Effects of anandamide on potassium channels in rat ventricular myocytes: a suppression of $I_{to}$ and augmentation of $K_{ATP}$ channels

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Li Q, Ma HJ, Song SL, Shi M, Ma HJ, Li DP, Zhang Y. Effects of anandamide on potassium channels in rat ventricular myocytes: a suppression of $I_{to}$, and augmentation of $K_{ATP}$ channels. Am J Physiol Cell Physiol 302: C924–C930, 2012. First published December 14, 2011; doi:10.1152/ajpcell.00228.2011.—Anandamide is an endocannabinoid that has antiarrhythmic effects through inhibition of L-type Ca$^{2+}$ channels in cardiomyocytes. In this study, we investigated the electrophysiological effects of anandamide on $K^+$ channels in rat ventricular myocytes. Whole cell patch-clamp technique was used to record $K^+$ currents, including transient outward potassium current ($I_{to}$), steady-state outward potassium current ($I_{to}$), inward rectifier potassium current ($I_{Kr}$), and ATP-sensitive potassium current ($I_{KATP}$) in isolated rat cardiac ventricular myocytes. Anandamide decreased $I_{to}$ while increasing $I_{KATP}$ in a concentration-dependent manner but had no effect on $I_{to}$ and $I_{Kr}$ in isolated ventricular myocytes. Furthermore, anandamide shifted steady-state inactivation curve of $I_{to}$ to the left and shifted the recovery curve of $I_{to}$ to the right. However, neither cannabidiol 1 (CB1) receptor antagonist AM251 nor CB2 receptor antagonist AM630 eliminated the inhibitory effect of anandamide on $I_{to}$. In addition, blockage of $I_{to}$ receptors, but not CB1 receptors, eliminated the augmentation effect of anandamide on $I_{KATP}$. These data suggest that anandamide suppresses $I_{to}$ through a non-CB1 and non-CB2 receptor-mediated pathway while augmenting $I_{KATP}$ through CB2 receptors in ventricular myocytes.

electrophysiology; endocannabinoid; receptor; cardiomyocyte; transient outward potassium current; ATP-sensitive potassium current

ENDOCANNABINOIDSS ARE A CLASS of signaling lipids consisting of amides and esters of long-chain polyunsaturated fatty acids. They are synthesized from lipid precursors in plasma membranes and exhibit cannabinoid-like actions by binding to cannabinoid receptors (the CB1 and CB2 receptors) (12). Endocannabinoids are importantly involved in hormonal regulation of food intake, cardiovascular, gastrointestinal, immune, and mammalian reproductive functions (24). Anandamide is one of the endogenous endocannabinoids and is involved in the regulation of cardiovascular function (12). Currently, at least two types of cannabinoid receptors, the CB1 receptor and CB2 receptor, have been cloned and found widely expressed in many tissues including cardiac myocytes (30). Anandamide limits the damage induced by ischemia-reperfusion in rat isolated hearts through different mechanisms (18, 38). Also, anandamide has been found to protect the heart from adrenalin-induced arrhythmias (37) or arrhythmias induced by ischemia-reperfusion (15). We have found recently that anandamide exerts antiarrhythmia action through suppression of action potential duration and blockade of L-type Ca$^{2+}$ channels in cardiomyocytes (19).

Cardiac $K^+$ channels play important roles in determining the firing frequency in sinus node pacemaker cells and resting potential and the shape and duration of action potential in cardiomyocytes (36). In mammalian cardiac cells, $K^+$ channels are categorized into voltage-gated and ligand-gated channels. The voltage-gated $K^+$ channels include the rapidly activating and inactivating transient outward current ($I_{to}$), the ultrarapid ($I_{Kur}$), rapid ($I_{Kr}$), and slow ($I_{Ks}$) components of the delayed rectifier and the inward rectifier ($I_{K1}$). The ligand-gated $K^+$ channels include adenosine triphosphate (ATP)-dependent K$^+$ ($I_{KATP}$), which is activated by a decrease in the intracellular concentration of ATP and $K_{ACb}$, which is activated by acetylcholine. Opening of $K^+$ channels results in efflux of $K^+$ and membrane repolarization. The Ca$^{2+}$ influx through L-type Ca$^{2+}$ channels delays the time course of the repolarization phase of action potential in cardiac myocytes (12a). Our recent study has shown that anandamide suppresses Ca$^{2+}$ influx through L-type Ca$^{2+}$ channels (19). However, the contribution of $K^+$ channels in the antiarrhythmia action of anandamide in cardiomyocytes remains unknown.

Although previous studies have revealed that endocannabinoids affect $K^+$ channels, the effects of anandamide on $K^+$ channels are still controversial. In this regard, anandamide blocks Kv4.3 channels expressed in Chinese hamster ovary (CHO) cells (1) and Kv1.5 channels (which generate the $I_{Kur}$) expressed in mouse fibroblasts [Ltk(-) cells] (2). Furthermore, Choisy et al. (6) have shown that anandamide inhibits steady-state outward potassium current ($I_{to}$) (which is proposed to reflect the activity of delayed rectifier $K^+$ channels) in rat cardiomyocytes. However, Zhang et al. (42) reported that anandamide had no effect on either $I_{K1}$ or $I_{K}$ in embryonic chick hearts. Although Krylatov et al. (15) found that blocking $I_{KATP}$ with glibenclamide did not abolish the antiarrhythmic effect of R-(+)-methanandamide, an enzyme-resistant analog of anandamide, the role of $I_{KATP}$ channels in the anandamide-induced antiarrhythmia effect cannot be ruled out. It has been shown that $I_{KATP}$ channels could be activated by arachidonic acid, a metabolite of anandamide, in rat ventricular myocytes (20). Interestingly, blocking $I_{KATP}$ channels with the blocker glibenclamide abolished cardioprotective effect of arachidonic acid during cardiac ischemia and reperfusion injuries (26). Recently, Reis et al. (33) reported that anandamide induces peripheral antinociception by activation of ATP-sensitive $K^+$ channels. Therefore, the effect of anandamide on $K^+$ channels may be dependent on the specific cellular organelle, cell type, and concentration of anandamide. The purpose of the present study was to investigate the effect of anandamide on $K^+$...
channels in ventricular myocytes by using whole cell patch-clamp technique.

MATERIALS AND METHODS

Animals. The animal experimental protocols employed in this study were approved by the Committee on the Use of Animals for Teaching and Research of Hebei Medical University. These protocols also conformed to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). Experiments were carried out in adult male Sprague-Dawley rats (230–280 g) obtained from the Experimental Animal Center of Hebei Province and received care in compliance with the requirements of Hebei Medical University and National Institutes of Health guidelines.

Isolation of ventricular myocytes. Cardiac ventricular myocytes were isolated by using enzymatic dissociation, as described previously (5). Briefly, the rat was anesthetized by intraperitoneal injection of pentobarbital sodium (60 mg/kg) and heparin (300 U/kg). The rat heart was excised and retrogradely perfused on a Langendorff apparatus with oxygenated ice-cold Ca2+-free Tyrode’s solution via the aorta at a perfusion rate of 4 ml/min for 5 min. Then, the heart was perfused with Ca2+-free Tyrode’s solution with addition of CaCl2 (34 μM) and collagenase II (300 mg/l) at 37°C for 12 min. Finally, the left ventricle was removed and teased into smaller pieces in Krebs solution. The isolated myocytes were harvested after filtration through a nylon mesh (pore size 200 μm) and stored in Krebs solution at room temperature for at least 1 h before recording was performed. The concentration of Ca2+ in Krebs solution was gradually increased to 1.0 mM.

Solutions and chemicals. The Ca2+-free Tyrode’s solution used for cell isolation contained (in mM) 135 NaCl, 5.4 KCl, 1.0 MgCl2, 0.33 NaH2PO4, 5 glucose, and 10 HEPES (pH adjusted to 7.4 with NaOH). The pipette solution for K+ currents recording consisted in (mM) 5.0 K2-ATP, 140.5 KCl, 10.0 MgCl2, 10.0 EGTA (pH 7.2 adjusted with KOH). The Tyrode’s solution for K+ currents recording consisted of (in mM) 135 NaCl, 5.4 KCl, 1.0 MgCl2, 1.8 CaCl2, 0.33 NaH2PO4, 5 glucose, and 10 HEPES (pH adjusted to 7.4 with NaOH). Krebs solution for cell storage contained (in mM) 80 KOH, 40 KCl, 25 NaH2PO4, 3 MgSO4, 50 glutamic acid, 20 taurine, 1.0 EGTA, 10 HEPES, and 10 glucose (pH adjusted to 7.5 with KOH).

During recording of K+ currents, tetrodotoxin (TTX, 10 μM) and CdCl2 (2 mM) were used to block sodium current (INa) and voltage-dependent Ca2+ channels, respectively. Dinitrophenol (DNP, 50 μM) was used to open Ios, BaCl2 (200 μM) and glibenclamide (50 μM) were used to block IK, and IATP, respectively, if needed. Anandamide, AM251, and AM630 were purchased from Cayman Chemical (Ann Arbor, MI); K2-ATP, TTX, CdCl2, DNP, BaCl2, and glibenclamide were obtained from Sigma-Aldrich (St. Louis, MO). Collagenase II was purchased from InVitrogen/GIBCO (Grand Island, NY). Anandamide was initially dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO during the experiment was <0.1%. In our pilot study, DMSO (up to 0.1%) alone had no significant effect on the electrophysiological characteristics of myocytes.

Recording of K+ current. Whole cell patch-clamp recordings were performed in isolated ventricular myocytes at room temperature. The myocytes were placed in the recording chamber (0.4 ml) mounted on the stage of an inverted microscope (CK2, Olympus, Japan). After settling to the bottom of the chamber, cells were perfused with external solution for 10 min at a rate of 2–3 ml/min. Transmembrane currents were recorded by an amplifier (Axopatch 200B, Molecular Devices). Glass recording pipettes were pulled using a microelectrode puller (model P-97, Sutter Instrument) and had a resistance of 2.0–4.0 MΩ when filled with internal pipette solution.

After the MΩ seal was formed, the cell membrane was ruptured with a brief negative pressure to obtain the whole cell voltage-clamp configuration. Membrane capacitance and series resistance were compensated after membrane rupture to minimize the contribution of capacitive currents. Compensated voltage pulses were programmed using pCLAMP 10.0 software (Axon Instruments). To measure IK, and Ios, serial test pulses from −80 to +60 mV in 10-mV increments and 500-ms duration were applied in the presence of 10 μM TTX, 2 mM CdCl2, and 200 μM BaCl2 to block Ios, L-type Ca2+ current (ICa,L) and IK, respectively. IK amplitude was measured as the peak outward current amplitude minus Ios, while IK amplitude was measured as the residual current at the end of the 500-ms pulses.

Voltage and time-dependent properties of IK were then determined in additional experiments. To calculate the steady-state activation curves, specific current conductance (G) was normalized to the maximal current conductance (Gmax) to yield G/Gmax. These values were normalized by Boltzmann distribution function: G/Gmax = 1/[1 + exp(V/2 – V0.5)], where V0.5 is membrane voltage, V/2 is voltage at half-maximal activation, and k is a slope factor at V/2 = V0.5. We next determined the steady-state inactivation curves by applying test pulses from −120 to +50 mV in duration of 500 ms before a test pulse of +60 mV (0.1 Hz, 500 ms). Steady-state inactivation curves were constructed by normalizing IK measured at each prepulse to the maximal IK (Ios) (Umax) and then plotting these values against prepulse voltage. These curves were also fitted by a Boltzmann distribution to derive steady-state inactivation parameters V1/2 and k. Finally, IK reactivation was analyzed in some cells by delivering test pulses. Once the IK was inactivated following membrane depolarization, a sufficient amount of hyperpolarizing time must elapse before the channel can recover and be fully activated again. To determine the kinetics of recovery from the inactivated channel state, two identical 500-ms depolarizing pulses from −80 mV to +60 mV, varying the interpulse interval from 50 to 3,000 ms (0.1 Hz), was applied. The mean normalized peak amplitude was plotted against interpulse intervals. A single exponential function was fitted to the plot, and the time constant of recovery from inactivation was then calculated. IK was recorded by 500-ms long test pulses ranging between −120 and 0 mV, in 10-mV increments, from a holding potential of −80 mV. A 100-ms long prepulse to −40 mV was applied to block Ios. Values obtained at the end of the 500-ms pulse were used for analysis.

To measure IKATP, a voltage-ramp test was used to record the currents in the presence and absence of DNP (50 μM) (7). The test protocol consisted of a prepulse from a holding potential of −80 mV to +60 mV and kept at +60 mV for 500 ms, to inactivate inward currents, and application of a voltage ramp from +60 to −100 mV at 4 mV/ms. Subtraction of the currents evoked in the presence and absence of DNP yields the pure IKATP currents.

For all experiments, anandamide was added to the superfusion solution 3 min before recording was performed. Time course studies (Fig. 1D) indicated that the maximal effects of anandamide can be measured within 8 min of initial exposure. The seal resistances were not altered during the whole time course of the experiments.

Data analysis. Data are expressed as means ± SE. The differences between groups were compared with one-way ANOVA followed by Dunnett’s post hoc test. Differences within the same group before and after drug application were analyzed by repeated ANOVA followed by Dunnett’s post hoc test. Statistical significance was accepted at P < 0.05.

RESULTS

Effects of anandamide on IK. We first determined the effect of anandamide (1, 10, and 100 nM) on the current-voltage relationship of peak IK. IK was recorded in serial test pulses from −80 to +60 mV in 10-mV increments and 500-ms duration that were applied in the presence of TTX, CdCl2, and BaCl2, as described in MATERIALS AND METHODS. Low concentrations (1 nM) of anandamide had little effect on peak current
density of $I_{to}$, while 10 and 100 nM anandamide reduced peak current density of $I_{to}$ (at +60 mV) from 22.8 ± 1.2 pA/pF to 17.7 ± 1.3 (n = 6; P < 0.05) and 14.6 ± 1.0 pA/pF (n = 6; P < 0.05), respectively (Fig. 1). Furthermore, 10 and 100 nM anandamide significantly inhibited the peak amplitude of $I_{to}$ at holding potentials ranging from −10 to +60 mV. The effects of anandamide were completely reversible within 5-min wash-out of the drug (data not shown).

We then determined the effect of anandamide on the kinetics of activation and inactivation of $I_{to}$. The voltage-dependent activation and inactivation curves of $I_{to}$ were compared before and after administration of 100 nM anandamide. The activation curves were derived from the current-voltage relationships (Fig. 1B). There were no significant differences in half-maximal activation potential ($V_{1/2-act}$) (−36.5 ± 2.0 mV vs. −36.9 ± 4.8 mV, n = 6, P > 0.05) and slope parameter (κ) (14.5 ± 1.7 mV vs. 20.1 ± 5.4 mV, n = 6, P > 0.05) of the activation of $I_{to}$ between the control and after application of 100 nM anandamide (Fig. 2A). The inactivation curves were plotted as voltages in the prepulses and currents in the test pulses. Anandamide (100 nM) significantly changed the half-maximal inactivation potential ($V_{1/2-inact}$) from −17.5 ± 1.2 to −36.2 ± 1.1 mV (n = 6, P < 0.05). However, slope parameter (κ) was not significantly changed (10.7 ± 0.83 mV vs. 13.0 ± 1.2 mV, n = 6, P > 0.05) (Fig. 2B).

We also examined the effect of anandamide on the recovery from the inactivation of $I_{to}$. The recovery of $I_{to}$ was analyzed by delivering two identical 500-ms depolarizing pulses from −80 mV to +60 mV and varying the interpulse interval from 50 to 3,000 ms (0.1 Hz). Anandamide (100 nM) shifted the recovery curve from inactivation of $I_{to}$ to the right (Fig. 3). The curve of recovery was well fitted by a single exponential function with a time constant of 180.6 ± 17.8 ms in the control condition and 437.3 ± 7.7 ms in the presence of 100 nM anandamide (n = 6, P < 0.05).

In addition, we determined the role of CB1 and CB2 receptors in mediating the effect of anandamide on $I_{to}$. Pretreatment of myocytes with AM251 (100 nM), an antagonist for CB1 receptors, or AM630 (100 nM), an antagonist for CB2 receptors, for 15 min had no effect on the inhibition of anandamide (100 nM) on $I_{to}$ (Fig. 4). However, anandamide at concentrations of 1–100 nM did not significantly change $I_{ss}$ and $I_K$ in isolated myocytes (Fig. 1C and Fig. 5).

**Effects of anandamide on $I_{KATP}$** To determine the effect of anandamide on the $I_{KATP}$, $I_{KATP}$ was measured by a voltage ramp from +60 to −100 mV at 4 mV/ms followed by a prepulse of holding the cell at +60 mV in 500 ms duration. The currents evoked in the presence of DNP (50 μM) subtracted from the current in the absence of DNP yielded the pure $I_{KATP}$ currents. Application of DNP (50 μM) induced a time-

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**Fig. 1.** Effect of anandamide on transient outward potassium current ($I_{to}$) and steady-state outward potassium current ($I_s$) in rat isolated ventricular myocytes. A: original recording showing the effect of anandamide on $I_{to}$ and $I_s$ in rat isolated ventricular myocytes. B and C: effects of anandamide on current-voltage curve of $I_{to}$ (B) and $I_s$ (C) in rat isolated ventricular myocytes. D: time dependence of 100 nM anandamide on $I_{to}$ during 500-ms depolarization from a holding potential of −80 to +60 mV in rat isolated ventricular myocytes (n = 6).
independent current and reached the maximum value at 15 min after DNP was added and remained constant for approximately 20–25 min. I_{K_{ATP}} was completely blocked by 50 μM glibenclamide. Anandamide was administrated to the chamber after I_{K_{ATP}} reached maximum value. Anandamide (1, 10, and 100 nM) significantly increased I_{K_{ATP}} in a dose-dependent manner: I_{K_{ATP}} (at -40 mV) was increased 17.4 ± 3.3%, 90.2 ± 5.6%, and 200.2 ± 2.5% by 1, 10, and 100 nM anandamide, respectively (Fig. 6).

Pretreatment of cells with AM251 (100 nM), an antagonist for CB1 receptors, had no effect on the enhancement of anandamide on I_{K_{ATP}}. However, pretreatment of cells with the CB2 receptor antagonist AM630 (100 nM) significantly inhibited the potentiating effect of anandamide on I_{K_{ATP}} in cardiac myocytes (n = 6, P < 0.05, Fig. 7).

**DISCUSSION**

This study has demonstrated that anandamide inhibits I_{to} in rat ventricular myocytes. We found that anandamide reduced I_{to} in a concentration-dependent manner through shifting the voltage dependence of I_{to} inactivation and suppression of I_{to} recovery from inactivation. Neither CB1 nor CB2 receptors mediated the inhibitory effect of anandamide on I_{to}. Furthermore, anandamide increased I_{K_{ATP}} in a concentration-dependent manner, and such effect was mediated by CB2 receptors, but not CB1 receptors in rat ventricular myocytes. On the contrary, anandamide had no effects on I_{K_1} and I_{ss} in rat ventricular myocytes.

Because cardiac I_{to} channels are critical to define resting membrane potential, heart rate, action potential shape, and duration in cardiac sinus node cells and cardiac myocytes (27), I_{to} is an important target of class III antiarrhythmic drugs (22). Inhibition of I_{to} channels leads to antiarrhythmic effects such as effectively preventing/suppressing re-entrant arrhythmias (35). It is well known that transient coronary artery occlusion induces malignant ventricular arrhythmias during ischemia and reperfusion (10). One prominent alteration induced by ischemia and reperfusion is that the electrophysiological heterogeneity is increased between the epicardium and the endocardium (13). The large epimyocardial transient outward K^+ current contributes to this electrical inhomogeneity (39) and leads to the genesis of ventricular arrhythmias. Blocking I_{to} with 4-aminopyridine reverses this high heterogeneity and exerts an antiarrhythmic effect (8). We found that anandamide suppressed I_{to} channels in cardiac myocytes. These data suggest that the inhibition of I_{to} may be involved in the antiarrhythmia action of anandamide. In our recent study, we found that anandamide shortened the duration of action potential and repolarization without changing the amplitude, overshoot, and maximal velocity in the depolarization phase of the action potential in ventricular myocytes (19). These findings are...
consistent with data shown in the present study, because K\textsuperscript{+} channels are mainly involved in the repolarization phase and Na\textsuperscript{+} channels are mainly involved in the amplitude and depolarization phase of action potentials.

We further analyzed the kinetics of \textit{I}_{\text{to}} in response to anandamide. Anandamide had no effect on the maximal activation but shifted the steady-state inactivation curve to the left, suggesting that the voltage-dependent steady-state inactivation of the \textit{I}_{\text{to}} channel was accelerated. It has been shown that the time course of recovery from inactivation is a major determinant of the functional role of \textit{I}_{\text{to}} in the action potential duration (27). In the present study, we found that anandamide markedly shifted the recovery curve of \textit{I}_{\text{to}} to the right, suggesting that anandamide attenuated the recovery of \textit{I}_{\text{to}} channels from inactivation. The attenuated recovery from inactivation may change frequency-dependent properties of \textit{I}_{\text{to}} channels. These findings provide substantial electrophysiological evidence that anandamide inhibits \textit{I}_{\text{to}} through facilitation of steady-state inactivation and attenuation of recovery from inactivation.

Two types of cannabinoid receptors, the CB\textsubscript{1} and CB\textsubscript{2}, have been cloned (21, 25) and are widely expressed in the cardiovascular system such as blood vessels and cardiac tissue (31). Anandamide is a natural constituent of the plasma membrane and is considered to be a CB\textsubscript{1} and CB\textsubscript{2} agonist because it exhibits pharmacological activities comparable to cannabinoids (9). Both CB\textsubscript{1} and CB\textsubscript{2} receptors are involved in the cardioprotective effect of anandamide. For instance, it has been shown that activation of CB\textsubscript{1} receptor reduces the infarct size induced by low-flow ischemia through production of nitric oxide in rat isolated hearts (18). In addition, blockade of CB\textsubscript{2} receptors eliminates the cardioprotective effect of endocannabinoids in rat isolated hearts exposed to low-flow ischemia and reperfusion (15, 17). However, we found that blocking both CB\textsubscript{1} receptor and CB\textsubscript{2} receptor with the selective CB\textsubscript{1} receptor antagonist AM251 and CB\textsubscript{2} receptor antagonist AM630 failed to affect the inhibitory effects of anandamide on \textit{I}_{\text{to}} in cardiac myocytes. These data suggest that neither CB\textsubscript{1} nor CB\textsubscript{2} receptors mediate the inhibitory effects of anandamide on \textit{I}_{\text{to}} in cardiac myocytes. In support of this notion, a previous study has shown that endocannabinoids modulated K\textsuperscript{+}, Na\textsuperscript{+}, and Ca\textsuperscript{2+} channel properties in a CB\textsubscript{1}/CB\textsubscript{2}-independent manner (28). It is possible that anandamide blocked cardiac Kv4.3 channels through direct action on the Kv4.3 \(\alpha\)-subunit (1), which is the predominant component of \textit{I}_{\text{to}}. Another possibility is that anandamide may act on non-CB\textsubscript{1}/CB\textsubscript{2} receptors (4, 38) to inhibit \textit{I}_{\text{to}} in cardiac myocytes. It is also possible that the effect of anandamide on the \textit{I}_{\text{to}} is a typical fatty acid-like effect on different ion channels. Thus, future studies are warranted to determine the structure-activity relationship for anandamide-related compounds on \textit{I}_{\text{to}} in cardiac myocytes.

The K\textsubscript{ATP} channels are critically involved in cellular energy metabolism and the regulation of membrane excitability by virtue of their involvement in hyperpolarizing cells. K\textsubscript{ATP} channels are widely distributed in a variety of tissues including cardiac myocytes (41). K\textsubscript{ATP} channels open when the intracellular ATP-to-ADP ratio decreases and close when this ratio increases (40). In the present study, we used DNP, a metabolic inhibitor, to open \textit{I}_{\text{KATP}} channels through decreasing intracellular ATP levels (7). It has been shown that K\textsubscript{ATP} channels play an important cardioprotective role during cardiac ischemia. In this regard, opening of K\textsubscript{ATP} shortens the action potential.
duration and decreases Ca\textsuperscript{2+} influx through L-type channels, which prevents cardiac Ca\textsuperscript{2+} overloading and preserves ATP level in cardiac myocytes (11). It has been shown that K\textsubscript{ATP} channels are activated by arachidonic acid, a substrate of anandamide degraded by the fatty acid amide hydrolase enzyme (3), in rat ventricular myocytes (20). Furthermore, activation of K\textsubscript{ATP} channels by arachidonic acid exerts antiarrhythmic and cardioprotective effects during cardiac ischemia (26). However, previous studies have shown that anandamide limits the cardiac damage induced by ischemia-reperfusion in rat isolated hearts through CB\textsubscript{2} receptor (16). Also, activation of CB\textsubscript{2} cannabinoid receptor produced a cardioprotective effect in a model of ischemia-reperfusion (23). Consistently, we found anandamide dose-dependently increased I\textsubscript{KATP} currents through CB\textsubscript{2} receptor but not CB\textsubscript{1} receptor in cardiac myocytes. These data suggest that anandamide-induced enhancement of I\textsubscript{KATP} is one of mechanisms underlying the antiarrhythmia or cardioprotective action of anandamide. This effect is likely mediated by CB\textsubscript{2} receptors.

In summary, this study provides direct evidence that anandamide attenuates I\textsubscript{to} and increases I\textsubscript{KATP}, but without affecting I\textsubscript{ss} and I\textsubscript{Kr}. This information is important to the understanding of the ionic mechanisms contributing to antiarrhythmic effect of anandamide. Furthermore, the antiarrhythmic effect of anandamide is dose-dependent. However, it is not clear whether there is a concentration window under which the antiarrhythmic effect of anandamide is dominant over its other physiological activity in other tissues since its receptors have a wide tissue distribution. Thus, it is necessary to obtain such information. In this aspect, this study provided valuable dose-dependent information about the anandamide-induced antiarrhythmic effect as well as the underlying mechanisms involved. This information is important for development of new pharmacological therapeutics for cardiac arrhythmia.

**Perspective.** It has been shown that oral administration of large-dose (−)−\textsuperscript{2}−tetrahydrocannabinol (\textsuperscript{2}−THC), the most psychoactive component of marijuana, causes reduced amplitude of T-wave with S-T elevation in electrocardiography (14). Because decreased T-wave represents the impaired repolarization of the ventricles, this finding is consistent with our data showing that anandamide suppressed I\textsubscript{to} in myocytes. In addition, the cannabinoid system is importantly involved in the control of cardiac rhythm. In this regard, acute administration of cannabinoids causes isolated tachycardia, whereas chronic use may lead to hypotension and bradycardia in humans (29, 32). Acute administration of cannabinoids in vivo suppresses ischemia-induced and epinephrine-induced arrhythmias (16, 37). It is not surprising that cannabinoids exert diverse effects in the control of cardiac rhythm, since cannabinoids are a group of pharmacologically active agents, which consist of phytocannabinoids (plant-derived), endocannabinoids (endogenous), and synthetic cannabinoids. Thus, future studies are needed to elucidate the mechanisms underlying the effects of anandamide on cardiac arrhythmia.
ANANDAMIDE AND POTASSIUM CHANNELS IN CARDIAC MYOCYTES

warranted to find cannabinoid components, which have beneficial effects in humans.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Q.L., H.-J.M, and Y.Z. conception and design of the research; Q.L. and H.-J.M. performed the experiments; Q.L., H.-J.M., and D.-P.L. analyzed the data; Q.L., S.-L.S., M.S., and H.-J.M. interpreted the results of the experiments; Q.L. prepared the figures; Q.L. drafted the manuscript; Q.L. and Y.Z. reviewed and revised the manuscript; Q.L., D.-P.L., and Y.Z. approved the final version of the manuscript.

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