Experimental approach for metabolic disorders as a tool to investigate impact of environment on human health

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Abstract
The epidemics of obesity and diabetes have occurred contemporaneously with increasing use and exposure to environmental endocrine disrupting chemicals. Metabolic disorders affect male reproductive potential due to low sperm count and quality, reduced sperm motility and suppression of testosterone production. In this respect our study aimed to evaluate testiscular cell populations and steroidogenic function in tandem with expression of cellular marker tACE (testiscular angiotensin converting enzyme) for germ cell development in experimental conditions of diabetes mellitus (DM) induced on day 1 (neonatally, NDM) or on day 10 (prepubertally, PDM) in rats; short and long high-fat diet (HFD) induced obesity in rats since puberty. Our data indicate that metabolic disorders (DM and HFD) affected macro-parameters (decreased gonado-somatic index, increased fat accumulation). Long-term obesity negatively influenced Leydig cell number and testosterone production. Expression of tACE in postmeiotic germ cells showed that prepubertal DM but not neonatal DM caused delay in the first spermatogenesis associated with suppressed Leydig cell development and steroidogenesis in adulthood. Our data indicate that metabolic syndrom involving obesity and diabetes exerts negative impact on male reproductive development and function and therefore environmental aspects of endocrine disorders should be considered as a risk factor for male reproductive health.
Keywords: Reproductive health, Endocrine disruptors, Metabolic disorders, Obesity, Diabetes Mellitus

1. Introduction

The epidemics of obesity and diabetes have occurred contemporaneously with increasing use of environmental chemicals, including chemicals that disrupt hormonal function (Legler , 2015). Metabolic syndrome is a global health problem in modern society and includes serious complications such as obesity, insulin resistance/diabetes, cardiovascular disease, neuropathies, hormonal imbalance, often associated with compromised hypothalamic-pituitary-gonadal axis (Khosravi, 2017). Early life represents the greatest window of vulnerability for developmental perturbations in physiology with long-term and potentially lifelong consequences (Legler, 2015). In this respect our study aimed to evaluate the environmental aspects of endocrine disorders occurred at an early stage with impact on male reproductive development and function in adulthood.

2. Materials and Methods

Diabetes mellitus (DM) was induced by single i.p. injection of streptozotocin (100 mg/kg) on day 1 (neonatally, NDM) or on day 10 (prepubertally, PDM). DM status was confirmed by blood glucose > 15 mmol/l on 2-3 days after injection. Serum levels of testosteron (T) were measured by RIA kits. Testes were sampled at day 50th, fixed in Bouin's fluid and embedded in paraffin. From day 21 male rats were fed with standard chow or with high fat diet (HFD). Animals were sacrificed 3 (short-term, sHFD) and 9 (long-term, lHFD) months after the start of their diets. Testicular testosteron levels were measured by ELISA kits.
Enumeration of Leydig cells was performed after immunostaining with rabbit polyclonal 3β-HSD antibody (1:200; Santa Cruz) for DM rats or goat polyclonal CYP-11 antibody (1:100; Santa Cruz). Testicular ACE (tACE) was visualized by ABC-HRP immunohistochemistry using a poly clonal antibody (1:500; Santa Cruz).

3. Results and Discussion

Hyperglicaemia was confirmed by significant elevation of blood glucose levels by 15% in adult NDM and by 35% in PDM rats (Table 1). In NDM both parameters were increased - body weight by 40% and testis weight by 15%. Nevertheless gonado-somatic index was decreased by 15%. PDM rats were with normal body weight but testis weight and gonado-somatic index were decreased

by 30%. (Table 1). Leydig cell number and T production were in normal range in adult NDM, whereas they were significantly decreased in PDM (Table 2).

Detailed immunohistochemical analysis in our previous studies revealed stage specific pattern of tACE expression in post meiotic germ cells in rat testis (Atanassova, 2016). In contrast to the control (Fig. 1A), in PDM spermatogenesis is not complete as there were no elongated spermatides in stages IV-VI, in early stages (I-VII) (Fig. 1B) and in some tubules there was complete absence at all the 14 stages of the cycle. The data indicate that PDM is a stronger risk factor for male reproduction than neonatally induced DM. Therefore, spermatogenesis is more vulnerable to hyperglycemia caused in the period of active proliferation as PDM is (4^{th}-12^{th} postnatal day) than after birth, when germ cells are still quiescent.

HFD status was confirmed by significant increase of percent of body fat in short- and long-HFD groups by 38% and 88%, respectively ($p < 0.01$). The values of epididymal and inguinal adipocyte diameter was significantly higher in sHFD animals (by 12% and 13%, $p < 0.05$), whereas in lHFD rats it was found for inguinal adipocyte only (by 12%, $p < 0.01$). Body weight was increased by 6.6% and 12.2% ($p < 0.05$) in sHFD and lHFD animals, respectively associated with decrease in relative testis weight in long HFD (22% reduction of gonado-somatic index, $p < 0.01$). In adult lHFD rats testicular T levels were significantly lower (by 31.5%, $p < 0.01$), whereas sHFD exposure had no significant effect compared to control. Further, we observed that long-term obesity reduced the number of Leydig cells (by 30.8%, $p < 0.01$), visualized by CYP-11 immunohistochemistry. The observed changes are associated with inhibited expression of key steroidogenic factors (StAR and Cyp11a1) in both obesity groups and with increased testicular levels of proinflammatory adipocytokine TNFa and the number of testicular macrophages in lHFD rats (Wagner, 2016).

Our results from HFD experimental model demonstrated that long-term negatively influenced the number of Leydig cells and testosterone production. Prepubertal DM but not neonatal DM suppressed Leydig cell development and steroidogenesis in adulthood. The tACE is a useful marker to evaluate the degree of suppression of post-meiotic germ cell differentiation in experimental conditions of metabolic disorders. In summary, we can conclude that that metabolic syndrom involving obesity and diabetes can influence testicular function by affecting germ cells and somatic cells (Sertoli cells in the seminiferous epithelium and Leydig cells in the interstitium). Therefore environmental aspects of endocrine disorders should be considered as a risk factor for male reproductive health.

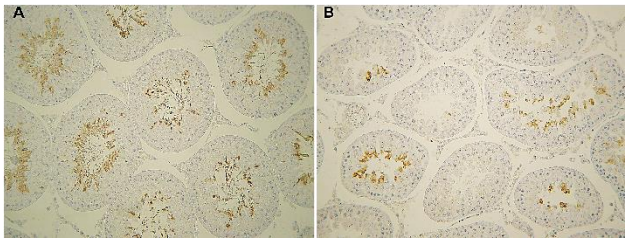

Fig. 1. Immunohistochemical staining of testicular ACE (brown) in testes from 50 day old rats - controls (A) and prepubertally induced diabetes – PDM (B). x 400.

Table 1. Macro-parameters and blood glucose of adult NDM and PDM rats. Data represent mean value ± SE.

	Body weight (g)	**Testis weight (g)**	**Gonado-somatic index**	**Blood glucose**
Control, n = 10	154 ± 6.5	0.88 ± 0.03	1.15 ± 0.03	8.32 ± 0.26
NDM, n = 18	211 ± 6.4 $p < 0.001$	1.0 ± 0.02 $p < 0.01$	0.96 ± 0.03 $p < 0.001$	9.37 ± 0.35 $p < 0.05$
PDM, n = 8	158 ± 3.3 not significant	0.64 ± 0.04 $p < 0.01$	0.81 ± 0.05 $p < 0.001$	11.21 ± 0.66 $p < 0.05$

Table 2. Leydig cell number and plasma T levels in adult NDM and PDM rats. Data represent mean value ± SE.

	Leydig cell absolute nuclear volume (mg)	**Plasma Testosterone(ng/ml)**
Control, n = 5	4.20 ± 0.33	2.18 ± 0.41
NDM, n = 5	3.99 ± 0.70 not significant	1.885 ± 0.64 not significant
PDM, n = 5	2.53 ± 0.31; $p < 0.01$	0.15 ± 0.03; $p < 0.01$

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