European Journal of Pharmacology 771 (2016) 247–254

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Endocrine pharmacology

Treatment effect of L-Norvaline on the sexual performance of male rats with streptozotocin induced diabetes

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ARTICLE INFO

Article history:
Received 21 April 2015
Received in revised form
1 December 2015
Accepted 4 December 2015
Available online 6 December 2015

Keywords:
Arginase
Erectile dysfunction
l-Arginine
l-Norvaline
Streptozotocin

ABSTRACT

Sexual impairment is an established risk factor in diabetes mellitus affecting about 75% of male diabetic population. In diabetes overexpression of arginase leads to decreased production of NO and diminished erectile response. Inhibition of arginase enzyme can lead to improvement in diabetes induced sexual dysfunction. In the present study diabetes mellitus was induced in adult male rats by intraperitoneal injection of single dose of streptozotocin (65 mg/kg) in 0.1 M Citrate buffer pH 4.5 and after 72 h fasting serum glucose level was checked by glucose oxidase–peroxidase method and those animals showing FSG above 250 mg/dl were selected. Diabetic animals were divided into four groups comprising six animals in each. L-Norvaline, potent arginase inhibitor was administered at a dose of 10 mg/kg ip to the different groups of diabetic animals for a period of 30 days. Sildenafil at a dose of 5 mg/kg orally was used as a standard drug. Mating behavior tests were performed at 0, 15th and 30th days. After 30 days, various biochemical and hormonal parameters (nitrates, LDH, urea, testosterone), testicular parameters (total protein, nitrates, LDH, total cholesterol, LDL, triglycerides, VLDL, HDL) were evaluated to find out the effect of L-Norvaline in sexual impairment. Sperm analysis was also carried out for the treated rats. L-Norvaline showed significant improvement in serum nitrates, urea, LDH, testosterone and testicular protein level as compared with diabetic group. It also improved sperm motility, count and viability in diabetic rats. Sildenafil showed no improvement in above parameters except restoration in serum nitrates level.

1. Introduction

Sexual impairment is a combined disorder related to endocrine, neurological and vascular functions. In male population the risk factor of diabetes is sexual impairment. This impairment leads to increased prevalence of erectile dysfunction upto three folds in diabetics as compared with non diabetic men (Maiorino et al., 2014, Giugliano et al., 2010). It is a quite common complication (Chew et al., 2013) associated with increase in age, diabetes mellitus, consumption of alcohol, drugs and with neurological damage (Fedele et al., 2001; Roth et al., 2003; Kleinman et al., 2000; Romeo et al., 2000). Moreover, diabetic patient with various clinical conditions such as obesity, overweight, atherogenic dyslipidemia, or cigarette smoking are themselves risk factors for sexual disorder in males (Maiorino et al., 2014, Lewis et al., 2010, Miner et al., 2012). Epidemiological data show that about 75% of men with diabetes mellitus affected with this major complication at an earlier age rather than in normal population (Hakim and Goldstein, 1996). Diabetes mellitus is characterized by poor metabolic control and altered carbohydrate homeostasis. It affects the endothelial functions along with endocrine control of spermatogenesis (Sexton and Jarrow, 1997) and ultimately leads to erectile and ejaculation dysfunction. Among the type I and type II diabetes it is still a controversial point of conflict that which subtypes of diabetes is majorly involved in sexual impairment. Erectile dysfunction is one of the most major complications of diabetes mellitus.

The physiopathology of sexual impairment in diabetes is multifactorial. In diabetes enhanced level of arginase enzyme reduces the availability of L-arginine as substrate to interact with eNOS, causing eNOS uncoupling and decreased synthesis of NO. In diabetes denovo synthesis of diacylglycerol leads to protein kinase C activation which further activates reactive oxygen species. Other mechanisms involved in the pathophysiology of sexual impairment are Rho A activation which ultimately increase arginase activity, increased endothelin-1 expression which causes vasoconstriction through ET_AR, generation of free radicals e.g. – peroxynitrate radicals which inactivates superoxide

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http://dx.doi.org/10.1016/j.ejphar.2015.12.008
0014-2999/

dismutase and reduces the availability of NO and/or increased formation of advanced glycated end product inducing vascular cell damage and reduction in cavernosal NO production (McKenzie and Ridley, 2007).

Generally penile erection is regulated by cavernosal nitric oxide synthesized from L-Arginine using NOS as enzyme. The Arginase activity has been upregulated in diabetic human corpus cavernosum smooth muscle due to overexpression of arginase and leads to decreased production of NO and diminished erectile response (Zhang et al., 2001). Moreover, arginase overexpression contributes to positive role in promoting cavernosum smooth muscle cell growth and proliferation which leads to vascular lesion. This lesion is further enhanced by decreased level of protective causing veno occlusive dysfunction of corpora cavernosum and inability to attain the required intracavernosal pressure for attaining stiffness as found majorly in Type 2 diabetic patient. Identification of two distinct genetic isoforms of human arginase shares more or less 60% amino acid sequence homology (Bivalacqua et al., 2001; Kim et al., 2001). Arginase I is highly expressed in liver where as arginase II is a mitochondrial enzyme, expressed in the prostate and kidney (Masuda, 2008). Moreover, both of these isoforms have also been localized in vascular endothelium and human cavernosum smooth muscle (Cox et al., 1999; Wei et al., 2000).

As arginase plays an important role in diabetes induced sexual impairment, inhibition of arginase can have promising role in improving sexual function. Arginase inhibition can cause improvement in endothelial cell functions or reduction of arginase activity via gene knockout in the penis and can restore sexual impairment in diabetes patients (Kim et al., 2009; Toque et al., 2011). Selective or non selective arginase inhibitors like (+)-S-2-amino-6 iodoacetamidohexanoic acid (AIHA), 2-(S)-amino-6-boronohexanoic acid (ABH), L-Norvaline, N-hydroxy-nor-L-arginine (nor-NOHA) etc. have been developed for treatment of various diseases related to vascular disorders such as erectile dysfunction, pulmonary hypertension, hypertension, atherosclerosis, renal disease, asthma, T-cell dysfunction, ischemia reperfusion injury, neurodegenerative diseases, wound healing, and fibrotic diseases (Segal et al., 2012; Bagnost et al., 2010, 2008; Ckless et al., 2008).

L-Norvaline, also known as 5-[(aminoiminomethyl)amino]- (mol. formula – $C_5H_{11}NO_2$, mol. weight – 117.15) is an amino acid and constitutional isomer with valine. This amino acid is often made synthetically. It is a white crystalline powder with melting point 303–307 °C and well soluble in water (10.5 g/100 ml), very soluble in hot water and dilute hydrochloric acid. It acts as drug intermediate of Perindropril, an ACE inhibitor. L-Norvaline has been proposed to inhibit the arginase enzyme and thus by increasing arginine concentrations the compound can restore the vasculopathic sexual functions in diabetes. Moreover no clinical studies regarding the effect of L-Norvaline in modifying the diabetes induced sexual impairment are available. Therefore the present study was aimed to determine whether the systemic administration of L-Norvaline could restore the sexual function in hyperglycemic state or not.

2. Materials and methods

2.1. Animals

Adult twelve-week-old inbred albino rats of Wistar strain of either sex ie, male (body weights ranging 225–250 g) and female (body weights ranging 150–180 g) were used for the present study. The animals were housed singly in standard polypropylene cages and maintained under controlled room temperature (24–28 °C) and relative humidity (60–70%) with 12:12 h light and dark cycle. All the animals were provided with commercially available rat normal pellet diet and de-mineralized drinking water ad libitum throughout the study. All the animals were allowed to acclimatize for a week with the laboratory environment before the beginning of the experiment. The guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Govt. of India were strictly followed and prior permission was granted from the Institutional Animal Ethics Committee (Reg. no. IAEC/273/CPCSEA/SBS/01/2013-2014) for conducting the experimental studies.

2.2. Reagents

L-Norvaline (N7627), Hank's Buffer salt solution (H9349) and Disodium phenyl phosphate (P7751) were supplied from Sigma Aldrich (India). Potassium phosphate Monobasic (MB050), Sodium hydroxide pellets (RM 467), Citric acid Monohydrate (RM 229), Fructose (RM 1355), Zinc sulfate (RM 1180), Ethylenediaminetetraacetic acid (RM 1279), Monobasic Sodium phosphate (RM 3964), Phenol crystalline (AS021) and N(1 napthyl ethylene diamine dihydrochloride) (RM 1073) were provided from HiMedia (India). Resorcinol (R0030), Leishman stain solution (L0060), Eosin Y stain (E0231) and Standard benzoic acid (B0181) were available from Rankem (India). Phosphoric Acid (61781505031730) was supplied from Merck, India. All analytical graded chemicals and reagents were used for the study.

2.3. Induction of Diabetes Mellitus

Diabetes Mellitus was induced in 16 h fasted adult male rats by intraperitoneal (i.p) injection of single dose of streptozotocin (65 mg/kg) in 0.1 M Citrate buffer pH 4.5 (Budin et al., 2011). The streptozotocin induced rats were supplied 5% glucose solution for 24 h to protect animals from initial hypoglycemic mortality. After 72 h blood was withdrawn from the animals by retro orbital puncture under chloroform anesthesia. Serum was separated out by centrifugation and fasting serum glucose (FSG) level was checked by glucose oxidase– peroxidase method using Seimen Diagnostic Kits. Animals showing FSG level above 250 mg/dl were considered as diabetic and included for the study.

2.4. Experimental design

The diabetic animals which showed FSG level above 250 mg/dl were randomly divided into four groups comprising six animals in each group and one group of normal non diabetic animals received the vehicle 0.1 M citrate buffer pH 4.5. L-Norvaline at a dose of 10 mg/kg was administered i.p to the different groups of animals for a period of 30 days. Group I represented control animals receiving 0.1 M citrate buffer pH 4.5, animals in group II, III and IV were diabetic and group II represented as diabetic positive control animals. Group III and IV received L-Norvaline (10 mg/kg body weight) i.p as treated drug and suspension of sildenafil citrate (5 mg/kg body weight) orally (Sekar et al., 2009) as reference drug, respectively. The study was continued for 30 days.

2.5. Mating behavior test

The test was carried out according to the method described by Dewsbury and Davis (1970) and Szechtman et al. (1981) modified by Amin et al. (1996). Healthy male albino animals with brisk sexually experienced were selected for the study. Before administration of the drug, the animals should be well adapted to the environment of copulatory arena provided with 1 w fluorescent tube in the laboratory of 14′ × 14′ regularly at the defined testing time for 3–6 days. Female rats showed maximum receptivity was confirmed for the test. As the female allowed mating only during