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3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, statins 對肺靜脈及心房心肌細胞之電生理與心律不整作用

研究計畫中英文摘要

中文摘要

心房顫動乃是臨床上常見之心律不整，且會造成心臟功能不良以及腦中風，雖然許多抗心律不整藥物已經被用於治療與預防心房顫動，然大多具有相當之副作用而無法長期使用。最近研究顯示 HMG-GA 還原酵素阻礙素 statins，乃是廣泛用於治療高血脂症之藥物，被發現可減少心房顫動之產生，然而其電生理機轉以及預防心房顫動的原因仍不清楚，再則，是否不同的 statins 會有不同的效果也未明瞭。

肺靜脈已知是引發心房顫動之病灶所在，過去的研究已知肺靜脈含心肌細胞且有其特有之電生理特性可引發心律不整活性，長時間心房電刺激，以及使用發炎物質都會增加肺靜脈心肌細胞引發心律不整活性，反之，一氧化氮則被發現可用抑制肺靜脈心肌心律不整之作用，由於 statins 已知會增加一氧化氮之生理活性以及明顯之抗發炎作用，這些結果顯示，statins 或可藉者抑制肺靜脈心肌之心律不整活性而達到其抑制心房顫動的效果，因此本研究旨在探討 statins 對肺靜脈之心律不整活性之作用。

方法: 傳統電極記錄記錄肺靜脈之動作電位，以及收縮力在接受 Simastatin 0.1 、 1μM 後之變化，以及在 1μM 之 Simastatin 下，使用 L-NAME 100μM 後的變化。

結果:Simastatin 在 1μM 的濃度下可以於 1 小時後抑制肺靜脈心肌細胞之自動性從 1.7±0.1Hz 到 1.5±0.1Hz，並在 2 小時後到達平穩的抑制狀態約 1.4±0.1 Hz。這個動作可以被 100μM 的 L-NAME 所抑制而回復肺靜脈之節律。再則 1μM Simastatin 可以稍微延長肺靜脈心肌之動作電位從 88±7 ms 到 93±7 ms。

結論：本實驗顯示 Simastatin 可以抑制肺靜脈引發心律不整活性，且此機轉與一氧化碳之產生有關。這些結果可能是造成 Statin 減少心房顫動的機轉。

英文摘要

Atrial fibrillation is the most common cardiac arrhythmia seen in clinical practice and induce cardiac dysfunction and stroke. Although several antiarrhythmic drugs have been used in treating and preventing atrial fibrillation, drugs with little adverse effects effectively to prevent atrial fibrillation. Recent studies have shown that use of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, statins reduce the occurrences of atrial fibrillation clinically. However, knowledge about the electrophysiological effects of statins on cardiomyocytes and the mechanisms of anti- fibrillation were limited and it is not clear whether different statins may have different cardiac effects. Pulmonary veins (PVs) were known to be important sources of ectopic beats with the initiation of paroxysmal atrial fibrillation. Our previous have found that PVs have cardiomyocytes with distinct electrophysiological characteristics and arrhythmogenic activities. Long-term rapid atrial pacing and inflammatory cytokine increased PV arrhythmogneic activity. In contrast, nitric oxide was found to decrease PV arrhythmogenic activity with the reduction of atrial fibrillation. Because statins increases nitric oxide bioavailability and has anti-infllammation effects, it is possible that the statins may inhibit atrial fibrillation through the decrease of PV arrhythmogenic activity. Therefore, the purposes of the present study are to investigate the effects of statins on the arrhythmogenic activity of PV cardiomyocytes.

Methods: Conventional microelectrodes were used to record the action potential

(AP) and contractility in isolated rabbit PV tissue specimens before and after the administration of simvastatin (0.1, 1 μM). L-NAME (100 μM)was administrated in the presence of simvastatin (1 μM).

Results: Simvastatin (1 μM, but not 0.1 μM) decrease the PV firing rates from 1.7±0.1 to 1.5±0.1 Hz at one hour and achieve steady state firing rates of 1.4±0.1 Hz at 2 hour. This effect is reversed after the administration of L-NAME (100 μM, inhibitor of nitric oxide production). Moreover, simvastatin (1 μM) mildly prolonged the action potential duration from 88±7 ms to 93±7 ms (n=5).

Conclusion

We demonstrated the simvastatin may decrease the PV arrhythmogenesis through the production of nitric oxide. These results may underlie the anti-arrhythmic potential of statin and result in the decrease of atrial fibrillation.

Key Word: Atrial fibrillation, electrophysiology, HMG-CoA, pulmonary vein,

Introduction

Atrial fibrillation is the most common cardiac arrhythmia seen in clinical practice and induce cardiac dysfunction and stroke [1-2]. Although several antiarrhythmic drugs have been used in treating and preventing atrial fibrillation, drugs with little adverse effects effectively to prevent atrial fibrillation, especially in the presence of heart failure and myocardial ischemia were still limited clinically. Recent studies have shown that use of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, statins reduces the occurrences of atrial fibrillation clinically [3-4] and administration of atorvastatin also reduce the occurrence of atrial fibrillation and increase of action potential duration in pericarditis animal model [5]. Moreover, statins may prevent atrial remodeling, reduce the occurrence of atrial fibrillation and attenuate the down-regulation of L-type calcium currents in the long-term atrial pacing animal models. [6] Atrial remodeling due to atrial tachyarrhythmias can alter atrial electrophysiology and promote AF, and these alterations are believed to contribute to both the occurrence and the persistence of the arrhythmia. [7-10] These findings suggest the potential antiarrhythmic effects of statins. However, knowledge about the electrophysiological effects of statins on cardiomyocytes and the mechanisms of anti- fibrillation were limited. Moreover, it is not clear whether different statins may have different cardiac effects.

Pulmonary veins (PVs) were known to be important sources of ectopic beats with the initiation of paroxysmal atrial fibrillation or the foci of ectopic atrial tachycardia and focal atrial fibrillation [11-13]. Other studies also suggested that PVs have a role in the maintenance of atrial fibrillation [14-15]. Previous anatomical and electrophysiological studies in isolated PV specimen have demonstrated that PVs contain a mixture of pacemaker cells and working myocardium [16-20]. Our previous studies have demonstrated the presence of spontaneous activities or high frequency irregular rhythms in isolated canine PVs, which may underlie the arrhythmogenic activity of these vessels [20]. After the isolation of single cardiomyocytes, PVs were found to have cardiomyocytes with distinct electrophysiological characteristics and arrhythmogenic activities [21-23]. In addition, long-term rapid atrial pacing could increase PV arrhythmogneic activity through the induction of triggered activities, shortening of action potential duration or enhancement of automaticity and contribute to the occurrence of atrial fibrillation [20-21] The administration of thyroid hormone also was demonstrated to increase PV arrhythmogenic activity [22]. All of these findings suggest the critical role of PVs in the genesis of maintenance of atrial fibrillation. Our previous studies have demonstrated that TNF-α increases PV arrhythmogenic activity and was suggested to be underlying the mechanism of inflammation induced-atrial fibrillation. [24] In addition, nitric oxide was been demonstrated to reduce PV arrhythmogenic activity and could be a potential antiarrhythmogenic drugs. [25] Previous studies have shown that statins may increase nitric oxide bioavailability and also have anti-infllammation effects [26-29], therefore, it is possible that

the statins may reduce the occurrence of atrial fibrillation through the decrease of PV arrhythmogenic activity with the production of nitric oxide and anti-inflammation. Therefore, the purposes of the present study are to investigate the effects of statins on PV arrhythmogenic activity.

Methods

Rabbit PV Tissue Preparations

The investigation conformed to the institutional *Guide for the Care and Use of Laboratory Animals.* Rabbits (1-1.5 Kg) were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). A mid-line thoracotomy was then performed and the heart with the lungs was removed. For dissection of the PVs, the left atrium was opened by an incision along mitral valve annulus extending from the coronary sinus to the septum in Tyrode's soluton with a composition (in mM) of 137 NaCl, 4 KCl, 15 $NaHCO_3$, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 2.7 $CaCl_2$, and 11 dextrose. The PVs were separated from the atrium at the left atrium-PV junction and separated from the lungs at the ending of the PV myocardial sleeves. One end of the preparation, consisting of the PVs and atrium-PV junction, was pinned with needles to the bottom of a tissue bath. The other end was connected to a Grass FT03C force transducer with a silk thread. The adventitia of the PVs faced upwards. The tissue was superfused at a constant rate (3 ml/min) with Tyrode's solution which was saturated with a 97% O_2-3% CO_2 gas mixture. The temperature was maintained constant at 37oC and the preparations were allowed to equilibrate for 1 hour before the electrophysiological study.

Electrophysiological and pharmacological studies

The transmembrane action potential (AP) of the PVs was recorded by

means of machine-pulled glass capillary microelectrodes filled with 3M of KCl and the PV preparation was connected to a WPI model FD223 electrometer under tension with 150 mg. The electrical and mechanical events were displayed simultaneously on a Gould 4072 oscilloscope and Gould TA11 recorder. The signals were recorded with DC coupling and a 10-KHz low-pass filter cutoff frequency using a data acquisition system. Signals were recorded digitally with 16-bit accuracy at a rate of 125 KHz. An electrical stimuli with a 10-ms duration and suprathreshold strength (30% above the threshold) were provided by a Grass S88 stimulator through a Grass SIU5B stimulus isolation unit. Different concentrations of simvastatin (0.1, 1 μM) were sequentially superfused to test the pharmacological responses. The 90% and 50% AP durations (APD_{90}, APD_{50}), AP amplitude (APA), and contractile force were measured during 2 Hz electrical stimuli before and after the drug administration.

Results

As the tracing shown in Figure 1, in the PVs with spontaneous activity, simvastatin (0.1 μM) did not change the PV firing rates significantly. However, the PV firing rates decreased from 1.7±0.1 to 1.5±0.1 Hz (n=3) after the administration of simvastatin (1 μM) for one hour. The firing rates were further decreased to 1.4±0.1 Hz after the administration of simvastatin (1 μM) for 2 hours. Figure 2 shows the tracing after the administration of simvastatin (1 μM).

In the PV without spontaneous activity, simvastatin (0.1 μM) did not change the AP duration in PV cardiomyocytes. However, simvastatin at the concentration of 1 μM prolonged the AP duration from 88±7 ms to 93±7 ms (n=5) after the administration for 3 hours. The simvastatin (0.1, 1 μM)

did not change the resting membrane potential, amplitudes of AP and contractility or vessel tone. Figure 3 shows the AP morphology before and after the administration of statin.

In order to evaluate the mechanism of statin on PV electrical activity, L-NAME was administrated in the PV treated with simvastatin (1 μM) for 3 hours. As the tracing show in Figure 4, the administration of L-NAME (100 μM) accelerated to the PV firings rates, which suggests that simvastatin may reduce PV electrical activity through the production of nitic oxide.

Discussion

Previous studies have shown that statin may have a beneficial effect on the prevention of atrial fibrillation [3-5]. Gaspo et al. have found that stain may attenuated the rapid atrial pacing-induced electrical remodeling, which suggested the anti-arrhythmic potential of statin [6]. However, vitamins C and vitamins E did not have effect on atrial electrical activity which suggested that the anti-arrhythmic potential of statin did not arise frome the anti-oxidants effects. In this study, for the first time, we demonstrated that statin may alter the PV electrical activity. Not only prolonged the AP duration, stain also decreases the PV firing rates. All of these findings may reduce PV arrhythmogeneis to result in the decrease of atrial fibrillation.

Previous studies have shown that nitic oxide may reduce PV arrhythmogenesis through the decrease of transient inward currents [25]. Statin has been shown to induce the occurrence of nitric oxide [26]. Therefore, it is possible that statin may reduce the PV arrhythmogenesis through the production of nitric oxide. In this study, administration of

L-NAME may reverse the inhibitory effects of statin. which suggests that statin may reduce PV arrhythmogenesis due to the production of nitric oxide.

Conclusion

We demonstrated the simvastatin may decrease the PV arrhythmogenesis through the production of nitric oxide. These results may underlie the anti-arrhythmic potential of statin and result in the decrease of atrial fibrillation.

References

1. Kannel WB, Abbott RD, Savage DD, McNamare PM. Epidemiologic features of chronic atrial fibrillation. N Engl J Med 1982;306:1018-1022.

2. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke. The Framingham Study. Stroke 1991;22:983-988.

3. Young-Xu Y, Jabbour S, Goldberg R, Blatt CM, Graboys T, Bilchik B, Ravid S. Usefulness of statin drugs in protecting against atrial fibrillation in patients with coronary artery disease. Am J Cardiol. 2003;92(12):1379-83

4. Siu CW, Lau CP, Tse HF. Prevention of atrial fibrillation recurrence by statin therapy in patients with lone atrial fibrillation after successful cardioversion. Am J Cardiol. 2003;92(11):1343-5.

5. Kumagai K, Nakashima H, Saku K. The HMG-CoA reductase inhibitor atorvastatin prevents atrial fibrillation by inhibiting inflammation in a canine sterile pericarditis model. Cardiovasc Res. 2004;62:105-11.

6. Gaspo R, Bosch RF, Talajic M, et al. Functional mechanisms underlying effect of simvastatin and antioxidant vitamins on atrial fibrillation promotion by atrial-tachycardia remodeling in dogs. Circulation. 2004;110:2313-9.

7. Lee SH, Yu WC, Cheng JJ, et al. Effect of verapamil on long-term tachycardia-induced atrial electrical remodeling. *Circulation.* 2000; 101: 200 - 206.

8. Wijffels MC, Kirchhof CJ, Dorland R, et al. Atrial fibrillation begets atrial fibrillation: a study in awake chronically instrumented goats. *Circulation.* 1995; 92: 1954 - 1968.

9. Morillo CA, Klein GJ, Jones DL, et al. Chronic rapid atrial pacing: structural, functional, and electrophysiological characteristics of a new model of sustained atrial fibrillation. *Circulation.* 1995; 91: 1588 - 1595.

10. Elvan A, Wylie K, Zipes DP. Pacing-induced chronic atrial fibrillation impairs sinus node function in dogs: electrophysiological remodeling. *Circulation.* 1996; 94: 2953 - 2960.

11. Walsh EP, Saul JP, Hulse E, et al. Transcatheter ablation of ectopic atrial tachycardia in young patients using radiofrequency current. Circulation 1992;86:1138-1146.

12. Haissaguerre M, Jais P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med 1998;339:659-666.

13. Chen SA, Hsieh MH, Tai CT, et al. Initiation of atrial fibrillation

by ectopic beatsoriginating from the pulmonary veins: Electrophysiological characteristics, pharmacological responses, and effects of radiofrequency ablation. Circulation 1999;100:1879-1886.

14. Pappone C, Rosanio S, Oreto G, et al. Circumferential radiofrequency ablation of pulmonary vein ostia: a new anatomic approach for curing atrial fibrillation. Circulation. 2000;102:2619-2628

15. Sueda T, Imai K, Ishii O, Orihashi K, Watari M, Okada K. Efficacy of pulmonary vein isolation for the elimination of chronic atrial fibrillation in cardiac valvular surgery. Ann Thorac Surg 2001;71:1189-1193.

16. Nathan H, Eliakim M. The junction between the left atrium and the pulmonary veins: an anatomic study of human hearts. Circulation 1966;34:412-422.

17. Saito T, Waki K, Becker AE. Left atrial myocardial extension onto pulmonary veins in humans: anatomic observations relevant for atrial arrhythmias. J Cardiovasc Electrophysiol 2000;11:888-894.

18. Blom NA, Gittenberger-de Groot AC, DeRuiter MC, Poelmann RE, Mentink MM, Ottenkamp J. Development of the cardiac conduction tissue in human embryos using HNK-1 antigen expression: possible relevance for understanding of abnormal atrial automaticity. Circulation 1999;99:800-806.

19. Cheung DW. Electrical activity of the pulmonary vein and its interaction with the right atrium in the guinea-pig. J Physiol (Lond) 1981;314:445-456.

20. Chen YJ, Chen SA, Chang MS, Lin CI. Arrhythmogenic activity of cardiac muscle in pulmonary vein of the dog: Implication for the genesis of atrial Fibrillation. Cardiovasc Res 2000;48:265-273.

21. Chen YJ, Chen SA, Chen YC, et al. Effects of rapid atrial pacing on the arrhythmogenic activity of single cardiomyocytes from pulmonary veins: implication in initiation of atrial fibrillation. Circulation 2001;104: 2849-2854.

22. Chen YC, Chen SA, Chen YJ, Chang MS, Chan P, Lin CI. Effects of thyroid hormone on the arrhythmogenic activity of pulmonary vein cardiomyocytes. J Am Coll Cardiol 2002;39:366-372.

23. Chen YJ, Chen SA, Chen YC, Yeh HI, Chang MS, Lin CI. Electrophysiology of single cardiomyocytes isolated from rabbit pulmonary veins: implication in initiation of focal atrial fibrillation. Basic Res Cardiol 2002;97:26-34

24. Chen YJ, Chen YC, Chan P, Lin CI, Chen SA. Do cytokines play a role in pulmonary vein arrhythmogenic activity. PACE 2003:26: 1106. (abstract)

25. Chen YJ, Chen YC, Shih-Lin Chang, Chan P, Lin CI, Chen SA. Nitric oxide regulates pulmonary vein arrhythmogenic activity. Circulation 2004;110: supplement III-350 (abstract).

26. Laufs U, La Fata V, Plutzky J, et al. Upregulation of endothelial nitric oxide synthase by HMG-CoA reductase inhibitors. *Circulation.* 1998; 97: 1129－1135.

27. Kureishi Y, Luo Y, Shiojima I, et al. The HMA CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes

angiogenesis in normocholesterolemic animals. *Nat Med.* 2000; 6: 1004-1010.

28. Dichtl W, Dulak J, Frick M, et al. HMG-CoA reductase ihnhibitos regulate inflammatory transcription factors in human endothelial and vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2003;23: 58-63.

29. Bustos C, Hernandez-Presa MA, Ortego M, et al. HMG-CoA reductase inhibition by atorvastatin reduces neointimal inflammation in a rabbit model of atherosclerosis. J Am Coll Cardiol 1998;32:2057-2064.

Figure Legends

Figure. 1. The tracings showing the PV firing rates before and after the administration of simvastatin (0.1 μM).

Figure 2. The tracings showing the PV firing rates before and after the administration of simvastatin (1 μM) at different time..

Figure 3. Effects of simvastatin (0.1, 1 μM) on the AP morphology of PV tissue specimen.

Figure 4. Effect of L-NAME on simvastatin-altered PV electrical activity.

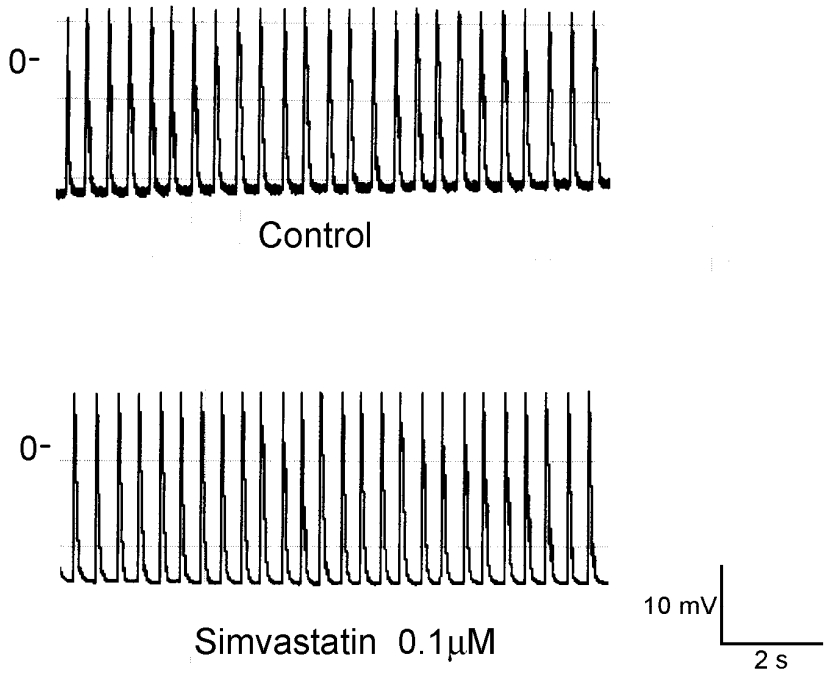

Figure 1.

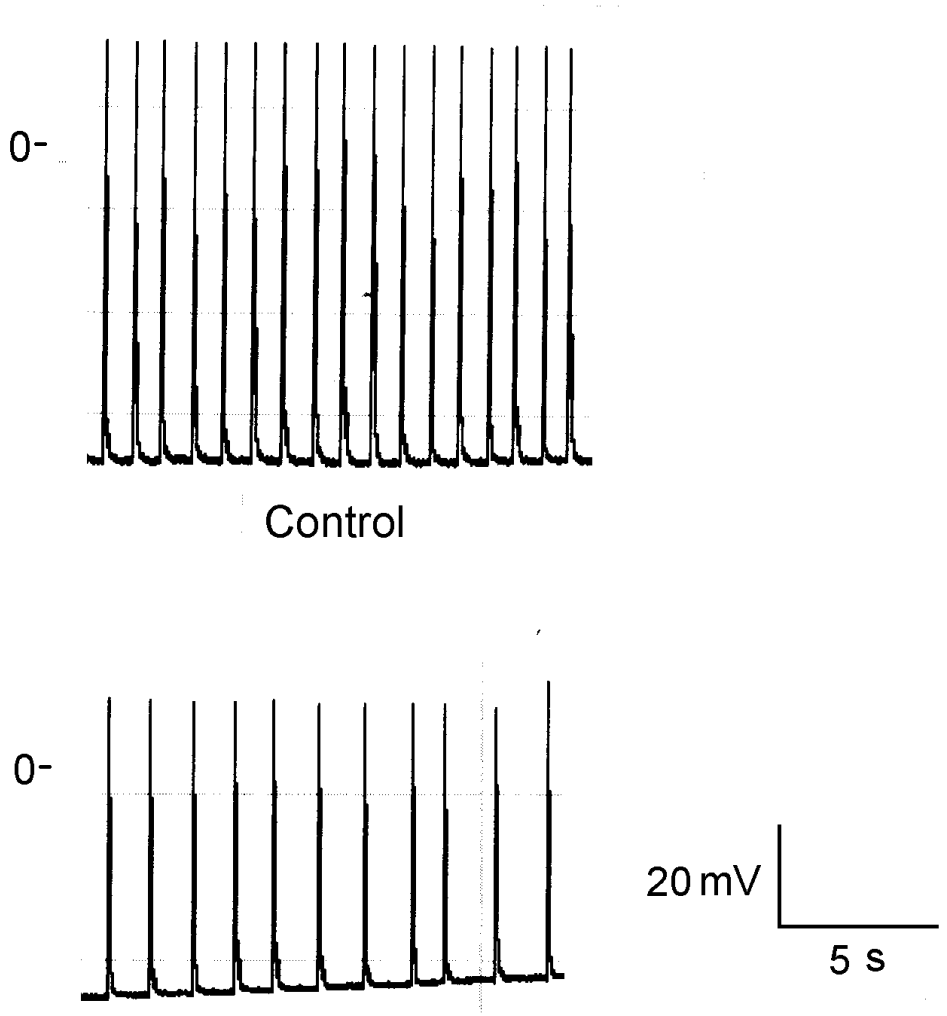

Figure 2

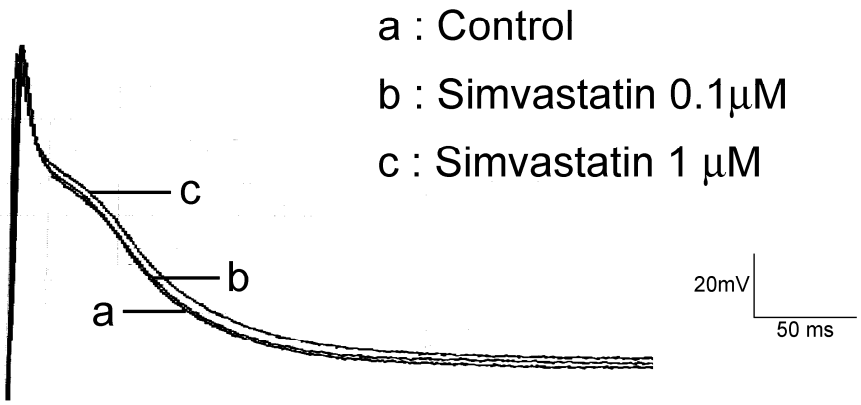
a : Control
b : Simvastatin 0.1μM
c : Simvastatin 1 μM
c
b
a
20mV
50 ms

Figure 3

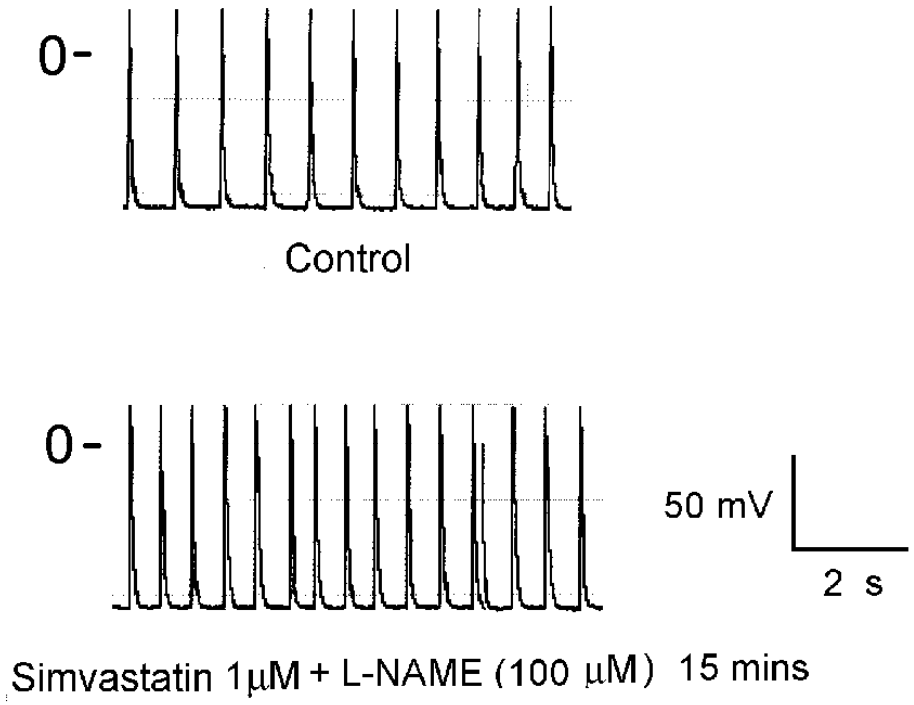

Figure 4