Cell Tissue Res
DOI 10.1007/s00441-007-0436-0

REGULAR ARTICLE

Distribution of cells expressing Jun and Fos proteins and synthesizing DNA in the adrenal cortex of hypophysectomized rats: regulation by ACTH and FGF2

Thompson Eusébio Pavan Torres · Claudimara F. P. Lotfi

Received: 16 January 2007 / Accepted: 7 May 2007

Abstract Protein expression of the early response genes, *jun* and *fos*, has been suggested to play an important role in the in vitro and in vivo proliferation of adrenal cells. To elucidate the immunolocalization of proliferative cells and the patterns of adrenal gland expression of members of the activating protein-1 (AP-1) family of oncogenes, we used hypophysectomized rats. The effects of adrenocorticotropic hormone (ACTH) and fibroblast growth factor 2 (FGF2) on Fos and Jun protein expression were investigated, and DNA synthesis was assessed by using bromodeoxyuridine (BrdU) incorporation. No change was detectable in the adrenal cortex at 2 days after hypophysectomy, although a reduction occurred in the number of BrdU-positive cells in the zona fasciculata. This hypophysectomy-induced early phase of adrenal cortex atrophy in the zona fasciculata was correlated with JunB protein induction, suggesting the formation of an inhibitory AP-1 complex. Accumulation of c-Jun/JunD and c-Fos/FosB, but not of JunB, in the zona fasciculata and zona reticularis implied that, after ACTH stimulation, these proteins were the principal AP-1 components in these zones. In these same zones, ACTH increased BrdU-positive cell counts, indicating that the composition of the AP-1 complex in these zones was proliferation-related. However, FGF2 induced an antagonistic modulation of the response to ACTH, by reducing the numbers of Jun-/Fos-positive cells and inhibiting DNA synthesis. Our results implicate the AP-1 family of transcription factors (in particular, the dynamics within the Jun protein family) in the regulation of cell control during ACTH-induced proliferation of the adrenal cortex.

Keywords Adrenal glands · Adrenocorticotropic hormone · Transcription factor AP-1 · Proto-oncogene proteins · c-Fos · c-Jun · Rat (Sprague Dawley, male)

This study received financial support in the form of grants and fellowships: C.F.P.L. is the recipient of grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, Foundation for the Support of Research in the State of São Paulo) and from the Pró-Reitoria de Pesquisa da Universidade de São Paulo (University of São Paulo Dean's Office for Research Projects); T.E.P.T. is the recipient of a Scientific Initiation fellowship from FAPESP.

T. E. P. Torres · C. F. P. Lotfi (✉)
Department of Anatomy, Institute of Biomedical Sciences,
University of São Paulo,
São Paulo 05508-900 SP, Brazil
e-mail: clotfi@usp.br

Introduction

The adrenal gland is composed of two distinct regions: the cortex and the medulla. The cortex consists of three concentric zones: the zona glomerulosa (ZG), the zona fasciculata (ZF), and the zona reticularis (ZR). Adrenocorticotropic hormone (ACTH) is the primary regulator of adult adrenal functions. The primary effect of ACTH on the adrenal cortex is to increase glucocorticoid synthesis and secretion via the G-protein-coupled receptor (Clark and Cammas 1996; Orth and Kovacs 1998). However, ACTH is also considered mitogenic because of the correlation between the levels of circulating ACTH and the size of the adrenal cortex (New 1998). Nevertheless, the regulation and mechanisms of cell proliferation/differentiation in the adrenal cortex have yet to be fully defined. Studies conducted in vitro have demonstrated that ACTH is a mitogenic hormone in the Y-1 mouse adrenocortical tumor cell line (Lotfi et al. 1997; Mattos and Lotfi 2005) and is an antimitogenic hormone in cell lines and primary cultures of

mouse, rat, and human adrenocortical cells. These observations suggest that, in vivo, ACTH has an indirect mitogenic effect (Hornsby 1985) and might be mediated by paracrine factors. However, the key regulatory genes are unknown, and their modulation by ACTH or by growth factors in the distinct cells that comprise the adrenal cortex is poorly understood. ACTH and fibroblast growth factor 2 (FGF2) have both been shown rapidly to induce the *c-fos* proto-oncogene in the Y-1 line (Kimura et al. 1993; Lotfi et al. 1997; Lotfi and Armelin 2001), in primary cultures (Viard et al. 1992; Mattos and Lotfi 2005) and in vivo (Yang et al. 1990; Imai et al. 1990). This suggests that these two factors regulate the activity of the transcription factor activator protein 1 (AP-1), which binds DNA and regulates gene expression in response to a diverse array of stimuli (Angel and Karin 1991; Shaulian and Karin 2001). Signals related to cell growth, cell differentiation, and environmental stress are transduced to the nucleus via activation of signaling pathways that induce the AP-1 complex (Shaulian and Karin 2001). The AP-1 dimers are comprised of Jun family protein (c-Jun, JunB, JunD) homodimers, Jun heterodimers with Fos (c-Fos, FosB, Fra-1, Fra-2), or activating transcription factor family proteins (Angel and Karin 1991; Shaulian and Karin 2001). The AP-1 complex can, therefore, consist of many different combinations of heterodimers and homodimers, and this combination determines the genes that are regulated by AP-1 (Eferl and Wagner 2003). The degree to which AP-1 determines cell fates depends on the relative abundance of AP-1 subunits, the composition of AP-1 dimers, the quality of the stimulus, the cell type, and the cellular environment (Hess et al. 2004).

Results previously obtained regarding the growth effects of ACTH appear contradictory (Zwermann et al. 2004). However, this is not surprising, since the final biological responses depend on the cell type and the responding state and on the relationships that the cell has with other tissue cells and signals in the environment. For these reasons, we have recently analyzed the effects that ACTH and FGF2 have on the immunolocalization of Fos and Jun proteins in rat adrenal infusion in situ (Baccaro et al. 2007). We have found that AP-1 components are correlated with in vivo ACTH and FGF2 proliferation in rats receiving dexamethasone.

In the present study, we used animals that had been hypophysectomized, thereby eliminating the influence of the pituitary gland but preserving the relationships among diverse physiological systems, especially among the various neuronal systems. We analyzed the effect of hypophysectomy on the proliferation and expression of c-Jun, JunB, JunD, c-Fos, FosB, Fra-1, and Fra-2 proteins in the different zones of the adrenal gland. In addition, we quantified ACTH/FGF2 regulation of *jun*/*fos* activity and DNA synthesis in cells obtained from hypophysectomized animals after a 12-h incorporation of 5-bromo-2-deoxyuridine (BrdU).

Materials and methods

Animals

Rats (male, Sprague Dawley, weighing 250±30 g) were obtained from the Biomedical Sciences Institute of the University of São Paulo and maintained in a temperature-controlled environment with a 12-h light/dark cycle. The Animal Experimentation Ethics Committee of the Biomedical Sciences Institute approved all protocols. All animals had free access to tap water and normal rat chow. All experiments were conducted between 9:00 a.m. and 11:00 a.m. At the indicated time, rats were killed by decapitation, and their adrenal glands were harvested. Trunk blood was collected in chilled tubes containing 3.8% EDTA. The blood was separated into cellular and plasma fractions (5,000 rpm for 10 min at 4°C), and the plasma was stored at –80°C until being analyzed by chemiluminescent immunoassay for ACTH (Immulite; Diagnostic Products, Los Angeles, Calif., USA).

Hypophysectomy

Animals were anesthetized by intraperitoneal injections of ketamine (5 mg/100 g body weight [BW]), acepromazine (0.2 mg/100 g BW), and xylazine (1 mg/100 g BW) and hypophysectomized by the parapharyngeal approach (Waynforth and Flecknell 1994) or sham-operated. After decapitation, the hypophyseal fossae of ostensibly hypophysectomized rats were visually inspected for the completeness of hypophysectomy. Only those rats deemed hypophysectomized (by visual inspection) and presenting low plasma ACTH levels were included in subsequent experiments.

Histological analysis of adrenal cortical zones after surgery

Hypophysectomized rats were allowed to survive, in groups of three, for various periods (2, 4, 5, 7, 9, or 10 days), during which time they were maintained on a normal diet. After the rats had been decapitated, the adrenal glands were removed, fixed in 4% paraformaldehyde in a solution of 0.1 M phosphate buffer (PBS; pH 7.4) for 8 h at room temperature, and then immersed in PBS plus 6% sucrose for 12 h. Fixed adrenal tissue was dehydrated and then embedded in paraffin. Transverse (3 μm thick) sections were cut and mounted on gelatin-coated glass slides. After deparaffinization (24 h at 60°C), sections were rehydrated in graded ethanol and sterile Milli-Q water and then stained with hematoxylin and eosin (H&E). The H&E-stained sections were examined by light microscopy under an objective with a 1-mm ocular grid in order to measure the length of the different cortical zones.

In vivo treatment and BrdU incorporation

At 72 h after the surgical procedure, the study-group rats were divided into five sub-groups: one group of sham-operated rats (Sham group) and four groups of hypophysectomized rats, all of which received single intraperitoneal injections of one of the following: 20 ng/ml saline (Hyp group); 10^{-7} M $ACTH_{39}$ (Hyp+ACTH group); 20 ng/ml bovine recombinant FGF2 (Hyp+FGF2 group); or 10^{-7} M $ACTH_{39}$ plus 20 ng/ml FGF2 (Hyp+ACTH+FGF2 group). At 12 h after the initial injections, all animals received single intraperitoneal injections of 100 mg BrdU (Sigma, St. Louis, Mo., USA) per 100 g BW, based on the protocols for in vitro BrdU incorporation described by Lotfi et al. (1997) and Mattos and Lotfi (2005). At 24 h after the initial injections, the rats were killed by decapitation. This protocol permitted the administered reagents to promote the transition of cells from the G1 to the S phase of the cell cycle and allowed 12 h of BrdU incorporation, resulting in the accumulation of S-phase cells (although BrdU incorporation does not prove that a cell has entered into mitosis, it is useful for identifying proliferative activity in tissue sections; Muskhelishvili et al. 2003). Each experimental group contained four or five rats. Adrenal glands were removed, fixed for 8 h in 10% formaldehyde, and embedded in paraffin.

In vivo treatment for expression of Fos and Jun proteins

At 72 h after the surgical procedure, the animals were divided into five groups (Hyp, Hyp+ACTH, Hyp+FGF2, Hyp+ACTH+FGF2, and Sham) and treated as described above. At 2 h after the injections, the rats were killed, after which the adrenal glands were removed and fixed as described in the histological analysis section.

Immunostaining for BrdU

After being fixed and deparaffinized, the sections were rehydrated in graded ethanol, sterile Milli-Q water, and PBS, followed by methanol with 1% hydrogen peroxide. Sections were incubated with monoclonal anti-mouse-BrdU antibody (Amersham Pharmacia Biotech, Uppsala, Sweden). The immune complexes were detected as described below. Adrenal sections incubated in pre-absorbed primary antibody with 100 mg/ml BrdU or in nonimmune primary sera tested negative (not shown). Jejunal sections were used as positive controls.

Immunohistochemistry for Fos and Jun proteins

After being fixed and deparaffinized, the sections were rehydrated in graded ethanol, sterile Milli-Q water, and PBS, followed by PBS with 0.5% hydrogen peroxide. Sections were incubated with anti-c-Fos, anti-FosB, anti-Fra-1, anti-Fra-2, anti-c-Jun, anti-JunB, or anti-JunD (Santa Cruz Biotechnology, Santa Cruz, Calif., USA), diluted 1:100 in PBS. They were then rinsed with PBS, after which the immune complexes were detected by immunoperoxidase staining with the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, Calif., USA) and diaminobenzidine (Sigma Fast; Sigma Aldrich, St. Louis, Mo., USA). The sections were washed in Milli-Q water, stained with Harris' hematoxylin, differentiated with a saturated solution of lithium carbonate, dehydrated, and mounted with Entellan (Merck, Darmstadt, Germany). Adrenal sections incubated in nonimmune primary sera yielded negative results (not shown).

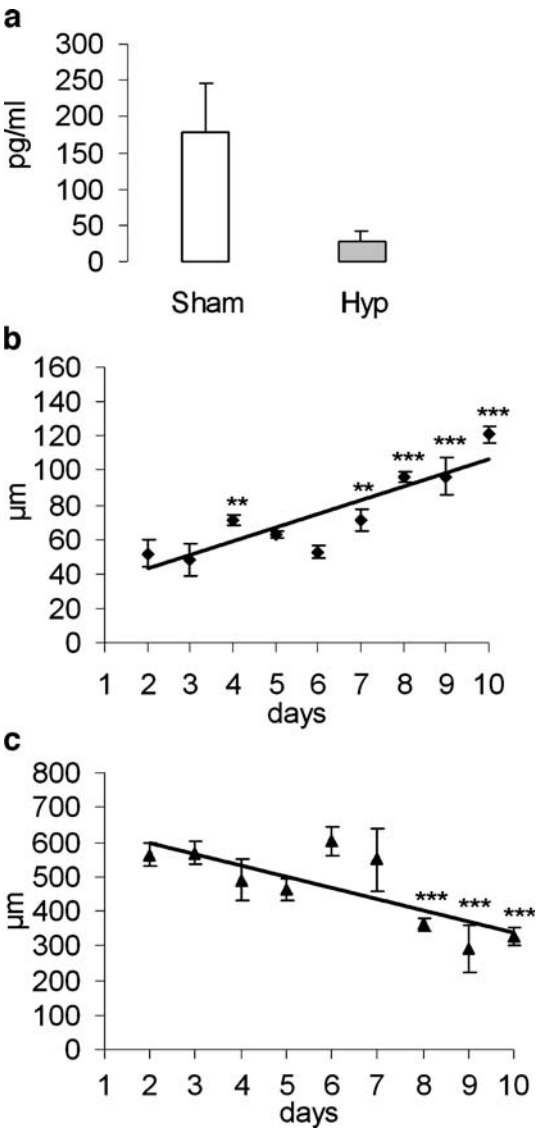

Fig. 1 **a** Effect of hypophysectomy on ACTH plasma concentrations in sham-operated (*Sham*) and hypophysectomized (*Hyp*) rats. **b** Effect of hypophysectomy in the zona glomerulosa. **c** Effect of hypophysectomy in the zona fasciculata and zona reticularis. The differences between post-hypophysectomy days were analyzed by an analysis of variance (ANOVA) and the Tukey-Kramer Multiple Comparisons Test. ***$P<0.001$ and **$P<0.01$ vs. post-hypophysectomy day 2

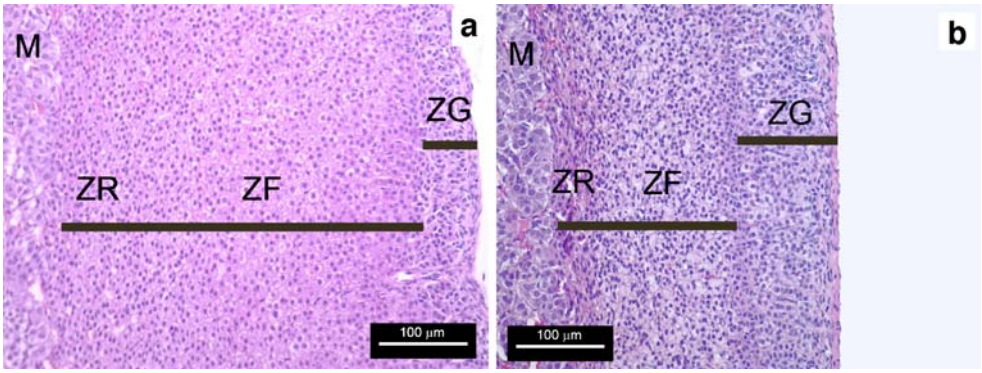

Fig. 2 Photomicrographs of rat adrenal glands stained with hematoxylin and eosin showing (**a**) post-hypophysectomy day 2 and (**b**) post-hypophysectomy day 10 (*ZG* zona glomerulosa, *ZF* zona fasciculata, *ZR* zona reticularis, *M* medulla)

Immunoreactive cell counts

The effects that the various treatments had on the expression of Fos and Jun proteins in the adrenal cortex was quantified by counting the number of immunostained cortical cells per unit area of the cortex. Three to five animals were used in each treatment. For each adrenal gland, three sections containing the medulla were randomly selected. In each section, five test fields of 0.75 mm^2 each were sampled randomly in the three zones of the adrenal cortex and examined with a 40× objective attached to a Nikon microscope equipped with a video camera. Images were digitalized by using Image-Pro Plus, and all immunoreactive nuclear profiles, regardless of the intensity of the immunostaining, were counted. For the quantification of BrdU incorporation, 10–15 areas (with 90–100 nuclei/area) from each section were sampled randomly in the three zones of the adrenal cortex by using the same equipment and image program described above. For each zone of each adrenal gland, the BrdU index, defined as the number of BrdU-positive nuclei and expressed as a percentage of the total number of nuclei, was determined. Together with the BrdU index, the morphology of the labeled cells was considered, as BrdU incorporation has also been seen in apoptosis.

Results

Hypophysectomy induces atrophy of ZF and ZR, together with an increase in the size of ZG

As shown in Fig. 1a, plasma ACTH levels were significantly lower ($P<0.0001$) in the Hyp group (26.8±15.1 pg/ml) than in the Sham group (179.5±66.7 pg/ml), confirming a hypophysectomy-induced decrease in endogenous ACTH. By post-hypophysectomy day 10, significant enlargement of the ZG (Fig. 1b) and marked atrophy in the ZF/ZR (Fig. 1c) had occurred. From post-hypophysectomy day 2 to post-hypophysectomy day 10, ZF/ZR atrophy increased by 42%, and the size of the ZG increased by 135% (Fig. 2).

Hypophysectomy inhibits S-phase entry in ZF of the rat adrenal cortex

In order to determine whether the changes in the adrenal cortex after hypophysectomy were attributable to altered S-phase entry in these zones, we used a 12-h incorporation of BrdU in Hyp rats to be killed on the post-hypophysectomy

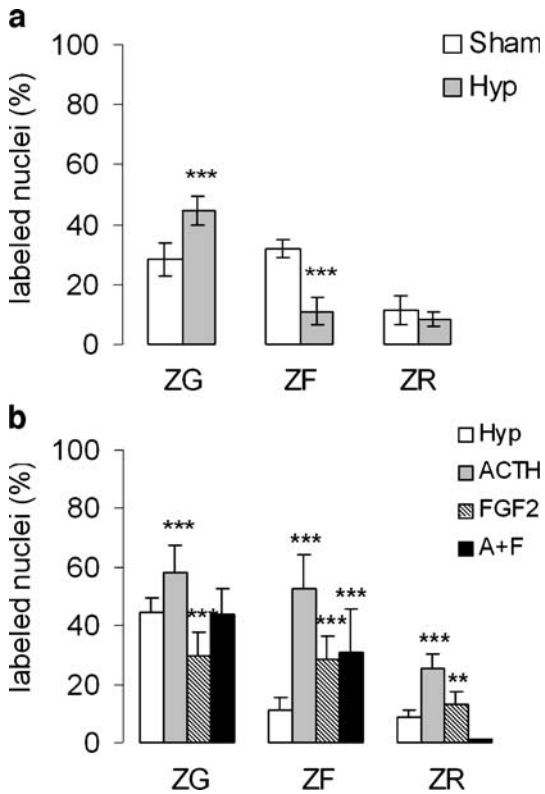

Fig. 3 **a** Effect of hypophysectomy on the percentage of BrdU-positive nuclei in the various zones of the adrenal cortex in sham-operated (*Sham*) and hypophysectomized (*Hyp*) rats (*ZG* zona glomerulosa, *ZF* zona fasciculata, *ZR* zona reticularis). **b** Effect of ACTH (10^{-7} M, *ACTH*), FGF2 (20 ng/ml, *FGF2*), and the ACTH-FGF2 combination (*A+F*) on the percentage of BrdU-positive nuclei in the various zones of the adrenal cortex in hypophysectomized (*Hyp*) rats. Values are given as the number of BrdU-positive nuclei expressed as a percentage of the total number of nuclei. The differences between values for BrdU incorporation of Sham versus Hyp rats and Hyp versus treated rats were analyzed by ANOVA and the Tukey-Kramer Multiple Comparisons Test. ***$P<0.001$, **$P<0.01$

day and in Sham rats (Fig. 3a). We found that the numbers of BrdU-positive nuclei in the ZF were significantly lower in the Hyp rats than in the Sham rats ($P<0.001$), whereas no significant alterations were observed in the ZR (Fig. 4a,b). However, the numbers of such cells in the ZG were significantly higher in the Hyp rats than in the Sham rats ($P<0.001$).

ACTH administration in hypophysectomized rats induces S-phase entry in all zones of adrenal cortex

In Hyp+ACTH rats, the percentages of BrdU-positive nuclei throughout the adrenal cortex were significantly greater ($P<0.001$) than those observed for Hyp rats (Fig. 3b): 1.3-times greater in the ZG, 4.8-times greater in the ZF, and 3.0-times greater in the ZR (Fig. 4c–e). In the ZF and ZR, BrdU uptake was significantly greater (2.6-times and 1.5-times, respectively) in the Hyp+FGF2 rats than in the Hyp rats (ZF: $P<0.001$; ZR: $P<0.01$). Therefore, ACTH and FGF2 both induced proliferation in the ZF and ZR, albeit to different degrees. Indeed, in these two zones, ACTH was nearly twice as efficient as FGF2 at inducing BrdU incorporation. In contrast, the percentage of BrdU-positive nuclei in the ZG was 1.5-times lower in the Hyp+FGF2 rats than in the Hyp rats. However, ACTH appeared to have a mitogenic effect in the ZG, whereas FGF2 inhibited S-phase entry in this zone. The combination of ACTH and FGF2 had an antagonist effect that inhibited or blocked ACTH-induced S-phase entry in all of the zones analyzed (decreased in the ZG and ZF; blocked in the ZR).

Hypophysectomy modulates immunoreactivity of early response genes in adrenal cortex

As seen in Fig. 5, the zonal distribution of Fos and Jun immunoreactivity was readily detected in the cortex cell nuclei of Sham and Hyp rats. The exception was for FosB protein, which was undetectable in the Sham rats, regardless of the zone analyzed (Fig. 5e). After hypophysectomy, the percentages of c-Jun-positive and JunB-positive cells in the ZG were moderately lower (46.2±7.2% and 34.8±4.2% lower, respectively) in the Hyp rats than in the Sham rats ($P<0.05$; Fig. 5a,b). However, ZG JunD immunoreactivity in the Hyp rats was comparable to that observed for the Sham rats (Fig. 5c). Although the numbers of c-Jun-positive cells in the ZG were lower in Hyp rats than in Sham rats, the numbers of c-Jun-positive cells in this zone were significantly higher than those of JunB-positive ($P<0.01$) and JunD-positive (c-Jun vs. JunB or JunD, $P<0.001$) cells. Greater variability was seen in the ZF and ZR. Although hypophysectomy had no effect on the numbers of c-Jun-positive cells in either of these two zones, we observed significantly higher percentages of JunB-positive cells (ZF: 45.5±6.7%, $P<0.001$; ZR: 13.9±3.7%, $P<0.05$, Fig. 6a,b) and significantly lower percentages of JunD-positive cells (ZF: 17.6±4.6%, $P<0.05$; ZR: 14.1±3.8, $P<0.001$).

Some similarities were seen between the Hyp and Sham rats in terms of the induction of Fos proteins in all adrenal cortex zones (Fig. 4d–g). No FosB-positive cells were detectable in the adrenal cortices of the Sham rats. However, after hypophysectomy, FosB protein (Fig. 6c,d)

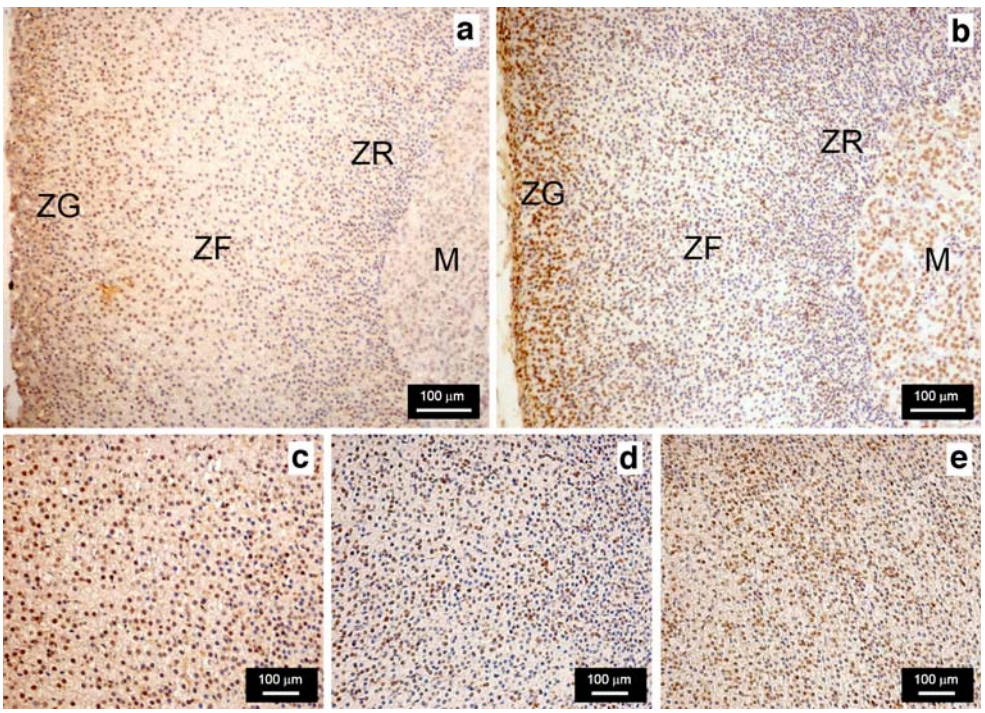

Fig. 4 Distribution of BrdU-labeled cells in the adrenal cortex. **a** Sham-operated rat. **b** Hypophysectomized rat. **c** Zona fasciculata of a sham-operated rat. **d** Zona fasciculata of a hypophysectomized rat. **e** Zona fasciculata of a hypophysectomized rat after administration of ACTH (10^{-7} M). BrdU (100 mg/100 g BW) was injected (i.p.) 12 h before rats were killed (*brown nuclei* BrdU incorporation into nuclei of S-phase cells). Sections were also stained with Harris' hematoxylin and differentiated with a saturated solution of lithium carbonate (*ZG* zona glomerulosa, *ZF* zona fasciculata, *ZR* zona reticularis, *M* medulla)

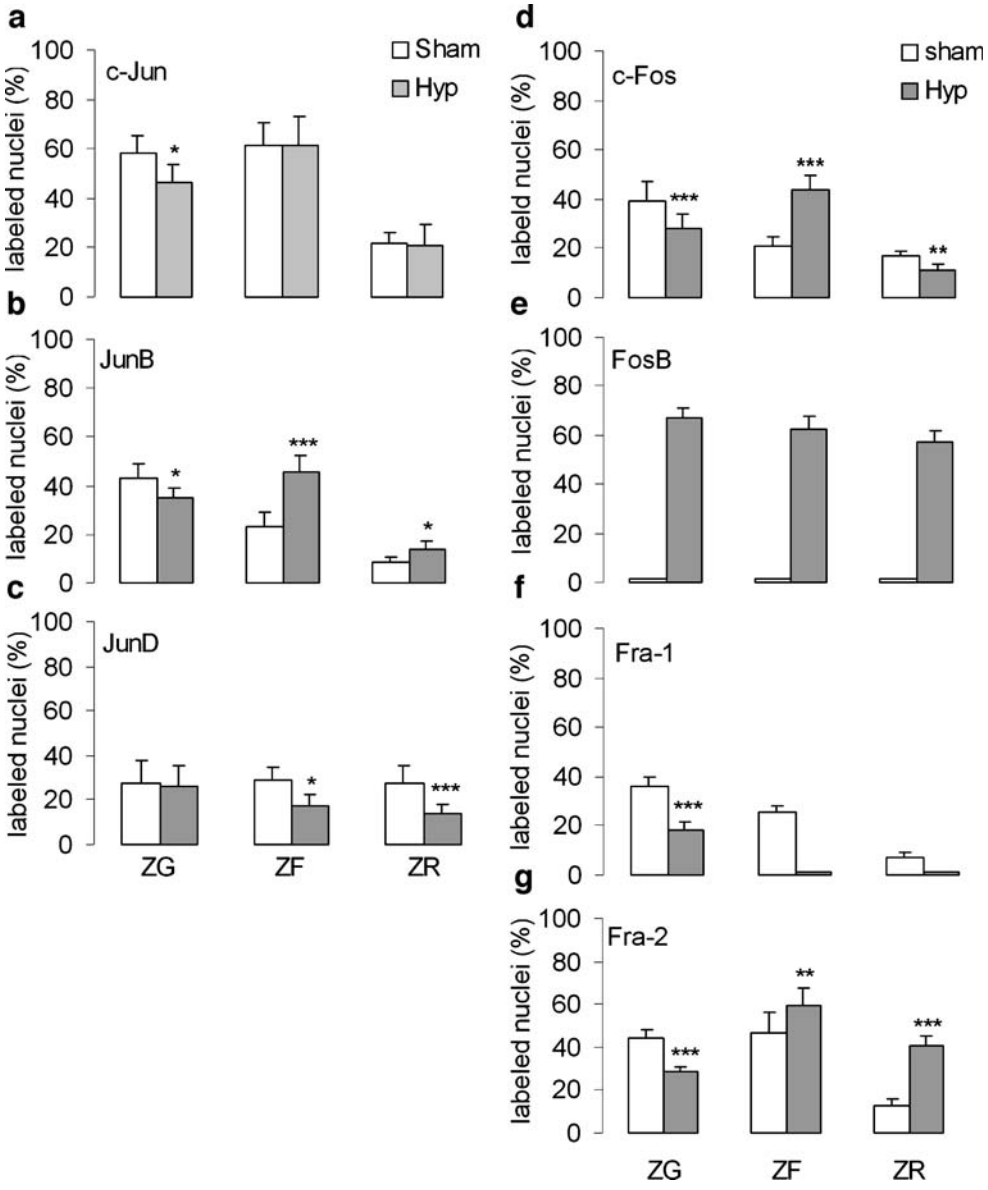

Fig. 5 Effect of hypophysectomy on immunoreactivity of (**a**–**c**) Jun family proteins and (**d**–**g**) Fos family proteins in sham-operated and hypophysectomized rats. Values are given as the number of immunoreactive nuclei expressed as a percentage of the total number of nuclei. The differences between sham-operated and hypophysectomized rats in terms of the Jun and Fos protein values were analyzed by using ANOVA and the Tukey-Kramer Multiple Comparisons Test. ***$P<0.001$, **$P<0.01$, *$P<0.05$ (*ZG* zona glomerulosa, *ZF* zona fasciculata, *ZR* zona reticularis)

was induced at the same level in all adrenal cortex zones (ZG: 66.9±3.9%; ZF: 62.6±5.2%; ZR: 57.3±4.4%). In contrast, the numbers of Fra-1-positive cells in Hyp rats were significantly lower (17.7% lower than in the Sham rats) in the ZG ($P<0.001$) and totally undetectable in the ZF and ZR. Comparing Hyp rats with Sham rats, the percentage of c-Fos-positive cells was higher in the ZF (43.6±6.1%) but lower in the ZG and ZR (28.3±5.5% and 11±2.6%, respectively). Despite the finding that the Hyp rats presented more c-Fos-positive cells in the ZF than did the Sham rats, there were significantly more FosB-positive and Fra-2-positive cells than c-Fos-positive cells in the ZF of the Hyp rats. In addition, the induction of Fra-2-positive cells in Hyp rats was greater than that seen in Sham rats in the ZF and ZR (by 59.7±8% and 40.8±4.1%, respectively), although not in the ZG, where the percentage of Fra-2-positive cells decreased by 28.2±2.8%. Correlations between the putative AP-1 composition and cell cycle progression in hypophysectomized rats are presented in Table 1.

Hypophysectomized rats receiving ACTH accumulate c-Jun-positive and JunD-positive cells but not JunB-positive cells in adrenal cortex

In Hyp+ACTH rats, c-Jun and JunB immunoreactivity was unaltered in the ZG and ZF (ZG: 49.8±4.6% and 37.8±6.6%; ZF: 53.2±9.6% and 37.9±8.1%; Fig. 7a,b). However, in these same zones, the numbers of JunD-positive nuclei were significantly greater in the Hyp+ACTH rats (ZG: 51.4±5.9%; ZF: 52.2±10%) than in the Hyp rats ($P<0.001$; Fig. 7c). In the ZR, administration of ACTH had no effect on the numbers of JunB-positive and JunD-positive cells, whereas

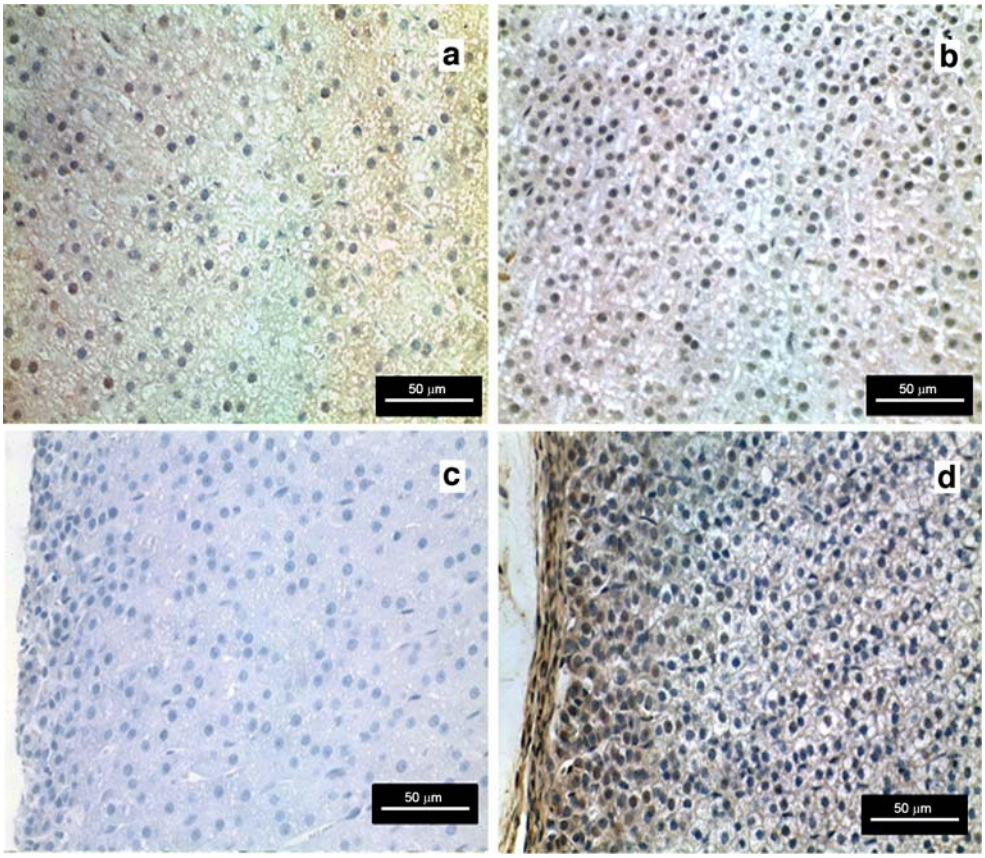

Fig. 6 Photomicrographs of JunB protein (**a**, **b**) and FosB protein (**c**, **d**) immunohistochemical staining in the zona fasciculata of the adrenal glands of sham-operated rats (**a**, **c**) and hypophysectomized rats (**b**, **d**)

the number of c-Jun-positive nuclei increased (42.1±11.4%; $P<0.001$). After ACTH administration, JunB protein expression remained the same in all adrenal cortical zones of the hypophysectomized rats. In addition, the overall number of JunB-positive cells was comparatively lower than that of c-Jun-positive or JunD-positive cells, regardless of the zone analyzed. Therefore, ACTH administration induced significant accumulation of c-Jun-positive cells in all three zones and significant accumulation of JunD-positive cells in the ZG and ZF (Fig. 8a,b). In contrast, and different from that observed in Hyp rats, the numbers of JunB-positive cells seen in the Hyp+ACTH rats remained lower than those of c-Jun-positive or JunD-positive cells in all zones.

Table 1 Correlations between the putative compositions of the AP-1 proteins and BrdU incorporation in hypophysectomized rats (ZG zona glomerulosa, ZF zona fasciculata, ZR zona reticularis

Zone	Jun[a]	Fos[a]	AP-1 complex	S phase entry[a]
ZG	↓ c-Jun*, ↓ JunB*, = JunD	↓ c-Fos*** ↑ FosB*** ↓ Fra-1*** ↓ Fra-2***	c-Jun, FosB	↑
ZF	= c-Jun, ↑ JunB***, ↓ JunD*	↑ c-Fos*** ↑ FosB*** Fra-1# ↑ Fra-2**	JunB, FosB/Fra-2	↓
ZR	= c-Jun, ↑ JunB*, ↓ JunD***	↓ c-Fos** ↑ FosB*** Fra-1# ↑ Fra-2***	JunB, FosB/Fra-2	=

[a] Percentage of labeled nuclei: ↑ higher in relation to hypophysectomized rats, ↓ lower in relation to hypophysectomized rats, = no alteration in relation to hypophysectomized rats, # no expression
***$P<0.001$, **$P<0.01$, *$P<0.05$

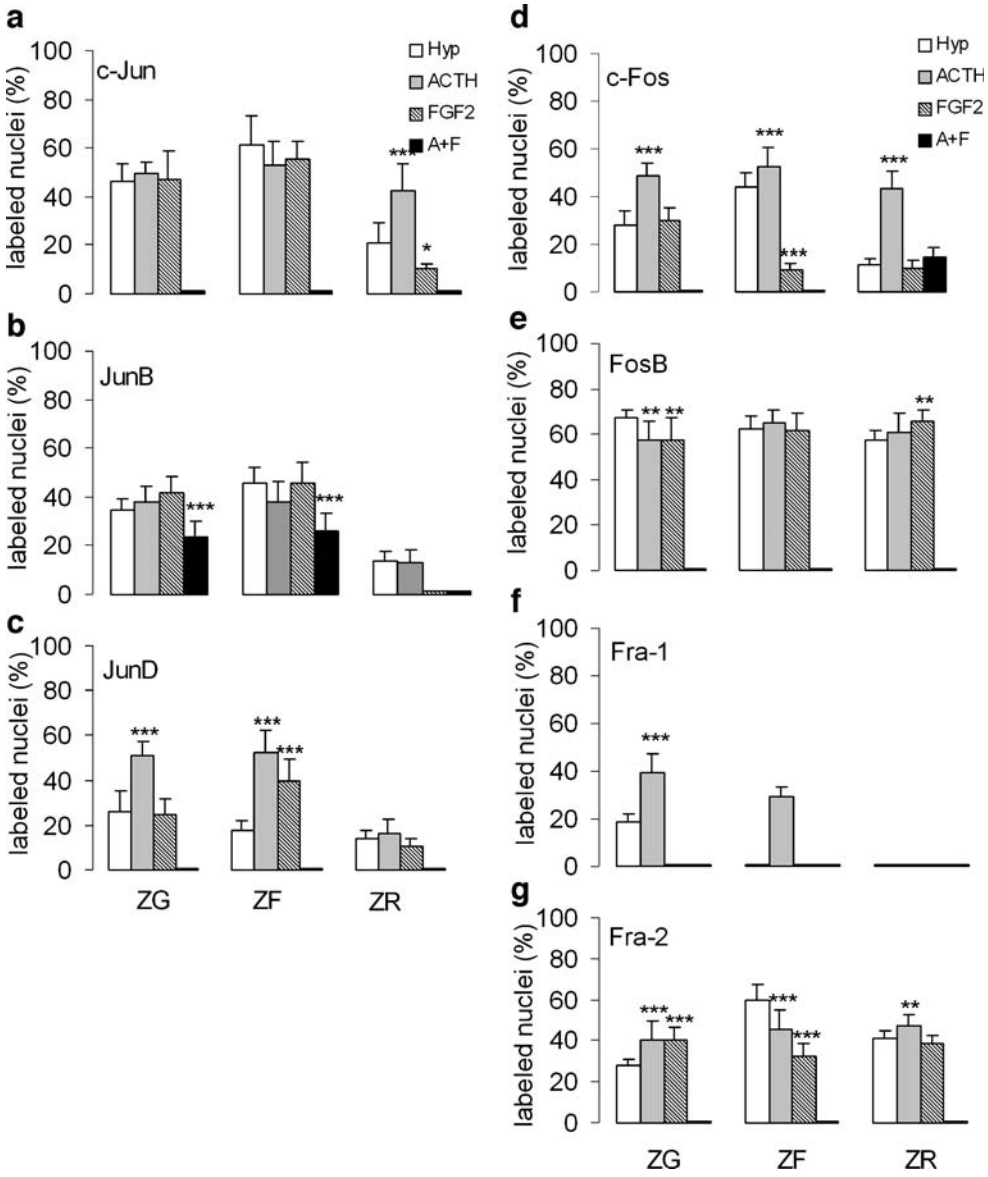

Fig. 7 Effect of administration of ACTH (10^{-7} M, *ACTH*), FGF2 (20 ng/ml, *FGF2*), and the ACTH-FGF2 combination (*A+F*) on the immunoreactivity of Jun family proteins (**a–c**) and Fos family proteins (**d–g**) in hypophysectomized rats (*Hyp*). Values are given as the number of immunoreactive nuclei expressed as a percentage of the total number of nuclei. The differences between hypophysectomized control rats and hypophysectomized study rats in terms of Jun and Fos protein values were analyzed by using ANOVA and the Tukey-Kramer Multiple Comparisons Test. ***P<0.001, **P<0.01, *P< 0.05 (*ZG* zona glomerulosa, *ZF* zona fasciculata, *ZR* zona reticularis)

In response to ACTH, c-Fos-positive cells were induced in all zones of the hypophysectomized rat adrenal cortex (ZG: 48.5±5.2%; ZF: 52.9±7.7%; ZR: 43.3±7.2%; Fig. 5d). However, FosB immunoreactivity was unaltered in the ZF and ZR, with only a modest decrease in the ZG. Indeed, after ACTH administration, the FosB-positive cell counts remained elevated and were at similar levels throughout the adrenal cortex (ZG: 57.1±8.2%; ZF: 65.2±5.0%; ZR: 60.9±8.1%; Fig. 7e). The Fra-1-positive cell counts increased significantly in the ZG (39.6±7.8%; $P<0.001$) and ZF (29.1±4.1%; $P<0.001$) but not in the ZR, in which no Fra-1 expression was observed in the Hyp+ACTH or Hyp+ACTH+FGF2 groups (Fig. 7f). In the ZG and ZR, induction of Fra-2-positive cells was significantly higher (P< 0.001) than was that of Fra-1-positive cells. In addition, induction of Fra-2-positive cells in the ZF was 45.8±9% lower in Hyp+ACTH rats than in Hyp rats (Fig. 7g). Therefore, in the ZG and ZF of Hyp+ACTH rats, there was significant accumulation of c-Fos-, FosB-, and Fra-2-positive cells (Fig. 8c,d) and of Fra-1-positive cells. However, in the ZR of those same rats, no Fra-1 protein induced.

Hypophysectomized rats receiving FGF2 present a pattern of Jun protein identical to that seen in response to ACTH only in ZF

In contrast to the result obtained with ACTH administration in hypophysectomized rats, FGF2 treatment did not alter the numbers of ZG nuclei that were immunoreactive to Jun proteins (Fig. 7a–c). In the ZG of Hyp+FGF2 rats, no change was observed in the numbers of c-Jun-positvie or JunB-positive nuclei (47.3±11.3% and 41.5±7%), whereas the JunD-positive cell counts were significantly lower than

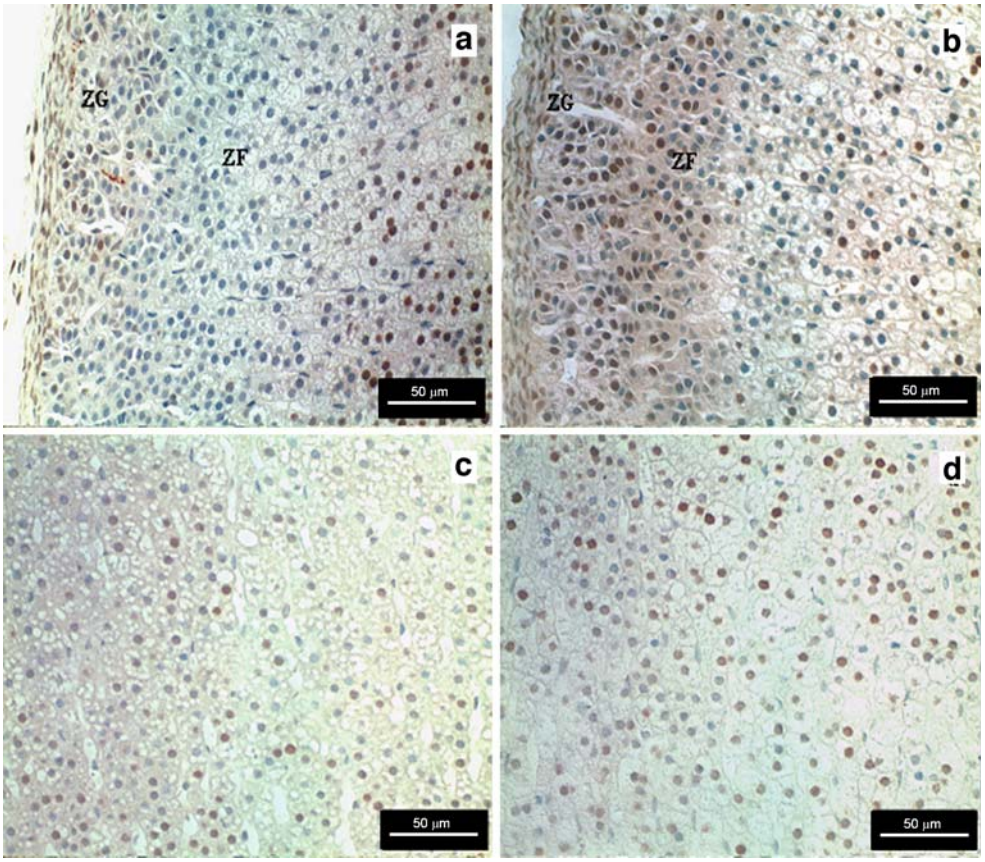

Fig. 8 Photomicrographs of immunoreactivity to JunB protein (**a**, **b**) and Fra-2 protein (**c**, **d**) in the adrenal glands of hypophysectomized rats (**a**, **c**) and hypophysectomized rats treated with ACTH (10^{-7} M; **b**, **d**)

in the Hyp+ACTH rats (24.6±7.2% vs. 51.3±5.9%; $P<0.001$). However, in the ZF, FGF2 administration resulted in a pattern of Jun protein immunoreactivity that was identical to that seen in response to ACTH. Both treatments significantly increased the number of JunD-positive cells (FGF2: 39.6±10.2%; ACTH: 52.1±10%; $P<0.001$) but did not significantly alter the numbers of c-Jun- or JunB-positive cells in relation to those seen in the Hyp rats. In the ZR, differences in Jun protein expression were more pronounced after FGF2 administration. In this zone, FGF2 administration resulted in significantly lower c-Jun-positive cell counts (10.3±1.9%; $P<0.05$) and the absence of any JunB-positive cells. The administration of FGF2 did not modify the immunoreactivity to JunD protein in the ZR. In the ZG, FGF2 administration induced significantly greater numbers of Fra-2-positive cells (40.5±6.3%; $P<0.001$; Fig. 7g), a modest but significant decrease in FosB protein expression (57±10%), and total inhibition of Fra-1 expression. In the ZF, FGF2 administration decreased protein expression of c-Fos and Fra-2 by 9.6±2.1% and 32.6±6.3%, respectively, but had no affect on the number of FosB-positive cells (61.6±7.3%). In contrast, FGF2 administration increased the number of FosB-positive cells in the ZR by 65.4±5.4%. However, Hyp+FGF2 rats presented Fra-1 protein expression that differed, in the ZR and in the ZF, from that seen in the Hyp rats.

ACTH-FGF2 combination has an antagonist effect on Jun and Fos expression in hypophysectomized rat adrenal cortex

Unlike the Hyp rats, the Hyp+ACTH+FGF2 rats presented no expression of Fos proteins in the adrenal cortex. Similarly, the ACTH-FGF2 combination resulted in no expression of Jun proteins in the ZR. In addition, total inhibition of c-Jun-positive and JunD-positive cells was also seen in the ZG and ZF of Hyp+ACTH+FGF2 rats. Furthermore, the antagonistic effect of the ACTH-FGF2 combination resulted in significant reductions in the numbers of JunB-positive cells in the ZG and ZF (23.5±6.9% and 26.3±6.9%; $P<0.001$).

AP-1 proteins are correlated with the regulation of cell proliferation after ACTH administration in hypophysectomized rats

Table 2 shows the presumed composition of the AP-1 complex and the increases in BrdU incorporation after administration of ACTH or FGF2. After administration of ACTH, the numbers of BrdU-positive cells were significantly higher in the ZF and ZR, where the likely composition of AP-1 was, respectively, c-Jun-JunD/c-Fos-FosB-Fra-2 and c-Jun/c-Fos-FosB-Fra-2, although, notably,

Table 2 Correlations between putative AP-1 complex compositions and BrdU incorporation in hypophysectomized rats receiving ACTH (10^{-7} M) or FGF2 (20 ng/ml) in the various zones (*ZG* zona glomerulosa, *ZF* zona fasciculata, *ZR* zona reticularis)

Zone	ACTH				FGF2			
	Jun[a]	Fos[a]	AP-1 complex	S phase entry	Jun[a]	Fos[a]	AP-1 complex	S phase entry
ZG	= c-Jun, = JunB, ↑ JunD***	↑ c-Fos*** ↓ FosB** ↑ Fra-1*** ↑ Fra-2***	c-Jun/ JunD, c-Fos/ FosB/ Fra-2	↑	= c-Jun, = JunB, = JunD	= c-Fos ↓ FosB** Fra-1# ↑ Fra-2***	c-Jun/ JunB, FosB/ Fra-2	↓
ZF	= c-Jun, = JunB, ↑ JunD***	↑ c-Fos*** = FosB ↑ Fra-1*** ↓ Fra-2***	c-Jun/ JunD, c-Fos/ FosB/ Fra-2	↑	= c-Jun, = JunB, ↑ JunD***	↓ c-Fos*** = FosB Fra-1# ↓ Fra-2***	c-Jun/ JunD, FosB	↑
ZR	↑ c-Jun***, = JunB, = JunD	↑ c-Fos*** = FosB Fra-1# ↑ Fra-2**	c-Jun, c-Fos/ FosB/ Fra-2	↑	↓ c-Jun*, JunB#, = JunD	= c-Fos ↑ FosB** Fra-1# = Fra-2	c-Jun, FosB	↑

[a] Percentage of labeled nuclei: ↑ higher in relation to hypophysectomized rats, ↓ lower in relation to hypophysectomized rats, = no alteration in relation to hypophysectomized rats, # no expression

***$P<0.001$, **$P<0.01$, *$P<0.05$

JunB expression was unaltered. Indeed, the number of JunB-positive cells remained lower than that of c-Jun-positive or JunD-positive cells. No such alteration in AP-1 proteins was observed in the ZG, in which the numbers of JunB-positive cells were lower in Hyp rats and unaltered in Hyp+ACTH rats. Indeed, cell proliferation was apparently less affected by ACTH. In contrast, FGF2 administration resulted in significantly lower DNA synthesis and higher numbers of c-Jun-positive and JunB-positive cells in the ZG. In the ZF and ZR, FGF2 administration increased BrdU incorporation. In addition, FGF2 administration led to significantly greater expression of c-Jun and JunD in the ZF and to significant inhibition of JunB in the ZR. Interestingly, in Hyp+ACTH+FGF2 rats, the antagonistic effect that the ACTH-FGF2 combination had on Jun and Fos protein expression was evidenced by the lower number of BrdU-positive nuclei in the ZG and, more markedly, in the ZR. In the ZR of Hyp+ACTH+FGF2 rats, there was marked inhibition of DNA synthesis, together with decreased Jun and Fos protein expression.

Discussion

In the present study, we have attempted to determine the effects of ACTH and FGF2 on the pattern of expression of AP-1 family members in the adrenal cortex after hypophysectomy. In addition, we have examined the correlations between the expression of the Jun and Fos proteins, together with the progression of adrenal cells from the G1-phase to S-phase of the cell cycle. Our results show that ACTH and FGF2 both regulate the expression of nuclear Jun and Fos proteins in hypophysectomized rats, which display differential patterns of Fos and Jun protein induction that are correlated with the regulation of cell proliferation.

The results presented herein corroborate those of studies reporting that hypophysectomy tends to cause atrophy of the inner zones of the adrenal cortex (Deane 1962; Tchen et al. 1977; Ceccatelli et al. 1995; Davis et al. 2000). These observations suggest that the atrophy of the inner zones is attributable to alterations in the G1/S transition phase of the adrenal cell cycle. In the present study, we have found that decreases in BrdU-positive cell counts are accompanied by an early hypophysectomy-induced alteration in the ZF. Therefore, we have demonstrated a direct relationship between the integrity and presence of the S-phase cell cycle. The same relationship in the ZF has been reported by our group in rats treated with dexamethasone (Baccaro et al. 2007). Conversely, we have observed that the ZG of hypophysectomized rats increases in size and presents larger numbers of BrdU-positive cells. These results are in agreement with those of Nussdorfer et al. (1973) and with those of Nickerson and Brownie (1975), whereas other authors have described few or no post-hypophysectomy alterations in the ZG (Deane 1962; Tchen et al. 1977; Ceccatelli et al. 1995; Davis et al. 2000). Nussdorfer et al. (1973) have reported that, by post-hypophysectomy day 7, the ZG increases significantly in width, despite absolute decreases in its volume, suggesting that a linear parameter

is not a reliable measure of ZG size. Nevertheless, the original mass of the ZG is restored by 1 month after hypophysectomy. The factors controlling the enlargement and restoration of the ZG volume remain unknown. However, the ZG now appears to be the site of formation of several factors that can act in a paracrine or autocrine fashion (Vinson 2003, 2004). In addition, the structure that we refer to as the ZG has been suggested to lie in the medulla (Bornstein et al. 1994; Renshaw and Hinson 2001), and the activities and responses of the ZG might therefore be subject to different interpretations. In agreement with the results obtained by using a combination of ACTH and dexamethasone in vivo (Baccaro et al. 2007), the rats in our Hyp+ACTH group present a BrdU uptake that is relatively ACTH-independent in the ZG, indicating that the ZG is maintained by other factors, such as angiotensin II or other paracrine components (Vinson 2003).

The lower BrdU incorporation in the ZG of the Hyp+FGF2 rats lends credence to the idea that the response of the ZG is distinct from that of the inner zones (Vinson 2004). These in vivo results of S-phase entry in glomerulosa cells treated with FGF2 are in contrast with observations described in isolated glomerulosa cells from adrenal rats, in which FGF2 has been found to induce increased BrdU uptake after 24 h of treatment (Mattos and Lotfi 2005). These contrasting data among different approaches are sufficiently intriguing to make us question the true biological role of the glomerulosa cells.

In the present study, administration of ACTH leads to the recuperation of the proliferative state of the ZF and an increase in the numbers of BrdU-positive cells in all adrenal zones. The response to the administration of FGF2 is similar in the ZF and ZR. However, the response to the combination of ACTH and FGF2 indicates that ACTH-induced BrdU uptake is modulated by FGF2, since the numbers of immunoreactive cells are reduced. Indeed, in all zones, the response to the ACTH-FGF2 combination is a slowing of the cell cycle progression. Our results suggest that FGF2 antagonizes ACTH, supporting the idea that ACTH controls the trophic effect regardless of exogenous FGF2. Recent studies have demonstrated the same effect in rats treated with dexamethasone and the ACTH-FGF2 combination (Baccaro et al. 2007).

A zone containing stem cells known as the undifferentiated cell zone has been reported (Mitani et al. 2003). This zone is located between the ZG and ZF in adult rats, especially in Sprague Dawley rats, and is associated with circadian-rhythm-related variations in plasma concentrations of ACTH (Miyamoto et al. 1999). Our results are inconsistent with those of these reports, since the appearance of the BrdU-positive cells in the ZG, ZF, and ZR after ACTH administration implies that ACTH activity is not limited to a specific zone. Conversely, our results are in agreement with those of Kobayashi et al. (2006), who have demonstrated that ACTH promotes the progression of the cell cycle in various types of adrenal cells.

Under sham-operated conditions, various members of the AP-1 immediate-response gene family, with the exception of FosB protein, are seen in the adrenal gland. The adrenal gland is unusual, since, in an untreated state, it expresses various AP-1 family members (Pennypacker et al. 1992; Baccaro et al. 2007). Hypophysectomy regulates the induction of Jun and Fos proteins differentially throughout the cortex, which might lead to the AP-1 complex being composed of a variety of proteins. Indeed, when the likely composition of the AP-1 complex is c-Jun/FosB, hypophysectomy induces a significantly larger number of BrdU-positive cells in the ZG. However, the inhibited BrdU incorporation in the ZF and the unaltered BrdU incorporation in the ZR are both accompanied by the strong expression of JunB, suggesting that the post-hypophysectomy composition of the AP-1 complex in these zones is JunB/FosB-Fra-2. Nevertheless, the limiting components that trigger proliferation seem to be the Jun protein family members, Fos protein having a weak effect (Shaulian and Karin 2001). In general, Fos family members play roles that are simultaneously overlapping and unique, functioning in a tissue-specific manner (Milde-Langosch 2005). The impairment of the JunB-AP-1 subunits in the ZG is in contrast with their significant induction in the ZF and ZR and suggests that, after hypophysectomy, c-Jun and JunB are the main components of the AP-1 complex in the ZG and ZF/ZR, respectively. The c-Jun protein is a positive regulator of proliferation and induces positive regulators of cell-cycle progression, although in vivo evidence indicates that JunB and JunD are negative regulators of cell proliferation (Szremska et al. 2003; Meixner et al. 2004). Indeed, only JunB antagonizes c-Jun (Passegue and Wagner 2000), whereas JunD protects cells from p53-dependent senescence and apoptosis responses, indicating that JunD is associated with survival (Weitzman et al. 2000; Hess et al. 2004). Therefore, the post-hypophysectomy abundance of JunB protein in the ZF suggests JunB involvement in an inhibitory mechanism of proliferation following adrenal cortex atrophy. Otherwise, JunB protein inhibition might be related to the increased BrdU uptake and the enlargement observed in the ZG.

In addition, the change in the c-Jun-JunD/c-Fos-containing AP-1 complex in the ZF and ZR, together with the lack of JunB protein expression after ACTH administration, suggests that proliferation occurs in these zones after ACTH stimulation. Indeed, in the ZF and ZR of Hyp+ACTH rats, DNA synthesis increases significantly, indicating that the AP-1 modification is related to cell cycle progression and proliferation. The Fos counterpart of AP-1 can be either c-Fos, FosB, or Fra-2. Like c-Fos, FosB

harbors a C-terminal transactivation domain and is related to cell cycle progression, whereas Fra-1 and Fra-2 lack this domain and might be involved in the progression of various types of tumors (Tulchinsky 2000). These results are in agreement with those of a previous study, in which (1) the c-Jun/c-Fos-containing AP-1 complex has been reported to be altered in the ZF, and (2) a lack of JunB protein expression has been noted throughout rat adrenal glands infused with ACTH (Baccaro et al. 2007).

In the ZG, ACTH administration differentially regulates the induction of Fos proteins, specifically c-Fos and Fra, but does not regulate the c-Jun/JunB protein ratio. Nevertheless, JunD-positive cells have been found in high percentages in the ZG. The abundance of c-Jun and JunD proteins found in the ZG, taken together with the notion that there is intense BrdU uptake in this zone, suggests that c-Jun and JunD proteins are involved in maintaining the integrity of the gland, independently of ACTH.

In all zones except for the ZG, the patterns of Fos induction, Jun induction, and DNA synthesis after hypophysectomy and FGF2 administration resemble those induced by ACTH. In the ZG, FGF2 does not regulate Jun protein expression, although it inhibits Fra-1 induction, FosB induction, and DNA synthesis. Hypophysectomy and FGF2 administration does not induce c-Fos in the ZG, although it has been shown to do so in cultures of primary rat adrenal cells (Mattos and Lotfi 2005).

After administration of the ACTH-FGF2 combination, the response of Jun/Fos proteins and in vivo BrdU incorporation to ACTH is apparently modulated by FGF2, since the numbers of immunoreactive cells are reduced in both experimental approaches. Our results suggest that FGF2 antagonizes ACTH, supporting the idea that ACTH controls the trophic effect regardless of exogenous FGF2, as described in Baccaro et al. (2007). Since FGF2 is a potent angiogenic and neurotrophic factor, it might be involved in the growth and maintenance of the vascularization of the adrenal cortex, although this has yet to be thoroughly investigated (Ho and Vinson 1997; Feige et al. 1998).

In summary, we have shown that hypophysectomy modulates the expression of members of the Jun and Fos protein family in the rat adrenal cortex. These results indicate that the JunB protein plays a role in the early mechanism of cell proliferation inhibition resulting from the discontinuation of pituitary hormone production and following atrophy of the adrenal cortex. In addition, our results regarding BrdU incorporation suggest that distinct members of the AP-1 family of transcription factors are implicated in the regulation of cell cycle progression and control in ACTH-induced proliferation. The effect of a single pharmacological dose of ACTH, which regulates the complex expression pattern of AP-1 transcription, seems to depend on the balance among the activities of various proteins, especially among those of the Jun protein. The differential expression and dimer composition of AP-1 proteins in response to extracellular stimuli is one of the major mechanisms modulating AP-1 activity (Chiu et al. 1989). However, the regulation of AP-1 activity is complex and occurs at different levels, including transcriptional and post-translational events and interactions with ancillary proteins (Eferl and Wagner 2003).

Acknowledgements We thank Prof. A. G. Gambarini, Department of Biochemistry, Chemistry Institute, University of São Paulo for generously providing the bovine recombinant FGF2.

References

Angel P, Karin M (1991) The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochem Biophys Acta 1072:129–157

Baccaro RBF, Mendonça POR, Torres TEP, Lotfi CFP (2007) Immunohistochemical Jun/Fos protein localization and DNA synthesis in rat adrenal cortex after treatment with ACTH or FGF2. Cell Tissue Res 328:7–18

Bornstein SR, Gonzalez-Hernandez JA, Ehrhart-Bornstein M, Adler G, Scherbaum WA (1994) Intimate contact of chromaffin and cortical cells within the human adrenal gland forms the cellular basis for important intraadrenal interactions. J Clin Endocrinol Metab 78:225–232

Ceccatelli S, Diana A, Villar MJ, Nicotera P (1995) Adrenocortical apoptosis in hypophysectomized rats is selectively reduced by ACTH. Neuroreport 6:342–344

Chiu R, Angel P, Karin M (1989) Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. Cell 59:979–986

Clark AJL, Cammas FM (1996) The ACTH receptor. Baillieres Clin Endocrinol Metab 10:29–47

Davis KT, McDuffie I, Mawhinney LA, Murray SA (2000) Hypophysectomy results in a loss of connexin gap junction protein from the adrenal cortex. Endocr Res 26:561–570

Deane HW (1962) The anatomy, chemistry and physiology of adrenocortical tissue. In: Deane HW (ed) Handbuch der experimentellen pharmakologie, vol 14. Springer, Berlin, pp 1–185

Eferl R, Wagner EF (2003) AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 3:859–868

Feige JJ, Keramidas M, Chambaz EM (1998) Hormonally regulated components of the adrenocortical cell environment and the control of adrenal cortex homeostasis. Horm Metab Res 30:421–425

Hess J, Angel P, Schorpp-Kistner M (2004) AP-1 subunits: quarrel and harmony among siblings. J Cell Sci 117:5965–5973

Ho MM, Vinson GP (1997) Peptide growth factors and the adrenal cortex. Microsc Res Tech 36:558–568

Hornsby PJ (1985) Regulation of adrenocortical cell proliferation in culture. Endocr Res 10:259–281

Imai T, Seo H, Murata Y, Ohno M, Satoh Y, Funahashi H, Takagi H, Matsui N (1990) Adrenocorticotropin increases expression of c-Fos and β-actin genes in the rat adrenals. Endocrinology 127:742–1747

Kimura E, Sonobe MH, Armelin MCS, Armelin HA (1993) Induction of Fos and Jun proteins by adrenocorticotropin and phorbol ester but not by 3′, 5′-cyclic adenosine monophosphate derivatives. Mol Endocrinol 7:1463–1471

Kobayashi H, Kambe F, Imai T, Hibi Y, Kikumori T, Ohmori S, Nakao A, Seo H (2006) Differential expression of cyclin-dependent kinase inhibitors, p27kip1 and p57Kip2, by corticotropin in rat adrenal cortex. J Endocrinol 189:671–679

Lotfi CFP, Armelin HA (2001) cFos and cJun antisense oligos block mitogenesis triggered by FGF2 and ACTH in mouse Y1 adrenocortica cells. J Endocrinol 168:381–389

Lotfi CFP, Todorovic Z, Armelin AH, Schimmer BP (1997) Unmasking a growth-promoting effect of the adrenocorticotropic hormone in Y1 mouse adrenocortical tumor cells. J Biol Chem 272:29886–29891

Mattos GE, Lotfi CFP (2005) Differences between the growth regulatory pathways in primary rat adrenal cells and mouse tumor cell line. Mol Cell Endocrinol 245:31–42

Meixner A, Karreth F, Kenner L, Wagner EF (2004) JunD regulates lymphocyte proliferation and T helper cell cytokine expression. EMBO J 23:1325–1335

Milde-Langosch K (2005) The Fos family of transcription factors and their role in tumourigenesis. Eur J Cancer 41:2449–2461

Mitani F, Mukai K, Miyamoto H, Suematsu M, Ishimura Y (2003) The undifferentiated cell zone is a stem cell zone in adult rat adrenal cortex. Biochim Biophys Acta 1619:317–324

Miyamoto H, Mitani F, Mukai K, Suematsu M, Ishimura Y (1999) Studies on cytogenesis in adult rat adrenal cortex: circadian and zonal variations and their modulation by adrenocorticotropic hormone. J Biochem (Tokyo) 126:1175–1183

Muskhelishvili L, Lantendresse JR, Kodell RL, Herderson EB (2003) Evaluation of cell proliferation in rat tissues with BrdU, PCNA, Ki-67 (MIB-5) immunohistochemistry and in situ hybridization for histone mRNA. J Histochem Cytochem 51:1681–1688

New MI (1998) Diagnosis and management of congenital adrenal hyperplasia. Annu Rev Med 49:311–328

Nickerson PA, Brownie AC (1975) Effect of hypophysectomy on the volume and ultrastructure of zona glomerulosa in rat adrenal. Endocrinol Exp 9:187–196

Nussdorfer GC, Mazzocchi G, Rebuffat P (1973) An ultrastructural stereologic study of the effects of ACTH and adenosine 3′,5′-cyclic monophosphate on the zona glomerulosa of rat adrenal cortex. Endocrinology 92:141–151

Orth DN, Kovacs WJ (1998) The adrenal cortex. In: Wilson JD (ed) Williams textbook of endocrinology, 9th edn. Saunders, Philadelphia

Passegue E, Wagner EF (2000) JunB suppresses cell proliferation by transcriptional activation of p16 (INK4a) expression. EMBO J 19:2969–2979

Pennypacker KR, Hong JS, Douglass J, McMillian MK (1992) Constitutive expression of AP-1 transcription factors in the rat adrenal. Effects of nicotine. J Biol Chem 267:20148–20152

Renshaw D, Hinson JP (2001) Neuropeptide Y and the adrenal gland: a review. Peptides 22:429–438

Shaulian E, Karin M (2001) AP-1 in cell proliferation and survival. Oncogene 20:2390–2400

Szremska AP, Kenner L, Weisz E, Ott RG, Passegue E, Artwohl M, Freissmuth M, Stoxreiter R, Theussl HC, Parzer SB, Moriggl R, Wagner EF, Sexl V (2003) JunB inhibits proliferation and transformation in B-lymphoid cells. Blood 102:4159–4165

Tchen TT, Chan SW, Kuo TH, Mostafapour KM, Drzewiecki VH (1977) Studies on the adrenal cortex of hypophysectomized rats: a model for abnormal cellular atrophy and death. Mol Cell Biochem 15:79–87

Tulchinsky E (2000) Fos family members: regulation, structure and role in oncogenic transformation. Histol Histopathol 15:921–928

Viard I, Hall SH, Jaillard C, Berthelon MC, Saez JM (1992) Regulation of c-Fos, c-JUN and JUN-B messenger ribonucleic acids by angiotensin-II and corticotropin in ovine and bovine adrenocortical cells. Endocrinology 130:1193–1200

Vinson GP (2003) Adrenocortical zonation and ACTH. Microsc Res Tech 61:227–239

Vinson GP (2004) Glomerulosa function and aldosterone synthesis in the rat. Mol Cell Endo 217:59–65

Waynforth HB, Flecknell PA (1994) Hypophysectomy. In: Waynforth HB, Flecknell PA (eds) Experimental and surgical technique in the rat, vol 2. Academic Press, London, pp 248–256

Weitzman JB, Fiette L, Matsuo K, Yaniv M (2000) JunD protects cells from p53-dependent senescence and apoptosis. Mol Cell 6:1109–1119

Yang GA, Koistinaho J, Iadarola M, Shenhua-Zhu, Hervonen A (1990) Administration of adrenocorticotropic hormone (ACTH) enhances Fos expression in the rat adrenal cortex. Regul Pept 30:21–31

Zwermann O, Beuschlein F, Klink A, Stahl M, Reincke M (2004) The role of the ACTH receptor in adrenal tumors: identification of a novel microsatellite marker. Horm Metab Res 6:406–410