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RESEARCH PAPER

Piceatannol, a derivative of resveratrol, moderately slows I_{Na} inactivation and exerts antiarrhythmic action in ischaemia-reperfused rat hearts

Wen-Pin Chen[1], Li-Man Hung[2], Chia-Hsiang Hsueh[1], Ling-Ping Lai[3] and Ming-Jai Su[1]

[1]*Institute of Pharmacology, National Taiwan University Medical College, Taipei, Taiwan,* [2]*Department of Life Science, College of Medicine, Chang Gung University, Kwei-Shan, Tao-Yuan, Taiwan, and* [3]*Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*

Background and purpose: Piceatannol is more potent than resveratrol in free radical scavenging in association with antiarrhythmic and cardioprotective activities in ischaemic-reperfused rat hearts. The present study aimed to investigate the antiarrhythmic efficacy and the underlying ionic mechanisms of piceatannol in rat hearts.
Experimental approach: Action potentials and membrane currents were recorded by the whole-cell patch clamp techniques. Fluo-3 fluorimetry was used to measure cellular Ca^{2+} transients. Antiarrhythmic activity was examined from isolated Langendorff-perfused rat hearts.
Key results: In rat ventricular cells, piceatannol (3–30 $\mu mol \cdot L^{-1}$) prolonged the action potential durations (APDs) and decreased the maximal rate of upstroke (V_{max}) without altering Ca^{2+} transients. Piceatannol decreased peak I_{Na} and slowed I_{Na} inactivation, rather than induced a persistent non-inactivating current, which could be reverted by lidocaine. Resveratrol (100 $\mu mol \cdot L^{-1}$) decreased peak I_{Na} without slowing I_{Na} inactivation. The inhibition of peak I_{Na} or V_{max} was associated with a negative shift of the voltage-dependent steady-state I_{Na} inactivation curve without altering the activation threshold. At the concentrations more than 30 $\mu mol \cdot L^{-1}$, piceatannol could inhibit $I_{Ca,L}$, I_{to}, I_{Kr}, Ca^{2+} transients and Na^+-Ca^{2+} exchange except I_{K1}. Piceatannol (1–10 $\mu mol \cdot L^{-1}$) exerted antiarrhythmic activity in isolated rat hearts subjected to ischaemia-reperfusion injury.
Conclusions and implications: The additional hydroxyl group on resveratrol makes piceatannol possessing more potent in I_{Na} inhibition and uniquely slowing I_{Na} inactivation, which may contribute to its antiarrhythmic actions at low concentrations less than 10 $\mu mol \cdot L^{-1}$.
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Keywords: piceatannol; resveratrol; sodium channel; APD prolongation; antiarrhythmia

Abbreviations: AP, action potential; APDs, action potential durations; $I_{Ca,L}$, L-type Ca^{2+} current; I_{K1}, inward rectifier K^+ current; I_{Kss}, steady-state outward K^+ current; I_{Na}, Na^+ current; I_{to}, transient outward K^+ current; V_{max}, maximal upstroke velocity of action potential

Introduction

Piceatannol (3,3′,4′,5-tetrahydroxystilbene, astringinin) is a polyphenolic stilbene phytochemical which is rich in the seeds of *Euphorbia lagascae* (Inamori *et al.*, 1984) and is also present in diets of plant-derived foods and beverage such as red wine (Waffo Teguo *et al.*, 1998). Piceatannol was identified as a selective inhibitor of non-receptor Syk tyrosine kinase (Oliver *et al.*, 1994) which plays a critical role in the regulation of immune and inflammatory responses of hematopoietic cells(Peters *et al.*, 1996; Sada *et al.*, 2001) and in maintaining vascular integrity (Abtahian *et al.*, 2003) in addition to playing the general physiological functions in a wide variety of non-hematopoietic cells (Yanagi *et al.*, 2001). It was found that piceatannol possesses multiple bioactivities such as anti-cancer (Ferrigni *et al.*, 1984; Potter *et al.*, 2002; Aggarwal *et al.*, 2006), anti-Epstein-Barr virus (Cooper and Longnecker, 2002), and cardioprotection associated with antiarrhythmia against ischaemia-reperfusion injury in rat hearts (Hung *et al.*, 2001).

Reperfusion is associated with potentially lethal arrhythmias that are rapidly and predictably induced within seconds

Correspondence: Ming-Jai Su, Institute of Pharmacology, College of Medicine, National Taiwan University, No. 1, Sec. 1, Jen-Ai Road, Taipei 100, Taiwan. E-mail: mingja@ntu.edu.tw
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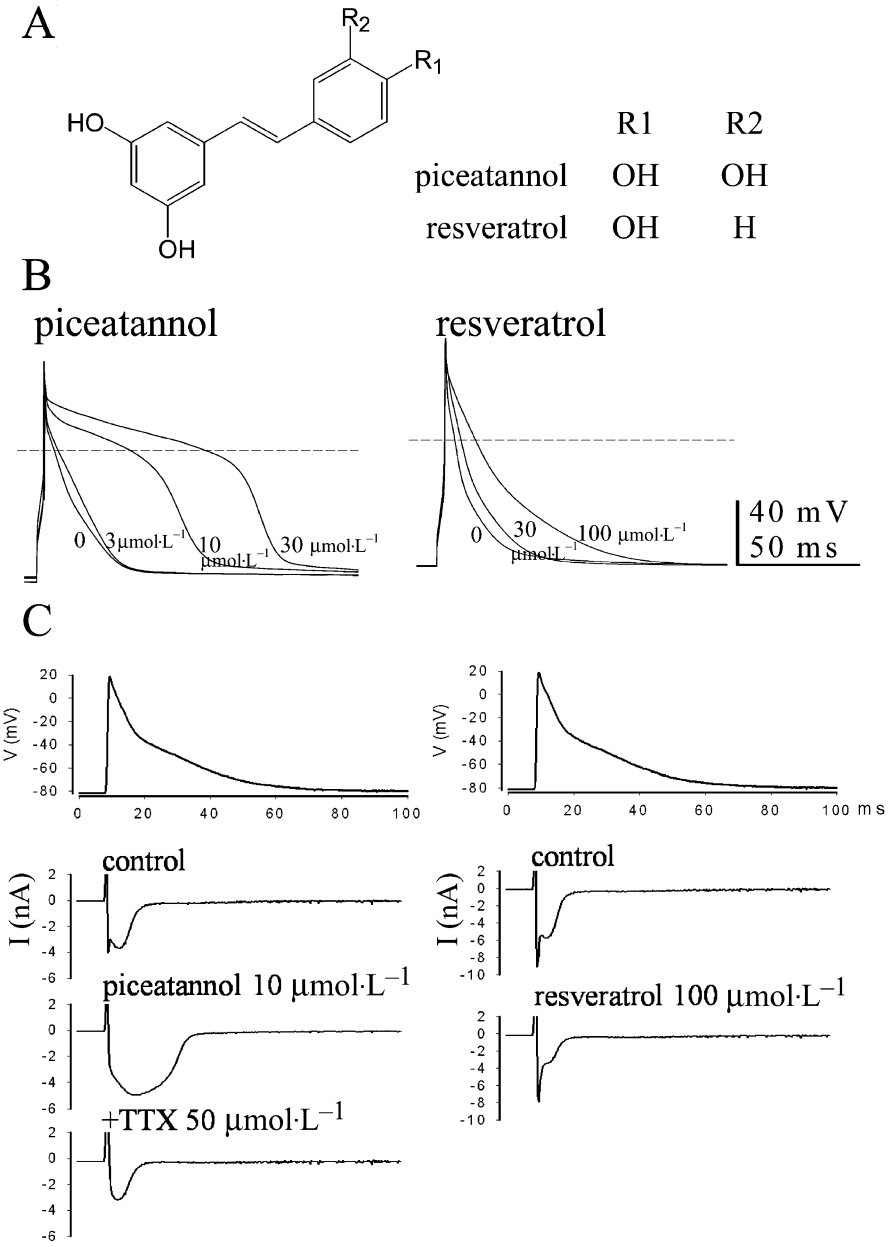

Figure 1 Comparison of (A) the structures between piceatannol and resveratrol and the different response to both polyphenols in (B) the APs and (C) the action-potential-clamp-elicited inward currents in rat ventricular cells. (B) The superimposed APs were elicited at 1 Hz in current clamp mode, and the steady-state recordings in the absence or presence of the cumulatively increasing doses of either piceatannol or resveratrol were shown. The dash lines indicate the membrane potential at zero. (C) The AP contour of rat papillary muscle stimulated at 1 Hz was employed as the voltage protocol to record the inward currents affected by either piceatannol or resveratrol. Cs^+ plus TEA-containing pipette internal solution was used to block potassium currents in addition to the presence of 10 mmol·L^{-1} Cs^+ in the bath solution.

of the onset of reflow (Manning *et al.*, 1984). Pretreatment of antioxidants or free radical scavengers can afford potential protection against ischaemia-induced or reperfusion-induced arrhythmias in experimental animals (Manning *et al.*, 1984; Bernier *et al.*, 1986). Piceatannol possesses an additional hydroxyl group on resveratrol (3,5,4′-trihydroxystilbene) as shown in Figure 1A and exerts higher radical scavenging activity (Fauconneau *et al.*, 1997) which was considered to contribute to the cardioprotective and antiarrhythmic effects in ischaemic and ischaemic-reperfused rat heart (Hung *et al.*, 2001). Our previous study found that resveratrol possesses weak cardiac ion channel blocking action which is in parallel with its less potent antiarrhythmic action in isolated rat hearts subjected to ischaemia-reperfusion injury (Chen *et al.*, 2007). It is unclear whether piceatannol can directly modulate cardiac ion channels and the relevance to its antiarrhythmic efficacy. Besides that, the overall health benefit of polyphenolic compounds, such as piceatannol, containing in our daily diets or fortified supplement, remains to be ascertained by clinical study (Halliwell, 2007) and the cardiac safety of piceatannol yet remains to be established. Therefore, the main objective of the present work is to characterize the

action of piceatannol on cardiac ion channels which could contribute to its antiarrhythmic activity or to raise the caution for the propensity to cause proarrhythmia. Although the expression of either *erg* mRNA or I_{Kr} channel is found in rat hearts (Wymore *et al*., 1997; Jones *et al*., 2004), the current amplitude is small in rat ventricular myocytes (Wymore *et al*., 1997). Hence, we examined the effect of piceatannol on I_{Kr} in HEK 293 cells transfected with hERG gene encoded I_{Kr} channel to evaluate the cardiac safety.

Methods

Ischaemia-reperfusion induced arrhythmia in isolated rat heart

Male Sprague-Dawley rats (250–300 g body weight, purchased from the Laboratory Animal Center, NTUMC) were intraperitoneally anaesthetized with pentobarbital (50 mg kg^{-1}) plus heparin (300 U kg^{-1}). The research was granted by National Taiwan University IACUC (approval No.:20070004), and was conducted in accordance with the Guide for the Care and Use of Laboratory Animals by US NIH. The rat hearts were excised and mounted immediately on the Langendorff apparatus, and retrogradely perfused via the aorta at a constant pressure (80 mm Hg) with 95% O_2 and 5% CO_2 gased Tyrode solution containing (mmol·L^{-1}) NaCl 137.0, KCl 5.4, $MgCl_2$ 1.22, $CaCl_2$ 1.8, $NaHCO_3$ 11.9, NaH_2PO_4 0.33, glucose 11.0, pH 7.4, at 37°C. After a stabilization period, the hearts were subjected to ligation of the left anterior descending (LAD) coronary artery for 20 min followed by reperfusion. The cardiac rhythm was recorded on a chart recorder (RS 3200, Gould Inc., Cleveland, OH, USA) during the ischaemia/reperfusion period. The polymorphic ventricular tachyarrhythmia (PVT) was defined as a run of three or more consecutive ventricular premature contractions. Reperfusion-induced PVT occurred after 20 min LAD occlusion. Once I-R-induced arrhythmia appeared, piceatannol (at the concentrations of 1, 3 and 10 μmol·L^{-1}) or resveratrol (at the concentrations of 3, 10, 30 and 100 μmol·L^{-1}) was administered respectively. No new tachyarrhythmia observed after drug administration was defined as a successful antiarrhythmic case.

Isolation of single ventricular myocytes

Single ventricular myocytes were enzymatically isolated from heart of adult male Sprague-Dawley rats weighing 250–300 g. Briefly, hearts were quickly removed from pentobarbital-anaesthetized rats, and mounted via the aorta on a Langendorff retrograde perfusion apparatus. Hearts were initially perfused with oxygenated Ca^{2+}-free HEPES solution [containing (mmol·L^{-1}) NaCl 137.0, KCl 5.4, KH_2PO_4 1.2, $MgCl_2$ 1.22, glucose 10, and HEPES 10, pH 7.4], and followed by the same solution containing 0.3 mg mL^{-1} collagenase (type II, Sigma) and 0.1 mg mL^{-1} protease (type XIV, Sigma). After 5–10 min digestion, enzymes were washed out with HEPES solution containing 0.05 mmol·L^{-1} Ca^{2+} for 5 min. The ventricles were separated and cut into small pieces, which were resuspended under gentle mechanical agitation and stored in HEPES solution containing 0.2 mmol·L^{-1} Ca^{2+} at room temperature.

Electrophysiological recordings

A droplet of the cell suspension was placed to a chamber (1 mL) mounted on the stage of an inverted microscope (Nikon, Diaphot, Japan). Cells were in 1.8 mmol·L^{-1} Ca^{2+}-containing HEPES-buffer. Membrane potentials and currents were recorded at room temperature by using a patch-clamp amplifier (WPC-100, E.S.F. Electronic, Göttingen, Germany) via a digital-to-analog converter (Digidata 1322, Axon Instruments) controlled by pClamp software. Patch electrodes were made from borosilicate glass (WPI, Sarasota, Fla., USA). For recording of action potential (AP), I_{to}, I_{Kr} and I_{K1}, the pipette was filled with the internal solution containing (mmol·L^{-1}): KCl 130, NaCl 10, Mg-ATP 5, $MgCl_2$ 2, EGTA 11, $CaCl_2$ 1, HEPES 10; pH was adjusted to 7.2 using KOH. For the measurement of I_{Na} and I_{Ca}, CsCl (130 mmol·L^{-1}) was used to replace KCl and TEA.Cl (15 mmol·L^{-1}) was added to block potassium currents in addition to adjusting pH 7.2 by CsOH. CsCl (10 mmol·L^{-1}) was always present in bath solution to block I_{K1}. $CoCl_2$ (1 mmol·L^{-1}) or $CdCl_2$ (50 μmol·L^{-1}) was used to block $I_{Ca,L}$. The general access resistance (Ra) was about 2–3 MΩ in most of our experimental condition. The series resistance was electronically compensated by 60–80% to reduce the voltage-clamp error. The voltage-drop error was reduced to be 0.8 ± 0.2 mV in $I_{Ca,L}$ measurement. In order to increase the clamp efficiency during measurement of I_{Na}, the external concentration of Na^+ was lowered to 40 mmol·L^{-1} using *N*-methyl-D-glucamine to replace Na^+ to decrease the maximum current amplitude of I_{Na} within 10 nA and the low resistance electrodes (Ra ≈ 1 MΩ) were used. Therefore, the voltage-drop error was reduced to be 3.7 ± 0.3 mV after electronical compensation. For recording the currents elicited by the AP clamp, the AP contour of rat papillary muscle, which was acquired by intracellular recording in the isolated papillary muscle horizontally fixed one end at bottom and hooked the other end to force transducer in a chamber (1.5 mL) perfused with normal Tyrode solution (37°C, gased with 95% O_2 and 5% CO_2) at the rate of 10 mL min^{-1} under isometric condition with a preload of 1 g to concomitantly measure the electrically paced contractile force at 1 Hz by Gould recorder and the APs by puncturing the microelectrode (filled with 3 M KCl, electrode resistance: 30 MΩ) into muscle to record APs by pClamp software via Axon clamp 2B amplifier in bridge mode, was employed as the voltage protocol to record the AP-clamp-elicited currents.

Measurement of intracellular Ca^{2+} transients

Isolated rat ventricular cells were loaded with fluo-3 by incubating with 0.5 mmol·L^{-1} Ca^{2+}-containing HEPES-buffer containing 5 μmol·L^{-1} fluo-3/AM and Pluronic F-127 (2%) for 30 min at room temperature. After washing out the excess fluo-3/AM, cells were then transferred to 1.8 mmol·L^{-1} Ca^{2+}-containing HEPES-buffer for another 30 min before beginning the experiments. Cells were electrically stimulated by 2 ms and twice-threshold pulses at 1 Hz. Fluorescent changes were detected and acquired by a Zeiss LSM-510 laser scanning confocal system (Carl Zeiss, MicroImaging GmbH, Germany) in line-scan mode. Fluo-3 was excited with a 488 nm argon laser, and fluorescence emission was measured using a 505–550 nm band-pass filter acquired by image acquisition system. The

intracellular Ca^{2+} transients are reported as F/F_0, where F is the fluorescence signal and F_0 is the resting fluorescence recorded at the start of the experiment.

Transfection of hERG in HEK293T cells

The cloned hERG-pEGFP-N2 vector was a gift from Dr L.P. Lai's laboratory. Lipofatamine 2000 (Invitrogen) was used as the reagent for transient transfection. HEK293T cells (4×10^5) were seeded into a 35 mm dish and grown in DMEM supplemented with 10% FBS and antibiotics at 37°C and 5% CO_2 the day before transfection. Onto the cell monolayer were added 2.5 μg plasmid and 5 μL Lipofatamine 2000 in 1 mL Opti-MEM (Invitrogen). The cells were trypsinized for patch clamp 48 h after transfection.

Drugs

Piceatannol and resveratrol were purchased from Sigma Chemicals, and were dissolved in DMSO.

Data analysis and statistics

Dose-response curves were fit by the Hill equation:

$$E/E_{max} = 1/\left[1 + (D/K_D)^n\right] \quad (1)$$

where D is the drug concentration, K_D is the concentration of drug for half-maximum effect and n is Hill's coefficient.

The voltage-dependent steady-state inactivation curves and activation curves of I_{Na} and $I_{Ca,L}$ were fitted by the Boltzmann equation:

$$I/I_{max} = 1/\{1 + \exp[(V_{0.5} - V_m)/s]\} \quad (2)$$

where V_m is the conditioning potential, $V_{0.5}$ is the potential of half inactivation, and *s* is the slope factor.

Data are presented as mean ± SEM. Two-tailed paired Student's *t*-test was taken to indicate statistical significance in data before and after drug treatment at the same preparation. Comparisons among groups were performed by one-way ANOVA with Duncan's new multiple range test. The effective antiarrhythmic concentrations of either resveratrol or piceatannol were determined by Yates' corrected chi-square test (two-sided).

Results

Effect of piceatannol on the APs and inward currents

Figure 1B compares the different potency in APD prolongation between piceatannol and resveratrol at the concentrations of 3–100 μmol·L^{-1} in rat ventricular cells stimulated at 1 Hz. Piceatannol markedly prolonged APDs in association with the decrease of V_{max} of AP in concentration-dependent manner (Table 1). Resveratrol significantly prolonged APDs at the concentration of 100 μmol·L^{-1} (APD_{90}: 19.9 ± 2.8 ms in control, n = 6; 50.2 ± 6.2 ms in resveratrol 100 μmol·L^{-1}, n = 6; $P < 0.05$).

Figure 1C shows the effect of piceatannol on AP-clamp-elicited inward currents and compares with that of resveratrol under the condition of blocking potassium channels. Piceatannol (10 μmol·L^{-1}) slightly inhibited the initial sodium current in concomitant with the pronounced increase of the late component of inward currents which was blocked by 50 μmol·L^{-1} tetrodotoxin, but not by calcium channel blocker nifedipine (3 μmol·L^{-1}, data not shown). However, resveratrol only inhibited both components of inward currents at the concentration of 100 μmol·L^{-1}.

Table 1 Action potential parameters of rat ventricular cells before and after exposure to cumulative increase of three concentrations of piceatannol at 1 Hz stimulation.

Piceatannol (μmol·L^{-1})	*RMP (mV)*	*V_{max} (v/s)*	*APD_{50} (ms)*	*APD_{90} (ms)*
0	–80.3 ± 1.0	337.5 ± 21.8	6.9 ± 1.5	16.6 ± 1.8
3	–80.2 ± 1.4	318.5 ± 22.0	15.4 ± 4.9	27.8 ± 6.5
10	–82.3 ± 1.7	242.5 ± 31.2*	53.0 ± 16.5*	63.7 ± 17.1*
30	–81.7 ± 1.4	161.3 ± 18.8*	82.6 ± 15.3*	95.8 ± 14.9*

Data are expressed as mean ± s.e.m. from different 6 cells. Asterisk indicates statistical significance ($P < 0.05$) as compared between control and drug-treated groups by paired t-test. RMP: resting membrane potential; V_{max}: the maximum upstroke velocity of action potential; APD_{50} and APD_{90}: action potential duration measured at 50 and 90% repolarization.

Effects of calcium channel and sodium channel blockers on piceatannol modified APs and inward currents

Ventricular AP (Figure 2A) and the AP-clamp-elicited currents (Figure 2B) were examined at the same cell by alternatively switching between current-clamp and voltage-clamp modes to acquire the respective signals. In the presence of Cd^{2+} (50 μmol·L^{-1}) to block $I_{Ca,L}$, APDs were shortened (Figure 2A-b) and the outward currents appeared in association with the suppression of inward currents (Figure 2B-b). In the presence of Cd^{2+}, piceatannol also prolonged the APs (Figure 2A-c) and induced an inward current (Figure 2B-c), which was markedly reversed by lidocaine 20 μmol·L^{-1} (Figure 2A-d,B-d).

Comparison of the different effect of piceatannol and resveratrol on cardiac sodium channel

Piceatannol dose-dependently inhibited the peak amplitude of I_{Na} in association with slowing I_{Na} inactivation, rather than produced a persistent component of I_{Na}, as shown in the left panel of Figure 3A. The inactivation rate of I_{Na} before and after exposure to piceatannol 10 μmol·L^{-1} was analysed in currents elicited by a long pulse from holding potential –80 mV to 0 mV for 100 ms, and $I_{Na,at\ 0\ mV}$ inactivation was fitted with a two exponential equation. The decay constants of fast and slow components of $I_{Na,at\ 0\ mV}$ were τ_{fast} = 0.8 ± 0.1 ms and τ_{slow} = 15.3 ± 0.1 ms in control (n = 4), and were τ_{fast} = 0.9 ± 0.1 ms and τ_{slow} = 40.1 ± 2.0 ms ($P < 0.05$ vs. control, n = 4) in the presence of piceatannol 10 μmol·L^{-1} respectively. The effect of resveratrol on I_{Na} was different from that of piceatannol as shown in the right panel of Figure 3A. Resveratrol inhibited I_{Na} without altering I_{Na} inactivation. The dose-response curves of piceatannol and resveratrol in I_{Na} inhibition are plotted in Figure 3B. IC_{50} and Hill's coefficient of I_{Na} inhibition at –30 mV of piceatannol were 19.5 ± 0.4 and 1.4 ± 0.1 μmol·L^{-1} (n = 6), and those of resveratrol were 83.3 ± 1.3 μmol·L^{-1} (n =

6, $P < 0.05$ vs. piceatannol) and 1.4 ± 0.1 μmol·L^{-1} ($n = 6$) respectively. Figure 3C shows that piceatannol significantly shifted the voltage-dependent steady-state inactivation curve of I_{Na} to more negative potential at the concentration of 30 μmol·L^{-1} without altering the I_{Na} activation threshold. The $V_{0.5}$ and the slope factor (s) of voltage-dependent steady-state inactivation of I_{Na} were –73.2 ± 0.4 and –7.5 ± 0.3 mV ($n = 6$) in control, –74.7 ± 1.6 and –9.6 ± 1.4 mV ($n = 6$) in piceatannol 10 μmol·L^{-1}, and –94.5 ± 1.7 mV ($n = 6$, $P < 0.05$ vs. control) and –11.9 ± 1.4 mV ($n = 6$) in piceatannol 30 μmol·L^{-1}. The $V_{0.5}$ and the slope factor (s) of I_{Na} activation curves were not significantly altered in the absence and presence of piceatannol [–45.0 ± 0.5 and 4.9 ± 0.4 mV in control ($n = 6$), –44.4 ± 0.8 and 3.8 ± 0.6 mV in piceatannol 10 μmol·L^{-1} ($n = 6$), and –42.0 ± 0.5 and 4.0 ± 0.4 mV in piceatannol 30 μmol·L^{-1} ($n = 6$)].

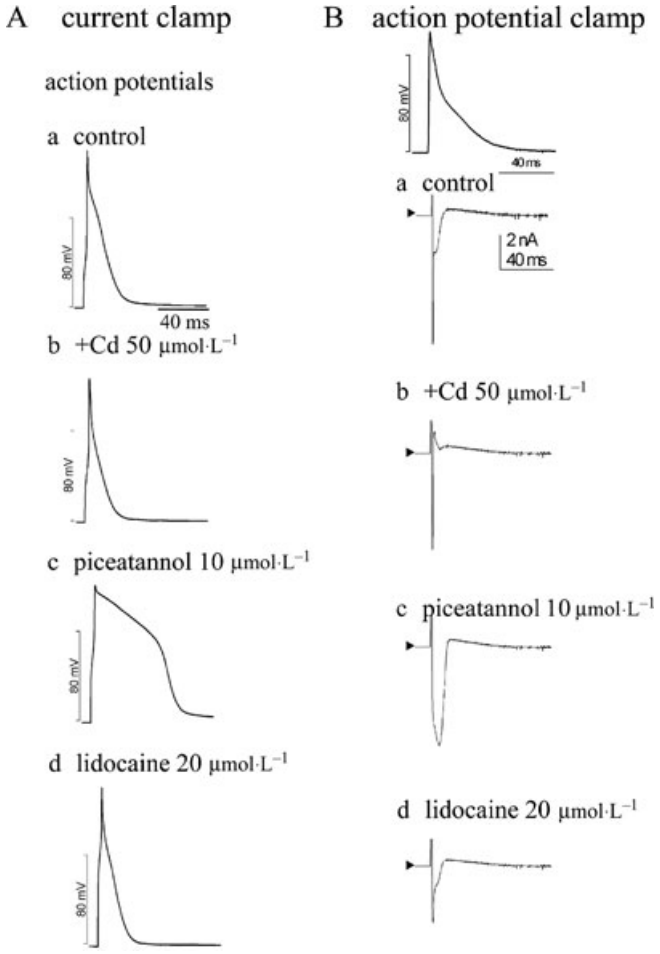

Figure 2 Effects of calcium channel and sodium channel inhibitors in piceatannol-produced modulation in (A) APs recorded in current mode and (B) the AP-clamp-elicited inward currents at the same cell under the conditions of using the normal K^+-containing pipette internal and bath solutions. The AP contour of rat papillary muscle stimulated at 1 Hz was employed as the voltage protocol in AP-clamp study. Cd^{2+} (50 μmol·L^{-1}) was used to block L-type calcium channel, and lidocaine (20 μmol·L^{-1}) to modulate the inactivation state of sodium channel. In the presence of Cd^{2+}, piceatannol can prolong APDs (A-b) associated with increasing inward current (B-b), which can be reversed by lidocaine (A-d and B-d).

Effect of piceatannol on aconitine-modulated sodium current

Aconitine could activate a persistent I_{Na} which resulted in an inward shift of holding membrane current (Figure 3D). The activation of a persistent I_{Na} was associated with a negative shift of the voltage-dependent activation curve from $V_{0.5}$ = –45.0 ± 0.5 mV ($n = 6$) in control to $V_{0.5}$ = –63.9 ± 0.4 mV ($n = 6$) in the presence of 3 μmol·L^{-1} aconitine (Figure 3E). The slope factor of I_{Na} activation curve was $s = 4.9 \pm 0.4$ ($n = 6$) in control and $s = 3.8 \pm 0.3$ in the presence of 3 μmol·L^{-1} aconitine. $V_{0.5}$ and the slope factor of voltage-dependent steady-state inactivation curve of I_{Na} was unaffected by 3 μmol·L^{-1} aconitine (control: $V_{0.5}$ = –73.2 ± 0.4 mV, $s = 7.5 \pm 0.3$, $n = 6$; aconitine 3 μmol·L^{-1}: $V_{0.5}$ = –73.1 ± 0.4 mV, $s = 7.4 \pm 0.3$, $n = 6$). Addition of piceatannol (10 μmol·L^{-1}) to aconitine-treated cells inhibited the amplitude of I_{Na} but increased the slowing inactivation component of I_{Na} as shown in the right panel of Figure 3D. I_{Na} activation curve of aconitine-treated cells was not altered by further addition of piceatannol ($V_{0.5}$ = –63.9 ± 0.4 mV, $s = 3.8 \pm 0.3$, $n = 6$), but $V_{0.5}$ of voltage-dependent steady-state inactivation of I_{Na} was shifted to –97.2 ± 0.2 mV ($P < 0.05$ vs. aconitine 3 μmol·L^{-1}, $n = 6$). *S* value remained at 7.1 ± 0.2 ($n = 6$).

Effect of piceatannol and resveratrol on L-type calcium current

Piceatannol inhibited $I_{Ca,L}$ at the concentrations of 30 μmol·L^{-1} (Figure 4A,B) in association with the right shift of $I_{Ca,L}$ activation curve (Figure 4C). The parameters of $I_{Ca,L}$ activation curves were $V_{0.5}$ = –20.3 ± 0.2 mV and $s = 4.8 \pm 0.2$ ($n = 6$) in control, $V_{0.5}$ = –15.3 ± 0.7 mV ($P < 0.05$ vs. control, $n = 6$) and $s = 5.3 \pm 0.6$ ($n = 6$) in piceatannol 30 μmol·L^{-1}, and $V_{0.5}$ = –14.6 ± 0.7 mV ($P < 0.05$ vs. control, $n = 6$) and $s = 5.3 \pm 0.6$ ($n = 6$) in piceatannol 100 μmol·L^{-1}. Figure 4D compares the dose-dependent inhibition of $I_{Ca,L}$ at 0 mV by piceatannol and resveratrol. The IC_{50} and Hill's coefficient of piceatannol were 137.9 ± 9.4 and 1.7 ± 0.2 μmol·L^{-1} ($n = 6$), and those of resveratrol were 121.1 ± 5.6 and 1.9 ± 0.2 μmol·L^{-1} ($n = 6$) respectively. There was no significant difference in the IC_{50} values between piceatannol and resveratrol.

Effect of piceatannol on potassium channels

Figure 5A shows that piceatannol inhibited transient outward current (I_{to}) and the slow inactivation component of the outward currents (I_{Kss}) in rat ventricular cells at the concentrations of 30 μmol·L^{-1}. I_{K1} was not significantly altered by piceatannol even at the concentration of 100 μmol·L^{-1}. The I-V curves of either peak I_{to} (I_{peak}) or I_{Kss} at 300 ms (I_{ss}) in response to the cumulative concentrations of piceatannol were plotted in the lower panel of Figure 5A, and the concentration-dependent inhibition curves in the upper right panel of Figure 5A. IC_{50} and Hill's coefficient of piceatannol were 44.1 ± 2.0 and 1.1 ± 0.1 μmol·L^{-1} ($n = 6$) in peak I_{to} inhibition and 118.7 ± 3.2 and 0.8 ± 0.1 μmol·L^{-1} ($n = 6$) in I_{Kss} inhibition, and those of resveratrol were 79.1 ± 0.1 and 1.2 ± 0.1 μmol·L^{-1} ($n = 6$) in peak I_{to} inhibition and 167.9 ± 0.1 and 0.9 ± 0.1 μmol·L^{-1} ($n = 6$) in I_{Kss} inhibition.

The effect of piceatannol on I_{Kr} was examined in HEK293T cells transfected with human ether-a-go-go-related gene (hERG) encoding the alpha-subunit of I_{Kr}. The typical current traces and the concentration-response curve of I_{Kr} inhibition

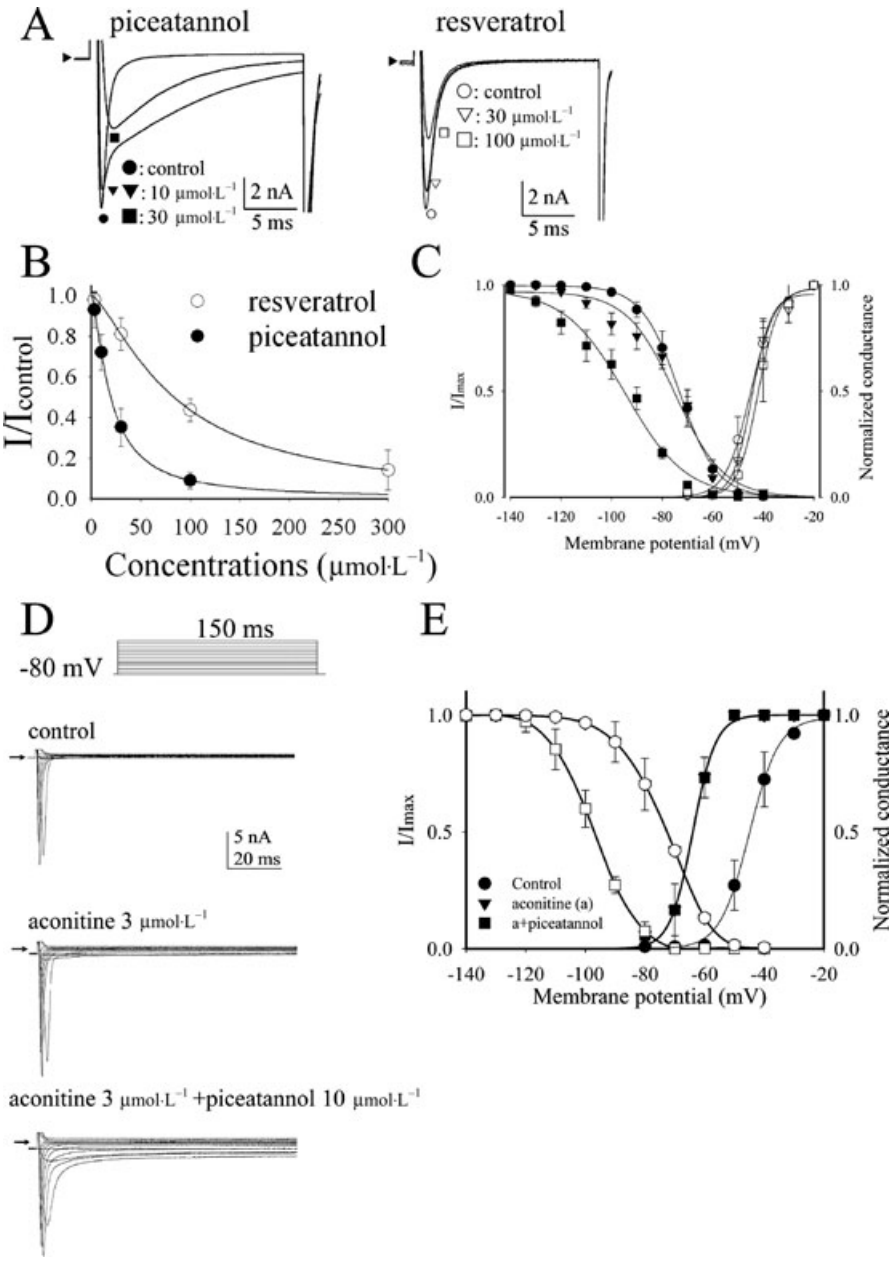

Figure 3 (A) Comparison of the different effect of piceatannol and resveratrol in rat cardiac sodium current elicited by a 20 ms step pulse form holding potential –80 mV to –10 mV. (B) The dose-response curves of I_{Na} inhibition by piceatannol and resveratrol. The curves are fitted with Hill equation (1). (C) The voltage-dependent steady-state inactivation and activation curves of sodium channel before and after exposure to piceatannol are plotted and the respective curves are fitted with Boltzman equations (2). Data are shown in mean ± SEM (n = 6). (D) Effect of piceatannol in aconitine-modulated sodium current. For measurement of the activation curves of I_{Na}, the membrane potential was held on –80 mV, and sodium currents were elicited by a family of depolarizing pulses for 150 ms from –70 mV stepped to +40 mV by 10 mV every step once per 5 s. The typical sodium current traces in response to aconitine (3 μmol·L^{-1}) in the absence and presence of piceatannol (10 μmol·L^{-1}). The arrowheads indicate the current level at zero. (E) For measurement of the steady-state inactivation curves of I_{Na}, I_{Na} was elicited by stepping to 0 mV for 20 ms from different holding potentials ranging from –140 mV to –40 mV increased by 10 mV every 10 s. The steady-state inactivation and activation curves of sodium channel in the conditions of control, aconitine (A) 3 μmol·L^{-1}, and aconitine plus piceatannol 10 μmol·L^{-1}. The curves are fitted with Boltzman equations (2).

by piceatannol was plotted in Figure 5B. IC_{50} and Hill's coefficient were 28.6 ± 1.1 and 1.0 ± 0.3 μmol·L^{-1} (n = 6).

Effect of piceatannol on electrical paced and caffeine-induced Ca^{2+} transients

The upper panel of Figure 6A shows the typical intracellular Ca^{2+} transients of ventricular cell paced at 1 Hz by field stimulation before and after applying piceatannol at the concentrations of 10 and 30 μmol·L^{-1}. From analysis of the averaged area under Ca^{2+} transients from consecutive five beatings in the steady-state condition of control or 5 min after exposure to piceatannol, the total cytosolic Ca^{2+} presented during every beating was significantly decreased by piceatannol 30 μmol·L^{-1} (the lower left panel of Figure 6A). The decay rate of intracellular Ca^{2+} transients was slowed by 22.5 ± 3.2% and

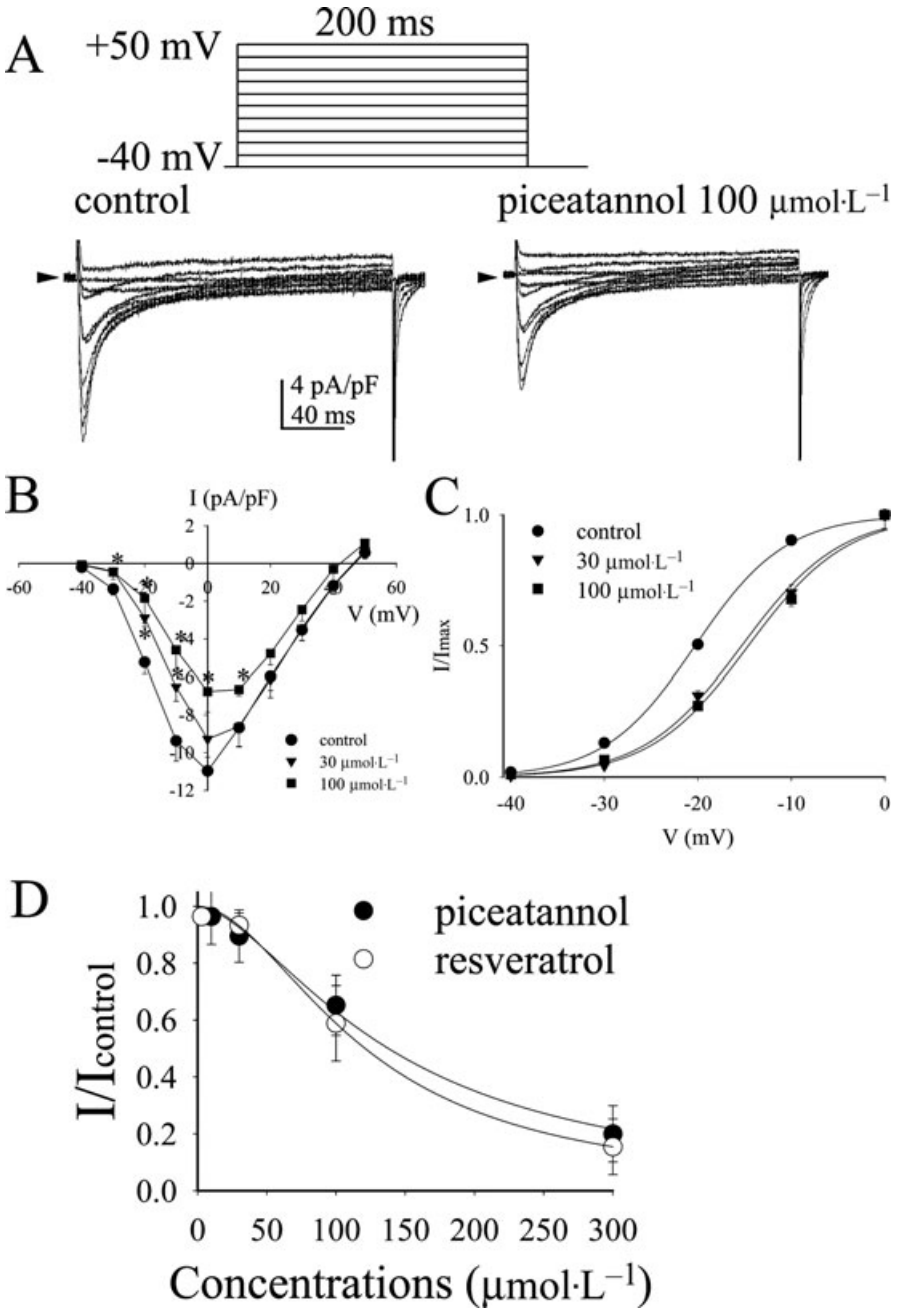

Figure 4 (A) Effect of piceatannol on L-type calcium current of rat ventricular cells. The typical current traces of a family of depolarizing pulses from the holding potential –40 mV to +50 mV by 10 mV every step for 200 ms in the absence and presence of piceatannol 100 μmol·L^{-1}. (B) The I-V curves and (C) the activation curves of L-type calcium channel in response to piceatannol at the concentrations of 30 μmol·L^{-1} and 100 μmol·L^{-1}. Data are shown as mean ± SEM (n = 6). *P < 0.05 (n = 6) versus control group by paired t-test. (D) Comparison of the dose-dependent inhibition of $I_{Ca,L}$ at 0 mV by piceatannol and resveratrol. The curves are fitted with Hill equation (1).

58.0 ± 11.4% in the presence of piceatannol 10 and 30 μmol·L^{-1} respectively (the right lower panel of Figure 6A). The effect of piceatannol on Na^+-Ca^{2+} exchanger was examined in resting cells applying caffeine (10 mmol·L^{-1}) to release intracellular Ca^{2+} from SR as shown in Figure 6B. Ni^{2+} (3 mmol·L^{-1}), a Na^+-Ca^{2+} exchanger inhibitor, markedly retarded the decline rate of caffeine-induced Ca^{2+} transient, which indicates Na^+-Ca^{2+} exchanger is a major contributor to diminish the elevated cytosolic Ca^{2+}. Piceatannol 30 μmol·L^{-1} also slowed the decline rate of caffeine-induced Ca^{2+} transient.

Comparison of the antiarrhythmic efficacy of piceatannol and resveratrol in ischaemia-reperfused rat hearts

Table 2 shows that reperfusion-induced PVT occurred in all animals of vehicle (DMSO 0.1%) group after 20 min LAD occlusion, and either piceatannol or resveratrol could convert reperfusion-induced PVT to normal sinus rhythm in concentration-dependent manner. Furthermore, PVT was completely abolished by 10 μmol·L^{-1} of piceatannol. The antiarrhythmic IC_{50} and Hill's coefficient of piceatannol were 3.1 ± 0.1 and 2.1 ± 1.1 μmol·L^{-1}, and those of resveratrol were

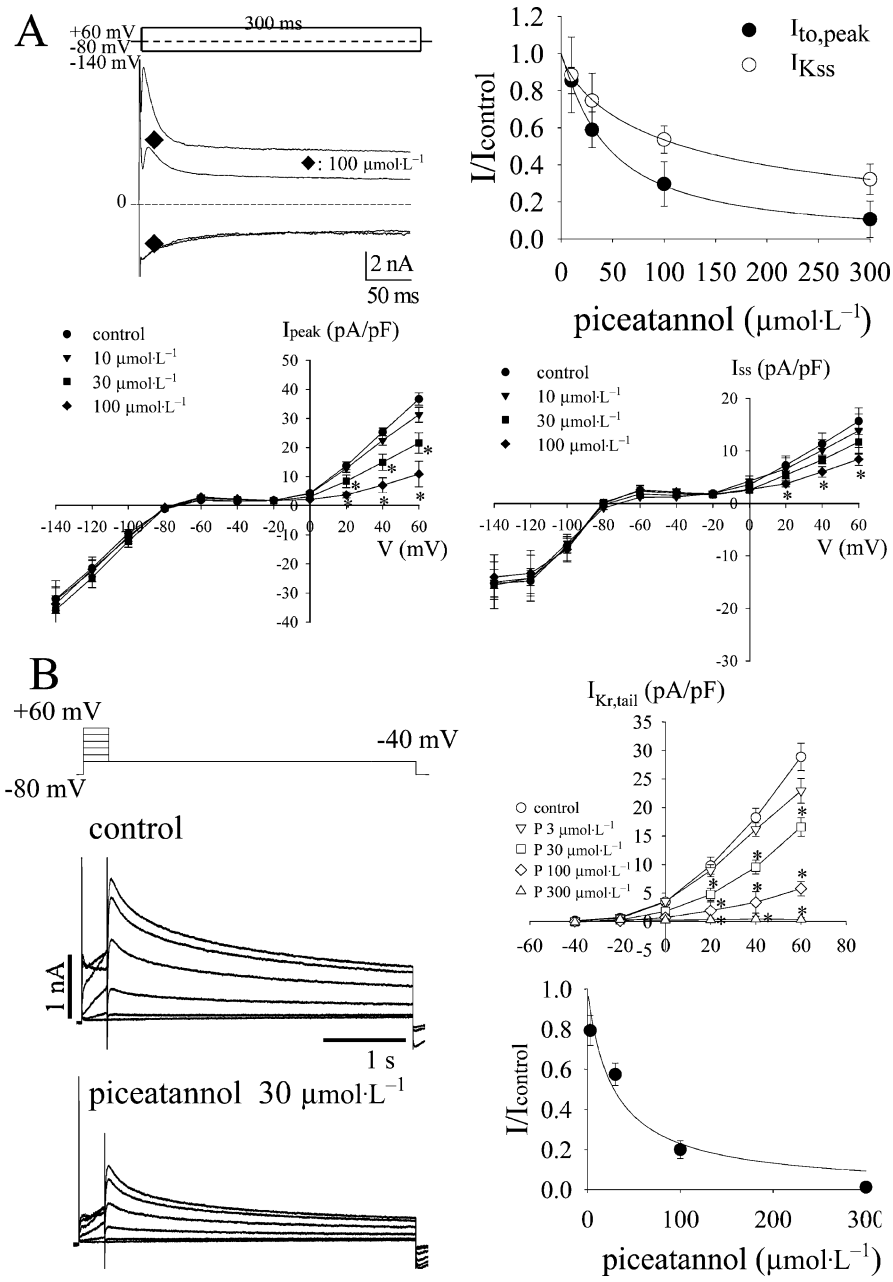

Figure 5 (A) Effect of piceatannol on potassium channels of rat ventricular cells. Co^{2+} (1 mmol·L^{-1}) was always present in the bath to block $I_{Ca,L}$. The left top shows the typical current traces of transient outward currents (I_{to}) followed by a sustained outward currents and the inwardly rectifying potassium current (I_{K1}) in the absence and presence of piceatannol at 100 μmol·L^{-1}. The membrane potential was held at –80 mV, and was either depolarized to +60 mV to activate the transient outward currents or hyperpolarized to –140 mV to elicit I_{K1} for 300 ms. The lower panael of (A) shows the I-V curves of the current density at peak (I_{peak}) and at the end of 300 ms (I_{ss}) in the absence and presence of piceatannol at the concentrations of 10, 30 and 100 μmol·L^{-1}. The outward currents were elicited by a family of depolarization steps from –60 mV to +60 mV increased by 10 mV every step, and a 20 ms depolarization pre-pulse was preceded to eliminate the contamination of sodium current. Data are shown in mean ± SEM ($n = 6$), and the asterisks indicate the statistical significance between the currents of control and drug-treated groups elicited at the same voltage level by one-way ANOVA with Duncan's new multiple range test. The right top shows the dose-dependent inhibition of I_{peak} and I_{ss} by piceatannol. The curves are fitted by the Hill equation (1). (B) Effect of piceatannol on I_{Kr} in HEK293T cells transfected with hERG encoding the alpha-subunit of I_{Kr}. I_{Kr} was elicited by a family of depolarization steps increased every 20 mV for 300 ms from holding potential at –80 mV, and followed by the repolarization at –40 mV to record the tail currents. The right top of (B) plots the I-V curves constructed by the peak amplitude of the tail currents elicited at the respective depolarization steps. Data are shown in mean ± SEM ($n = 6$), and the asterisks indicate the statistical significance between the currents of control and drug-treated groups elicited at the same voltage level by one-way ANOVA with Duncan's new multiple range test. The right bottom of (B) shows the dose-dependent inhibition of $I_{Kr,tail}$ elicited at +60 mV by piceatannol. The curve is fitted with the Hill equation (1).

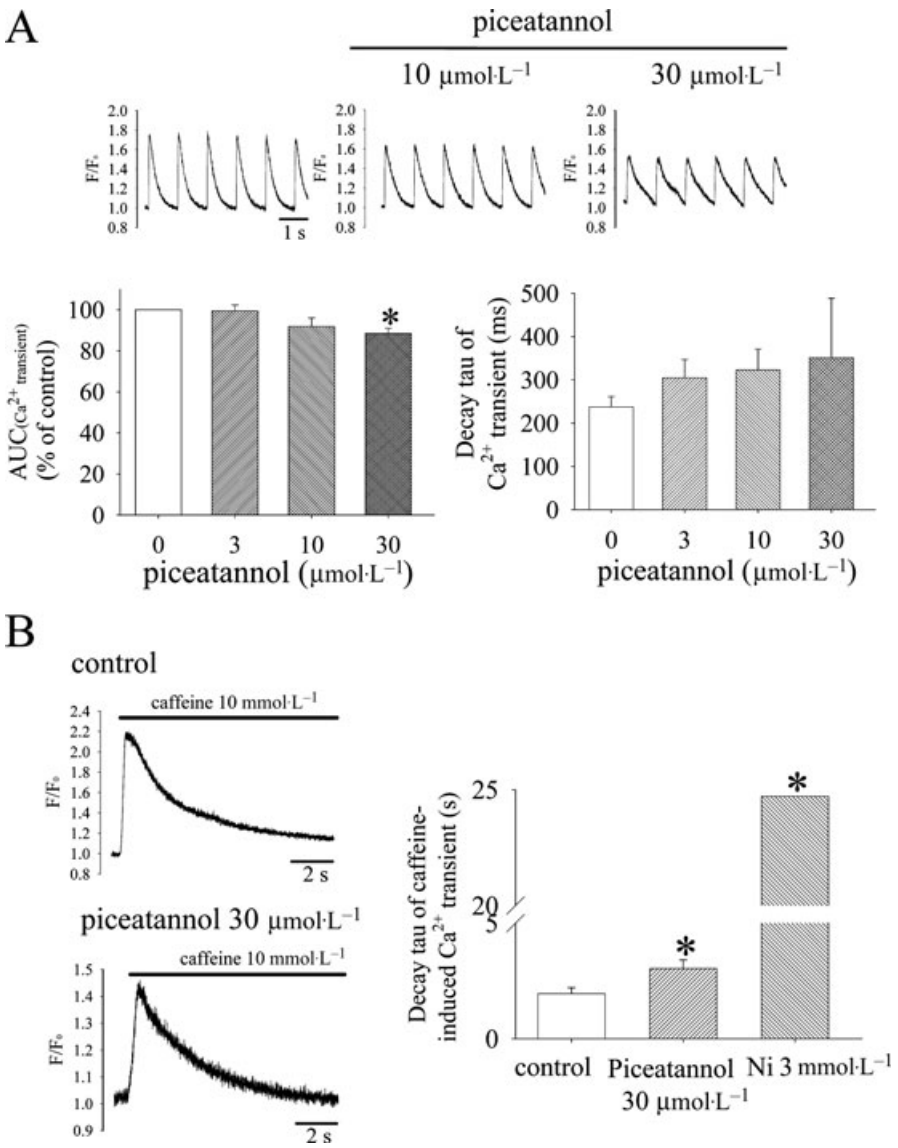

Figure 6 (A) Effect of piceatannol in the intracellular Ca^{2+} transients of electrical paced rat ventricular cells and the decay rate of caffeine-induced Ca^{2+} transients. The area under Ca^{2+} transient (AUC) was integrated and averaged from consecutive five beatings in the steady-state condition of control or after applying piceatannol for 5 min from 10 different cells. The decay rate of Ca^{2+} transient was analysed by simple one-exponential equation ($n = 10$) as shown in the right bottom of (A). (B) Caffeine 10 mmol·L^{-1} was applied to elicit intracellular Ca^{2+} release in quiet cells which previously had electric pacing to a steady state in the absence and presence of piceatannol as shown in the left panel of (B). The decay rate of caffeine-released intracellular Ca^{2+} was also analysed by simple one-exponential equation. $NiCl_2$ (3 mmol·L^{-1}) was used to block Na^+-Ca^{2+} exchange which markedly prolonged the decay time of caffeine-elicited Ca^{2+} transient as shown the measured decay tau in the right panel of (B). Piceatannol 30 μmol·L^{-1} significantly slowed the decay rate. Data are shown as mean ± SEM ($n = 6$). *$P < 0.05$ versus control group by one-way ANOVA with Duncan's new multiple range test.

33.7 ± 8.6 and 0.8 ± 0.2 μmol·L^{-1}. The antiarrhythmic efficacy of piceatannol was more potent than that of resveratrol.

Discussion and conclusions

Polyphenolic antioxidants of red wine, including resveratrol and piceatannol, are thought to be responsible for the cardiovascular benefits associated with moderate wine consumption (Das *et al.*, 1999). Piceatannol possesses an additional hydroxyl group in the 3′ position of resveratrol, which makes piceatannol exerting the unique action to moderately slow I_{Na} inactivation, rather than to induce a persistent non-inactivation I_{Na}, in association with the decrease of peak I_{Na} at the concentration of 3 μmol·L^{-1}. The potency of cardiac ion channel modulation by piceatannol is in the order of $I_{Na} > I_{Kr} > I_{to} > I_{Kss} > I_{Ca,L}$. At the antiarrhythmic concentrations within 10 μmol·L^{-1}, piceatannol could moderately prolong APD via slowing I_{Na} inactivation to increase the effective refractory period in association with the decrease of V_{max} of AP via decreasing I_{Na} to stabilize the membrane excitability, which may contribute to the ionic mechanisms of antiarrhythmic actions in ischaemic-reperfused rat hearts. Furthermore, piceatannol is more potent than resveratrol in cardiac ion

Table 2 Comparison of antiarrhythmic efficacy between piceatannol and resveratrol in isolated Langendorff-perfused rat hearts subjected to ischemia-reperfusion

	PVT	*Converted to normal rhythm*
Piceatannol ($\mu mol \cdot L^{-1}$)		
0	13	0
1	13	0
3	9	4
10†‡	0	13
Resveratrol ($\mu mol \cdot L^{-1}$)		
0	14	0
3	13	1
10*	9	5
30*	8	6
100*‡	4	10

PVT: polymorphic ventricular tachyarrhythmia.
Piceatannol or resveratrol was administered 5 min after reperfusion-induced arrhythmia. Data are the animal number of the arrhythmia without treatment or the successful case number which was converted to regular rhythm 1h after exposure to piceatannol or resveratrol at the different concentrations. The total animal number at every concentration of the respective treatment was 13 in piceatannol-treated group and was 14 in resveratrol-treated group. The symbols on the right side of the treated groups indicate the significant differences among the treatments in the number of both PVT and converted to normal rhythm. †$P < 0.01$ drug-treated group vs vehicle (DMSO 0.1%), *$P < 0.05$ drug-treated group vs vehicle (DMSO 0.1%), and ‡$P < 0.05$ 3 $\mu mol \cdot L^{-1}$ vs other concentrations by Yates' corrected Chi-square test.

channel inhibition, including I_{Na}, I_{to} and I_{Kss}, in parallel with the antiarrhythmic activity. Our present study provides the electrophysiological evidence to support the findings of better antiarrhythmic activity of piceatannol than resveratrol in ischaemia-reperfused rat hearts.

Piceatannol moderately slowed I_{Na} inactivation, which could be reverted by lidocaine. The result indicates that piceatannol may fix Na^+ channel in a state of inactivation which shunts rapidly between inactivation state and open state. Lidocaine may modify piceatannol-modulated sodium channel into another inactivation state that could not shunt rapidly between inactivation and open state. Besides that, the modulation of piceatannol on I_{Na} was different from that of aconitine. Aconitine shifted the sodium channel activation threshold to negative potentials and thus produced a persistent I_{Na} at resting membrane potential of cardiomyocytes to induce arrhythmia. Aconitine can bind inside the pore of Na^+ channel to cause easier and longer opening of Na^+ channel (Tikhonov and Zhorov, 2005). Piceatannol did not reverse aconitine-activated Na^+ channel, and produced an additive effect in increasing the slow inactivation component of I_{Na}; nevertheless, piceatannol could decrease the window of I_{Na} by left shifting the voltage-dependent inactivation curves of aconitine-modified I_{Na}. It indicates that the action model of piceatannol in Na^+ channel is far from that of aconitine. Furthermore, piceatannol did not increase intracellular Ca^{2+} transients and could inhibit the forward mode of Na^+-Ca^{2+} exchanger to decrease triggered activity, which was different from other Na^+ channel modulators, such as DPI201-106 and BDF 9198 (Gwathmey *et al.*, 1988; Yuill *et al.*, 2000), which could enhance proarrhythmia.

Resveratrol and piceatannol have been detected in red wine and have long been associated with the cardioprotection of the 'French paradox' (Renaud and de Lorgeril, 1992; Hung *et al.*, 2000; 2001). The pharmacokinetics of piceatannol appears to be qualitatively similar to resveratrol in the rat, which is absorbed quickly, distributed widely in heart, liver and kidney tissues, and is eliminated quickly with a plasma elimination half-life of 30 min (Marier *et al.*, 2002; Roupe *et al.*, 2004; 2006a,b; Abd El-Mohsen *et al.*, 2006). Though the plasma concentrations of oral piceatannol in humans remain to be established, the blood levels of resveratrol could achieve 2.4 $\mu mol \cdot L^{-1}$ in a phase I study of oral resveratrol 5 g in healthy volunteers (Boocock *et al.*, 2007). Besides that, piceatannol can be a metabolite of resveratrol via the cytochrome P450 1A2 and 1B1 enzymes (Potter *et al.*, 2002; Piver *et al.*, 2004), which have led investigators to postulate that resveratrol may act as a pro-drug for the production of piceatannol. The present study offers important information that piceatannol at the concentration of 3 $\mu mol \cdot L^{-1}$ can modulate I_{Na} and produces antiarrhythmic effect in ischaemia-reperfused rat hearts. Furthermore, piceatannol is more potent than resveratrol in the inhibition of ion channels, including I_{Na}, I_{to} and I_{Kss}, which is in parallel with the more potent antiarrhythmic efficacy of piceatannol than that of resveratrol. Because the decay time constant of piceatannol-modified I_{Na} at 0 mV was about 40 ms which is less than normal human ventricular APDs, piceatannol may not influence the normal AP and do not have the risk to induce long QT at the concentrations less than 10 $\mu mol \cdot L^{-1}$. Therefore, piceatannol is safe for healthy human as present in daily food or drink. However, piceatannol at the higher concentrations (more than 30 $\mu mol \cdot L^{-1}$) can markedly inhibit I_{Kr} and I_{Na}. Blocking I_{Kr} can cause long QT syndrome and induce Torsades de pointes. The excessive inhibition of I_{Na} can interfere with cardiac conduction and cause Brugada-like syndrome. Hence, it is not recommended taking high doses of piceatannol as daily supplement.

In conclusion, piceatannol was more potent than resveratrol in cardiac ion channel inhibition which was also in parallel with its potent antiarrhythmic efficacy in ischaemia-reperfused rat hearts. Piceatannol-mediated modulation on cardiac sodium channel may contribute to its antiarrhythmic action at concentrations less than 10 $\mu mol \cdot L^{-1}$.

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Conflict of interest

None.

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