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A study of male reproductive form and function in a rat model

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ABSTRACT

Objective: This investigation was undertaken in the hope of delineating the effects of four different treatments on male reproductive biology in a rat model. The effect of cryptorchidism, gonadectomy, pharmacological ablation of Leydig cell function and androgen-treatment was examined; these four treatments illustrate four different factors influencing and controlling male sexual function in a reproducible animal model. **Methods:** Total body weight, androgen concentration, gonad weight and secondary sex organ weights were measured for the four-abovementioned treatment groups and correlated to function. **Results:** It was demonstrated that total body weight decreased for all treatment options. The testicular-derived testosterone concentration for EDS-treated and castrated rats was determined to be zero, although adrenal production continues. Testis weight was shown to decrease following testosterone administration as a consequence of feedback inhibition. Both the prostate and seminal vesicles are highly androgen-sensitive, and as such both experienced an increase in weight following testosterone administration. **Conclusions:** This experimental setup was successful in elucidating the physiological mechanisms involved in male reproductive function. Body weight, the weights of the testes and accessory sex organs and androgen levels were intrinsically related to basal androgen levels.

1. Introduction

This study investigated the effects of four treatments on male reproductive function in a rat model. The effect of cryptorchidism, gonadectomy (castration) and pharmacological ablation of Leydig cell function, as well as an examination of the effects of physiological (androgen) agents, was delineated experimentally. Body weight (g), testosterone concentration (ng/mL), testis weight (g), epididymis weight (g), vas deferens weight (g), seminal vesicle weight (g) and prostate weight (g) were measured for a control group, a castrated group, an ethane dimethane sulphonate (EDS)-treated group, a cryptorchid group and a testosterone-treated group.

The prostate is dependent on a continuous supply of androgens[1]. Castration of an adult male rat will lead to extensive tissue regression in association with apoptosis in the majority of ventral prostate epithelial cells[2]. This study will examine the androgen dependence of both the prostate and the seminal vesicles. Conversely, testosterone administration increases total serum androgen concentration, leading to the hypertrophy of the prostate and seminal vesicles[3].

Cryptorchidism results in a progressive decrease in testicular weight and the concomitant loss of germ cells associated with increasing plasma levels of FSH and LH [4]. These findings indicate that cryptorchidism disrupts the secretion of inhibin and interferes with general Sertoli cell function[5]. On the other hand, EDS has been shown to selectively obliterate Leydig cells, thereby decreasing androgen production in adult rats[6]. Others have proposed that EDS, which is a cytotoxic agent, leads to the degeneration and

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spontaneous apoptosis of spermatogenic cells, especially spermatocytes and spermatids[7].

The aim of the experiment was to elucidate the physiological mechanisms involved in male reproductive function. As a result, the experiment attempted to quantitatively assess the characteristics of the male gonads and accessory sex organs, as well as total body weight and androgen levels in relation to four different treatments. It is hypothesised that total body weight will decrease for all treatment options; that the testosterone concentration for the castrated groups will be effectively zero; that the testosterone concentration for the cryptorchid groups will be lower than the control because of the higher core temperature in the abdomen; that the testicular-derived testosterone concentration for EDS-treated rats will also be effectively zero; that testis weight will decrease following testosterone administration because of interference of the feedback mechanism of the hypothalamic-pituitary-testicular axis; that the prostate and seminal vesicles, as strongly androgen-dependent organs, will experience an increase in total weight following testosterone administration; and that the prostate and seminal vesicles will both decrease in size and weight following castration and EDS administration.

2. Materials and methods

2.1. Animals

A rat model was used in the experimental procedure. Male Sprague-Dawley rats were used in all treatment groups. The rats were specifically bred for research purposes (following ethics approval through the Department of Physiology, Monash University) and were kept in pens in a controlled environment. In the pens, the rats were supplied adequate sustenance.

2.2. Experimental design

The experimental procedures attempted to clarify the dependence and interaction of the two functional regions of the testis; the androgen-producing Leydig cells and the sperm-producing Sertoli cells. The experiment consisted of five groups of rats. There were four treatment group rats and one control rat. There were 18 groups, designated A through to R. Each group received four treated rats and one control rat. In total there were 72 treated rats and 18 control rats. The groups were:

1. Control rats.
2. Those rats that have undergone sham surgery and been made cryptorchid.
3. Those rats that have undergone surgical castration.
4. Those rats that have been given a single injection of ethane dimethane sulphonate (EDS)
5. Those rats that have received a number of testosterone injections.

Data from the four treatment groups and the control for each group, A though to R, was collected and analysed. Mean values for body weight (g) for the five groups was determined, as was the standard deviation for that particular group. The average testosterone concentration (ng/mL) and the standard deviation for the four treatment groups and the control for each group, A though to R, was then determined. Two-tailed *t*-tests for the different groups were then calculated signifying the level of significance between the variables. If the calculated *t* value is above the chosen threshold for statistical significance, i.e., it gives a *p* value > 0.05, the null hypothesis stating that the two treatment groups are not different is rejected in preference for an alternative hypothesis, which usually states that the groups do differ. If the calculated *t* value is below the threshold for statistical significance, i.e., it gives a *P* value < 0.05, the hypothesis is supported and a significant difference exists between the groups.

2.3. Treatments

1. Control rats: These rats did not undergo any treatment and should be considered a normal rat population.
2. Cryptorchid rats: These rats were made cryptorchid before the beginning of the academic year at the university because it takes approximately three weeks for the effects of cryptorchidism to be fully demonstrable in the rat model.
3. Castrated rats: These rats were castrated before the beginning of the academic year.
4. The EDS-treated rats were given a single intraperitoneal injection of dose 75 mg/kg EDS one week before they were examined.
5. The testosterone-treated rats were injected with a dose of 5 000 mcg/kg testosterone propionate subcutaneously. The first of four doses was given two days apart.

2.4. Analysis

Total body weight for the five groups (control, castrated, EDS-treated, cryptorchid and testosterone-treated) was first determined following cardiac puncture under anaesthesia. Testosterone concentration (ng/mL) for the five groups was determined via a competitive protein-binding assay (radioimmunoassay) of collected plasma samples. Using this technique, the antibody is immobilised to the wall of the polypropylene tube. Radioactively labelled testosterone (using a radioactive isotope of iodine) competes

with testosterone in the plasma sample for antibody sites. By isolating the antibody-bound fraction of the labelled androgen, the concentration of testosterone in each sample was determined from the resulting standard curve.

3. Results

Results of the *t*-tests for the control compared to the four treatment groups – Control *vs.* castrated: 9.72118E-10; control *vs.* EDS-treated: 3.94633E-11; control *vs.* cryptorchid: 9.34938E-07; control *vs.* testosterone-treated: 3.09966E-05. For the control *vs.* castrated groups, there was an increased significant difference between the numbers. For the control *vs.* EDS-treated, there was an increased significant difference between the groups. For the control *vs.* cryptorchid there was an increased significant difference, but it was less than the castrated and EDS-treated groups. For the control *vs.* testosterone-treated group, there was a decreased significant difference.

It can be seen from Figure 1 that the mean body weight (g) for the four treatment groups is lower than the control. The castrated and EDS-treated groups are both lower than the cryptorchid and testosterone-treated groups.

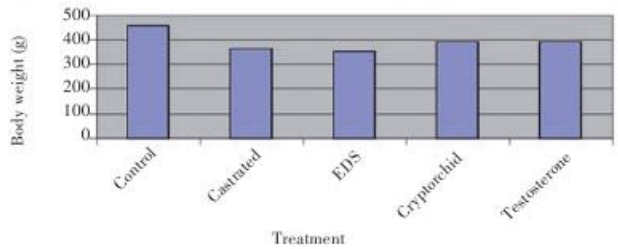

Figure 1. Body weight *vs.* treatment.

It can be seen from Figure 2 that the mean testosterone concentration (ng/mL) for the four treatment groups shows significant variability. The castrated and EDS-treated groups have a mean testosterone concentration (ng/mL) of zero. The mean testosterone concentration (ng/mL) of the cryptorchid group is lower than the control, whereas the mean concentration for the testosterone-treated group is higher than the control concentration.

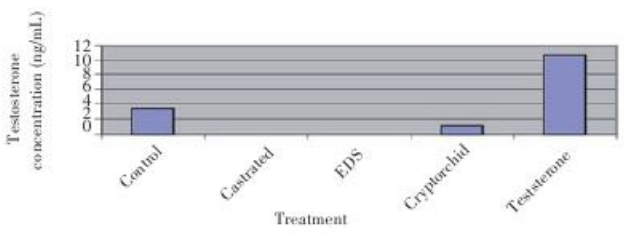

Figure 2. Testosterone concentration (ng/mL) *vs.* treatment.

It can be seen from Figure 3 that the mean testis weight (g) for the castrated group is zero. Obviously there were no testes in these animals. The other three groups all showed a decrease in mean testis weight (g). However, the decrease for the testosterone-treated group was less significant than the EDS-treated and cryptorchid groups.

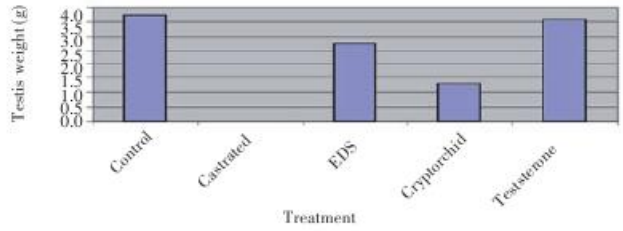

Figure 3. Testis weight *vs.* treatment.

It can be seen from Fig 4 that the mean epididymis weight (g) for the castrated group is zero. There should be no epididymides in these animals. The other three groups all showed a decrease in mean epididymis weight (g). However, the decrease for the testosterone-treated group was less significant than the EDS-treated and cryptorchid groups.

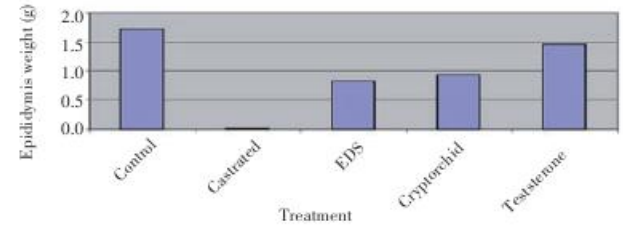

Figure 4. Epididymis weight *vs.* treatment.

It can be seen from Figure 5 that the mean vas deferens weight (g) for the castrated group is significantly lower than the other groups. The EDS-treated group is also lower than the control. The mean vas deferens weight (g) for the testosterone-treated group is the same as the control. On the other hand, the cryptorchid group displays an above average vas deferens weight (g).

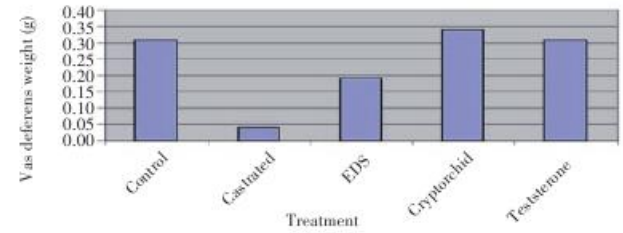

Figure 5. Vas deferens weight *vs.* treatment.

It can be seen from Figure 6 that the mean seminal vesicle weight (g) for the castrated group is significantly lower than the other groups. The EDS-treated and cryptorchid groups also display a below average seminal vesicle weight (g). On the other hand, the mean seminal vesicle weight (g) is above the mean for the testosterone-treated group.

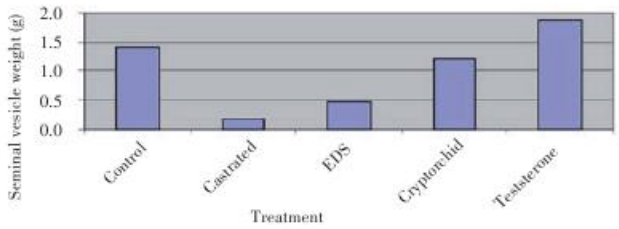

Figure 6. seminal vesicle weight vs. treatment.

It can be seen from Figure 7 that the mean prostate weight (g) for the castrated and EDS-treated groups is significantly lower than the other groups The cryptorchid group also displays a below average prostate weight (g). On the other hand, the mean prostate weight (g) is above the mean for the testosterone-treated group.

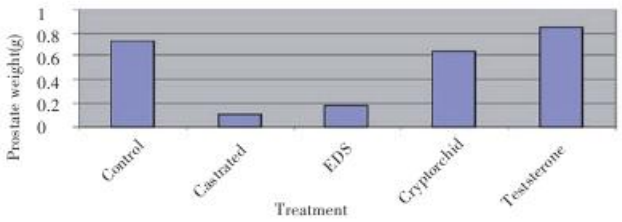

Figure 7. Prostate weight vs. treatment.

4. Discussion

The aim of this experiment was to elucidate the physiological mechanisms involved in male reproductive function. Total body weight did decrease for all treatment options as seen in Figure 1. This result supports the findings of other groups and indicates that total body weight is dependent on a dynamic interplay between hormonal factors[8,9]. The testosterone concentration for the castrated groups was zero as seen in Figure 2. Testicular-derived androgens have been removed from the system, and for the purposes of this experiment, can be considered zero. Nevertheless, the concentration of total androgens in the blood is a small non-zero value because the zona reticularis of the adrenal cortex remains an ongoing source of weak androgens[10].

The testosterone concentration for the cryptorchid groups was lower than the control as seen in Figure 2. Cryptorchidism, in which there is failure of the testes to descend from the abdomen to the scrotum, does not provide an environment conducive to spermatogenesis[11,12]. The cooler conditions for optimal spermatogenesis are provided by testicular descent in early mammalian life, and this is seen across species. This condition exposes the testes to the higher body temperature, inhibiting normal spermatogenesis; indeed, other groups provide a pathophysiologic mechanism and maintain that the anatomical location of the cryptorchid testis is unfavorable for spermatogenesis owing to the gradual degeneration of the germinal epithelium of the intra-testicular space[12,13].

Cryptorchidism results in a progressive decrease in testicular weight and, as others have elucidated, the concomitant loss of germ cells associated with increasing plasma levels of FSH and LH; a consequence of the normal feedback loop[4]. These findings indicate that cryptorchidism disrupts the secretion of inhibin and interferes with general Sertoli cell function[13]. No sperm were found upon microscopic examination, further supporting the conclusions of the Courtens and Ploen group[4]. In the control animals, motile sperm were found in the tail of the epididymis as expected, and this sperm was deemed to be viable in form and function.

The testicular-derived testosterone concentration for EDS-treated rats was effectively zero. In the EDS treated rats the Leydig cells have been destroyed[10-13]. As a result of this obliteration, there is no testosterone produced by the testes as seen in Figure 2. No sperm was found upon microscopic examination, indicating the significance of testosterone in initiating and maintaining spermatogenesis. The fertility of the EDS treated rats will be affected, as testosterone is needed for the production of sperm by the germinal cells. The libido of the rats will be affected as testosterone regulates sexual drive. The effect of the EDS treatment was seen within a week (five to six days). One group propose that EDS, which is a cytotoxic agent, leads to the degeneration and spontaneous apoptosis of spermatogenic cells, especially spermatocytes and spermatids, and the evidence supports this claim[7,14,15]

Testis weight did decrease following testosterone administration as see in Figure 3. However, the decrease wasn't as significant as expected. The time for the effects of exogenous testosterone to become apparent in testis weight (g) is longer than this experiment could provide. Interference of the feedback mechanism of the hypothalamic-pituitary-testicular axis decreases testis weight. This system consists of three components; the hypothalamus, the anterior pituitary and the testis. If exogenous androgens are provided, the Leydig cells no longer produce testosterone

upon LH and FSH stimulation; the testes subsequently atrophy[10]. As a consequence, very few sperm were found in the epididymides of these rats.

It was found that the prostate and seminal vesicles, as strongly androgen-dependent organs, both experienced an increase in total weight following testosterone administration as seen in Figure 6 and 7. The prostate and seminal vesicles will both decrease in size and weight following castration and EDS administration. The prostate and seminal vesicles are dependent upon a continuous supply of androgens[1-4]. Castration of an adult male rat will lead to extensive tissue regression in association with apoptosis in the majority of ventral prostate epithelial cells. According to the Hayek group, acute vasoconstriction is a significant early event associated with prostate regression in the rat. It appears likely that this process occurs in response to castration and the concomitant removal of androgens from the circulation, thus contributing to the regression of the tissue in vivo. Conversely, testosterone administration increases total androgen concentration in the blood, leading to the hypertrophy of the prostate and seminal vesicles[8].

This experimental setup was successful in elucidating the physiological mechanisms involved in male reproductive function. Body weight, the weights of the testes and accessory sex organs and androgen levels were compared for four different treatments. Minor procedural faults such the loss of tissue prior to weighing did not markedly hinder the experiment. However, future studies delineating testis atrophy over a longer time period following testosterone administration would be beneficial, as would studies investigating Leydig cell regeneration following EDS administration.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

[1] Banerjee PP, Banerjee S, Dorsey R, Zurkin BR, Brown TR. Age- and lobe-specific responses of the brown Norway rat prostate to androgen. *Biol Reprod* 1994; **51**: 675-684.

[2] Colombel M, Buttyan R. Hormonal control of apoptosis: the rat ventral prostate gland as a model system. *Methods Cell Biol* 1995; **46**: 369-385.

[3] McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de Kretser DM, Pratis K, et al. Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys and man. *Recent Prog Horm Res* 2002; **57**: 149-179.

[4] Courtens JL, Ploen L. Improvement of spermatogenesis in adult cryptorchid rat testis by intratesticular infusion of lactate. *Biol Reprod* 1999; **61**: 154-161.

[5] Teerds KJ, de Rooij DG, Rommerts FFG, Wensing CJG. The regulation of the proliferation and the differentiation of rat Leydig cell precursors after EDS administration or daily hCG treatment. *J Androl* 1988; **9**: 343-351.

[6] Morris ID, Philips DM, Bardin CW. Ethylene dimethane sulfonate destroys Leydig cells in the rat testis. *Endocrinology* 1986; **118**: 709-719.

[7] Sumathi R, Sriraman V, Kurkalli BS, Rommerts FFG, Jagannadha Rao A. Ethane dimethane sulphonate selectively destroys Leydig cells in the adult bonnet monkeys (Macaca radiata). *Asian J Androl* 1999; **1**: 115-120.

[8] Hayek OR, Shabeigh A, Kaplan SA, Kiss AJ, Chen MW, Burchardt T, et al. Castration induces acute vasoconstriction of blood vessels in the rat prostate concomitant with a reduction of prostatic nitric oxide synthase activity. *J Urol* 1999; **162**: 1527-1531.

[9] Baksalska M, Atanassova N, Angelova P, Koeva I, Nikolov B, Davidoff M. Degeneration and restoration of spermatogenesis in relation to the changes in Leydig cell population following ethane dimethane sulphonate treatment in adult rats. *Endoc Regul* 2001; **35**: 209-215.

[10]Hardy MP, Kelce WR., Klinefelter GR, Ewing LL. Differentiation of Leydig cell precursors in vitro: a role for androgen. *Endocrinology* 1990; **127**: 488-490.

[11]Kerr JB, Donachie K, Rommerts FFG. Selective destruction and regeneration of rat Leydig cells *in vivo*. *Cell Tissue Res* 1985; **242**: 145-156.

[12]Kerr JB, Bartlett JMS, Donachie K, Sharpe RM. Origin of regenerating Leydig cells in the testis of the adult rat. An ultrastructural, morphometric and hormonal assay study. *Cell Tissue Res* 1987; **249**: 367-377.

[13]Risbridger GP, Kerr JB, de Kretser DM. Influence of the cryptorchid testis on the regeneration of rat Leydig cells after administration of ethane dimethane sulphonate. *J Endocrinol* 1987; **112**: 197-204.

[14]Risbridger GP, Robertson DM, Drummond AE, Hedger MP, Clements JA, Li H, et al. Interactions between the seminiferous tubules and Leydig cells of the testis. *Reprod Fert Develop* 1990; **2**: 219-223.

[15]Teerds KJ, Closset J, Rommerts FFG, de Rooij DG, Stocco DM, Colenbrander B, et al. Effects of pure FSH and LH preparations on the number and function of Leydig cells in immature hypophysectomized rats. *J Endocrinol* 1989; **120**: 97-106.