Mechanisms of adrenal gland growth: signal integration by extracellular signal regulated kinases 1/2

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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...
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 bioologically 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 H295R 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 those 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 adrenal glands after hypophysectomy (Estivariz et al. 2008).
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 (Oris 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 I (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 over-expressing 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/IGF1 system potently 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 phosphoinositols-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 adenomedullary 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

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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. 2003). This finding was discussed in context with 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

Figure 2 Control of ERK1/2 phosphorylation in adrenocortical cells. ERK1/2 phosphorylation is controlled by a multitude of endocrine or non-endocrine factors. 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.
(GH secretagogue receptor, GHSR) are abundantly expressed in the adrenal cortex, suggesting autocrine/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, Kozun 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 (Lotth 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 (Cammarota 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, dehydroepiandrosterenedione (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 urocoritin-2 (Nemoto et al. 2005) were found...
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. 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), 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.
Sos (son of sevenless), Ras, Raf, and MEK (mitogen activated protein kinase kinase). However, RTKs can also be transactivated by GPCRs via activated EGF and the EGF receptor (Daub et al. 1996). Furthermore, RTKs can also be transactivated by GPCRs via an inhibition of phosphatases or activation by Src (Wetzker & Bohmer 2003). In addition, GPCRs 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. 2007)) but also environmental or psychosocial factors induce cell proliferation by direct 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.

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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.

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Signal integration by ERK1/2 in the adrenals · A. HOEFFLICH and M. BIELOHUBY


Received in final form 27 November 2008

Accepted 2 December 2008

Made available online as an Accepted Preprint 3 December 2008