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Short Communication

Glucocorticoid receptor number and affinity differ between peripheral blood mononuclear cells and granulocytes in domestic pigs

L.C. Engert*, U. Weiler, V. Stefanski, S.S. Schmucker

Behavioral Physiology of Livestock, Institute of Animal Science, University of Hohenheim, Garbenstr. 17, 70599 Stuttgart, Germany

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ABSTRACT

The aim of the present study was to characterize the number and affinity of glucocorticoid receptors (GR) in peripheral blood mononuclear cells (PBMC) and granulocytes of domestic pigs because glucocorticoid signaling is considered important for animal health and welfare. To investigate GR binding characteristics in intact porcine immune cells, blood samples of 6 castrated male pigs were collected via indwelling vein catheters. Porcine PBMC and granulocytes were isolated using two-layer density gradients, followed by radioligand binding assays to determine the number of GR sites per cell and the dissociation constant K_d as a measure for GR binding affinity. The present study revealed a greater number of GR sites per cell ($P = 0.039$) in PBMC (mean ± SEM: 1,953 ± 207 sites/cell) compared to granulocytes (1,561 ± 159 sites/cell) in domestic pigs. Furthermore, porcine PBMC had a higher GR binding affinity than porcine granulocytes ($P = 0.003$) as the dissociation constant K_d of PBMC (1.8 ± 0.2 nM) was lower than that of granulocytes (3.5 ± 0.4 nM). Our results point to differences in underlying mechanisms of glucocorticoid signaling in different porcine leukocyte populations.

1. Introduction

Glucocorticoids are a central link between the neuroendocrine and the immune systems by influencing distribution and function of leukocytes [1,2]. Whereas their diurnal rhythmic release controls homeostasis of physiological functions on a daily basis, they are also known to be released in response to stressor exposure [3]. Especially when chronic, stress is associated with an impaired immune competence [4]. In modern pig husbandry, animals face many stressors, such as weaning, transportation, or repeated mixing of animal groups, and glucocorticoid signaling is therefore considered as important factor influencing animal health and welfare [5,6].

Glucocorticoids exert their immunomodulating effects via glucocorticoid receptors (GR) [1,7]. They act primarily through binding to cytosolic GR, which translocate into the nucleus after ligand binding and exert their influence on gene expression through binding as homodimers to glucocorticoid response elements (GRE) or via the interaction with other transcription factors [1]. Therefore, glucocorticoid signaling depends not only on available glucocorticoids but also on GR number and affinity. Differences in GR number or affinity might therefore be related to variations found in glucocorticoid responsiveness among different leukocyte types [2,7]. So far, only a few studies directly compare GR binding characteristics in different leukocyte populations [8–11]. These studies suggest differences in the GR number between peripheral blood mononuclear cells (PBMC) and granulocytes in humans [8,9]. It should be mentioned that PBMC consist of lymphocytes, monocytes, and a minor proportion of dendritic cells, whereas the total population of granulocytes

* Corresponding author. Tel.: +49 711 459 22536; fax: +49 711 459 22498.
E-mail address: Larissa.Engert@uni-hohenheim.de (L.C. Engert).

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consists of primarily neutrophils and a minor proportion of eosinophils. Thereby, different leukocyte types have specific functions, for example, neutrophils belong to the innate arm of the immune system and represent the first line of defense against pathogens, whereas T and B lymphocytes belong to the adaptive immunity with the capability of forming an immunological memory [12].

Until now, number and affinity of the GR have been characterized in various porcine tissues (eg, preadipocytes, liver, kidney, different brain regions) as well as in porcine PBMC [13–15]. However, assessment of GR binding characteristics in other populations of porcine leukocytes remains limited. Therefore, the aim of the present study was to characterize GR number and affinity in PBMC and granulocytes of domestic pigs. This was carried out using radioligand binding assays to analyze GR binding characteristics in intact immune cells. Thus, not only the number of GR sites per cell was assessed but also the functioning of the GR in terms of receptor-ligand affinity.

2. Materials and methods

2.1. Animals and sampling

All procedures were conducted according to the ethical and animal care guidelines and approved by the local authority for animal care and use (Regional Council Stuttgart, Germany; V309/13TH). Six castrated male pigs (Piétrain × German Landrace, 10–11 months old, BW range 157–172 kg) were included in the study and were housed individually in the experimental unit of the department in pens, which were cleaned and littered daily with dust-free wood shavings. The pigs had ad libitum access to hay and water and were fed concentrate twice daily. The pigs were surgically catheterized with indwelling vein catheters (*vena cava cranialis*) to enable blood collection without disturbing the animals as previously described [16]. To circumvent anesthesia-related effects on glucocorticoid signaling, surgery was conducted at least 2 wk before sampling [17,18]. Blood samples (60 mL per animal) were collected into lithium heparin tubes (Sarstedt, Nümbrecht, Germany) at 0800 h in the morning and processed immediately for the analysis of GR number and affinity. Preceding leukocyte isolation, plasma was separated by centrifuging an aliquot of 1 mL of each blood sample for 10 min at 1,000 × *g* at 4°C and stored at −80°C until cortisol analysis.

2.2. Isolation of PBMC and granulocytes

Using a two-layer density gradient, PBMC and granulocytes were isolated simultaneously from whole blood. The upper layer had a density of 1.077 g/mL (Biocoll separating solution, Biochrom, Berlin, Germany) and the lower layer was adjusted to a density of 1.097 g/mL by diluting 1.100 g/mL-Biocoll (Biochrom) with PBS (phosphate buffered saline, Biochrom). The heparinized blood (59 mL) was diluted 1:2 with PBS, carefully layered onto the gradient and centrifuged for 35 min at 500 × *g* at 20°C. Then, PBMC were collected from the top of the upper layer and granulocytes were collected from the top of the lower layer. Isolated cells were washed twice in PBS by centrifuging for 10 min at 300 × *g* at 20°C to remove endogenous cortisol molecules from the cell suspensions. Preceding flow cytometric experiments showed that this method leads to a cell purity of at least 95% for PBMC and granulocytes, respectively (data not shown). Finally, cells were resuspended in RPMI 1640 (Biochrom) without addition of any serum. Cell numbers were determined using a Z2 Coulter Counter (Beckman Coulter, Krefeld, Germany). Cell viability (determined by PI staining and flow cytometric analysis) was at least 95% and did not differ significantly between PBMC and granulocytes.

2.3. Glucocorticoid receptor binding assay

After leukocyte isolation, GR binding assays with intact cells were carried out according to Sartori et al [19] with modifications. In detail, to support dissociation of bound endogenous cortisol from GR, suspensions of PBMC and granulocytes were preincubated in 50-mL tubes at a concentration of 6.25×10^6 cells/mL for 90 min at pig-specific physiological conditions of 39°C and 5% CO_2 [19]. Binding assays were carried out in duplicate. After preincubation, the cells were transferred to 96-well flat-bottom plates (1×10^6 cells/well) and incubated in a final volume of 200 μL/well under continuous shaking for 60 min at 39°C and 5% CO_2 in the presence of 8 different concentrations (0.25, 0.5, 0.75, 1, 1.25, 2.5, 5, 10 nM) of [1,2,4,6,7-^{3}H]-dexamethasone (77 Ci/mmol, American Radiolabeled Chemicals, St. Louis, MO, USA) dissolved in PBS. Nonspecific binding was assessed at three different concentrations of [^{3}H]-dexamethasone (0.25, 2.5, 10 nM) with at least 200-fold excess of unlabeled dexamethasone (2 μM, Sigma-Aldrich, Munich, Germany) dissolved in RPMI 1640. Furthermore, blank controls were prepared without addition of dexamethasone. The incubation was slowed by placing the plates on ice. Free ligand was washed out by harvesting the cells with a cell harvester (Titertek instruments, Huntsville, AL, USA) with deionized water onto Whatman 934-AH glass microfiber filters (pore size 1.5 μm, Sigma-Aldrich). Filters were dried overnight at room temperature and then 3.6 mL Irga-Safe Plus (PerkinElmer, Groningen, The Netherlands) was added to each vial (Pico Prias Vial, PerkinElmer). Radioactivity was measured 24 h later using a Tri-Carb 2800 TR liquid scintillation analyzer (PerkinElmer). The amount of inserted [^{3}H]-dexamethasone in each assay was determined by measuring duplicate aliquots of each concentration level directly in Irga-Safe Plus. Each assay was corrected for the inserted amount of radioligand for each concentration level and the rate of radioactive decay. Background noise for each sample was removed by including the blank controls into analysis. Binding data were analyzed using GraphPad Prism 5 (version 5.04, GraphPad Software, La Jolla, CA, USA) with nonlinear regression to reach greater accuracy than with Scatchard plot analysis [20]. The results are characterized by the number of GR sites per cell and the dissociation constant K_d, which specifies the binding affinity of the GR toward the ligand [^{3}H]-dexamethasone (lower K_d signifies higher affinity). Intra-assay variability was 9.6% for the