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Determinations of mRNA's For TGFβ$_{2, 3}$ in Mouse Testes and Ovaries After a Five days Injection With Glycine or a Serine and Threonine Mixture

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Abstract: This study is presented to test the effect of injecting a mixture of Serine/Threonine or Glycine amino acids to determine and measure the changes in mRNAs expression for TGFβ$_{2, 3}$ within the mouse testes and the ovaries. Male (n = 30) and female (n = 30) mice (CD-1 strain) were injected, once a day for 5 days, with 0.2 mL Saline (control), 0.2 mL Saline with a mixture of Serine/Threonine (test) containing 0.26 μg L Threonine, 0.13 μg L Serine or 38 ng Glycine (Test). Total RNA was extracted and transferred on to a nylon membrane then probed with ^{32}P-lebelled cDNA probes. The expression of mRNA for TGFβ$_2$ increased in male mice while there were no differences in female mice. This study shows that TGFβ$_{2, 3}$ are expressed both in the ovary and testis and their expressions were increased by the injection of Serine/Threonine or glycine.

Key words: Mice, RNA, TGFβ, serine, threonine, glycine

INTRODUCTION

Transforming Growth Factor-β (TGF-β) like peptides are produced in both male and female gonads (Avallet *et al*., 1997; Mamluk *et al*., 1998). TGF-β$_2$ and β$_3$ play autocrine and a paracrine role in the regulation of both ovary and testis function (Gautier *et al*., 1997). They exert their effect using a variety of possible mechanisms including direct mitotic action, modulation of steroidogenesis, signal transduction and gonadotrophin receptor induction (Erickson and Danforth, 1995).

Serine and Threonine amino acids are subsituted within the intracellular proteins and phosphorylated by protein kinases. Many cellular functions such as transcription, translation, ion transport, cell re-modelling, mitotic activity and the cell cycle are regulated by phosphorylation of Serine/Threonine in target proteins (Alberts *et al*., 1994; Lodish *et al*., 1995). Unfortunately, no work has been conducted to confirm the effect of serine and threonine on the synthesis of TGF-βs. But, some works have been carried out on arginine. In a study, the effect of L-Arginine on the expression of TGFβ1 mRNA was examined by Narita *et al*. (1995) in rat. According to their results, decreasing the quantity of L-Arginine in diet resulted in decreased expression of TGFβ1 mRNA expression and TGFβ1 protein. The ffect of arginine on mRNA expression for TGF β$_{2,3}$ heve not been reported. Therefore, the aim of this research is to show the presence of TFG-β$_{2, 3}$ in mice gonads and to measure the effect of injecting a mixture of Serine/Threonine or Glycine on the expression of mRNAs for these peptides.

MATERIALS AND METHODS

Animals, injections, isolation of total RNA and quantification: Male (n = 30) and female (n = 30) , 21 days old CD-1 strain mice, were daily injected (intra-peritonealy) with 0.2 mL Saline (control) or Saline containing 0.26 μg L- Threonine and 0.13 μg L-Serine (test) or 38 ng Glycine (test) for 5 days. Following the last injection the mice were killed then ovaries and testes were removed. Total RNA was extructed and quantified by using a RNA/DNA spectrophotometer (Mod; 80-2103-98, Ser; 66884, Pharmacia Biotech. Cambridge, England).

Preparation of cDNA probes by RT-PCR: From the total RNA, cDNA was synthesized by reverse transcription (Avantage RT-PCR kit, Cat; K1402-1, Colontech, Palo Alto, USA). Then an aliquot of that cDNA was used for PCR amplification.It was carried out by using specific primers for TGFβ$_2$ and β$_3$. Primers for TGFβ$_2$ and β$_3$ provided by Dr G. Zaman, Royal Veterinary College, Molecular Biology Unit, Royal Collage St. London).

Northern blotting

Making up the gel (1.2%) and electrophoresis: Agarose (4.8g) was dissolved in 10X MOPS. In a fume cupboard, 20 mL formaldehyde was added to the gel at 60°C and mixed; it was then poured into an electrophoresis tank then and left until the gel set. When the gel set solid, the gel-running buffer was added to cover the gel. This was prepared 2h before loading the samples. Fifteen microgram of total RNA from each experimental group w as placed in

0.2 mL nuclease free micro tubes (Cat; AB-0337, Advanced Biotechnologies) and the volume made up to 15 μL with sample buffer. The tubes, containing RNA plus sample buffer, were placed in a water bath at 61°C for 5 min and immediately placed on ice. Fifteen micro liter was loaded into the wells of the gel and run at 65 V for 5 h at room temperature.

Transfer of total RNA to menbrane, labelling of probes and hiyridization: Total RNA was transfered to nylon membrane (Hybond-N+, cat; I6435, Amersham, Buckinghamshire, England) by upward transfer for 18 h. After the transfer, the membrane was sandwiched between Whatman filter paper and left at room temperature for 10 min to dry. After 10 min, the membrane was placed in an oven at 80°C for 2h to fix the RNA. To label the probes, 15 ng of a DNA probe (for labelling) was dissolved in 16.27μL of distilled and filtered water, denatured at 100°C for 5 min and then chilled on ice. The tube was mixed and then centrifuged to concentrate the contents at the bottom. The tube placed in a water bath at 37°C, the water bath was then switched off and left overnight for labelling. Hybridisation performed using a commercial kit (Cat; RPN, 1600Y, Amersham. Buckinghamshire, England).

Quantification: Quantification was done by using molecular analyst software (Copyright 1992-1995, Bio-Rad Laboratories, Hercules, USA).

RESULTS AND DISCUSSION

The injection of Serine/Threonine or Glycine did not influce mRNA expression for TGF-β_2 in the female but its expression was increased in the male. In female, none of the mRNA species for TGF-β_2 were detected after the injection of glycine. In male mice, mRNA species of 6.2, 6.0, 5 and 4.0 kb for TGF-β_2 were increased after the injection of Serine/Threonine or Glycine injection (Fig. 1). The expression of mRNA species of 4.5 and 3.8 kb for TGF-β3 were increased by by Serine/Threonine injection in female mice. In male mRNA species of 3.8 and 2.4 kb were increased by the injection of Serine/Threonine. Injection of Glycine to male mice caused increases in mRNA species of 7.5 and 9.5 kb for TGF-β_3 (Fig. 2).

TGF-β superfamily of growth factor initiates diverse cellular responses by bindig to cell surface receptors that have SerineThreonine kinase activity. When the ligand bids to its receptors, the phosphorylation of receptor regulated smad proteins on serine residues is stimulated (Dijke and Hill, 2004). Some other intra-cellular proteins

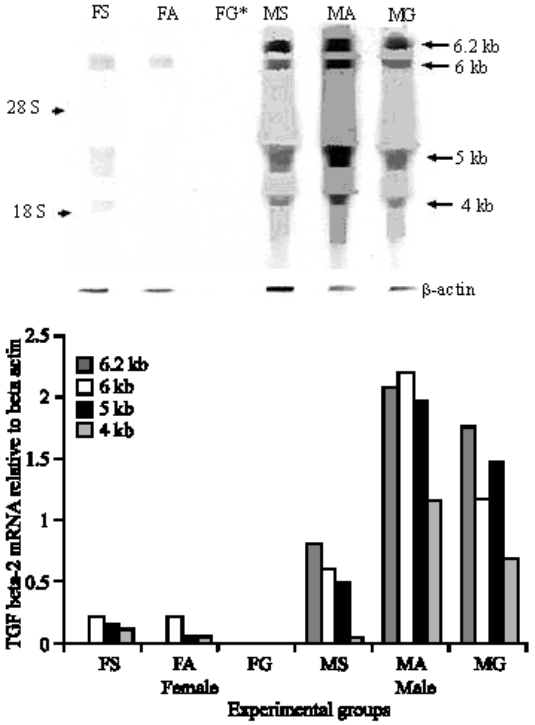

Fig. 1: Four different, 6.2, 6, 5 and 4 kb of mRNA species for TGF-$\beta_2$were detected in mice tests and ovary. The expression of mRNA for TGF-β_2 was not effected by Serin/Threonine (A) and Glycine (G) in female mice (F) whereas four different mRNA species was increated in male by Serne/Threonine (A) and glycine injection

that cause gene expression and hormone secretion are phosphorylated on Serine and Threonine residues, examples are the cAMP-Responsive Element Binding proteins (CREB) and the cAMP-Responsive Enhancer Element Modulator (CREM) (Potchinsky *et al*., 1997). TGF-β2 mRNA has been shown to be variably expressed in ovarian theca and granulosa cells from pig, rat, human and hamster (Muhleron and Scomberg, 1990; May *et al*., 1995). Three isoform of TGFβ mRNA expressed in immature rat testes and their expressions decrease as pubertal development progress (Mullaney and Skinner, 1993).

In this study 4 different TGF-β2 mRNA transcript of 6.2, 5.8, 5.0 and 4.0 were detected. The simillar transcipts of mRNA for TGF-β2 were obtained from mouse epithelial cells after a 24h treatment with 5 ng mL^{-1} of TGF-β2 or TGF-β3 (O'Reilly *et al*., 1992). Therefore the gene of interest is the same. The expression of mRNA for TGF-β3 in mouse testes and ovary has not been documented. In this study, 5 different species (2.4, 3.8, 4.5, 7.5 and 9.5 kb) of mRNA for TGF-β3 have been detected.

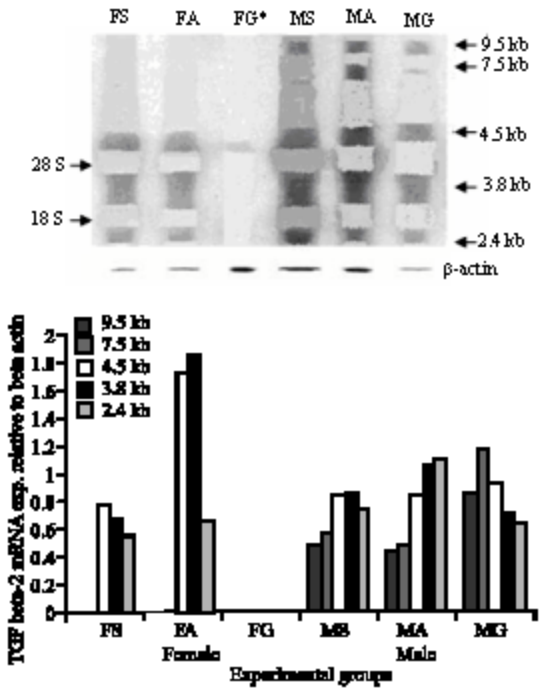

Fig. 2: Five different mRNA species were detected in mouse tests and ovaries after the injection. The injection of Serin/Threoninecaused an increase in 4.5 and 3.8 kb mRNA species of mRNA expression for TGF-β_2 in female and male mice. Increated of glycine increased mRNA species of 7.5 and 9.5 kb in male mice

It has been reported that restriction of diatery arginine resulted with a decrease in expression of mRNA for TGF-β1 (Narita *et al*., 1995). In another experiment, Arg-Gly-Asp-Ser containing protein (RGDS) added into the human mesengial cell culture and it has been reported that increased mRNA for TGF-β1 through the activation of integrin linked kinase which is a type of Serine and Threonine kinase. This enzyme directly phoshorilates proteins in cytosol namely protein kinase B, glycine synthase kinase 3 on serine residues (Velazquez *et al*., 2003).

Increase in expression of TGF-β influces gametogenesis within the gonads. Transforming Growth Factor-β can stimulate preantral follicle growth. In adult mice, TGF-β caused a significant increase in follicular growth and inhibin production in a dose dependent manner (Liu *et al*., 1999). It has been reported that TGF-β2 and 3 decreased testosterone production and the number of spermatids in the testes of adult mice (Ohta *et al*., 1996).

This experiment shows that TGF- β2 and β3 are expressed in mouse testes and in mouse ovaries and their expression is effected by Serine/Threonine or glycine injection. The reasons for these increases might be just a nutritional positive effect of these amino acids or a positive indirect effect of Serine/Threonine and glycine injection on protein kinase C activation and consequent increase in mRNA expression of TGF-beta.

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