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Effect of Serum Immunoglobulin G on Norepinephrine Transporter Expression in Mouse Sympathetic Neurons

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JB and XD participated in conceiving the design of the study and collecting and reviewing the data and coordination of project. LG, JW and CG participated in doing literature review, collecting the data and analysis and in preparing the manuscript. JW and CG helped in critical revision and finalizing the manuscript. All authors read, revised, and approved the final manuscript.

Key words
Prostatitis, Autoantibody, Sympathetic neurons, Norepinephrine transporter

ABSTRACT

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) patients with lower urinary tract symptoms (LUTS) and erectile dysfunction (ED) eventually show increased sympathetic tension. Therefore, this study aimed to assess the effects of serum Immunoglobulin G (IgG) on secreted norepinephrine (NE) amounts and norepinephrine transporter (NET) expression in sympathetic neurons. The purified serum IgG from the patient group Control, CP/CPPS+ED, CP/CPPS and ED was administered to cultured superior cervical sympathetic ganglions from nude mice, and secreted NE was quantitated by radioimmunoassay. Quantitative real-time PCR and Western blot were performed to assess NET expression at the mRNA and protein levels, respectively. There was no significant difference in secreted NE levels among the four groups afterincubating mouse sympathetic neurons with serum IgG ($P>0.05$). However, NET mRNA expression levels in mouse sympathetic neurons were significantly higher after administration of serum IgG from the CP/CPPS+ED and CP/CPPS groups compared with the ED and control groups ($P<0.05$); the ED and control groups showed comparable values ($P<0.05$). Meanwhile, NET protein expression levels were significantly higher ($P<0.05$) in the CP/CPPS+ED group compared with the CP/CPPS, ED and control groups, which showed similar values ($P>0.05$). Serum IgG from LUTS-dominated CP/CPPS patients accompanied by ED affects NET expression in mouse sympathetic neurons.

INTRODUCTION

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) represents a chronic illness featuring multiple symptoms such as persistent and widespread pain, which can be accompanied by psychosocial or psychiatric comorbidities, severely decreasing the quality of life in men (Huang *et al*., 2020). CP/CPPS prevalence is estimated at 2.2-13.8% in the general population (Zhang *et al*., 2016). Besides psycho-psychological parameters, autoimmune factors also play an important role in the developing CP/CPPS (Jang and Schaeffer, 2003; Bai *et al*., 2010; Ramzan and Ramzan, 2017). Previous findings have revealed T lymphocyte proliferation response targeting prostate-specific antigen and prostatic acid phosphatase in CP/CPPS patients (Motrich *et al*., 2005). An animal model of autoimmune prostatitis confirmed the presence of an immune response against autoantigens in autoimmune animals (Pontari and Ruggieri, 2008). Aclinical study recently detected autoantibodies against multifunctional seminal vesicle proteins in CP/CPPS patients (Hou *et al*., 2009). The above reports supported the notion that autoantibodies play an important role in the pathogenesis of CP/CPPS.

The symptoms of lower urinary tract symptoms (LUTS) and erectile dysfunction (ED) in CP/CPPS patients might show direct correlations (Umbrăveanu *et al*., 2018). The bladder sphincter, bladder detrusor and prostate sphincter, as well as the smooth muscle of the cavernous body of the penis, were rich in adrenergic nerve endings (Jung *et al*., 2012; Dean and Lue, 2005). Consequently, long-term mental stress, such as anxiety and depression,

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increases the activity of sympathetic nerves innervating the aforementioned smooth muscles, causing spasms or contractions in the bladder neck, prostate urethra and penile cavernous body, resulting in obstructive urination symptoms and erectile dysfunction (ED). Meanwhile, neuroendocrine changes caused by mental stress, including alterations of the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenal (HPA) axis, are likely to affect the human immune system (Stephens and Wand, 2012; Ménard *et al*., 2017).

Norepinephrine transporter (NET), found in the presynaptic membrane of sympathetic nerves, mainly regulates the concentration of norepinephrine (NE) released by sympathetic nerve impulses, playing a very important role in receptor expression and functions (Bristow, 2000; Zahniser and Doolen, 2001; Eisenhofer, 2001).

Our previous study confirmed that changes in ANS function play a major role in LUTS-dominated CP/CPPS patients accompanied by ED. In addition, compared with healthy individuals, CP/CPPS patients with LUTS and ED eventually show increased sympathetic tension (Breser *et al*., 2017). However, whether the patient's serum antibodies could bind to sympathetic neurons and trigger the corresponding function change, thereby causing or aggravating the symptoms of LUT and ED, remains unknown. Therefore, the aim of the present study was to explore whether serum immunoglobulin G (IgG) from CP/CPPS patients with LUTS and ED could affect the function of sympathetic neurons by binding to them.

MATERIALS AND METHODS

In this study, CP/CPPS and ED patients who visited the Urology Male Clinic of our hospital from March 2018 to June 2020 were enrolled. Taking the salivary cortisol levels of the CP/CPPS+ED, CP/CPPS, ED, and Control groups in our previous study as the main observation index, the sample size was estimated, and each group needed at least 21 cases. Therefore, 25 cases were finally included per group.

Inclusion criteria were CP/CPPS diagnosis according to the National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) items (Wagenlehner *et al*., 2013), including frequent urination, urinary discomfort, perineal pain and discomfort, and varying degrees of sexual dysfunction for at least 3 months; ED diagnosed based on diagnostic criteria of the National Institutes of Health, with a course of disease longer than 3 months and a score of 5-21 in the International Index of Erectile Function-5 (IIEF-5) system (Rosen *et al*., 1999), no conscious penile erection, poor hardness or non-lasting erection, and inability to complete normal sexual life; >18 years of age; married or long-term regular sexual partners; genital examination showing no obvious developmental deformity; normal development of secondary sexual characteristics.

Exclusion criteria were other lower urinary tract diseases such as urinary tract infections, tuberculosis, renal calculus, benign prostatic hyperplasia, prostate cancer, urethral strictures, bladder tumors, and neurogenic bladder; sexual dysfunction or abnormal sex hormone test; psychosis; diabetes; peripheral vascular disease; spinal cord injury; hypertension; coronary heart disease; a history of alcohol or drug abuse. The patients did not use drugs affecting sexual function in the past 6 months, and were not administered drugs or other methods for ED treatment within 3 months.

All subjects provided signed informed consent. This study was approved by the medical ethics committee of our hospital. Subjects in the CP/CPPS+ED group were CP/CPPS cases with no pain symptoms, but accompanied by LUTS and ED. The severity of LUTS was assessed by the international prostate symptom score (IPSS), one of the commonly used questionnaires. The CP/CPPS group encompassed CP/CPPS cases accompanied by only LUTS, with no pain symptoms or ED. The ED group comprised ED cases with no CP/CPPS symptoms. Healthy individuals were assessed as the normal control group.

Affinity chromatography purification of serum IgG

A total of 10 mL of fresh venous blood was collected per patient from the elbow vein on an empty stomach in the morning. The serum was then immediately collected by centrifugation and stored at -70℃. The Bio-Rad Econo affinity chromatography HPLC purification system (Bio-Rad, California, USA) was used to purify serum IgG. Briefly, 5 mL of the serum sample was diluted to 25 mL with 20 mM phosphate-buffered saline (PBS, pH 7.0) and loaded onto the Hitrap Protein A affinity column (MabSelect Sure; GE Healthcare, Amersham, UK). Elution was performed at a flow rate of 1 mL/min, with a UV monitoring OD280 of 1.0 (Thermo Scientific, Massachusetts, USA). Impurities were eluted with 12 column volumes (CVs) of 20 mM PBS and 2CVs gradient elution (20 mM PBS [pH7.0] +0.1 M citric acid [pH4.0]). IgG elution was performed with 10 CVs of citric acid (pH4.0) at a flow rate of 2 mL/min. Protein content was calculated according to the following formula: Protein amounts (mg/mL)= 1.45×OD280 nm-0.74×OD260 nm. IgG purity was detected by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE; Thermo-Fisher Life Technologies, Massachusetts, USA).

Isolation of sympathetic neurons and co-culture with IgG

Healthy kunming mice (10 male and 10 female, weight 20± 2g, aged 4 weeks) were provided by the Experimental Animal Center of Tongji Hospital (License Number: SCXK 20150009, Hubei). All the mice were housed in polypropylene cages using a standard environment (25±2°C, humidity 45%±5%, 12 h light-dark cycle, free feeding). Food and water were freely assessible to the mice. The experiments on mice were done after approval from the Ethics Committee of Laboratory Animals of Tongji Hospital which also follows the guidelines for Ethical Conduct in the Care and Use of Animals.

Nude mice on the day of birth were euthanized and fixed on a plastic foam board with the abdomen facing upward under sterile conditions. The common carotid arteries on both sides of the trachea were identified under a dissecting microscope. As a mark, the superior cervical sympathetic section was found at the intersection where the common carotid artery was branched into internal and external carotid arteries. The superior cervical sympathetic section was extracted and placed in D-Hank's balanced salt solution (HBSS) for cleaning. After peeling off the outer membrane of the ganglion, it was placed in a 2-mL mixed digestive solution containing 1% collagenase and 1% dispase (37℃, 1h) for digestion and dispersion. Then, dilution with culture medium (Sigma-Aldrich, Missouri, USA) was performed to achieve a density of 0.2×105 cells/mL. Sympathetic neurons were co-cultured with IgG (1mg/mL), and the cultures were incubated in a 5% carbon dioxide incubator at 37℃ for 2h. After the incubation, supernatant liquids were collected for radioimmunoassay of NE. Western blot was conducted to analyze the protein level of NET; qPCR was conducted to detect NET mRNA level.

NE quantitation by radioimmunoassay

NE quantitation in culture supernatants was performed essentially as described in a previous report (Denfeld *et al*., 2018).

Western blot

The harvested cells were lysed with the RIPA lysis buffer (Boster, Wuhan, China), and quantified by a BCA kit (Boster, Wuhan, China). Equal amounts of protein were separated by 10% or 12% SDS-PAGE (Thermo-Fisher Life Technologies, Massachusetts, USA), and transferred onto PVDF membranes, which were blocked with blocking solution containing 5% skimmed milk at room temperature for 1.5~2h. After blocking, mouse anti-GAPDH (dilution 1:1000, sc-32233, Santa Cruz Biotechnology, Texas, USA) and rabbit anti-NET (dilution 1:1000, ab41559, Abcam, Cambridge, UK) primary antibodies were added for incubation at 4℃ overnight. Then, the samples were washed with tris-buffered saline-Tween (TBST) and incubated with secondary antibodies for 2h at room temperature. Enhanced chemiluminescence (ECL) reagents (Super Signal West Pico, Thermo Scientific, USA) were added for color development. Protein bands were acquired by densitometry, using Quantity-one 4.6.6 Image Analyzer software (Bio-Rad, California, USA) and quantified by ImageJ (NIH, USA), and the gray level ratio of the target protein to that of GAPDH was used as relative expression levels.

qRT-PCR

After treatment, cells were collected, and total RNA was extracted by the TRIZOL reagent (Invitrogen, California, USA). RNA amounts were assessed, and cDNA was obtained with a reverse transcription kit (Clontech, California, USA). Real-time PCR was performed using 5 μL of the cDNA prepared by the RT reaction and SYBR Green master mix (Stratagene, Amsterdam, the Netherlands). qPCR was performed as follow: UDG enzyme activation at 50℃ for 2 min; pre-denaturation at 95℃ for 2 min; 40 cycles of denaturation at 95℃ for 15 s and annealing/extension at 72℃ for 45 s. NET mRNA expression levels were analyzed by the $2^{-\triangle\triangle}$Ct methods with the following primers: sense 5'-CAT TGT TAT ATG CCG TTG AAA-3' and antisense 5'-GAT AGG CCA CTC TAG GAC GAA-3'. Data were normalized to the expression of β-actin, whose primers were: sense 5'-AAG ACC TGT ACG CCA ACA-3' and antisense 5'-CGG AGT ACT TGC GCT CAG-3'. All primers were designed and synthesized by Sangon Biotech (Shanghai, China). Each group had 5 samples, and the absorbance of each PCR band was measured and analyzed with the ImageMaster VDS analysis software (Amersham Biosciences, Buckinghamshire, UK).

Statistical analysis

GraphPad 5.0 and SPSS 21.0 were used for analysis. Measurement data conforming to normal distribution are mean ± standard deviation (SD), and were compared by one-way analysis of variance (ANOVA) for multiple groups. The LSD-t test was used for pairwise comparisons. Measurement data with non-normal distribution were expressed as median and compared among groups by the Wilcoxon's rank sum test. $P<0.05$ was considered statistically significant.

RESULTS

NIH-CPSI, IPSS and IIEF-5 scores served as criteria for assignment to the CP/CPPS+ED, CP/CPPS, ED, and control groups (Table I).

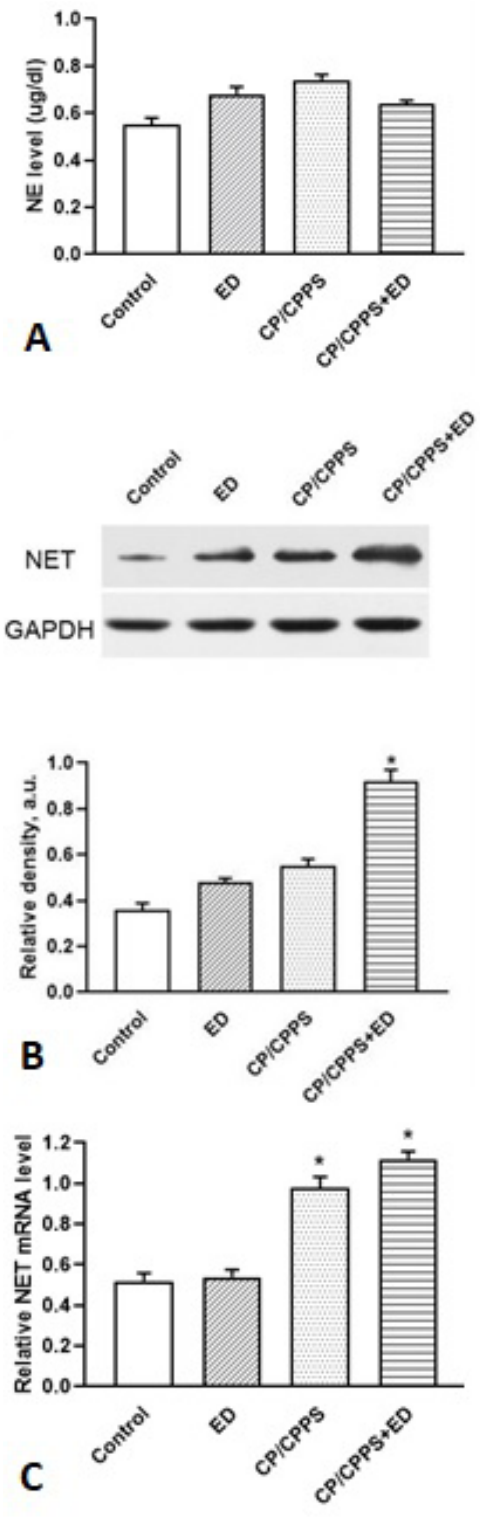

Fig. 1. Effect of serum IgG on norepinephrine (A), norepinephrine transporter (B) and Relative NET mRNA levels in mouse sympathetic neurons (C).

Table I. Disease scores in various groups.

Group	CP/CPPS+ED	CP/CPPS	ED	Control
Sample size	25	25	25	25
NIH-CPSI	≥5	≥5	<5	<5
IPSS	≥8	≥8	0	0
IIEF-5	<12	≥22	<12	≥22

Radioimmunoassay yielded no significant difference in NE levels among the four groups. After treatment of mouse sympathetic neurons with serum IgG from the CP/CPPS+ED, CP/CPPS, ED and Control groups, NE amounts in supernatants were 0.64±0.07μg/dl, 0.74±0.12μg/dl, 0.68±0.16μg/dl and 0.55±0.15μg/dl, respectively (P>0.05; Fig. 1A). These results indicated that cultured mouse sympathetic neurons secreted similar NE amounts upon treatment with IgG from different patient groups.

WB showed that NET protein levels were significantly higher in the CP/CPPS+ED group of mouse sympathetic neurons compared with the CP/CPPS, ED and Control groups (P<0.05; Fig. 1B). Meanwhile, there was no significant difference in NET protein amounts among the CP/CPPS, ED and Control groups (P>0.05). The relative expression levels of the NET protein were 0.92±0.25, 0.55±0.16, 0.48±0.09 and 0.36±0.15 in the CP/CPPS+ED, CP/CPPS, ED and Control groups, respectively (Fig. 1B). These results suggested that IgG from CP/CPPS+ED patients increased NET protein amounts in mouse sympathetic neurons.

qPCR indicated that NET mRNA expression levels were significantly higher in mouse sympathetic neurons treated with serum IgG from the CP/CPPS+ED and CP/CPPS groups compared with the ED and Control groups (P<0.05). Meanwhile, the ED and Control groups showed similar values (P>0.05). The relative NET gene expression levels in the CP/CPPS+ED, CP/CPPS, ED and Control groups were 1.12±0.19, 0.98±0.26, 0.54±0.18 and 0.52±0.20, respectively (Fig. 1C).

DISCUSSION

The present work demonstrated that serum IgG from LUTS-dominated CP/CPPS patients accompanied by ED could regulate NET expression in cultured mouse sympathetic neurons.

In this study, serum IgG from the CP/CPPS+ED, CP/CPPS, ED and Control groups, respectively, were administered to sympathetic neurons from neonate (p0) mice, and NE levels measured in supernatants were not significantly different among the four groups. However,

NET mRNA and protein expression levels in the CP/CPPS+ED group were significantly higher than control values. This suggested that when the LUTS-dominated CP/CPPS patients have concurrent ED, NE release and reuptake by peripheral sympathetic neurons may be enhanced in the short term, so that NE levels in the interstitial space of effector cells could still remain normal. However, long-term enhancement of NE reuptake might reduce interstitial NE amounts, eventually leading to the upregulation of NE receptors and increased sensitivity, which is manifested as a tension advantage for sympathetic neurons relative to parasympathetic neurons (Li *et al*., 2001; Bascks *et al*., 2001).

The above effects were not observed for individuals with CP/CPPS or ED alone, as there was no significant difference in NET protein amounts among the CP/CPPS, ED and Control groups ($P>0.05$), as shown above. These findings indicated that CP/CPPS and ED might act synergistically to produce IgG in amounts necessary to increase NET protein amounts in mouse sympathetic neurons.

Although NET mRNA expression levels were significantly higher in mouse sympathetic neurons treated with serum IgG from the CP/CPPS group in comparison with the ED and Control groups ($P<0.05$), such changes were not noted at the protein level. This could be explained by post-transcriptional regulation of NET expression. Alternatively, the changes in gene expression in the CP/CPPS group might have been insufficient to result in significant change at the protein level. Further investigation is warranted to verify these hypotheses. Rats with experimental autoimmune prostatitis, which mimics CP/CPPS in humans (features of depression, reduced sexual drive, premature ejaculation, and elevated threshold of penile erection), show significantly elevated prostatic interstitial infiltration by inflammatory cells and serum IL-1β and TNF-α amounts, alongside altered expression patterns of serotonin system regulators (Zhang *et al*., 2020). Finally, it is believed that multifaceted interactions exist between adaptive immunity and the central nervous system, which modulates the immune response by hormone secretion and neurotransmitters' effects on the immune system (Kipnis, 2016, Ransohoff and Brown, 2012).

The present study had limitations. First, whether other co-existing factors could promote the increase of sympathetic neuron excitability was not assessed. In addition, sympathetic nerve function was not directly assessed. Finally, mouse neurons were evaluated in vitro, and this may not faithfully reflect the human physiology. Therefore, further studies are needed to address these shortcomings.

CONCLUSION

The levels of the neurotransmitter NE secreted by sympathetic neurons is normal after treatment with serum IgG from LUTS-dominated CP/CPPS patients with ED, although NET expression is significantly increased. These findings suggest that the function of long-term NE reuptake by the presynaptic membrane of sympathetic nerves is enhanced. This reduces the levels of NE in the interstitial space of effector cells, leading to increased sensitivity of NE receptors, and ultimately increasing the excitability of sympathetic neurons.

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Ethical statement

This study was approved by the medical ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Approval no: TJ-IRB20180416).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Backs, J., Haunstetter, A., Gerber, S.H., Metz, J., Borst, M.M., Strasser, R.H., Kübler, W. and Haass, M., 2001. The neuronal norepinephrine transporter in experimental heart failure: Evidence for a posttranscriptional downregulation. *J. mol. cell Cardiol*., **33**: 461-472. https://doi.org/10.1006/jmcc.2000.1319

Bai, J., Wang, S., Liu, J., Ye, Z., Yu, X., Xi, Q., Hu, D. and Su, S., 2010. Characterization of circulating CD4+CD25high regulatory T cells in men with chronic prostatitis/chronic pelvic pain syndrome. *Urology*, **75**: 938-942. https://doi.org/10.1016/j.urology.2009.07.1229

Breser, M.L., Salazar, F.C., Rivero, V.E. and Motrich, R.D., 2017. Immunological mechanisms underlying chronic pelvic pain and prostate inflammation in chronic pelvic pain syndrome. *Front. Immunol*., **8**:898. https://doi.org/10.3389/fimmu.2017.00898

Bristow, M.R., 2000. Beta-adrenergic receptor blockade in chronic heart failure. *Circulation*, **101**: 558-569. https://doi.org/10.1161/01.CIR.101.5.558

Dean, R.C. and Lue, TF., 2005. Physiology of penile erection and pathophysiology of erectile dysfunction. *Urol. Clin. North Am*., **32**: 379-395. https://doi.org/10.1016/j.ucl.2005.08.007

Denfeld, Q.E., Habecker, B.A. and Woodward, W.R., 2018. Measurement of plasma norepinephrine and 3, 4-dihydroxyphenylglycol: Method development for a translational research study. *BMC Res. Notes*, **11**: 248. https://doi.org/10.1186/s13104-018-3352-3

Eisenhofer, G., 2001. The role of neuronal and extra neuronal plasma membrane transporters in the inactivation of peripheral catecholamines. *Pharmacol. Ther*., **91**: 35-62. https://doi.org/10.1016/S0163-7258(01)00144-9

Hou, Y., DeVoss, J., Dao, V., Kwek, S., Simko, J.P., McNeel, D.G., Anderson, M.S. and Fong, L., 2009. An aberrant prostate antigen-specific immune response causes prostatitis in mice and is associated with chronic prostatitis in humans. *J. clin. Invest*., **119**: 2031-2041. https://doi.org/10.1172/JCI38332

Huang, X., Qin, Z., Cui, H., Chen, J., Liu, T., Zhu, Y. and Yuan, S., 2020. Psychological factors and pain catastrophizing in men with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS): A meta-analysis. *Transl. Androl. Urol*., **9**: 485-493. https://doi.org/10.21037/tau.2020.01.25

Jang, T.L. and Schaeffer, A.J., 2003. The role of cytokines in prostatitis. *World J. Urol*., **21**: 95-99. https://doi.org/10.1007/s00345-003-0335-2

Jung, J., Ahn, H.K. and Huh, Y., 2012. Clinical and functional anatomy of the urethral sphincter. *Int. Neurourol. J*., **16**: 102-106. https://doi.org/10.5213/inj.2012.16.3.102

Kipnis, J., 2016. Multifaceted interactions between adaptive immunity and the central nervous system. *Science*, **353**: 766-771. https://doi.org/10.1126/science.aag2638

Li, H., Ma, S.K., Hu, X.P., Zhang, G.Y. and Fei, J., 2001. Norepinephrine transporter (NET) is expressed in cardiac sympathetic ganglia of adult rat. *Cell Res*., **11**: 317-320. https://doi.org/10.1038/sj.cr.7290102

Ménard, C., Pfau, M.L., Hodes, G.E. and Russo, S.J., 2017. Immune and neuroendocrine mechanisms of stress vulnerability and resilience. *Neuropsychopharmacology*, **42**: 62-80. https://doi.org/10.1038/npp.2016.90

Motrich, R.D., Maccioni, M., Molina, R., Tissera, A., Olmedo, J., Riera, C.M. and Rivero, V.E., 2005. Presence of INFgamma-secreting lymphocytes specific to prostate antigens in a group of chronic prostatitis patients. *Clin. Immunol*., **116**: 149-157. https://doi.org/10.1016/j.clim.2005.03.011

Pontari, M.A. and Ruggieri, M.R., 2008. Mechanisms in prostatitis/chronic pelvic pain syndrome. *J. Urol.*, **179**: S61-S67. https://doi.org/10.1016/j.juro.2008.03.139

Ramzan, F. and Ramzan, M.H., 2017. Effect of variable kisspeptin doses on prostatic citric acid levels in male mice. *Pakistan J. Zool.*, **49**: 379-380. https://doi.org/10.17582/journal.pjz/2017.49.1.sc2

Ransohoff, R.M. and Brown, M.A., 2012. Brown MA. Innate immunity in the central nervous system. *J. clin. Invest*., **122**: 1164-1171. https://doi.org/10.1172/JCI58644

Rosen, R.C., Cappelleri, J.C., Smith, M.D., Lipsky, J. and Pena, B.M., 1999. Development and evaluation of an abridged, 5-item version of the international index of erectile function (IIEF-5) as a diagnostic tool for erectile dysfunction. *Int. J. Impot. Res*., **11**: 319-326. https://doi.org/10.1038/sj.ijir.3900472

Stephens, M.A.C. and Wand, G., 2012. Stress and the HPA axis: Role of glucocorticoids in alcohol dependence. *Alcohol. Res*., **34**:468-83. https://psycnet.apa.org/record/2013-28231-011

Umbrăveanu, I., Ceban, E. and Banov, P., 2018. Lower urinary tract symptoms and erectile dysfunction in men from the Republic of Moldova. *J. Med. Life*, **11**: 153-159. https://doi.org/10.1016/j.eururo.2012.10.042

Wagenlehner, F.M., van Till, J.O., Magri, V., Perletti, G., Houbiers, J.G., Weidner, W. and Nickel, J.C., 2013. National institutes of health chronic prostatitis symptom index (NIH-CPSI) symptom evaluation in multinational cohorts of patients with chronic prostatitis/chronic pelvic pain syndrome. *Eur. Urol*., **63**: 953-959.

Zahniser, N.R. and Doolen, S., 2001. Chronic and acute regulation of Na+/Cl- dependent neurotransmitter transporters: Drugs, substrates, presynaptic receptors, and signaling systems. *Pharmacol. Ther*., **92**: 21-55. https://doi.org/10.1016/S0163-7258(01)00158-9

Zhang, Y., Li, X., Zhou, K., Zhou, M., Xia, K., Xu, Y., Sun, X., Zhu, Y., Cui, C. and Deng, C., 2020. Influence of experimental autoimmune prostatitis

on sexual function and the anti-inflammatory efficacy of celecoxib in a rat model. *Front. Immunol*., **11**: 574212. https://doi.org/10.3389/fimmu.2020.574212

Zhang, Y., Zheng, T., Tu, X.A., Chen, X., Wang, Z., Chen, S., Yang, Q., Wan, Z., Han, D., Xiao, H. and Sun, X., 2016. Erectile dysfunction in chronic prostatitis/chronic pelvic pain syndrome: Outcomes from a multi-center study and risk factor analysis in a single center. *PLoS One*, **11**: e0153054. https://doi.org/10.1371/journal.pone.0153054