responsiveness to ET-1 in cultured cardiac myocytes. *Biochem. Biophysiol. Res. Comm.* 202: 1612-1618, 1994.
15. Kaibara, M., and M. Kameyama. Inhibition of the calcium channel by intracellular protons in single ventricular myocytes of the guinea-pig. *J. Physiol.* 403: 621-640, 1988.
16. Kleber, A.G. Resting membrane potential, extracellular potassium activity, and intracellular sodium activity during acute global ischemia in isolated perfused guinea pig hearts. *Circ. Res.* 52: 442-450, 1983.
17. Lee, C.O. Ionic activities in cardiac muscle cells and application of ion-selective microelectrodes. *Am. J. Physiol.* 241 (Heart Circ Physiol 10): H459-H478, 1981.
18. MacLeod, K.T. Effects of hypoxia and metabolic inhibition on the intracellular sodium activity of mammalian ventricular muscle. *J. Physiol.* 416: 455-468, 1989.
19. McDonald, T.F., and D.P. MacLeod. Metabolism and the electrical activity of anoxic ventricular muscle. *J. Physiol.* 229: 559-582, 1973.
20. Nakaya, H., S. Kimura, and M. Kanno. Intracellular K^+ and Na^+ activities under hypoxia, acidosis, and no glucose in dog hearts. *Am. J. Physiol.* 249 (Heart Circ. Physiol. 18): H1078-H1085, 1985.
21. Orchard, C.H., and J.C. Kentish. Effects of changes of pH on the contractile function of cardiac muscle. *Am. J. Physiol.* 258 (Cell Physiol. 27): C967-C981, 1990.
22. Portman, M.A., T.A. Standaert, and X.H. Ning. Relation of myocardial oxygen consumption and function to high energy phosphate utilization during graded hypoxia and reoxygenation in sheep in vivo. *J. Clin. Invest.* 95: 2134-2142, 1995.
23. Sato, R., A.Noma, Y. Kurachi, and H. Irisawa. Effects of intracellular acidification on membrane currents in ventricular cells of the guinea-pig. *Circ. Res.* 57: 553-561,1985.
24. Schonekess, B.O., M.F. Allard, S.L. Henning, R.B. Wambolt, and G.D. Lopaschuk. Contribution of glycogen and Exogenous glucose to glucose metabolism during ischemia in the hypertropies rat heart. *Circ. Res.* 81: 540549, 1997.
25. Seki, S., and K.T. MacLeod. Effects of anoxia on intracellular Ca^{2+} and contraction in isolated guinea pig cardiac myocytes. *Am. J. Physiol.* 268 (Heart Circ Physiol 37): H1045-H1052, 1995.
26. Silverman, H.S., M.D. Stern, and E.G. Lakatta. Contrasting effects of anoxia and graded hypoxia on single cardiac myocyte function. *Am. J. Cardiovasc. Pathol.* 4: 256-264, 1992.
27. Ten Eick, R.E., D.W. Whalley, and H.H. Rasmussen. Connections: heart disease, cellular electrophysiology, and ion channels. *FASEB J.* 6: 2568-2580, 1992.
28. Than, N., N. Shah, J. White, J.A. Lee, and C.H. Orchard. Effects of acidosis and hypoxia on the response of isolated ferret heart muscle to inotropic agents. *Cardiovasc. Res.* 28: 1209-1217, 1994.
29. Wilde, A.A.M., and A.G. Kleber. The combined effects of hypoxia, high K^+, and acidosis on the intracellular sodium activity and resting potential in guinea pig papillary muscle. *Circ. Res.* 58: 249-256, 1986.
30. Yang, J.M., and K.T. Chung. Epinephrine stimulates Na-K pump by mediation of α- and β-adrenoceptors in canine Purkinje fibers. *Chin. J. Physiol.* 36: 19-25, 1993.
31. Yang, J.M., K.T. Chung, and C.I. Lin. Simultaneous effects of carbachol on intracellular Na^+ activities, action potential, and twitch tension in guinea-pig cardiac ventricular papillary muscles. *Jpn. J. Physiol.* 46: 225-230, 1996.