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Evaluation of adrenocorticotropin regulated glucocorticoid synthesis pathway in adrenal of different breeds of pigs

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ABSTRACT

Variation in the hypothalamic-pituitary-adrenal (HPA) axis has been reported in numerous species and has been implicated in the differences of adrenal response to ACTH observed between different pig breeds; however, few studies have been conducted in indigenous Chinese pig breeds. The present study, we have investigated ACTH regulated adrenal glucocorticoid synthesis pathway in five breeds of pig: four indigenous Chinese pig breeds (Bama, Huanjiang, Lantang and Ningxiang) and an alien breed (Landrace) which were under same conditions. As a result, the ACTH concentrations were significantly greater than others in Lantang and significantly lesser than others in Bama, and they were also significantly lesser in Ningxiang than in Landrace. On the contrary, the cortisol concentrations in Lantang were significantly lesser than those in Bama. According to the opposite pattern on serum concentrations of ACTH and cortisol, Bama, Lantang and Landrace were chosen for further research. The protein contents of MC2R and StAR in adrenal were significantly lesser in Lantang than in Landrace, and the MC2R levels of Bama were also significantly greater than those of Lantang. The StAR protein levels in Bama were greater than those in Lantang as well, and this difference approached significance. Compared with Landrace, the number of mRNA transcripts for StAR, POMC and PC1 in Lantang were significantly lesser than in Landrace. These results indicate Lantang exhibited lower cortisol synthesis capacity under a relatively greater serum ACTH concentration, while Bama and Landrace exhibited just the opposite. These results at least reveals ACTH regulated glucocorticoid synthesis pathway in adrenal of different breeds of pigs, which can provided reference data for further breeding work.

1. Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is a neuroendocrine system that plays an important role in responses to stress. The activation of the HPA axis by stresses culminates in the synthesis and secretion of glucocorticoids (GCs), which are a class of steroid hormones (*e.g.*, cortisol in primates and corticosterone in rodents (Papadimitriou and Priftis, 2009)) and are the major stress hormones in animals. These hormones are secreted in the adrenal cortex under the regulation of adrenocorticotropin (ACTH) from the pituitary gland (Herman et al., 2012). The ACTH is a proopiomelanocortin (POMC)-derived peptide, and the posttranslational proteolytic processing of POMC in the pituitary gland is performed by the enzymes prohormone convertase 1 (PC1) and prohormone

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convertase 2 (PC2). The production and secretion of GCs from the adrenal cortex are regulated by ACTH via the type 2 melanocortin receptor (MC2R), which transduces the ACTH signal by stimulating the cAMP-PKA pathway to phosphorylate a cAMP response element binding protein (CREB) (Penhoat et al., 2001). In turn, this protein activates the steroidogenic acute regulatory protein (StAR) (Lehoux et al., 1998) and initiates steroidogenesis. The resulting cascade reactions of steroidogenesis involve a series of enzymes, such as the cholesterol side-chain cleavage cytochrome P450 (P450scc).

Theoretically, when serum ACTH concentrations are elevated, more cortisol will be synthesized and secreted. However, previous studies have shown that pigs with high concentrations of serum GCs did not all exhibit high serum ACTH concentrations. Désautés et al. (1997) demonstrated that, compared with Large White pigs, Meishan pigs exhibited greater basal and post-stress cortisol concentrations but not different basal ACTH concentrations; these results are consistent with the research of Li et al. (2008a), whose animal models were Erhualian and Pietrain pigs. However, the results of Mormède et al. (1984) showed that MS exhibited greater basal and post-stress cortisol concentrations as well as greater basal ACTH concentrations when compared with Western breeds. Désautés et al. (1999) hypothesized that the adrenal cortices of Meishan pigs were hyperresponsive to ACTH, while Li et al. (2008b) demonstrated that the increasted plasma cortisol concentrations of Erhualian pigs were caused by an enhanced cAMP/PKA/pCREB-signaling system and the augmented expression of StAR and steroidogenic enzymes.

Actually, there are dozens of indigenous pig breeds in China, including Bama (BM), Huanjiang (HJ), Lantang (LT), Ningxiang (NX), and so on. As mini-type breeds, BM and HJ are both from south China, while LT and NX are lard type breeds from south and central China, respectively. Most Chinese indigenous pig breeds are characterized by smaller body weight, lower growth rate, higher fat deposition, lesser stress response compared with Western pig breeds. And no previous research exists comparing the adrenal ACTH signaling pathways of other indigenous Chinese pig breeds to those of Meishan and Erhualian.

In the present study, samples were collected from pigs two weeks in age for three south Chinese pig breeds (BM, LT and HJ), a central Chinese pig breed (NX) and the alien pig breed Landrace (LR). Serum concentrations of ACTH and cortisol, along with the expression levels of the genes in the related pathways in the adrenal and pituitary gland, were measured to characterize the ACTH regulated glucocorticoid synthesis pathway in adrenal.

2. Materials and methods

The experimental procedures were approved by the Animal Care Committee of the South China Agricultural University (Guangzhou).

2.1. Animals

Three south Chinese pig breeds (BM, HJ and LT), a central Chinese pig breed (NX) and an alien pig breed (LR) were used in the present study. Twelve 14-day-old pigs (six males and six females, weighted with BM: 1.73 ± 0.09 kg, HJ: 1.74 ± 0.08 kg, LT: 2.30 ± 0.11 kg, NX: 1.62 ± 0.10 kg and LR: 4.80 ± 0.28 kg) were sampled from each breed.

2.2. Slaughter and tissue collection

Sixty pigs of different breeds were euthanized with an overdose intravenous injection of 10% sodium pentobarbital before sampling. Blood samples of jugular vein were collected in tubes and immediately placed on boxes with ice. After centrifugation at 3000*g* for 30 min at 4 °C, serum samples were collected and stored at −20 °C. Whole adrenal and pituitary samples were taken immediately and rapidly frozen in liquid nitrogen, then stored at −80 °C until analysis.

2.3. Radioimmunoassay for hormone

Serum concentrations of ACTH and cortisol were measured in duplicate using commercially available ^{125}I-RIA kits (Beijing North Institute of Biotechnology Technology, China) according to the manufacturer's guidelines. The intra and inter-assay coefficients of variation were $< 10\%$ and $< 15\%$ for ACTH and cortisol. The minimum detectable concentration of the kits were 2.5 pg/mL for the ACTH and 10 ng/mL for cortisol.

2.4. RNA extraction and cDNA synthesis

The adrenal and pituitary samples were ground in liquid nitrogen using a mortar and pestle. Total RNA was isolated from the adrenal or pituitary tissues using RNA-Solv® Reagent (Omega Bio-tek, Norcross, GA, USA) and treated with DNase I (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. To verify the integrity of the RNA, it was subjected to electrophoresis through a 1.5% agarose gel stained with 10 mg/mL ethidium bromide. The RNA had an OD_{260}:OD_{280} ratio between 1.8 and 2.0, as measured by a NanoDrop 2000 UV–vis spectrophotometer (Thermo, Wilmington, USA).

After the RNase-Free DNase treatment, 2 μg of total RNA was reverse-transcribed to complementary DNA (cDNA) using a mixture of Moloney Murine leukemia virus reverse transcriptase (M-MLV) (Promega, Madison, WI, USA), N10 (N=A, T, G, C) random primers (Sangon, Shanghai, China), M-MLV buffer, deoxynucleotide triphosphate mix (TaKaRa) and ribonuclease inhibitor (TaKaRa), incubated at 65 °C for 5 min, 37 °C for 60 min, and 80 °C for 5 min to end the reaction. The products remained frozen (−30 °C) until use.

2.5. Real-time RT-PCR Analyses

Real-time RT-PCR was performed with the Stratagene MxPro 3005P apparatus (Agilent Technologies, Santa Clara, CA, USA) using SYBR® Green Realtime PCR Master Mix (TOYOBO, Tokyo, Japan). The standard PCR protocol was as follows: (1) 1 denaturation cycle (95 °C, 60 s); (2) 35 amplification cycles (95 °C, 15 s; 58 °C, 15 s and 72 °C,