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Undernutrition during foetal to prepubertal life affects aquaporin 9 but not aquaporins 1 and 2 expression in the male genital tract of adult rats

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Abstract

Expression of aquaporin water channels (AQPs) in the male excurrent ducts, is of major importance for local water movements. To study the influence of pre- and postnatal undernutrition on AQP-expression in the adult male genital tract, 4 pregnant female rats were fed *ad libitum* (control group) and 4 with 33.5% of gestational feed requirements (underfed group). Feeding restriction of underfed group pups continued up to weaning (25 days of age), then all pups were fed *ad libitum* until slaughtered at 100 days of age. Epididymides were sampled and processed for aquaporin immunohistochemistry. Expression of AQP1 was similar either in the control and underfed groups of rats, strongly evidenced at the apical and lateral plasma membrane of the efferent ducts non-ciliated cells, in the smooth muscle cells surrounding epididymal duct and in blood vessel endothelium throughout the epididymis. AQP2-immunoreactivity was present in the corpus and cauda regions, strongly expressed in the principal cells of both groups of rats. In contrast, AQP9 expression was modified by early life undernourishment, as it was weakly evidenced at the microvilli in the principal cells and strongly diminished or completely lacked in the clear cells of the cauda, in underfed group epididymides. Since it is known that clear cells are involved in luminal fluid acidification, this function might be altered in adult animals, which were underfed during early life.

Keywords: AQP (aquaporin); Efferent ducts; Epididymis; Vas deferens; Foetal programming

1. Introduction

Although Robert A. McCance in 1962 [1] was the first to demonstrate, in animals, that early nutrition could also have lifetime "programming" effects, growing interest about the concept of foetal and developmental programming began to rise only twenty years ago after the epidemiological observations of David J. Barker and his co-workers, demonstrating in the human an inverse relation between weight in infancy and death from ischemic heart disease [2]. Since those observations, Barker developed a theory, now known as the "Barker hypothesis" [3], proposing that adverse events *in utero* induce compensatory responses in the foetus that reflect "developmental plasticity" during this critical period [4] and persist permanently. Each individual's phenotype, even if based on a specific genetic programme, is influenced by epigenetic and environmental factors. During development there are critical windows of vulnerability to suboptimal conditions when programming may permanently modify adult phenotype and disease susceptibility [5] and the altered phenotype will persist throughout a lifetime.

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Alterations in foetal developmental programming can direct adult health consequences by way of epigenetic factors acting in diversified ways [6]. Programming involves structural changes in different organs, such as altered cell number, imbalance in distribution of different cell types within the organ, and altered blood supply or receptor numbers. Compensatory efforts made after birth to reverse the consequences of programming may have their own unwanted consequences, thus carrying a price, as postnatal conditions can be different from those for which the foetus prepared. Effects of programming may pass across generations by mechanisms that do not necessarily involve structural gene changes. Programming often has different effects in males and females [5].

Maternal undernutrition and, under certain circumstances, overnutrition, before or during pregnancy or during early postnatal life can alter reproductive function of the offspring [7]. Developmental maturation of the hypothalamic-pituitary-gonadal axis has been shown to be susceptible to foetal programming, too [8,9]. It is suggested that single nutrients and/or metabolites are unlikely to have direct impacts on the pattern of development of the reproductive system and it is postulated that multiple endocrine and metabolic signals are involved. Early life nutritional influences modify many components of the hypothalamic-pituitary-gonadal system, such as gonadotrophins and GnRH secretion. The normal ontogeny of gonadal development and function can be disrupted by undernutrition or by the influence of endocrine-disrupting compounds either in a direct way or by an altered endocrine status. Hormones such as insulin, triiodothyronine (T_3) and leptin have the potential to directly mediate the effects of nutrition on the gonads or to influence programming of the developing brain of the foetus, and in particular the hypothalamus, with potential indirect consequences for gonadal development and function [9].

Aquaporins (AQPs) are channel proteins facilitating the rapid passive movement of water throughout the cell membranes [10]. Aquaporin water channels are found in a subset of epithelia with a 10- to 100-fold higher capacity for water permeation in comparison with those devoid of these proteins [11]. The presence of AQPs in the male genital tract, where significant movements of fluids and molecules useful to sperm maturation are known to take place [12], is therefore of primary importance [13]. Many AQPs are known to be present in the rat male genital tract (AQP1, AQP2, AQP5, AQP8) [14–18], also belonging to the aquaglyceroporin group (AQP3, AQP9, AQP10) [19,14–16], known to be permeable even to small solutes such as urea and glycerol. Since some AQP molecules are known to be sexual steroid-dependent [20,14,21–23], it is possible that their expression could be induced by androgens present during prenatal and perinatal development, either in the plasma or *in situ* in the epididymis [21]. Therefore it is of particular interest to study their expression in undernourished rats, in which affected testicular structure and reduced number of Sertoli cells have already been demonstrated [24]. Thus the aim of this research was to study if undernutrition during foetal to prepubertal life up to weaning affects aquaporin expression in the male genital tract of adult rats.

2. Materials and methods

2.1. Animals

Animal breeding and experimental procedures are described in detail in [24]. Briefly, twelve adult (5 month aged) virgin Sprague–Dawley rats (mean body weight 272.5 ± 28.74 g) housed at the 'Instituto de Investigaciones Biológicas Clemente Estable', Montevideo, Uruguay, were bred all with the same male (body weight 257.5 g) in groups of 1 male and 3 females until all became pregnant. Pregnant rats (gestation diagnosed by vaginal smears) were divided into two groups. Control group (CG, n = 4), kept in individual cages, were fed a standard rat diet (3 Cal/g, 21% protein, 10% fibre, 7% total minerals) *ad libitum*, during gestation and lactation (until 25 day post-partum). Litters were adjusted to eight pups per lactating dam. Another group of pregnant dams was underfed (UG, n = 7) by 33.5% of *ad libitum* intake of CG pregnant rats. Water was always offered *ad libitum* to dams. All the rats were weighed weekly. After parturition, dams were offered feed *ad libitum*, but litters in UG (which could not reach feed) were adjusted to 14 pups. All pups (from both CG and UG) were weighed daily until weaning. At 25 days of age pups were weaned and housed (4 animals per cage), fed *ad libitum* and weighed weekly until 100 days of age.

At 100 days of age, male pups (CG, n = 10; UG, n = 7) were weighed and euthanized with 25 mg Sodium thiopental i/p. Animal management and euthanasia procedures were approved by the Animal Welfare Committee at the Veterinary Faculty, University of Uruguay. Testes were promptly collected and the epididymides were gently dissected free. Both organs were weighed, then fragments of caput (including efferent ducts, EDs), corpus, cauda epididymis, and proximal vas deferens were dissected free from testis, and