Glucocorticoid Regulation of Cardiac K⁺ Currents and L-Type Ca²⁺ Current in Neonatal Mice

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Abstract—Previous studies have reported that dexamethasone (Dex) prolongs cardiac action potential repolarization in mice and rats. However, the cellular mechanisms of this effect have not been addressed. Because action potential duration is influenced by a complex interplay of both inward and outward currents, this study evaluated the role of K⁺ currents and the L-type Ca²⁺ current in response to chronic in vivo Dex treatment. Accordingly, neonatal mice were randomly allocated to treatment with Dex (1 mg/kg per day) or placebo (saline) given subcutaneously for 5 days. At 14 to 15 days of age, the L-type Ca²⁺ current and K⁺ currents were recorded in ventricular myocytes using whole-cell patch-clamp techniques. The density of peak outward K⁺ currents was significantly decreased in the chronic Dex-treated group, but the current measured at the end of a 1-second depolarization pulse was similar in both groups. We further measured the magnitudes of the fast-inactivating (Iₒ) and the slowly inactivating (Iₙₒ) currents that contribute to the peak outward K⁺ currents. Iₒ was reduced from 17.5±3.0 pA/pF (control) to 10.6±2.5 pA/pF (Dex) at +50 mV (P<0.05), but Iₙₒ was not significantly different. These data suggest that downregulation of Iₒ is responsible for the reduced peak outward current. Time courses of the onset and offset of in vivo Dex effects were also assessed. A period of 3 days of treatment was required to observe the Dex effect on peak outward K⁺ currents, whereas a 7-day period after discontinuation of Dex was required to recover the baseline current density. Acute in vitro treatment with Dex (1 μmol/L) had no effect on K⁺ current densities. In addition, chronic Dex treatment significantly increased the density of the L-type Ca²⁺ current (Iₒ) from −7.2±0.5 pA/pF of control to −8.9±0.6 pA/pF of Dex at +10 mV, P<0.05. In conclusion, chronic in vivo Dex treatment decreases Iₒ and increases Iₒ in neonatal mouse ventricular myocytes, both of which contribute to the prolongation of cardiac action potential repolarization induced by glucocorticoids. (Circ Res. 1999;85:168-173.)

Key Words: dexamethasone • K⁺ current • L-type Ca²⁺ current • action potential duration • mice

Previous studies have shown that treatment with adrenal glucocorticoids exerts important effects on cardiac action potential duration (APD) in neonatal rats and mice.¹⁻³ A balance of inward depolarizing and outward repolarizing currents determines APD in heart. If an imbalance of the inward and outward forces occurs, a change in APD will result. Therefore, an increase in the inward depolarizing L-type Ca²⁺ current or a decrease in the outward repolarizing K⁺ currents may contribute to glucocorticoid-induced action potential prolongation. Recently, Takimoto et al.⁴ have reported that mRNA encoding the cardiac α₁C isoform of L-type Ca²⁺ channel is upregulated by chronic in vivo dexamethasone (Dex) treatment. In parallel, Dex also significantly increased the dihydropyridine binding site density in rat ventricle,⁵ which raised the possibility of an increase in L-type Ca²⁺ current density. In addition, Takimoto and Levitan also reported that in vivo glucocorticoid treatment upregulates expression of Kv1.5 mRNA in rat heart.⁶ However, no previous studies have explored the mechanisms of glucocorticoid-induced prolongation of APD at the functional channel level by measuring depolarizing or repolarizing currents. Therefore, the purpose of this study was to examine the effects of glucocorticoids on cardiac K⁺ currents and L-type Ca²⁺ current in mouse ventricular myocytes during postnatal development. Accordingly, pairs of neonatal mice were randomly allocated to chronic in vivo Dex (1 mg/kg) or placebo treatment. At 14 to 15 days of life, K⁺ currents and L-type Ca²⁺ current were recorded from cardiac ventricular myocytes using a whole-cell patch-clamp technique. Herein we report that chronic in vivo Dex treatment decreases the density of the fast-inactivating current (Iₒ) and increases the density of the L-type Ca²⁺ current (Iₒ), both of which contribute in a complementary manner to prolongation of APD induced by glucocorticoids in neonatal mice.

Materials and Methods

In Vivo Drug Treatment Protocols
CD-1 mice (Charles River, St. Constant, Quebec, Canada) were used in this study. Pairs of neonatal mice at day 7 of age were randomly
assigned to receive either subcutaneous placebo (saline) or Dex (1 mg/kg per day) treatment for 5 days. The dose and duration of Dex used in this study are similar to those used previously. To assess the time course of the onset of the Dex effect, a variable duration of the treatment ranging from 1 to 3 and 5 days was used. To assess the time course of the offset of the Dex effect, all mice were treated with Dex for 5 days; then, whole-cell patch clamp experiments were performed at variable intervals of 1, 3, and 5 days after discontinuation of Dex. To carry out the experiments using mice of similar age, we adjusted the age at which the injections started for the onset/offset studies. All mice were euthanized at 14 to 15 days of age except for the offset studies, with 5- and 7-day intervals after treatment discontinuation. Those mice were studied at 20 days of age. Note that the density of the peak outward currents was similar between 14- and 20-day control mice.

Whole-Cell Patch-Clamp Recording

Single ventricular myocytes were enzymatically isolated from neonatal mice by using a previously described Langendorff perfusion technique. Macroscopic $I_{\text{Ko}}$ currents and $I_{\text{Ca-L}}$ were recorded by whole-cell patch-clamp method with an Axopatch 200 amplifier (Axon Instruments). For $K^+$ current recordings, the ventricular myocytes were perfused with HEPES-buffered Tyrode solution containing (in mmol/L) NaCl 140, KCl 4, MgCl$_2$ 1, CaCl$_2$ 1, glucose 5.5, and HEPES 10, pH 7.4 adjusted with NaOH. L-type Ca$^{2+}$ current was blocked by CdCl$_2$ (0.3 mmol/L). Tetrodotoxin (TTX; 20 $\mu$mol/L) was used to block $I_{\text{Na}}$ in our preliminary study, showing that $I_{\text{Na}}$ density and kinetics were similar in the presence and absence of TTX; thus, TTX was not routinely included in the external solution. The pipette solution was composed of (in mmol/L) potassium aspartate 110, MgCl$_2$ 4, K$_2$-ATP 4.2, CaCl$_2$ 1, NaCl 8, HEPES 5, and EGTA 10, pH 7.2 adjusted with KOH. For L-type Ca$^{2+}$ current recordings, the ventricular myocytes were perfused with a Na$^+$- and K$^+$-free solution modified from that of Aggarwal and Boyden containing (in mmol/L) tetraethylammonium chloride 130, CaCl$_2$ 2, MgCl$_2$ 1, 4-aminopyridine 2, glucose 10, and HEPES 10, pH 7.4 adjusted with CsOH. The pipette solution was composed of (in mmol/L) CsOH 110, aspartic acid 110, Mg-ATP 3, CaCl$_2$ 1, Na$_2$-phosphocreatine 3.6, tetraethylammonium chloride 20, EGTA 10, and HEPES 10, pH 7.2 adjusted with CsOH. To minimize the time-dependent rundown effect, all measurements of $I_{\text{Ca-L}}$ were carried out between 10 and 20 minutes after whole-cell membrane rupture. All recordings were conducted at room temperature (22°C to 23°C), and external solutions were bubbled with 100% O$_2$.

Electrodes had tip resistances of 2 to 4 MΩ when filled with internal solutions. Cell capacitance was calculated from the uncompensated capacity current transients elicited by a 10-mV hyperpolarizing voltage step from a holding potential of –80 mV to test potentials from –40 to 200 mV in 10-mV increments. The interpulse interval was 20 seconds. The amplitude of $I_{\text{Ca-L}}$ in the cell isolated from Dex-treated mice (A) was decreased compared with that recorded from control cell (B).

Results

Density and Inactivation Kinetics of the Outward $K^+$ Currents

Figure 1 displays the whole-cell voltage-clamp recordings of depolarization-activated outward $K^+$ currents in ventricular myocytes isolated from control (1A) and chronic Dex-treated (1B) mice. The amplitude of peak outward $K^+$ current ($I_{\text{peak}}$) at all test potentials was substantially lower in the cells isolated from Dex-treated mouse (Figure 1B) than from control mouse (Figure 1A). Because the cell capacitance was smaller in chronic Dex-treated mice (75.7±18.2 pF, n=38) than that in control mice (91.8±14.9 pF, n=40, P<0.01), the current amplitude was normalized to the cell capacitance and then expressed as current density. The $I_{\text{peak}}$ density was significantly reduced in cells isolated from the chronic Dex-treated mice (23.4±3.8 pA/pF) compared with control mice (34.6±7.4 pA/pF, P<0.05). In contrast, the current density measured at the end of 1-second voltage steps was not significantly different in the cells isolated from control (13.1±3.2 pA/pF) and Dex-treated (11.5±2.3 pA/pF) mice.

As reported previously, the decay phases of $I_{\text{peak}}$ in mouse ventricular myocytes consist of 2 inactivating components: the fast-inactivating current, $I_{\text{fast}}$, and the slowly inactivating current, $I_{\text{slow}}$. To assess their contribution to the reduced $I_{\text{peak}}$ in Dex-treated cells, the amplitudes of $I_{\text{fast}}$ and $I_{\text{slow}}$ were determined by biexponential fit using the Clampfit program. Figure 2 shows examples of current traces and exponential fittings in ventricular myocytes isolated from control (Figure 2A) and chronic Dex-treated (Figure 2B) mice. Dolph treatment...
did not affect the inactivation kinetics of $I_{to}$ and $I_{slow}$ (Figure 2C). However, as shown in Figure 2D, chronic Dex treatment selectively decreased the density of $I_{to}$ but did not significantly affect the density of $I_{slow}$.

In addition, we have directly applied Dex (0.01 to 1 μmol/L) to in vitro neonatal mouse ventricular myocytes for up to 20 minutes. Even at the concentration of 1 μmol/L, Dex failed to alter the $K^+$ current densities (n=6) (data not shown).

**Steady-State Inactivation of the Outward $K^+$ Currents**

Figure 3 displays representative current traces elicited by a typical double-pulse protocol for steady-state inactivation in cells isolated from control (Figure 3A) and Dex-treated (Figure 3B) mice. Outward $K^+$ currents were evoked during 3-second depolarization to +50 mV. Before each depolarization to +50 mV, the cell was held for 5 seconds at a conditioning potential between –100 and 0 mV. The amplitudes of $I_{to}$ and $I_{slow}$ evoked from each conditioning potential were measured in individual cells and normalized to the amplitudes evoked from a conditioning potential of –100 mV. The mean values of half-inactivation potential ($V_h$) and slope factor ($k$) for $I_{to}$ were –46.4±9.5 mV and –4.5±1.5 mV for control (n=6) and –47.2±9.9 mV and –4.4±1.3 mV for Dex treatment, respectively (n=5; NS). The mean values of $V_h$ and $k$ for $I_{slow}$ were –38.8±6.4 mV and –10.1±2.1 mV for control (n=6) and –39.4±7.2 mV and –10.8±2.3 mV for Dex treatment (n=5; NS). Therefore, shifts in steady-state inactivation could not account for the differences in reduced $I_{to}$ density in the ventricular myocytes isolated from Dex-treated mice.

**Time Courses for the Onset and Offset Effects of Dex on the Outward $K^+$ Currents**

To assess whether the reduced magnitudes of the peak outward $K^+$ current vary as a function of the duration of in vivo Dex treatment, the ventricular myocytes were isolated...
from neonatal mice after 1, 3, and 5 days of Dex treatment, and $I_{\text{peak}}$ was measured. As shown in Figure 4A, Dex treatment for 1 day was not sufficient to alter the magnitude of the peak current density. Both 3 and 5 days of Dex treatments significantly decreased the density of $I_{\text{peak}}$.

To assess the reversibility of Dex-induced effects on cardiac $K^+$ currents, all mice were treated with Dex for 5 days. Then, the density of $I_{\text{peak}}$ was examined at 1-, 3-, 5-, and 7-day intervals after discontinuation of Dex. The results are summarized in Figure 4B. Partial recovery was observed after termination of the treatment for 5 days, and full recovery occurred at 7 days after termination of the treatment.

**Inwardly Rectifying $K^+$ Current ($I_{\text{K1}}$)**

We also examined the effect of in vivo Dex treatment on $I_{\text{K1}}$. The family of $K^+$ currents shown in Figure 5 was evoked from a holding potential of $-50$ mV to test potentials ranging from $-110$ to $+40$ mV in 10-mV increments for 1 second. This protocol enables a comparison of the effect of Dex treatment on both depolarization-activated outward $K^+$ currents and the inwardly rectifying $K^+$ current, $I_{\text{K1}}$, in the same cell. Note that the magnitude of the peak transient outward current was substantially reduced by chronic Dex treatment (Figure 5B), whereas the magnitude of $I_{\text{K1}}$ was increased in the same cell. Figure 5C shows the mean current density-voltage relationships of $I_{\text{K1}}$.

**Current Density and Voltage Relationship of $I_{\text{Ca-L}}$**

To assess whether chronic in vivo treatment with Dex affects $I_{\text{Ca-L}}$, whole-cell $I_{\text{Ca-L}}$ was recorded in ventricular myocytes isolated from control and Dex-treated mice. For $I_{\text{Ca-L}}$ measurements, the holding potential was at $-50$ mV, a membrane potential at which T-type Ca$^{2+}$ current is inactivated. Figure 6 shows representative examples of $I_{\text{Ca-L}}$ tracings recorded from control (Figure 6A) and Dex-treated (Figure 6B) ventricular myocytes. The $I_{\text{Ca-L}}$ elicited from $-50$ mV was completely blocked by a selective L-type Ca$^{2+}$ blocker, nisoldipine, at a concentration of 0.4 μmol/L (data not shown). The mean current density-voltage relations are illustrated in Figure 6C. In both control and Dex-treated ventricular myocytes, $I_{\text{Ca-L}}$ activation-threshold was approximately $-25$ mV, and the current peaked around $+10$ mV. Moreover, the average density of $I_{\text{Ca-L}}$ was significantly increased in ventricular myocytes isolated from Dex-treated mice ($n=10$) as compared with those from control mice ($n=12$, $P<0.05$).
These data suggest that Dex-induced prolongation of action potential duration likely relates to the combination of an increase in $I_{Ca-L}$ density and a reduction of $I_{to}$ density.

**Action Potential Configuration**

The ventricular action potential configuration of control and Dex-treated mice was recorded at 37°C using a conventional microelectrode technique under physiological conditions as described previously. Representative examples of cardiac action potentials from control and Dex-treated neonatal mice are shown in Figure 7A and 7B, respectively. As expected, chronic in vivo Dex treatment significantly prolonged APD$_{50}$ from $14.7 \pm 1.4$ (control, $n=6$) to $20.3 \pm 6.3$ ms (Dex, $n=17$), $P<0.05$, and APD$_{90}$ from $38.7 \pm 6.7$ (control) to $50.2 \pm 11.7$ ms (Dex), $P<0.05$.

**Discussion**

In the present study, we have demonstrated that chronic in vivo Dex treatment decreases the density of $I_{to}$ but does not alter the density of $I_{slow}$ in neonatal mouse ventricular myocytes. The effect of chronic in vivo Dex treatment on $I_{to}$ is time dependent and completely reversible. In addition, the density of $I_{Ca-L}$ is significantly increased in the ventricular myocytes isolated from chronic in vivo Dex-treated neonatal mice. Direct application of Dex to the isolated ventricular myocytes does not affect the channel properties. These data suggest that Dex-induced alteration in current densities occurs likely through the regulation of gene expression.

**Comparison to Previous Work**

Maternal glucocorticoid levels decline sharply before birth and continue to decline in the neonatal rat until the third postnatal week. During this developmental period, a substantial shortening of cardiac action potential was observed. To correlate the relation between decreased glucocorticoid level and action potential shortening during postnatal development, Penefsky and McCann reported that pretreatment of neonatal rats with Dex largely inhibited the developmental shortening of phase 1 of APD. Initial rapid repolarization (phase 1) of APD is largely determined by $I_{to}$, which indicates that the level of circulating glucocorticoids may affect $I_{to}$ channel expression. In this study, we observe that pretreatment of neonatal mice with Dex results in a significant decrease in $I_{to}$ density without alteration of the biophysical properties. This finding is in keeping with the work of Penefsky and McCann and is also consistent with our...
previous report that developmental increase in \( I_{to} \) contributes to developmental shortening of APD in neonatal mice.\(^\text{6}\) Because \( I_{to} \) density is decreased by Dex, a prolonged APD would be expected. Indeed, APD is longer in Dex-treated neonatal mice compared with that in control mice. The significant increase in \( I_{Ki} \) density was observed only at negative potentials and therefore likely does not contribute to the observed APD change.

Takimoto and Levitan\(^\text{5}\) found that glucocorticoids caused an induction of Kv1.5 channel gene expression in ventricles of adrenalectomized adult rat. Recent studies indicate that Kv1.5 channel gene may contribute to \( I_{slow} \) in mouse ventricular myocytes.\(^\text{9,10}\) However, our study shows no significant effect on \( I_{slow} \) in ventricular myocytes isolated from neonatal mice pretreated with Dex. This discrepancy may relate to different ages of the experimental animals and species.

In terms of regulation of Ca\(^{2+}\) channel expression by glucocorticoids, the results obtained from the present study are in keeping with the previous biochemical studies. Takimoto et al\(^\text{4}\) have reported that Dex produces an increase in mRNA levels encoding \( \alpha_{1C} \) isoform of the L-type Ca\(^{2+}\) channel paralleled by an increase in the dihydropyridine binding site density in rat ventricle. In keeping with those findings, we have shown in this study that the density of L-type Ca\(^{2+}\) current is significantly increased in the ventricular myocytes isolated from neonatal mice pretreated with Dex.

In conclusion, upregulation of \( I_{Ca-L} \) and downregulation of \( I_{to} \) contribute to the glucocorticoid-induced action potential prolongation in neonatal mice.

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