Dexamethasone treatment causes resistance to insulin-stimulated cellular potassium uptake in the rat

Michael S. Rhee, Anjana Perianayagam, Pei Chen, Jang H. Youn, and Alicia A. McDonough
Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California, Los Angeles, California 90033

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Rhee, Michael S., Anjana Perianayagam, Pei Chen, Jang H. Youn, and Alicia A. McDonough. Dexamethasone treatment causes resistance to insulin-stimulated cellular potassium uptake in the rat. Am J Physiol Cell Physiol 287: C1229–C1237, 2004. First published June 22, 2004; doi:10.1152/ajpcell.00111.2004.—Patients treated with glucocorticoids have elevated skeletal muscle ouabain binding sites. The major Na\(^+\)-K\(^+\)-ATPase (NKA) isoform proteins found in muscle, \(\alpha_2\) and \(\beta_1\), are increased by 50% in rats treated for 14 days with the synthetic glucocorticoid dexamethasone (DEX). This study addressed whether the DEX-induced increase in the muscle NKA pool leads to increased insulin-stimulated cellular K\(^+\) uptake that could precipitate hypokalemia. Rats were treated with DEX or vehicle via osmotic minipumps at one of two doses: 0.02 mg·kg\(^{-1}\)·day\(^{-1}\) for 14 days (low DEX; \(n = 5\) pairs) or 0.1 mg·kg\(^{-1}\)·day\(^{-1}\) for 7 days (high DEX; \(n = 6\) pairs). Insulin was infused at a rate of 5 mU·kg\(^{-1}\)·min\(^{-1}\) over 2.5 h in conscious rats. Insulin-stimulated cellular K\(^+\) and glucose uptake rates were assessed in vivo by measuring the exogenous K\(^+\) infusion (\(K_{inf}^+\)) and glucose infusion (\(G_{inf}\)) rates needed to maintain constant plasma K\(^+\) and glucose concentrations during insulin infusion. DEX at both doses decreased insulin-stimulated cellular K\(^+\) uptake as previously reported. \(G_{inf}\) (in mmol·kg\(^{-1}\)·h\(^{-1}\)) was 10.2 ± 0.6 in vehicle-treated rats, 5.8 ± 0.8 in low-DEX-treated rats, and 5.2 ± 0.6 in high-DEX-treated rats. High DEX treatment also reduced insulin-stimulated K\(^+\) uptake. K\(_{inf}^+\) (in mmol·kg\(^{-1}\)·h\(^{-1}\)) was 0.53 ± 0.08 in vehicle-treated rats, 0.49 ± 0.14 in low-DEX-treated rats, and 0.27 ± 0.08 in high-DEX-treated rats. DEX treatment did not alter urinary K\(^+\) excretion. NKA \(\alpha_2\)-isoform levels in the low-DEX-treated group, measured by immunoblotting, were unchanged, but they increased by 38 ± 15% (soleus) and by 67 ± 3% (gastrocnemius) in the high-DEX treatment group. The NKA \(\alpha_1\)-isoform level was unchanged. These results provide novel evidence for the insulin resistance of K\(^+\) clearance during chronic DEX treatment. Insulin-stimulated cellular K\(^+\) uptake was significantly depressed despite increased muscle sodium pump pool size.

skeletal muscle; sodium pump; Na\(^+\)-K\(^+\)-ATPase.

SKELETAL MUSCLE CONTAINS the body’s largest pool of sodium pumps, and the activity of these pumps can regulate extracellular K\(^+\) concentration (26). Skeletal muscle sodium pump activity is acutely increased by insulin, which clears postprandial K\(^+\) from the plasma, and by catecholamines, which returns K\(^+\) to the muscle intracellular fluid after exercise (8, 19, 22, 26), both of which are important in maintaining a normal K\(^+\) gradient and membrane potential across the plasma membrane of excitable cells. The clinical use of \(\beta_2\)-agonists for asthma attacks has been reported to occasionally provoke cardiac arrhythmias accompanied by (and perhaps driven by) hypokalemia, consistent with acute activation of muscle sodium pumps in vivo (18, 28, 30).

Chronic glucocorticoid treatment in patients, experimental subjects, or animals leads to an increase in pool size of skeletal muscle ouabain binding sites (15, 31). Investigators at our laboratory previously examined which sodium pump isoforms contribute to the increase in ouabain binding sites (35). In rats treated for 14 days, the synthetic glucocorticoid dexamethasone (DEX) caused >50% increases in Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\)- and \(\beta_1\)-isoform (the major isoforms in muscle) protein levels in all muscle groups while having no significant effect on \(\alpha_2\)- or \(\beta_1\)-isoform levels. The change in pool size is driven by increased synthesis as \(\alpha_2\) and \(\beta_1\) mRNA levels increase in parallel. Despite the increase in sodium pump pool size, skeletal muscle levels of Na\(^+\) and K\(^+\) are unchanged by DEX treatment (14, 15). Thus it is not known whether the increased abundance of \(\alpha_2\)- and \(\beta_1\)-subunits and ouabain binding predict increased muscle Na\(^+\)-K\(^+\)-ATPase activity in vivo. Theoretically, an increase in functional muscle sodium pumps would increase acute insulin-driven cellular K\(^+\) uptake after a meal as well as acute catecholamine-driven cellular K\(^+\) uptake during an asthma attack. This question is clinically important because glucocorticoids are routinely administered for a variety of medical conditions, including asthma, and it is important to know whether insulin action or treatment in glucocorticoid-treated patients carries an extra risk of hypokalemia and ensuing arrhythmias and cardiac arrest (43).

In this study, we aimed to test the hypothesis that the DEX-induced increase in pool size of skeletal muscle Na\(^+\)-K\(^+\)-ATPase causes an increase in insulin-driven cellular uptake of K\(^+\). To address this question, we applied a technique recently developed at our laboratory for quantifying in vivo insulin action on cellular K\(^+\) uptake, termed the “K\(^+\) clamp” (6, 26). In this method, plasma K\(^+\) is maintained constant during insulin infusion by varying exogenous K\(^+\) infusion (\(K_{inf}^+\)), and the K\(^+\) infusion rate required to clamp plasma K\(^+\) is a measure of insulin action to increase cellular K\(^+\) uptake. This approach is analogous to the glucose-clamp technique (12), which has been used widely in studies of insulin action on glucose metabolism in vivo. Of the many homeostatic systems of the body, the K\(^+\) and glucose homeostatic systems are unique in that they share acute regulation by insulin. That DEX causes insulin resistance to glucose uptake is well established (4, 13, 32, 34, 38–40), but whether it causes a change in insulin-stimulated K\(^+\) uptake has not, to our knowledge, been investigated.

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METHODS

DEX treatment. Male Sprague-Dawley rats, 9–10 wk of age (180–200 g), were maintained on standard chow at a constant temperature (21°C) under a 12:12-h artificial light-dark cycle with unlimited access to water. DEX or vehicle (polyethylene glycol 400) was continuously infused via dorsally implanted osmotic minipumps (model 2002; Alzet, Palo Alto, CA). Two DEX protocols were studied: 0.02 mg·kg⁻¹·day⁻¹ for 14 days (low DEX) and 0.1 mg·kg⁻¹·day⁻¹ for 7 days (high DEX).

Catheterization. Three days before the experiments, animals were placed in individual cages with wire floors. The distal one-third of each rat’s tail was drawn through a hole placed low on the side of the cage and secured there with a rubber stopper. This arrangement was required to protect tail blood vessel catheters during experiments (42). Animals were free to move about and allowed unrestricted access to food and water. Two infusion catheters were inserted into the tail vein on the day before the experiment, and one arterial blood sampling catheter was inserted into the tail 6 h before the start of experiments (~0700). Catheters were placed percutaneously during local anesthesia with lidocaine while the rat’s torso was restrained in a towel. The tail was drawn through a hole placed low on the side of the cage. The tail was fixed to the cylinder of the cage with a block of aluminum, and stored at ~70°C for later analysis.

K⁺ and glucose clamps. Food was removed from the cage at ~0700. At ~1300, basal blood samples were taken from the tail artery. Experiments began with a constant infusion of porcine insulin (5 mU·kg⁻¹·min⁻¹; Novo Nordisk, Princeton, NJ) through a tail vein catheter for 2.5 h. Insulin infused via a Y connector through a tail vein catheter at variable rates placed in individual cages with wire floors. Animals were anesthetized with pentobarbital sodium and skeletal muscles were rapidly dissected out, frozen using liquid N₂-cooled aluminum blocks, and stored at ~70°C for later analysis.

Urine. Urine was collected from the floor of the cage as previously described (42), both during 2.5 h before the clamp and during the 2.5-h clamp. In addition, urine was collected from the urinary bladder while the rats were under anesthesia at the end of the clamp. The urinary bladder was not emptied at the beginning of the experiment, so it is possible that urine in the bladder before the clamp was included with urine collected during the clamp. Although this would overestimate urinary K⁺ excretion during the clamp, the error appears to be small because the amount of K⁺ in the bladder is typically ~15% of the total amount collected during the clamp. To avoid the contamination of urine excreted during the experiments by fecal K⁺, a mesh screen was placed underneath the wire floor to separate feces from urine.

Na⁺–K⁺–ATPase α-subunit levels. Na⁺–K⁺–ATPase α-subunit immunoreactivity was determined as previously described (35, 36). In brief, frozen skeletal muscles (soleus and whole gastrocnemius) were defrosted and homogenized with a Polytron homogenizer 1:20 (wt/vol) in 5% sorbitol, 25 mM histidine-imidazole (pH 7.4), 0.5 mM Na₂-EDTA, and proteolytic enzyme inhibitors: 0.5 mM phenylmethysulfonyl fluoride, 1 μg/ml leupeptin, and 1 mM 4-aminobenzamidine dichloride. Protein concentration was measured with the bicinchoninic acid assay kit (Pierce Technology, Iselin, NJ). Homogenate protein was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto Immobilon-P membranes (Millipore, Bedford, MA). Blots were incubated for 4 h with either monoclonal anti-α, C464.6 (1:100 dilution; provided by M. Kashgarian, Dept. of Pathology, Yale Univ. School of Medicine, New Haven, CT) or polyclonal anti-α-2McHERED [1:500 dilution; prepared in our laboratory according to the protocol of Pressley (29)]. Antibody-antigen complexes were detected with Alexa 680-labeled goat anti-rabbit or goat anti-mouse secondary probe (Molecular Probes, Eugene, OR) and then detected with an Odyssey infrared imaging system (LI-COR Biotechnology, Lincoln, NE) and quantitated using the accompanying LI-COR software. Linearity was verified by assaying each sample at 12 and 24 μg of protein/lane on the same blot.

RESULTS

Effect of DEX treatment on body weight and food consumption. DEX is known to depress weight gain over time (24). The impact of the low- and high-DEX treatment regimes on weight at the time of the simultaneous K⁺ and glucose clamps is summarized in Fig. 1. The low-DEX regime (0.02 mg·kg⁻¹·day⁻¹ for 14 days) produced a lower rate of weight gain (~35 g gain) than did the vehicle (~80-g gain) over the 14-day treatment period (Fig 1A). The high-DEX regime (0.1 mg·kg⁻¹·day⁻¹ for 7 days) actually caused rats to lose weight (~20 g) (Fig 1B). To minimize the difference in weight between the DEX- and vehicle-treated groups at the time of the clamps, DEX-treated rats were paired at day 0 with vehicle-infused rats that weighed 35–40 g less than they did. The vehicle-treated group gained an average of 56 g during the 7-day treatment period; thus the difference between the groups at the time of K⁺ clamps was 35–40 g (Fig 1B). Food

Fig. 1. Effect of dexamethasone (DEX) treatment on weight gain in the rat. A: effect of 0.02 mg·kg⁻¹·day⁻¹ DEX or vehicle for 14 days. Both groups started at same initial body weight range. B: effect of 0.1 mg·kg⁻¹·day⁻¹ DEX or vehicle for 7 days. DEX-infused rats were paired with vehicle-infused rats of a lighter body weight (175 g) at the start of the DEX treatment so that their body weights would be more similar at the end of the DEX treatment period. Values represent means ± SE. *P < 0.05 vs. vehicle controls.
consumption was $\sim 20\%$ lower in the high-DEX-treated group (135 ± 9 g) than in the vehicle-infused group (167 ± 5 g). K$^+$-clamp results were normalized to body weight in recognition of these differences.

**Simultaneous glucose and K$^+$ clamps during insulin infusion.** Insulin stimulates K$^+$ transport into insulin-sensitive tissues, such as skeletal muscle and liver (11, 23), primarily via Na$^+$-K$^+$-ATPase pumps, although there are reports that there may be bumetanide-sensitive Na$^+$-K$^+$-2Cl or K$^+$-2Cl cotransporters expressed in muscle that also could potentially mediate cellular K$^+$ uptake (41). Investigators at our laboratory (35) have shown that expression of the Na$^+$-K$^+$-ATPase $\alpha_2$-isoform, not the $\alpha_1$-isoform, was significantly increased in skeletal muscles after DEX treatment, in agreement with a previous study demonstrating that DEX treatment increases ouabain binding sites (15). We predicted that if insulin-mediated cellular K$^+$ uptake is primarily mediated by Na$^+$-K$^+$-ATPase, and if skeletal muscle is the major tissue involved in insulin-mediated K$^+$ uptake in vivo, then in vivo insulin-mediated K$^+$ uptake would be increased substantially, secondary to increased sodium pump number, in DEX-treated rats. To test this hypothesis, we performed the K$^+$-clamp experiment in rats that had been infused with either a low dose of DEX for 14 days or a higher dose for 7 days, with each animal paired with a vehicle-infused control as described in METHODS (Fig. 2; see also Fig. 3). Basal (time 0) plasma K$^+$ and glucose concentrations were not altered by low- or high-DEX infusion. The ability to technically clamp plasma K$^+$ and glucose is demonstrated in the figures summarizing plasma K$^+$ and glucose concentrations over the 150-min clamp period.

**Insulin-mediated net glucose and K$^+$ uptake after low-dose DEX treatment.** Following the low-DEX regime (Fig. 2), insulin-stimulated net glucose clearance, equivalent to the amount of glucose infusion to clamp plasma glucose levels at baseline (G inf in $\mu$mol·kg$^{-1}$·min$^{-1}$), was significantly suppressed ($P \leq 0.05$) (see Fig. 5) as previously reported (39, 40). There was no significant increase in the magnitude of the insulin-stimulated net K$^+$ clearance ($K_{inf}$) at this dosage, even though this low-DEX regime was reported to increase ouabain binding, a measure of sodium pump $\alpha_2$ sites in the rat, by 30% in skeletal muscles (15). To determine whether the sodium pump $\alpha_2$ levels were elevated in this set, immunoblotting of the soleus, a typical slow oxidative muscle, and the whole gastrocnemius, a mixed-fiber muscle, were conducted in samples from the same animals used for the K$^+$ and glucose clamps. Na$^+$-K$^+$-ATPase $\alpha_2$ levels in soleus muscle total homogenate, shown in Fig. 3A, were not significantly increased by low-DEX treatment (1.17 ± 0.11 vs. 1.00 ± 0.13 in vehicle-treated controls, in normalized density units). Similarly, $\alpha_3$ levels were not significantly increased by low-dose DEX treatment in whole gastrocnemius (1.38 ± 0.27 vs. 1.00 ± 0.12 in vehicle control; blots not shown), and there was no change in Na$^+$-K$^+$-ATPase $\alpha_1$ abundance in either muscle (data not shown). In summary, after the low-dose DEX treatment, we observed the expected decrease in insulin-sensitive net glucose uptake, evidence for the efficacy of the DEX, but no significant DEX-stimulated increase in either muscle Na$^+$-K$^+$-ATPase $\alpha_2$ levels or insulin-stimulated net K$^+$ uptake. In the next series of experiments, we used a higher dose of DEX to significantly increase the muscle Na$^+$-K$^+$-ATPase $\alpha_2$ abundance.

**Insulin-mediated net glucose and K$^+$ uptake after high-dose DEX treatment.** Following the high-DEX regime, sodium pump $\alpha_2$ levels were significantly increased [38 ± 15% in soleus (Fig. 3B) and 67 ± 3% in gastrocnemius (data not shown)] as measured in muscles taken from these animals at the end of the clamp ($P \leq 0.05$ for both). These findings confirm those in a previous study at our laboratory (35) detailing the effects of 14-day high-DEX treatment on ouabain binding and $\alpha_2$ levels in soleus, extensor digitorum longus (EDL), whole gastrocnemius, and diaphragm: ouabain binding increased by 22–48%, $\alpha_2$ levels increased by 53–78%, and $\beta_1$ levels increased $\sim 50\%$ across this panel of muscles, and $\alpha_1$ levels increased by 65 ± 7% only in diaphragm. Figure 4 summarizes the hyperinsulinemic (5 mU·kg$^{-1}$·min$^{-1}$) glucose and K$^+$ clamps in vehicle-infused control rats, and DEX infused at 0.02 mg·kg$^{-1}$·day$^{-1}$ for 14 days. Values are means ± SE for 5 paired experiments. Steady-state values of K$^+$ infusion (K inf) and glucose infusion (G inf) during final 60 min and significance of observed differences are analyzed and summarized in Fig. 5.
Urinary K⁺ excretion during insulin infusion. Because a decrease in \( K_{\text{inf}}^+ \) during insulin infusion could reflect a decrease in insulin-stimulated K⁺ urinary excretion rather than (or in addition to) a decrease in insulin-stimulated net cellular uptake, urine was collected before and during the clamps in the high-DEX experiment, as described in Methods. As summarized in Table 1, in the baseline 2.5-h period before the clamp, there was no significant difference in K⁺ excretion between the two groups. From this and the unchanged plasma K⁺ concentration (Fig. 4), we conclude that DEX has no chronic effect on K⁺ excretion. During the clamp, there was a similar increase in K⁺ excretion in both groups. The increase may be attributed to at least a couple of factors: 1) the clamp involved the infusion of insulin, glucose, and K⁺, which may stimulate fluid output, and 2) the clamp was conducted in conscious animals, and we observed that they slept more during the preclamp period and were awake more (and thus more likely to empty their bladders) during the clamp period because of the sampling activity in the room every 10 min. Nonetheless, there was no decrease in urinary K⁺ excretion during insulin infusion in the DEX-treated group; the difference between the vehicle- and DEX-treated groups was insignificant, although there was a tendency for clamp-stimulated K⁺ excretion to actually increase. Thus we attribute the suppression of \( K_{\text{inf}}^+ \) in the DEX-treated group to a decrease in insulin-stimulated net cellular uptake because there was no evidence for a decrease in urinary K⁺ excretion during insulin infusion.

Figure 5 summarizes net cellular glucose uptake (\( G_{\text{inf}} \)) and net K⁺ uptake (\( K_{\text{inf}}^+ \)) during the final hour of the clamps, when equilibrium was established, as well as Na⁺-K⁺-ATPase \( \alpha_2 \) abundance in DEX-treated normalized to vehicle-treated groups. The magnitude of the decrease in insulin-stimulated net glucose uptake was equivalent at both low-DEX and high-DEX treatment groups (~40%), while the decrease in insulin-stimulated net K⁺ uptake (~50%) was evident only in the high-DEX treatment group and was accompanied, paradoxically, by an increase in the abundance of muscle Na⁺-K⁺-ATPase \( \alpha_2 \), the main route of cellular K⁺ uptake.
GLUCOCORTICOIDS INDUCE INSULIN RESISTANCE TO K⁺ UPTAKE

DISCUSSION

Glucocorticoids are administered chronically for a variety of medical conditions. Ravn and Dørup (31) demonstrated that chronic DEX treatment of patients with chronic obstructive lung disease (COLD) in an intensive care unit (ICU; mean dose of 42.7 mg of prednisone/day) resulted in a 40% increase in muscle sodium pump number assessed by [3 H]ouabain binding. In rats, DEX infusion for 7–14 days at a lower dose (0.1–0.1 mg·kg⁻¹·day⁻¹) (present study and Ref. 35) than that used in the patients with COLD in the ICU increased muscle sodium pump α₂-isofrom levels by 40–70%, a magnitude similar to that seen in the COLD study. These findings raised the concern that an increase in pool size of functional muscle sodium pumps could increase acute insulin-driven cellular K⁺ uptake after a meal or catecholamine-driven cellular K⁺ uptake during an asthma attack, thus precipitating acute hypokalemia and ensuing arrhythmias and cardiac arrest (43). The results of the present study contradict this prediction (in the case of insulin) and establish that there is less, rather than more, insulin-resistant cellular K⁺ uptake in DEX-treated rats. That glucocorticoid-treated rats exhibit insulin resistance to cellular uptake of glucose is well established (39, 40); the present study establishes that glucocorticoid treatment also provokes insulin resistance to insulin-stimulated cellular K⁺ uptake. Whether catecholamine activation of cellular uptake of K⁺ is greater in glucocorticoid-treated animals or patients remains to be determined.

Skeletal muscle contains the body’s largest pool of sodium pumps (Na⁺/K⁺-ATPase) (9, 26). The α₁- and α₂-isoforms of Na⁺/K⁺-ATPase predominate in skeletal muscle; the relative ratio of the isoforms is muscle dependent. The α₂-total protein ratios are quite similar across muscle types from soleus to white gastrocnemius, but α₁ expression is fourfold greater in soleus than in white gastrocnemius or EDL (36). The ratio of α₂ to total α in EDL, independent of β expression, is estimated to be 75–85% (20, 21); the concentration of α₂ in soleus would be significantly lower because of the fourfold greater expression of α₁ compared with EDL. Because β-subunits are expressed in a muscle fiber-specific manner (soleus only β₁, white gastrocnemius only β₂), β abundance can be used as a measure of total αβ heteromers (36). DEX was previously shown to increase both β₁ and β₂ 1.5-fold in soleus and EDL, respectively, which is likely a reasonable estimate of the change in total αβ pool size (35).

Muscle sodium pump activity can shift the ratio of K⁺ in extracellular fluid vs. intracellular fluid, and activity can, in theory, be regulated by changing Na⁺/K⁺-ATPase abundance and/or activity in the plasma membrane. Total muscle sodium pump abundance has been shown to be increased in rats by thyroid hormone (T3) treatment (2, 19), which increases α₂ and β₂ levels two- to threefold (3), and by glucocorticoid treatment, which increases α₂ and β₁ levels 1.5-fold (35). These increases are mediated by pretranslational stimulation of synthesis because T3 and glucocorticoids increase muscle α₂ mRNA levels five- and sixfold, respectively (3, 35). In isolated muscles in vitro, sodium pump activity, measured as ouabain-sensitive K⁺ uptake, has been shown to be increased acutely by insulin or electrical stimulation (8, 27). The central hypothesis tested in this study is that the DEX-stimulated increase in α₂ abundance in muscle provokes an increase in net cellular K⁺ uptake in vivo when sodium pump activity is acutely stimulated. We addressed this question by applying the K⁺-clamp technique, a method of measuring insulin-stimulated net cellular K⁺ uptake in conscious rats (6, 26). The results did not support the

Table 1. Urinary K⁺ excretion in high-DEX-treated rats before and during K⁺ clamp with insulin infusion

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>K⁺ excreted, μmol/kg</th>
<th>Vehicle</th>
<th>DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preclamp, 2.5 h</td>
<td>782±76</td>
<td>659±114</td>
<td></td>
</tr>
<tr>
<td>Clamp, 2.5 h</td>
<td>1,446±120</td>
<td>1,659±182</td>
<td></td>
</tr>
<tr>
<td>Clamp-stimulated, clamp − preclamp</td>
<td>663±174</td>
<td>1,001±197</td>
<td></td>
</tr>
</tbody>
</table>

High-DEX-treated rats were infused at a rate of 0.1 mg·kg⁻¹·day⁻¹. Values are means ± SE. DEX, dexamethasone.

Fig. 5. Comparison of low- vs. high-DEX doses on insulin-mediated plasma glucose disappearance (Ginf) during the last 60 min of the clamp (A), insulin-mediated plasma K⁺ disappearance (Kinf) during the last 60 min of the clamp (B), and Na⁺/K⁺-ATPase α₂-subunit abundance, assessed by immunoblotting in a constant amount of homogenate protein (C). Vehicle-infused group (solid bars) is compared with DEX-infused group (hatched bars). Values are means ± SE for 5 or 6 experiments. *P < 0.05 vs. vehicle-treated controls.
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hypothesis, and, to the contrary, DEX treatment decreased insulin-stimulated clearance of K⁺ from the plasma despite the significant elevation in muscle α2 levels. The suppression of K⁺\textsubscript{inf} in the DEX-treated group is attributed to a decrease in insulin-stimulated net cellular uptake because there is no evidence for a decrease in urinary K⁺ excretion during insulin infusion. In parallel, DEX treatment decreased insulin-stimulated net clearance of glucose from the plasma, a phenomenon that has been well studied (39, 40). Whether there is any change in the cellular efflux rate of glucose or K⁺ during the clamp was not assayed, so the results are expressed as net uptake or clearance.

There was a clear and consistent decrease in K⁺\textsubscript{inf} and G\textsubscript{inf}, and very good clamping of glucose and K⁺ concentrations during the last 90 min of the clamp in the DEX-treated group vs. the control group (Fig. 4), but during the first 30-40 min, there was no apparent difference in insulin-stimulated G\textsubscript{inf} or K⁺\textsubscript{inf}. There are at least two potential explanations for these results: 1) the clamp is an empirical manual technique, and during the pre-steady-state first 30 min, there was a small underestimate of G\textsubscript{inf} and K⁺\textsubscript{inf} in controls, evidenced by the drop in both glucose and K⁺ concentrations, along with a small overestimate of G\textsubscript{inf} and K⁺\textsubscript{inf} in DEX-treated animals, evidenced by the rise in both glucose and K⁺ concentrations; and 2) in the early phase, the splanchnic bed may be the predominant route of cellular K⁺ uptake, whereas after 1 h of insulin infusion (steady state), skeletal muscle may be the predominant route of cellular K⁺ uptake (11). Thus the time dependency may indicate specific suppression of insulin-stimulated uptake into muscle, not into splanchnic bed. A similar observation was made in previous studies at our laboratory regarding insulin stimulation in K⁺-deprived rats (6).

This study establishes that DEX induces insulin resistance of not only glucose uptake but also K⁺ uptake. However, the two effects can be dissociated by the dose of DEX administered: a low dose of DEX (0.02 mg·kg\(^{-1}\)·day\(^{-1}\) for 14 days) reduced insulin-stimulated cellular glucose uptake but not K⁺ uptake, while a higher dose of DEX (0.1 mg·kg\(^{-1}\)·day\(^{-1}\)) had no further effect on insulin-stimulated glucose uptake but significantly suppressed insulin-stimulated K⁺ uptake. In other words, the effect on insulin-stimulated glucose uptake was fully expressed after a low-DEX treatment that had no measurable effect on K⁺ uptake, indicating that the effects were quite distinct. Insulin-stimulated cellular K⁺ uptake decreased at the high dose of DEX, even though an important route of K⁺ uptake from the plasma, namely, muscle sodium pumps, increased in total abundance (increase in α2, no change in α1 isofrom), in agreement with previous reports regarding the stimulatory effects of DEX on ouabain binding and α2 synthesis and abundance (15, 35). At the higher dose of glucocorticoids, the animals did not gain or maintain their body weight, but the dose infused (0.1 mg·kg\(^{-1}\)·day\(^{-1}\)) was lower than that reported in patients treated in the ICU for COLD (42.7 mg/day), which is equivalent to 0.61 mg·kg\(^{-1}\)·day\(^{-1}\) in a 70-kg patient. Glucocorticoids have many actions, and this weight loss may be attributed, at least in part, to the insulin resistance to cellular uptake of both glucose and K⁺.

Whether there is any functional connection between the coincident decrease in insulin-stimulated cellular K⁺ uptake and the increase in Na⁺-K⁺-ATPase α2 levels remains to be established. A previous study at our laboratory suggested that high-dose DEX exerts a direct stimulatory effect on α2 synthesis (35). A second possibility is that plasma insulin levels per se may increase Na⁺-K⁺-ATPase abundance. DEX treatment at doses higher than those used in the present study leads to hyperinsulinemia (e.g., Ref. 38). In a thorough study of insulin-treated patients with non-insulin-dependent diabetes mellitus and hyperinsulinemia, Schmidt et al. (33) demonstrated that the pool size of [\(^3\)H]ouabain binding sites in vastus lateralis was increased by ∼20% vs. controls, with a significant correlation between plasma insulin and the content of [\(^3\)H]ouabain binding sites among these patients. In the same report, it was shown that in rats with streptozotocin diabetes, [\(^3\)H]ouabain binding sites in soleus were reduced by 12-21% and insulin treatment increased the binding sites to 18-26% above controls (33). Because insulin levels were not measured in this study (although plasma glucose levels were unaltered; see Fig. 4), whether hyperinsulinemia accompanying DEX treatment contributes to the increase in muscle Na⁺-K⁺-ATPase α2 levels remains to be determined. A third possible hypothesis is that DEX primarily blunts the pathway of insulin-stimulated cellular K⁺ uptake, which itself stimulates an increase in the pool size of Na⁺-K⁺-ATPase α2 as a homeostatic mechanism to normalize cellular K⁺ uptake and Na⁺ extrusion. In other words, the effect of insulin resistance to cellular K⁺ uptake would presumably be more severe if the number of sodium pumps did not climb significantly above baseline.

The study by Dørup and Clausen (15) demonstrated that DEX treatment increased ouabain binding to muscle biopsy samples (a measure of functional α2-type pumps on the cell surface) but did not alter cellular Na⁺ or K⁺ levels. These results could be interpreted to indicate that the elevated levels of pumps are needed to maintain normal cell electrolytes or, alternatively, that the elevated levels do not influence cell electrolytes. That there is no apparent change in Na⁺ or K⁺ content implies that the Na⁺ and K⁺ leaks balance the Na⁺ and K⁺ pumping after DEX treatment, as was described previously during thyroid hormone treatment in muscle (17). Dørup and Clausen (15) also measured the effect of 4-h DEX treatment (10⁻⁸ to 10⁻⁶ M) in vitro on 22Na influx, and no significant difference was detected. Whether long-term DEX treatment alters Na⁺ or K⁺ leaks remains to be determined. After 2-wk high-dose DEX treatment (35), no significant increase in total V\textsubscript{max} Na⁺-K⁺-ATPase activity was detected, despite 53-78% increases in α2 and β1 levels, indicating less activity per pump, that the DEX-induced subunits were not assembled as functional pumps, or that the pumps were allosterically modified in a manner that depressed activity. However, the coincident increase in ouabain binding during high-DEX treatment, a measure of functional pumps (15), indicates that the increased pool of α2-subunits are indeed assembled and functional on the cell surface. It remains to be determined whether the inhibitory influence of DEX on cellular K⁺ uptake is restricted to insulin stimulation, or whether it extends to other acute stimulators of sodium pump activity, such as catecholamines, which may augment cellular K⁺ uptake secondary to the increase in sodium pump abundance. Whether other routes of K⁺ transport are affected also remains to be determined.

DEX-induced insulin resistance to glucose uptake has been studied extensively. Weinstein et al. (39) showed that DEX induced not only insulin resistance to glucose uptake
in muscle but also resistance to the stimulatory effects of IGF-I and hypoxia leading to the postulate that glucocorticoids alter the kinetics of glucose transporter GLUT4 subcellular trafficking in favor of increased partitioning to the intracellular membranes. Investigators in a number of studies have determined that depressed glucose uptake in the DEX-treated rat is not secondary to decreased total cellular abundance of GLUT4; rather, some studies (38, 40) have reported a 25% increase in GLUT4, while others have reported no change (13, 34). In our own studies (data not shown), DEX did not significantly increase GLUT4 protein levels in soleus, EDL, gastrocnemius, or diaphragm. Dimtriadis et al. (13), using a cell surface biotinylation approach, and Weinstein et al. (40), using cell surface photoaffinity labeling of the transporter, conducted studies to test the hypothesis that insulin-stimulated recruitment of GLUT4 to the cell surface is impaired during DEX treatment. Both studies concluded that DEX treatment reduced GLUT4 translocation >50%. Related studies in adipocytes cultured in DEX confirmed reduced translocation of GLUT4 during insulin stimulation (4, 32), while tyrosine phosphorylation of the insulin receptors was normal (4, 32). Weinstein et al. (38) showed that thiazolidine-2,4-diones could partially ameliorate glucocorticoid-induced insulin resistance to muscle glucose uptake, and Thomas et al. (34) showed an analogous effect of the anti-hyperglycemic agent metformin. These drugs did not prevent the catabolic effect of DEX or alter GLUT1 or GLUT4 levels, suggesting more transport activity and/or transporter, perhaps mediated by improved insulin-stimulated translocation to the plasma membrane. Whether these drugs ameliorate the insulin resistance to K+ uptake is a future direction of this line of study.

Is the mechanism for DEX-mediated insulin resistance to K+ uptake analogous to the insulin resistance to glucose uptake, that is, a blunting of translocation of the pumps? There is strong evidence both for and against insulin-stimulated translocation of sodium pumps to the plasma membrane in skeletal muscle. In support of translocation, Marette et al. (25), in a morphological study, used immunoelectron microscopy, first, to show that Na+•K+-ATPase α2-subunits could be detected in intratubular and vesicular structures as well as in plasma membrane, and, second, to measure an either 3.7- or 1.5-fold increase in the density of gold particles per length of plasma membrane (depending on protocol variables). The authors acknowledged that identifying the location of intracellular stores of α2 is complicated because of the mild fixation requisite to retain antigenicity and because α2-subunit labeling was also observed in tubular structures and in the triad region of transverse tubules. Thus whether a portion of the α2 identified as intracellular was actually in membranes contiguous with the plasma membrane is difficult to determine. Also in support of translocation, Al-Khalili et al. (1) incubated epimyocellular muscles with or without insulin in vitro and then labeled the cell surface by biotinylation at 4°C. Biotinylated proteins purified from a constant amount of solubilized muscle protein with streptavidin beads were analyzed by immunoblotting. Insulin increased the pool of biotinylated α2 by 51% and of α2 by 74%; coincident incubation with phos- phatidylinositol 3-kinase inhibitor or PKC inhibitor prevents the increases. Whether the results indicated translocation or an insulin-induced increase in overall efficacy of cell surface biotinylation was not addressed. In the same study, analysis of translocation by subcellular fractionation on sucrose gradients showed a 41% increase in α2 and no increase in α3 in the plasma membrane enriched fraction after insulin. These authors conceded that <10% of plasma membranes were recovered after subcellular fractionation and that subfractions of plasma membrane may not be recovered on the sucrose gradients, criticisms that may apply to the previous reports of translocation studied by subcellular fractionation (22).

Studies that refute the conclusion of insulin-stimulated translocation of sodium pumps are based on the hypothesis that a significant translocation of Na+•K+-ATPase α2 to the plasma membrane should be measurable as an increase in [3H]ouabain binding to isolated muscles because ouabain binds to the extracellular face of the pump, has very high specificity for the rat α2-isofrm, and binds only to active sodium pumps. Studies of rat soleus by Clausen and Hansen (7, 10) and Weil et al. (37) showed that insulin doubled the initial rate of ouabain binding (at 10 min) but not that of steady-state binding (at 3 h). The initial report (16) of an increase in ouabain binding to frog muscles incubated in insulin likely reflects a technical error because the duration of incubation was insufficient to allow full occupancy of all the sodium pumps: what appeared to be an increase in binding capacity of the intact muscle arose from the early increase in the rate of pumping. More recently, McKenna et al. (27) revisited the question of translocation of Na+•K+-ATPase in muscle and showed that insulin incubation of soleus increased 86Rb uptake by 23% and decreased intracellular Na+ by 27%, but [3H]ouabain binding was unchanged. It is generally accepted that ouabain does not cross the plasma membrane and detects only cell surface pumps. In support of this, at supersaturating concentration (10^{-3} M), ouabain occupies the same space as the extracellular space marker [14C]sucrose (10). Because the molecular mechanism of insulin stimulation of Na+•K+-ATPase activity remains unresolved, it is not easy to postulate how DEX treatment blunts the effect. The most straightforward future direction of studying this phenomenon is to use cell surface biotinylation, including internal plasma membrane marker controls, to determine whether there is, in fact, detectable translocation blunted by DEX treatment.

In previous studies at our laboratory, investigators discovered that the insulin sensitivity of cellular K+ uptake can be regulated independently of the insulin sensitivity of cellular glucose uptake. In the first K+-clamp study, investigators at our laboratory demonstrated that in rats deprived of dietary K+, cellular K+ uptake became resistant to insulin stimulation (even before a decrease in sodium pump abundance), while insulin stimulation of cellular glucose uptake remained normal (6). The decrease in insulin-stimulated K+ uptake mediated by a K+-deficient diet (~80%) is greater than the decrease seen after DEX treatment (~50%). In contrast, in rats fed a high-fat diet with K+ intake paired to that of controls on a standard chow, insulin-stimulated K+ uptake was normal, while insulin-stimulated cellular glucose uptake was decreased (5). In the current study, low-dose DEX suppressed insulin stimulation of glucose uptake.
cellular uptake of glucose (but not of K\(^+\)), while at high doses, it suppressed both glucose and K\(^+\) uptake. Taken together, these studies indicate that insulin sensitivity of glucose and K\(^+\) transport can be regulated independently; with profound insulin resistance to K\(^+\) uptake, there can be normal insulin-sensitive glucose uptake and vice versa. Finally, this study demonstrates that insulin resistance to K\(^+\) uptake coincided with an increase in the abundance of the main cellular K\(^+\) uptake pathway, Na\(^+\)-K\(^+\)-ATPase \(\alpha_2\)-type pumps, suggesting that the increase in \(\alpha_2\) may be a homeostatic mechanism to compensate for the DEX-mediated insulin resistance to K\(^+\) uptake.

GRANTS

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REFERENCES


