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The level of adrenomedullin immunoreactivity in seminal fluid is higher in oligozoospermic subjects and correlates with semen biochemical parameters

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Abstract

Objective: The newly discovered vasoactive peptide, adrenomedullin, and its receptors are widely distributed in various non-vascular tissues. Recent studies have suggested the possible regulatory role of adrenomedullin (AM) at several levels of the pituitary-gonadal axis. We determined the level of adrenomedullin-like immunoreactivity in the seminal fluid and examined its possible correlation with routine semen parameters, semen biochemical levels or plasma levels of FSH, LH, testosterone or prolactin.
Materials and methods: A total of 51 males were divided into three groups according to semen analysis: (i) normospermic ($n = 19$); (ii) oligozoospermic ($n = 17$); (iii) azoospermic ($n = 15$). All the subjects were submitted to hormone analysis (LH, FSH, testosterone, prolactin), routine semen parameters and semen biochemical levels (fructosio, citric acid, L-carnitine, nitric oxide) evaluation. AM was determined in plasma and seminal fluid using a specific radioimmunoassay.
Results: Mean AM concentration in seminal plasma was higher in oligozoospermic subjects than in normospermic males. In patients with non-obstructive azoospermia AM in semen was significantly lower than in patients with obstructive azoospermia. Semen AM levels correlated negatively with citric acid concentrations in oligozoospermic subjects. In patients with obstructive azoospermia AM in seminal fluid was correlated with citric acid levels. There was a relationship between plasma AM and prolactin.
Conclusions: We conclude that in human seminal fluid AM concentration is increased in infertile oligozoospermic patients and derives very likely from the prostate. Its role in the regulation of male fertility, however has to be understood.

Keywords: Male infertility; Adrenomedullin; Seminal fluid; Gonadotrophins; Citric acid; Nitric oxide

1. Introduction

Adrenomedullin (AM) is a novel peptide that belongs to the calcitonin gene-related peptide (CGRP) family. It consists of 52 amino acids, first isolated from human pheochromocytoma it elicits a long-lasting vasorelaxant activity [1]. The wide distribution of AM-like immunoreactivity and of mRNA for pre–pro-AM and AM receptor suggests diverse actions in various non-vascular tissues [2]. In studies of the pituitary-gonadal axis, AM was detected in human and rat pituitary [3] and in the ovary and follicular fluid [4,5]. Not surprisingly then, there is now strong evidence supporting a role for AM in modulating sexual and reproductive functions in mammalian species. Plasma AM concentrations fluctuate during menstrual cycle and correlate with gonadotrophins [6]. AM is expressed in rat granulosa cells and enhances the effects of FSH treatment, acting additionally to produce cAMP in the cells [7]. AM and its receptor [8] have been found in the human granulosa lutein cells at the preovulatory stage and at the midluteal phase demonstrating that AM acts as a local factor to

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enhance progesterone production by these cells. The expression of AM in human endometrium is conditioned by sex steroids [9], and AM transcript is up regulated in the oviducts of mice after mating with fertile males [10].

Recently, studies in animals have suggested a potential role for AM in male fertility, not only because of its vasodilator activity, participating in the penile erection as demonstrated for the CGRP [11], but also in the paracrine regulation of testicular function [12]. AM is present in human testis [13] and prostate [14] and large amount of AM has been found in human seminal fluid where it correlates with sperm motility [15]. Although not definitive, such a localization suggests a role for AM in spermatogenesis and sperm maturation.

To better understand whether AM may be implicated in male fertility we measured AM in human seminal fluid samples studying the relationship between the level of seminal fluid AM and the sperm count, sperm motility, semen biochemical parameters of epididymis function (carnitine), seminal vesicles function (fructose), prostate function (citric acid), and plasma levels of FSH, LH, prolactin, testosterone.

2. Materials and methods

2.1. Study population

Fifty-one healthy males aged 25–41 years, attending the infertility clinic because of male or female factors or both, participated in the study, after giving informed consent. Retrieval, analysis and classification of the ejaculate were performed according to the World Health Organization (WHO) recommendations [16]. All semen samples were collected following 3–5 days of sexual abstinence by masturbation and allowed to liquefy at room temperature for 30–40 min. On the basis of the semen analysis, the subjects were divided into normospermic ($n = 19$); oligozoospermic ($n = 17$); azoospermic ($n = 15$). Azoospermic subjects were further sub-grouped in accordance to obstructive ($n = 7$) and non-obstructive azoospermia ($n = 8$). No patient had vasectomy, varicocele, drug or alcohol abuse, ongoing medical treatment, diabetes or hypertension. Samples with a semen leukocyte concentration of $>1 \times 10^6$ cells/ml were excluded.

All patients underwent semen analysis and blood sampling at the same visit to clinic (within 3 h). AM concentration and different biochemical parameters including nitric oxide (NO) metabolites were assayed in seminal plasma. Furthermore, in peripheral plasma FSH, LH, testosterone and prolactin in addition to AM were assayed.

The study was approved by the University Ethical Committee.

2.2. Semen analysis

Automated semen analysis was performed using Superimposed Images Analysis System (Delta Sistemi, Italy). Sperm count, motility and morphology as well sperm trajectory characteristics (amplitude of lateral displacement, curvilinear velocity and straight-line velocity) were recorded. Semen volume was determined by a graded cylinder.

Fructose, citric acid and carnitine were measured in the supernatant obtained after centrifugation at $3000 \times g$ for 15 min. Fructose was determined according to the hexokinase method and citric acid with the ultraviolet method using citrate lyase catalysed reaction. L-carnitine was determined according to a radiometrical method. With this method, carnitine, after addition of [^{14}C]acetylcoenzime A and carnitine acetyltrasferase, was completely transformed into acetylcarnitine. Subsequent treatment of the reaction mixture with anion exchange resin Dowex 1-X10 removed the highly negatively charged [^{14}C]acetyl-CoA. The positively charged [^{14}C]acetylcarnitine remained in solution and was determined using an LKB-Wallac 1410 liquid scintillation isotope counter. A reference curve was used to obtain the carnitine content of the sample by calibration. In our laboratory the normal range was—fructose: 155–455 mg/dl; citric acid: 350–670 mg/dl; L-carnitine: 3–7 mg/dl.

A 1 ml of seminal plasma for NO and AM measurement was separated from sperm by centrifugation at $1500 \times g$ for 15 min and stored at -80 °C until assayed.

NO concentrations were assessed by measuring seminal plasma levels of the stable oxidation products of NO metabolism (nitrate and nitrite). The concentrations of NO metabolites were assayed with the Greiss reaction procedures previously described [17].

2.3. Blood samples

Circulating blood samples were collected at the time of sperm collection from antecubital vein in tubes containing EDTA and aprotinin. Samples were centrifuged at $650 \times g$ for 15 min at 4 °C, the plasma was divided into aliquots and stored at -80 °C until assayed.

Serum LH, FSH, prolactin and testosterone were measured by specific commercially available immunoassay kits (Radim, Pomezia, Rome, Italy). For all these methods the maximal intra-assay and inter-assay coefficient of variability was 5.8 and 10.3%, respectively.

2.4. AM measurement

AM was assayed on seminal plasma and blood samples after extraction as reported [18]. Briefly, 1 ml of samples were applied to conditioned Sep-pak C18 cartridges (Millipore Corp., Waters Chromatography, Mildford, MA, USA) and the column was sequentially washed with 5 ml of isotonic saline, 5 ml of 0.1% trifluoracetic acid and 5 ml of 20% acetonitrile in 0.1% trifluoracetic acid. The absorbed material was eluted with 4 ml of 50% acetonitrile and the elute was lyophilized. After lyophilization samples were