Biol Trace Elem Res (2010) 133:171–180
DOI 10.1007/s12011-009-8433-7

Effects of Chromium(III) Picolinate on Cortisol and DHEAs Secretion in H295R Human Adrenocortical Cells

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Received: 18 May 2009 / Accepted: 7 June 2009 /
Published online: 24 June 2009

Abstract Dietary chromium(III) picolinate (CrPic) effects on circulating steroid hormones have been reported in various experimental animals. However, direct effects of CrPic on adrenocortical steroidogenesis are uncertain. Therefore, the objective was to determine the effects of CrPic on cortisol and dehydroepiandrosterone sulfate (DHEAs) secretion from H295R cells. In experiment 1, a 24-h exposure to CrPic (0 to 200 μM) had both linear ($p<0.001$) and quadratic ($p<0.001$) effects on cortisol secretion from forskolin-stimulated cells with the highest cortisol secretion at 0.1 μM of CrPic and the lowest at 200 μM of CrPic. In experiment 2, a 48-h exposure to CrPic (200 μM) decreased cortisol ($p<0.07$) release from forskolin-stimulated cells during a 24-h collection period. In experiment 3, a 48-h exposure to CrPic (100 μM) decreased cortisol ($p<0.05$) and DHEAs ($p<0.01$) from forskolin-stimulated cells during a 24-h sampling period. In experiment 4, a 24-h exposure to forskolin followed by a 24-h exposure to both forskolin and CrPic (100 and 200 μM) decreased both cortisol and DHEAs secretion ($p<0.01$). This study suggests that at high concentrations, CrPic inhibits aspects of steroidogenesis in agonist-stimulated adrenocortical cells.

Keywords Chromium · Cortisol · DHEAs · Steroid · H295R · Adrenocorticoid

Introduction

Trivalent chromium (Cr) is an essential micronutrient for humans and animals [1, 2]. Since the first report on the essentiality of Cr in rats [3], the role of Cr has been widely studied in humans, animals, and in vitro models. A well-known function of Cr is its ability to

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potentiate the actions of insulin [4, 5]. Chromium(III) picolinate (CrPic) has been demonstrated to facilitate insulin internalization in muscle cells [6].

The effects of a variety of dietary Cr sources on adrenal steroid hormones have been investigated, but results are inconclusive. Dietary Cr decreased circulating adrenal steroids in young stressed calves [7, 8], in epinephrine challenged lambs [9], in stressed dairy cows [10], and in postmenopausal women [11]. In other studies, however, dietary Cr had no major effects on adrenal steroids [12–15].

The effects of Cr on insulin secretion from islets of Langerhans [16] and on catecholamine secretion from bovine medullary cells were reported [17]. However, direct effects of Cr on adrenocortical steroidogenesis have not been reported. Therefore, the objective was to investigate the effect of CrPic on cortisol and dehydroepiandrosterone sulfate (DHEAs) secretion from H295R adrenocortical cells. For these studies, we used the well-characterized human adrenocortical carcinoma cell, NCI-H295, which maintains hormonal and enzymatic characteristics similar to those of normal human adrenocortical cells [18–20]. The H295R is a subpopulation of H295 that forms a monolayer in culture [21] and has been extensively used as a model system in adrenal steroid metabolism investigations [22–25].

Materials and Methods

Materials

Human NCI-H295R adrenocortical cell line derived from human adrenocortical cells was obtained from the American Type Culture Collection (ATCC, Manassas, VA). The sources for the other materials are given below.

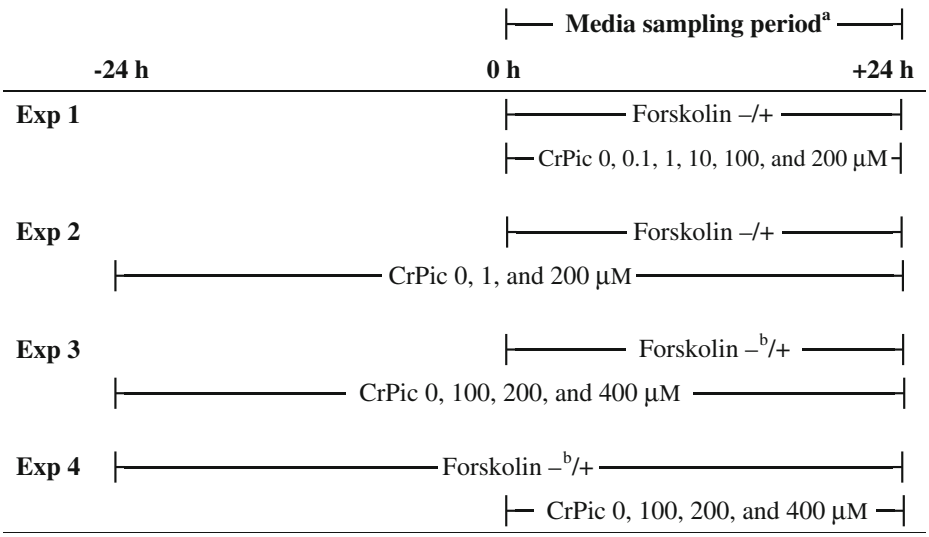
Cell Culture

Cells were cultured in an equal mixture (vol/vol) of Dulbecco's Modified Eagle's Medium and Ham's F-12 medium (DMEM/F-12, Invitrogen, Chicago, IL) containing HEPES (15 mM), sodium pyruvate (0.5 mM), and sodium bicarbonate (1.2 g/L) supplemented with 1% ITS Plus (insulin, 6.25 μg/mL; transferrin, 6.25 μg/mL; selenium, 6.25 ng/mL; bovine serum albumin, 1.25 mg/mL; linoleic acid, 5.35 μg/mL, BD Bioscience, Bedford, MA) and Nu-Serum (2.5%, BD Bioscience). Cells were grown in 75-cm^2 flasks under a humidified atmosphere of 95% air/5% carbon dioxide at 37°C. The medium was changed every 3 days, and cells were detached weekly using a solution of 0.25% trypsin-EDTA for subcultures at a ratio of 1:4.

Treatment Structure

The treatment structure for each experiment is illustrated in Table 1. In experiment 1, treatments included six levels of CrPic (0, 0.1, 1, 10, 100, and 200 μM) with or without forskolin (10 μM) in the media for 24 h of incubation. In experiment 2, three levels of CrPic (0, 1, and 200 μM) were used for 48 h of incubation with or without forskolin in the media for the last 24 h. In experiment 3, treatments included negative control (media) without forskolin and four levels of CrPic (0, 100, 200, and 400 μM) for 48 h of incubation with forskolin during the last 24 h. In experiment 4, treatments included negative control (media) and 24-h exposure to forskolin followed by 24-h exposure to both forskolin and

Table 1 Treatment Structure for Experiments 1 to 4



[a] Media samples were collected after the last 24 h in all the experiments

[b] Forskolin-free medium without CrPic treatment was used as a negative control group. In experiments 3 and 4, therefore, five treatment groups included a negative control and four CrPic levels with forskolin (10 μM) stimulation

four levels of CrPic (0, 100, 200, and 400 μM). In experiments 2, 3, and 4, media samples for steroid assay were collected from the last 24 h of incubation and kept frozen at −20°C until steroid analysis.

Forskolin was used as an agonist in the present study, and this agonist at 10 μM dramatically increased the steroid secretion from H295R cells (Figs. 1, 2, 3, 4), consistent with previous reports [19, 26, 27].

Analysis of Cortisol and DHEAs

The cortisol and DHEAs concentrations in the sample media collected from each well were determined using coated tube radioimmunoassays from Diagnostic Products Corporation (Los Angeles, CA). Briefly, steroid standard solution was made by dissolving cortisol and DHEAs (Steraloids, Newport, RI) to DMEM/F-12. Media samples or standards were added to antibody-coated tubes. Radiolabeled cortisol (^{125}I) was added to tubes and incubated for 45 (cortisol assay) or 30 (DHEAs assay) minutes at 37°C using a waterbath. Then, all moisture in the tube was thoroughly removed and the radioactivity was measured using a Packard Cobra Auto-gamma counter (Packard Instrument Co., Meriden, CT). Calibrators and samples were analyzed in triplicate and duplicate, respectively.

Protein Determination

After removing media, the cells in each well were solubilized in sodium dodecyl sulfate (SDS, 5%), and protein content was determined using the colorimetric detergent compatible

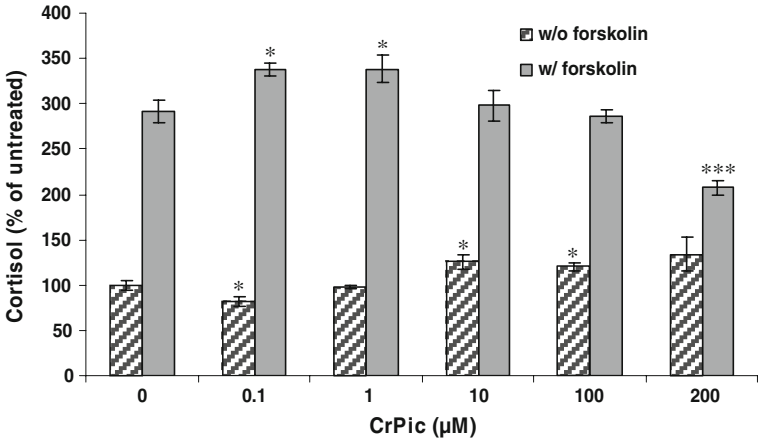


Fig. 1 Effects of CrPic on the cortisol secretion by agonist-stimulated or nonstimulated H295R cells (n=8). In the absence of forskolin, the exposure to CrPic had a linear (p<0.05) effect on cortisol secretion. When forskolin (10 μM) was included in the media, CrPic had both a linear (p<0.001) and quadratic (p<0.001) effect on cortisol secretion. Differences significant from 0 μM CrPic treatment of same forskolin inclusion level are indicated: *p<0.05; ***p<0.001

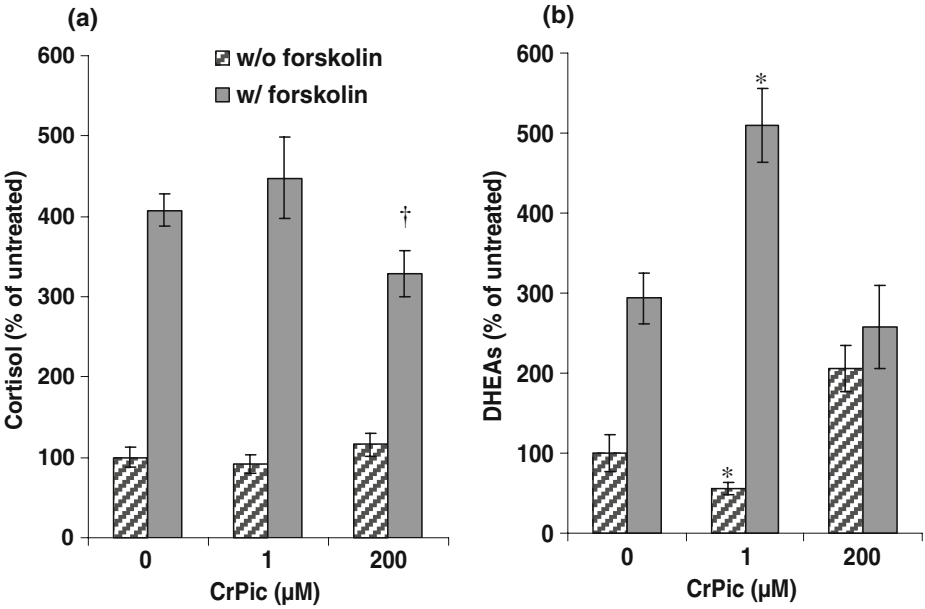


Fig. 2 Effects of CrPic on the cortisol (**a**) and DHEAs (**b**) secretion by agonist-stimulated or nonstimulated H295R cells (24 h pretreatment of CrPic; n=4). The exposure to CrPic had linear (p<0.05) and quadratic (p< 0.05) effects on cortisol secretion from forskolin-stimulated cells. The secretion of DHEAs was quadratically (p<0.001) affected by CrPic in forskolin-stimulated cells. Differences significant from 0 μM CrPic treatment of same forskolin inclusion level are indicated: †p<0.07; *p<0.05

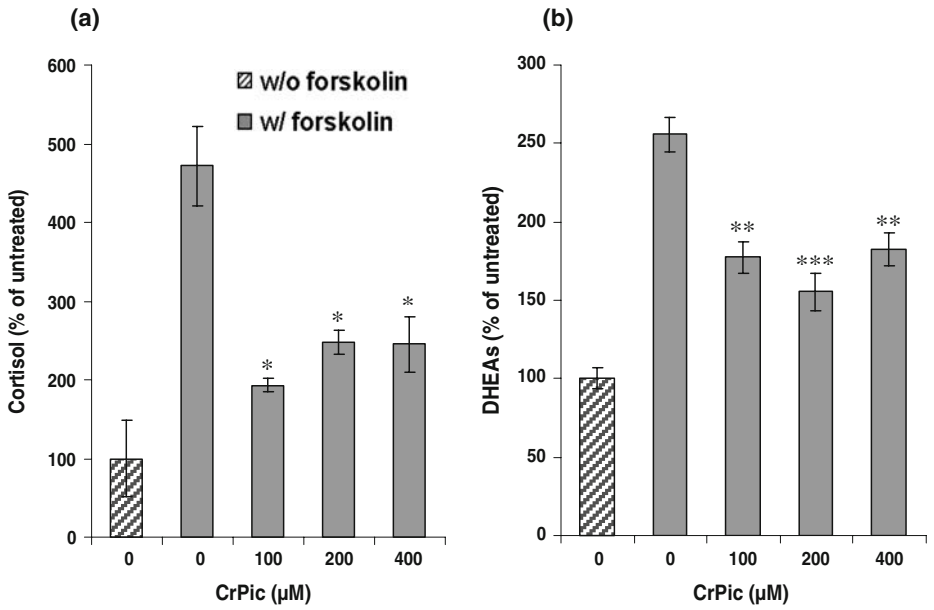


Fig. 3 Effects of CrPic on the cortisol (**a**) and DHEAs (**b**) secretion by agonist-stimulated H295R cells (24 h pretreatment of CrPic; $n=4$). The exposure to CrPic had linear ($p<0.001$) effects on cortisol secretion. The effects of CrPic on DHEAs secretion were linear ($p<0.001$) and quadratic ($p<0.01$). Differences significant from 0 μM CrPic with forskolin are indicated: *$p<0.05$; **$p<0.01$; ***$p<0.001$

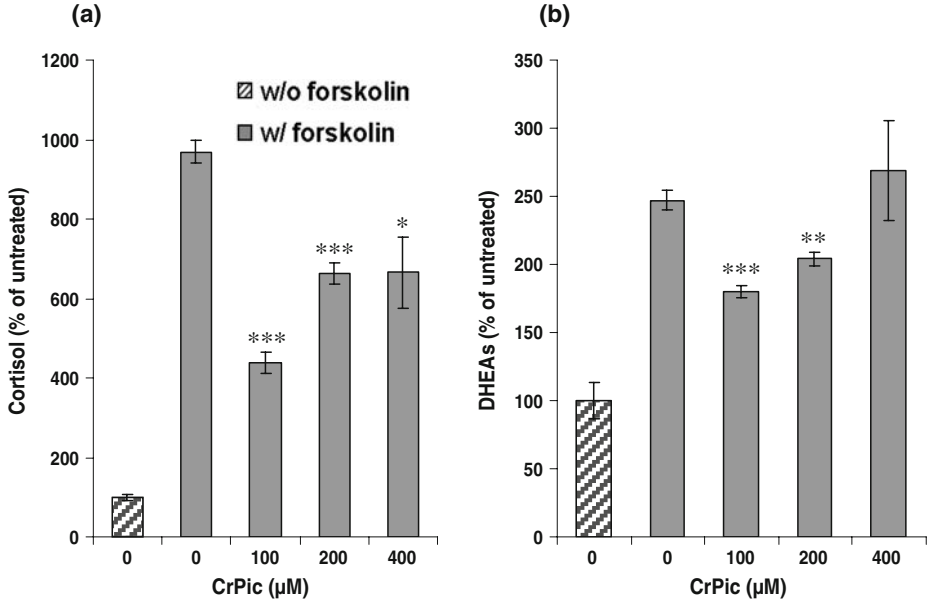


Fig. 4 Effects of CrPic on the cortisol (**a**) and DHEAs (**b**) secretion by agonist-stimulated H295R cells (24 h pretreatment of forskolin; $n=4$). The exposure to CrPic had linear effects on cortisol ($p<0.001$) and DHEAs ($p<0.01$). Differences significant from 0 μM CrPic with forskolin are indicated: *$p<0.05$; **$p<0.01$; ***$p<0.001$

(DC) protein assay kit (Bio-Rad, Hercules, CA). The DC protein assay technique is similar to Lowry protein analysis. Bovine serum albumin was used as a standard.

Calculations and Statistical Analysis

The steroid analysis results were standardized to the concentration of steroid per milligram of protein using the protein concentration of each well. The standardized steroid concentration was divided by the result from the CrPic and forskolin-free media (i.e., the control treatment). Values were expressed as mean ± SE. Each well was considered an experimental unit. Orthogonal polynomial contrasts were conducted to test linear and quadratic effects of CrPic on cortisol secretion using SAS [28]. Appropriate coefficients for unequally spaced log concentration of CrPic were obtained using the interactive matrix language procedure (Proc IML) of SAS [28]. Because the logarithm of zero is undefined, 0.01 μM, one log unit lower than the lowest concentration (0.1 μM) in experiment 1, was used for zero concentration of CrPic in Proc IML. The unpaired Student's *t* test was applied to compare the means of the different CrPic treatments.

Results

In experiment 1, 24 h of exposure to CrPic (0, 0.1, 1, 10, 100, and 200 μM) affected cortisol production (Fig. 1). In the absence of forskolin, a linear increase of cortisol was observed ($p<0.05$). When forskolin (10 μM) was included in the incubation media, CrPic had both a linear ($p<0.001$) and quadratic ($p<0.001$) effect on cortisol secretion. At 0.1 and 1 μM, CrPic potentiated stimulatory effects of forskolin on cortisol release ($p<0.05$), but at 200 μM, CrPic diminished effects of forskolin ($p<0.001$).

In experiments 2 and 3, cells were pretreated with CrPic to mimic a situation of CrPic administration followed by stress. In experiment 2, cells were pretreated with CrPic for 24 h, and the CrPic treatment concentration maintained during a following 24-h sampling period. The doses of CrPic were based on the results in experiment 1. A 48-h exposure to CrPic (0, 1, and 200 μM) had linear ($p<0.05$) and quadratic ($p<0.05$) effects on cortisol secretion from forskolin-stimulated cells during a 24-h sampling period (Fig. 2a). The lowest cortisol release was at 200 μM of CrPic ($p<0.07$). In the absence of forskolin, however, cortisol production was unaffected by CrPic. The secretion of DHEAs was quadratically ($p<0.001$) affected by CrPic in forskolin-stimulated cells with the highest DHEAs release at 1 μM of CrPic ($p<0.05$; Fig. 2b). In the forskolin-free media, CrPic affected DHEAs secretion linearly ($p<0.05$) and quadratically ($p<0.07$). The secretion of DHEAs was lowest at 1 μM of CrPic ($p<0.05$).

Experiment 3 was designed to address the effects of high concentration of CrPic (100, 200, and 400 μM) on cortisol and DHEAs secretion from agonist-stimulated cells. A 48-h exposure to high concentration of CrPic decreased cortisol ($p<0.05$) secretion from forskolin-stimulated cells during a 24-h collection period (Fig. 3a). The dose-dependent effect of CrPic on cortisol secretion was linear ($p<0.001$). In the same experiment, DHEAs secretion in agonist-stimulated cells was also decreased ($p<0.01$) in high concentration of CrPic groups (100, 200, and 400 μM; Fig. 3b). The dose-dependent effect of CrPic on DHEAs secretion was linear ($p<0.001$) and quadratic ($p<0.01$).

In experiment 4, cells were pretreated with forskolin to mimic a situation of stress occurrence followed by CrPic administration. A 24-h exposure to forskolin followed by a

24-h exposure to both forskolin and CrPic (100 and 200 μM) decreased both cortisol ($p<0.001$) and DHEAs ($p<0.01$) secretion (Fig. 4). The linear effects of CrPic were observed on cortisol ($p<0.001$) and DHEAs ($p<0.01$) secretion.

Discussion

In the present study, H295R cells were used as a model system and forskolin as an agonist (stressor) for adrenocortical steroid secretion. In stress situations, the hypothalamic-pituitary-adrenal axis is known to be activated. The pituitary adrenocorticotropic hormone (ACTH) released from the pituitary regulates glucocorticoid synthesis in the adrenal cortex. In the human adrenocortical cell line, H295R, however, ACTH effects on steroidogenesis are relatively insignificant perhaps due to the lack of ACTH receptors [19, 29] whereas forskolin bypasses ACTH receptors and triggers steroid synthesis [19]. Thus, forskolin was employed as an agonist in the present study, and this agonist (10 μM) dramatically increased cortisol and DHEAs secretion from H295R cells (Figs. 1, 2, 3, 4) in agreement with other studies [19, 26, 27]. Forskolin is known to activate adenylate cyclase [30] followed by increased cyclic adenosine monophosphate (cAMP) in the cell and the activation of the protein kinase A (PKA) signal transduction pathway [19, 31]. The effect of forskolin is also induced through the PKA-independent pathway [32].

The results of experiment 1 indicate that the low concentration of CrPic (0.1 and 1 μM) increases the secretion of cortisol, but high concentration of CrPic (200 μM) dramatically inhibits the secretion of cortisol from agonist-stimulated adrenocortical cells. In contrast, when adrenocortical cells were cultured in forskolin-free media, CrPic (10 and 100 μM) slightly increased the cortisol secretion (Fig. 1). Similar dual effects of Cr were observed in catecholamine secretion from bovine adrenal medullary cells [17]. High concentrations of $CrCl_3$ (500 to 1,000 μM) significantly inhibited catecholamine release stimulated by 56 mM of K^+ from adrenal medullary cells. Similarly, when 10 μM of 1,1-dimethyl-4-phenylpiperazinium was used as an agonist, $CrCl_3$ (5 to 1,000 μM) significantly inhibited catecholamine release. In the adrenal medullary cells cultured without agonist, a slight increase of catecholamine secretion by $CrCl_3$ was reported. Insulin release from isolated rat islet of Langerhans was also inhibited by high concentration of $CrCl_3$ (250 to 1,500 μM) in a dose-dependent manner [16].

The decreased cortisol release from agonist-stimulated adrenocortical cells by high concentration of CrPic was confirmed in the following experiments (Figs. 2, 3, 4). In experiments 2, 3, and 4, cells were pretreated with CrPic or forskolin to mimic either a situation of CrPic administration followed by stress or a situation of stress occurrence followed by CrPic administration, respectively. In experiment 2, cells were pretreated with CrPic for 24 h, and the CrPic treatment concentrations continued during the following sampling period. The doses of CrPic (0, 1, 200 μM) were based on the results in experiment 1. As anticipated, CrPic affected cortisol release from agonist-stimulated cells in a linear and quadratic manner consistent with experiment 1. However, further enhancement of CrPic effects by pretreatment relative to cotreatment was not observed. In experiment 3, a 48-h treatment of CrPic at 100, 200, or 400 μM inhibited cortisol secretion from forskolin-stimulated cells. In contrast, in experiment 1, a 24-h treatment of CrPic at 100 μM had no effects on cortisol release from agonist-stimulated cells. These observations are consistent with the concept of an effect being related to both dose and time.

The present study employed CrPic as a source of Cr. Although the potential effects of picolinic acid on the hormonal secretion from adrenocortical cells should not be completely

ruled out, the effects of CrPic in the literature were more likely by the action of Cr rather than picolinic acid. In a study by Evans and Bowman [6], membrane fluidity was increased by CrPic but not by zinc picolinate. In an animal experiment [33], dietary CrPic but not picolinic acid increased lean growth and decreased fat retention.

The inhibitory effect of CrPic on cortisol secretion observed in the current study may partially explain decreased blood cortisol concentration in stressed calves [7, 8, 34] and dairy cows [10] by dietary supplementation of Cr-yeast or chelated Cr. In other studies, however, supplemental dietary Cr had no effect on circulating cortisol concentration in immune stressed pigs fed 0.2 mg/kg Cr from $CrCl_3$, CrPic, or Cr-nicotinate [12], weaning stressed piglets fed 0.3 mg/kg Cr from Cr yeast [13], thermal stressed pig fed 1 or 2 mg/kg Cr from CrPic [15], and steers fed 0.2 or 0.4 mg/kg Cr from Cr yeast [14]. The discrepancy of cortisol responses by dietary Cr in animal studies may be attributed to the dosage and source of Cr, duration of dietary Cr treatment, source and intensity of stress, animal species, and environmental variation. It may be that the most important factors on the effects of dietary Cr are the animal species and the dosage of Cr as most of stressed ruminants had lower circulating cortisol concentration with high dose (>0.4 mg/kg) supplementation of dietary Cr.

Because the antagonistic effects of CrPic were observed at relatively high concentrations (100 μM or greater), in vivo situations may not be completely represented. However, animals fed CrPic for a longer period may exhibit similar results due to the retention of Cr in the kidney [35–37]. The renal concentration of Cr in rats fed 5 mg/kg Cr from CrPic for 3 weeks was greater (368 vs. 23 μg/kg dry weight) than in those fed the control diet containing 0.03 mg/kg of Cr, and the Cr concentration in the kidney of the rats fed CrPic was greater than in other tissues including the liver, spleen, lung, and gastrocnemius muscle [35]. A similar response was observed in pigs fed 5 mg/kg Cr from CrPic for 75 days [36]. In addition, the concentration of Cr in the adrenal gland linearly increased with increased dietary CrPic (0 to 1 mg/kg Cr) fed to reproducing pigs for approximately 11 months [37]. Moreover, the normal Cr concentration in human plasma ranges from 0.1 to 2.1 μg/ml (2 to 40 μM) [38] which is beginning to approach the level used in the present study.

The increase of cortisol secretion from the cells pretreated with forskolin is notable (Fig. 4a). Forskolin stimulation during the 24 h of media collection period increased cortisol secretion by three- to five-folds (Figs. 1, 2a, 3a). Pretreatment of forskolin for 24 h induced a ten-fold increase in cortisol secretion compared with non-forskolin-treated cells (Fig. 4a). The higher fold change of cortisol secretion by pretreatment of forskolin implies that the second 24 h of cortisol secretion is higher than the first 24 h of that in the cortical cells cultured for 48 h with forskolin. In agreement, compared with 24 h of incubation with 10 μM forskolin, approximately three times higher cortisol concentration was reported in 48-h incubation with forskolin [18, 39]. This result may be attributed to intracellular cAMP accumulated during the pretreatment period.

The release of DHEAs was also inhibited by a high concentration of cortisol in agonist-stimulated adrenocortical cells (Figs. 3b, 4b). In postmenopausal women, dietary Cr tended to decrease serum DHEAs and cortisol [11].

The present study indicates that CrPic can appreciably regulate steroid secretion from agonist-stimulated cells, but minimally from non-stimulated cells, thus the sites of action for CrPic may be closely related to the sites affected by forskolin. H295R cells stimulated with forskolin have been reported to induce increases in cAMP, steroidogenic acute regulatory protein (StAR), several enzymes involved in the adrenal steroidogenic pathway, and organic anion transporter 3 (OAT3) [19, 24, 40–42]. Because a high-concentration CrPic inhibited the secretion of both cortisol and DHEAs from agonist-stimulated cells in the present study, it is postulate that CrPic affects the factors related to cortisol and DHEAs

synthesis in common. These factors include cAMP, StAR, cytochrome P450 enzyme (CYP) 11A1, and CYP17A1. Cholesterol transport is triggered by StAR, which is the rate limiting step in the steroid synthesis. Desmolase, also called CYP11A1, converts cholesterol to pregnenolone, a common precursor of adrenal steroidogenic pathway. This precursor can be converted to 17α-hydroxy pregnenolone by CYP17A1, which can be transformed to either cortisol or DHEAs by further steps. Oskarsson et al. [24] reported that the mRNA of CYP17A1 was increased 5-fold by 24 h of forskolin exposure to H295R cells, and CYP17A1 expression was the highest compared to the other enzymes. These steroidogenesis regulating factors are affected by cAMP through PKA pathway. Asif et al. [42] observed that OAT3 expression, involved in cortisol and DHEAs transport, was markedly potentiated by forskolin exposure, but the enzymes of steroidogeneis were increased to a lesser extent. Further studies to elucidate detailed mechanism of CrPic on steroidogenesis in the adrenal cortex seem warranted.

This study suggests that at high concentrations, CrPic inhibits aspects of steroidogenesis in agonist-stimulated adrenocortical cells.

Acknowledgements The authors thank Mrs. S. Hayes, University of Kentucky, for assistance in radioimmunoassay, and Dr. R. A. Anderson, USDA-ARS, and Dr. J. C. Matthews, University of Kentucky, for helpful comments. Special appreciation is expressed to Nutrition 21, Purchase, NY, for partial support for this study. During this research, B. G. Kim was a recipient of the Presidential Graduate Fellowship and the Kentucky Opportunity Fellowship at the University of Kentucky.

References

1. Anderson RA (1987) Chromium. In: Mertz W (ed) Trace elements in human and animal nutrition. Academic, New York, pp 225–244
2. Offenbacher EG, Pi-Sunyer FX (1988) Chromium in human nutrition. Annu Rev Nutr 8:543–563
3. Schwarz K, Mertz W (1959) Chromium(III) and the glucose tolerance factor. Arch Biochem Biophys 85:292–295
4. Mertz W, Toepfer EW, Roginski EE, Polansky MM (1974) Present knowledge of the role of chromium. Fed Proc 33:2275–2280
5. Vincent JB (2000) The biochemistry of chromium. J Nutr 130:715–718
6. Evans GW, Bowman TD (1992) Chromium picolinate increases membrane fluidity and rate of insulin internalization. J Inorg Biochem 46:243–250
7. Moonsie-Shageer S, Mowat DN (1993) Effect of level of supplemental chromium on performance, serum constituents, and immune response of stressed feeder steer calves. J Anim Sci 71:232–238
8. Mowat DN, Chang X, Yang WZ (1993) Chelated chromium for stressed feeder calves. Can J Anim Sci 73:49–55
9. Gentry LR, Fernandez JM, Ward TL, White TW, Southern LL, Bidner TD, Thompson DL, Horohov DW, Chapa AM, Sahlu T (1999) Dietary protein and chromium tripicolinate in Suffolk wether lambs: Effects on production characteristics, metabolic and hormonal responses, and immune status. J Anim Sci 77:1284–1294
10. Pechova A, Pavlata L, Illek J (2002) Metabolic effects of chromium administration to dairy cows in the period of stress. Czech J Anim Sci 47:1–7
11. Roussel AM, Bureau I, Favier M, Polansky MM, Bryden NA, Anderson RA (2002) Beneficial effects of hormonal replacement therapy on chromium status and glucose and lipid metabolism in postmenopausal women. Maturitas 42:63–69
12. van Heugten E, Spears JW (1997) Immune response and growth of stressed weanling pigs fed diets supplemented with organic or inorganic forms of chromium. J Anim Sci 75:409–416
13. Baldi A, Bontempo V, Dell'Orto V, Cheli F, Fantuz F, Savoini G (1999) Effects of dietary chromium-yeast in weaning-stressed piglets. Can J Anim Sci 79:369–374
14. Pollard GV, Richardson CR, Kamezos TP (2002) Effects of supplemental organic chromium on growth, feed efficiency and carcass characteristics of feedlot steers. Anim Feed Sci Technol 98:121–128
15. Kim BG, Lindemann MD, Cromwell GL (2009) The effects of dietary chromium(III) picolinate on growth performance, blood measurements, and respiratory rate in pigs kept in high and low ambient temperature. J Anim Sci 87:1695–1704

16. Ghafghazi T, McDaniel ML, Lacey PE (1980) Chromium-induced inhibition of insulin secretion from isolated islets of Langerhans. Diabetologia 18:229–232
17. Liu P, Lin M (1997) Biphasic effects of chromium compounds on catecholamine secretion from bovine adrenal medullary cells. Toxicol 117:45–53
18. Gazdar AF, Oie HK, Shackleton CH, Chen TR, Triche TJ, Myers CE, Chrousos GP, Brennan MF, Stein CA, La Rocca RV (1990) Establishment and characterization of a human adrenocortical carcinoma cell line that expresses multiple pathways of steroid biosynthesis. Cancer Res 50:5488–5496
19. Rainey WE, Bird IM, Sawetawan C, Hanley NA, McCarthy JL, McGee EA, Wester R, Mason JI (1993) Regulation of human adrenal carcinoma cell (NCI-H295) production of C19 steroids. J Clin Endocrinol Metab 77:731–737
20. Staels B, Hum DW, Miller WL (1993) Regulation of steroidogenesis in NCI-H295 cells—a cellular-model of the human fetal adrenal. Mol Endocrinol 7:423–433
21. Rainey WE, Bird IM, Mason JI (1994) The NCI-H295 cell line: a pluripotent model for human adrenocortical studies. Mol Cell Endocrinol 100:45–50
22. Rainey WE, Saner K, Schimmer BP (2004) Adrenocortical cell lines. Mol Cell Endocrinol 228:23–38
23. Vakharia K, Hinson JP (2005) Lipopolysaccharide directly stimulates cortisol secretion by human adrenal cells by a cyclooxygenase-dependent mechanism. Endocrinol 146:1398–1402
24. Oskarsson A, Ulleras E, Plant KE, Hinson JP, Goldfarb PS (2006) Steroidogenic gene expression in H295R cells and the human adrenal gland: adrenotoxic effects of lindane in vitro. J Appl Toxicol 26:484–492
25. Mikhaylova IV, Kuulasmaa T, Jaaskelainen J, Voutilainen R (2007) Tumor necrosis factor-alpha regulates steroidogenesis, apoptosis, and cell viability in the human adrenocortical cell line NCI-H295R. Endocrinol 148:386–392
26. Gell JS, Oh J, Rainey WE, Carr BR (1988) Effect of estradiol on DHEAS production in the human adrenocortical cell line, H295R. Reprod Sci 5:144–148
27. Ehrhart-Bornstein M, Lamounier-Zepter V, Schraven A, Langenbach J, Willenberg HS, Barthel A, Hauner H, McCann SM, Scherbaum WA, Bornstein SR (2003) Human adipocytes secrete mineralocorticoid-releasing factors. Proc Natl Acad Sci U S A 100:14211–14216
28. Institute SAS (1996) SAS Users Guide. SAS institute, Cary
29. Penhoat A, Naville D, El Mourabit H, Buronfosse A, Durand P, Begeot M (2000) Functional expression of the human ACTH receptor gene. Endocr Res 26:549–557
30. Seamon KB, Padgett W, Daly JW (1981) Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc Natl Acad Sci U S A 78:3363–3367
31. Litvin Y, Pasmantier R, Fleischer N, Erlichman J (1984) Hormonal activation of the cAMP-dependent protein-kinases in Att20 cells—preferential activation of protein kinase-I by corticotropin releasing-factor, isoproterenol, and forskolin. J Biol Chem 259:296–302
32. Ding X, Staudinger JL (2005) Induction of drug metabolism by forskolin: the role of the pregnane X receptor and the protein kinase A signal transduction pathway. J Pharmacol Exp Ther 312:849–856
33. Page TG, Southern LL, Ward TL, Thompson DL Jr (1993) Effect of chromium picolinate on growth and serum and carcass traits of growing-finishing pigs. J Anim Sci 71:656–662
34. Chang X, Mowat DN (1992) Supplemental chromium for stressed and growing feeder calves. J Anim Sci 70:559–565
35. Anderson RA, Bryden NA, Polansky MM, Gautschi K (1996) Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. J Trace Elem Exp Med 9:11–25
36. Lindemann MD, Cromwell GL, Monegue HJ, Purser KW (2008) Effect of chromium source on tissue concentration of chromium in pigs. J Anim Sci 86:2971–2978
37. Lindemann MD, Carter SD, Chiba LI, Dove CR, LeMieux FM, Southern LL (2004) A regional evaluation of chromium tripicolinate supplementation of diets fed to reproducing sows. J Anim Sci 82:2972–2977
38. Cerulli J, Grabe DW, Gauthier I, Malone M, McGoldrick MD (1998) Chromium picolinate toxicity. Ann Pharmacother 32:428–431
39. Asif AR (2004) Role of transport systems in cortisol release from human adrenal cells, PhD dissertation. Georg-August-Universität, Göttingen, Germany
40. Bird IM, Mason JI, Rainey WE (1998) Battle of the kinases: integration of adrenal responses to cAMP, DG and Ca^{2+} at the level of steroidogenic cytochromes P450 and 3betaHSD expression in H295R cells. Endocr Res 24:345–354
41. Zenkert S, Schubert B, Fassnacht M, Beuschlein F, Allolio B, Reincke M (2000) Steroidogenic acute regulatory protein mRNA expression in adrenal tumours. Eur J Endocrinol 142:294–299
42. Asif AR, Ljubojevic M, Sabolic I, Shnitsar V, Metten M, Anzai N, Muller GA, Burckhardt G, Hagos Y (2006) Regulation of steroid hormone biosynthesis enzymes and organic anion transporters by forskolin and DHEA-S treatment in adrenocortical cells. Am J Physiol Endocrinol Metab 291:E1351–E1359