

## Research Paper

# Inhibitory effects of endocannabinoid on the action potential of pacemaker cells in sinoatrial nodes of rabbits

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**Abstract:** Endocannabinoid anandamide (AEA) has protective effect on the heart against ischemia/reperfusion injury and arrhythmia, but the electrophysiological mechanism is unclear yet. In this study, the sinoatrial node (SAN) samples from New Zealand rabbits were prepared, and intracellular recording technique was used to elucidate the effect of AEA on the action potential (AP) of SAN pacemaker cells of rabbits and the mechanism. Different concentrations of AEA (1, 10, 100, 200, 500 nmol/L) were applied cumulatively. For some SAN samples, cannabinoid type 1 (CB1) receptor antagonist AM251, cannabinoid type 2 (CB2) receptor antagonist AM630, potassium channel blocker tetraethylammonium (TEA) and nitric oxide (NO) synthase inhibitor *L*-nitro-arginine methylester (*L*-NAME) were used before AEA treatment, respectively. We found that: (1) AEA (100, 200 and 500 nmol/L) not only shortened AP duration (APD), but also decreased AP amplitude (APA) ( $P < 0.05$ ). (2) AM251, but not AM630, abolished the effect of AEA on APD shortening. (3) TEA and *L*-NAME had no influence on the AEA effect. These findings suggest that anandamide can decrease APA and shorten APD in SAN pacemaker cells of rabbits, which may be mediated by activation of CB1 receptors, and is related to blockade of calcium channels but not potassium channels and NO.

**Key words:** anandamide; action potential; cannabinoid type 1 receptor; cannabinoid type 2 receptor; sinoatrial node; rabbit

## 内源性大麻素物质对家兔窦房结自律细胞动作电位的抑制作用

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**摘要:** 内源性大麻素物质(endocannabinoid anandamide, AEA)可对抗缺血/再灌注所致心脏损伤和心律失常, 但其电生理机制尚未完全明了。本文旨在利用细胞内微电极记录方法观察AEA对家兔窦房结自律细胞动作电位的影响, 并探讨其机制。采用新西兰大白兔制备离体窦房结标本并记录动作电位, 通过累计给药法给予窦房结标本不同浓度(1、10、100、200和500 nmol/L)的AEA处理, 部分标本在AEA (200 nmol/L)处理前, 分别给予大麻素1型(CB1)受体阻断剂AM251、大麻素2型(CB2)受体阻断剂AM630、非特异性K<sup>+</sup>通道阻滞剂tetraethylammonium (TEA)和NO合酶抑制剂*L*-nitro-arginine methylester (*L*-NAME)预处理。结果显示: (1) AEA (100、200和500 nmol/L)可缩短家兔窦房结自律细胞的动作电位时程, 降低动作电位幅度( $P < 0.05$ ); (2) AM251可消除AEA缩短动作电位时程的作用, 而AM630对此无影响; (3) TEA和*L*-NAME对AEA的作用无影响。结果提示, AEA可通过CB1受体降低家兔窦房结自律细胞动作电位幅度、缩短动作电位时程, 此作用可能通过阻断Ca<sup>2+</sup>通道所实现, 与K<sup>+</sup>通道及NO无关。

**关键词:** 内源性大麻素物质; 动作电位; 大麻素1型受体; 大麻素2型受体; 窦房结; 家兔

**中图分类号:** Q463, R331.3

Received 2012-07-02 Accepted 2012-10-31

This work was supported by the National Natural Science Foundation of China (No. 31100823) and Medical Scientific Research Funds of Health Bureau, Hebei Province, China (No. 20110039).

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Cardiac arrhythmia is one of the major public problems in the world and a common cause of cardiac sudden death [1]. It has been reported that over 90% of cardiac sudden deaths are resulted from malignant arrhythmia, such as ventricular tachycardia, ventricular fibrillation and atrial fibrillation [2]. Many pathological changes of myocardial electrophysiological characteristics result in arrhythmia. For instance, myocardial ischemia and electrolyte disturbances can cause various kinds of tachyarrhythmia because of abnormal increase of myocardial automaticity. Also the abnormality of impulse conduction that may create reentrance can generate persistent and violent arrhythmia [3]. Generally the abnormality of myocardial electrophysiological characteristics is resulted from the pathological changes of different channels [4]. For example, myocardial ischemia or anoxia increases persistent  $\text{Na}^+$  influx in ventricular myocytes to induce intracellular sodium overload, leading to intracellular calcium overload through enhancing the exchange of sodium and calcium, and as a result triggering arrhythmia [5]. Again, arrhythmia is easy to occur in different ionic channel diseases of myocardium, such as ATP-sensitive potassium ( $\text{K}_{\text{ATP}}$ ) channel pathological changes are often related to adrenaline-induced atrial fibrillation [6].

A lot of researches indicate that endocannabinoids participate in various physiological and pathophysiological processes, such as neuronal activity, functional stress in gastrointestinal tract, anxiety, and cardiovascular function [7–10]. N-arachidonylethanolamine (endocannabinoid anandamide, AEA) is one of the endocannabinoids originally identified [11]. It has been detected that two types of cannabinoid receptors, CB1 and CB2, exist in the heart and peripheral blood vessels [12]. Endocannabinoids play an important role in the regulation of cardiovascular function and participate in the protection of the heart in some pathological conditions. For example, AEA can evoke a complex triphase reaction in blood pressure and bradycardia in anesthetized rats [13]. AEA reduces the incidence of arrhythmia and infarct size induced by myocardial ischemia-reperfusion [14]. Our recent electrophysiological studies demonstrate that AEA may suppress action potential (AP) in ventricular papillary muscle, and inhibit L-type  $\text{Ca}^{2+}$  channel and transient outward potassium channels in ventricular myocytes via activation of CB1 receptors, but activate  $\text{K}_{\text{ATP}}$  channels in ventricular myocytes in rats [15,16]. So far, the mechanism of the anti-arrhythmic

effect of AEA has not been understood completely, and the effect of AEA on the electrophysiological activity of cardiac pacemaker cells remains unknown. The aim of the present study was to investigate the effect of AEA on the AP of the pacemaker cells in sinoatrial nodes (SAN) of rabbits and the potential mechanism using intracellular recording technique.

## 1 MATERIALS AND METHODS

### 1.1 Animal and preparation of SAN

New Zealand rabbits, male ( $n = 27$ ), weighing ( $2.1 \pm 0.1$ ) kg, were provided by the Experimental Animal Center of Hebei Province (Grade II, Certificate No. 2008-1-003). The SAN was prepared as previous literature [17]. The preparations were pinned down on a thin silicon disc on the base of perfusing chamber and equilibrated with modified Tyrode solution for 1 h before recording. The composition of modified Tyrode solution was (in mmol/L): NaCl, 136.8;  $\text{NaHCO}_3$ , 1.2; KCl, 5.4;  $\text{MgCl}_2$ , 1.05;  $\text{CaCl}_2$ , 1.08; glucose, 11.0 and Tris, 5.0 with pH of  $7.40 \pm 0.05$ . The solution was oxygenated with 100%  $\text{O}_2$  and maintained at ( $36.0 \pm 0.5$ ) °C.

### 1.2 AP recording

The transmembrane AP was recorded by micropipettes (with a tip resistance of 10–20 M $\Omega$ ) filled with 3 mol/L KCl and coupled to a high input impedance amplifier (SWF-2W, Chengdu Instrument Factory, Chengdu, China). The amplified signals were fed to a microcomputer via A/D converter, and processed and analyzed using a specific analyzing program (Chengdu Instrument Factory, Chengdu, China). The parameters of AP include the amplitude of AP (APA), the maximal rate of depolarization ( $V_{\text{max}}$ ), AP duration at 50% and 90% repolarization ( $\text{APD}_{50}$  and  $\text{APD}_{90}$ ), the rate of pacemaker firing (RPF), and the velocity of diastolic (phase 4) depolarization (VDD).

### 1.3 Experimental protocols

The experiments were divided into two parts. In the first part of the experiments, SAN preparations were treated with different concentrations of AEA (1, 10, 100, 200, 500 nmol/L) cumulatively after 30 min of equilibration in modified Tyrode's solution, and the AP was recorded for 10 min before and after each concentration of AEA. In the second part of experiment, CB1 receptor antagonist AM251, CB2 receptor antagonist AM630, potassium channel blocker tetraethylammonium (TEA), and NO synthase inhibitor *L*-nitro-arginine

methylester (*L*-NAME) were used before 200 nmol/L AEA treatment, respectively.

#### 1.4 Statistics

All data were expressed as means  $\pm$  SD. The data before and after AEA application were compared with one-way analysis of variance followed by Dunnett's *post hoc* test. The data before and after AM251, AM630, TEA or *L*-NAME pretreatments were compared with paired Student's *t*-test. Statistical significance was accepted at  $P < 0.05$ .

## 2 RESULTS

### 2.1 Effect of AEA on AP in SAN of rabbits

AEA (100, 200, 500 nmol/L) not only shortened APD<sub>50</sub> and APD<sub>90</sub>, but also decreased APA and Vmax, and reduced RPF and VDD. At a 200 nmol/L concentration of AEA, APD<sub>50</sub> and APD<sub>90</sub> were shortened by 9.7%, and 10.6%, respectively; APA and Vmax were decreased by 11% and 8.2%, respectively; and RPF and VDD were reduced by 36.2% and 19%, respectively ( $P < 0.05$ , Table 1 and Fig. 1).

### 2.2 Effects of AM251 and AM630 on AEA action

CB1 receptor antagonist AM251 (100 nmol/L) or CB2 receptor antagonist AM630 (100 nmol/L) alone had no

effect on baseline parameters of AP of rabbit SAN, including APD<sub>50</sub>, APD<sub>90</sub>, APA, RPF, Vmax and VDD ( $P > 0.05$ ). The action of AEA (200 nmol/L) on AP was eliminated by 15 min of pretreatment with 100 nmol/L AM251 ( $P < 0.05$ , Table 2), but not by 100 nmol/L AM630 ( $P > 0.05$ , Table 3).

### 2.3 Effect of TEA on AEA action

Nonspecific antagonist TEA (20 nmol/L) alone pro-

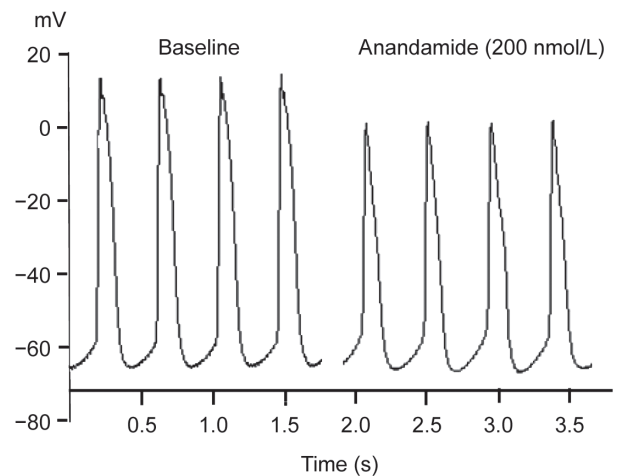


Fig.1. Original recording of action potential before and after anandamide treatment in rabbit SA nodes.

Table 1. Effects of anandamide on transmembrane action potential in rabbit sinoatrial nodes

	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)	Vmax (V/s)	APA (mV)	RPF (beat/min)	VDD (mV/s)
Baseline	75.8 $\pm$ 7.4	126.1 $\pm$ 11.6	4.8 $\pm$ 1.5	77.6 $\pm$ 5.0	140.4 $\pm$ 7.0	68.4 $\pm$ 8.5
Anandamide (nmol/L)						
1	70.6 $\pm$ 9.2	124.9 $\pm$ 11.6	4.5 $\pm$ 1.7	78.0 $\pm$ 5.0	138.6 $\pm$ 8.7	63.4 $\pm$ 12.6
10	71.3 $\pm$ 8.8	121.6 $\pm$ 11.0	4.4 $\pm$ 1.7	78.8 $\pm$ 4.7	136.9 $\pm$ 7.3	65.1 $\pm$ 13.7
100	66.0 $\pm$ 6.2*	111.1 $\pm$ 4.3*	3.2 $\pm$ 0.8*	70.9 $\pm$ 4.2*	127.9 $\pm$ 6.3*	53.9 $\pm$ 10.0*
200	65.2 $\pm$ 5.8**	107.5 $\pm$ 4.0**	3.0 $\pm$ 0.5*	69.0 $\pm$ 7.1**	125.1 $\pm$ 5.5**	52.4 $\pm$ 14.4*
500	64.4 $\pm$ 8.4**	106.4 $\pm$ 8.4**	2.8 $\pm$ 0.4**	68.4 $\pm$ 8.9**	120.8 $\pm$ 6.7***	47.8 $\pm$ 11.8**

Data were expressed as means  $\pm$  SD,  $n = 6$ . APD<sub>50</sub>: action potential duration at 50% depolarization; APD<sub>90</sub>: action potential duration at 90% depolarization; Vmax: maximal rate of depolarization; APA: amplitude of action potential; RPF: rate of pacemaker firing; VDD: velocity of phase 4 depolarization. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs Baseline.

Table 2. Influence of AM251 on the electrophysiological effect of anandamide in rabbit sinoatrial nodes

	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)	Vmax (V/s)	APA (mV)	RPF (beat/min)	VDD (mV/s)
Baseline	73.1 $\pm$ 6.4	129.4 $\pm$ 10.8	5.0 $\pm$ 2.0	71.7 $\pm$ 2.7	145.8 $\pm$ 9.1	60.2 $\pm$ 4.9
Anandamide (200 nmol/L)	65.3 $\pm$ 4.1*	113.5 $\pm$ 10.8*	2.8 $\pm$ 0.6*	63.8 $\pm$ 5.1*	128.2 $\pm$ 11.8*	45.0 $\pm$ 6.3**
AM251 (100 nmol/L)	73.1 $\pm$ 6.1	128.6 $\pm$ 10.1	5.7 $\pm$ 2.4	72.4 $\pm$ 4.6	144.8 $\pm$ 9.9	62.0 $\pm$ 5.8
AM251 (100 nmol/L) + anandamide (200 nmol/L)	73.5 $\pm$ 5.3#	129.7 $\pm$ 11.2#	6.0 $\pm$ 2.6#	72.2 $\pm$ 6.3#	144.8 $\pm$ 9.3#	60.5 $\pm$ 5.8##

Data were expressed as means  $\pm$  SD,  $n = 6$ . APD<sub>50</sub>: action potential duration at 50% depolarization; APD<sub>90</sub>: action potential duration at 90% depolarization; Vmax: maximal rate of depolarization; APA: amplitude of action potential; RPF: rate of pacemaker firing; VDD: velocity of phase 4 depolarization. \* $P < 0.05$ , \*\* $P < 0.01$  vs Baseline. # $P < 0.05$ , ## $P < 0.01$  vs Anandamide.

Table 3. Influence of AM630 on the electrophysiological effect of anandamide in rabbit sinoatrial nodes

	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)	Vmax (V/s)	APA (mV)	RPF (beat/min)	VDD (mV/s)
Baseline	80.3 ± 3.1	140.1 ± 11.3	6.7 ± 1.2	76.9 ± 5.4	140.8 ± 7.2	76.9 ± 5.4
Anandamide (200 nmol/L)	63.4 ± 6.1***	112.7 ± 13.2**	3.0 ± 0.5***	69.4 ± 5.3*	126.3 ± 10.7*	69.4 ± 5.3*
AM630 (100 nmol/L)	80.3 ± 3.0	139.8 ± 9.0	6.7 ± 1.7	77.5 ± 5.7	141.0 ± 7.1	77.5 ± 5.7
AM630 (100 nmol/L) + anandamide (200 nmol/L)	67.8 ± 5.2	125.1 ± 9.1	3.5 ± 1.0	69.4 ± 4.6	126.2 ± 5.9	68.4 ± 4.6

Data were expressed as means ± SD,  $n = 6$ . APD<sub>50</sub>: action potential duration at 50% depolarization; APD<sub>90</sub>: action potential duration at 90% depolarization; Vmax: maximal rate of depolarization; APA: amplitude of action potential; RPF: rate of pacemaker firing; VDD: velocity of phase 4 depolarization. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs Baseline.

Table 4. Influence of TEA on the electrophysiological effect of anandamide in rabbit sinoatrial nodes

	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)	Vmax (V/s)	APA (mV)	RPF (beat/min)	VDD (mV/s)
Baseline	74.0 ± 5.5	125.9 ± 2.2	6.4 ± 0.8	73.7 ± 7.4	138.4 ± 6.6	69.7 ± 2.5
Anandamide (200 nmol/L)	63.7 ± 6.9*	112.0 ± 11.9*	3.0 ± 0.6***	65.5 ± 2.2*	123.2 ± 8.1*	51.0 ± 16.6*
TEA (20 nmol/L)	79.6 ± 5.5*	139.9 ± 5.2**	5.9 ± 0.8	65.8 ± 12.1	142.0 ± 4.7	66.0 ± 2.1*
TEA (20 nmol/L) + anandamide (200 nmol/L)	66.0 ± 4.9	117.2 ± 5.0	3.2 ± 0.8	69.5 ± 12.1	134.8 ± 12.5	61.0 ± 3.3

Data were expressed as means ± SD,  $n = 6$ . APD<sub>50</sub>: action potential duration at 50% depolarization; APD<sub>90</sub>: action potential duration at 90% depolarization; Vmax: maximal rate of depolarization; APA: amplitude of action potential; RPF: rate of pacemaker firing; VDD: velocity of phase 4 depolarization. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  vs Baseline.

Table 5. Influence of *L*-NAME on the electrophysiological effect of anandamide in rabbit sinoatrial nodes

	APD <sub>50</sub> (ms)	APD <sub>90</sub> (ms)	Vmax (V/s)	APA (mV)	RPF (beat/min)	VDD (mV/s)
Baseline	78.6 ± 10.7	139.8 ± 4.5	7.9 ± 1.3	79.7 ± 13.3	137.6 ± 2.9	68.3 ± 6.8
Anandamide (200 nmol/L)	62.3 ± 6.2*	119.3 ± 15.2*	3.0 ± 0.6*	63.3 ± 4.7*	124.2 ± 10.3*	47.8 ± 10.4**
<i>L</i> -NAME (1 mmol/L)	74.6 ± 10.5	134.5 ± 4.5	7.4 ± 2.0	79.1 ± 14.2	131.5 ± 5.7	65.9 ± 3.6
<i>L</i> -NAME (1 mmol/L) + anandamide (200 nmol/L)	67.3 ± 10.1	129.0 ± 3.0	3.3 ± 0.6	76.0 ± 14.4	122.3 ± 4.7	57.2 ± 4.5

Data were expressed as mean ± SD,  $n = 6$ . APD<sub>50</sub>: action potential duration at 50% depolarization; APD<sub>90</sub>: action potential duration at 90% depolarization; Vmax: maximal rate of depolarization; APA: amplitude of action potential; RPF: rate of pacemaker firing; VDD: velocity of phase 4 depolarization; MDP: maximal diastolic potential. \* $P < 0.05$ , \*\* $P < 0.01$  vs Baseline.

longed APD<sub>50</sub> and APD<sub>90</sub> ( $P < 0.05$ ), but had no significant effect on APA, RPF, Vmax and VDD of AP ( $P > 0.05$ ). Pretreatment with TEA had no effect on the action of AEA (200 nmol/L) on AP ( $P > 0.05$ , Table 4).

#### 2.4 Effect of *L*-NAME on AEA action

NO synthase inhibitor *L*-NAME (1 mmol/L) alone had no significant effect on APD<sub>50</sub>, APD<sub>90</sub>, APA, RPF, Vmax and VDD of AP ( $P > 0.05$ ). And, pretreatment with *L*-NAME had no effect on the action of AEA (200 nmol/L) on AP ( $P > 0.05$ , Table 5).

### 3 DISCUSSION

In the present study, we examined the effect of AEA on the AP of the pacemaker cells in rabbit SAN by intracellular microelectrode recording technique. The result showed for the first time that AEA shortened APD,

decreased APA, RPF, Vmax and VDD of AP in SAN cells of rabbits. The effect of AEA on AP in SAN cells of rabbits was abolished by CB1 receptor antagonist AM251, which suggests CB1 receptors are involved in the electrophysiological effect of AEA on the pacemaker cells of rabbit SAN.

It is well known that transmembrane AP in the pacemaker cells of SAN can be divided into three phases: phase 0 depolarization, phase 3 repolarization and phase 4 spontaneous depolarization. Phase 0 depolarization is resulted from inward current owing to the influx of calcium ions through L-type calcium channels and phase 3 repolarization is resulted from an outward current owing to the outflow of potassium ions through potassium channels. Phase 4 spontaneous depolarization, the major feature of the AP of pacemaker cells, is the ion basis of autorhythmicity generation, which is com-



posed of several different ion currents including a progressively decreasing outward potassium current, a gradually increasing inward calcium current owing to the influx of calcium ions through T-type calcium channels, and the  $I_f$  pacemaker current mediated by sodium ions<sup>[18]</sup>. Any agents that are able to block calcium channels and/or activate potassium channels can shorten the APD<sup>[19]</sup>. In this study, TEA, a non-specific blocker of potassium channels, did not prevent the effect of AEA on the APD of SAN cells, suggesting that the shortening of APD by AEA was not resulted from the activation of potassium channels. Our results revealed that AEA not only shortened APD, but also reduced APA and VDD, suggesting that AEA may have caused these effects via blockade of calcium channels in rabbit SAN pacemaker cells.

Consistently, one of our previous study on papillary muscle and ventricular myocytes in rats showed that AEA could shorten APD and suppress L-type calcium current<sup>[15]</sup>. Based on the results of this previous study and those of the present study, we can declare that AEA has a blocking effect on the calcium channels of the cardiac myocytes (working cells and autorhythmic cells) in different animals (rabbit and rat). There are reports that calcium channel blockers are one class of anti-arrhythmia medicine<sup>[3]</sup>; and blockade of L-type calcium channels in cardiac myocytes has anti-arrhythmic effect<sup>[20]</sup>. The blockade of calcium channels by AEA might be one of the ionic mechanisms of its anti-arrhythmic effect.

Lots of researches demonstrated that there are two types of cannabinoid receptors in cardiovascular system: CB1 and CB2 receptors<sup>[21]</sup>. Some studies showed that the activation of CB1 receptors in anesthetized animals could decrease blood pressure and cardiac contractility, and cause bradycardia<sup>[22]</sup>, while another study showed that the activation of CB2 receptors using a specific CB2 receptor agonist JWH-133 during ischemia decreased the infarct size after myocardial ischemia/reperfusion in rats<sup>[23]</sup>. One of our previous study showed that AEA produced an anti-arrhythmic effect through CB1 receptors<sup>[15]</sup>, and this study found the effect of AEA on AP in SAN pacemaker cells was eliminated by CB1 receptor antagonist AM251, which confirmed that the effects of AEA on the AP of SAN pacemaker cells were mediated by CB1 receptor.

NO is a vital messenger member and is involved in many physiological and pathophysiological processes, and abundant studies demonstrated that NO took part in the cardiovascular effect of AEA. For example, AEA had a protective action through increasing the activity

of constructive nitric oxide synthase (cNOS) and promoting the release of NO<sup>[24]</sup>. In spontaneously hypertensive rats, long-time suppression of CB1 receptors enhanced the expression of vessel endothelial nitric oxide synthase (eNOS), thus reduced blood pressure<sup>[25]</sup>. Our results showed that NO synthase inhibitor L-NAME had no effect on AEA's effect, which suggests that the effect of AEA on the AP of pacemaker cells is independent of NO.

In conclusion, this study demonstrates for the first time that anandamide can decrease APA and shorten APD in SAN pacemaker cells of rabbits, which is mediated by activation of CB1 receptors, and related to blockade of calcium channels but not potassium channels, and is independent of NO. This might be one of the mechanisms for the anti-arrhythmic effect of AEA.

## REFERENCES

- 1 Seidl K, Senges J. Worldwide utilization of implantable cardioverter/defibrillators now and in the future. *Card Electrophysiol Rev* 2003; 7(1): 5–13.
- 2 Yang BF (杨宝峰), Cai BZ. Advances in the study of arrhythmogenic mechanisms. *J Int Pharm Res (国际药理学研究杂志)* 2001; 37(2): 81–88 (Chinese, English abstract).
- 3 Lu ZY (陆再英), Zhong NS. *Internal Medicine*. Beijing: People's Medical Publishing House (人民卫生出版社) 2008, 182–228 (Chinese).
- 4 Yue P, Zhang Y, Du Z, Xiao J, Pan Z, Wang N, Yu H, Ma W, Qin H, Wang WH, Lin DH, Yang B. Ischemia impairs the association between connexin 43 and M3 subtype of acetylcholine muscarinic receptor (M3-mAChR) in ventricular myocytes. *Cell Physiol Biochem* 2006; 17(3–4): 129–136.
- 5 Saint DA. The role of the persistent  $\text{Na}^+$  current during cardiac ischemia and hypoxia. *J Cardiovasc Electrophysiol* 2006; 17(1): 96–103.
- 6 Olson TM, Terzic A. Human KATP channelopathies: diseases of metabolic homeostasis. *Pflugers Arch* 2010; 460(2): 295–306.
- 7 Ralevic V, Kendall DA, Randall MD, Smart D. Cannabinoid modulation of sensory neuron transmission via cannabinoid and vanilloid receptors: roles in regulation of cardiovascular function. *Life Sci* 2002; 71(22): 2577–2594.
- 8 Hiley CR, Ford WR. Cannabinoid pharmacology in the cardiovascular system: potential protective mechanisms through lipid signaling. *Biol Rev Camb Philos Soc* 2004; 79(1): 187–205.
- 9 Grant I, Cahn BR. Cannabis and endocannabinoid modulators: therapeutic promises and challenges. *Clin Neurosci Res* 2005; 5(2–4): 185–199.
- 10 Hiley CR. Endocannabinoids and the heart. *J Cardiovasc*

- Pharmacol 2009; 53(4): 267–276.
- 11 Wang PF (王鹏飞), He B. The endocannabinoid system and myocardial ischemia/reperfusion injury. *Chin J Arterioscler (中国动脉硬化杂志)* 2010; 18(8): 669–704 (Chinese, English abstract).
  - 12 Ma SY (马世玉), Ma YX. Effects of endocannabinoids on cardiovascular system. *Prog Physiol Sci (生理科学进展)* 2006; 37(4): 297–301 (Chinese, English abstract).
  - 13 Varga K, Lake K, Martin BR, Kunos G. Novel antagonist implicates the CB1 cannabinoid receptor in the hypotensive action of anandamide. *Eur J Pharmacol* 1995; 278(3): 279–283.
  - 14 Krylatov AV, Ugdyzhekova DS, Bernatskaya NA, Maslov LN, Mekhoulam R, Pertwee RG, Stephano GB. Activation of type II cannabinoid receptors improves myocardial tolerance to arrhythmogenic effects of coronary occlusion and reperfusion. *Bull Exp Biol Med* 2001; 131(6): 523–525.
  - 15 Li Q, Ma HJ, Zhang H, Qi Z, Guan Y, Zhang Y. Electrophysiological effects of anandamide on rat myocardium. *Br J Pharmacol* 2009; 158 (8): 2022–2029.
  - 16 Li Q, Ma HJ, Song SL, Shi M, Ma HJ, Li DP, Zhang Y. Effects of anandamide on potassium channels in rat ventricular myocytes. *Am J Physiol Cell Physiol* 2012; 302(6): C924–C930.
  - 17 Ma T, Fan ZZ, He RR. Electrophysiological effects of phytoestrogen genistein on pacemaker cells in sinoatrial nodes of rabbits. *Acta Pharmacol Sin* 2002; 3 (4): 367–370.
  - 18 Zhu MZ (朱妙章), Yuan WJ, Wu BW, Zhang WJ, Zhu DN. *Cardiovascular Physiology and Clinical*. Beijing: Higher Education Press (高等教育出版社), 2004, 20–35 (Chinese).
  - 19 Robert SK, Abriel H, Rivolta I. Ion channels in the heart. In: Sperelakis N, Kurachi Y, Terzic A, Cohen MV, eds. *Heart Physiology and Pathophysiology*, 4th ed. San Diego: Academic Press, 2001, 1137–1151.
  - 20 Triggle DJ. Calcium channel antagonists: clinical uses – past, present and future. *Biochem Pharmacol* 2007; 74 (1): 1–9.
  - 21 Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* 1997; 74(2): 129–180.
  - 22 Pacher P, Batkai S, Kunos G. Cardiovascular pharmacology of cannabinoids. *Handb Exp Pharmacol* 2005; (168): 599–625.
  - 23 Montecucco F, Lenglet S, Braunersreuther V, Burger F, Pelli G, Bertolotto M, Mach F, Steffens S. CB2 cannabinoid receptor activation is cardioprotective in a mouse model of ischemia/reperfusion. *J Mol Cell Cardiol* 2009; 46(5): 612–620.
  - 24 Xu LM (许雷鸣), Fu Q, Hu BR, Tang Q, Xiang ZZ. Effects of anandamide on the cardiac function, nitric oxide content and nitric oxide synthase activity in the myocardium of the rat. *Herald Med (医药导报)* 2006; 25(12): 1241–1246 (Chinese, English abstract).
  - 25 Yang DC (杨大春), Chen XP, Wang LJ, Cao YB, Ya H, Feng XL, Zhu ZM, Liu DL. Effect of long-term inhibition of CB1 receptor on blood pressure and vascular reactivity in spontaneously hypertensive rats. *Chin J Geriatr Heart Brain Vessel Dis (中华老年心脑血管病杂志)* 2008; 10(5): 364–367 (Chinese, English abstract).