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Molecular Genetic Analysis of Glucocorticoid Signalling in Development

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A null mutation of the glucocorticoid receptor was generated by homologous recombination. Mutant newborn mice showed impaired lung development, hypertrophy of the adrenal cortex and a strongly reduced size of the adrenal medulla. Phenylethanolamine *N*-methyltransferase (PNMT) was undetectable in the adrenals of the mutant mice. Serum levels of corticosterone were moderately and ACTH levels were strongly elevated in the mutants. A weaker but significant increase of corticosterone and ACTH was observed already in heterozygous animals. This points to a dysregulation of the HPA axis due to defective feedback regulation via the glucocorticoid receptor. Liver gluconeogenetic enzymes were reduced to a variable degree. Whereas survival of heterozygous mutants was not affected, most of the homozygous mutant mice died during the perinatal period.

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Steroid hormones regulate a number of developmental and physiological processes in vertebrates by specifically controlling the transcriptional activity of genes in target tissues [1]. One aim of our studies is to understand how genes are selectively activated in specific cells and tissues, at the appropriate time in development, and in dependence of external signals, such as steroid hormones. The signal transduction pathways which are responsible for glucocorticoid action are present in many different, possibly even all cells. Thus the question arises how these ubiquitous signalling pathways lead to selective responses; that is how these signal transduction pathways are integrated into mechanisms determining cell-specific gene activation.

The ability of target cells to respond to glucocorticoids requires the presence of specific receptors which mediate hormone action within cells. In the inactive state, these receptors exist within the cell in association with other proteins, e.g. hsp90 [1]. Upon hormone binding, the hormone–receptor complex is released from this inactivate complex and binds as a dimer to a specific DNA sequence, the so-called glucocorticoid responsive element, GRE [1, 2]. The effects of glucocorticoids are mediated by two highly homologous receptors, the glucocorticoid (type II) and the mineralocorticoid (type I) receptor which operate at different concentrations of glucocorticoids and which differ in their transactivation potential [3, 4]. To investigate the role of the glucocorticoid receptors during development and adult life, we have generated, by gene targeting, a mouse with a disrupted glucocorticoid receptor gene.

Glucocorticoid hormones are produced in the adrenal cortex and are known to control many key steps in metabolism and development [5]. These include the perinatal activation of genes involved in gluconeogenesis [5] and fetal developmental processes, such as differentiation of the adrenal medulla [6] and lung maturation [7]. In the past, we have been interested primarily in the role of the glucocorticoid receptor in perinatal activation of liver-specific genes which are involved in gluconeogenesis. We have defined the key regulatory elements in one of those genes involved in gluconeogenesis, the tyrosine aminotransferase (TAT) gene [8, 9] and characterized the complex molecular structure of the hormone-dependent signal transduction pathways that lead to activation of TAT gene transcription [9, 10]. These studies have now been further advanced by generation of a mutation in the gene coding for the glucocorticoid receptor (T. Cole *et al.*, submitted). A brief summary of the results we have obtained during the analysis of this mutation is described below.

Some of the known functions of glucocorticoids are summarized in Fig. 1. Glucocorticoids are involved in

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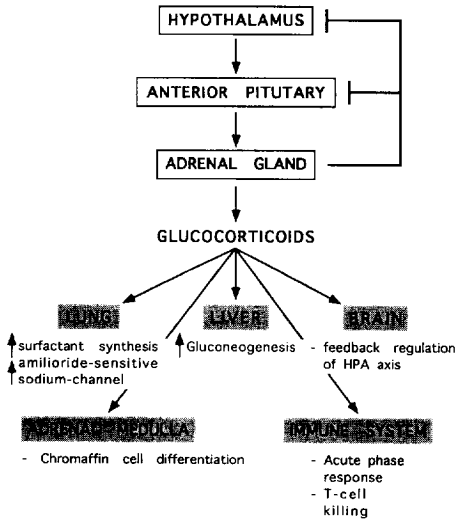

Fig. 1. Physiological effects of glucocorticoids.

perinatal activation of the gluconeogenic pathway in liver and in kidney. The hypoglycemia following birth leads to an increased secretion of glucocorticoids and glucagon. These two signal pathways lead to concerted activation of several genes involved in gluconeogenesis [5]. Glucocorticoids also play an important role in lung maturation, possibly by influencing the synthesis of surfactants [7]. The surfactants are important for the reduction of the surface tension of the alveolar membrane, thus promoting inflation and ventilation of the lung. Furthermore, glucocorticoids are thought to control the developmental switch from a neural-crest derived bipotential precursor cell to produce chromaffin cells in the adrenal medulla [6].

It was quickly noted that only a minority of the mice carrying a disruption in the glucocorticoid receptor gene on both alleles survived the first 4 weeks after birth. It was suspected that this occurred in the perinatal period, and therefore animals were genotyped at birth (Table 1). The Mendelian frequency of homozygous and heterozygous offspring was as expected, but most of the homozygous mutant animals died within the first hours after birth due to severe respiratory distress. This is due to inefficient inflation of the lung leading to large atelectatic segments. We believe that lung atelectasis is the primary cause of death. The levels of surfactant proteins A and C are not affected significantly, but the level of a Na^+ channel protein which is thought to be involved in liquid resorption in the lung [11] is substantially reduced.

Table 1. *Genotypes of progeny of heterozygous intercrosses*

Progeny genotypes	+/+	+/−	−/−
Out-bred			
4 weeks	188 (35%)	331 (61%)	25 (4.6%)
(C57BL/6-129/J)			
At birth	26 (21%)	69 (56%)	28 (23%)
Alive after 4 hr	26	69	2

During perinatal hypoglycemia, the induction of gluconeogenic genes is mediated by both glucocorticoids and glucagon, the action of the latter being mediated by cAMP. We analyzed the level of the mRNAs for several enzymes involved in gluconeogenesis such as glucose-6-phosphatase, phosphoenolpyruvate carboxykinase, serine dehydratase and tyrosine aminotransferase. The mRNAs coding for these enzymes are strongly reduced in the mutant, indicating that glucocorticoid signalling is important for activation of the gluconeogenic pathway at birth.

Glucocorticoid synthesis and secretion in the zona fasciculata of the adrenal cortex is controlled by the hypothalamic–pituitary-axis via a negative feedback loop. Therefore, the levels of glucocorticoids and ACTH in serum of animals of various genotypes were determined. We find a strong elevation of corticosterone (3-fold) and ACTH (15–20-fold) in the homozygous mutants and a lower but significant increase in heterozygous mice. This result clearly shows that feedback regulation is mediated by the glucocorticoid receptor. Feed-back control may occur directly on the level of POMC gene expression where a negative GRE has been characterized in its control region [12].

Glucocorticoids are implicated in determination of the differentiation of chromaffin cells from bipotential sympatho-adrenal progenitors. It is thought that glucocorticoids inhibit progression to the neuronal phenotype and promote differentiation towards chromaffin cells [13]. In mice, differentiated chromaffin cells are a mixed population of adrenergic phenylethanolamine *N*-methyltransferase (PNMT)-positive (75%) and noradrenergic, PNMT-negative cells (25%) [14]. In mutant embryos using antibodies directed against synaptophysin (a neuronal cell marker) and tyrosine hydroxylase (a key enzyme in catecholamine synthesis), a strong reduction of the number of cells expressing synaptophysin and tyrosine hydroxylase was found. Cells expressing PNMT were completely absent. A strong reduction of the number of TH-positive medullary cells comparable to the reduction seen in neonate adrenals was observed already at day 13.5 p.c. Based on these studies, we conclude that in the absence of GR the noradrenaline-producing chromaffin cell lineage can proliferate and differentiate normally. As the number of chromaffin precursor cells is already diminished at a very early stage of development we believe that the cell lineage which gives rise to adrenaline-producing cells is derived from a precursor which strictly requires GR for its survival and/or proliferation.

CONCLUSION

Generation of a null mutation in the glucocorticoid receptor gene has demonstrated the role of the glucocorticoid receptor in mouse embryonic development

and postnatal adaptation to self-sustaining extrauterine life. We find several striking defects in glucocorticoid receptor-deficient mice. They show developmental defects in the lung and in both the adrenal cortex and medulla, impairment of induction of gluconeogenic enzymes, and, due to defective feedback regulation, strongly elevated levels of circulating corticosterone and ACTH. The few surviving animals will be very useful for an understanding of the role glucocorticoids play in these and other developmental processes and in many physiological and pathological processes, such as the acute phase response, the response to acute and chronic stress and behavioural adaptations.

REFERENCES

1. Beato M.: Gene regulation by steroid hormones. *Cell* **56** (1989) 335–344.
2. Strähle U., Klock G. and Schütz G.: A DNA sequence of 15 base pairs is sufficient to mediate both glucocorticoid and progesterone induction of gene expression. *Proc. Natn. Acad. Sci. U.S.A.* **84** (1987) 7871–7875.
3. Evans R. M. and Arriza J. L.: A molecular framework for the actions of glucocorticoid hormones in the nervous system. *Neuron* **2** (1989) 1105–1112.
4. Rupprecht R., Arriza J. L., Sprengler D., Reul J. M. H. M., Evans R. M., Holsboer F. and Damm K.: Transactivation and synergistic properties of the mineralocorticoid receptor: relationship to the glucocorticoid receptor. *Molec. Endocr.* 7 (1993) 597–603.
5. Pilkis S. J. and Granner D. K.: Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *A. Rev. Physiol.* 54 (1992) 885–909.
6. Michelsohn A. M. and Anderson D. J.: Changes in competence determine the timing of two sequential glucocorticoid effects on sympathoadrenal progenitors. *Neuron* **8** (1992) 589–604.
7. Mendelson C. R. and Boggaram V.: Hormonal control of the surfactant system in fetal lung. *A. Rev. Physiol.* **53** (1991) 415–440.
8. Jantzen H-M., Strähle U., Gloss B., Stewart F., Schmid W., Boshart M., Miksicek R. and Schütz G.: Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. *Cell* **49** (1987) 29–38.
9. Boshart M., Weih F., Schmidt A., Fournier R. E. K. and Schütz G.: A cyclic AMP response element mediates repression of tyrosine aminotransferase gene transcription by the tissue-specific extinguisher locus Tse-1. *Cell* **61** (1990) 905–916.
10. Nitsch D., Boshart M. and Schütz G.: Activation of the tyrosine aminotransferase gene is dependent on synergy between liver-specific and hormone-responsive elements. *Proc. Natn. Acad. Sci. U.S.A.* **90** (1993) 5479–5483.
11. Champigny G., Voilley N., Lingueglia E., Friend V., Barbry P. and Lazdunski M.: Regulation of expression of the lung amiloride-sensitive Na^+ channel by steroid hormones. *EMBO J.* **13** (1994) 2177–2181.
12. Drouin J., Sun Y. L., Chamberland M., Gauthier Y., De Lean A., Nemer M. and Schmidt T. J.: Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *EMBO J.* **12** (1993) 145–156.
13. Michelsohn A. M. and Anderson D. J.: Changes in competence determine the timing of two sequential glucocorticoid effects on sympathoadrenal progenitors. *Neuron* **8** (1992) 589–604.
14. Coupland R. E.: *The Natural History of the Chromaffin Cell.* Longmans Press, Edinburgh (1965) pp. 250–251.