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·Original Article·

Beta-endorphin in serum and seminal plasma in infertile men

Shawky El-Haggar[1], Salah El-Ashmawy[2], Ahmed Attia[1], Taymour Mostafa[1], M. M. Farid Roaiah[1], Ashraf Fayez[1], Sherif Ghazi[1], Wael Zohdy[1], Nagwa Roshdy[3]

[1]Andrology Department, [2]Clinical Pathology Department, [3]Medical Biochemistry Department, Faculty of Medicine, Cairo University, Cairo 12311, Egypt

Abstract

Aim: To access beta-endorphin levels in serum as well as seminal plasma in different infertile male groups. **Methods:** Beta-endorphin was estimated in the serum and seminal plasma by enzyme-linked immunosorbent assay (ELISA) method in 80 infertile men equally divided into four groups: non-obstructive azoospermia (NOA), obstructive azoospermia (OA), congenital bilateral absent vas deferens (CBVAD) and asthenozoospermia. The results were compared to those of 20 normozoospermic proven fertile men. **Results:** There was a decrease in the mean levels of beta-endorphin in the seminal plasma of all successive infertile groups (mean ± SD: NOA 51.30 ± 27.37, OA 51.88 ± 9.47, CBAVD 20.36 ± 13.39, asthenozoospermia 49.26 ± 12.49 pg/mL, respectively) compared to the normozoospermic fertile control (87.23 ± 29.55 pg/mL). This relation was not present in mean serum level of beta-endorphin between four infertile groups (51.09 ± 14.71, 49.76 ± 12.4, 33.96 ± 7.2, 69.1 ± 16.57 pg/mL, respectively) and the fertile control group (49.26 ± 31.32 pg/mL). The CBVAD group showed the lowest seminal plasma mean level of beta-endorphin. Testicular contribution of seminal beta-endorphin was estimated to be approximately 40%. Seminal beta-endorphin showed significant correlation with the sperm concentration ($r = 0.699$, $P = 0.0188$) and nonsignificant correlation with its serum level ($r = 0.375$, $P = 0.185$) or with the sperm motility percentage ($r = 0.470$, $P = 0.899$). **Conclusion:** The estimation of beta-endorphin alone is not conclusive to evaluate male reproduction as there are many other opiates acting at the hypothalamic pituitary gonadal axis. *(Asian J Androl 2006 Nov; 8: 709–712)*

Keywords: azoospermia; beta-endorphin; male infertility; opioid peptides; semen; seminal plasma

1 Introduction

The narcotic analgesics field has always held both promise and frustration. In ancient Chinese culture, therapeutic uses for opioids were already known. The discovery of the two pentapeptides, methionine enkephalin and leucine enkephalin, was merely the opening of the flood gates. Since then a never-ending deluge of opioid peptides has been recognized [1].

The presence of beta-endorphin in the semen of normal men was first reported in 1981 by Sharp and Pekary [2]. Further reports showed that Leydig cells were not the only source for this peptide in the semen, but so were the epithelium of the epididymis, vas deferens, seminal vesicles and prostate [3–6] . Zalata *et al*. [7] added that beta-endorphin in seminal plasma plays an immune suppressive role.

The fact that immunostainable beta-endorphin and

Correspondence to: Dr Taymour Mostafa, Andrology Department, Faculty of Medicine, Cairo University, Cairo 12311, Egypt.
Tel: +20-1051-50297
E-mail: taymour1155@link.net
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other pro-opiomelanocortin (POMC)-derived peptides in Leydig cells increase during periods of testosterone synthesis in fetal life and again at puberty, suggests that the expression of these peptides might depend on gonadotropin secretion [8]. The finding of immunoreactive N-acetylated endorphins in the testis was suggested to be of potential importance as it might be a marker of spermatocytes development or it might have a physiological role. Because N-acetylation renders endogenous opiate receptors inactive, the process in germ cells might be a disposal mechanism to block what might be physiologically unwanted effects of opiate active peptides from germ cells [9].

2 Materials and methods

2.1 Subjects

Eighty infertile males, selected prospectively after consent from the Andrology Outpatient Clinic of Cairo University Hosptial, were included in the present study. They were equally divided into four groups according to their sperm counts and clinical examination: non-obstructive azoospermia (NOA), obstructive azoospermia (OA), congenital bilateral absent vas deferens (CBAVD) and asthenozoospermia. The results were compared to those obtained from 20 normozoospermic proven fertile men. NOA cases were selected from spermatogenic maturation arrest cases diagnosed previously by testicular biopsy. OA cases (scheduled for epididymo-vasosotomy operations) were diagnosed beforehand by normal testicular size, normal serum follicle stimulating hormone (FSH), and full epididymis with nodular tail. CBAVD was verified clinically and was confirmed by absent seminal fructose. Asthenozoospermic cases demonstrated sperm forward motility < 50% (mean sperm count 27.63 ± 8.00 × 10^6/mL and mean sperm motility 27.13 ± 11.29%). Fertile cases had fathered a child within the last year (mean sperm count 46.88 ± 14.14 × 10^6/mL and mean sperm forward motility 58.13 ± 4.23%).

2.2 *Estimation of beta-endorphin*

Blood samples were collected between 08:00 and 10:00 into Lavender Vacutaner tubes containing EDTA, and gently rocked immediately after collection for anticoagu-lation. Blood was transferred to centrifuge tubes containing aprotinin (0.6 TIU/mL blood) and gently rocked to inhibit the activity of proteinases, then centrifuged at 1 600 × *g* for 15 min at 4ºC to collect the plasma that was kept at –70ºC. All semen samples were collected after 4 days of sexual abstinence. Conventional semen analysis according to WHO [10] was carried out (normally: sperm count > 20 × 10^6 sperm/mL, sperm motility > 50%; abnormal: sperm morphology < 70%; vitality > 75% and leukocytes < 10^6/mL). Azoospermia was verified after three different analyses and centrifugation. Seminal plasma was separated at 1 200 × *g* immediately after complete liquefaction. All samples were stored at –20ºC till use. Beta-endorphin estimations in the serum and seminal plasma were done by enzyme-linked immunosorbent assay (ELISA) (MD Biosciences, Zürich, Switzerland). The test sensitivity was 0.18 pg/mL, intra-assay variation: < 5%, inter-assay variation: < 14%.

2.3 Statistical analysis

Numerical data were expressed as mean ± SD and range. Comparisons were performed by Student's test. Correlations were tested by Spearman's test. Comparisons and correlations were considered statistically significant when $P < 0.05$.

3 Results

The mean levels of beta-endorphin in serum and seminal serum in four different infertile groups and in the corresponding fertile control group were shown in Table 1.

Comparison between the mean levels of seminal beta-endorphin of different studied groups (Table 1) showed that the fertile control group had the highest mean with significant difference compared to all other groups. The lowest mean level was present in the CBAVD group in both serum and seminal plasma with a significant difference compared to other groups. Correlation between different parameters showed that seminal beta-endorphins had a nonsignificant relation with its serum levels ($r = 0.375$, $P = 0.185$), a nonsignificant relation with sperm motility percent ($r = 0.470$, $P = 0.899$) and a significant relation with the sperm concentration ($r = 0.699$, $P = 0.0188$).

4 Discussion

In our study, the mean level of beta-endorphin in seminal plasma was higher than that in the serum in the normozoospermic fertile men, suggesting active secretion of this peptide in semen. Reported sites for its synthesis were epididymal epithelium, vas deferens, seminal

Table 1. Comparison of mean seminal and serum endorphin (pg/mL) levels bewteen different studied groups. P_1, comparison between non-obstructive azoospermia (NOA) group and other groups. P_2, comparison of obstructive azoospermia (OA) group and other groups. P_3, comparison of congenital bilateral absent vas deferens (CBAVD) and other groups. P_4, comparison of asthenozoospermia group and other groups. $P < 0.05$ was considered significant.

		NOA	OA	CBAVD	Asthenospermia	Fertile (control)
Serum	Mean ± SD	51.09 ± 14.71	49.76 ± 12.40	33.96 ± 7.20	69.10 ± 16.57	49.60 ± 31.32
	(Range)	(32.6 – 79.5)	(40.7 – 72.6)	(28.6 – 46.7)	(48.4 – 93.4)	(14.5 – 102.4)
	P_1		> 0.05	< 0.05	< 0.05	> 0.05
	P_2			< 0.05	< 0.05	> 0.05
	P_3				< 0.05	< 0.05
	P_4					< 0.05
Semen	Mean ± SD	51.30 ± 27.37	51.88 ± 9.47	20.36 ± 13.39	23.13 ± 4.7	87.23 ± 29.55
	(Range)	(12.1 – 92.7)	(40.8 – 69.2)	(5.3 – 41.5)	(15.2 – 29.0)	(22.1 – 130.5)
	P_1		> 0.05	< 0.05	< 0.05	< 0.05
	P_2			< 0.05	< 0.05	< 0.05
	P_3				> 0.05	< 0.05
	P_4					< 0.05

vesicles and prostate [5, 11, 12]. Demonstrating decreased mean beta-endorphin seminal plasma levels in both OA cases compared to fertile controls denoted that the testicular prostatic contribution for this peptide in semen reaches approximately 40% (95% confidence interval). Singer *et al*. [13] demonstrate human seminal beta-endorphin in normozoospermic, oligozoospermic and azoospermic human semen. The mean amount in normozoospermic specimens was 278.6 pg/mL, whereas only 191.1 pg/mL in the others [13]. Both values were significantly higher than those present in the blood in their study.

Previous reports demonstrate a decrease of luteinizing hormone (LH) level after beta-endorphin administration in humans [14, 15]. A significant finding is the presence in testis of the major regulator of pituitary beta-endorphin, corticotropin-releasing factor (CRF) which stimulates beta-endorphin production from Leydig cells and inhibits hCG-induced testosterone production. CRF has inhibitory effects on the brain [16] and the hypothalamus-pituitary axis, inhibiting both sexual behavior and LH secretion, respectively. CRF is also the principal neurohormone in the initiation of the stress response [17], therefore, it is conceivable that the productive effect of stress might be mediated through a multilevel activation of the CRF beta-endorphin system. In the testis this activation would lead to a direct CRF inhibition of gonadotropin-stimulated testosterone production and indirect inhibitory effects on the tubular compartment by opioid-mediated inhibition of Sertoli cell function and by reduction of androgen production in the Leydig cell necessary for optimal spermatogenesis in the seminiferous tubule.

Fabbri *et al*. [12] pointed out that the arrest of spermatogenesis is mostly idiopathic and that alterations of testicular paracrine factors can be involved in determining the disease. It is of interest that in an azoospermic spermatid arrest case of unknown cause with intense staining for beta-endorphin in Leydig cells, therapy with long acting opiate antagonist reversed the case up to fertility. In a study of Ragni *et al*. [18], male heroin addicts' semen is shown to be always abnormal; asthenozoospermia and oligozoospermia were present in 100% and 17% of these patients, respectively. Such seminal pathology might be an indication of heroin toxicity to the male reproductive tract, suggesting local testicular effects of the opiates. In the present study, the levels of seminal beta-endorphin in the different infertile groups were lower than those in the control group, which might be explained by Leydig cell dysfunction, activation of beta-endorphin metabolic degradation pathway or deviation of POMC mRNA to the formation of other active peptides.

Correlation between different parameters shows that seminal beta-endorphins have significant correlation with sperm concentration and nonsignificant relation with its serum levels and sperm forward motility percentage. Graczykowski *et al*. [19] demonstrate the absence of

any direct effect of beta-endorphin or calcitonin on human sperm motility. In contrast, Fraioli *et al.* [3] demonstrate that beta-endorphin and calcitonin might act as potent motility inhibitors, with certain concentrations of beta-endorphin sperms being affected. Singer *et al.* [20] suggest that the high cellular beta-endorphin and calcitonin levels would be involved in the process of motility through their effect on calcium transport.

The estimation of beta-endorphin does not precisely correlate with the male reproductive function in humans as it comes from different sources, for example, the pituitary, which yields the major source in peripheral circulation, the hypothalamus, the gastrointestinal tract, the adrenal gland, the pancreas, the lymphocytes, the erythrocytes leucocytes and the reproductive system. Also, estimation of this peptide alone was not conclusive to evaluate male reproduction as there are many other opiates believed to act at the hypothalamic pituitary gonadal axis.

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