EFFECT OF SIMULTANEOUS VERSUS APART ADMINISTRATION OF DEXAMETHASONE AND ALPHAA-KETOGLUTARATE ON GROWTH HORMONE, CORTISOL AND INSULIN-LIKE GROWTH FACTOR-I IN PIGLETS

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Abstract

The aim of this study was to determine the influence of dexamethasone and α-ketoglutarate administered simultaneously and separately during both prenatal and postnatal life on the serum level of cortisol, growth hormone, and insulin-like growth factor-I (IGF-I) in 30-d-old piglets. The experimental procedure was conducted from the 91st d of pregnancy to the parturition. The sows were administered orally AKG at the dosage of 0.4 g/kg b. w./d (AKG group), injected i. m. with dexamethasone at the dosage of 3 mg/sow/48 h (Dex group), and administered AKG simultaneously with dexamethasone (Dex+AKG group), similarly to the other groups. Newborns were divided into groups according to their mothers treatment, to continue dexamethasone and AKG administration. The first group of neonatal piglets was administered orally AKG at the dosage of 0.4 g/kg b. w./d (AKG group) and the second group was injected i.m. with dexamethasone (Dex group) at the dosage of 0.5 mg/ kg b.w./48 h. The third group of piglets received dexamethasone simultaneously with AKG (Dex+AKG group), similarly to the other experimental piglets’ groups. Experiment lasted up to 30 d of piglets’ postnatal life. Piglets being under influence of dexamethasone during 24 last d of pregnancy and 30 d of their postnatal life showed final body weight lower by 38% compared with AKG treated piglets and by 35% compared with the Dex+AKG group. Moreover, the levels of growth hormone, IGF-I and cortisol were the lowest in the Dex group. There was significant correlation between body weight and IGF-I serum level (r = - 0.92). The concentrations of IGF-I and growth hormone were the highest in AKG group. Levels of GH and IGF-I were significantly correlated in AKG group (r = 0.7) and Dex+AKG (r = 0.64). Moreover, the body weight and IGF-I were positively and significantly correlated in AKG group (r = 0.67). Simultaneous administration of α-ketoglutarate with dexamethasone improved body weight gain and enhanced the cortisol serum level of piglets compared with animals treated with alone dexamethasone which significantly decreased cortisol serum level.

Key words: swine, dexamethasone, α-ketoglutarate, growth hormone, cortisol, insulin-like growth factor-I.

The development and metabolism in mammals depend on hormonal function with complex feedback and control mechanisms. The somatotropic axis, essentially including growth hormone (GH), insulin-like growth factor-I (IGF-I) and cortisol, their associated carrier proteins and receptors, plays a role in the regulation and control of physiological processes. The function of the hypothalamo-pituitary-adrenal axis depends on integration between genetic factors and endocrine system as well as nutritional components. Growth hormone, IGF-I and cortisol are involved in developmental processes not only during prenatal life but influence postnatal growth and reflect the adaptation to environmental conditions (2, 13, 34). Among these growth factors, cortisol determines foetal growth and development of essential organs as well as metabolism with lasting consequences for the offspring (25). Rapid brain growth linked to neuroendocrine development also occurs in late foetal life in animals and humans (12). In the pig, the major developmental increase in adrenocortical function occurs in late gestation, when endogenous glucocorticoid concentration in foetal blood is the highest. At this high level it lasts until the term what was observed in other species (5). Glucocorticoid concentration decreases in neonates and show only minimal changes around the time of weaning in pigs. These hormones are the key in the regulation of glucose homeostasis as well as in the development of metabolic pathways, stress and immune responses, neurobiology, and programmed cell death. In addition, corticosteroids are widely used drugs, primarily for their anti-inflammatory and immunosuppressive action. Dexamethasone is a synthetic glucocorticoid used for in
vitro and in vivo studies indicating its effects on a number of different cellular and physiological responses. Dexamethasone therapy may stimulate the somatotropic axis in foetuses around birth (2, 5). Glucocorticoids are very often used in serious problems concerning children with rheumatoid arthritis and other systemic diseases. Glucocorticoid therapy is needed for children and youths with asthma as well. The drugs are administered during pregnancy in woman in order to improve the lung morphology in premature foetus. Repeated prenatal exposure to corticosteroids causes short-term and long-term modification of neuroendocrine function and behaviour increasing in pituitary-adrenocortical responses to stress (26). However, there is still lack of information about the influence of both maternal and neonatal administration of glucocorticoids on GH, IGF-I, and cortisol in infants.

Alpha-ketoglutarate (AKG) is a precursor of glutamine and as a nutrient factor improves protein synthesis, plays important role in the maintenance of metabolic function and activity of the immune system. Its role in prenatal programming of hormonal activity is still unclear. Our earlier studies showed reverse influence of dexamethasone and AKG on piglets’ skeletal system when were administered during the last 24 d of their prenatal life, when their growth is very intensive, and after further administration during 14 d of neonatal time (28-30). AKG showed protective effect on the action of dexamethasone on bone mineral density, when both were administered simultaneously during prenatal or neonatal time (28-30). Their role in programming hormonal state of piglets at the end of weaning, a very stressful period, is unknown and not determined. Considering negative effects of glucocorticoids treatment, especially during prenatal and early neonatal development, when individual sensitivity to metabolic factors is very high, it is important to investigate factors diminishing or eliminating these disadvantages in the skeletal and hormonal systems. According to these earlier results it was aimed to investigate the effect of simultaneous versus apart administration of dexamethasone and AKG during the last 24 d of prenatal life and further administration during the whole time of nursing until 30 d of postnatal piglets’ life on their growth hormone, insulin-like growth factor-I, and cortisol level. The lack of information about the effects of long-term simultaneous treatment with dexamethasone and AKG on the hormonal state in piglets inspired us to perform this study.

**Material and Methods**

**Sows.** The experiment was carried out on 9 sows of Large Polish White breed. The sows were housed under standard rearing conditions (temperature and humidity) with free access to fresh water and were fed standard commercial diet for pregnant sows. The sows were fed at 7.00 a.m. and 3.00 p.m. The experimental procedure was conducted from the 91st d of pregnancy to the parturition. The time of parturition was calculated from the date of the mating. The sows were divided into 3 groups. The first group (dex group), being control for other groups, was injected with dexamethasone (dexamethasone sodium phosphate as Dexamethasone pro inj. 0.2%, Eurovet Animal Health, the Netherlands), whereas sows of the second group (AKG group) were treated per os with AKG (Table 1). Powdered AKG (Gramineer Int. AB, Sweden), with a purity of 99%, was mixed with saline and adjusted to final pH of 7.3 by the addition of NaOH. The third group received dexamethasone and AKG simultaneously (AKG+Dex group), similarly to the other groups (Table 1).

**Piglets.** Two newborn piglets just after their birth were chosen randomly from every sow and then were divided into 3 groups according to their mothers treatment to continue dexamethasone and AKG administration. Every group consisted of 3 male and 3 female piglets hold with sows and fed sow milk. The first group of piglets was injected i. m. with dexamethasone (Dex group); the second group was administered orally AKG (AKG group), and the third group received dexamethasone and AKG simultaneously (AKG + Dex group), similarly to the other experimental piglets’ groups (Table 1). The experiment on piglets lasted up to day 30 of their postnatal life. Total time of prenatal and postnatal experiment was 54 d.

**Table 1**
The prenatal and postnatal procedure with piglets

<table>
<thead>
<tr>
<th></th>
<th>Dex group</th>
<th>AKG group</th>
<th>Dex+AKG group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal time</td>
<td>the last 24 d of pregnancy</td>
<td>dexamethasone - 3 mg/sow/48 h</td>
<td>dexamethasone - 3 mg/sow/48 h + AKG - 0.4 g/kg b.w./d</td>
</tr>
<tr>
<td>Prenatal treatment</td>
<td>dexamethasone - 3 mg/sow/48 h</td>
<td>AKG - 0.4 g/kg b.w./d</td>
<td>AKG - 0.4 g/kg b.w./d</td>
</tr>
<tr>
<td>Postnatal time</td>
<td>1 – 30 d of postnatal life</td>
<td>dexamethasone - 0.5 mg/kg b.w./48 h</td>
<td>dexamethasone - 0.5 mg/kg b.w./48 h + AKG - 0.4 g/kg b.w./d</td>
</tr>
<tr>
<td>Postnatal treatment</td>
<td>dexamethasone - 0.5 mg/kg b.w./48 h</td>
<td>AKG - 0.4 g/kg b.w./d</td>
<td>AKG - 0.4 g/kg b.w./d</td>
</tr>
<tr>
<td>Total time of treatment</td>
<td>54 d</td>
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**Blood samples.** The blood samples were collected from 30 d old piglets in the morning. All the piglets were not fasted before blood collection. Blood samples were collected from the subclavian vein. After centrifugation (3 000 g for 15 min) the obtained serum samples were rapidly frozen at −25°C and kept at this temperature until the analysis of serum hormone level. Serum growth hormone, morning serum cortisol, and IGF-I were measured using the commercial ELISA kit. Kits for human serum cortisol (Cortisol Elisa Kit – IBL Gesellschaft für Immunchemie und Immunbiologie, Germany) and IGF-I (Human IGF-I Elisa Kit - R&D, USA) were validated for analysis of extracted pig serum. Validation of these assays was conducted by verifying parallelism of dilutions of serum samples with the standard curve. Since these kits were for human serum, we verified that pig serum was suitable matrix. The intra-assay coefficient of variation for cortisol was 5% and the inter-assay was 8%. The intra-assay coefficient of variation for IGF-I was 6.3% and the inter-assay was 6.8%. Cross-reactivity for cortisol and IGF-I was 72% and 78%, respectively. Porcine GH Elisa Kit (Diagnostic Systems Laboratories, Inc, USA) was used for analysis of growth hormone level.

**Statistical analysis.** All results are expressed as means ± SEM. Differences between means were tested for statistical significance with the use of one-way ANOVA and post hoc Duncan test with the aid of STATISTICA 6.0 software. Data were found to be normally distributed and have equal variance. The level of statistic significance was set at P ≤ 0.05 for all comparisons. The degree of linear relationship between two variables reflects Pearson’s correlation.

**Results**

Piglets treated with dexamethasone during the last 24 d of their prenatal life and then for 30 d after birth showed the lowest mean body weight for P = 0.002 versus AKG group and for P = 0.001 versus Dex+AKG, while those which received AKG and were born by sows treated with AKG had the highest mean body weight. Piglets from Dex+AKG group had lower mean body weight than those in the AKG group, but higher than in the Dex group (Table 2).

Growth hormone level was the highest in the AKG group (P = 0.01 versus Dex group; Table 1), lower in the Dex+AKG group and the lowest in piglets being under treatment with dexamethasone during prenatal and postnatal life. The morning serum cortisol level of piglets at 30 d of age in the Dex group was the lowest for P = 0.02 versus AKG group and for P = 0.03 versus Dex+AKG group, whereas the highest level was in AKG group (P = 0.03). The serum level of IGF-I was the highest in the AKG group, lower in the Dex+AKG group, and the lowest in the Dex group for P = 0.006 versus AKG group. The results of the serum parameters in piglets at 30 d of age are presented in Table 2 and the values of Pearson’s correlation between IGF-I and GH and between IGF-I and body weight are presented in Table 3.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Body weight and serum levels of hormones in piglets at 30 d of age treated with dexamethasone (Dex group) and α-ketoglutarate (AKG ) during prenatal and postnatal time, mean ± SEM values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dex group</td>
</tr>
<tr>
<td>Number of piglets</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>5244 ± 485&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GH (pg/ml)</td>
<td>2.18 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cortisol (µg/ml)</td>
<td>12.17 ± 0.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IGF-I (µg/ml)</td>
<td>211.5 ± 48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b</sup> - statistically significant differences (P ≤ 0.05) between groups.

<table>
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<tr>
<th>Table 3</th>
<th>The value of Pearson’s correlation between IGF-I and GH or body weight</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Dex group</td>
</tr>
<tr>
<td>Body weight &amp; IGF-I</td>
<td>-0.92</td>
</tr>
<tr>
<td>IGF-I &amp; GH</td>
<td>-0.43</td>
</tr>
</tbody>
</table>
Discussion

Environmental influences during prenatal or early postnatal life may programme developmental processes and influence the health of adult animals and humans. Growth retardation is a complication often associated with glucocorticoid therapy used in the treatment of pregnant women and children with chronic diseases (32). Dexamethasone affects through the glucocorticoid receptors located in the pituitary, hypothalamus and hippocampus (8). Synthetic glucocorticoids bind to those receptors and the development following prenatal glucocorticoid administration is mediated at the level of the brain, which induces alteration in hormonal state in adult offspring (15, 24). In the glucocorticoid treated animals, less weight gain was observed (3, 27-30). It is known that chronic long-term administration of dexamethasone, as a potent steroid, hinders the development of the whole organism (24). General catabolic effect of dexamethasone on the development was observed in neonatal rats and piglets (1-4, 17, 24). The highest decrease was in protein synthesis and increase in protein degradation in the intestine and lower effect was on skeletal muscles (1, 17). The influence of dexamethasone on other tissues, including skeletal system, was found out in our various studies (27-31). It decreased bone mineral density and bone mineral contents as well as the serum level of alkaline phosphatase. Newborn piglets treated with dexamethasone during their prenatal and neonatal life had shorter femora and humeri (25, 28). The inhibitory actions of dexamethasone on growth is mediated by somatomedin like IGF-I, the most known growth factor involved in the regulation of foetal growth and postnatal development (2, 13, 16, 18). Many studies in animals showed that dexamethasone treatment increased protein catabolism via decreasing the circulating IGF-I (2, 16, 25). IGFs are considered to be the primary regulators of postnatal somatic growth not only in pigs (13, 24). It is produced in multiple tissues, mainly in the liver, present in the circulation acts in endocrine and autocrine ways and for this reason is important for longitudinal growth and muscle mass (6, 7, 10, 13). The gut is one of the most sensitive IGF-I target organ. IGF-I is present within the submucosa of the newborn mouse ileum (20). The foetal small intestine undergoes rapid maturation during the latter third of gestation by increasing the activity of digestive enzymes, villus length, mucosal thickness, cell number, lumen diameter, and smooth muscle thickness (9). Dexamethasone exposure accelerates the maturation of the small intestine but reduces IGF-I localization within the lamina propria and extracellular space beneath the epithelia (9). Submucosal growth and thickness in the neonatal mouse ileum is mediated by IGF-I and diminished by dexamethasone treatment resulting in submucosal thinning (11). Glucocorticoids are known to reduce serum concentrations of IGF-I in human neonates and submucosal IGF-I in newborn mice (11). Growth hormone stimulates the expression of IGF-I and enhances its synthesis in the liver (9, 21, 33). There is a link between IGF-I concentration and growth of the body (3, 10, 24).

GH affects multiple functional and structural processes in numerous tissues and organs, especially observed in growing animals (13, 21). It influences the metabolism of carbohydrates, lipids, proteins and minerals (34). This hormone is a single factor in a complex described as the somatotropic axis playing a pivotal role in controlling metabolism. The major role of GH is regulation of skeletal growth by the proliferation and function of chondrocytes and osteoblasts as well as enhancing bone formation. It improved bone metabolism and linear growth in animals treated with glucocorticoids (6, 33). The present experiment showed that dexamethasone administered during few last weeks of prenatal life and 30 d of postnatal life reduced IGF-I and GH levels in the investigated piglets. However, the levels of GH and IGF-I significantly increased in piglets treated with AKG. This experiment demonstrated significantly positive correlation between the concentration of GH and IGF-I in AKG + Dex group (r = 0.64) and in AKG group (r = 0.7) as well as between the body weight and the concentration of IGF-I in AKG group (r = 0.67) and in Dex group (r = - 0.92). A tendency to higher values of serum IGF-I and GH in simultaneously treated piglets was observed. AKG might be protective on hormonal metabolism hindered by glucocorticoid therapy. Moreover, our earlier experiment proved anabolic effect of AKG administered during the last 3 weeks of pregnancy on body weight of newborn piglets. We observed increase in cortisol and IGF-I serum levels in these newborns investigated within the first hour after the birth as well as the mean body weight (30). AKG elevated GH serum level in piglets treated additionally with dexamethasone (30). The role of glutamine in protein metabolism is well known. This mechanism is mediated by glutamate and α-ketoglutarate which is stable in solution and because of that it can be used as dietary supplement (14, 19). Considering the results obtained, prenatal administration of AKG continued during 30 d of postnatal life to piglets increased their body weight. AKG is a factor which influences positively the growth of the whole organism. It is a precursor not only glutamine, but as well of other amino acids, such as proline, arginine and asparagine involved in the synthesis of protein. Moreover, the present experiment showed that AKG given together with dexamethasone improved weight gain of piglets which had higher mean body weight compared to piglets being under the influence of dexamethasone alone. Many hormones and growth factors are potential developmental regulators of prenatal and postnatal weight gain. Among them, cortisol is the main hormone which regulates development of the foetal essential organs such as lung, liver, and kidneys (3, 4, 22, 23). It is a dominant glucocorticoid in humans and swine synthesized from progesterone in the adrenal cortex, involved in stress adaptation. It elevates blood pressure and Na+ uptake, gives numerous effects to the immune system, and stimulates fat and carbohydrate metabolism for fast energy. The result of this action is stimulation of appetite and weight gain. Cortisol plays a major function
in glutamine degradation by extracting it from the liver and utilizing for energy and its secretion is controlled by classical negative feedback loops. Corticotropin (ACTH) released from the hypothalamus leads to biosynthesis of cortisol in the adrenals with pulsatory secretion. Typically, the highest level is in the morning and the lowest at night. In the present study the morning level of cortisol was significantly lower under the dexamethasone treatment compared with its simultaneous with AKG or apart administration. Our earlier experiment showed that prenatal administration of glucocorticoids increased cortisol level in newborns investigated just after their birth (31). Administration of exogenous glucocorticoid like dexamethasone during postnatal life inhibits the adrenal function which was observed in this experiment. The hormonal function has genetic as well as nutritional components, because the serum level of cortisol in piglets under simultaneous treatment with AKG and dexamethasone was higher than that in piglets treated with dexamethasone alone. The role of maternal nutrition in the causal pathway for long-term effects after delivery requires further considerations. The effects are depending on the nature, intensity, and duration of the factors as well as on the time of the pregnancy at which the factor is effective. It is worth to underline that the investigations of physiological and nutritional aspects in both humans and animals with the action and role of AKG in the whole metabolism, especially on hormonal level, are very attractive.

References


