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SUPPLEMENT – PROCEEDINGS OF THE 8TH NATIONAL CONFERENCE WITH INTERNATIONAL PARTICIPATION "MORPHOLOGICAL DAYS"

Impact of Diabetes Mellitus Induced in Early Postnatal Life on Bax Protein Expression in Rat Testes

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The aim of our study was to investigate the impact of experimentally induced hyperglycemia on Bax expression in the testes of pubertal and mature rats. Diabetes mellitus (DM) was induced by single i.p. streptozotocin injection at dose of 100 mg/kg b.w. on day 1 (neonatally, NDM) or day 10 (prepubertally, PDM). Treated animals were sacrificed on day 18 or day 50 and testes and blood were sampled. Serum glucose was evaluated. Testis lysates were used to determine expression of pro-apoptotic Bax protein by SDS-PAGE followed by Western blot. Our results revealed prominent expression of pro-apoptotic Bax protein with most intensive reaction in adult PDM testes. Experimentally induced DM in early life increased pro-apoptotic Bax expression in pubertal and mature rat testes that is probably involved in mechanisms underlying reproductive disorders in diabetic males.

Key words: Diabetes mellitus, streptozotocin, testis, apoptosis, Bax

Introduction

Diabetes mellitus (DM) can lead to male infertility via action at multiple levels including altered sperm count and quality, degeneration and apoptosis of germ cells,

impaired glucose metabolism in Sertoli cells, compromised testosterone production and secretion [6, 8]. Investigation of apoptotic protein expression in conditions of hyperglycemia would contribute to elucidate the mechanisms underlying reproductive disorders in diabetic males. Bax is a multidomain, pro-apoptotic member of the Bcl-2 family that is required for normal spermatogenesis in different mammalian species. The ratio of Bax/Bcl-2 family members is a critical determinant of cell fate: elevated Bcl-2 favors extended survival of cells whereas increasing levels of Bax expression accelerates cell death [2]. Bax is found in all mouse and human testicular cell types [1]. In this regard the aim of our study was to investigate the Bax protein expression in pubertal and mature rat testes after experimentally induced hyperglycemia in early postnatal life of rats.

Materials and Methods

Diabetes mellitus was induced by single intraperitoneal injection of streptozotocin at dose of 100 mg/kg body weight on day 1, to induce neonatal DM (NDM) or on day 10, to induce prepubertal DM (PDM) in male rat pups. Animals were sacrificed at day 18 (early puberty) or day 50 (maturity) and testes and blood were sampled. Serum glucose was evaluated. Testis lysates were used to detect changes in expression of the pro-apoptotic Bax protein (1:1000) by SDS-PAGE followed by Western blot. As internal reference control α-smooth muscle actin (1:5000) was used. The experiment was carried out in accordance with guidelines EU Directive 2010/63/EU for animal experiments.

Results

Diabetic status of rats was validated by elevation of serum glucose in both ages. More pronounced increase was detected in prepubertal diabetic rats than in neonatal DM. On day 18 glucose was 75% and 6% higher than control, respectively. On day 50 fasting glucose was 69% and 26% higher than control, in PDM and NDM respectively. In early puberty Western blotting revealed increased but not consistent reaction of antibody against pro-apoptotic Bax protein. In the control group Bax expression was not detected. In most of the NDM and PDM samples bands on the membrane correspond to 21 kDa Bax protein and protein levels in both diabetic groups increased compared to control. On day 50 Bax expression in NDM and PDM was increased compared to controls but reaction in PDM testes was more intensive than in NDM lysates (**Fig. 1**). In both diabetic groups on day 50 Bax reaction was stronger compared to early pubertal diabetic testes.

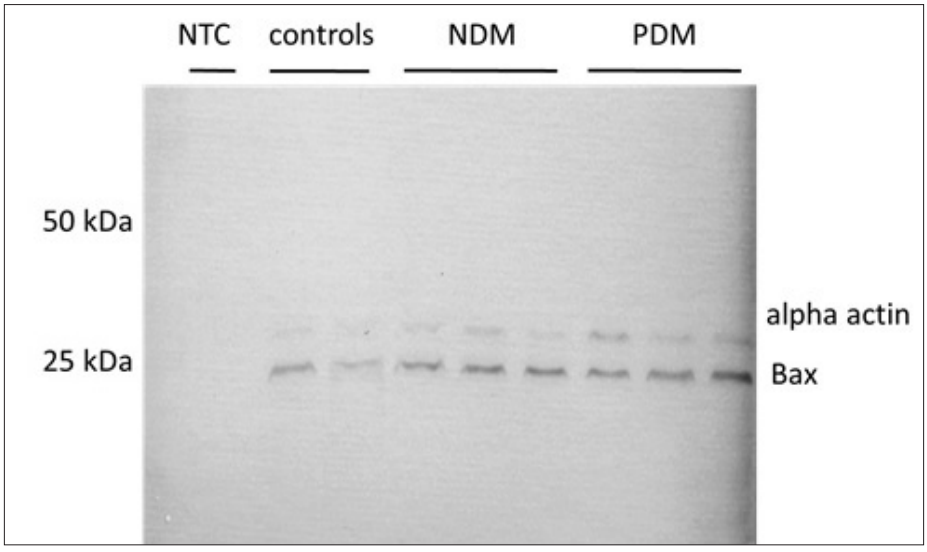

Fig. 1. Western blot expression of Bax in adult (day 50) control and diabetic testis homogenates. NDM – Neonatally induced diabetes mellitus; PDM – Prepubertally induced diabetes mellitus; NTC – negative control; α-smooth muscle actin as internal reference control.

Discussion

The high proliferative capacity of the seminiferous epithelium is accompanied by spontaneous apoptosis during development. Germ cell apoptosis can be induced under stress caused by radiation, hormonal imbalance, temperature and chemical toxicants [6]. Glucose metabolism is an important factor for normal proceeding of spermatogenesis. In this study we provide new data that high glucose levels at adulthood due to DM induced in early life, caused elevated testicular expression of proapoptotic Bax protein in 50 day old rats, more pronounced in PDM than in NDM animals. Koh et al. and Zha et al. [3, 7] reported increased apoptosis (phosphorylation of JNK, elevated Bax expression and TUNEL positive cells, decreased Bcl-2 expression) in diabetic condition induced in adult animals. The results from current study correspond to our previous data for significant reduction of serum testosterone levels and gonado-somatic index (testis weight to body weight ratio) in mature rats [4]. According to Zha et al. [7] hyperglycaemia induced in adulthood is associated by lower testosterone production. At day 18 we did not find any increase in testosterone levels (unpublished data) in both PDM and NDM animals that could explain why increase in testicular Bax protein expression is not consistent at that age. It is well known that elevated germ cell apoptosis can be responsible for reduced germ cell number that in turn is directly related to lower testis weight. Summarizing our observations it seems that prepubertally induced diabetes significantly increased glucose levels in mature animals that is associated with reduced testosterone levels and elevated germ cell apoptosis (evidenced by increased Bax expression) in the testis.

Conclusions

Our results indicate that Diabetes mellitus exerts more adverse impact on the testis if induced at the beginning of puberty manifested by increased expression of pro-apoptotic Bax protein in pubertal and adult rat testes.

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