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Dexamethasone and cardiac potassium currents in the diabetic rat

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1 Experiments were designed to compare effects of dexamethasone on transient (I_{peak}) and sustained (I_{sus}) K^+ currents in control and diabetic rat myocytes. Ventricular myocytes were isolated from control or type 1 streptozotocin (STZ)-induced diabetic male and female rats. Currents were measured using whole-cell voltage-clamp methods.

2 Incubation of cells from control males or females with 100 nM dexamethasone (5–9 h) significantly ($P<0.005$) augmented I_{sus} (by 28–31%). I_{peak} was unchanged. I_{sus} augmentation was abolished by cycloheximide or cytochalasin D, but not by inhibition of protein kinases A or C. Inhibition of tyrosine kinases by genistein (but not its inactive analog genistin) prevented the increase of I_{sus} by dexamethasone. In marked contrast, dexamethasone had a significantly ($P<0.015$) smaller effect on I_{sus} (11% increase) in cells from male STZ-diabetic rats, as compared to control cells. However, I_{sus} augmentation in cells from female STZ-diabetic rats was normal (31% increase). In ovariectomized-diabetic rats, I_{sus} was unchanged by dexamethasone. The reduced effect in diabetic males might be due to preactivation of tyrosine kinases linking dexamethasone to current modulation.

3 In conclusion, type I diabetes is associated with gender-specific changes in sensitivity of K^+ currents to glucocorticoids, linked to alterations in tyrosine-phosphorylated proteins.
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Abbreviations: ANG II, angiotensin II; HPA, hypothalamic-pituitary-adrenal; PKA, protein kinase A; PKC, protein kinase C; RAS, renin–angiotensin system; STZ, streptozotocin

Introduction

Diabetes mellitus is associated with various cardiovascular complications (Grundy *et al*., 1999; Mooradian, 2003), including cardiac contractile dysfunction (Tomlinson *et al*., 1992) and a higher incidence of some cardiac arrhythmias (Robillon *et al*., 1999; Rossing *et al*., 2001). Arrhythmogenesis may be related to an attenuation of repolarizing K^+ currents (Magyar *et al*., 1992; Shimoni *et al*., 1999). Diabetes is associated with the activation of a cardiac renin–angiotensin system (RAS; Fiordaliso *et al*., 2000). We showed that this contributes to the attenuation of a transient and a sustained K^+ current in the streptozotocin (STZ)-induced (type I) diabetic rat and in the (type II) db/db diabetic mouse (Shimoni, 2001). *In vitro* inhibition of the formation or action of ANG II increases these currents by enabling synthesis of new channel protein (Shimoni & Liu, 2003a). This effect is gender dependent, with less attenuation of currents and less sensitivity to ANG II inhibition in females (Shimoni & Liu, 2003b). We also demonstrated that cardiac levels of angiotensin II (ANG II) are elevated in male but not female diabetic rats, associated with the differences in attenuation of K^+ currents (Shimoni & Liu, 2004). Some of the transduction pathways linking ANG II activation to K^+ current attenuation also exhibit gender dependence (Shimoni & Liu, 2004). A possible link between the activation of cardiac RAS and glucocorticoid action has not been studied.

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The activation of the hypothalamic-pituitary-adrenal (HPA) axis contributes to pathogenesis of the metabolic syndrome and induction of diabetes (Andrews & Walker, 1999; Reynolds *et al*., 2003). At high doses, glucocorticoids induce insulin resistance, modulate insulin action, and affect glucose metabolism (Andrews & Walker, 1999; Bernal-Mizrachi *et al*., 2003).

Although circulating cortisol levels may not be altered in diabetes, peripheral sensitivity to glucocorticoids may be altered (Walker, 1996). Glucocorticoids can be synthesized locally in cardiac cells (Silvestre *et al*., 1998). Importantly, a major stimulus for glucocorticoid synthesis in the heart is ANG II (Silvestre *et al*., 1998).

Glucocorticoids regulate many cellular functions, with both genomic and nongenomic effects (Falkenstein *et al*., 2000). Some interaction between these modes has been suggested (Moyer *et al*., 1993; Nordeen *et al*., 1994). The interaction with signaling pathways is complex, with different mediators implicated in glucocorticoid action (Chen & Qiu, 1999). It was shown (Takimoto & Levitan, 1994) that the synthetic glucocorticoid dexamethasone increases mRNA levels and the expression of Kv1.5, a channel protein underlying some of the delayed rectifier K^+ currents in rat ventricle (Nerbonne, 2000). A corresponding increase in outward current was shown in GH3 pituitary cells (Takimoto *et al*., 1993).

The response to glucocorticoids may vary in control and pathological settings. The phosphorylation of several proteins by glucocorticoids in rats is different in control and in STZ-induced diabetes (Giorgino *et al*., 1993). Cortisol regulation of

α-adrenergic-evoked contractility of smooth muscle is suppressed in hypoxic conditions (Xiao *et al*., 2004). Modulation of cell signaling pathways can enhance or impair glucocorticoid gene expression in some systems (Moyer *et al*., 1993). In some tissues, glucocorticoid actions are blocked by inhibition of protein kinase A (PKA) but not of protein kinase C (PKC) (Urbach *et al*., 2002), while in others the converse is true (Park *et al*., 2001). Glucocorticoids may activate different PKC isoforms or inhibit PKA (Dwivedi & Pandey, 2000; Kajita *et al*., 2001).

There are also indications that secretion, activation or action of glucocorticoids may be gender dependent under some conditions (Walker *et al*., 2000; Fisher *et al*., 2001; Traustadottir *et al*., 2003).

The aims of the present study were three-fold: (a) to investigate the effects of dexamethasone on K^+ currents in control and diabetic conditions; (b) to establish some of the signaling pathways involved in these effects; and (c) to determine if effects of dexamethasone on K^+ currents are gender dependent and, if so, whether they can be related to differential effects on signal transduction pathways.

Methods

This study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Animals

The study was carried out on control and diabetic male and female Sprague–Dawley rats (250–300 g). Insulin-deficient diabetes was induced with a single intravenous injection of STZ (100 mg kg^{-1}), given 7–14 days before cell isolation. The diabetic state (elevated plasma glucose and reduced insulin) was confirmed in our earlier studies (Shimoni *et al*., 1999). Ovariectomized females were also used. STZ diabetes was induced 2–3 weeks after ovariectomy, and cells were isolated 7–14 days after STZ injection.

Cell isolation

Ventricular myocytes were obtained by enzymatic dispersion. Rats were anesthetized by CO_2 inhalation and killed by cervical dislocation. The hearts were removed and the aortas cannulated on a Langendorff apparatus for coronary perfusion. Hearts were perfused for 3–5 min (37°C, bubbled with 95% O_2–5% CO_2) with a solution consisting of (in mM): 121 NaCl; 5.4 KCl; 2.8 Na-acetate; 1 $MgSO_4$; 5 Na_2HPO_4; 24 $NaHCO_3$; 5 glucose, 1 $CaCl_2$ (brought to pH 7.4 with NaOH). This was switched to a calcium-free solution (other constituents unchanged) for 10 min, followed by the same solution containing collagenase (Yakult Honsha, 0.015 mg ml^{-1}), protease (Sigma type XIV, 0.0075 mg ml^{-1}), 20 mM taurine, 40 μM $CaCl_2$). After 8 min, the free wall of the right ventricle was cut into pieces for further incubation in a shaker bath (at 37°C), in a solution containing 0.3 mg ml^{-1} collagenase, 0.15 mg ml^{-1} protease, 20 mM taurine, and 10 mg ml^{-1} albumin. Aliquots of cells were removed over 10–20 min and stored at room temperature in a solution containing no enzymes, 20 mM taurine, 5 mg ml^{-1} albumin, and 0.1 mM $CaCl_2$. Cells were divided into groups, consisting of untreated cells or cells incubated with drugs. Incubation periods lasted from 5 to 9 h. We have previously shown (Shimoni & Rattner, 2001) that sustained outward current (I_{sus}) is stable over this period. Experiments were carried out at 21–22°C, due to the long incubation times required to obtain effects (at warmer temperatures, cells did not maintain viability).

For each experiment, treated and untreated cells were compared, allowing for variations in the degree of diabetes. Results were pooled from different days, and are given as means ± s.e.m.

Current recording

Cells were placed on a stage of an inverted microscope and perfused with a solution containing (in mM): 150 NaCl; 5.4 KCl; 1 $CaCl_2$; 1 $MgCl_2$; 5 HEPES; 5 glucose (brought to pH 7.4 with NaOH). $CdCl_2$ (0.3 mM) was added to block L-type calcium currents. The whole-cell voltage-clamp method was used to record currents, elicited by 500 ms pulses to membrane potentials ranging from −110 to +50 mV. Currents were digitized at 2 kHz and normalized to cell size by dividing current amplitude by cell capacitance, measured by integrating current traces (digitized at 10 kHz) obtained in response to 5 mV steps from −80 mV. Recording pipettes (2–3 MΩ resistance) contained solutions consisting of (in mM): 120 K-aspartate; 30 KCl; 4 Na_2ATP; 10 HEPES; 10 EGTA; 1 $CaCl_2$; 1 $MgCl_2$ (pH 7.2, KOH). Peak outward current (I_{peak}) and I_{sus} (measured 500 ms after initiation of the voltage step) were measured. The increase in I_{sus} in some cases (e.g. Figures 1, 4, and 6) produced an apparent slowing of the decay of the transient current (since both currents are present for the first 100–200 ms). However, measuring current magnitude 500 ms after the onset of depolarization ensured that only I_{sus} was present, as the transient current is completely inactivated at that time, as shown in our earlier work (Thorneloe *et al*., 2001, Figure 2).

Statistics

Mean values were compared using *t*-test or ANOVA, with the Student–Newman–Keuls multiple comparisons test. $P<0.05$ was considered significant.

Results

Control myocytes

We first examined the effects of dexamethasone on outward currents in myocytes from control males. Current densities were compared in untreated cells and in cells incubated with (100 nM) dexamethasone. Concordant with other interventions (Shimoni *et al*., 1999; Shimoni, 2001), effects were only seen after a lag of 4–5 h. After 5–9 h, dexamethasone significantly enhanced I_{sus}, with no effect on I_{peak}. These effects of dexamethasone are shown in Figure 1. Panel a shows current traces from an untreated cell (left) and from a cell treated for 6 h with dexamethasone (right). Figure 1b shows the current–voltage relationships, with mean densities of I_{peak} (left) and I_{sus} (right). I_{peak} was not significantly altered ($P>0.05$), whereas I_{sus} was significantly ($P<0.025$–<0.002) augmented between −30 and +50 mV. At negative potentials (−60 to −110 mV,

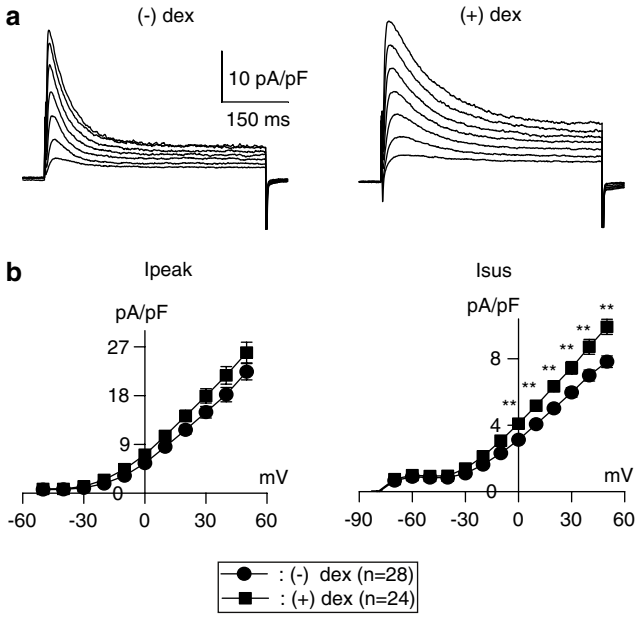

Figure 1 Effects of dexamethasone (100 nM) on outward K^+ currents in rat ventricular myocytes. (a) Sample current traces obtained from male control rats in the absence (left) or after 6 h in 100 nM dexamethasone (right). Currents are in response to 500 ms pulses from −80 mV to potentials ranging from −10 to +50 mV. (b) Mean current densities as a function of membrane potentials (circles in the absence, squares in the presence of 100 nM dexamethasone, 5–8 h). The current–voltage relationships for I_{peak} (left) and I_{sus} (right, measured at 500 ms) show that only I_{sus} is altered, with significant augmentation ($P<0.005$, denoted by ** here and in subsequent figures) between −30 and +50 mV.

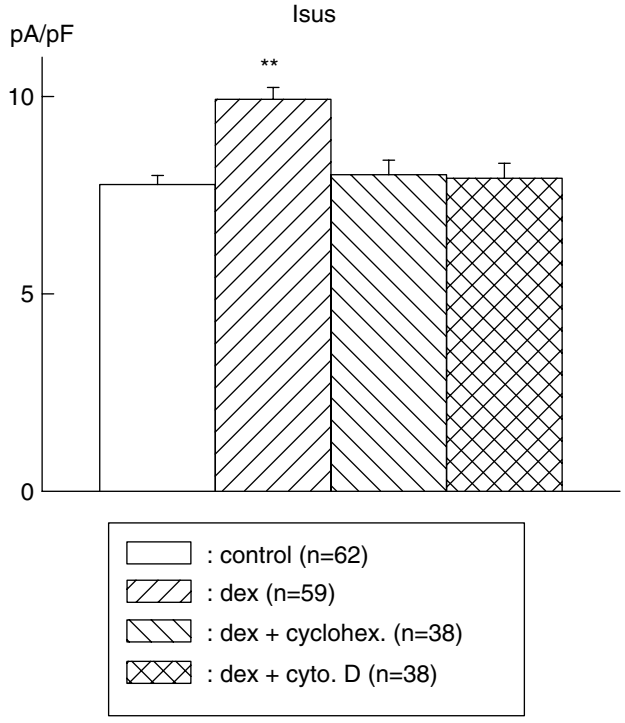

Figure 2 Cycloheximide and cytochalasin D abolish the augmentation of I_{sus} by dexamethasone. Mean (±s.e.m.) I_{sus} density at +50 mV is shown in the absence of drugs, with dexamethasone alone (100 nM, 5–8 h), and with dexamethasone and either cycloheximide (2 μM) or cytochalasin D (1 μM). These were added 1 or 2 h before dexamethasone, respectively.

not shown) where currents reflect the background current I_{K1}, no effects of dexamethasone were observed.

The lag time required for current augmentation, as well as the increased expression of Kv1.5 protein (Takimoto & Levitan, 1994) suggested augmented channel protein synthesis. When dexamethasone was added in the presence of the protein synthesis inhibitor cycloheximide (2 μM, added 1 h before dexamethasone), the augmentation of I_{sus} was abolished, as shown in Figure 2. In earlier work, we found that disruption of actin microfilaments by cytochalasin D prevented I_{sus} augmentation by insulin (Shimoni *et al*., 1999). In this study, we found that the effects of dexamethasone were also abolished by cytochalasin D (1 μM, added 2 h before dexamethasone), suggesting that new channels induced by dexamethasone require the actin network to become functionally expressed in the membrane. The mean current densities at +50 mV were 7.8 ± 0.3 ($n=62$), 9.5 ± 0.3 ($n=59$), 8.0 ± 0.4 ($n=38$) and 7.9 ± 0.4 pA pF^{-1} ($n=38$) in the absence of drug, with dexamethasone, and with dexamethasone with either cycloheximide or cytochalasin D, respectively. These results are shown in Figure 2.

The lag time for dexamethasone action on I_{sus} and its sensitivity to cycloheximide suggest genomic actions, as proposed by Takimoto & Levitan (1994). These are mediated by transcription factors (Falkenstein *et al*., 2000), which interact in a complex manner with cell-specific phosphorylation events (Nordeen *et al*., 1994). We therefore examined whether PKC or PKA play a role in mediating the actions of dexamethasone on I_{sus}.

Cells were incubated with the PKC inhibitor bis-indolylmaleimide (100 nM) for 30 min prior to the addition of (100 nM) dexamethasone (PKC inhibitor maintained). PKC inhibition did not prevent augmentation of I_{sus}, measured after 5–7 h. In this group of cells, I_{sus} densities (at +50 mV) were 7.1 ± 0.3 ($n=25$) and 11.0 ± 0.7 pA pF^{-1} ($n=23$) in the absence or presence of the drug combination, respectively ($P<0.0001$). In control experiments, addition of bis-indolylmaleimide on its own (100 nM, 5–9 h) had no effect on I_{sus}. In these experiments, the mean density of I_{sus} (at +50 mV) was 6.8 ± 0.3 ($n=20$) and 6.9 ± 0.3 pA pF^{-1} ($n=18$) in the absence or presence of the PKC inhibitor, respectively ($P>0.05$).

The specific inhibitor of PKA RpCAMPS (100 μM, added 30 min before dexamethasone) also did not prevent the increase in I_{sus}, with mean I_{sus} densities (at +50 mV) of 7.3 ± 0.3 ($n=28$) and 9.3 ± 0.6 pA pF^{-1} ($n=24$) in the absence or presence of drugs, respectively ($P<0.003$). These results are shown in Figure 3.

Glucocorticoid actions may also be mediated by tyrosine kinases (Giorgino *et al*., 1993; Koukouritaki *et al*., 1999; Sakoda *et al*., 2000). We incubated cells with the nonspecific tyrosine kinase inhibitor genistein prior to adding dexamethasone. The augmentation of I_{sus} was partially (but significantly, $P<0.001$) blunted by 50 μM genistein, whereas 100 μM completely prevented I_{sus} augmentation. In contrast, the inactive analog genistin (100 μM) did not prevent I_{sus} augmentation. Mean I_{sus} values (at +50 mV) were 9.8 ± 0.4 ($n=32$), 11.9 ± 0.6 ($n=22$), 8.9 ± 0.5 ($n=26$) and 11.8 ± 0.8 pA pF^{-1} ($n=16$) in the absence of drugs, with dexamethasone alone, or with dexamethasone and either genistein or genistin (100 μM for both drugs), respectively, as shown in Figure 4.

Diabetic myocytes

Subsequently, we tested the effects of dexamethasone on I_{sus} in cells from STZ-diabetic rats. I_{sus} is attenuated in diabetic conditions, more prominently in males than females (Shimoni & Liu, 2003b). In contrast to control myocytes, I_{sus} augmentation by dexamethasone was nearly abolished in cells from male diabetic rats. Mean densities (at $+50$ mV) were 5.2 ± 0.1 ($n=66$) and 5.8 ± 0.3 pA pF^{-1} ($n=57$). This slight increase (11%, $P<0.04$) was much smaller ($P<0.015$) than the 28% increase of I_{sus} in control myocytes. Sample current traces and current–voltage relationships for I_{peak} and I_{sus} in the absence or presence of dexamethasone in cells from STZ-diabetic male rats are shown in Figure 5.

In earlier work, we showed that many of the electrophysiological consequences of STZ-diabetic conditions are reduced or absent in females (Shimoni & Liu, 2003b; 2004). In the present work, we tested the effects of dexamethasone on I_{sus} in cells from diabetic females. In marked contrast to the greatly diminished effect in diabetic males, dexamethasone significantly augmented I_{sus} in cells from diabetic females, to a similar extent (by 31%) as in control males. The mean densities of I_{sus} at $+50$ mV in these experiments were 4.7 ± 0.3 ($n=21$) and 6.1 ± 0.3 pA pF^{-1} ($n=27$) ($P<0.002$). I_{peak} was unchanged by dexamethasone in these cells. Sample current traces and current–voltage relationships for I_{peak} and I_{sus} are shown in Figure 6.

In an attempt to define some of the mechanisms underlying these gender differences, we tested the role of estrogen. The effects of dexamethasone on I_{sus} in cells from diabetic ovariectomized females were measured. In these cells, dexamethasone had no effect on I_{sus}, as in diabetic males. Mean densities at $+50$ mV were 6.3 ± 0.3 ($n=21$) and 5.9 ± 0.3 pA pF^{-1} ($n=22$) ($P>0.05$) in the absence and presence of dexamethasone, respectively. Finally, we measured the effects of dexamethasone on I_{sus} in control, nondiabetic females. Under control conditions, dexamethasone augmented I_{sus} in females as in males. In these cells, I_{sus} (at $+50$ mV) was significantly augmented from 7.4 ± 0.3 ($n=25$) to 8.9 ± 0.4 pA pF^{-1} ($n=23$)

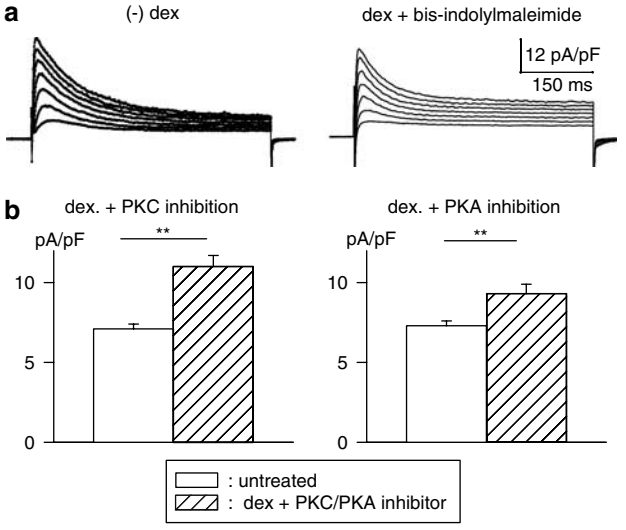

Figure 3 Effects of dexamethasone are maintained in the presence of PKC and PKA inhibition. (a) Sample current traces (same protocol as Figure 1) obtained from two cells, in the absence of dexamethasone (left), or (on the right) following 5 h in (100 nM) dexamethasone and the PKC inhibitor bis-indolylmaleimide (100 nM, added 30 min before dexamethasone). (b) Mean I_{sus} densities (at $+50$ mV) in the absence of drugs (open bars) or with dexamethasone and the PKC inhibitor (left, hatched bar) or (on the right) with dexamethasone and the PKA inhibitor RpCAMPS (100 μM) added 30 min before dexamethasone (hatched bar).

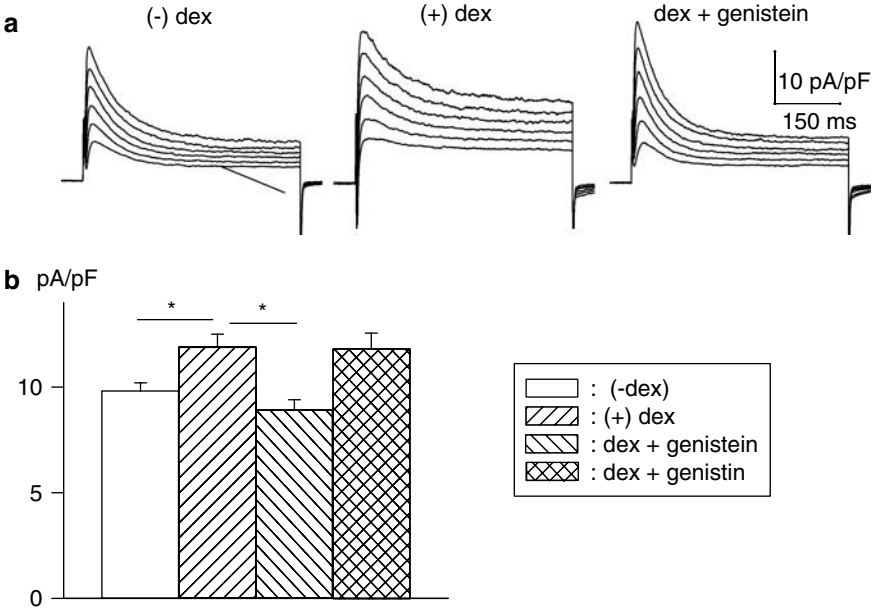

Figure 4 Genistein inhibits the augmentation of I_{sus} by dexamethasone. (a) Sample current traces, obtained in response to pulses from -80 to potentials ranging from 0 to $+50$ mV, in an untreated cell (left), a cell treated (5 h) with 100 nM dexamethasone (center) and a cell treated with dexamethasone (for 5.5 h) and 100 μM genistein, added 30 min before dexamethasone (right). (b) Summary data showing mean current densities at $+50$ mV in the absence or presence of dexamethasone, and with dexamethasone and either genistein (100 μM) or the inactive analog genistin (100 μM). Whereas genistein significantly ($P<0.005$) blunts I_{sus} augmentation by dexamethasone, genistin has no effect, suggesting that tyrosine kinases mediate the effects on I_{sus}.

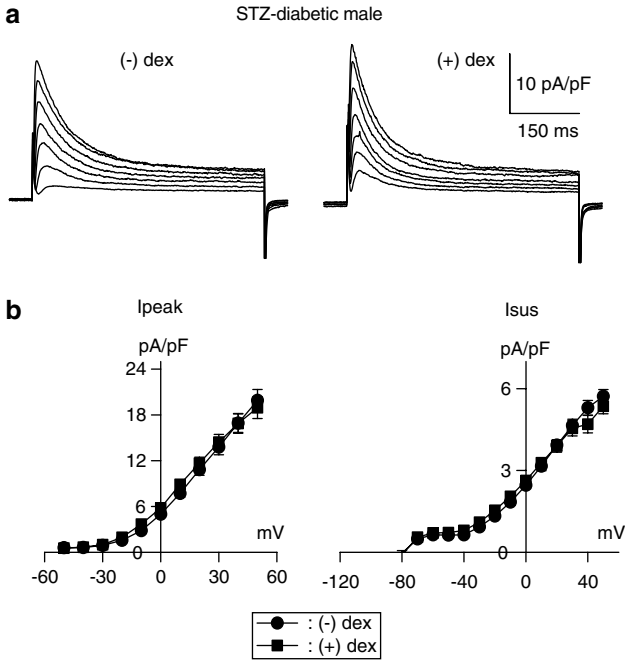

Figure 5 Absence of I_{sus} augmentation by dexamethasone in cells from STZ-diabetic males. (a) Sample current traces (voltage steps from −80 to potentials ranging from −10 to +50 mV) in two cells from diabetic rats, in the absence (left) or after 7 h in (100 nM) dexamethasone (right). (b) Current–voltage relationships for I_{peak} (left) and I_{sus} (right), showing no significant differences in mean current densities in the absence (circles) or presence (5–9 h) of dexamethasone (squares).

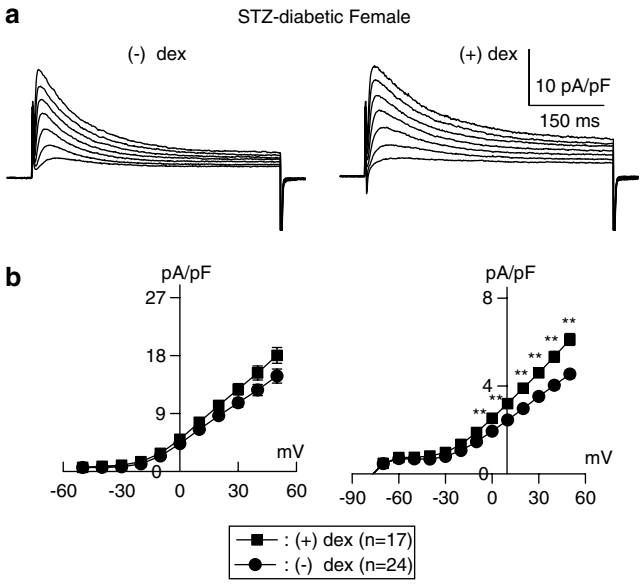

Figure 6 Augmentation of I_{sus} by dexamethasone in STZ-diabetic females. (a) Sample current traces (in response to −10 to +50 mV voltage steps) in two cells from diabetic females, either untreated (left) or following 5.5 h in (100 nM) dexamethasone. (b) Current–voltage relationships for I_{peak} (left) and I_{sus} (right), showing the augmentation of I_{sus}, which was highly significant ($P<0.005$) between −10 and +50 mV.

($P<0.002$). Thus, gender differences are not seen in control, but are elicited under diabetic conditions.

The relative effects of dexamethasone were compared in all these groups using ANOVA. For each group, the current density in response to dexamethasone in each cell was divided by the mean current density obtained in untreated cells. This gave a population of individual ratios indicating the degree of I_{sus} augmentation in treated cells relative to a baseline mean I_{sus}. These ratios were averaged, and the effects of dexamethasone compared in control and diabetic males and females. The mean augmentation of I_{sus} in males (by 1.28 ± 0.04, $n=70$) and in females (by 1.21 ± 0.05, $n=23$) was not significantly different ($P>0.05$). In diabetic cells, the mean augmentation in males was by 1.11 ± 0.05 ($n=57$), which is smaller than in control females ($P<0.015$) and smaller ($P<0.025$) than in diabetic females, where the augmentation was by 1.31 ± 0.07 ($n=27$). In diabetic ovariectomized (ovx) females, the augmentation was by 1.08 ± 0.04 ($n=21$), similar to diabetic males and smaller than in diabetic females ($P<0.015$). This suggests that estrogen plays a role in the differential response to dexamethasone in diabetic hearts (see Discussion).

Discussion

Summary

Several novel findings are presented. First (Figures 1–4), a sensitivity of a cardiac K^+ current to glucocorticoids is present under normal but not under pathological (diabetic) conditions in male rats (Figure 5). Secondly, females exhibit sensitivity in both control and diabetic conditions. Thus, although no gender differences are present under normal conditions, the onset of diabetes leads to gender-specific alterations in glucocorticoid sensitivity (Figure 6).

Interpretation and significance

Glucocorticoids play a central role in cellular function. They have been implicated in the induction of insulin resistance, altered glucose metabolism and diabetes (Andrews & Walker, 1999; Sakoda *et al*., 2000; Bernal-Mizrachi *et al*., 2003). Their effects on K^+ channels have been recognized (Takimoto & Levitan, 1994; Wang *et al*., 1995; Tian *et al*., 2001), but underlying mechanisms are poorly understood. In neonatal mice, chronic dexamethasone attenuated a transient outward current, with no effect on sustained currents (Wang *et al*., 1995). Species and developmental differences probably underlie the differences with regard to the present work. Since the major effect in control cells was on I_{sus}, I_{peak} was not analyzed in detail in the present work, and comparisons relate to currents measured at the end of 500 ms pulses, when the transient component is completely inactivated (Shimoni & Rattner, 2001).

Localized cardiac autocrine mechanisms are activated in several cardiac pathologies such as heart failure and diabetes (Wollert & Drexler, 1999; Fiordaliso *et al*., 2000; Barlucchi *et al*., 2001). Our previous work (Shimoni, 2001; Shimoni & Liu, 2004) demonstrated that local elevation in ANG II in diabetic hearts contributes to alterations in the repolarization of the cardiac action potential, with potential arrhythmogenic implications. However, these mechanisms seem to be less activated in female diabetic hearts (Shimoni & Liu, 2003b; 2004). This presumably results from an inhibition of autocrine

mechanisms by estrogen, since in ovariectomized rats the effects are similar to those in males. Estrogen regulates various components of RAS (Gallagher *et al*., 1999), which supports this interpretation.

Extra-adrenal production of corticosteroids is established (Davies & MacKenzie, 2003), with ANG II as a major stimulant in cardiac tissue (Silvestre *et al*., 1998). The present results suggest that elevated ANG II levels in the diabetic state may activate tyrosine kinases. Such persistent preactivation would preclude I_{sus} augmentation by the addition of dexamethasone. Our earlier work showed a loss of sensitivity to PKC activation in cells from diabetic rats, presumably due to chronic activation of PKC (Shimoni, 1999).

In diabetic females, cardiac ANG II levels are unchanged (Shimoni & Liu, 2004), presumably with no activation of tyrosine kinases, so that I_{sus} is still sensitive to dexamethasone. In ovx-diabetic rats, cardiac ANG II is elevated as in males (Shimoni & Liu, 2004), and, concordantly, the sensitivity to dexamethasone is lost. It should be appreciated that many factors act in concert to determine I_{sus} magnitude. Thus, I_{sus} can be attenuated in male diabetic myocytes despite the chronic activation of tyrosine kinases, since there is a concomitant insulin deficiency and elevated ANG II, both of which attenuate K^+ currents (Magyar *et al*., 1992; Shimoni *et al*., 1999; Yu *et al*., 2000; Shimoni, 2001), presumably outweighing potential I_{sus} enhancement by glucocorticoids.

It should be noted that although the effects of dexamethasone reported here are relatively small, they could have profound impact on repolarization of the action potential. In other studies, it was shown that attenuation of K^+ currents (at membrane potentials corresponding to the action potential plateau) by $\sim$30% significantly prolongs the action potential (e.g. Magyar *et al*., 1992; Shimoni, 2001). In addition, a corresponding prolongation of the QT interval of the electrocardiogram was measured in diabetic rats (D'Amico *et al*., 2001), similar to the QT prolongation commonly measured in human diabetic patients (Robillon *et al*., 1999; Rossing *et al*., 2001). Conversely, an increase in delayed rectifier currents such as I_{sus} would be expected to shorten the action potential. This may occur in larger mammals, in which action potential durations are of the order of 300–400 ms. Control rat action potentials are considerably shorter than in larger mammals (Shimoni, 2001), so that measuring a further abbreviation is not feasible. Nevertheless, glucocorticoid action in rat (and possibly in human) ventricular cells could stabilize action potential duration, by prevention of prolongation. A potentially arrhythmogenic prolongation of the cardiac action potential often occurs during cardiac pathologies such as diabetes, hypertrophy or heart failure (Magyar *et al*., 1992; Li *et al*., 2002). Thus, changes in tonic modulation of K^+ currents by glucocorticoids in different pathologies may have antiarrhythmogenic consequences. Glucocorticoid protection against some types of arrhythmias has indeed been reported in humans and rats (Gorelik *et al*., 2003; Dernellis & Panaretou, 2004). Unfortunately, although changes in the electrocardiogram in human diabetics are very similar to those found in the STZ-diabetic rat (Robillon *et al*., 1999; D'Amico *et al*., 2001), there are no reports in the literature of gender differences in the ECG of human diabetics, or in incidence of diabetes-related arrhythmias. However, it is of interest that under conditions of coronary artery disease, a very common complication of diabetes (Mooradian, 2003), females show a significantly smaller tendency to develop ventricular arrhythmias (Lampert *et al*., 2004).

The results presented here suggest that the effects of glucocorticoids may be gender selective, possibly underlying some gender differences observed in some cardiac arrhythmias (Larsen & Kadish, 1998; Albert *et al*., 2003). This study does not resolve the components of I_{sus}, although the main candidates are Kv1.2, Kv2.1, and Kv1.5 (Nerbonne, 2000). These channels exist in humans and rodents (Nerbonne, 2000), although the HERG channel plays a more dominant role in humans (Mitcheson & Sanguinetti, 1999). The effects of glucocorticoids on HERG are unknown, but an increase in Kv1.5 protein expression in rat ventricle was reported (Takimoto & Levitan, 1994). It is not known at present if this also occurs in humans, in which Kv1.5 protein levels were shown to decrease in atrial fibrillation (Van Wagoner *et al*., 1997). Thus, glucocorticoids may have positive impact on atrial fibrillation, as indeed found by Dernellis & Panaretou (2004).

It is important to emphasize that although no gender differences in glucocorticoid action exist in control conditions, the onset of cardiac pathology may elicit such differences.

Limitations

One limitation of this work is that the tyrosine-phosphorylated proteins that are altered in male (but not female) diabetic hearts have not been identified. The family of tyrosine kinases is very large, and several receptor-associated or receptor-independent kinases may link glucocorticoid receptor activation to channel protein synthesis. In addition, the sustained current reflects a mixed contribute of several channel isoforms. At present, we have not identified which isoforms are affected, or whether alpha or beta subunits are altered. These issues will be determined in future work.

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