Insulin resistance and the modulation of rat cardiac $K^+$ currents

Y. SHIMONI,1 D. SEVERSON,2 AND H. S. EWART2

1Department of Physiology and Biophysics and 2Department of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Received 9 November 1999; accepted in final form 7 February 2000

Resistance to insulin has traditionally been defined in terms of an impairment in the signaling pathway linking insulin to its metabolic effects. This is manifested as a reduction in the stimulation of glucose uptake by insulin, with accompanying changes in the distribution of glucose transporter isoforms (34). However, in recent years it has become clear that insulin exerts many other (nonmetabolic) effects on cardiovascular tissue, resulting in changes in blood flow (25) and in cardiac performance (9). Direct effects on ion channels, such as the L-type calcium channel, have also been suggested (2). Of particular interest are recent findings (46) showing that insulin directly affects the dispersion of ventricular action potential repolarization (as measured by Q-T intervals in the electrocardiogram). This is of great importance because Q-T dispersion is a major determinant of cardiac arrhythmias (42).

We and others have identified changes occurring in two cardiac $K^+$ currents under both insulin-deficient (23, 39) and hyperinsulinemic (39) conditions. Thus, under insulin-deficient conditions, there is an attenuation in both a transient ($I_t$) and a sustained (steady-state) $K^+$ current ($I_{ss}$). Elevated insulin levels augment $I_{ss}$ (but not $I_t$). Our results in animal models of diabetes, as well as results in humans (46), suggest that insulin probably has a tonic modulatory role in determining the magnitude of $K^+$ currents (40), which underlies some of the direct cardiac effects of insulin.

In view of the emerging importance of resistance to the metabolic actions of insulin as a key event in cardiac pathology, it was considered important and timely to investigate whether insulin resistance can also be identified in terms of its actions on cardiac ion channels. Any such changes in cardiac function on $K^+$ channels would affect the cardiac action potential repolarization process, with possible arrhythmogenic implications (42). The major aim of the present study was to determine whether the effects of insulin on $K^+$ currents were diminished or lost under conditions in which effects on glucose metabolism are known to be compromised. A second aim was to establish whether any insulin resistance observed for cardiac $K^+$ currents...
METHODS

Vanadate compounds are known to be insulin mimetic and are used both clinically and in animal models to either reverse (attenuate) insulin resistance or to mimic the effects of insulin (3, 5, 8, 13, 20).

Two rat models known to exhibit different degrees of insulin resistance were used in the present study. One model, used in earlier studies (39), was the fructose-fed rat. A high-carbohydrate diet (60% caloric intake) of fructose or sucrose leads to development of moderate hyperinsulinemia, insulin resistance, and hypertension within several weeks in both rats and dogs (21, 29). The second model used in the present study was a strain of genetically corpulent rats (JCR:LA-cp), which are markedly obese with very high plasma levels of insulin (28). These rats, however, are normotensive. In addition to being very resistant to the metabolic actions of insulin, homozygous cp/cp rats develop cardiac lesions. Some aspects of their cardiac mechanical function are compromised (28, 35).

Our results indicate that in both models of insulin resistance there is an attenuation or abolition of the augmenting effect of insulin on the steady-state K⁺ current \( I_{\text{ss}} \). Furthermore, this resistance to insulin action can be reversed by insulin-sensitizing drugs.

METHODS

All the experiments described here were done in accordance with the guidelines of the Animal Care Committee of the University of Calgary.

Animal models. Two different experimental models of insulin resistance were used in the present study. One was the fructose-fed rat, in which normal Sprague-Dawley (SD) rats were fed a diet enriched with fructose (60% caloric intake; Harlan Teklad, Madison, WI). Within 3–4 wk these rats develop hyperinsulinemia and insulin resistance, with normal or slightly elevated plasma glucose levels, compared with untreated rats (21, 39). SD rats fed a normal chow diet served as the control for fructose feeding. A more severe model of genetic insulin resistance was the JCR:LA-cp rat. The JCR:LA-cp rat, when homozygous for the cp gene (cp/cp), develops obesity and severe hyperinsulinemia with normal or modest elevations of glucose levels (28). In plasma collected at the time the rat was euthanized, the mean glucose and insulin concentrations were 9.8 ± 0.6 mM (means ± SE, \( n = 13 \)) and 446.8 ± 31.4 μM (\( n = 15 \)), respectively. In lean counterparts (cp/+ heterozygotes or homozygous normal), the respective concentrations were 9.4 ± 0.5 mM (\( n = 11 \)) and 115.6 ± 14.2 μM (\( n = 13 \)). In additional experiments, vanadyl sulfate (obtained from Fisher Scientific) was added to the drinking water (0.75 g/l) of cp/cp rats as well as to fructose-fed and control SD rats. In a final subset of experiments, cp/cp rats were given 1,1-dimethylbiguanide (metformin, obtained from Sigma) in the drinking water at 1.4 g/l for 5 days, followed by 4.2 g/l for a further 3–4 wk (37, 44).

Myocyte preparation. In all experiments cells were obtained as described previously (39). Rats were heparinized (2,400 U/kg ip), anesthetized by methoxyflurane inhalation, and then killed by cervical dislocation. The hearts were removed, and the aortas were cannulated on a Langendorff apparatus, followed by retrograde coronary perfusion (at 70 cmH₂O pressure, 37°C) with a solution of the following composition (in mM): 121 NaCl, 5.4 KCl, 2.8 sodium acetate, 1 MgCl₂, 1 CaCl₂, 5 Na₂HPO₄, 24 NaHCO₃, and 5 glucose. After 5 min, this was changed to the same solution with calcium omitted for 10 min, followed by the same solution with the addition of 40 μM CaCl₂, 20 mM taurine, 10 U/ml collagenase (Yakult Honsha, Tokyo, Japan), and 0.01 mg/ml protease (type XIV, Sigma) for 7–8 min. The free wall of the right ventricle was then dissected into smaller pieces, which were further incubated in a shaker bath at 37°C in the calcium-free solution that also contained 0.1 mM CaCl₂, 20 mM taurine, 10 U/ml collagenase, 0.1 mg/ml protease, and 10 mg/ml albumin. Cells were collected over the next 30–60 min and stored in the same basic solution, with no enzymes, with 0.1 mM CaCl₂ and 20 mM taurine.

Recording of currents. Cells were placed in a 1-ml chamber on the stage of an inverted microscope and perfused with a solution containing (in mM) 150 NaCl, 5.4 KCl, 1.0 MgCl₂, 1.0 CaCl₂, 5 HEPES, and 5.5 glucose (titrated to pH 7.4 with NaOH). In all experiments 0.3 mM CdCl₂ was added to block the L-type calcium current. Currents were recorded (at 20–22°C) using the whole cell suction electrode method. Electrodes were pulled from borosilicate glass and filled with a solution containing (in mM) 110 potassium aspartate, 30 KCl, 4 Na₂ATP, 1 MgCl₂, 1 CaCl₂, 10 EGTA, and 5 HEPES (adjusted to pH 7.2 with KOH). Series resistance was minimized by low-resistance electrodes (2–4 MΩ) and by partial electronic compensation (30–40%). When series resistance changed by >10% during recordings the results were discarded. Three K⁺ currents were recorded: I₁, the transient, calcium-independent outward current \( I_{\text{t}} \), obtained in response to depolarizing steps to membrane potentials between −30 and +50 mV and measured as the peak outward current; I₂, the slowly inactivating, delayed rectifier, quasi-steady-state current labeled \( I_{\text{ss}} \), measured at the end of 500-ms pulses to potentials between −30 and +50 mV; and I₃, the background inward rectifier current, \( I_{\text{K₁}} \), measured at the end of 500-ms pulses to potentials between −110 and −30 mV. The main focus in this study was \( I_{\text{ss}} \), which was found previously to be enhanced by elevated insulin levels and attenuated in insulin-deficient conditions (39). \( I_{\text{t}} \) is not affected by elevated insulin but is reduced by insulin deficiency (39). \( I_{\text{K₁}} \) is unchanged by alterations in insulin levels (39). Currents were recorded with an EPC-7 amplifier, digitized (at 2 kHz), and stored for subsequent measurement. To account for differences in cell size, cell capacitance was measured by integration of currents in response to 5-mV steps from −80 mV. Current densities (in pA/pF) were obtained by dividing currents by cell capacitance.

Statistics. Student’s unpaired t-test was used for comparison between different groups with differences showing \( P < 0.05 \) considered significant. Each subgroup of treatments contained its own control group.

RESULTS

Separation of currents. The main focus of this study was to investigate changes in \( I_{\text{ss}} \). Unlike \( I_{\text{t}} \), \( I_{\text{ss}} \) is very insensitive to inhibition by 4-aminopyridine (1) and even very high concentrations of tetraethylammonium do not completely block it (1). Thus it was considered of importance to establish whether \( I_{\text{ss}} \) can be reliably measured without interference from the inactivating \( I_{\text{t}} \). \( I_{\text{t}} \) can be inactivated by short depolarizing pulses to potentials in which it is activated. Thus, by comparing the currents elicited without and with a prepulse that inactivates \( I_{\text{t}} \), it is possible to establish how long the
prepulse (with $I_C$ fact that chronic insulin deficiency attenuates augmented. This was presumed to be a result of the diet for several weeks, the calcium-independent in ventricular cells from rats fed an enriched fructose 500 ms (Fig. 1B). A subtraction of the current with the prepulse to 0 mV (Fig. 1A). These rats, when homozygous for the $cp$ gene, spontaneously develop obesity, hyperinsulinemia, and hyperlipidemia (35). Plasma insulin levels in these rats are very high, up to 10-fold higher than normal. Glucose levels are normal or only modestly elevated, indicating severe resistance to the effects of insulin on glucose uptake into target tissues.

In the first series of experiments, we characterized the potassium currents in cells from these obese (cp/cp) rats, because this had not been done previously. Our results show that in contrast to myocytes from the control cells or to cells from the fructose-fed rats. The data indicate that there may be a saturating magnitude of $I_{ss}$, which is attainable by acute (5–9 h) insulin addition to normal cells or to cells from the fructose-fed rats.

Fructose-fed model. We (39) previously showed that in ventricular cells from rats fed an enriched fructose diet for several weeks, the calcium-independent $I_C$ was unchanged as was the background $I_{K1}$, whereas $I_{ss}$ was augmented. This was presumed to be a result of the chronic elevation in plasma insulin levels, based on the fact that chronic insulin deficiency attenuates $I_{ss}$ (39) and on the finding that in vitro incubation of cells from normal rats with insulin enhances $I_{ss}$ (39).

The fructose-fed model has been shown to be resistant to the metabolic effects of insulin (3). The main question addressed in the present study was whether the augmented steady-state current $I_{ss}$ was still susceptible to insulin stimulation in cardiac cells from these rats. Single ventricular myocytes from these rats were exposed to 100 nM insulin for 5–9 h, and the current magnitudes at the end of 500-ms pulses were measured for voltage steps ranging from −110 to +50 mV.

As shown in Fig. 2, despite the fact that $I_{ss}$ was already larger than normal in these cells, insulin still produced a small but significant ($P < 0.05$) enhancement of the current. Figure 2A shows sample traces from a cell without insulin (left) and from a cell exposed to insulin (right), in which a clear augmentation of the steady-state current is evident. Figure 2B shows summary data indicating that $I_1$ and $I_{K1}$ are unaffected by insulin (as is the case in control cells), whereas $I_{ss}$, despite a larger baseline magnitude in fructose-fed conditions, can still be further significantly ($P < 0.05$) augmented by acute incubation with insulin. At +50 mV, $I_{ss}$ was enhanced from 10.4 ± 0.4 pA/pF (means ± SE, $n = 35$) to 11.7 ± 0.5 pA/pF ($n = 34$). Figure 2C shows a comparison of the effects of insulin on $I_{ss}$ in control cells [8.7 ± 0.4 and 12.1 ± 0.7 pA/pF for cells incubated without (n = 34) and with (n = 22) insulin, respectively]. Also shown are the effects of insulin in cells from the fructose-fed rats. The data indicate that there may be a saturating magnitude of $I_{ss}$, which is attainable by acute (5–9 h) insulin addition to normal cells or to cells from the fructose-fed rats.

Corpulent rats. These rats, when homozygous for the $cp$ gene, spontaneously develop obesity, hyperinsulinemia, and hyperlipidemia (35). Plasma insulin levels in these rats are very high, up to 10-fold higher than normal. Glucose levels are normal or only modestly elevated, indicating severe resistance to the effects of insulin on glucose uptake into target tissues.

In the first series of experiments, we characterized the potassium currents in cells from these obese (cp/cp) rats, because this had not been done previously. Our results show that in contrast to myocytes from the modestly hyperinsulinemic fructose-fed rats, there were no changes in $I_{ss}$. The magnitudes (current densities) of both $I_1$ and $I_{ss}$ were compared with two different controls. A comparison was made to the lean counterparts of these rats, which are either homozygous normal (+/+ or heterozygous for the $cp$ gene (cp/+) in addition. A comparison of current densities was made to the normal SD rats used as controls in all our other studies, including fructose feeding. Because the corpulent rats are typically much larger than age-matched control rats (400–550 g body wt as opposed to 200–300 g body wt), cells were also obtained from larger (−400 g body wt) SD rats, and current densities were compared between these and the corpulent (cp/cp) rats. Figure 3 shows that in either case, the currents in the cp/cp rats are unchanged despite very high levels of circulating insulin. Figure 3A shows sample current traces, obtained from myocytes taken from a lean (left) and corpulent (right) rat, in response to depolarizing voltage steps. Figure 3B (left) shows the mean (± SE) peak $I_{ss}$ densities in the corpulent and large SD rats, as well as (right) the densities for the steady-state currents, measured at the end of 500-ms pulses to voltages

![Fig. 1. Separation of steady-state ($I_{ss}$) and transient outward ($I_t$) currents. A: voltage protocol where 500-ms pulses were given from −80 to +50 mV with (*) or without a prepulse to 0 mV (100 ms). B: prepulse inactivates $I_t$ (current trace with prepulse). The current magnitudes are similar at pulse end, with or without prepulse (arrow), indicating that only $I_{ss}$ is present at 500 ms, when its magnitude was measured. C: difference current with and without prepulse. This gives the uncontaminated transient current, which is seen to completely decay by the end of the depolarizing step.](http://ajpheart.physiology.org/Downloadedfrom)
ranging from −110 to +50 mV. At the negative voltages the currents reflect $I_{K1}$, whereas at the positive potentials the currents reflect $I_{ss}$. In both cases there are no changes, with the comparison in this case made to the lean counterparts of the corpulent rats.

The fact that $I_{ss}$ is unaltered in the obese cp/cp rats, despite the very high levels of insulin in their plasma, suggested that whatever mechanism(s) determine resistance to the metabolic effects of insulin may also cause a complete resistance to the stimulatory effect of insulin on $K_1$ channels in this model. This was tested directly by incubating cells from these rats with 100 nM insulin for 5–9 h. In contrast to myocytes from SD rats, in which insulin enhances $I_{ss}$ (Fig. 2), there was no stimulatory effect of insulin in myocytes from the corpulent rats, as shown in Fig. 3C. Figure 3C (left) shows summary data from cells from the lean counterparts, in which the normal enhancing effect of insulin is observed. Insulin (100 nM, 5–9 h) significantly ($P < 0.05$) increased $I_{ss}$ from 7.9 ± 0.2 pA/pF in the absence of insulin ($n = 27$) to $9.0 ± 0.3$ pA/pF in the presence of insulin ($n = 25$). However, Fig. 3C (right) shows that insulin did not enhance $I_{ss}$ in cells from the corpulent rats. The mean density of $I_{ss}$ (at +50 mV) in this group was $7.5 ± 0.3$ pA/pF in the absence of insulin ($n = 23$) and $7.3 ± 0.3$ pA/pF after incubation for 5–9 h with 100 nM insulin ($n = 24$). In either lean or obese rats, insulin did not enhance $I_{K1}$. The mean density of this current at −110 mV was $9.4 ± 0.4$ pA/pF ($n = 27$) and $10.0 ± 0.5$ pA/pF ($n = 25$) in the absence and presence of 100 nM insulin (>5 h), respectively, in the lean rats. The corresponding values in the corpulent rats were $10.0 ± 0.6$ ($n = 23$) and $10.4 ± 0.4$ ($n = 24$) pA/pF.

The preceding results suggest that insulin resistance can be exhibited in terms of the effects of insulin on cardiac ion channels, as well as metabolic resistance. In addition, there appears to be a gradation in the degree of resistance, with the fructose-fed rats showing responsiveness to the elevated plasma insulin levels in vivo, so that the baseline $I_{ss}$ magnitude is larger. In addition, there is a further enhancing effect of insulin added in vitro, although it is reduced relative to control cells. In the corpulent rats there are no effects of insulin either in vivo or in vitro, suggesting a much more pronounced resistance to effects of insulin on this ion channel.

Modulation of insulin sensitivity. One of the major developments in recent years has been the discovery of drugs that can either mimic the actions of insulin or increase tissue sensitivity to the effects of insulin. Such
drugs are obviously of great potential therapeutic benefit in both Type 1 (insulin-deficient) diabetes, and more so in Type 2 (non-insulin-dependent) diabetes, in which insulin resistance is a major complication. In this context, it has been established that vanadium compounds act as both insulin-mimetic agents and compounds that sensitize tissues to insulin (13, 18, 38). In addition to normalizing hyperglycemia in several models of non-insulin-dependent diabetes (30), vanadate was found to prevent the decline in cardiac performance associated with insulin-deficient diabetes (20).

The actions of vanadate compounds is of particular interest because they have been shown to bypass early events associated with the activation of the insulin receptor (18), with possibly two distinct tyrosine phosphorylation pathways (22). It has also been suggested that, unlike insulin, vanadate does not modify muscle protein synthesis or degradation (7). However, vanadate compounds have been shown to activate transcription factors and to stimulate mitogenesis and cell growth under some conditions (13). Interestingly, mitogen-activated protein (MAP) kinases, which we showed to be involved in the action of insulin on K^+ channels (39), have been shown to be directly activated by vanadate, independently of insulin receptor autophosphorylation (11).

In the present study, experiments with vanadyl sulfate were conducted based on the fact that previous work had demonstrated that oral vanadate decreases muscle insulin resistance in both rodents and human diabetic patients (5, 8). Furthermore, vanadyl sulfate was shown to prevent the development of hyperinsulinemic, insulin-resistant conditions or to restore insulin sensitivity after induction of insulin-resistance by fructose feeding (3). We therefore attempted to see whether adding vanadyl sulfate to the drinking water of fructose-fed rats prevented the augmentation in I_{ss} associated with this model.

**Effects of vanadyl sulfate.** In these experiments rats received vanadyl sulfate (0.75g/l in their drinking water) for 1 wk before and during the 3–4 wk fructose feeding. However, in contrast to experiments showing that vanadyl sulfate prevents the effects of high-fructose feeding on glucose metabolism (3), in myocytes isolated from these rats I_{ss} was still significantly enhanced compared with untreated rats and was comparable to the augmented magnitude seen after fructose feeding with no vanadyl sulfate (I_{ss} was similar in all groups). Thus I_{ss} density (at +50 mV) is 8.7 ± 0.4
pA/pF, n control (C), fructose fed (F), fructose fed 1 I the maximal density of value in fructose-fed rats without vanadyl (10.8 45), which was not significantly different from the vitro incubation with insulin (VF
1 2 110 mV) in this group was 2 12.5 6 pA/pF in control myocytes (left) and from a myocyte obtained a control myocyte (right). Rat was fructose fed for 4 wk; VS was started 1 wk before fructose feeding and maintained throughout. Downward traces are currents obtained in response to pulses to −110 mV and reflect I_{K1}, which is not significantly different in the two groups. Arrow: zero-current level. See RESULTS for details. B: mean (± SE) I_{ss} densities in control cells, fructose-fed rats, in fructose-fed+VS-treated rats, and fructose-fed+VS treated rat cells incubated with insulin (100 nM) for 5–9 h. I_{ss} in all treated groups was significantly (P < 0.05) larger than control group, but differences between treatments were not significant. A trend toward enhancement of I_{ss} by VS, without and with insulin, can be seen.

pA/pF in control myocytes (n = 34), 10.5 ± 0.5 pA/pF in fructose-fed rats (n = 34), and 11.3 ± 0.4 pA/pF in fructose-fed rats with vanadyl sulfate (n = 46). Furthermore, incubating cells from the fructose + vanadyl sulfate rats with 100 nM insulin for 5–9 h produced a further small (but not significant) augmentation of I_{ss}, to 12.5 ± 0.6 pA/pF (n = 43). These results are shown in Fig. 4. Figure 4A shows sample current traces from a control myocyte (left) and from a myocyte obtained from a rat treated with vanadyl sulfate for 4 wk (right). Figure 4B shows the current-voltage relationship for the control and vanadyl sulfate-treated group (P < 0.005). Note that at negative potentials, at which the current reflects I_{K1}, there are no differences. No differences were observed in the magnitude of I_{1} in the two groups (not shown). Figure 5C shows the summary data for I_{ss} densities in the two groups as well as results for vanadyl sulfate-treated cells incubated (in vitro) with insulin.

An important control experiment was subsequently conducted to rule out a possible direct enhancing effect of vanadyl sulfate on the I_{ss} channel. In this context, a comparison was made with the effects of insulin on this current. Insulin was previously found to augment I_{ss} only after at least 5 h of incubation (39). This result, as well as the inhibition of the effects of insulin by cycloheximide, suggested that insulin acts by inducing the formation of new channels. In the present study, it was found that addition of a high concentration (100 μM) of vanadyl sulfate directly to the solution-perfusing control cells failed to augment I_{ss} acutely (up to 20 min exposure), whereas after 5 h I_{ss} was augmented. In this group, I_{ss} density without vanadyl sulfate (at +50 mV) was 7.3 ± 0.4 pA/pF (n = 22), whereas after vanadyl sulfate for >5 h I_{ss} density was significantly (P < 0.0001) enhanced to 11.6 ± 0.4 pA/pF (n = 27). This effect could be blocked by adding 2 μM cycloheximide to the vanadyl sulfate solution. In this group, I_{ss} density (at +50 mV) was 8.7 ± 0.9 pA/pF in untreated myocytes (n = 17) and was significantly (P < 0.005) enhanced by vanadyl sulfate (100 μM, >5 h) to 12.6 ± 0.5 pA/pF (n = 19). Cycloheximide (with vanadyl sulfate) significantly (P < 0.005) attenuated I_{ss} to 9.9 ±
0.6 pA/pF ($n = 19$). These results are shown in Fig. 6. Figure 6A shows sample current traces from a control myocyte (left) and from a myocyte after $5 \text{ min}$ of vanadyl sulfate (right), illustrating the augmented $I_{\text{ss}}$. Figure 6B shows that addition of vanadyl sulfate has no effects on $I_t$ or $I_{\text{ss}}$ magnitudes during the first 10 min of exposure. Figure 6C shows that the significant enhancement of $I_{\text{ss}}$ by vanadyl sulfate (left) is abolished by cycloheximide. $I_t$ (right) was not affected by any of these treatments.

These results suggest that vanadyl sulfate has an enhancing effect on $I_{\text{ss}}$, presumably by inducing the formation of new channels, in a manner similar to the effects previously reported for insulin (39). Thus it is not possible to demonstrate a reversal of the effects of fructose feeding (with the accompanying elevated insulin levels) with vanadyl sulfate. However, because vanadate compounds act by (at least partly) different pathways than insulin (although a common final pathway is suggested), the following issue was considered: because vanadate improves insulin sensitivity and reduces insulin resistance (5, 8), it was still of interest to investigate whether vanadyl sulfate could affect $I_{\text{ss}}$ in the more pronounced insulin-resistant model. Thus, in another set of experiments, corpulent cp/cp rats were given vanadyl sulfate in their drinking water for 3–4 wk. In myocytes obtained from these rats, $I_{\text{ss}}$ was unaffected (results not shown), implying that the severe resistance to insulin is also expressed as resistance to the effects of vanadyl sulfate. $I_{\text{ss}}$ density after vanadyl treatment was $7.3 \pm 0.4$ pA/pF ($n = 27$), compared with $7.5 \pm 0.3$ pA/pF for untreated cp/cp myocytes ($n = 23$) and $7.3 \pm 0.3$ pA/pF for cp/cp cells ($n = 24$) treated with insulin in vitro (Fig. 3).

Thus the mechanism(s) or alterations in the signaling pathways that lead to severe resistance to the effects of insulin in cp/cp rats also prevent the effects of vanadyl sulfate. The insulin-sensitizing effects of vanadate compounds that have been reported in the context of glucose homeostasis are not apparent as a sensitizing to the effects of insulin on $I_{\text{ss}}$. However, other insulin-sensitizing drugs are commonly used clinically to alleviate the complications of insulin resistance and improve glucose handling (26). In the final set of experiments, we attempted to reverse the extreme insulin resistance of the corpulent rats with one such drug, the biguanide drug metformin.
Effects of metformin. Metformin decreases insulin resistance and improves insulin sensitivity, which is expressed as peripheral glucose uptake (37). Metformin was also found to decrease insulin levels in spontaneously hypertensive rats (44). At high concentrations, metformin enhances glucose uptake of cardiac cells, although this may not be its main mode of action (16) because other actions have also been identified. These include suppression of hepatic glucose output (10) and changes unrelated to glycemic control, such as alterations in lipid profiles (32). In isolated hearts from insulin-deficient (streptozotocin-induced) diabetic rats, cardiac function was improved by metformin treatment, in addition to lowering plasma glucose levels (45).

In this set of experiments, metformin was added to the drinking water of the corpulent rats; 1.4 g/l for the first 5 days, followed by 4.2 g/l for 3–4 additional wk (based on Refs. 37, 44). This treatment had no effect on $I_t$, with a mean density (at +50 mV) of $25.9 \pm 1.8 \text{pA/pF}$, similar to values in untreated myocytes. $I_{K1}$ was also unaffected, with a mean density (at $-110 \text{mV}$) of $-9.6 \pm 0.5 \text{pA/pF}$. In this group, $I_{ss}$ density (at +50 mV) was slightly lower than in untreated myocytes, with a mean value of $6.0 \pm 0.2 \text{pA/pF}$ ($n = 26$). However, incubation of myocytes from metformin-treated cp/cp rats with 100 nM insulin ($>5$ h) produced a very significant ($P < 0.005$) enhancement of $I_{ss}$. The mean density at $+50 \text{mV}$ after incubation with insulin was $8.1 \pm 0.4 \text{pA/pF}$ ($n = 29$). This result is shown in Fig. 7A, which shows sample current traces from two myocytes from a metformin-treated cp/cp rat in the absence of insulin (left) and after in vitro incubation with insulin for 6 h (right). Figure 7B shows the steady-state current-voltage relationship for cells in the absence or presence of insulin. Insulin significantly enhances $I_{ss}$ at membrane potentials between $+10$ and $+50 \text{mV}$. Note that at negative potentials, no differences are observed, indicating no effect of insulin on $I_{K1}$. The summary data for the effects of insulin on $I_t$, $I_{ss}$, and $I_{K1}$ are shown in Fig. 7C.

**DISCUSSION**

**Summary of findings.** The results presented here show for the first time that resistance of cardiac tissue to the effects of insulin can be manifested as changes in the function of an ion channel, in addition to the more
elaborate previously defined resistance to the metabolic effects of insulin. Thus both mild and severe resistance to the normal ability of insulin to enhance a delayed rectifier $K^+$ current, $I_{ss}$, were observed with the two rat models used in this study. In the fructose-fed model, the mild hyperinsulinemia and modest insulin resistance is accompanied by a chronic elevation of $I_{ss}$ magnitudes, with a further slight augmentation obtained by incubation with 100 nM insulin (Fig. 2). In the cp/cp model, which is extremely hyperinsulinemic with severe insulin resistance, no changes in basal $I_{ss}$ were found and incubation with 100 nM insulin had no stimulatory effects (Fig. 3). Vanadyl sulfate, an insulin mimetic that had been found to prevent the metabolic consequences of insulin action in the fructose-fed model, did not reverse the augmentation of $I_{ss}$ (Fig. 4). This was due to a direct insulin-like action of vanadyl sulfate on $I_{ss}$ as seen in both in vivo treatment and in vitro incubation (Figs. 5 and 6). However, the in vitro stimulatory effect required several hours and was inhibited by cycloheximide (Fig. 6), similar to the in vitro effects of insulin (39). Addition of insulin did not further augment $I_{ss}$ (Fig. 5). Chronic exposure to vanadyl sulfate had no effect on $I_{ss}$ in the corpulent rats. Finally, the severe insulin resistance in the corpulent rats was partly reversible by the biguanide drug metformin (Fig. 7).

Potential mechanisms and significance. Insulin resistance, which is of prime clinical importance due to its prevalence in a variety of common clinical disorders (14, 34), has been the subject of intense investigation (17, 24). Defects in the action of insulin have been detected at both receptor and postreceptor levels (36). Insulin signaling is extremely complex, with multiple pathways activated subsequent to receptor binding (6). Many components have been implicated in the changes occurring before or after the development of insulin resistance, such as the receptor itself (17) as well as the receptor substrates IRS-1 and IRS-2 (17, 47). Changes in downstream targets and effectors, such as MAP kinase and glucose transporters, are also involved in the development of insulin resistance (12, 19, 41). The enhancement of $K^+$ currents in ventricular cells by insulin is cycloheximide sensitive, dependent on activation of MAP kinase, and presumably reflects the synthesis of new channels (39, 40). However, the precise signaling pathway for this effect is unknown. In this context, the results with vanadyl sulfate were of interest. Vanadyl sulfate and insulin actions were occlusive (nonadditive). Thus, even though vanadyl sulfate bypasses the insulin receptor and its activation of receptor substrates (38), our results suggest that the signaling pathways of insulin and vanadyl sulfate converge at some point. The common actions probably involve the consequences of altered tyrosine phosphorylation (13). Interestingly, vanadyl sulfate was also shown (11) to stimulate MAP kinase, which is an essential step leading to $I_{ss}$ augmentation. Conversely,
severe insulin resistance in cp/cp rats (manifested as insensitivity to both in vivo elevation of insulin and to in vitro insulin incubation) was also expressed as resistance to the action of vanadyl sulfate, which suggests that the resistance to the effects of insulin on $I_{ss}$ lies (at least partly) downstream to the point of convergence of the vanadyl sulfate and insulin pathways.

The results with metformin were of interest as well, showing that even severe insulin resistance (in terms of the effects of insulin and vanadyl sulfate on ion channels in cells from corpulent rats) can be at least partly reversed by a drug that sensitizes the tissue to the metabolic effects of insulin. The precise mechanism of metformin action is unknown, but these results suggest that a key step (or steps) in the signaling pathway of insulin, common to both metabolic and electrophysiological effects of insulin, is altered when insulin resistance is established.

Obviously, much more work is required to elucidate the precise components that are altered in conditions of resistance to the actions of insulin on $K^+$ channels. It is not yet clear how insulin resistance affects cardiac performance. Although there is some evidence that the mechanical performance is unimpaired in some conditions of insulin resistance (35), there is contrasting evidence showing that contractility is altered (28). Furthermore, the ventricular function of non-insulin-dependent diabetic patients was found to be impaired, with significant reductions in cardiac output (15). Insulin resistance has also been implicated in the deterioration of cardiac failure (43). Changes in tonic modulatory effects of insulin on a repolarizing current may affect action potential duration and thereby indirectly affect contraction through changes in action potential duration (4). Furthermore, resistance to modulation of $K^+$ currents by insulin may also alter the repolarization of the cardiac action potential. Changes in ventricular repolarization and dispersion of repolarization have been shown to be a major cause of cardiac arrhythmias and mortality (42), which are more common in insulin-dependent and non-insulin-dependent diabetes mellitus (27). Insulin has recently been shown to directly affect dispersion of repolarization (46), which suggests that changes in sensitivity to insulin may directly alter the susceptibility to cardiac arrhythmias. The connection among insulin, insulin resistance, and cardiac arrhythmias obviously requires further studies.

**Limitations.** The major limitation of this study is shared by other studies of insulin resistance: namely, that this condition is complex, with multiple alterations in a very complex signaling system. The exact pathway leading from insulin binding (or vanadyl sulfate action) to the modulation of potassium currents is less well understood than other aspects of insulin action, making it more difficult at present to determine which components may be altered in the pathway leading to alterations in potassium current modulation.

A further limitation is that the molecular identity of $I_{ss}$ is unknown at present. It would obviously be very important to establish changes in mRNA and protein levels, in parallel to changes in current magnitude. This must await further developments.

This work was supported by a grant (to Y. Shimoni) from the Canadian Diabetes Association in honor of Mary Selina Jamieson.

**REFERENCES**

11. D’Onofrio F, Le MQU, Chiasson JL, and Srivastava AK. Activation of mitogen activated protein (MAP) kinases by vana


