General and Comparative Endocrinology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Research paper

Impairment of microvascular angiogenesis is associated with delay in prostatic development in rat offspring of maternal protein malnutrition

Ketlin T. Colombelli, Sérgio A.A. Santos, Ana C.L. Camargo, Flávia B. Constantino, Caroline N. Barquilha, Jaqueline C. Rinaldi, Sérgio L. Felisbino, Luis A. Justulin *

Department of Morphology, Institute of Biosciences, Sao Paulo State University, Botucatu, SP, Brazil

ARTICLE INFO

Article history:
Received 1 October 2016
Revised 13 December 2016
Accepted 28 December 2016
Available online xxxx

Keywords:
Maternal protein malnutrition
Prostate morphogenesis
Angiogenesis
VEGF signaling
AQP1
Insulin/IGF

ABSTRACT

Experimental data demonstrated the negative impact of maternal protein malnutrition (MPM) on rat prostate development, but the mechanism behind the impairment of prostate growth has not been well understood. Male Sprague Dawley rats, borned to dams fed a normal protein diet (CTR group, 17% protein diet), were compared with those borned from dams fed a low protein diet (6% protein diet) during gestation (GLP group) or gestation and lactation (GLLP). The ventral prostate lobes (VP) were removed at post-natal day (PND) 10 and 21, and analyzed via different methods. The main findings were low birth weight, a reduction in ano-genital distance (AGD, a testosterone-dependent parameter), and an impairment of prostate development. A delay in prostate morphogenesis was associated with a reduced testosterone levels and angiogenic process through downregulation of aquaporin-1 (AQP-1), insulin/IGF-1 axis and VEGF signaling pathway. Depletion of the microvascular network, which occurs in parallel to the impairment of proliferation and differentiation of the epithelial cells, affects the bidirectional flux between blood vessels impacting prostatic development. In conclusion, our data support the hypothesis that a reduction in microvascular angiogenesis, especially in the subepithelial compartment, is associated to the impairment of prostate morphogenesis in the offspring of MPM dams.

1. Introduction

Malnutrition is a global problem, affecting mainly developing countries. Among the population, pregnant women and newborns are the most affected by malnutrition, leading to increased risks of morbidity and mortality for newborn infants (Gao et al., 2012). The idea that maternal exposure to adverse conditions impacts offspring health has been defined as a developmental origin of adult disease, also called the "Barker hypothesis" (Barker et al., 1989). It states that poor conditions during intrauterine life can result in permanent changes in physiology and metabolism. Due to obviously ethical considerations, there are few data about the effects of fetal programming on human health. Thus, the use of animal models is an important research tool in an attempt to elucidate the relationship between intrauterine malnutrition and diseases in adult life. In this sense, one of the most studied models is dams fed a low protein diet (Cezar de Oliveira et al., 2016; Ozanne, 2001; Ozanne et al., 1999; Pinho et al., 2014; Rinaldi et al., 2013; Sene et al., 2013; Vega et al., 2016).

Although the effects of maternal protein malnutrition (MPM) have been first described with regard to the cardiorespiratory system in the last decade, experimental models have demonstrated that MPM also affects offspring reproductive parameters, such as decreased reproductive capacity in female and male rats (Guzmán et al., 2006, 2014) or impairment of sexual accessory gland development associated with reduction in circulating testosterone levels (Ramos et al., 2010; Rinaldi et al., 2013). Ramos et al. (2010) demonstrated a delay in dorsal prostate maturation in the offspring of rats fed a low protein diet during lactation. Other authors subsequently demonstrated impairment in prostate morphogenesis at post-natal day (PND) 1(Pinho et al., 2014) or maturation at PND30 and 120 in the offspring of dams fed a low protein diet during gestation (Rinaldi et al., 2013).

In addition to prostate androgenic dependence (Cunha et al., 1987), other factors such as insulin-like growth factors (IGFs), cytokines and adhesion molecules are also important to development, maturation and maintenance of glandular function (Timms et al., 1994; Prins and Putz, 2008). Another key factor is the nutritional support and oxygen supplied by blood vessels (Wong et al.,

* Corresponding author at: São Paulo State University (UNESP), Institute of Biosciences of Botucatu, SP 18618-689, Brazil.
E-mail address: justulin@ibb.unesp.br (L.A. Justulin).

http://dx.doi.org/10.1016/j.ygcen.2016.12.016
0016-6480/

2014). Vasculature is also responsible for delivery signaling molecules for prostate morphogenesis (Carmeliet, 2005). Development of the microvessel network and growth/elongation of the prostatic buds during glandular morphogenesis are closely related, both spatially and temporally (Wong et al., 2014). Among the regulatory genes involved in the angiogenesis process, the most studied is the vascular endothelial growth factor (VEGF) and its receptor VEGFR (Ferrara et al., 2003).

Although endothelial cells do not express androgen receptors (AR), studies have demonstrated the indirect involvement of androgens in the maintenance of the vascular structure and increased expression of angiogenic factors such as VEGF, VEGF-B, PLGF, and FGF-2, which act in a paracrine fashion, inducing endothelial proliferation (Prins et al., 1991). Considering the importance of nutritional and oxygen supplied by blood vessels for prostate morphogenesis, maturation and maintenance, we assessed whether maternal protein malnutrition impacts the process of angiogenesis in the ventral prostate of rat offspring. We demonstrated that, in parallel to the impairment of proliferation and differentiation of the epithelial cells, there is an intense reduction in prostate microvascular angiogenesis in offspring from MPM, especially in the subepithelial compartment, collaborating for the delay of ventral prostate morphogenesis.

2. Material and methods

2.1. Animals and experimental design

In our experiment, we used an established low protein diet (Pinho et al., 2014; Rinaldi et al., 2013; Sene et al., 2013), provided by PragSoluções (PragSoluções, SP, Brazil). All procedures described in this study were approved by the Biosciences Institute/UNESP Ethics Committee for Animal Experimentation (Protocol number 670). Adult female (90 days of age, n = 30) and male (90 days of age, n = 10) *Sprague Dawley* rats were obtained from the Central Stock breeder at the State University of Campinas (Campinas, SP, Brazil). The animals were maintained under controlled temperature conditions (22 to 25 °C), relative humidity (55%), and a 12 h photoperiod, with free access to water and chow.

Virgin female rats were mated overnight with established male breeders and after confirmation of mating; pregnant rats were housed individually in standard rat cages. They were fed an isocaloric and normosodic normal diet (17% protein) or low protein diet (6% protein) during gestation or during gestation and lactation (Table 1). To maximizing lactation performance, litters were reduced to eight pups at PND1: four males and four females (Fischbeck and Rasmussen, 1987; Rinaldi et al., 2013). Thus, the dams were divided into three experimental groups: Control (CTR), fed normal protein diet; Gestational low protein (GLP), fed low protein diet during gestation; and Gestational and lactational low protein (GLLP), fed low protein diet during gestation and lactation.

Maternal body weight was determined at the beginning of gestation (GD1) and at the end of the gestational period (GD21). The difference between body weight at GD1 and GD21 was used to demonstrate maternal bodyweight variation. Food intake of the dams and pups was measured throughout the experimental period. To calculate the relative food intake, the value of ingested food was divided by the bodyweight of the rats. The ano-genital distance (AGD) of male offspring was determined using a digital caliper (Digimess®, Brazil) on PND1, 10 and 21. On PND 10 and 21, male rats from the CTR, GLP and GLLP groups (n = 15/group) were weighed and then euthanized using sodium pentobarbital anesthesia (30 mg/kg, i.p.) followed by decapitation. Blood samples were collected from ruptured cervical vessels. At PND10 and PND21, the VP lobes were removed, weighed and processed as described below. Such as prostate development in rodents starts at the end of the gestational period and continues after birth, the ingestion of a low protein diet during pregnancy and lactation affects the period of intense prostate development (Prins and Putz, 2008).

Table 1
Composition of the control and low protein diets.

Ingredientes (g/kg)	Control diet 17% of protein	Low protein diet 6% of protein[#]
Cornstarch	397	480
Caseín (84%)	202	71.5
Dextrin (90–94%)	130.5	159
Sucrose	100	121
Soybean oil	70	70
Fiber	50	50
Mineral mix (AIN 93%)[*]	35	35[†]
Vitamin mix (AIN 93)[*]	10	10
L-Cystine	3	1
Choline bitartrate	2.5	2.5
Total energy (kcal g^{-1})	3.76	3.76

[#] The low protein diet was prepared by PragSoluções (PragSoluções, SP, Brazil). Diets were supplemented with L-Cystine as sulfur amino acid.
[*] Vitamin and mineral mixtures were formulated to meet the American Institute of Nutrition AIN-93G recommendation for rodent diets (Reeves et al., 1993).
[†] Potassium phosphate, monobasic, was added to the salt mix of this diet to maintain phosphorus at the levels found in the control casein diet (3 g/kg of diet) and the calcium:phosphorus ratio has been kept at 1.3 in both diets.

2.2. Hormone assay

For hormones measurement, blood samples (n = 6/group) from each animal were collected at the time of euthanasia. Serum was obtained after centrifugation (2400*g* for 20 min) and stored at −20 °C. The concentrations of testosterone (Abcam, ab178663, CA, UK) at PND10, IGF-1 (R&D, MG100, MA, USA) at PND10 and 21 and insulin (Millipore, EZRMI-13 k, MA, USA) at PND10 and 21 were determined by colorimetric method following the protocol of the manufacturers. The sensitivities of these assays were 0.06 pg/mL for testosterone, 0.1 ng/mL for insulin and 3.5 pg/mL for IGF-1. The intra-assay and inter-assay variations were <10% for testosterone, 3.6 and 9.2% for the insulin and 5.6% and 9.1% for IGF-1.

2.3. Histological procedure

Samples of VP from different experimental groups (n = 5/group) were fixed for 4 h in Methacarn (70% methanol + 20% chloroform + 10% acetic acid). The samples were then dehydrated in ethanol, diaphanized in xylene and embedded in Paraplast (Sigma Co, Saint Louis, MO). 5 µm sections were produced in rotative microtome, collected in silanized slides and stored until the time of use. The slides were stained with hematoxylin-eosin (HE) for morphological and stereological analyses. The sections were analyzed using a Leica DMLB 80 microscope connected to a Leica DC300FX camera. The digitalized images were analyzed using Leica Q-win software Version 3 for Windows.

The relative proportion of the VP components (epithelium, stroma and lumen) was determined by stereological analysis (Weibel et al., 1966). Random measurements were performed in 10 different fields (400X) and from five different individual prostatic lobe sections. The relative values were determined by counting the coincident points of the test grid and dividing them by the total number of points. The results were expressed as a percentage of each component and a proportion of the total area analyzed.

Please cite this article in press as: Colombelli, K.T., et al. Impairment of microvascular angiogenesis is associated with delay in prostatic development in rat offspring of maternal protein malnutrition. Gen. Comp. Endocrinol. (2017), http://dx.doi.org/10.1016/j.ygcen.2016.12.016