Neuroscience Letters 602 (2015) 133–138

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

The effects of intracerebroventricular infusion of apelin-13 on reproductive function in male rats

Suleyman Sandal[a,*], Suat Tekin[a], Fatma Burcu Seker[b], Ali Beytur[c], Nigar Vardi[d], Cemil Colak[e], Tuba Tapan[a], Sedat Yildiz[a], Bayram Yilmaz[b]

[a] Inonu University, Faculty of Medicine, Department of Physiology, Malatya 44280 Turkey
[b] Yeditepe University, Faculty of Medicine, Department of Physiology, Istanbul, Turkey
[c] Inonu University, Faculty of Medicine, Department of Urology, Malatya, Turkey
[d] Inonu University, Faculty of Medicine, Department of Histology and Embryology, Malatya, Turkey
[e] Inonu University, Faculty of Medicine, Department of Biostatistics and Medical Informatics, Malatya, Turkey

HIGHLIGHTS

- Infusion of central apelin-13 can suppress LH pulse.
- Apelin-13 decreases serum testosterone levels.
- Apelin-13 may cause infertility in male rats.

ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 22 June 2015
Accepted 30 June 2015
Available online 3 July 2015

Keywords:
Apelin
FSH
LH
Testosterone
Leydig cell
Reproduction function

ABSTRACT

Apelin is a novel bioactive peptide as the endogenous ligand for APJ. Apelin and APJ have also been identified in the testis, hypothalamic nuclei such as arcuate, supraoptic and paraventricular nuclei, implicating roles in the control of reproduction.

Therefore, the present study was designed to investigate the effects of chronic central infusion of apelin-13 on LH, FSH and testosterone levels and testis morphology. 21 Wistar–Albino male rats received continuous intracerebroventricular infusion via Alzet osmotic mini pumps filled artificial cerebrospinal fluid (vehicle) or apelin-13 at concentrations of 1 or 10 nmol (10 μl/h) for seven days. At the last 90 min of the infusion period, the blood samples were collected at 15 min intervals (0–90 min) for LH and FSH analyses. At the last sampling point, the blood samples were analyzed for testosterone levels.

Infusion of high dose apelin-13 significantly suppressed LH release compared with the vehicle values at 30, 60 and 75 min ($p < 0.05$). However, FSH levels did not significantly differ among the groups. Serum testosterone levels in high dose apelin-13 group were statistically lower than the control group ($p < 0.05$). In addition, histological examination showed that infusion of high dose apelin-13 significantly decreased the number of Leydig cells compared with the control and lower dose apelin-13 groups ($p < 0.05$, $p < 0.01$).

Our results suggest that apelin-13 may play a role in the central regulation and decreases testosterone release by suppressing LH secretion. Thus, antagonists of the apelin receptor may, therefore, be useful for pharmaceuticals in the treatment of infertility.

1. Introduction

Gonadotropin-releasing hormone (GnRH) is a neuropeptide utilized as messenger molecules in the systems of nervous and neuroendocrine to control many physiological processes containing reproduction and sexual behavior. The secretion of GnRH from the hypothalamus is necessary for the synthesis and secretion of ideal gonadotropin (luteinizing hormone; LH and follicle stimulating hormone; FSH), and finally for normal reproductive behavior [18,21,27]. Reproductive functions start with the secretion of GnRH from the hypothalamus; this process is followed by releasing the hormones of LH and FSH from the anterior pituitary [12]. Testosterone secretion from the cells of Leydig in the testis improves with LH stimulation [36]. In addition to the system of central GnRH, there

* Corresponding author.
E-mail address: suleyman.sandal@inonu.edu.tr (S. Sandal).

http://dx.doi.org/10.1016/j.neulet.2015.06.059
0304-3940/

is available evidence for the peripheral production for GnRH in testicular cells [9], ovarian granulosa cells [26], and in the pituitary gland [2].

In 1993, described to APJ as a putative receptor protein related to AT1 in a human gene [23]. Further evidence suggested important roles for these receptors in the regulation of fluid homeostasis [28,32] and cardiovascular function [14]. Successively, apelin, endogenous ligand for APJ, was identified in human and bovine tissue that it is derived from a 77-amino-acid precursor [17,19,30].

The varying forms of the apelin peptide including apelin-13, apelin-17 and apelin-36 have been shown in different tissues in order to activate the APJ. Apelin-13 is the main circulatory form, and its biological activity is greater than apelin-17 and 36 [33,35]. It has been shown that apelin and its receptor APJ are widely expressed in several tissues such as lung, heart, kidney, ovary, testis, mammary gland, gastric mucosa, adipose tissue and central nervous system [15,19,20].

There are studies showing both large number of apelin positive cells and large amount of mRNA expression of APJ in the hypothalamus area in the rat brain, especially in the paraventricular (PVN) and the supraoptic (SON) nuclei [7,8,22]. The intense labeling in anterior and intermediate lobes of the pituitary has also been exhibited [8].

The presence of APJ in the brain areas related to reproduction such as hypothalamus and hypophysis suggests that apelin can play a specific role in the control of LH and FSH release from the adenohypophysis. In addition, distribution of APJs in the ovary and testicles brings to mind that apelin may affect regulation of reproduction through hypothalamus–hypophyseal–gonadal axis.

Therefore, the current study was designed to investigate the effects of central infusion of apelin-13 on plasma LH, FSH and serum testosterone levels and testis morphology in the male rats.

2. Materials and methods

2.1. Animals and experimental design

In the present study, was used to 21 adult Wistar–Albino male rats (weighing 300–350 g), and all protocols were approved by the local ethics committee of Inonu University. Rats were housed in a temperature controlled room at 22 ± 1 °C with a 12:12 h light/dark cycle and they were fed as ad libitum. Twenty one rats were randomly divided into three groups, control (vehicle), low (1 nmol) and high (10 nmol) doses of apelin-13 group ($n = 7$ in each group).

Animals were anesthetized with ketamine and xylazine and placed in a sterotaxic frame (Harvard Apparatus, USA) for intracerebroventricular (icv) injection. Right lateral ventricle coordinates were decided from the rat brain stereotaxic coordinates (1.4 mm lateral, 0.8 mm posterior and 4.8 mm vertical from bregma) [25]. The brain infusion kits were placed in right lateral ventricle and fixed by dental cement. Subsequently, an Alzet osmotic mini pump was implanted by insertion under the skin.

All rats received continuous icv infusion via osmotic mini pumps filled artificial cerebrospinal fluid (aCSF in mmol/l: KCl 3.4, NaCl 133.3, $MgCl_2$ 1.2, $CaCl_2$ 1.3, $NaHCO_3$ 32.0, NaH_2PO_4 0.6 and glucose 3.4, pH 7.4) [13] for control (10 μl/h) or apelin-13 at concentrations of 1 or 10 nmol (10 μl/h) for experimental group for seven days.

Rats were re-anesthetized and implanted a catheter into femoral vein after seven days from implantation of osmotic mini pumps. Blood samples were collected at the 15 min intervals (0, 15, 30, 45, 60, 75 and 90 min) for LH and FSH analysis. At the last sampling point (90 min), blood samples were analyzed serum testosterone levels. The testis were taken for histological examination after the rats sacrificed.

2.2. LH, FSH and Testosterone enzyme immunoassays

The levels of LH and FSH were analyzed based on Pappa et al. with some alterations [24]. 96-well immunoplates (Nunc, Denmark) were coated with rat LH or rat FSH. Standards or plasma samples were preincubated with primer antibodies and were then transferred into coated plates for competition with antigens on the solid phase. Plates were washed, and the secondary antibodies conjugated to streptavidine peroxidase was added into each well, and color was developed by using tetramethylbenzidine as a substrate. Plates were read at 450 nm using a plate reader (Tecan Spectra III, Austria). Rat LH, rat FSH and primer antibodies (rabbit anti-rat LH and rabbit anti-rat FSH) were achieved from Dr. A.F. Parlow (NIDDK, NIH, USA) and secondary antibodies (goat anti-rabbit IgG) conjugated to streptavidin peroxidase was bought from Sigma (Sigma–Aldrich, Germany). Serum testosterone levels of rats was identified by EIA (Testosterone EIA kit; Cayman Chemical Company, USA), in pursuance of the manual.

2.3. Histological examination

For histological study, testis were fixed in formalin%10, dehydrated in ethyl alcohol, cleared in xylol and embedded in paraffin wax. 5 μm thickness sections were cut, and stained with haematoxylin-eosin. We measured the diameter of the seminiferous tubule (hundred tubules for each testis). The Leydig cells from twenty diverse interstitial regions of each testis were counted. The sections were examined using a Leica Q Win plus Image Analysis System (Leica Micros Imaging Solutions Ltd., UK), respectively, at 10X and 100X.

2.4. Statistical analysis

Statistical analysis was performed using SPSS software (version 22.0). The experimental results were reported as mean ± SEM (standard error of means). Normality assumption was confirmed by Kolmogorov Smirnov test. One-way analysis of variance (ANOVA) was employed to compare the experimental groups with the controls. Multiple comparisons were carried out using post hoc Tukey HSD test. In addition, histological results were compared with Kruskal–Wallis variance analysis. Where differences among the groups were detected, groups were compared using the Mann–Whitney U with Bonferroni correction. Values of $p < 0.05$ were considered significant.

3. Results

3.1. The effect of icv apelin-13infusion on LH and FSH release

After the icv apelin-13 infusion, the plasma LH and FSH levels changes in the groups were shown in Figs. 1 and 2, , respectively. The LH levels of animals showed an increase in all groups that apelin-13 was administered. However, infusion of both low and high dose apelin-13 significantly suppressed LH release ($p < 0.05$) compared with the vehicle values at 30, 60 and 75 min (Fig. 1). However, FSH levels did not significantly differ among the groups (Fig. 2).

3.2. The effect of icv apelin-13 infusion on serum testosterone levels

Effects of icv apelin-13 administration on serum testosterone levels were shown in Fig 3. Low dose apelin-13 suppressed serum testosterone, but this was not statistically significant. In contrast,