Strain-specific steroidal control of pituitary function

Sang-Nam Lee, Bonnie Peng1, Roxane Desjardins2, John E Pintar1, Robert Day2 and Iris Lindberg

Department of Biochemistry and Molecular Biology, Louisiana State University Health Sciences Center, New Orleans, 1901 Perdido Street, New Orleans, Louisiana 70112, USA
1Department of Neuroscience and Cell Biology, University of Medicine and Dentistry of NJ, Fiscataway, New Jersey 08854, USA
2Département de Pharmacologie, Faculté de Médecine et Institut de Pharmacologie de Sherbrooke, Université de Sherbrooke, Québec, Canada J1H 5N4

(Requests for offprints should be addressed to I Lindberg; Email: ilindb@lsuhsc.edu)

Abstract

We have previously shown that 7B2 null mice on the 129/SvEvTac (129) genetic background die at 5 weeks of age with hypercorticotosteronemia due to a Cushing's-like disease unless they are rescued by adrenalectomy; however, 7B2 nulls on the C57BL/6NTac (B6) background remain healthy, with normal steroid levels. Since background exerts such a profound influence on the phenotype of this mutation, we have evaluated whether these two different mouse strains respond differently to high circulating steroids by chronically treating wild-type 129 and B6 mice with the synthetic steroid dexamethasone (Dex). Dex treatment decreased the dopamine content of the neurointermediate lobes (NIL) of 129 mice, leading to NIL enlargement and increased total D3R mRNA in the 129, but not the B6, NIL. Despite the decrease in this inhibitory transmitter, Dex-treated 129 mice exhibited reduced circulating α-melanocyte-stimulating hormone (α-MSH) along with reduced POMC-derived peptides compared with controls, possibly due to reduced POMC content in the NIL. In contrast, Dex-treated B6 mice showed lowered cellular ACTH, unchanged α-MSH and β-endorphin, and increased circulating α-MSH, most likely due to increased cleavage of NIL ACTH by increased PC2. Dex-treated 129 mice exhibited hyperinsulinemia and lowered blood glucose, whereas Dex-treated B6 mice showed slightly increased glucose levels despite their considerably increased insulin levels. Taken together, our results suggest that the endocrinological response of 129 mice to chronic Dex treatment is very different from that of B6 mice. These strain-dependent differences in steroid sensitivity must be taken into account when comparing different lines of transgenic or knockout mice.


Introduction

Prohormone convertase 1 (PC1/3) along with prohormone convertase 2 (PC2), members of the family of calcium-dependent subtilisin-like endoproteases, is predominantly expressed in neuronal and endocrine cells (Seidah et al. 1990, Day et al. 1992). Both PC1/3 and PC2 generate bioactive peptides from prohormones such as proopiomelanocortin (POMC; Benjamet et al. 1991), proglucagon (Rouille et al. 1997), and proinsulin (Bennett et al. 1992) by endoproteolytic processing at paired basic residues (reviewed by Seidah et al. 1994). PC2 and PC1/3 are both synthesized as proenzymes; the propeptide must be cleaved to generate an enzymatically active mature species (Muller & Lindberg 1999). Unlike PC1/3, the PC2 precursor requires a neuroendocrine-binding partner, 7B2, in order to generate an active enzyme molecule (Muller et al. 1997, Muller & Lindberg 1999). While the molecular mechanism of the PC2 and 7B2 interaction remains unclear, three subdomains within proPC2 and a 36-residue peptide within 7B2 are known to participate (Muller et al. 1999), and our present working hypothesis is that 7B2 blocks proPC2 from assuming a non-activatable conformer (S-N Lee & I Lindberg, unpublished data).

The POMC precursor is expressed in both the corticotrophs of the anterior lobe (AL) and the melanotrophs of the pituitary neurointermediate lobe (NIL). However, corticotrophs express mainly PC1 mRNA with low levels of PC2 mRNA, while melanotrophs express high levels of PC2 mRNA with appreciable PC1 mRNA levels (Seidah et al. 1990, Day et al. 1992). Since the specificity of PC1/3 and PC2 is not identical, processing of the POMC precursor differs greatly between corticotrophs and melanotrophs (Mains & Eipper 2000). In AL corticotrophs, POMC is processed mainly into adreno–corticotropic hormone (ACTH) and β-lipoprotein as well as into lesser amounts of β-endorphin, while in NIL melanotrophs, POMC-derived peptides are cleaved to a variety of smaller bioactive peptides, such as α-melanocyte-stimulating hormone (α-MSH) and β-endorphin (Mains & Eipper 2000). Secretion of POMC-derived peptides in both cell types is stimulated by both the hypothalamic peptides, arginine vasopressin and corticotropin-releasing factor (CRF; Vale et al. 1981, Lundblad & Roberts 1988). Control of
expression and secretion of POMC-derived peptides is negatively regulated in a cell type-dependent manner; in the AL, glucocorticoids exert a negative feedback effect by a direct action on corticotrophs (Raymond et al. 1979) and by inhibiting hypothalamic CRF (Suda et al. 1984). By contrast, in the NIL, POMC expression and secretion are primarily under direct tonic inhibitory control by A14 periventricular-hypophysial dopaminergic neurons (Goudreau et al. 1995).

In vivo chronic administration with D_2R agonists decreases melanotroph POMC biosynthesis, the secretion of α-MSH and β-endorphin (Chen et al. 1983, Beaulieu et al. 1984), and the melanotroph proliferation rate (Chronwall et al. 1987). In addition, removal of dopaminergic axons within the rat intermediate lobe by the administration of 6-hydroxydopamine increases melanotroph proliferation (Rychter & Stepien 1977, Gary & Chronwall 1992). Thus, POMC expression is controlled differently in the two lobes of the pituitary.

Previous data obtained using the PC2 and 7B2 null mouse models have shown that both nulls show defective prohormone processing in vivo (Furuta et al. 1998, Westphal et al. 1999, Laurent et al. 2002). Interestingly, PC2 and 7B2 null mice exhibit exceedingly different phenotypes; PC2 null mice are healthy except for slight hypoglycemia and running (Furuta et al. 1997), while 7B2 null mice die at 5 weeks due to a Cushing’s disease-like disorder (Westphal et al. 1999).

Cushing’s disease normally arises from adrenal hypercorticosteronism as a result of ACTH hypersecretion from anterior pituitary (reviewed by Shomali & Hussain 2000); the 7B2 null instead develops a Cushing’s-like disease via greatly enhanced secretion of ACTH from the NIL (Westphal et al. 1999). Hypercorticosteronism is directly involved in the lethal phenotype, as adrenalectomy rescues the 7B2 129 null from death (Laurent et al. 2002).

However, the PC2 and 7B2 nulls were not generated on identical backgrounds; the PC2 nulls are in a mixed 129X129 background, while the 7B2 nulls are in a pure 129 genetic background. The profound effect of background on the 7B2 mutation was clearly demonstrated by transfer of the 7B2 null onto a pure B6 background, where it does not exhibit signs of disease (Peinado et al. 2005). Conversely, the movement of the PC2 null to a 129 background results in the development of illness similar to the 7B2 null after six generations of backbreeding (Peinado et al. 2005). Since ACTH hypersecretion and hypercorticosteronism have been directly implicated in the development of illness, we speculated that the two strains are differentially sensitive to ACTH and steroids. Indeed, we have confirmed that 129 mice possess a more sensitive adrenocortical response to ACTH than B6 mice using ACTH challenge experiments (Peinado et al. 2005).

In order to test for other strain-dependent effects of excess circulating steroids, in this study we have treated wild-type 129 and B6 mice with chronic dexamethasone (Dex) to reproduce the effect of excess circulating steroid in a manner similar to that observed in 7B2 nulls. Dex is a potent synthetic corticosteroid that mimics the action of endogenous steroids in the suppression of AL ACTH synthesis and secretion (Murphy 1991). We have examined the effect of Dex on the hypothalamo–pituitary–adrenal (HPA) axis in both 129 and B6 wild-type mice and determined cellular and/or circulating plasma levels of ACTH, α-MSH, β-endorphin, dopamine, and insulin using RIA, and blood glucose levels. Lastly, we have performed ultrastructural analyses of melanotrophs and in situ hybridization of D_3R expression levels in NIL. Our results, described below, reveal considerable strain differences in the response to steroids in most of the parameters measured.

Materials and Methods

Animals

Four-week-old 129/SvEvTac and C57BL/6NTac wild type (WT) males were commercially purchased from Taconic Farms (Germantown, NY, USA). Mice were individually housed in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal care facility. Killing procedures were approved by the Louisiana State University Health Sciences Center (LSUHSC) animal care committee.

Dexamethasone administration

Four-week-old mice were given 10 μg/ml Dex (Sigma) in the drinking water for a week before they are killed; this timing was chosen to approximate the steroid susceptibility of the 129 7B2 null in the week prior to death. Pituitaries were removed and dissected into ALs and NILs. Separate lobes were individually homogenized via sonication either in 60 μl 0-5 M HClO_4 containing 10 mg/ml sodium bisulfite for dopamine assay or in 250 μl ice-cold 5 M acetic acid with 2 mg/ml BSA for ACTH and α-MSH assays. The samples were centrifuged for 15 min at 17 383 g at 4 °C and then clear supernatants were individually harvested into fresh tubes. Serum was prepared from trunk blood obtained from mice killed by decapitation at the same time of the day (1030–1230 h). Clotted blood was centrifuged briefly to separate the serum from cells and then collected individually in fresh tubes. All samples were stored frozen at –70 °C until use.

ACTH assays

Ten to twenty microliters of a 1/200 dilution of pituitary samples (ALs or NILs) or 50–100 μl sera prepared from either 129 control mice, Dex-treated 129 mice, B6 mice, or Dex-treated B6 mice were assayed in duplicate using the two-site Nichols human ACTH 1–39 assay kit (Nichols Institute, San Juan Capistrano, CA, USA). The 125I-ACTH antibody used in this kit is directed to both N-terminal and C-terminal regions of intact ACTH molecule and does not recognize ACTH cleavage products. Dilutions were performed in RIA buffer (100 mM sodium phosphate, pH 7.4, containing 0-1% heat-treated BSA, 50 mM NaCl, and 0-1% sodium azide).
Radioactivity was determined using a Wallac 1470 Wizard gamma counter (Perkin-Elmer Life and Analytical Sciences, Shelton, CT, USA).

Corticosterone assays

Five to ten microliters of sera were assayed using the Immuchem Double Antibody corticosterone $^{125}$I RIA kit (M P Biomedicals, Orangeburg, NY, USA).

α-MSH and β-endorphin assays

Twenty-five microliters of sera (insulin assay) or 50–100 μl sera (glucagon assay) were assayed using the Linco RIA kit (St Charles, MI, USA).

Insulin and glucagon assays

All antisera were raised in rabbits against peptides conjugated to keyhole limpet hemocyanin. The antiserum against PC2 (LS18) was directed against the COOH-terminus of mature PC2 (Shen et al. 1993). The antiserum against 7B2 (LS13) was raised against residues 23–39 of 7B2 (Zhu & Lindberg 1995). The antiserum against PC1 (LS2) was directed against the amino terminus of mature PC1 (Vindrola & Lindberg 1992, Zhu & Lindberg 1995). The antiserum against POMC (LS41) was directed against ACTH(1–24) (Fortenberry et al. 2002). Thirty microliters of pituitary (ALs or NILs) samples solubilized directly in Laemmli sample buffer were applied to the gels. Samples were subjected to electrophoresis on 10% Criterion Tris–HCl (Bio–Rad) SDS gels, followed by western blotting using the respective antisera. Proteins were transferred from gels to nitrocellulose membranes, and the membranes were preincubated in 5% non-fat milk in TBS for 30 min at room temperature prior to incubation overnight at 4 °C with antiserum 2B6 diluted 1:1000 in milk. Membranes were washed thrice with tris buffer saline (TBS) containing 0.05% Tween followed by incubation at room temperature for 1 h with secondary antibody (goat anti-rabbit IgG coupled to alkaline phosphatase) diluted 1:10 000 in milk. Membranes were then washed once with TBS containing 0.05% Tween and twice with TBS alone, and then developed with 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt/p-nitro blue tetrazolium chloride. For western blotting using horseradish peroxidase (HRP) conjugate as secondary antibody, membranes were incubated with SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL, USA) for 1 min, and the chemiluminescent bands were revealed using a Fluor–S Max MultiImager System.

**In situ hybridization**

Pituitaries were removed, embedded in OCT compound (Miles, Elkhart, IN, USA), frozen in 2-methylbutane on dry ice powder, and stored at −20 °C before sectioning. Sections (10 μm) were mounted onto Superfrost/Plus slides (Fisher Scientific, Fairlawn, NJ, USA) and stored at 70 °C until use.

The specific D2R complementary RNA and sense-strand control probes were transcribed using $^{35}$S-labeled UTP (New England Nuclear, Boston, MA, USA), Riboprobe System II transcription buffers (Promega), either T3 or T7 RNA polymerase (Stratagene, La Jolla, CA, USA), and a linearized plasmid construct that contains a 295 bp cDNA fragment corresponding to the D2R exon 2 coding region.

**Western blotting**

Twenty-five microliters of sera (insulin assay) or 50–100 μl sera (glucagon assay) were assayed using the Linco RIA kit (St Charles, MI, USA).
(sense-strand cRNA probes) in adjacent sections yielded only low background.

**Electron microscopy**

Sample preparation for electron microscopy was carried out using methods described previously (Day *et al.* 1987). Pituitaries from animals were removed, fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.4), embedded in Epon, and sectioned. The sectioned samples were stained with 1% uranyl acetate and 6% lead citrate (pH 10). Pictures of identical surface areas were taken at a magnification of ×7000.

**Statistical analysis**

Data were analyzed using Student’s *t*-test. Results are expressed as the mean ± S.E.M. of the number of samples indicated.

**Results**

We treated 5-week-old 129 and B6 mice with the synthetic glucocorticoid Dex for 1 week, mimicking the effect of high circulating corticosteroid levels found in 7B2 nulls just prior to their death. Following dissection of the AL and NIL from whole pituitary, the levels of various pituitary and circulating hormones were determined. It was important to analyze the two pituitary lobes separately, given the fact that the release and content of POMC-derived peptides differs significantly between the two lobes. Most of the Dex-treated 129 animals showed symptoms of illness such as slower growth, fragile, thin skin, and poor hair coat condition. Dex-treated B6 mice showed similar, but much less severe signs of illness. Both 129 and B6 mice treated with Dex exhibited growth retardation compared with controls, as indicated by reduced body weights (Fig. 1). Although the volumes consumed by Dex-treated 129 mice were slightly more than other groups of mice, this effect did not reach statistical significance (Fig. 1).

Circulating α-MSH levels were decreased approximately 2.5-fold in Dex-treated 129 mice when compared with their controls (Table 1). In contrast, Dex-treated B6 mice showed increased circulating α-MSH levels (twofold) compared with controls (Table 1).

Dexamethasone treatment results in decreased ACTH, α-MSH, β-endorphin, and POMC levels in 129 NIL

To test whether Dex treatment affects the pituitary contents of POMC-derived peptides, we performed RIAs of ACTH, α-MSH, and β-endorphin on pituitary lobe extracts; in addition, we performed western blotting for POMC, PC1, and PC2 in 129 and B6 NIL.

Although circulating ACTH levels in 129 WT controls were higher than those in B6 WT mice (Table 1), AL ACTH levels were lower in 129 mice than in B6 mice, potentially indicating a higher secretion rate in 129 AL (Table 2). Dex treatment resulted in considerably decreased ACTH levels in the AL of both strains via the normal response of glucocorticoid feedback inhibition (Table 2). Additionally, β-endorphin was detected in B6 AL (Table 2), but not in 129 AL. This result indicates that the β-endorphin content of the B6 AL is considerably higher than that of the 129 AL and confirms previous data on differential processing of POMC in different strains (Crabbe *et al.* 1981).
Total POMC contents were significantly higher in 129 mice than in B6 mice (Fig. 2B), possibly because the surface area of 129 NIL is fourfold larger than that of B6 NIL (Fig. 3A).

In Dex-treated 129 NILs, the POMC content was decreased, and PC1 and PC2 contents were unaltered (Fig. 2). In Dex-treated 129 ALs, the contents of POMC and both enzymes were unaltered (data not shown). In contrast, Dex treatment resulted in significantly decreased POMC, but increased PC2 content in B6 NIL (Fig. 2), suggesting that in B6 mice, Dex treatment facilitates the processing of ACTH to α-MSH via an increase in PC2.

Taken together with the RIA data presented in Table 1, these data imply that the decreased plasma α-MSH levels observed in Dex-treated 129 mice might result from the decreased POMC content in the NIL rather than via decreased release of α-MSH. On the other hand, in B6 NIL, Dex may increase POMC processing by upregulating the content of the prohormone convertase PC2.

In summary, Dex treatment decreased both circulating (Table 1) and pituitary (Table 2) levels of POMC-derived peptides in 129 mice (similar to the results described in Chen et al. 1983), but many of these effects on POMC peptides were not recapitulated in B6 mice.

### Table 1 Comparison of the serum levels of ACTH, corticosterone, and α-MSH in 129 and B6 mice treated with dexamethasone or vehicle (Ctr). Data represent the mean ± S.E.M. of the number of animals shown

<table>
<thead>
<tr>
<th></th>
<th>129</th>
<th>B6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ctr Dex</td>
<td>Ctr Dex</td>
</tr>
<tr>
<td>ACTH</td>
<td>pg/ml</td>
<td>pg/ml</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>28.9±2.5±* N=14</td>
<td>ND</td>
</tr>
<tr>
<td>α-MSH</td>
<td>pg/ml</td>
<td>pg/ml</td>
</tr>
</tbody>
</table>

ND, not detectable (below the limit of detection, 1.0 pg/ml for ACTH, 2.5 ng/ml for corticosterone). *Significant statistical differences in ACTH levels were observed between 129 Ctr and B6 Ctr mice (P<0.0012); †, between 129 Ctr and B6 Ctr mice (P<0.0001). Significant statistical differences in α-MSH levels were observed; ‡, between Ctr and Dex in 129 mice (P<0.0001); §, between Ctr and Dex in 129 mice (P=0.0005); ‌, between 129 Ctr and B6 Ctr mice (P<0.0001).

### Table 2 Comparison of the pituitary levels of ACTH, α-MSH, and β-endorphin (µg/pituitary) in 129 and 129 mice treated with dexamethasone or vehicle (Ctr). Data represent the mean ± S.E.M. of the number of animals shown

<table>
<thead>
<tr>
<th></th>
<th>129</th>
<th>B6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL NIL</td>
<td>AL NIL</td>
</tr>
<tr>
<td>ACTH</td>
<td>µg/pituitary</td>
<td>µg/pituitary</td>
</tr>
<tr>
<td>α-MSH</td>
<td>µg/pituitary</td>
<td>µg/pituitary</td>
</tr>
<tr>
<td>β-endorphin</td>
<td>µg/pituitary</td>
<td>µg/pituitary</td>
</tr>
</tbody>
</table>

ND, not detectable (<0.05 µg/pituitary). Significant statistical differences in the AL ACTH levels were observed; *, between Ctr and Dex of 129 mice (P<0.0001); †, between Ctr and Dex of B6 mice (P<0.0001); ‡, between 129 Ctr and 129 B6 Ctr mice (P<0.0001). Significant statistical differences in the NIL ACTH levels were observed; †, between 129 Ctr and 129 B6 Ctr mice (P<0.0001); §, between Ctr and Dex of B6 mice (P=0.0005); †, between Ctr and Dex of 129 mice (P<0.0001); ‡, between 129 Ctr and B6 Ctr mice (P<0.0001). Significant statistical differences in the NIL α-MSH levels were observed; ‡, between Ctr and Dex of 129 mice (P<0.0001); §, between Ctr and Dex of 129 mice (P=0.0132); ‌, between 129 Ctr and B6 Ctr mice (P<0.0002).

---

[1](#1)www.endocrinology-journals.org
Dexamethasone administration decreases dopamine levels in 129 NIL and induces a prominent ER–Golgi complex in 129 melanotrophs; total D2R mRNA levels are slightly increased in Dex-treated 129 NIL.

Interestingly, total NIL surface area was increased in Dex-treated 129 NIL (1.5-fold), indicating that 1 week of Dex administration causes melanotroph proliferation in 129 mice, but not in B6 mice (Fig. 3A). Since dopamine has a key role in the control of cell proliferation and in the maintenance of the melanotroph phenotype (Saiardi & Borelli 1998), we determined total dopamine levels and D2R mRNA densities (grains per mm²) in the NILs of Dex-treated 129 and B6 mice using RIA and in situ hybridization respectively. In addition, we studied the morphological effect of Dex at the ultrastructural level using electron microscopy.

Basal dopamine levels in the NILs of 129 mice were significantly higher than those of B6 NILs (Fig. 3B), possibly due to the different sizes of the tissue in each strain (Fig. 3A). Dex treatment produced a lower dopaminergic tone in 129 NIL (1.3-fold), but not in B6 NIL (Fig. 3B), indicating differential steroid control. However, the dopamine level in the AL of both the strains of mice was unaltered following Dex treatment (data not shown). Since the synthesis and release of α-MSH from the NIL are under inhibitory control by dopamine (Goudreau et al. 1995), these decreased dopamine levels in the Dex-treated 129 NIL would be expected to result in enhanced release and synthesis of α-MSH; this was clearly not the case (Table 1). However, melanotrophs in Dex-treated 129 mice, but not in B6 mice, contained a prominent endoplasmic reticulum and Golgi complex, a phenotype associated with dopaminergic loss (Fig. 4; Gary & Chronwall 1992, Chronwall et al. 1996).

Dex treatment resulted in a trend (P=0.056) toward decreased D2R mRNA densities in 129 NIL, but not in B6 NIL (Fig. 3C). However, when the values of D2R mRNA levels in Dex-treated 129 NIL were slightly increased (1.2-fold) are observed. This result is consistent with our finding of decreased dopamine levels in Dex-treated 129 NIL (Fig. 3B). In vivo chronic treatment with D2R antagonists is also known to increase D2R mRNA expression (Autelitano et al. 1989).
Dexamethasone treatment of 129 animals results in considerably decreased glucose levels

We previously found that 7B2 nulls on the 129 background exhibit severe hypoglycemia; this contributes greatly to lethality (Sarac et al. 2002). In the work reported here, we observed that approximately 15% of the Dex-treated 129 mice unexpectedly died at about day 5, while all B6 mice always survived Dex treatment. We determined serum glucose,
insulin, and glucagon levels in the surviving mice. Dex-treated 129 mice exhibited lowered blood sugar levels and strikingly elevated insulin levels, but only slightly increased levels of glucagon (Fig. 5). This result suggests that the lethality observed in Dex-treated 129 mice could occur, at least in part, due to considerably decreased glucose levels. In contrast, Dex treatment of B6 mice resulted in highly increased insulin and glucagon levels (Fig. 5); however, this did not result in lowered blood sugar levels, suggesting greater resilience and/or compensatory mechanisms in this strain.

Discussion

We have previously reported that the Cushing’s disease-like symptoms generated by intermediate lobe ACTH hypersecretion and adrenal hypercorticosteronism in 7B2 nulls are heavily influenced by background (Peinado et al. 2005). In addition, we found that hypercorticosteronism is directly involved in the lethal phenotype of the 7B2 null, as adrenalectomy rescues this null from early death (Laurent et al. 2002). Since we observed a 3-5-fold drop in pituitary dopamine (an inhibitory transmitter controlling NIL peptide release) in 129 7B2 null mice, but not in B6 7B2 nulls, and since dopamine levels return to normal in adrenalectomized animals (Laurent et al. 2002), we hypothesized that steroids could somehow be involved in the dopaminergic disinhibition phenomenon, generating increased intermediate lobe peptide release. Further, this effect would have to occur in a strain-specific manner, since it does not occur in B6 7B2 nulls. This led us to the present investigation of potential differential strain effects of glucocorticoids on the HPA axis.

Dex treatment results in differential effects on pituitary dopamine and POMC processing in 129 and B6 mice

We observed that chronic Dex treatment indeed reduces pituitary dopamine in 129 mice, but not in B6 mice, upholding our previous results on strain-specific steroidal control of pituitary dopamine levels (Laurent et al. 2002). Interestingly, we observed a distinct enlargement of the NILs in Dex-treated 129 mice, accompanied by extensive rough endoplasmic reticulum and a prominent Golgi apparatus in melanotrophs. Earlier studies by others had shown that administration of D2R antagonists in vivo results in proliferation of melanotrophs. D2R-deficient mice on the mixed 129/Sv × C57BL/6 genetic background, with a 75% contribution of C57BL/6 background, show enhanced

---

Figure 5 Dex-treated 129 animals exhibit lowered blood sugar levels and are hyperinsulinemic and hyperglucagonemic compared with their WT controls (Ctr). (A) Glucose levels (*significant differences between Ctr and Dex-treated 129 mice, \( P < 0.0001 \)). (B) Insulin levels (†significant differences between Ctr and Dex-treated 129 mice, \( P < 0.0001 \); ‡significant differences between Ctr and Dex-treated B6 mice, \( P < 0.0001 \).) (C) Glucagon levels (§significant differences between Ctr and Dex-treated 129 mice, \( P = 0.0071 \); ‡significant differences between Ctr and Dex-treated B6 mice, \( P < 0.0001 \); \( n \geq 10 \)).
melanotroph numbers (Saiardi & Borelli 1998), and removal of dopaminergic axons by the administration of 6-hydroxydopamine results in an increase in melanotroph proliferation in rat pituitary intermediate lobe (Gary & Chronwall 1992), linking downregulation of dopaminergic control to melanotroph proliferation. Therefore, the melanotroph proliferation exhibited by Dex-treated 129 mice is likely to be caused by the decrease in dopamine. It is interesting that these effects do not occur in B6 mice, a strain resistant to Dex-induced changes in dopamine.

Since dopamine is an inhibitory transmitter in the NIL, we hypothesized that the decreased dopaminergic tone generated by Dex treatment of 129 mice would result in increased circulating POMC-derived peptide levels; however, Dex treatment of 129 mice resulted in significantly decreased rather than increased circulating α-MSH, the intermediate lobe ACTH-derived peptide product in wild-type mice. Since Dex-treated 129 mice also exhibited reduced NIL POMC, α-MSH, and β-endorphin levels, clearly Dex affects POMC synthesis in 129 NIL; if there is a Dex effect on NIL release, it is overshadowed by Dex effects on synthesis. Previous studies have shown that Dex treatment results in a reduction in NIL POMC mRNA in both intact and adrenalectomized rats (Roberts et al. 1982, Schachter et al. 1982). It has also been found that long-term Dex treatment of rats abolishes the haloperidol-induced increase in ir-β-endorphin, but further increases the haloperidol-induced rise in POMC mRNA (Autelitano et al. 1987), supporting the idea that circulating glucocorticoids can have complex and potent effects on NIL POMC expression and secretion.

The mechanism by which glucocorticoids can affect NIL POMC is not well understood. It has been demonstrated that the expression of GR in adult rat melanotropin cells is under negative dopaminergic control (Antakly et al. 1987). Schimchowitsch et al. (1994) reported the presence of functional GR, which negatively regulates melanotropin activity in the rabbit pituitary intermediate lobe. We performed western blotting to observe potentially induced changes in pituitary GR expression levels, but it proved impossible to detect any GR bands (data not shown). In situ hybridization analysis using PCR-amplified GR signals is needed to elucidate whether the reduction of dopamine levels has an effect on GR mRNA expression. In conclusion, since we could not recapitulate the NIL hypersecretion phenomenon using Dex treatment of wild-type mice, but did observe the expected dopamine decrease, the reduction in dopamine in the 129 7B2 null is unlikely to represent a primary cause of its NIL ACTH hypersecretion.

Dex treatment of B6 mice resulted in different cellular and circulating profiles of POMC-derived peptides when compared with 129 mice. While we also observed reduced NIL ACTH, α-MSH, and β-endorphin in this strain, dopamine levels were unaltered, and an increase in circulating α-MSH was found. Interestingly, Dex treatment also resulted in significantly increased PC2 levels in the B6 (but not in the 129) NIL; since this enzyme is responsible for processing ACTH to α-MSH, this increase may directly explain the decreased tissue ACTH and increased circulating MSH levels. Other studies have shown that Dex treatment increases PC2 expression in the ocular ciliary epithelium (Ortego et al. 2002) and the endocrine cell line MTC 6–23 (Barbero & Kitabgi 1999). In contrast, the 129 NIL seemed to be fairly resistant to PC2 induction by Dex, indicating strain-specific differences in control of PC2 expression. In summary, the ability of Dex to control the synthesis and release of POMC-derived peptides in either the 129 or B6 NIL appears to occur directly through effects on POMC and PC2 expression rather than via dopaminergic mechanisms.

Dex treatment uncovers strain differences in glucose regulation

In addition to strain-dependent effects of Dex on pituitary peptides, we observed differences in glucose regulation. For example, Dex-treated 129 animals exhibited lowered blood sugar levels and highly increased insulin levels. In contrast, Dex-treated B6 animals, while also showing considerably increased insulin levels, had slightly increased glucose levels relative to controls. Recently, Goren et al. (2004) reported that male C57BL/6 animals are susceptible to glucose intolerance, which is associated with impaired insulin sensitivity in liver and fat tissues, whereas the liver and fat tissues from 129×1 animals exhibit greater insulin sensitivity than the same tissues from other strains. Therefore, the Dex-induced decrease in glucose levels in 129 mice might be due to strain-dependent insulin hypersensitivity, a hypothesis which requires testing through additional experiments. In addition, we observed that although insulin-induced hypoglycemia induces a rapid increase in plasma levels of hormones (glucagon, epinephrine, growth hormone, and cortisol) related to the glucose counterregulatory response (Cryer 1993), the increase in insulin was significantly higher in Dex-treated 129 mice when compared with Dex-treated B6 mice, while glucagon levels were lower in 129 mice after Dex treatment than in B6 mice. The lowered blood sugar levels seen in Dex-treated 129 mice support our previous data showing that older 7B2 nulls on the 129 background exhibit severe hypoglycemia, which contributes greatly to lethality (Sarac et al. 2002).

In conclusion, our study suggests that although both strains of mice can expand their insulin supply in response to increased Dex, only 129 mice exhibit glucocorticoid hypersensitivity, and these mice may consequently be more susceptible to the increased functional stress imposed by chronic corticosteronemia, resulting in their much greater morbidity when challenged with Dex administration. The exaggerated Dex response in 129 mice appears to be due to the failure of normal regulatory mechanisms, which modulate hormonal secretion and/or deficits in compensatory responses to impaired glucose homeostasis. In addition to the data showing that Dex-treated 129 NILs exhibit blunted inhibitory dopaminergic control of POMC expression and secretion, our results imply that the 129 mouse is already
predisposed to Cushing's disease and Dex treatment induces further iatrogenic Cushing's disease. Therefore, we clearly demonstrate here that the lethal phenotype observed in 129 7B2 nulls (Laurent et al. 2002), but not in B6 7B2 nulls (Peinado et al. 2005), is caused by strain-dependent differences in steroid sensitivity. These considerable strain differences between 129 and B6 mice may provide a promising model system for studying steroidal effects on the HPA axis, and must certainly be taken into account when comparing the hormonal status of nulls constructed on different backgrounds.

Acknowledgements

We thank Gregory Hubbard and Jan Dufrene for assistance with animal handling. This work was supported by grants from NIH DK 49703 to I L, DA-08622 to J E P, and CIHR MOP-79283 and MOP-57870 to R D. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


Barbero P & Kitabgi P 1999 Protein 7B2 is essential for the targeting and activation of PC2 into the regulated secretory pathway of mTMC 6–23 cells. Biochemical and Biophysical Research Communications 257 473–479.


Benjannet S, Rondeau N, Day R, Chretien M & Seidah NG 1991 PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. PNAS 88 3564–3568.


Chen CL, Dionne FT & Roberts JL 1983 Regulation of the pro-opiomelanocortin mRNA levels in rat pituitary by dopaminergic compounds. PNAS 80 2211–2215.


Goren HJ, Kulkarni RN & Kahn CR 2004 Glucose homeostasis and tissue transcript content of insulin signaling intermediates in four inbred strains of mice: C57BL/6, C57BL/KsJ, DBA/2, and 129X1. Endocrinology 145 3307–3323.


S-N LEE and others · Strain differences in steroid response

Downloaded from Bioscientifica.com at 11/06/2018 05:54:48PM via free access


Sarac MS, Zieske AW & Lindberg I 2002 The lethal form of Cushing’s in 7B2 null mice is caused by multiple metabolic and hormonal abnormalities. Endocrinology 143 2324–2332.


Seidah NG, Gaspar L, Mion P, Marcinkiewicz M, Mbiakay M & Chretien M 1990 cDNA sequence of two distinct pituitary proteins homologous to Kex2 and furin gene products: tissue-specific mRNAs encoding candidates for pro- hormone processing proteinases. DNA and Cell Biology 9 789.


Vindrola O & Lindberg I 1992 Biosynthesis of the prohormone convertase mPC1 in AtF-20 cells. Molecular Endocrinology 6 1088–1094.


Received in final form 30 November 2006
Accepted 6 December 2006
Made available online as an Accepted Preprint 27 December 2006