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Potential impact of urocortin I on sperm count, motility and sex hormone profiles in normal adult rats

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Urocortin I (Ucn I), Ucn II and Ucn III are corticotropin-releasing factor (CRF) - like peptides. Ucn I has a high affinity for CRF_1 and CRF_2 receptors. The function of Ucn in male reproduction has not yet been elucidated. Ucn I is present in rat testis with lower levels of Ucn II and III mRNA gene expressions. Therefore, it is suggested that Ucn I may play a role in the regulation of male reproductive action. This study is designed to find out the endocrine and reproductive effects of Ucn I in normal adult male rats through investigation of exogenous Ucn I effects on epididymal sperm count, sperm motility, testicular weight and sex hormone profiles. 24 normal adult male albino rats of 175 to 200 gm initial body weight were implemented in this study. Randomly, the rats were subdivided into four equal groups. Group (I): Vehicle-treated group in which control rats received 0.1 mL of normal saline for 42 days as Ucn I-treated groups. Rats in group II were divided into three equal subgroups. Group (IIA), (IIB) and (IIC): Ucn I-treated groups in which rats were given daily intraperitoneal injections of rat Ucn I at doses of 5, 10 and 20 µg/kg body weight respectively for 42 days. Rats were weighed. Serum testosterone and levels of Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) were measured, in addition to epididymal sperm count, sperm motility and testicular weight. Administration of Ucn I in dose dependant manner induced significant reducing effects upon body weight, testes weight, epididymal sperm count, sperm motility and serum testosterone levels of normal adult rats. Ucn I may possibly affect male infertility by induction of a significant decrease in epididymal sperm count, motility and serum testosterone levels that might be due to a direct effect of Ucn I on testicular tissue and the germ cells without direct involvement of hypothalamic–pituitary–gonadal axis.

Key words: Urocortin I, epididymal sperm count, sperm motility, testosterone, male rat.

INTRODUCTION

Urocortin (Ucn) I, II and III and corticotropin releasing hormone (CRH) are peptide hormones that belong to the

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corticotropin releasing hormone family of peptides. Ucn I has a high affinity for CRF_1 and CRF_2 receptors. However, Ucn II and Ucn III show selective CRF_2 affinity (Hsu and Hsueh, 2001, Karteris et al., 2004).

Ucn I, II and III have been detected in the central nervous system (Bittencourt et al., 1999) as well as in many peripheral tissues (Kageyama et al., 1999; Lewis et al., 2001, Oki and Sasano, 2004). Ucns have roles in diverse physiologic processes such as regulation of the cardiovascular, gastrointestinal, reproductive, immune functions, body weight, food intake, and behavioral responses to stressors (Rodriguez et al., 1983, Fekete and Zorrilla, 2007).

Ucn I is composed of 40 amino acids with 45% sequence identity to CRF and 63% sequence identity to fish urotensin. It was first isolated from rat midbrain (Vaughan et al., 1995; Zhao et al., 1998). The major site of brain Ucn I synthesis is the Edinger-Westphal nucleus (Bittencourt et al., 1999).

The reproductive function of Ucns in male is still unclear. However, both Ucn mRNA and peptide were expressed in mature spermatozoa. Moreover, the expressions of CRF_1 and CRF_2 receptors in spermatocytes and spermatogonia respectively suggested that Ucns may play a role in the germ cell division, differentiation and spermatogenesis (Tao et al., 2007; Rivier, 2008).

Ucn I gene is located in rat testis with lower detectable levels of Ucn II and III mRNA gene expressions indicating the predominance of Ucn I signals. Ucn I gene expression appeared restricted to interstitial Leydig cells (Lee et al., 2011). Hence, the testis is a main target for Ucn I interactions. The prostate is a main source of local Ucn I. The secreted Ucn in the seminal fluid could activate the myometrial contractility in the female genital tract. So, it is suggested that Ucn I may participate in the physiology of fertilization, pregnancy and parturition (Petraglia et al., 1999; Yutaka and Hironobu., 2004, Lee et al., 2011).

Therefore, this study was designed to find out the endocrinal and reproductive function of Ucn I in male through evaluating the effects of exogenous Ucn I on epididymal sperm count, motility and sex hormone profiles in normal adult rats.

MATERIALS AND METHODS

24 adult albino male rats weighing 175 - 200 gm were obtained from the animal house of Faculty of Veterinary Medicine- Zagazig University. They were put under well controlled light and temperature conditions before experiments in the physiology animal house in Faculty of Medicine - Zagazig University. Animals were not manipulated except for feeding or cleaning of houses and were allowed to eat and drink ad libitum. Animals were handled with principles for the care and use of research as adopted by the National Institutes of Health and the approval from Animal Ethic Committee of the institution (Egypt). After one week of acclimatization, the rats were randomly and equally divided into four groups. Group (I): Vehicle-treated group in which control rats received 0.1 mL of normal saline for 42 days. Rats in group II were divided into three equal subgroups. Group (IIA), (IIB) and (IIC): Ucn I-treated groups in which rats were given daily intraperitoneal injections of rat Ucn I at doses of 5, 10 and 20 µg / kg body weight respectively for 42 days (Haron et al., 2010). Rat Ucn I (1 mg, powder form, Sigma Chemical Co., St. Louis, USA) was dissolved with 1% acetate solution and frozen at -80°C until use (Kihara et al., 2001). All injections were performed intraperitoneally in a volume of 0.1 ml/rat.

Samples collection

24 h after the last injection of Ucn I, blood samples were taken from retro-orbital venous plexus. Serum was separated by centrifugation of blood at 3000 rpm for 20 minutes and kept deep frozen at (-20°C). Serum Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) and testosterone levels were measured. Under mild ether anesthesia, the animals were sacrificed by cervical dislocation. Testes were weighed and epididymis was used for the evaluation of sperm parameters. Repeated freezing and thawing of samples were avoided.

Hormonal assays

Serum FSH levels were measured using FSH enzyme immunoassay test kits (BioCheck, CA 94404) according to the method described by Rebar et al. (1982). Both serum LH levels and testosterone levels were measured using LH enzyme immunoassay test kits and testosterone enzyme immunoassay test kits respectively (BioCheck, CA 94404) (Tietz, 1995). All are measured by spectrophotometer (spectronic 3000 Array, Germany) at 450 nm.

Sperm count and motility analysis

For each rat, the right epididymis was dissected, removed and minced in 2 ml of Hank's buffer salt solution (HBSS) (Sigma-Aldrich Co.-USA) at 37°C (Idris et al., 2012). The cauda epididymis sperm was determined using the standard hemocytometer method after 5 min incubation at 37°C, then the epididymal fluid was drawn up to the 0.5 mark of pipette White Blood Cell (WBC) and the semen diluting fluid (sodium bicarbonate 5 g, formalin 1 ml, distilled water 99.0 ml) was drawn up to '11' mark, and subsequently mixed well. One drop was kept in haemocytometer chamber in humid place for 1 h. Then, under the light microscope, the number of spermatozoa in the appropriate haemocytometer squares was counted according to the formula; sperm count =No. of spermatozoa counted x dilution factor x volume factor/ No. of areas counted (Belsey et al., 1980), the sperm concentration refers to the number of spermatozoa / ml fluid was gauged.

Using the number of live sperm cells over the total number of sperm cells (either motile which displayed some movement or non-motile that not move at all), the sperm motility percentage was calculated (Khaki et al., 2009).

Statistical analysis

The statistical analysis of results was done by using Statistical Package for Social Science (SPSS) program, version 18, for windows XP professional. The biochemical data were expressed as Mean ± SD. Statistical analysis were performed using one way analysis of variance (ANOVA) followed by post-hoc multiple comparisons (Tukey test). P value < 0.05 was considered

Table 1. Serum LH, FSH, T levels in Ucn I-treated rats and controls after a duration of 42 days.

Variable	FSH (µUL/mL)	LH (µUL/mL)	T (ng/mL)
Group I (Control rats)	0.43±0.04	0.41±0.07	4.39±0.79
Group IIA	0.40±0.03	0.39±0.05	3.5±0.47
Group IIB	0.38±0.04	0.40±0.04	$2.7±0.34^{p1,p2}$
Group IIC	0.37±0.04	0.37±0.073	$1.87±0.41^{p1,\ p2}$

p1 = significant difference when compared with negative control group, p2 significant when compared with group IIa, p3 when compared with group IIb

Table 2. Epididymal sperm count, motility and testis weight in Ucn I-treated rats and controls after a duration of 42 days.

Variable	Epididymal sperm count (millions/ml)	Total sperm motility (%)	Progressive motility (%)	Testis weight (g)
Group I (Control rats)	53.1±5.9	85.9±9.8	78.9±8.86	1.84±0.3
Group IIA	$43.7±5.5^{p1}$	$70.3±7.4^{p1}$	$61.72±8.1^{p1}$	$1.48±0.17^{p1}$
Group IIB	$40.7±5.5^{p1}$	$67.1±10.6^{p1}$	$59.4±11.\ 1^{p1}$	$1.17±0.16^{p1}$
Group IIC	$35.2±6.1^{p1}$	$60.1±9.2^{p1}$	$56.31±11.35^{p1}$	$0.87\ ±0.13^{p1}$

p1 = significant difference when compared with negative control group, p2 significant when compared with group IIa, p3 when compared with group IIb
Total Motility = Progressive Motility + Non-progressive Motility; Progressive motility: sperms moving actively, either linearly or in large circle regardless of speed; Non-progressive motility: all other motility patterns with an absence of progression (i.e. swimming in a small circle or when only a flagellar beat can be observed); Immotile sperms: no movement.

statistically significant at confidence interval 95 %.

RESULTS

Hormone assays

Table 1 shows the effects of Ucn I on hormones in the studied groups. Following treatment with different doses of Ucn I, the results showed no significant differences were evident in serum FSH and LH levels between rats given Ucn I and their age-matched controls (p1>0.05). However, T was significantly lower in rats treated with 10 and 20 µg / kg (p2<0.04, p2<0.001 respectively) compared to those treated by 5 µg / kg of Ucn I in same group and their age-matched controls (p1 <0.001, p1 <0.001 respectively). As presented in Table 2 Epididymal sperm count, motility and testis weight were significantly lower in rats given Ucn I at doses of 5 µg (p1<0.05, p1<0.05, p1<0.04 respectively), 10 µg / kg body weight (p1<0.006, p1<0.01, p1<0.001 respectively) and 20 µg / kg body weight (p1<0.001, p1<0.001, p1<0.001 respectively) compared to their age-matched controls.

DISCUSSION

Male infertility is a major health problem that represents approximately 30% of all infertilities (Carlsen et al., 1992; Isidori et al., 2006). While stress activates the hypothalamic–pituitary–adrenal (HPA) axis, it suppresses the hypothalamic–pituitary–gonadal (HPG) axis (Kageyama, 2013, Bhongade et al., 2015). Stress profoundly inhibits the reproductive function by suppressing the pulsatile release of hypothalamic gonadotropin-releasing hormone (GnRH) and consequently luteinizing hormone (LH), at least in part via the corticotrophin-releasing factor (CRF) system as well as through the GABAergic system (Lin et al., 2012, Bhongade et al., 2015).

The release of CRF in response to various stressors suppresses of the HPG axis, especially the GnRH pulse generator in the hypothalamus, and also decreases GnRH mRNA levels via the CRF_1 receptors (Kagayama, 2013). Locally, CRF was found to exert an inhibitory effect on Leydig cell activity in rat testes (Dufau et al., 1993). However, Urocortin I (Ucn I), rather than CRF, is located in rat Leydig cells (Hardy et al., 2005).

Ucn I is an endogenously secreted corticotrophin-releasing factor (CRF)-related peptide. Ucn I is a 40-amino acid peptide that shares 45% homology with CRF (Vaughan et al., 1995; Zhao et al., 1998). In addition, Ucn I exerts its biological activity through CRF_1 and CRF_2 receptors and binds to both types with high affinity (Hsu and Hsueh, 2001). Neurons in the centrally projecting Edinger-Westphal nucleus are the main site of Ucn I synthesis in the mammalian brain, and are assumed to play a role in the stress response. Acute and chronic stress resulted in an increase in Ucn I content of the

Edinger-Westphal nucleus (Derks et al., 2012).

Both CRF and Ucn I contribute to stress responses, cardiovascular and gonadal functions via G protein-coupled seven transmembrane receptors (Vale et al., 1997; Kageyama et al., 1999a; Suda et al., 2004). It is well known that anxiety has a detrimental effect on fertility (Demyttenaere et al., 1988). Ucn I was found to elicit an increase in anxiogenic behavior and potentiate the anxiogenic action of ghrelin as well (Currie et al., 2014).

Therefore, this study is implemented to find out the probable endocrine and reproductive actions of Ucn I in adult male rats via investigation of the effects of exogenous Ucn I on epididymal sperm count, motility, testicular weight and sex hormone profiles. The study of the current hypothesis is that CRF-related peptides act within the gonads, rather than in the periphery, and that their influence on Leydig cells activity is at least partly due to rapid decreases in levels of the steroidogenic enzymes (Herman and Rivier, 2006).

In the present study, Ucn I administration for 42 days significantly decreased the testis weight, epididymal sperm count and motility. These findings are collaborated by the findings of Tao et al. (2007) who hold that Ucn I significantly inhibited the sperm motility and ascrosome reaction in a concentration-dependent manner. It inhibited T-type calcium channels in mouse spermatogenic cells, sperm motility and progesterone-evoked sperm acrosome reaction, indicating that inhibition of Ca^{2+} channels may be a mechanism for the inhibitory effects of Ucn I on male reproductive functions. Ucn I might decrease Ca^{2+} via inhibiting T-type calcium channels directly in male reproductive cells, instead of binding to its receptors firstly (Tao et al., 2005).

The duration of treatment was documented by Haron et al. (2010). As the germ cells are arranged in specific cell associations, called the stages of the cycle of the seminiferous epithelium. For the rat, the fourteen different stages of each cycle of the seminiferous epithelium last approximately 13 days. The germ cell traverses the different stages of the cycle four times for its complete development, which takes all together 52 days (Clermont, 1972; Karl et al., 1991).

Intraperitoneal administration of Ucn I in the present study significantly decreased the serum testosterone levels. These results are in consistent with findings of Rivier (2008) who reported that intratesticular administration of CRF and Ucn I significantly inhibited the testosterone response to LH-like molecules such as human chorionic gonadotropin (hCG). Ucn I was more effective than CRF in inhibiting Leydig cell responsiveness.

This study further suggested that the intraperitoneal Ucn I administration may be an effective route in inhibition of the release of testosterone from Leydig cells. Ucn I may penetrate the testes after its intraperitoneal injection and acted via a testicularly mediated site of action. Blood-borne compounds readily penetrate the intensely vascularized testes.

In the present study, no significant differences were evident in serum FSH and LH levels between Ucn I-treated rats and their age-matched controls. These results are in agreement with findings of Rivier (2008) who reported that blockade of endogenous LH before Ucn I injection did not alter the inhibitory effect of this peptide regardless of whether it was administered into the general circulation or into the testes.

In addition, it was suggested that the inhibitory influence of CRF-related peptides on testosterone response to gonadotropins is primarily exerted through CRF_1 receptor activation (Li et al., 2005). It is Ucn I, rather than CRF, that is located in rat Leydig cells (Hardy et al., 2005). However, stress-induced suppression of LH pulses was mediated by CRF_2 receptors probably through pituitary Ucn II (Nemoto et al., 2010).

Conclusion

The results of this study indicate that Ucn I administration significantly reduces testis weight, sperm count, motility and serum testosterone level which does not seem to directly involve the hypothalamic–pituitary–gonadal axis but might be due to a direct effect of Ucn I on testicular tissue and the germ cells.

Conflict of Interests

The authors have not declared any conflict of interests.

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