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EARNEST

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The role of glucocorticoids in fetal programming

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Abstract:

Perinatal stress may influence early programming of adult diseases leading to metabolic and behavioral disorders. The mechanisms by which perinatal events increase the risk of developing diseases are unclear but may involve stress induced glucocorticoid release. To address this question, we developed animal models that are either deprived of, or overexpress the glucocorticoid receptor gene (GR), a transcription factor, in targeted cell populations of the pancreas or the brain. We demonstrated that fetuses lacking the GR in pancreatic precursors show increased ß-cell fraction which is not affected by increased GC levels induced by maternal undernutrition. In contrast, a later recombination of GR gene, in differentiated ß-cells did not affect ß cell mass. This demonstrates that the absence of GR signaling in precursor cells protects the fetal beta-cell mass from the deleterious effect of undernutrition. GCs effect requires the activation of Pdx-1 a master gene for pancreas development. This conclusion was reinforced by the establishment and study of mice overexpressing GR in pancreatic precursors that show a decreased ß-cell mass. Perinatal stress can influence adult behaviour. We showed that GR overexpression in the forebrain affects anxiety and despair. Blockade of GR overexpression in the adult reverted anxiety but not despair suggesting that this behavior was modified by glucocorticoids during perinatal period.

Low birth-weight is strongly predictive of hypertension, cardiovascular diseases, obesity, insulin resistance, glucose intolerance and Type 2 diabetes. From these epidemiological studies the concept of early programming of adult diseases arose. More recently, preclinical data suggested that perinatal stress may also influence adult behavior and behavioural disorders. The mechanisms by which perinatal events increase the risk of developing diseases are unclear. A dysfunction of stress response, including modified circulating glucocorticoids levels, is suspected in the aetiology of several metabolic and behavioural disorders. Glucocorticoids activate the Glucocorticoid Receptor (GR), a transcription factor that controls the expression of a large spectrum of genes. We investigated the implication of the GR during fetal life and studied its effect on Pdx-1 gene expression.

Beta cell mass was studied on mouse fetuses with specific deletions of the GR in pancreatic precursors (GR^{PdxCre} mice) or mature beta cells (GR^{RIPCre} mice) under different nutritional conditions. Binding of the GR to Pdx-1 promoter and genes regulated by GC were assessed in beta cell lines or mouse islets. We demonstrated that fetuses lacking the GR in pancreatic precursors show increased beta-cell fraction which is not affected by increased GC levels induced by maternal undernutrition. In contrast, a later recombination of GR gene, in differentiated ß-cells did not affect ß cell mass. Suggesting that GR acting at an early stage of ß-cell differentiation may influence ß-cell mass. This demonstrates that the absence of GR signaling in precursor cells protects the fetal beta-cell mass from the deleterious effect of undernutrition. In beta-cell lines and islets, dexamethasone treatment inhibits major beta-cell transcription factor expression levels. Protein and promoter activity levels were decreased for Pdx-1, a master gene for pancreas development and beta-cell function. Chromatin immunoprecipitation and deletion mutants indicate that GC effect implicates the binding of GR on area II, a DNA region of Pdx1 promoter, crucial for its activity. In contrast, dexamethasone treatment increased the expression of Ppargc1a, a transcriptional co-activator of the GR and its forced overexpression also downregulates Pdx-1

expression. Together, our results define the glucocorticoid receptor as a crucial regulator of beta-cell development and Pdx-1 expression and suggest Ppargc1a as a likely participant in these effects.
A protection against induced diabetes was expected in the mutant mice lacking the GR in the precursors cells due to the doubling of their beta-cell mass. On the contrary, metabolic and insulin secretion studies from isolated islets showed that these mice had impaired glucose tolerance and beta cells not responsive to glucose, stressing out that the presence of GR during early life in precursor cells determines a proper insulin secretion and response to glucose in adult life.

To understand the molecular mechanisms that underlye GR function in pancreatic islet cells, partners 23 and 24 used GR^{PdxCre} mutant mice to identify the GR-target genes by comparing the transcriptomes of mutant and control mice, in collaboration with Arndt Benecke. We identified a subset of 150 genes that are directly, or indirectly, controlled by GR transcription factor.

In order to mimic a situation of chronically elevated glucocorticoids levels, such as in the case of perinatal malnutrition, we developped animal models in which the Glucocorticoid Receptor (GR) gene is overexpressed in pancreatic precursor cells or mature beta cells. This was achieved in transgenic mice using the "Tetracycline system". This approach requires a transgenic line expressing a synthetic transactivator (tTA) in a precise cell population, and a transgenic line carrying the transgene of interest (GR in this case) under the control of a tTA-dependent promoter. In animals carrying both transgenes, the GR will be overexpressed in the desired cell populations. The presence of doxycycline in the drinking water will block tTA binding to DNA and will stop GR gene overexpression. There was no line expressing tTA in pancreatic precursors or mature ß-cells with sufficient efficiency. We established these two models using large DNA segments (Bacterial Artificial Chromosomes) encompassing Pdx1 gene and insulin-1 gene, respectively, to ensure correct expression of the tTA, in place of these two genes, either in pancreatic progenitor cells expressing the transcription factor *Pdx1*, or in mature beta cells expressing the *insulin-1* gene.
To establish these models, we first generated several transgenic lines carrying tTA transgenes and screened them using a reporter line that expresses the LacZ gene under the control of a tTA-dependant promoter. The spatio-temporal expression analysis of the LacZ reporter gene shows that in the BAC-Pdx-tTA mice, the transgene is expressed as early as embryonic (E) day 11.5 in the two pancreatic buds and at E13.5 and E15.5 in all pancreatic precursor cells. In the adult mouse pancreas, the transgene is expressed in mature beta, delta and PP cells and in some acinar cells. Transgene expression was also detected in the duodenum and stomach. In the BAC-Ins1-tTA mice, the transgene is expressed from E13.5 but is restricted to beta cells. Four weeks administration of Doxycycline, in drinking water of adult mice, suppressed tTA-activated transgene expression. Using BAC-Pdx-tTA mice, we also showed that transgene overexpression can be blocked from development to postnatal age, by doxycycline treatment, and can be induced upon doxycycline removal.
Using these animal models, we demonstrated that GR gene overexpression in precursor cells leads to decreased beta-cell fraction in the adult mice with no alteration of glucose tolerance. This is coherent with our starting hypothesis that GR overstimulation, during stress response, has a similar effect. This mirrors the situation of the mice carrying a specific deletion of the GR gene in the same pancreatic populations that display, indeed, a mirror phenotype with regards to beta-cell mass expansion. In contrast, GR overexpression in mature beta cells had no consequences on beta-cell fraction but impaired glucose tolerance due to insufficient insulin secretion.

In conclusion, we have generated transgenic mice in which the transgene is correctly targeted to precursor cells, mature beta and some acinar cells for Pdx-tTA mice and only to beta cells for Ins-tTA mice. The transgene overexpression can be controlled in time, thus providing new useful tools to overexpress genes in pancreatic cells during defined development periods. These mice are exclusive tools to control transgene expression in the pancreas.

Long-term changes of the neuroendocrine axis that controls glucocorticoid release, associated with behavioral changes, are conditioned by early life experiences exerted by environmental variations on the embryo or the young animal. In some situations, such as the increase of anxiety in adult animals that were submitted to maternal restraint stress during embryonal life, GCs seem to be directly involved since adrenalectomy have a protective effect. In other situations, such as maternal separations or variations in maternal cares, long-term HPA axis modifications are observed.
The aim of this third project was first to define the implication of the GR in the emotional behavior and second to dissociate the GCs young and adult effects on the emotional behavior. To specifically address the role of GR activation in forebrain neurones, we generated a genetic mouse model that overexpresses the GR in targeted tissues (FBhGR mice). We used the tetracyclin system tTA to overexpress in the forebrain the human GR gene. This model was obtained by crossing a tTA-dependant GR transgenic line previously developped by partner 23 with the CamKIItTa transgenic line. The transgene was largely expressed in the main forebrain structures (hippocampus, striatum, cortex and olfactive bulbs). The FBhGR mice are characteriezd by an increased state of anxiety in the open field test and in the dark light transition test and a reduced despair the second day of the forced swim test. The tTA system enables us to inhibit the transgene expression with a doxycycline treatment. We stopped transgene expression at the adult stage (6 weeks old) to dissociate the young (before 6 weeks) and adult GCs (after 6 weeks) effects on the emotional behavior. Our results show that the extinction of transgene expression makes disappearing the anxiety phenotype of the FBhGR mice but do not the depression one. These results point out the fact that the increased anxiety state of FBhGR mice is due to the adult hGR expression whereas their reduced depression state is due to the young hGR expression. We quantified the expression of BDNF which is one of the potential target gene of the GR. BDNF is implicated in the emotional and cognitive behavior and stress influences its expression. We found a decreased BDNF protein expression in the hippocampus of the FBhGR mice. The emotional phenotype of FBhGR mice could be due to changes in BDNF expression. In conclusion, an early overstimulation of GR transcription factor influences some behavioral responses in the adult rodent.