Effects of platelet activating factor on action potentials and potassium channels in guinea-pig ventricular myocytes

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Abstract: This study was designed to investigate the effects of platelet activating factor (PAF) on the action potential and potassium currents in guinea-pig ventricular myocytes. Whole cell patch clamp techniques were used. With 5 mmol/L ATP in the pipette electrode (mimic normal condition), 1 µmol/L PAF increased APD90 from 225.8±23.3 to 352.8±29.8 ms (n=5, P<0.05), decreased I_K1 and I_K tail currents from -6.1±1.3 to -5.6±1.1 nA (n=5, P<0.05) at -120 mV and from 173.5±16.7 to 152.1±11.5 pA (P<0.05, n=4) at +30 mV, respectively. But PAF had no effect on I_K1 at potentials within the normal range of membrane potentials (between -90 mV and +20 mV).

In the contrary, without ATP in the pipette electrode by which I_K·ATP was activated (mimic ischemic condition), 1 µmol/L PAF shortened APD90 from 153±24.6 to 88.2±19.4 ms (n=5, P<0.01). Incubation of myocytes with 1 µmol/L glibenclamide, a blocker of I_K·ATP could restore prolongation of APD induced by PAF. In conclusion, in guinea-pig ventricular myocytes, with 5 mmol/L ATP in the pipette PAF could prolong APD partly due to the inhibition of I_K; while with 0 mmol/L ATP in the pipette, PAF could induce an activation of I_K·ATP, hence a decrease in APD. It is suggested that PAF may amplify the heterogeneity between ischemic and normal cardiac myocytes during ischemia/reperfusion, which may play a vital role in the pathogenesis of the arrhythmias induced by ischemia/reperfusion.

Key words: platelet activating factor; cardiac myocyte; action potential; potassium channels; patch clamp technique
profound effects on various tissues including the heart\[1\]. Several reports have demonstrated that PAF caused arrhythmias and was an important arrhythmogenic factor during ischemia and reperfusion\[2,3\]. However, the ionic mechanism of its proarrhythmic action is unclear yet.

Delayed rectifier potassium current (\(I_k\)) and inward rectifier potassium current (\(I_{k1}\)) are major components in the process of repolarization and therefore may be the important targets for proarrhythmic action\[4\]. ATP-sensitive K\(^+\) channel currents (\(I_{KATP}\)) have been found and studied in the heart. A number of reports have shown that the opening of ATP-sensitive K\(^+\) channels might be involved in ischemia/reperfusion induced-arrhythmias\[5,6\]. So in this study, we intended to examine the effects of PAF on action potential and the underlying ionic currents, \(I_k\), \(I_{k1}\), and \(I_{KATP}\) with whole-cell patch-clamp recording technique in guinea-pig ventricular myocytes, and to explore its proarrhythmic mechanisms.

1 Materials and methods

1.1 Cell isolation. Guinea-pig (300–400 g) ventricular myocytes were isolated by an enzymatic dissociation method similar to that previously described\[7\]. Briefly, the heart was rapidly excised and mounted in a Langendoff-type apparatus for retrograde coronary perfusion. The heart was initially perfused with Tyrode solution for 2–3 min, followed by Ca\(^2+\)-free Tyrode solution for 5–10 min, then it was enzymatically digested for 10 min with a Ca\(^2+\)-free Tyrode solution containing 50–100 U/ml collagenase (Yakult, Tokyo, Japan). All solutions were gassed with 100% O\(_2\) and warmed to 37ºC. Isolated ventricular myocytes were stored in KB (Kraftbrühe) medium at 4ºC and studied within 8 h. After 2 h of incubation at 4ºC, a few drops of the cell suspension were placed in a 0.3 ml perfusion chamber mounted on an inverted microscope stage. Only rod-shaped noncontracting cells with clear cross striations were used for the whole-cell patch-clamp studies.

1.2 Electrophysiological recording. The whole-cell patch-clamp technique was used to evaluate individual ionic currents with an EPC-9 amplifier (HEKA, electronic, Lambrecht, Germany) connected to a Macintosh Quadra 840AV computer (Apple Computer, Inc., Cupertino, CA). Pipette electrodes were made from borosilicate glass capillaries (USA) with a vertical pipette puller (Narishige PP-83, Tokyo, Japan), having a resistance of 1–2 \(\Omega\)M, when filled with the internal solution. Action potentials were elicited in current-clamp mode by applying test pulses (10 ms of duration at 0.5 Hz). \(I_k\) was determined by measuring the outward tail currents elicited on the repolarization to −40 mV after depolarizing step potentials in 10 mV increments for 2000 ms from 0 mV to +80 mV. The amplitude of the tail current was measured as the difference between the peak outward tail current and the steady state current at −40 mV. The membrane potential was held at −40 mV. \(I_{k1}\) was activated by 200 ms test pulses from a holding potential of −40 mV to inactivate the sodium channel. Calcium current was also blocked by the addition of 0.2 mmol/L CdCl. The voltage dependence of \(I_{k1}\) was determined by stepwise 10 mV increases in the test voltage, ranging between −120 mV and +20 mV. \(I_{KATP}\) steady-state currents were measured at the end of each test pulse.

1.3 Solutions and Drugs. Normal Tyrode solution contained (in mmol/L): NaCl 135, KCl 4.0, NaH\(_2\)PO\(_4\) 0.33, MgCl\(_2\) 1.0, CaCl\(_2\) 1.8, D-Glucose 10, HEPES 5.5, HEPES-Na 4.5 (pH=7.4 at 37ºC). The KB medium contained (in mmol/L): KOH 90, L-Glutamic acid 70, Taurine 20, KCl 30, KH\(_2\)PO\(_4\) 10, HEPES 10, D-glucose 10, EGTA 0.5 and pH was adjusted to 7.3 with KOH. The pipette solution contained (in mmol/L): K-Aspartate 110, HEPES 10, EGTA 5.0, K\(_2\)ATP 5.0, KCl 20, MgCl\(_2\) 2, CaCl\(_2\) 4 and pH was adjusted to 7.2 with KOH. To activate the \(I_{KATP}\) experiment was performed with 0 mmol/L ATP in the pipette solution.

PAF (Sigma) was dissolved in 50/50 (V/V) water and DMSO to make a 1 mmol/L stock solution and kept frozen. Glibenclamide (Sigma) was dissolved in DMSO to make a 2 mmol/L stock solution. Both PAF and glibenclamide were diluted with Tyrode solution just before used. Glibenclamide (1 µmol/L) resulted in 100% inhibition of \(I_{KATP}\) within 1–2 min after application of the drug\[8\]. The final concentration of DMSO (<0.05%) did not affect currents. All chemicals and solvents were of analytical reagent grade.

1.4 Data analysis. Data were digitally filtered at 2 kHz, then analyzed with Pulse/Pulsefit (HEKA electronic), IGOR (Wave Metrics Inc., Lake Oswego, OR) and Kaleida Graph (Synergy Software) on the Macintosh computer. Values were expressed as mean±SE. Statistical analysis was performed using t-test. \(P<0.05\) was considered statistically significant.

2 RESULTS

2.1 Effect of PAF on action potential with 5 mmol/L ATP in the pipette

Initial experiments were done to investigate the effect of PAF on action potential duration in isolated guinea pig
The effect of PAF on action potential was an increase in the action potential duration. 5 min after application of 1µmol/L PAF action potential duration measured at 90% repolarization (APD$_{90}$) was markedly increased from 225.8±23.3 ms to 352.8±29.8 (n=5, P<0.05). The effect of PAF was partially reversible 10 min after washing out. And the resting potential ($E_m$) was not significantly affected by PAF (from 79.4±0.2 mV to 78.9±0.3 mV, n=5, P>0.05).

2.2 Effect of PAF on $I_k$ with 5 mmol/L ATP in the pipette

The effect of PAF (1 and 3 µmol/L) on the $I_k$ was studied. Typical $I_k$ tail current records obtained from a guinea pig ventricular myocyte in several test potentials were shown in Fig. 2A. In Fig. 2B, tail currents were plotted as a function of membrane potential. 1 µmol/L PAF decreased the peak tail currents only at the membrane potential less than +40 mV. But peak tail currents were clearly suppressed at all potentials after perfusion with 3 µmol/L PAF. At +30 mV, PAF at 1 and 3 µmol/L decreased $I_k$ tail currents from 173.5±16.7 pA to 152.1±11.5 nA and 138.5±8.9 nA (P<0.05, n=4), respectively.

2.3 Effect of PAF on $I_{k1}$ with 5 mmol/L ATP in the pipette

The effect of PAF (1 and 3 µmol/L) on the $I_{k1}$ was studied. At −120 mV, 1 and 3 µmol/L PAF decreased $I_{k1}$ from −6.1±1.3 to −5.6±1.1 nA (P<0.05, n=5) and −4.7±0.8 nA (P<0.05) respectively, while did not change the outward current significantly. Figure 3A showed original currents elicited by a test potentials of every 10 mV step ranging from −120 to +20 mV for 200 ms before and during perfusion with PAF (1 and 3 µmol/L). The holding potential is −40 mV. The inhibitory effect was concentration-dependent. Figure 3B showed an example the sup-
pression of $I_{k1}$ by PAF at different potentials, which revealed that. The inhibitory effect was not occurring during normal membrane potential range (between $-90$ and $+20$ mV). And the reverse potential of $I_{k1}$ was unaffected (Fig. 3B).

2.4 Effect of PAF on action potential with 0 mmol/L ATP in the pipette

We recorded the action potential of guinea-pig ventricular myocytes with 0 mmol/L ATP in the pipette, at which $I_{k,ATP}$ was activated\(^9\). Figure 4 showed a typical record, in which action potential duration was rapidly shortened. When a nearly stable level of action potential had reached, 1 µmol/L PAF was applied, APD$_{90}$ was further decreased from 153±24.6 ms to 88.2±19.4 ms ($n=5$, $P<0.01$) and partly recovered after washing out.

To investigate whether $I_{k,ATP}$ contributes to the PAF-induced AP duration shortening with 0 mmol/L ATP in the pipette, the experiments were performed to test the effect of $I_{k,ATP}$ blocker. Results of a typical experiment were shown as Fig. 5. Incubation with 1 µmol/L glibenclamide, a specific $I_{k,ATP}$ blocker, restored the PAF-induced prolongation of the action potential duration from 242.7±22.8 ms to 311.5±34.1 ms ($n=4$, $P<0.05$).

3 DISCUSSION

PAF was found to produce lethal arrhythmias in several animal models\(^2\)\(^-\)\(^3\). But the studies examining the electrophysiological actions of PAF at the cellular level are contradictory\(^10\)\^-\)\(^12\). PAF has been reported to prolong, shorten or have no effect on action potential duration. Because some of the confusion might arise from the presence of
different cell types in this multicellular preparation, we decided to examine the effects of PAF on single ventricular cells isolated from guinea pig hearts. This protocol would preclude the possible interference due to the interaction of different cell types.

In the present study, we compared the effects of PAF on electrical activity of guinea-pig ventricular myocytes with 5 mmol/L ATP in the pipette electrode (mimic normal conditions) and with 0 mmol/L ATP in the pipette electrode where $I_{K,ATP}$ was activated (mimic ischemic condition).

Our results showed that with 5 mmol/L ATP in the pipette electrode, 1 µmol/L PAF prolonged the action potential significantly without affecting the resting membrane potential (Fig.1). Prolongation of APD is often associated with the development of early afterdepolarizations probably responsible for induction of Torsade de Pointes ventricular arrhythmias.

Delayed rectifier potassium channels play a key role in regulating cardiac APD. Analysis of the activation of $I_{K1}$ is difficult because of additional currents, which may be simultaneously activated by depolarizing pulses. Upon repolarization from activating voltage steps, an outward tail current was observed which is considered to represent the slow deactivation of $I_{K}$ and serves as a more reliable index of the $I_{K}$ current. Therefore the effect of PAF on the $I_{K}$ tail current was studied. Our results showed that PAF exerts an inhibitory effect on $I_{K}$ in a concentration-dependent manner. 1 µmol/L PAF decreased the peak tail currents only at the membrane potential less than +40 mV. But peak tail currents were clearly suppressed at all potentials after perfusion with 3 µmol/L PAF (Fig. 2). However, it has reported that PAF has no effect on delayed outward potassium current in human and chick ventricular myocytes.

The differences between the results of the two studies may reflect the different species used. Since $I_{K}$ play a critical role in the repolarization process of cardiac action potential, inhibition of $I_{K}$ by PAF must induce the prolongation of APD in guinea-pig ventricular myocytes.

We found that PAF inhibited $I_{K1}$ in a concentration-dependent manner; it is similar to the report of Wehler et al.[14]. The $I_{K1}$ channel not only is responsible for maintaining a stable cardiac resting potential near K⁺ equilibrium potential ($E_{K}$), but also determines the time course of rapid repolarization of cardiac action potential. The observation that PAF did not alter resting membrane potential ($E_{m}$) could not by itself exclude an inhibitory effect on $I_{K}$ because normally only a fraction of $I_{K1}$ is required to maintain the membrane potential around $E_{m}$[15]. Indeed, current-voltage relations disclosed a unsignificant affected by PAF of the current observed at potentials between −90 mV and +20 mV. Whether blockade of $I_{K1}$ induces action potential prolongation is unsettled issue because “pure” $I_{K1}$ blockers such as the benzopyran compounds RP 58866 and its active enantiomer terikalant, turned out to be also potent blockers of $I_{K1}$[16]. So inhibition of $I_{K1}$ may not contribute to the prolongation of APD in guinea-pig myocytes. But it may involve in ischemia/reperfusion induced arrhythmia.

$I_{K,ATP}$, which is glibenclamide-sensitive, is activated by low [ATP], and may be responsible for the increase in K⁺ efflux and the shortening of the action potential duration. Therefore, opening of this channel may result in cardio-protective as well as proarrhythmic effects. The opening of $I_{K,ATP}$ might involve in ischemia/reperfusion induced-arrhythmia. However, it is unclear whether or not PAF have effects on $I_{K,ATP}$. We investigated the effect of PAF on action potential with 0 mmol/L ATP in the pipette. In this condition, $I_{K,ATP}$ can be activated. We found the action potential duration was decreased. When a nearly stable level of action potential had reached, 1 µmol/L PAF was applied, the APD$_{90}$ was further decreased (from 153±24.6 to 88.2±19.4 ms, $n=5$), which was similar to previous reports[10]. Upon washing out of PAF, APD$_{90}$ partially reversed back to control values. Incubation with 1 µmol/L glibenclamide, a specific $I_{K,ATP}$ blocker, restored the PAF-induced prolongation of the action potential duration from 242.7±22.8 ms to 311.5±34.1 ms ($n=4$), which was similar to previous reports[10]. These findings demonstrate that $I_{K,ATP}$ is the main current causing PAF induced AP shortening in guinea-pig myocytes in the presence of very low intracellular ATP concentration.

In summary, in guinea-pig ventricular myocytes, with 5 mmol/L ATP in the pipette APD was lengthened by PAF partly due to PAF-induced inhibition of $I_{K}$ and $I_{K1}$, while with 0 mmol/L ATP in the pipette, APD was further decreased by PAF because of PAF-induced activation of $I_{K,ATP}$. Our results suggest that PAF might amplify the heterogeneity between ischemic and normal zones during ischemia/reperfusion, which might play a vital role in ischemia/reperfusion induced arrhythmias.

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