REVIEW

Mechanisms of adrenal gland growth: signal integration by extracellular signal regulated kinases1/2

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Abstract

The adrenal gland influences a multitude of processes during stress response, but also potently affects the immune system, glucose metabolism, electrolyte or water homeostasis, and cardiovascular functions. According to the present understanding, the adrenal cortex is tightly controlled by the hypothalamic–pituitary–adrenal axis. This axis involves hypothalamic CRH and pituitary ACTH which determine processes of adrenocortical growth and function. However, control of the adrenal gland comprises a plethora of additional endogenous or exogenous factors. Among those are diverse hormones, psychosocial parameters, physiological stress, secondary plant products, or even environmental pollutants. In the present review, we summarize the current view of endocrine growth control in the adrenal gland. We then discuss intracellular mechanisms of adrenal growth control and focus on extracellular signal regulated kinases 1/2 (ERK1/2), which have been demonstrated to be controlled by not only ACTH or angiotensin II, but also by a large number of additional effectors. On the basis of these multiple exogenous or endogenous factors which impact on the adrenal gland through ERK1/2 activity, we speculate on a mechanism by which ERK1/2 act as a central integrative growth regulatory elements in the adrenal gland.

Journal of Molecular Endocrinology (2009) **42**, 191–203

Introduction

In mammals, the adrenal cortex and medulla are formed during embryogenesis by two distinct cell populations deriving from mesodermal and neuroectodermal origins (Hammer *et al.* 2005). Both cell populations are encapsulated before birth and establish the cortical and the medullary compartments. In mice, zonation of the cortex occurs soon after birth. Although the classical view of a concentric arrangement and a strict separation of the different adrenal zones is oversimplified (Whitworth *et al.* 2003), the adrenal cortex can roughly be divided into three different zones: the outer zona glomerulosa, followed by the zona fasciculata and the zona reticularis which directly surrounds the medulla. In mice, a functional zona reticularis is absent, instead, the so-called X-Zone can be found during certain postnatal developmental stages in mice.

Up to an age of about 7 weeks the adrenal glands of male and female mice grow rapidly in order to grant for the appropriate supply of adrenal hormones to the increasing blood volume and body size. Between week 7 and 9 of age exclusively in male mice reductions of the

adrenal gland mass can occur which, in combination with a higher growth activity of the adrenal gland in female mice, contribute to the phenotype of sexually dimorphic adrenal weights (Bielohuby *et al.* 2007). At adult stages growth of the adrenal gland is controlled by the principle of action and reaction since a series of exogenous or endogenous factors including the psychosocial environment or the immune system and e.g., soluble cytokines have been demonstrated to affect adrenal growth and function (Bornstein & Rutkowski 2002, Harbuz 2002).

For the adrenal gland, growth and function can be interpreted by an integrative concept (Otis *et al.* 2008), since the actual number and size of adrenal cells directly impact upon its function (Fig. 1). This concept takes into account that the adrenal gland is a highly dynamic tissue since mechanisms of adrenal gland growth require the perception or integration of factors including sex, age or location within the adrenal cortex but also a great number of physiological or psychosocial conditions. Interestingly, very recently, conditional inactivation demonstrated that not only for adrenal development but also for the maintenance of adrenal mass in adult mice the presence of β -catenin is required

Figure 1 Interaction between adrenal gland growth and function. The adrenal gland size or the volume of a particular zone respectively impact upon adrenal function through the number and size of cells present. Simultaneously, also the secreted hormones influence adrenal gland growth through partly superordinate feedback mechanisms such as the corticosterone/ACTH feedback loop. In addition, some growth factors primarily stimulate adrenal growth, e.g., the GH/IGF1 system. Other factors, such as physical or psychological stress, primarily affect adrenal gland function. However, these factors primarily affecting size or function, subsequently lead to secondary alterations of adrenal size or function respectively.

(Kim *et al.* 2008). The permanent perception of diverse factors affecting adrenal growth and function argues in favor of one common intracellular target, which coordinates and integrates the different stimuli and which further represents a potent effector of central cellular responses like proliferation, survival, apoptosis, or differentiation. As a potential candidate for an intracellular target in adrenal cells extracellular signal regulated kinases 1/2 (ERK1/2) have been demonstrated to affect cell proliferation (Morooka & Nishida 1998, Andreis *et al.* 2000, Lepique *et al.* 2000, Lotfi *et al.* 2000, Whitworth *et al.* 2002, Ferreira *et al.* 2007), apoptosis (Mazzocchi *et al.* 2004, Edwin & Patel 2008) cell survival (Ziegler *et al.* 2006), cell migration (Ho *et al.* 2001, 2005), or synthesis and secretion of cortical (Wu *et al.* 2002, Otis *et al.* 2005, Kempná *et al.* 2007, Chang *et al.* 2008) or medullary hormones (Cox & Parsons 1997, Shibuya *et al.* 2002). Thus, in fact, ERK1/2 have the potential and format to represent such common targets with multiple biological effects in the adrenal gland as proposed before (Chabre *et al.* 1995). In the first part of the present review, we will summarize present concepts of extracellular (hormonal) adrenal growth mechanisms. In the second part of the review, we demonstrate that a high number of extracellular hormones or additional factors are tracking ERK1/2 within the cells. We thus provide solid evidence to support the existing hypothesis (Chabre *et al.* 1995) of ERK1/2 as potential sites of intracellular signal integration in the adrenal gland, linking a large

number of extracellular and intracellular signals to specific biological responses in the adrenal gland.

Models to study adrenal growth

Different models to study adrenal growth have been discussed in a recent review (Kempná & Flück 2008). In general, classical approaches to study adrenal gland growth *in vivo* include the characterization of adrenal growth in rodents of different genetic backgrounds and developmental stages or interspecies comparisons. In addition, administration of pharmacological substances *in vitro* (e.g. Armelin *et al.* 1996) or *in vivo* (e.g. Zwermann *et al.* 2005) has provided precious insights into regulatory circuits during adrenal cell growth control. Another powerful tool to analyze adrenal cortical growth processes is the experimental model of unilateral adrenalectomy (uADX) and the resulting compensatory growth of the remaining adrenal gland (Engeland *et al.* 2005). By use of this technique, the remaining adrenal gland undergoes hyperplasia and hypertrophy. This meanwhile well-defined model allowed the identification of a number of new adrenal growth regulating factors, like the further below mentioned serine protease (AsP). But also the roles of several steroids in compensatory growth (Phillips *et al.* 1985), or factors that were formerly thought to only affect adrenal steroidogenesis, like nuclear receptor subfamily 5, group A, member 1 (Nr5a1; Beuschlein *et al.* 2002), were identified through the uADX model.

The combination of different experimental models (Bielohuby *et al.* 2008) to analyze adrenal growth and the rapidly advancing field of genetic engineering (Kim *et al.* 2008) possess enormous potential – many path breaking findings evolved through these approaches in the past, yet still more are to come in the future.

Extracellular control of adrenal growth

The effect of ACTH

The 39-amino acid peptide pituitary proopiomelanocortin (POMC)-derived ACTH is regarded as the principal regulator of postnatal adrenal gland growth and function. CRH, arginine vasopressin and oxytocin are the main stimuli for corticotroph cells of the anterior pituitary to release ACTH (Nakamura *et al.* 2008). ACTH in turn stimulates the adrenal cortex to produce cortisol or corticosterone in rodents respectively. High corticosterone levels suppress the disposal of further, above-mentioned, ACTH-releasing agents (Tanimura & Watts 2001). Already after 3 days of exogenous ACTH treatment, the adrenal mass of rats increased by about 4–5%, while 36 days of ACTH treatment resulted in an about 70% increase of the adrenal mass in rats (Nussdorfer *et al.* 1974). uADX in hypophysectomized rats demonstrated that the remaining adrenal gland still gained weight (Engeland *et al.* 1975). This implied already in the 1970s that additional factors for adrenal growth may exist. Notably, ACTH induced growth occurred both on the level of cell number and cell size in the rat adrenal gland: exogenous ACTH resulted in a hypertrophic effect of cells from the inner zona fasciculata and medulla but had hyperplastic effects in cells from the outer region of the zona fasciculata indicating also site specific effects of ACTH (Ulrich-Lai *et al.* 2006). On the other hand, reductions in ACTH, by hypophysectomy (Cater & Stack-Dunne 1953) or by continuous activation of the negative feedback loop through glucocorticoid administration, lead to decreased adrenal gland weights, which was thought to be mainly due to an induction of apoptosis (Wyllie *et al.* 1973, Tchen *et al.* 1977). Although ACTH is a central regulatory element in the hypothalamic–pituitary–adrenal axis, lately other POMC-derived peptides have sparked interest. The polypeptide precursor POMC is actively transcribed in different tissues including anterior pituitary corticotrophs, neurons of the hypothalamic arcuate nucleus, cells in the dermis, and the lymphoid system. POMC is cleaved in a tissue specific manner into a number of smaller biologically active peptides (Bertagna 1994, Raffin-Sanson *et al.* 2003). Pro- γ -MSH is one of these smaller peptides and coreleased during stress response. Although pro- γ -MSH has no mitogenic effect on

adrenal cells itself, smaller peptide fragments derived from pro- γ -MSH digestion experiments showed a potent mitogenic effect on the adrenal cells (Estivariz *et al.* 1982). Furthermore, peripheral delivery via osmotic mini pumps or s.c. injections of purified 1–28 POMC partially prevented the atrophy of regenerating adrenal glands after hypophysectomy (Estivariz *et al.* 1988). Cell culture experiments using adrenocortical carcinoma cells also showed that 1–28 POMC with correctly aligned disulphide bridges stimulated cell proliferation in a comparable magnitude to what was seen with other adrenal mitogens, such as IGF1. By contrast, an even reduced proliferation was seen in these experiments when the cells were treated with ACTH only (Fassnacht *et al.* 2003). However, the lack of proliferation after ACTH stimulation in the aforementioned experiment was probably also due to the experimental cell line used, since it is known, that H295 cells show only little or no responsiveness to ACTH (Parmar *et al.* 2008). In cultured adrenocortical cells in culture and in the mouse Y-1 adrenocortical tumor cell line, ACTH inhibited growth of the cells. Armelin *et al.* (1996) found a dual effect of ACTH treatment on proliferation in Y-1 cells: 2 h of ACTH treatment stimulated DNA synthesis, but longer treatments inhibited S-phase entry in these cells. Apart from cell line specific factors, such as the dominant inhibitory mutations in the cAMP-dependent protein kinase (PKA) of Y1 adrenal tumor cells (Olson *et al.* 1993), the paradoxical effect of ACTH action on cell proliferation between *in vivo* and *in vitro* studies suggested that additional factors must be involved *in vivo*, that overcome the growth inhibitory influence of ACTH (Lotfi *et al.* 1997). As shown in this review, a multitude of different factors are capable to influence adrenal growth *in vivo* and thus probably override the growth inhibitory effects of ACTH.

In 2001, a serine protease was characterized that is expressed in the outer adrenal cortex and is capable to cleave circulating pro- γ -MSH into the smaller, mitogenic peptide N-POMC 1–52 (Bicknell *et al.* 2001, Bicknell 2002). This important finding closed the gap between circulating pro- γ -MSH and the question of how smaller POMC-derived peptides may stimulate adrenal growth *in vivo*. Three years after its initial discovery, this protease has been identified as a short secretory isoform of the transmembrane airway trypsin-like protease (AsP; Hansen *et al.* 2004). POMC-derived peptides are not required for prenatal adrenal growth since homozygous POMC^{−/−} mutant mice showed normal adrenal weights and morphology at birth (Karpac *et al.* 2005). However, in the postnatal period, the adrenal glands from POMC^{−/−} mice gradually atrophied and lost a clear zonation (Karpac *et al.* 2005, 2007). Adrenal gland size and function of POMC^{−/−} mutant mice were restored by either transplanting adrenal glands of 1-week-old POMC^{−/−} mice to an

environment containing POMC peptides (Karpac *et al.* 2005) or by injection of ACTH (Coll *et al.* 2004). In the experiments by Coll and colleagues, the increase in adrenal size was due to an increase in cell size. Therefore, it has been suggested that ACTH particularly induces differentiation and hypertrophy (Zwermann *et al.* 2005) and that the other POMC peptides were mainly required for cell proliferation (Karpac *et al.* 2007).

Angiotensin II

The role of angiotensin II on zona glomerulosa function is well documented (Otis *et al.* 2007a). Moreover, systemic and local angiotensin II levels might also influence adrenal growth processes, as an interaction between functional aspects and growth events of the adrenal gland can be assumed (Fig. 1). In rodents, the angiotensin II type 1 (AT1) receptor has been shown to be the predominant adrenal receptor for angiotensin II (Chiu *et al.* 1989, Lehoux *et al.* 1997). *In vitro* experiments in bovine zona glomerulosa cells have shown AT1 receptor mediated mitogenic (Tian *et al.* 1995) and proto-oncogene expression stimulatory properties for this hormone (Viard *et al.* 1992). *In vivo*, 2-week infusion of angiotensin II via osmotic mini pumps to male Wistar rats induced proliferation (BrdU-positive cell nuclei) in zona glomerulosa cells. (McEwan *et al.* 1999). Similarly, 4-week infusion of angiotensin II resulted in a massive adrenal enlargement (up to +60%) in genetically hypertensive Lyon rats. The authors found that the enlargement was due to volume increases of the adrenal cortex only (Aguilar *et al.* 2004). Despite the prevailing view that the AT1 receptor promotes growth (Nakajima *et al.* 1995), Elijovich and colleagues proposed an antiproliferative function for this receptor *in vivo*. Therefore, they specifically blocked the AT1 receptor in rats by losartan and found increased adrenal gland weights in the treatment group. They therefore suggested that angiotensin II exerts a growth inhibitory effect on the adrenal gland via the AT1 receptor (Elijovich *et al.* 1997). The difference between the studies possibly derives from systemic versus local effects of angiotensin II on the adrenal gland, since systemic angiotensin II has also been shown to stimulate ACTH secretion (Sobel & Vagnucci 1982, Coiro *et al.* 1998). In addition, genetic differences of the rat strains used must be taken into consideration.

Sex hormones

At birth, adrenal gland weights are indistinguishable between male and female mice (Moog *et al.* 1954). However, within the first weeks postnatally a marked

gender-dependent adrenal phenotype is established and adrenal gland weights in female mice are about twofold higher at an age of 11 weeks when compared to male littermates (Bielohuby *et al.* 2007). In ovariectomized rats, estrogen treatment resulted in increased adrenal gland weights (Saruhan & Ozdemir 2005), which also points out the importance of sex steroids for adrenal gland growth regulation. One factor contributing to the larger adrenal glands in female mice is the persisting X-zone. Growth of this zone has been shown to be affected by androgens, as testosterone treatment in female mice resulted in X-zone regression and gonadectomy in male mice resulted in X-zone regrowth (Hershkovitz *et al.* 2007). At the organ levels, the overall volume of the X-zone is relatively small and is not sufficient to explain the dimorphic adrenal phenotype. Here, especially volume increases of the largest adrenal zone, the zona fasciculata, are responsible for the increased adrenal weights in female mice (Bielohuby *et al.* 2007). Apart from direct effects of sex steroids on adrenal weight, it has also been proposed that sex steroids modify adrenal function through influences on ACTH release (for review see Viau 2002). Testosterone, for example, was found to inhibit stress induced ACTH release (Viau & Meaney 1996), possibly also through interaction with the estrogen receptor (Lund *et al.* 2004). Thus, sex steroids clearly affect adrenal function and potentially subsequent or direct growth events in rodents.

LH and TSH

LH and TSH also influence adrenocortical growth and function. Adrenal glands of transgenic mice overexpressing LH were characterized by an 80% increase in size (Kero *et al.* 2000). Simultaneously, these mice showed drastically increased corticosterone levels. Furthermore, LH was identified as an important tumor promoter in adrenal tumorigenesis (Mikola *et al.* 2003). However, from the above-mentioned experiments, it seems that very high circulating LH levels are required to induce expression of the LH receptor in the adrenal cortex. Thus, LH could primarily play a role on adrenal growth promotion in the context of chronic, pathophysiologically high LH levels. Moreover, it is difficult to dissect direct from secondary (via sex steroids) LH growth effects on the adrenal gland.

TSH, the main regulator of thyroid gland growth and function may exert effects on adrenal growth as well. Already in 1979 increased adrenal cAMP production was described after TSH binding to the adrenal gland (Trokoudes *et al.* 1979). Later on, the TSH receptor (TSHR) was found to be expressed in the adrenal gland (Dutton *et al.* 1997) and recently, mice overexpressing glycoprotein hormone beta 5 (GPHB5), an activator of

the TSHR, showed increased adrenal gland weights. Similar increases in adrenal weight were observed when mice were treated with exogenous thyroxine (Okada *et al.* 2006). Taken together, direct and indirect effects of LH and TSH on adrenal gland growth and function can be assumed.

Annexin A1

It has been suggested that annexin A1 (ANXA1), a paracrine/juxtacrine mediator of the non-genomic actions of glucocorticoids in the neuroendocrine system (John *et al.* 2004), has a role in adrenal cell proliferation and acts as an adrenal mitogen. ANXA1 knockout mice showed unchanged baseline ACTH and corticosterone levels in plasma (Morris *et al.* 2006). However, morphometric studies in ANXA1 null mice suggested that ANXA1 has a function in adrenocortical growth, since adrenal glands were considerably smaller in these knockout mice (Davies *et al.* 2007). In addition, ANXA1 expression has been found to be restricted to the subcapsular layer of the adrenal gland (Davies *et al.* 2007), thus potentially influencing growth processes in this highly active zone of adrenal cell proliferation.

GH/IGF

The growth hormone/insulin-like growth factor 1 (GH/IGF1) system plays an important role in the regulation of adrenal growth and glucocorticoid biosynthesis (Fottner *et al.* 2004). GH overexpression in mice resulted in up to twofold increased adrenal gland weights and markedly increased levels of corticosterone (Cecim *et al.* 1991, Blackburn *et al.* 1997, Hoefflich *et al.* 2002). IGF binding protein 2 (IGFBP2) is a presumed inhibitor of IGF1 action *in vivo*. GH transgenic mice, simultaneously overexpressing IGFBP2 showed significantly reduced adrenal gland weights when compared with GH transgenic littermates (Hoefflich *et al.* 2002). Interestingly, GH positively affects both cell size and cell number in the adrenal cortex, whereas the growth inhibitory effect of IGFBP2 exclusively blocked hypertrophic but not the hyperplastic effect exerted by increased GH/IGF1 expression as demonstrated by coexpression of IGFBP2 in GH transgenic mice. In fact, the isolated infusion of IGF1 in fetal sheep resulted in a marked hypertrophy of adrenocortical cells but did not affect steroidogenesis during late gestation (Ross *et al.* 2007). Also IGF2 seems to have specific effects for adrenal growth and function. Although body weight was not significantly affected in mice overexpressing IGF2, the adrenal glands in these transgenic mice showed significant increases in weight (Wolf *et al.* 1994, Weber *et al.* 1999). In summary, several components of the

GH/IGF1 system potentially influence growth and function of the mouse adrenal gland.

Intracellular control of adrenal growth

ERK1/2 has been linked to growth and function both in the adrenal cortex and medulla. While growth is achieved by an increase of cell number and cell size, the effects of ERK1/2 seem to specifically affect the cell number. By contrast, the control of cell size in zona fasciculata cells has been attributed to the phosphoinositol-3-kinase pathway (Lawlor *et al.* 2002). The effects of ERK1/2 on cell number can be exerted by different mechanisms: A) an induction of cell proliferation (Morooka & Nishida 1998, Andreis *et al.* 2000, 2003, Lin *et al.* 2002, Whitworth *et al.* 2002, Mazzocchi *et al.* 2004, Ho *et al.* 2005, Ferreira *et al.* 2007), B) by blockade of apoptosis (Mazzocchi *et al.* 2004, Edwin & Patel 2008) or C) by promoting cell survival (Ziegler *et al.* 2006). Although it is widely accepted that ERK activity is required for cell proliferation and mitosis (Chambard *et al.* 2007), it has been shown recently that too high ERK1/2 activity also can block the entry into mitosis (Rahmouni *et al.* 2006). In line with this provocative hypothesis, activation of ERK1/2 resulted in a growth arrest of PC12 cells and induced neuronal differentiation in that cellular system (Morooka & Nishida 1998). Also in rat adrenal zona glomerulosa cells ERK1/2 activation blocked cell proliferation and stimulated steroidogenesis (Otis *et al.* 2005, Otis & Gallo-Payet 2007). A role of ERK1/2 for functional aspects of cortical (Wu *et al.* 2002, Chang *et al.* 2008) or medullary cells (Cox & Parsons 1997, Takekoshi *et al.* 2001, Shibuya *et al.* 2002, Yanagihara *et al.* 2005, Shinkai *et al.* 2007) has also been provided by others. In addition to growth and function also migration is under control by ERK1/2 in adrenomedullary cells (Ho *et al.* 2001, 2005).

Effectors of ERK1/2 activation in adrenocortical cells

In concert with ACTH a network of utmost complexity impacts on the activity of p44/42 MAPK in the adrenal cortex (Fig. 2). A crosstalk between several hormones (ACTH, angiotensin II, and FGF2) and different pathways (inositide-, cAMP, and growth factor dependent tyrosine kinase pathways) in adrenocortical cells was originally suggested by Chabre *et al.* (1995). Concerning ERK1/2 in zona glomerulosa cells, angiotensin II represents a potent effector of MAPK activity as reviewed recently (Otis & Gallo-Payet 2007). For the human adrenocortical carcinoma cell line H295R it has been suggested that angiotensin II stimulated mitogenesis might occur via ERK1/2 activation (Watanabe *et al.* 1996). By contrast, in non-malignant rat adrenal

glomerulosa cells, angiotensin II mediated induction of ERK1/2 phosphorylation resulted in an inhibition of cell proliferation (Otis *et al.* 2005). Since in the same system angiotensin II stimulated hypertrophy, protein synthesis and steroidogenesis via a mechanism involving both ERK1/2 and p38 MAPK, the effects on growth seem to be related to functional aspects of zona glomerulosa cells (Otis & Gallo-Payet 2006). In addition to its tropic effects angiotensin II dependent activation of ERK1/2 resulted in an activation of the hormone-sensitive lipase (cholesterol ester hydrolase) in adrenal zona glomerulosa cells, which supports a role of ERK1/2 for steroidogenesis, as activation of this lipase initializes cholesterol mobilization to the outer mitochondrial membrane (Cherradi *et al.* 2003). The function of angiotensin II as one important regulator of adrenal ERK1/2 activity is further substantiated by the existence of a number of co-effectors of angiotensin II dependent ERK1/2 phosphorylation like ACTH (Chabre *et al.* 1995), integrins (Campbell *et al.* 2003, Otis *et al.* 2007b) or BMP-6 (Suzuki *et al.* 2004, Inagaki *et al.* 2006). The effect of BMP-6 is thought to be exerted in an autocrine fashion via SMAD proteins, which are known modulators of ERK1/2 activity (Kano *et al.* 2005). However, as this review demonstrates, ERK1/2 activity is affected by a plethora of additional endocrine or environmental factors.

Also FGF2 is capable of modulating ERK1/2 activity in adrenal cells (Chabre *et al.* 1995, Lepique *et al.* 2000,

Lotfi *et al.* 2000, Le & Schimmer 2001, Rocha *et al.* 2003, Mattos & Lotfi 2005). Proliferative effects of FGF2 were found in zona glomerulosa cells but not in cells derived from the zona fasciculata or zona reticularis (Mattos & Lotfi 2005). This finding was discussed in context with the hypothesis that the zona glomerulosa is the primary site of growth factor stimulated cell division in the adrenal gland. Furthermore, this finding might support the so-called migration theory, according to which pluripotent adrenal stem cells located in the highly proliferative adrenal periphery migrate centripetally to differentiate into specialized adrenocortical cell types (Belloni *et al.* 1978). However, growth stimulatory signals for zona glomerulosa cells might also derive from the adrenal medulla. It has been demonstrated that adrenomedullin (Andreis *et al.* 2000) and proadrenomedullin (Rebuffat *et al.* 2001), which are synthesized in a variety of tissues including the adrenal medulla, specifically activate ERK1/2 and thereby stimulate cell proliferation in zona glomerulosa cells. This effect is highly specific, since in other cell types (neuroblastoma cells or teratocarcinoma cells) the effect was antiproliferative and other signaling systems in zona glomerulosa cells (cAMP/PKA/PKC) were not involved in adrenomedullin induced activation of ERK1/2. ERK1/2 activation was restricted to the zona glomerulosa but undetectable in the zona fasciculata/reticularis (Semplicini *et al.* 2001). The same group demonstrated that ghrelin and its receptor

(GH secretagogue receptor, GHSR) are abundantly expressed in the adrenal cortex, suggesting auto-/paracrine growth regulation (Andreis *et al.* 2003). Andreis *et al.* (2003) have further provided evidence that ghrelin enhances the proliferative rate and that this effect involved ERK1/2 activation but did not affect aldosterone or corticosterone secretion from zona glomerulosa or zona fasciculata cells respectively.

Another example of intricate complexity present in MAPK activation is constituted by orexin A and orexin B, two hypothalamic peptides, which originate from posttranslational cleavage of a common precursor (Sakurai *et al.* 1998). Orexin A exerted proliferative effects in cultured rat adrenocortical cells whereas orexin B showed antiproliferative effects in the same experimental system (Spinazzi *et al.* 2005). By use of specific inhibitors the effect of orexin A or B was shown to depend on ERK1/2 or p38 MAPK activity respectively. As demonstrated recently, orexins also stimulate steroidogenic acute regulatory protein expression (Ramanjaneya *et al.* 2008) and thus impact on steroid biosynthesis, which is exerted through multiple pathways (ERK1/2, p38 MAPK, PKA, and PKC).

The IGF system has been implicated in malignant growth of adrenal cell systems as documented by a number of comprehensive reviews (Fottner *et al.* 2004, Beuschlein & Reincke 2006, Stratakis & Boikos 2007). In this context, IGF-dependent signal transduction was investigated recently (Giulia *et al.* 2008). In that study, therapeutic intervention using a peroxisome proliferator-activated receptor gamma (PPARG) ligand (rosiglitazone) in adrenocortical tumor cells resulted in decreased levels of IGF1 dependent ERK1/2 and Akt phosphorylation, suggesting an involvement of both MAPK and PI3K pathways in the mitogenic effects of IGF1. In addition to these factors, many other agents (Fig. 2) including neuropeptides and sex steroids have been demonstrated to affect the activity of ERK1/2 (Cote *et al.* 1998, Mazzocchi *et al.* 2000, McNeill & Vinson 2000, Whitworth *et al.* 2002, Chien *et al.* 2005, McNeill *et al.* 2005, Shah *et al.* 2005, 2006, Brizuela *et al.* 2007, Kovzun 2007, Keramidas *et al.* 2008), which might suggest cross activation by receptor tyrosine kinase (RTK) and G-protein-coupled receptors (GPCR; see below).

ACTH as a key regulator of adrenal gland growth and function affects several intracellular signaling cascades which have been reviewed (Gallo-Payet & Payet 2003, Forti *et al.* 2006, Otis *et al.* 2007b) and which cannot be discussed here in a comprehensive manner. Concerning the effects of ACTH on ERK activation, positive (Le & Schimmer 2001, Ferreira *et al.* 2004, 2007, McNeill *et al.* 2005), weak (Lotfi *et al.* 1997, 2000, Lepique *et al.* 2000), negative (Watanabe *et al.* 1997), or no effects have been described (Cote *et al.* 1998). In this context it is important to mention that the effects of ACTH on ERK1/2 can also be indirect since it has been

demonstrated that ACTH blocks angiotensin II or FGF2 dependent ERK1/2 activation (Chabre *et al.* 1995). Interestingly, also integrins co-regulate ACTH effects (Otis *et al.* 2007b). From these interactions a core regulatory system can be established consisting of ACTH as the principal regulator and angiotensin II. Both hormones control ERK1/2 and have temporarily shifted activities of ERK inactivation by MKP-1 as a common phosphatase as discussed later. An up to now unsolved question is the relevance of time dependent activation of ERK1/2 by ACTH or by other effectors (Katz *et al.* 2007). To date, only isolated attempts have been made in order to unravel the temporary sequence of signal transduction including activation and deactivation of ERK1/2 in response to ACTH (Rocha *et al.* 2003).

Effectors of ERK1/2 activation in cells from the adrenal medulla

ERK1/2 activation in the adrenal medulla (Fig. 3) seems to be affected by a completely different set of factors with one exception: also in medullary cells from the adrenal gland angiotensin II promoted ERK1/2 phosphorylation (Cammara *et al.* 2001). Since the MEK inhibitor U0126 blocked CREB phosphorylation it was concluded that ERK1/2 activation but not PKA or Ca^{2+} /calmodulin-dependent protein kinases (CaMKs) mediate phosphorylation of CREB initiated by angiotensin II. However, the first growth factor that was described to activate ERKs in medullary PC12 cells was nerve growth factor (NGF; Gomez & Cohen 1991), which has also been confirmed by other groups later on (Morooka & Nishida 1998, Ho *et al.* 2001, 2005, Ziegler *et al.* 2006). Interestingly, dehydroepiandrosterone (DHEA) blocked NGF induced ERK activation and NGF dependent survival in PC12 cells (Ziegler *et al.* 2008). Also the effects of leptin on catecholamine synthesis from medullary cells are mediated in part by ERKs (Utsunomiya *et al.* 2001, Shibuya *et al.* 2002). Clearly, in medullary cells sex steroids impact on the level of ERK activity as demonstrated by different studies (Brown *et al.* 2001, Yanagihara *et al.* 2005). It is tempting to speculate that this co-regulation of ERK activity by sex steroids might also contribute to the sexually dimorphic phenotype of adrenal gland size. Importantly, also estrogenic pollutants (Yanagihara *et al.* 2005) and phytoestrogens (Yanagihara *et al.* 2008) have been shown to stimulate or block catecholamine synthesis via ERKs depending on their respective concentration. A number of additional growth factors, like epidermal growth factor (EGF; Morooka & Nishida 1998, Ho *et al.* 2005), insulin (Sugano *et al.* 2006), vasoactive intestinal peptide (VIP; Whitworth *et al.* 2002) and urocortin-2 (Nemoto *et al.* 2005) were found

Figure 3 Control of ERK1/2 phosphorylation in adrenomedullary cells. Positive effects are indicated by arrows, whereas negative interferences are visualized by blunted arrows. Each interaction is indexed by a number, which identifies the respective reference. See text for abbreviations and a detailed discussion.

to affect ERK activation in different medullary cell systems. In addition to the aforementioned growth factors also pharmaceutical agents such as milnacipran (Shinkai *et al.* 2007), chemical factors such as histamine (Cammarota *et al.* 2003), and nicotine (Cox *et al.* 1996), or even environmental pollutants such as cadmium (Leal *et al.* 2007) impact on medullary ERK activation and in part also catecholamine secretion. Thus, also in the medullary compartment signals from multiple systems (tropic, metabolic, stress, environment) can be perceived on the level of ERK1/2 activity.

Effectors of MAPK dephosphorylation

As we have demonstrated in a current study partial inactivation of ERK1/2 was observed in three independent mouse models (sex-related, GH/IGF1 induced on ACTH induced growth) of elevated adrenal gland growth (Bielohuby *et al.* 2008). Thus, high ERK1/2 activation is not necessarily associated with high growth activity in the adrenal gland. Instead, as discussed earlier high ERK1/2 activation was demonstrated potentially even to block the cell cycle (Rahmouni *et al.* 2006). Thus, timely inactivation of MAPKs seems to be important for correct biological responses. MAPKs are inactivated via dephosphorylation by a family of MAPK phosphatases (MKPs) in various tissues (Kondoh & Nishida 2007, Owens & Keyse 2007) including the adrenal gland (Gorostizaga *et al.* 2007). This family presently consists of seven members (MKP-1 to -5, MKP-7, and MKP-X).

However, to date, only MKP-1 has been detected in adrenal cells. MKP-1 is specific for p38 MAPK, JNK, and ERK1/2 dephosphorylation and thought to be rapidly induced by growth factors and stress (Keyse 2000). In fact, both effector types have been found in adrenal cells since MKP-1 expression is controlled by ACTH (Bey *et al.* 2003), angiotensin II (Casal *et al.* 2007), and heat stress (Gorostizaga *et al.* 2004). It is interesting to note that MKP-1 induction by angiotensin II lagged behind angiotensin II induction of ERK1/2 activation (Casal *et al.* 2007). Thus, angiotensin II has a biphasic effect on ERK1/2 phosphorylation with an initial stimulation of ERK1/2 phosphorylation followed by a terminating signal through dephosphorylation by MKP-1. In a mechanistic sense induction of MKP-1 gene expression depended on PKA (Sewer & Waterman 2003). Although direct effects of MKP-1 in adrenal cells have not been tested to our knowledge, it is clear from other cellular systems, that MKP-1 cannot simply be regarded as an inhibitor of growth since forced expression in transformed and untransformed breast cells was associated with a suppression of JNK activation and conferred chemoresistance (Small *et al.* 2007).

Integration of intracellular signaling cascades by ERK1/2

One important pathway, which activates ERK1/2 after RTK activation, involves Shc (Src-homology-2-containing), Grb2 (growth factor receptor bound-2),

Sos (son of sevenless), Ras, Raf, and MEK (mitogen activated protein kinase kinase). However, RTKs can also be transactivated by GPCR via activated EGF and the EGF receptor (Daub *et al.* 1996). Furthermore, RTKs can also be transactivated by GPCR via an inhibition of phosphatases or activation by Src (Wetzker & Bohmer 2003). In addition, GPCR have direct access to ERK1/2 activation by mechanisms that employ PI3K, PKC, Rap1B-Raf (Wetzker & Bohmer 2003). According to present concepts (Delcourt *et al.* 2007) transactivation can also occur in the opposite direction leading to activation of GPCR-dependent pathways by RTKs. Thus, very clearly the former categorization of GPCR having postmitotic effects, while RTKs are mediating mitotic functions can definitely be termed obsolete (Rozengurt 2007). It is clear that many GPCR agonists (angiotensin, VIP, vasopressin) but also environmental or psychosocial factors induce cell proliferation by direct activation of ERK1/2 or in a synergistic manner with RTKs (Rozengurt 2007). In addition to RTKs and GPCRs also cytokine receptors e.g., the GH receptor (Brooks *et al.* 2008) and integrins (Yee *et al.* 2008) impact on ERK1/2. From the multiple activation of ERK1/2 in the different adrenal compartments we may assume that a large number of signals is tracking ERK1/2 also in the adrenal gland suggesting a role in adrenal growth and function, which hardly can be overestimated.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors express their gratitude for Sandra Hinrichs for her kind help with the figures.

References

- Aguilar F, Lo M, Claustrat B, Saez JM, Sassard J & Li JY 2004 Hypersensitivity of the adrenal cortex to trophic and secretory effects of angiotensin II in Lyon genetically-hypertensive rats. *Hypertension* **43** 87–93.
- Andreis PG, Markowska A, Champion HC, Mazzocchi G, Malendowicz LK & Nussdorfer GG 2000 Adrenomedullin enhances cell proliferation and deoxyribonucleic acid synthesis in rat adrenal zona glomerulosa: receptor subtype involved and signaling mechanism. *Endocrinology* **141** 2098–2104.
- Andreis PG, Malendowicz LK, Trejter M, Neri G, Spinazzi R, Rossi GP & Nussdorfer GG 2003 Ghrelin and growth hormone secretagogue receptor are expressed in the rat adrenal cortex: evidence that ghrelin stimulates the growth, but not the secretory activity of adrenal cells. *FEBS Letters* **536** 173–179.
- Armelin HA, Lotfi CF & Lepique AP 1996 Regulation of growth by ACTH in the Y-1 line of mouse adrenocortical cells. *Endocrine Research* **22** 373–383.
- Belloni AS, Mazzocchi G, Meneghelli V & Nussdorfer GG 1978 Cytogenesis in the rat adrenal cortex: evidence for an ACTH-induced centripetal cell migration from the zona glomerulosa. *Archives d'Anatomie, d'Histologie et d'Embryologie Normales et Expérimentales* **61** 195–205.
- Bertagna X 1994 Proopiomelanocortin-derived peptides. *Endocrinology and Metabolism Clinics of North America* **23** 467–485.
- Beuschlein F & Reincke M 2006 Adrenocortical tumorigenesis. *Annals of the New York Academy of Sciences* **1088** 319–334.
- Beuschlein F, Mutch C, Bavers DL, Ulrich-Lai YM, Engeland WC, Keegan C & Hammer GD 2002 Steroidogenic factor-1 is essential for compensatory adrenal growth following unilateral adrenalectomy. *Endocrinology* **143** 3122–3135.
- Bey P, Gorostizaga AB, Maloberti PM, Lozano RC, Poderoso C, Maciel FC, Podesta EJ & Paz C 2003 Adrenocorticotropin induces mitogen-activated protein kinase phosphatase 1 in Y1 mouse adrenocortical tumor cells. *Endocrinology* **144** 1399–1406.
- Bicknell AB 2002 Identification of the adrenal protease that cleaves pro-gamma-MSH: the dawning of a new era in adrenal physiology? *Journal of Endocrinology* **172** 405–410.
- Bicknell AB, Lomthaisong K, Woods RJ, Hutchinson EG, Bennett HP, Gladwell RT & Lowry PJ 2001 Characterization of a serine protease that cleaves pro-gamma-melanotropin at the adrenal to stimulate growth. *Cell* **105** 903–912.
- Bielohuby M, Herbach N, Wanke R, Maser-Gluth C, Beuschlein F, Wolf E & Hoeflich A 2007 Growth analysis of the mouse adrenal gland from weaning to adulthood: time- and gender-dependent alterations of cell size and number in the cortical compartment. *American Journal of Physiology. Endocrinology and Metabolism* **293** E139–E146.
- Bielohuby M, Sawitzky M, Johnsen I, Wittenburg D, Beuschlein F, Wolf E & Hoeflich A 2008 Decreased p44/42 MAPK phosphorylation in gender- or hormone-related but not during age-related adrenal gland growth in mice. *Endocrinology* (Epub ahead of print).
- Blackburn A, Schmitt A, Schmidt P, Wanke R, Hermanns W, Brem G & Wolf E 1997 Actions and interactions of growth hormone and insulin-like growth factor-II: body and organ growth of transgenic mice. *Transgenic Research* **6** 213–222.
- Bornstein SR & Rutkowski H 2002 The adrenal hormone metabolism in the immune/inflammatory reaction. *Endocrine Research* **28** 719–728.
- Brizuela L, Rábano M, Gangoiti P, Narbona N, Macarulla JM, Trueba M & Gómez-Muñoz A 2007 Sphingosine-1-phosphate stimulates aldosterone secretion through a mechanism involving the PI3K/PKB and MEK/ERK 1/2 pathways. *Journal of Lipid Research* **48** 2264–2274.
- Brooks AJ, Wooh JW, Tunny KA & Waters MJ 2008 Growth hormone receptor; mechanism of action. *International Journal of Biochemistry and Cell Biology* **40** 1984–1989.
- Brown JW, Kesler CT, Neary T & Fishman LM 2001 Effects of androgens and estrogens and catechol and methoxy-estrogen derivatives on mitogen-activated protein kinase (ERK(1,2)) activity in SW-13 human adrenal carcinoma cells. *Hormone and Metabolic Research* **33** 127–130.
- Cammarota M, Bevilacqua LR, Dunkley PR & Rostas JA 2001 Angiotensin II promotes the phosphorylation of cyclic AMP-responsive element binding protein (CREB) at Ser133 through an ERK1/2-dependent mechanism. *Journal of Neurochemistry* **79** 1122–1128.

- Cammarota M, Bevilacqua LRM, Rostas JAP & Dunkley PR 2003 Histamine activates tyrosine hydroxylase in bovine adrenal chromaffin cells through a pathway that involves ERK1/2 but not p38 or JNK. *Journal of Neurochemistry* **84** 453–458.
- Campbell S, Otis M, Cote M, Gallo-Payet N & Payet MD 2003 Connection between integrins and cell activation in rat adrenal glomerulosa cells: a role for Arg-Gly-Asp peptide in the activation of the p42/p44(mapk) pathway and intracellular calcium. *Endocrinology* **144** 1486–1495.
- Casal AJ, Ryser S, Capponi AM & Wang-Buholzer CF 2007 Angiotensin II-induced mitogen-activated protein kinase phosphatase-1 expression in bovine adrenal glomerulosa cells: implications in mineralocorticoid biosynthesis. *Endocrinology* **148** 5573–5581.
- Cater DB & Stack-Dunne MP 1953 The histological changes in the adrenal of the hypophysectomised rat after treatment with pituitary preparations. *Journal of Pathology and Bacteriology* **66** 119–133.
- Cecim M, Ghosh PK, Esquifino AI, Began T, Wagner TE, Yun JS & Bartke A 1991 Elevated corticosterone levels in transgenic mice expressing human or bovine growth hormone genes. *Neuroendocrinology* **53** 313–316.
- Chabre O, Cornillon F, Bottari SP, Chambaz EM & Vilgrain I 1995 Hormonal regulation of mitogen-activated protein kinase activity in bovine adrenocortical cells: cross-talk between phosphoinositides, adenosine 3',5'-monophosphate, and tyrosine kinase receptor pathways. *Endocrinology* **136** 956–964.
- Chambard JC, Lefloch R, Pouyssegur J & Lenormand P 2007 ERK implication in cell cycle regulation. *Biochimica et Biophysica Acta* **1773** 1299–1310.
- Chang LL, Wun WS & Wang PS 2008 Effects of dehydroepiandrosterone on aldosterone release in rat zona glomerulosa cells. *Journal of Biomedical Science* **15** 463–470.
- Cherradi N, Pardo B, Greenberg AS, Kraemer FB & Capponi AM 2003 Angiotensin II activates cholesterol ester hydrolase in bovine adrenal glomerulosa cells through phosphorylation mediated by p42/p44 mitogen-activated protein kinase. *Endocrinology* **144** 4905–4915.
- Chien CL, Chen YC, Chang MF, Greenberg AS & Wang SM 2005 Magnolol induces the distributional changes of p160 and adipose differentiation-related protein in adrenal cells. *Histochemistry and Cell Biology* **123** 429–439.
- Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson AL *et al.* 1989 Identification of angiotensin II receptor subtypes. *Biochemical and Biophysical Research Communications* **165** 196–203.
- Coiro V, Volpi R, Capretti L, Caffarri G, Colla R, Giuliani N & Chiodera P 1998 Stimulation of ACTH and GH release by angiotensin II in normal men is mediated by the AT1 receptor subtype. *Regulatory Peptides* **74** 27–30.
- Coll AP, Challis BG, Yeo GS, Snell K, Piper SJ, Halsall D, Thresher RR & O'Rahilly S 2004 The effects of proopiomelanocortin deficiency on murine adrenal development and responsiveness to adrenocorticotropin. *Endocrinology* **145** 4721–4727.
- Cote M, Muyldermans J, Chouinard L & Gallo-Payet N 1998 Involvement of tyrosine phosphorylation and MAPK activation in the mechanism of action of ACTH, angiotensin II and vasopressin. *Endocrine Research* **24** 415–419.
- Cox ME & Parsons SJ 1997 Roles for protein kinase C and mitogen-activated protein kinase in nicotine-induced secretion from bovine adrenal chromaffin cells. *Journal of Neurochemistry* **69** 1119–1130.
- Cox ME, Ely CM, Catling AD, Weber MJ & Parsons SJ 1996 Tyrosine kinases are required for catecholamine secretion and mitogen-activated protein kinase activation in bovine adrenal chromaffin cells. *Journal of Neurochemistry* **66** 1103–1112.
- Daub H, Weiss FU, Wallasch C & Ullrich A 1996 Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* **379** 557–560.
- Davies E, Omer S, Buckingham JC, Morris JF & Christian HC 2007 Expression and externalization of annexin 1 in the adrenal gland: structure and function of the adrenal gland in annexin 1-null mutant mice. *Endocrinology* **148** 1030–1038.
- Delcourt N, Bockaert J & Marin P 2007 GPCR-jacking: from a new route in RTK signalling to a new concept in GPCR activation. *Trends in Pharmacological Sciences* **28** 602–607.
- Dutton CM, Joba W, Spitzweg C, Heufelder AE & Bahn RS 1997 Thyrotropin receptor expression in adrenal, kidney, and thymus. *Thyroid* **7** 879–884.
- Edwin F & Patel TB 2008 A novel role of Sprouty 2 in regulating cellular apoptosis. *Journal of Biological Chemistry* **283** 3181–3190.
- Elijovich F, Zhao HW, Laffer CL, Du Y, DiPette DJ, Inagami T & Wang DH 1997 Regulation of growth of the adrenal gland in DOC-salt hypertension. Role of angiotensin II receptor subtypes. *Hypertension* **29** 408–413.
- Engeland WC, Shinsako J & Dallman MF 1975 Corticosteroids and ACTH are not required for compensatory adrenal growth. *American Journal of Physiology* **229** 1461–1464.
- Engeland WC, Ennen WB, Elayaperumal A, Durand DA & Levay-Young BK 2005 Zone-specific cell proliferation during compensatory adrenal growth in rats. *American Journal of Physiology. Endocrinology and Metabolism* **288** E298–E306.
- Estivarez FE, Iturriza F, McLean C, Hope J & Lowry PJ 1982 Stimulation of adrenal mitogenesis by N-terminal proopiomelanocortin peptides. *Nature* **297** 419–422.
- Estivarez FE, Carino M, Lowry PJ & Jackson S 1988 Further evidence that N-terminal pro-opiomelanocortin peptides are involved in adrenal mitogenesis. *Journal of Endocrinology* **116** 201–206.
- Fassnacht M, Hahner S, Hansen IA, Kreutzberger T, Zink M, Adermann K, Jakob F, Troppmair J & Allolio B 2003 N-terminal proopiomelanocortin acts as a mitogen in adrenocortical tumor cells and decreases adrenal steroidogenesis. *Journal of Clinical Endocrinology and Metabolism* **88** 2171–2179.
- Ferreira JG, Cruz C, Vinson GP & Pignatelli D 2004 ACTH modulates ERK phosphorylation in the adrenal gland in a time-dependent manner. *Endocrine Research* **30** 661–666.
- Ferreira JG, Cruz CD, Neves D & Pignatelli D 2007 Increased extracellular signal regulated kinases phosphorylation in the adrenal gland in response to chronic ACTH treatment. *Journal of Endocrinology* **192** 647–658.
- Forti FL, Dias MH & Armelin HA 2006 ACTH receptor: ectopic expression, activity and signaling. *Molecular and Cellular Biochemistry* **293** 147–160.
- Fottner C, Hoeflich A, Wolf E & Weber MM 2004 Role of the insulin-like growth factor system in adrenocortical growth control and carcinogenesis. *Hormone and Metabolic Research* **36** 397–405.
- Gallo-Payet N & Payet MD 2003 Mechanism of action of ACTH: beyond cAMP. *Microscopic Research and Technique* **61** 275–287.
- Giulia C, Adriana L, Elisabetta P, Poli G, Elisabetta C, Sara M, Tonino E, Andrea G, Mario S, Massimo M *et al.* 2008 Rosiglitazone inhibits adrenocortical cancer cell proliferation by interfering with the IGF-IR intracellular signaling. *PPAR Research* **2008** 904041.
- Gomez N & Cohen P 1991 Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases. *Nature* **353** 170–173.
- Gorostizaga A, Brion L, Maloberti P, Poderoso C, Podestá EJ, Maciel FC & Paz C 2004 Molecular events triggered by heat shock in Y1 adrenocortical cells. *Endocrine Research* **30** 655–659.
- Gorostizaga A, Cornejo Maciel F, Brion L, Maloberti P, Podestá EJ & Paz C 2007 Tyrosine phosphatases in steroidogenic cells: regulation and function. *Molecular and Cellular Endocrinology* **266** 131–137.
- Hammer GD, Parker KL & Schimmer BP 2005 Minireview: transcriptional regulation of adrenocortical development. *Endocrinology* **146** 1018–1024.
- Hansen IA, Fassnacht M, Hahner S, Hammer F, Schammann M, Meyer SR, Bicknell AB & Allolio B 2004 The adrenal secretory

- serine protease AsP is a short secretory isoform of the transmembrane airway trypsin-like protease. *Endocrinology* **145** 1898–1905.
- Harbuz M 2002 Neuroendocrine function and chronic inflammatory stress. *Experimental Physiology* **87** 519–525.
- Hershkovitz L, Beuschlein F, Klammer S, Krup M & Weinstein Y 2007 Adrenal 20 α -hydroxysteroid dehydrogenase in the mouse catabolizes progesterone and 11-deoxycorticosterone and is restricted to the X-zone. *Endocrinology* **148** 976–988.
- Ho W, Uniyal S, Meakin SO, Morris VL & Chan BM 2001 A differential role of extracellular signal-regulated kinase in stimulated PC12 pheochromocytoma cell movement. *Experimental Cell Research* **263** 254–264.
- Ho WC, Uniyal S, Zhou H, Morris VL & Chan BMC 2005 Threshold levels of ERK activation for chemotactic migration differ for NGF and EGF in rat pheochromocytoma PC12 cells. *Molecular and Cellular Biochemistry* **271** 29–41.
- Hoeflich A, Weber MM, Fisch T, Nedbal S, Fottner C, Elmlinger MW, Wanke R & Wolf E 2002 Insulin-like growth factor binding protein 2 (IGFBP-2) separates hypertrophic and hyperplastic effects of growth hormone (GH)/IGF-I excess on adrenocortical cells *in vivo*. *FASEB Journal* **16** 1721–1731.
- Inagaki K, Otsuka F, Suzuki J, Kano Y, Takeda M, Miyoshi T, Otani H, Mimura Y, Ogura T & Makino H 2006 Involvement of bone morphogenetic protein-6 in differential regulation of aldosterone production by angiotensin II and potassium in human adrenocortical cells. *Endocrinology* **147** 2681–2689.
- John CD, Christian HC, Morris JF, Flower RJ, Solito E & Buckingham JC 2004 Annexin I and the regulation of endocrine function. *Trends in Endocrinology and Metabolism* **15** 103–109.
- Kano Y, Otsuka F, Takeda M, Suzuki J, Inagaki K, Miyoshi T, Miyamoto M, Otani H, Ogura T & Makino H 2005 Regulatory roles of bone morphogenetic proteins and glucocorticoids in catecholamine production by rat pheochromocytoma cells. *Endocrinology* **146** 5332–5340.
- Karpac J, Ostwald D, Bui S, Hunnewell P, Shankar M & Hochgeschwender U 2005 Development, maintenance, and function of the adrenal gland in early postnatal proopiomelanocortin-null mutant mice. *Endocrinology* **146** 2555–2561.
- Karpac J, Kern A & Hochgeschwender U 2007 Pro-opiomelanocortin peptides and the adrenal gland. *Molecular and Cellular Endocrinology* **265–266** 29–33.
- Katz M, Amit I & Yarden Y 2007 Regulation of MAPKs by growth factors and receptor tyrosine kinases. *Biochimica et Biophysica Acta* **1773** 1161–1176.
- Kempná P & Flück CE 2008 Adrenal gland development and defects. *Best Practice and Research. Clinical Endocrinology and Metabolism* **22** 77–93.
- Kempná P, Hofer G, Mullis PE & Flück CE 2007 Pioglitazone inhibits androgen production in NCI-H295R cells by regulating gene expression of CYP17 and HSD3B2. *Molecular Pharmacology* **71** 787–798.
- Keramidas M, Faudot C, Cibiel A, Feige JJ & Thomas M 2008 Mitogenic functions of endocrine gland-derived vascular endothelial growth factor and Bombina variegata 8 on steroidogenic adrenocortical cells. *Journal of Endocrinology* **196** 473–482.
- Kero J, Poutanen M, Zhang FP, Rahman N, McNicol AM, Nilson JH, Keri RA & Huhtaniemi IT 2000 Elevated luteinizing hormone induces expression of its receptor and promotes steroidogenesis in the adrenal cortex. *Journal of Clinical Investigation* **105** 633–641.
- Keyse SM 2000 Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. *Current Opinion in Cell Biology* **12** 186–192.
- Kim AC, Reuter AL, Zubair M, Else T, Serecky K, Bingham NC, Lavery GG, Parker KL & Hammer GD 2008 Targeted disruption of β -catenin in Sfl-expressing cells impairs development and maintenance of the adrenal cortex. *Development* **135** 2593–2602.
- Kondoh K & Nishida E 2007 Regulation of MAP kinases by MAP kinase phosphatases. *Biochimica et Biophysica Acta* **1773** 1227–1237.
- Kovzun OI 2007 Effect of mitogen-activated protein kinases and transcriptional factor c-Fos on estradiol signal transduction in the rat adrenocortical cells. *Fiziologicheskii Zhurnal* **53** 46–51.
- Lawlor MA, Mora A, Ashby PR, Williams MR, Murray-Tait V, Malone L, Prescott AR, Lucocq JM & Alessi DR 2002 Essential role of PDK1 in regulating cell size and development in mice. *EMBO Journal* **21** 3728–3738.
- Le T & Schimmer BP 2001 The regulation of MAPKs in Y1 mouse adrenocortical tumor cells. *Endocrinology* **142** 4282–4287.
- Leal RB, Posser T, Rigon AP, Oliveira CS, Goncalves CA, Gelain DP & Dunkley PR 2007 Cadmium stimulates MAPKs and Hsp27 phosphorylation in bovine adrenal chromaffin cells. *Toxicology* **234** 34–43.
- Lehoux JG, Bird IM, Briere N, Martel D & Ducharme L 1997 Influence of dietary sodium restriction on angiotensin II receptors in rat adrenals. *Endocrinology* **138** 5238–5247.
- Lepique AP, Forti FL, Moraes MS & Armelin HA 2000 Signal transduction in G0/G1-arrested mouse Y1 adrenocortical cells stimulated by ACTH and FGF2. *Endocrine Research* **26** 825–832.
- Lin R, LeCouter J, Kowalski J & Ferrara N 2002 Characterization of endocrine gland-derived vascular endothelial growth factor signaling in adrenal cortex capillary endothelial cells. *Journal of Biological Chemistry* **277** 8724–8729.
- Lotfi CF, Todorovic Z, Armelin HA & Schimmer BP 1997 Unmasking a growth-promoting effect of the adrenocorticotrophic hormone in Y1 mouse adrenocortical tumor cells. *Journal of Biological Chemistry* **272** 29886–29891.
- Lotfi CF, Costa ET, Schwindt TT & Armelin HA 2000 Role of ERK/MAP kinase in mitogenic interaction between ACTH and FGF2 in mouse Y1 adrenocortical tumor cells. *Endocrine Research* **26** 873–877.
- Lund TD, Munson DJ, Haldy ME & Handa RJ 2004 Dihydrotestosterone may inhibit hypothalamo–pituitary–adrenal activity by acting through estrogen receptor in the male mouse. *Neuroscience Letters* **365** 43–47.
- Mattos GE & Lotfi CFP 2005 Differences between the growth regulatory pathways in primary rat adrenal cells and mouse tumor cell line. *Molecular and Cellular Endocrinology* **245** 31–42.
- Mazzocchi G, Rossi GP, Malendowicz LK, Champion HC & Nussdorfer GG 2000 Endothelin-1 [1–31], acting as an ETA-receptor selective agonist, stimulates proliferation of cultured rat zona glomerulosa cells. *FEBS Letters* **487** 194–198.
- Mazzocchi G, Neri G, Rucinski M, Rebuffat P, Spinazzi R, Malendowicz LK & Nussdorfer GG 2004 Ghrelin enhances the growth of cultured human adrenal zona glomerulosa cells by exerting MAPK-mediated proliferogenic and antiapoptotic effects. *Peptides* **25** 1269–1277.
- McEwan PE, Vinson GP & Kenyon CJ 1999 Control of adrenal cell proliferation by AT1 receptors in response to angiotensin II and low-sodium diet. *American Journal of Physiology* **276** E303–E309.
- McNeill H & Vinson GP 2000 Regulation of MAPK activity in response to dietary sodium in the rat adrenal gland. *Endocrine Research* **26** 879–883.
- McNeill H, Whitworth E, Vinson GP & Hinson JP 2005 Distribution of extracellular signal-regulated protein kinases 1 and 2 in the rat adrenal and their activation by angiotensin II. *Journal of Endocrinology* **187** 149–157.
- Mendoza IE, Schmachtenberg O, Tonk E, Fuentealba J, Díaz-Raya P, Lagos VL, García AG & Cárdenas AM 2003 Depolarization-induced ERK phosphorylation depends on the cytosolic Ca^{2+} level rather than on the Ca^{2+} channel subtype of chromaffin cells. *Journal of Neurochemistry* **86** 1477–1486.
- Mikola M, Kero J, Nilson JH, Keri RA, Poutanen M & Huhtaniemi I 2003 High levels of luteinizing hormone analog stimulate gonadal

- and adrenal tumorigenesis in mice transgenic for the mouse inhibin- α -subunit promoter/Simian virus 40 T-antigen fusion gene. *Oncogene* **22** 3269–3278.
- Moog F, Bennett CJ & Dean CM Jr 1954 Growth and cytochemistry of the adrenal gland of the mouse from birth to maturity. *Anatomical Record* **120** 873–891.
- Morooka T & Nishida E 1998 Requirement of p38 mitogen-activated protein kinase for neuronal differentiation in PC12 cells. *Journal of Biological Chemistry* **273** 24285–24288.
- Morris JF, Omer S, Davies E, Wang E, John C, Afzal T, Wain S, Buckingham JC, Flower RJ & Christian HC 2006 Lack of annexin I results in an increase in corticotroph number in male but not female mice. *Journal of Neuroendocrinology* **18** 835–846.
- Nakajima M, Hutchinson HG, Fujinaga M, Hayashida W, Morishita R, Zhang L, Horiuchi M, Pratt RE & Dzau VJ 1995 The angiotensin II type 2 (AT₂) receptor antagonizes the growth effects of the AT₁ receptor: gain-of-function study using gene transfer. *PNAS* **92** 10663–10667.
- Nakamura K, Fujiwara Y, Mizutani R, Sanbe A, Miyauchi N, Hiroyama M, Yamauchi J, Yamashita T, Nakamura S, Mori T *et al.* 2008 Effects of vasopressin v1b receptor deficiency on adrenocorticotropin release from anterior pituitary cells in response to oxytocin stimulation. *Endocrinology* **149** 4883–4891.
- Nemoto T, Mano-Otagiri A & Shibasaki T 2005 Urocortin 2 induces tyrosine hydroxylase phosphorylation in PC12 cells. *Biochemical and Biophysical Research Communications* **330** 821–831.
- Nussdorfer GG, Rebuffat P, Mazzocchi G, Belloni AS & Meneghelli V 1974 Investigations on adrenocortical mitochondria turnover. I. Effect of chronic treatment with ACTH on the size and number of rat zona fasciculata mitochondria. *Cell and Tissue Research* **150** 79–94.
- Okada SL, Ellsworth JL, Durnam DM, Haugen HS, Holloway JL, Kelley ML, Lewis KE, Ren H, Sheppard PO, Storey HM *et al.* 2006 A glycoprotein hormone expressed in corticotrophs exhibits unique binding properties on thyroid-stimulating hormone receptor. *Molecular Endocrinology* **20** 414–425.
- Olson MF, Krolczyk AJ, Gorman KB, Steinberg RA & Schimmer BP 1993 Molecular basis for the 3',5'-cyclic adenosine monophosphate resistance of Kin mutant Y1 adrenocortical tumor cells. *Molecular Endocrinology* **7** 477–487.
- Otis M & Gallo-Payet N 2006 Differential involvement of cytoskeleton and rho-guanosine 5'-triphosphatases in growth-promoting effects of angiotensin II in rat adrenal glomerulosa cells. *Endocrinology* **147** 5460–5469.
- Otis M & Gallo-Payet N 2007 Role of MAPKs in angiotensin II-induced steroidogenesis in rat glomerulosa cells. *Molecular and Cellular Endocrinology* **265–266** 126–130.
- Otis M, Campbell S, Payet MD & Gallo-Payet N 2005 Angiotensin II stimulates protein synthesis and inhibits proliferation in primary cultures of rat adrenal glomerulosa cells. *Endocrinology* **146** 633–642.
- Otis M, Campbell S, Payet MD & Gallo-Payet N 2007a The growth-promoting effects of angiotensin II in adrenal glomerulosa cells: an interactive tale. *Molecular and Cellular Endocrinology* **273** 1–5.
- Otis M, Campbell S, Payet MD & Gallo-Payet N 2007b Expression of extracellular matrix proteins and integrins in rat adrenal gland: importance for ACTH-associated functions. *Journal of Endocrinology* **193** 331–347.
- Otis M, Battista MC, Provencher M, Campbell S, Roberge C, Payet MD & Gallo-Payet N 2008 From integrative signalling to metabolic disorders. *Journal of Steroid Biochemistry and Molecular Biology* **109** 224–229.
- Owens DM & Keyse SM 2007 Differential regulation of MAP kinase signalling by dual-specificity protein phosphatases. *Oncogene* **26** 3203–3213.
- Parmar J, Key RE & Rainey WE 2008 Development of an adrenocorticotropin-responsive human adrenocortical carcinoma cell line. *Journal of Clinical Endocrinology and Metabolism* **93** 4542–4546.
- Phillips RW, Crock C & Funder J 1985 Effects of mineralocorticoids and glucocorticoids on compensatory adrenal growth in rats. *American Journal of Physiology. Endocrinology and Metabolism* **248** E450–E456.
- Raffin-Sanson ML, de Keyser Y & Bertagna X 2003 Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. *European Journal of Endocrinology* **149** 79–90.
- Rahmouni S, Cerignoli F, Alonso A, Tsutji T, Henkens R, Zhu C, Louisdit-Sully C, Moutschen M, Jiang W & Mustelin T 2006 Loss of the VHR dual-specific phosphatase causes cell-cycle arrest and senescence. *Nature Cell Biology* **8** 524–531.
- Ramanjaneya M, Conner AC, Chen J, Stanfield PR & Rande HS 2008 Orexins stimulate steroidogenic acute regulatory protein expression through multiple signaling pathways in human adrenal H295R cells. *Endocrinology* **149** 4106–4115.
- Rebuffat P, Gottardo L, Malendowicz LK, Neri G & Nussdorfer GG 2001 Proadrenomedullin N-terminal 20 peptide (PAMP) enhances proliferation of rat zona glomerulosa cells by activating MAPK cascade. *Peptides* **22** 1909–1912.
- Rocha KM, Forti FL, Lepique AP & Armelin HA 2003 Deconstructing the molecular mechanisms of cell cycle control in a mouse adrenocortical cell line: roles of ACTH. *Microscopic Research and Technique* **61** 268–274.
- Ross JT, McMillen IC, Lok F, Thiel AG, Owens JA & Coulter CL 2007 Intrafetal insulin-like growth factor-I infusion stimulates adrenal growth but not steroidogenesis in the sheep fetus during late gestation. *Endocrinology* **148** 5424–5432.
- Rozengurt E 2007 Mitogenic signaling pathways induced by G protein-coupled receptors. *Journal of Cellular Biology* **213** 589–602.
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S *et al.* 1998 Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* **92** 573–585.
- Saruhan BG & Ozdemir N 2005 Effect of ovariectomy and of estrogen treatment on the adrenal gland and body weight in rats. *Saudi Medical Journal* **26** 131.
- Semplicini A, Ceolotto G, Baritono E, Malendowicz LK, Andreis PG, Sartori M, Rossi GP & Nussdorfer GG 2001 Adrenomedullin stimulates DNA synthesis of rat adrenal zona glomerulosa cells through activation of the mitogen-activated protein kinase-dependent cascade. *Journal of Hypertension* **19** 599–602.
- Sewer MB & Waterman MR 2003 CAMP-dependent protein kinase enhances CYP17 transcription via MKP-1 activation in H295R human adrenocortical cells. *Journal of Biological Chemistry* **278** 8106–8111.
- Shah BH, Baukal AJ, Shah FB & Catt KJ 2005 Mechanisms of extracellularly regulated kinases 1/2 activation in adrenal glomerulosa cells by lysophosphatidic acid and epidermal growth factor. *Molecular Endocrinology* **19** 2535–2548.
- Shah BH, Baukal AJ, Chen HD, Shah AB & Catt KJ 2006 Mechanisms of endothelin-1-induced MAP kinase activation in adrenal glomerulosa cells. *Journal of Steroid Biochemistry and Molecular Biology* **102** 79–88.
- Shibuya I, Utsunomiya K, Toyohira Y, Ueno S, Tsutsui M, Cheah TB, Ueta Y, Izumi F & Yanagihara N 2002 Regulation of catecholamine synthesis by leptin. *Annals of the New York Academy of Sciences* **971** 522–527.
- Shinkai K, Toyohira Y, Yoshimura R, Tsutsui M, Ueno S, Nakamura J & Yanagihara N 2007 Stimulation of catecholamine synthesis via activation of p44/42 MAPK in cultured bovine adrenal medullary cells by milnacipran. *Naunyn-Schmiedeberg's Archives of Pharmacology* **375** 65–72.
- Small GW, Shi YY, Higgins LS & Orlowski RZ 2007 Mitogen-activated protein kinase phosphatase-1 is a mediator of breast cancer chemoresistance. *Cancer Research* **67** 4459–4466.

- Sobel D & Vagnucci A 1982 Angiotensin II mediated ACTH release in rat pituitary cell culture. *Life Sciences* **30** 1281–1286.
- Spinazzi R, Ziolkowska A, Neri G, Nowak M, Rebuffat P, Nussdorfer GG, Andreis PG & Malendowicz LK 2005 Orexins modulate the growth of cultured rat adrenocortical cells, acting through type 1 and type 2 receptors coupled to the MAPK p42/p44- and p38-dependent cascades. *International Journal of Molecular Medicine* **15** 847–852.
- Stratakis CA & Boikos SA 2007 Genetics of adrenal tumors associated with Cushing's syndrome: a new classification for bilateral adrenocortical hyperplasias. *Nature Clinical Practice. Endocrinology & Metabolism* **3** 748–757.
- Sugano T, Yanagita T, Yokoo H, Satoh S, Kobayashi H & Wada A 2006 Enhancement of insulin-induced PI3K/Akt/GSK-3 β and ERK signaling by neuronal nicotinic receptor/PKC- α /ERK pathway: up-regulation of IRS-1/-2 mRNA and protein in adrenal chromaffin cells. *Journal of Neurochemistry* **98** 20–33.
- Suzuki J, Otsuka F, Inagaki K, Takeda M, Ogura T & Makino H 2004 Novel action of activin and bone morphogenetic protein in regulating aldosterone production by human adrenocortical cells. *Endocrinology* **145** 639–649.
- Takekoshi K, Ishii K, Nanmoku T, Shibuya S, Kawakami Y, Isobe K & Nakai T 2001 Leptin stimulates catecholamine synthesis in a PKC-dependent manner in cultured porcine adrenal medullary chromaffin cells. *Endocrinology* **142** 4861–4871.
- Tanimura SM & Watts AG 2001 Corticosterone modulation of ACTH secretagogue gene expression in the paraventricular nucleus. *Peptides* **22** 775–783.
- 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. *Molecular and Cellular Biochemistry* **15** 79–87.
- Tian Y, Balla T, Baukal AJ & Catt KJ 1995 Growth responses to angiotensin II in bovine adrenal glomerulosa cells. *American Journal of Physiology* **268** E135–E144.
- Trokoudes KM, Sugeno A, Hazani E, Row VV & Volpe R 1979 Thyroid-stimulating hormone (TSH) binding to extrathyroidal human tissues: TSH binding to extrathyroidal human tissues: TSH and thyroid-stimulating immunoglobulin effects on adenosine 3',5'-monophosphate in testicular and adrenal tissues. *Journal of Clinical Endocrinology and Metabolism* **48** 919–923.
- Ulrich-Lai YM, Figueiredo HF, Ostrander MM, Choi DC, Engeland WC & Herman JP 2006 Chronic stress induces adrenal hyperplasia and hypertrophy in a subregion-specific manner. *American Journal of Physiology. Endocrinology and Metabolism* **291** E965–E973.
- Utsunomiya K, Yanagihara N, Tachikawa E, Cheah TB, Kajiwar K, Toyohira Y, Ueno S & Izumi F 2001 Stimulation of catecholamine synthesis in cultured bovine adrenal medullary cells by leptin. *Journal of Neurochemistry* **76** 926–934.
- Viard I, Jaillard C, Ouali R & Saez JM 1992 Angiotensin-II-induced expression of proto-oncogene (c-fos, jun-B and c-jun) mRNA in bovine adrenocortical fasciculata cells (BAC) is mediated by AT-1 receptors. *FEBS Letters* **313** 43–46.
- Viau V 2002 Functional cross-talk between the hypothalamic–pituitary–gonadal and -adrenal axes. *Journal of Neuroendocrinology* **14** 506–513.
- Viau V & Meaney MJ 1996 The inhibitory effect of testosterone on hypothalamic–pituitary–adrenal responses to stress is mediated by the medial preoptic area. *Journal of Neuroscience* **16** 1866–1876.
- Watanabe G, Lee RJ, Albanese C, Rainey WE, Batlle D & Pestell RG 1996 Angiotensin II activation of cyclin D1-dependent kinase activity. *Journal of Biological Chemistry* **271** 22570–22577.
- Watanabe G, Pena P, Albanese C, Wilsbacher LD, Young JB & Pestell RG 1997 Adrenocorticotropin induction of stress-activated protein kinase in the adrenal cortex *in vivo*. *Journal of Biological Chemistry* **272** 20063–20069.
- Weber MM, Fottner C, Schmidt P, Brodowski KM, Gittner K, Lahm H, Engelhardt D & Wolf E 1999 Postnatal overexpression of insulin-like growth factor II in transgenic mice is associated with adrenocortical hyperplasia and enhanced steroidogenesis. *Endocrinology* **140** 1537–1543.
- Wetzer R & Bohmer FD 2003 Transactivation joins multiple tracks to the ERK/MAPK cascade. *Nature Reviews. Molecular Cell Biology* **4** 651–657.
- Whitworth EJ, Hinson JP & Vinson GP 2002 Neuropeptides and adrenocortical proliferation *in vitro*. *Endocrine Research* **28** 677–681.
- Whitworth EJ, Kosti O, Renshaw D & Hinson JP 2003 Adrenal neuropeptides: regulation and interaction with ACTH and other adrenal regulators. *Microscopic Research and Technique* **61** 259–267.
- Wolf E, Kramer R, Blum WF, Foll J & Brem G 1994 Consequences of postnatally elevated insulin-like growth factor-II in transgenic mice: endocrine changes and effects on body and organ growth. *Endocrinology* **135** 1877–1886.
- Wu CH, Chen YF, Wang JY, Hsieh MC, Yeh CS, Lian ST, Shin SJ & Lin SR 2002 Mutant K-ras oncogene regulates steroidogenesis of normal human adrenocortical cells by the RAF-MEK-MAPK pathway. *British Journal of Cancer* **87** 1000–1005.
- Wyllie AH, Kerr JF, Macaskill IA & Currie AR 1973 Adrenocortical cell deletion: the role of ACTH. *Journal of Pathology* **111** 85–94.
- Yanagihara N, Toyohira Y, Ueno S, Tsutsui M, Utsunomiya K, Liu M & Tanaka K 2005 Stimulation of catecholamine synthesis by environmental estrogenic pollutants. *Endocrinology* **146** 265–272.
- Yanagihara N, Toyohira Y & Shinohara Y 2008 Insights into the pharmacological potential of estrogens and phytoestrogens on catecholamine signaling. *Annals of the New York Academy of Sciences* **1129** 96–104.
- Yee KL, Weaver VM & Hammer DA 2008 Integrin-mediated signalling through the MAP-kinase pathway. *IET Systems Biology* **2** 8–15.
- Ziegler CG, Sicard F, Sperber S, Ehrhart-Bornstein M, Bornstein SR & Krug AW 2006 DHEA reduces NGF-mediated cell survival in serum-deprived PC12 cells. *Annals of the New York Academy of Sciences* **1073** 306–311.
- Ziegler CG, Sicard F, Lattke P, Bornstein SR, Ehrhart-Bornstein M & Krug AW 2008 Dehydroepiandrosterone induces a neuroendocrine phenotype in nerve growth factor-stimulated chromaffin pheochromocytoma PC12 cells. *Endocrinology* **149** 320–328.
- Zwermann O, Schulte DM, Reincke M & Beuschlein F 2005 ACTH1-24 inhibits proliferation of adrenocortical tumors *in vivo*. *European Journal of Endocrinology* **153** 434–444.

Received in final form 27 November 2008

Accepted 2 December 2008

Made available online as an Accepted Preprint 3 December 2008