血脂異常誘發心臟電氣生理及機械功能失全之病生理機轉 (**主 持 人：吳造中**)

中文摘要：

關鍵詞: **血脂異常**, **電氣-機械功能**, **動作電位**, **鈣離子孔道**, **鉀離子孔道。**

本研究計劃乃比較由正常血脂或高膽固醇血症兔子分離出的單一心肌細胞，在接受電刺激時其動作電位，鉀離子流，及鈣離子流的變化，藉以探討血脂異常所造成的心臟電氣-機械功能失全的基礎細胞生理機轉。

實驗結果發現高膽固醇血症兔子的心肌細胞較(162±8.2 pF (n=118) vs. 108±8.2 pF (n=125), p<0.05)**，動作電位的零期去極化較慢** (80.7±16.2 V/s vs. 54.8±32.1 V/s, p<0.02)**，及動作電位期間較長** (APD: 206.2±77.7 vs. 137.6±74.7 ms, APD_{90}: 196.9±79.0 vs. 135.0±70.5 ms, APD_{50}: 173.2±82.2 vs. 112.5±68.9 ms, p **值均**<0.05)。 **高膽固醇血症兔子的心肌細胞具有較大的** I_{Ca} **離子流 ，然而由於其心肌細胞較大，故** I_{Ca} **離子流密度兩者間並無顯著之差異。**I_{to} **離子流兩者間並無顯著之差異，然因高膽固醇血症兔子的肌細胞較大，故其心肌細胞具有較小的** I_{to} **離子流 密度。總結而論，高膽固醇血症所引起的心肌細胞動作電位的變化，乃由其具有較小的** I_{to} **與** I_{Na} **離子流 密度。**

英文摘要：

Keywords：Dyslipidemia, Electromechanical function, Action potential, Calcium channel, Potassium channel, Intracellular calcium..

In this study project, single ventricular myocytes isolated from hyper- & normocholesterolemic rabbits were used to define the amplitude and duration of action potential in hypercholesterolemic status and to study the hyperlipidemic effect on the magnitude of calcium inward and potassium outward currents, which are two major currents responsible for the duration of action potential.

The cell capacitance was 108±8.2 pF in normolipidemic myocytes (n=125) and 162±8.2 pF (n=118) in hypercholesterolemic cells (p<0.05). There were no significant difference of the resting membrane potential (-70.4±9.4 vs. –73.4±12.3 mV, p=NS) and the amplitude of action potential (115.2±24.1 vs. 13.2±16.7 mV, p=NS) between the hypercholesterolemic myocytes (n=18) and normal controls (n=11). However, the phase 0 depolarization of action potential was slower (54.8±32.1 vs. 80.7±16.2 V/s, p<0.02) and the action potential duration was longer (APD: 206.2±77.7 vs. 137.6±74.7 ms, APD_{90}: 196.9±79.0 vs. 135.0±70.5 ms, and APD_{50}: 173.2±82.2 vs. 112.5±68.9 ms, respectively, p<0.05). While the peak I_{Ca} was larger in the hypercholesterolemic myocytes, capacitative surface area was also larger; thus there were no differences in the peak current densities between these two groups. While there was no difference in the peak currents, I_{to} density was significantly smaller in the hypercholesterolemic myocytes.

In conclusion, the findings of the present experiments suggested that hypercholesterolemia associated changes in action potential resulted from the decrease in I_{Na} and I_{to} density.

Background

Several recently published clinical trials have established that lipid-lowering interventions are associated with reduced coronary artery disease events and mortality.[1, 2, 3, 4] However, whether dyslipidemias influence the severity of myocardial infarction (MI) is still uncertain. In the Scandinavian Simvastatin Survival Study (4S),[1] the reduction in the risk of coronary death (42%) is greater than the reduction in the risk of major coronary events (34%), mostly MI, in the simvastatin group. The mortality rate of definite acute MI was 23% in the placebo group and 18% in the simvastatin group. Besides, the sudden cardiac death (SCD) rate was also markedly reduced in the simvastatin group. These findings suggest that dyslipidemias may have an adverse impact on the evolution of MI. The change in myocardial vulnerability by dyslipidemias develops much earlier than the change in the severity of coronary atherosclerosis, which usually takes years to occur.

There is considerable evidence which suggests that change in the lipid composition of biological membranes is fundamental to alterations in their function as it is generally agreed that the ‘fluidity’ of membrane lipids provides a dynamic mileau for enzyme and receptor function.[5, 6] In the heart it has

been suggested that dietary induced changes in the fatty acid composition of cardiac muscle cell membranes are closely associated with the development of arrhythmia and the extent of ischaemic damage.[7, 8] For example, hypercholesterolemia can increase the extent of myocardial injury during coronary occlusion.[9, 10] In papillary muscles from hypercholesterolemic rabbits, mechanical dysfunction was observed and suggested to be resulted from the defect in Ca2+ transport of the cardiac cells.[11] Manipulation of the cholesterol content of isolated membranes from a variety of tissues has caused alterations in the activities of Na^+-K^+ ATPase[12, 13] and Ca^{2+}-Mg^{2+} ATPase.[14] Our previous studies have shown that the sodium current density on hyperlipidemic ventricular sarcolemma was significantly lower than that of normolipidemic one,[15] and dyslipidemias had a detrimental effect on left ventricular systolic function in patients with a first acute myocardial infarction.[16] However, the effect of hypercholesterolemia on the action potential of cardiac myocytes has never been studied. Thus, in this study, single ventricular myocytes isolated from hyper- and normocholesterolemic rabbits were used to define the amplitude and duration of action potential in hypercholesterolemic status and to study the hyperlipidemic effect on the magnitudes of calcium inward and potassium outward currents, which are the two major currents responsible for the duration of action potential.

Materials and Methods

Animals and diets Twenty 3-month-old male New Zealand white rabbits were randomized into two groups. Control group was fed with a laboratory standard rabbit chow (Purina 5321, St. Louis, Mo, U.S.A.) for 3 months. In addition to the standard chow, 40% of the total energy source of the experimental group was derived from a high cholesterol (0.5% cholesterol; Wako Co., Japan) high fat (coconut oil; Yeali Co., Taiwan) diet.

Biochemical measurements Serum total cholesterol and triglyceride levels were determined by automated enzymatic methods (Merck: 14366 and 14354, respectively) before the animals were sacrificed.

Cell isolation Rabbits were sacrificed after heparinization and anesthetization with pentobarbital. The heart cells was enzymatically isolated with minor modification of the procedures described previously by Mitra and Morad. The left and right ventricles were separated from atria. The tissue was dispersed and stored separately in KB medium at a temperature of around 25^{o}C before the electrophysiologic studies. Only rod-like relaxed, Ca^{2+}-tolerant cells showing clear striation were used for the experiments.

Electrophysiologic study Transmembrane voltages and currents were studied in the whole-cell clamp configuration. Action potentials were elicited by intracellularly applied stimuli (4 ms duration of 1 nA) through a heat-polished patch electrodes with a resistance of 2-5 megaohm. Capacitance of the cell was measured by total charge movement of the capacitative transient in response to a 5 mV hyperpolarizing voltage clamp pulse. Comparisons of currents were made after normalizing data by the cellular capacitance. During the measurement of potassium outward currents, the contamination of calcium inward current (I_{Ca}) was prevented by adding 1 mM Co^{2+} to the bathing medium. In order to eliminate the contamination of inward current completely, 30 uM TTX was added to inhibit the I_{Na}. During measurement of I_{Ca}, the potassium current was prevented by adding 2-4 mM Cs^+ to the bathing medium and internal dialysis of the cells with Cs^+-containing internal solution. The I_{Na} was inactivated by the first step depolarization of membrane potential to -40 mV, and the I_{Ca} could then be activated by the second step depolarization to levels positive to -20 mV.

Recording and data analysis Recording will be made through a Dagan 8900 voltage clamp amplifier (Dagan Co., Minneapolis, MN, U.S.A.), displayed on a storage oscilloscope (Model 511A, Tektronix Inc., Beaverton, OR, U.S.A.) and photographed subsequent analysis. All values will be expressed as mean$\pm$SD. Comparisons between two means will be made by Student's t test for unpaired data. Statistical significance will be established at $p<0.05$.

Results

The serum cholesterol and triglyceride levels were remarkably higher in cholesterol-fed rabbits (2342$\pm$320 vs. 118$\pm$23 mg/dl and 304$\pm$35 vs. 102$\pm$16 mg/dl, repectively, each $p<0.001$).

The cell capacitance was 108±8.2 pF in normolipidemic myocytes (n=125) and 162±8.2 pF (n=118) in hypercholesterolemic cells ($p<0.05$). There were no significant difference of the resting membrane potential (-70.4±9.4 vs. –73.4±12.3 mV, p=NS) and the amplitude of action potential (115.2±24.1 vs. 13.2±16.7 mV, p=NS) between the hypercholesterolemic myocytes (n=18) and normal controls (n=11). However, the phase 0 depolarization of action potential was slower (54.8±32.1 vs. 80.7±16.2 V/s, $p<0.02$) and the action potential duration was longer (APD: 206.2±77.7 vs. 137.6±74.7 ms, APD_{90}: 196.9±79.0 vs. 135.0±70.5 ms, and APD_{50}: 173.2±82.2 vs. 112.5±68.9 ms, respectively, $p<0.05$). While the peak I_{Ca} was larger in the hypercholesterolemic myocytes, capacitative surface area was also larger; thus there were no differences in the peak current densities between these two groups. While there was no difference in the peak currents, I_{to} density was significantly smaller in the hypercholesterolemic myocytes.

Discussion

The results of the present study showed that hypercholeterolemia had significant effect on the electrophysiological characteristics of cardiac myocytes. It slowed the phase 0 depolarization and prolonged the duration of action potential. Our previous study has shown that the sodium inward current was decreased in hypercholesterolemic state, which was mainly due to the functional change of the sodium channel on the myocardial membrane. This must be the pathophysiological bases for the slower phase 0 depolarization and had the clinical implication that hyperlipidemic patients might have a slower myocardial conduction velocity, which might predispose the formation of conduction defect or reentry tachycardia.

The present experiments showed that I_{Ca} was larger in hyperlipidemic myocytes but, once normalised for variations in capacitative surface area, the difference disappeared. These data suggested that as cells enlarged with hypercholesterolemia there was a proportional increase in the number of calcium channels such that calcium current density was maintained. The possibility that changes in I_{to} contributed to the action potential changes with hyperlipidemia was also examined. There was no major change in the overall magnitude of I_{to} with hyperlipidemia but, since hypercholesterolemic cells were larger, there was a hyperlipidemia related decrease in the current density of I_{to}. This finding suggested that the synthesis of I_{to} channels did not increase in proportion to cellular enlargement in hyperlipidemia. This alteration in I_{to} should contribute to the action potential duration changes that occurred with hyperlipidemia. These hyperlipidemia associated changes in action potential were similar to the changes during aging process. Perhaps, hyperlipidemia would aggravate the functional changes of aging hearts.

In summary, the findings of the present experiments suggested that hypercholesterolemia associated changes in action potential resulted from the decrease in I_{Na} and I_{to} density.

References

1. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering therapy in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383-1389.
2. Shepherd J, Cobbe SM, Isles CG, Lorimer AR, MacFarlane PN, Mckillop JH, Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. The West of Scotland Coronary Prevention Study Group. N Engl J Med. 1995;333:1301-1307.
3. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JMO, Wun CC, Davis BR, Braunwald E, for the Cholesterol and Recurrent Events Trial Investigators. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. N Engl J Med 1996;335:1001-1009.
4. Brown G, Albers JJ, Fisher LD, Sheafer SAM, Lin JT, Kaplan C, Zhao XQ, Bisson BD Fitzpatrick VF, Dodge HT. Regression of coronary artery disease as a result of intensive lipid lowering therapy in men with high levels of apolipoprotein B. N Engl J Med 1990;323:1289-1298.
5. Chapman D, Gomez-Fernandez JC, Goni FM. Intrinsic protein-lipid interactions: physical and biomedical evidence. FEBS Lett 1979;98:211-223.

6. King ME, Spector AA. Effect of specific fatty acyl enrichments on membrane physical properties detected with a spin label probe. J Biol Chem 1978;253:6493-6501.

7. Dhalla NS, Ziegelhoffer A, Harrow JA. Regulatory role of membrane systems in heart function. Can J Physiol Pharmacol 1977;55:1211-1234.

8. Katz AM, Messineo FC. Lipid-membrane interactions and the pathogenesis of ischemic damage in the myocardium. Circ Res1981;48:1-16.

9. Golino P, Maroko PR, Carew TE. The effect of acute hypercholesterolemia on myocardial infarct size and the no-reflow phenomenon during coronary occlusion-reperfusion. Circulation 1987;75: 292-298.

10. Chen MF, Lee YT, Hsu HC, Yeh PC, Liau CS, Huang PC. Effects of dietary supplementation with fish oil on atherosclerosis and myocardial injury during acute coronary occlusion-reperfusion in diet-induced hypercholesterolemic rabbits. Int J Cardiol 1992;35;323-331.

11. Peterson DW, Napolitano CA, Griffith Jr DW. Spontaneous mechanical alternans in papillary muscles from atherosclerotic rabbits. Am J Physiol 1980;239:H674-680.

12. Claret M, Garay R, Giraud F. The effect of membrane cholesterol on the sodium pump in red blood cells. J Physiol 1978;274:247-263.

13. Sinensky M, Pinkerton F, Sutherland B, Simon FR. Rate limitation of (Na^+, K^+)-stimulated adenosinetriphosphatase by membrane acyl chain ordering. Proc Natl Acad Sci USA 1979;76:4893-4897.

14. Warren GB, Housley MD, Metcalfe JC, Birdsalt NJM. Cholesterol is excluded from the phospholipid annulus surrounding an active calcium transport protein. Nature 1975;255:684-687.

15. Wu CC, Su MJ, Chi JF, Chen WJ, Hsu HC, Lee YT. The effect of hypercholesterolemia on the sodium inward currents in cardiac myocyte. *J Mol Cell Cardiol* 1995;27:1263-1269.

16. Wang TD, Wu CC, Chen WJ, Lee CM, Chen MF, Liau CS, Sung FC, Lee YT. Dyslipidemias have a detrimental effect on left ventricular systolic function in patients with a first acute myocardial infarction. Am J Cardiol 1998;81:531-537.