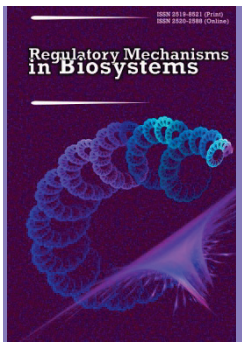

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The diagnostic value of the NO-synthase, Ca^{2+}- and Na^{+}-dependent ATP-hydrolase systems and the therapeutic potential of NO-stimulators in erectile dysfunction of men injured as a result of combat operations (combat trauma)

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Erectile dysfunction (ED), as a sexual disorder, is characterized by persistent inability to achieve and maintain an erection sufficient for satisfactory sexual intercourse. Among the numerous pathological conditions that precede ED or are complicated with it, neurotic disorders, metabolic disorders, blood vessels diseases, partial androgen deficiency, combat injuries, etc. take precedence. An injury is not only a physical, but also a psychological trauma, which is often stretched over some period of time. Post-traumatic stress disorder, depressive states, and post-traumatic chronic pain may develop as a result of a combat wound. These conditions also affect sexual function. The search for sensitive indicators that would reflect both the physiological status of the organism and metabolic changes inside the cell is an urgent issue of modern biochemistry, physiology and medicine in general. This study involved results of examination and treatment of 136 men, participants in hostilities, with sexual dysfunction and leading complaints of erectile dysfunction. The control group consisted of 48 clinically healthy men without complaints of sexual dysfunction or cardiac, neurological or endocrinological pathology. According to the form of ED, patients were divided into groups: patients with psychogenic ED after a combat injury (n = 84) and patients with ED of mixed genesis, which included participants in hostilities with endothelial dysfunction, metabolic syndrome, dyslipidemia, hypertension, coronary heart disease, late hypogonadism (n = 52). It was shown that the development of psychogenic and mixed forms of ED is mostly associated with stress hyperprolactinemia with normal indicators of cavernous dopplerography, lipid metabolism and androgen levels. The Na^{+},K^{+}-ATPase activity of peripheral blood lymphocytes was significantly decreased in men of different age groups with a mixed form of ED. The Ca^{2+},Mg^{2+}-ATPase activity of plasma membrane and endoplasmic reticulum decrease was significantly decreased in men with ED of both psychogenic and mixed forms, but a more pronounced decrease was observed with increasing age of patients. Complex treatment of patients with a mixed form of ED with sildenafil leads to a significant increase in Na^{+},K^{+}-ATPase activity and Ca^{2+},Mg^{2+}-ATPase activity of endoplasmic reticulum in lymphocytes in patients with ED of mixed genesis. Complex treatment of young age patients with psychogenic ED led to a decrease in the arginase activity in patients with both psychogenic and mixed ED. A decrease in cNOS activity was accompanied by a corresponding increase in iNOS activity in patients with mixed form of ED. Treatment of patients with the use of sildenafil led to non significant increase in cNOS activity in the lymphocytes of patients of both age groups.

Keywords: combat trauma; erectile dysfunction; lymphocytes; Na^{+},K^{+}-ATPase; Ca^{2+},Mg^{2+}-ATPase; arginase; NO-synthase.

Introduction

Erectile dysfunction (ED) is a stress factor for the patients, which negatively affects the quality of life and relationships. Male sexual disorders are often not a separate nosology, but reflect manifestations of diseases of the whole body. ED, as a sexual disorder, is characterized by persistent inability to achieve and maintain an erection sufficient for satisfactory sexual intercourse. ED is closely related to the state of physical and psychological well-being and has a significant impact on the quality of life not only of the patients themselves but their partners and family members (Breyer et al., 2014; Castillo et al., 2022). Recent epidemiological studies have revealed a significant increase of ED cases worldwide (Breyer et al., 2014; Snoga et al., 2022). Erection is a neurovascular phenomenon that is under hormonal control, which includes arterial dilatation, relaxation of trabecular smooth muscles and activation of the corporal venoocclusive mechanism (Ho et al., 2019; Kim et al., 2021; Souza et al., 2022). Among the numerous pathological conditions that precede ED or are complicated with it, neurotic disorders, metabolic disorders, blood vessel diseases, partial androgen deficiency, combat injuries, etc. take precedence (Breyer et al., 2014; Ho et al., 2019; Bird et al., 2021; Snoga et al., 2022). An injury is not only a physical, but also a psychological trauma, which is often stretched over some period of time. It also should not be forgotten that the drugs prescribed by the doctor can affect libido and erectile function. Post-traumatic stress disorder, depressive states, and post-traumatic chronic pain may arise as a result of a combat injury (Breyer et al., 2014; Salman et al., 2016; Snoga et al., 2022). These conditions also affect sexuality.

The search for sensitive indicators that would reflect both the physiological status of the organism and metabolic changes inside the cell is an urgent issue of modern biochemistry, physiology and medicine in general. One of such indicators can be ATPases, which form an ion gradient across membranes, membrane potential, regulate water-salt exchange and cell metabolism (Kosterin et al., 2016; Clausen et al., 2017; Dyla et al., 2020; Xu & Van Remman, 2021; Staehr et al., 2023; Contreras et al., 2024). Living cells maintain a low intracellular concentration of Na^{+} (5–15 mM) despite the high concentration of this ion in the extracellular medium (~145 мМ). The transmembrane gradient of Na^{+} ions is supported by

Na^+,K^+-ATPase. In many pathological conditions, for example, myocardial ischemia, diabetic cardiomyopathy, the growth of cancer tumors, etc., an increase in the intracellular concentration of Na^+ has been noted (Alevizopoulos et al., 2014; Babsky, 2014). The most important reasons for this phenomenon are energy deficit with the subsequent decrease in Na^+,K^+-ATPase activity, as well as cellular acidification, which stimulates the Na^+/H^+ exchanger in the plasma membrane and the influx of Na^+ ions into the cell. Na^+ ions are also involved in other transport mechanisms, in particular, those that regulate intracellular pH and, hence, the functioning of mitochondrial H^+-ATPase (Clausen et al., 2017; Cao et al., 2020; Contreras et al., 2024). Ca^{2+} homeostasis is an important indicator of the cell's functional state. Ca^{2+} is an intracellular messenger which regulates almost all cellular functions, including muscle contraction and relaxation (Kosterin et al., 2016; Dyla et al., 2020; Xu, Van Remman, 2021; Kho, 2023). The functioning of Ca^{2+},Mg^{2+}-ATPases of the plasma membrane and endoplasmic reticulum, which ensure the maintenance of intracellular Ca^{2+} homeostasis, is modulated by both extracellular and intracellular factors.

On the other hand, nitric oxide (NO) is a key signaling molecule involved in the regulation of many biological and physiological processes in mammals. Extensive experimental and clinical experience indicates that impaired bioavailability and response to endogenous NO confers risk for cardiovascular, pulmonary, endothelial, renal, and hepatic diseases, as well as ED. Some of these diseases are currently treated with organic nitrates (such as trinitroglycerol), other NO donors, or nitrovasodilators that release NO by decomposition or bioconversion to activate soluble guanylyl cyclase (sGC) (Danylovych et al., 2014; Opelt et al., 2018; Pearson, Butler, 2021; Souza et al., 2022). However, the use of these medicines is limited (Salvagno et al., 2024). Despite symptomatic improvement after the use of organic nitrates in patients with cardiovascular disease, there is no objective evidence that such treatment reduces mortality. Therefore, drugs that activate sGC in NO-independent way may have significant advantages over current therapies. Recent discoveries of substances that stimulate or activate sGC independently of NO release have allowed the development of entirely different approaches to achieving this pharmacological goal (Andersson, 2018). NO-independent but heme-dependent sGC stimulators, as well as NO- and heme-independent sGC activators, are valuable tools that can help elucidate the physiology and pathophysiology of NO-sGC-cGMP pathways in more detail. It is expected that these agents will be widely used in the treatment of relevant diseases based on *in vitro* and *in vivo* clinical studies.

Penile erection is caused by relaxation of cavernous smooth muscles, increased blood flow, and cessation of venous outflow. These processes are regulated by spinal reflexes, which depend on visual, imaginal, and olfactory stimuli generated through the central nervous system, as well as tactile stimulation of the penis. Medicines can have an enhancing or inhibiting effect both on the nervous regulation of this reflex and on the cavernous tissue. The balance between contraction and relaxation factors governs penile detumescence/tumescence states. Drugs can increase the level of cytosolic calcium, preventing an erection. In contrast, agents that reduce the concentration of cytosolic calcium relax smooth myocytes and initiate an erection. The effectiveness of ED treatment with phosphodiesterase inhibitors, especially type 5, α-adrenoceptor antagonists, as well as dopamine agonists, is due to these mechanisms of action through penile tissue or the central nervous system. Cavernous nerves contain neuronal NO synthase, which synthesizes NO from L-arginine (Andersson, 2018). NO is produced during stimulation of the cavernous nerve. PDE-5 inhibitors can affect noradrenergic tone through effects of NO on nerve endings (Ostroff, 2018; Zhu et al., 2020).

There is a serious debate about the role of androgens in the development and maintenance of erectile function (Aversa et al., 2019; Erkon et al., 2019; Zhu et al., 2020). Recent studies indicate that testosterone plays a key role in erectile function. Without an adequate level of testosterone, NOS and PDE-5 gene expression is disrupted (Aversa et al., 2019; Zhu et al., 2020). Most researchers adhere to the theory that testosterone is more responsible for sexual desire than for the direct physiology of ED (Azevedo et al., 2024). Therefore, testosterone concentration is more related to psychogenic rather than organic erectile function. Thus, ATPases, NO-synthases, and other enzymes can be used as markers to determine general "vascular" health, preventing the manifestation of disease and providing monitoring of treatment. The purpose of the work is to assess changes in the activities of Na^+,K^+-ATPase, Ca^{2+},Mg^{2+}-ATPase, arginase, NO-synthase in peripheral blood lymphocytes and the possibilities of their correction during complex treatment with the use of sildenafil.

Materials and methods

Patients and bioethics approval. This study involved results of examination and treatment of 136 men, participants in hostilities, with sexual dysfunction and leading complaints of ED. The control group consisted of 48 clinically healthy men. They were somatically healthy and had no sexual dysfunction, cardiac, endocrinological or neurological pathology. The control group was divided into two age subgroups: 30 men aged 29–39 and 18 men aged 40–53. All patients underwent a comprehensive clinical, laboratory and instrumental examination at the urology clinic of the Danylo Halytsky Lviv National Medical University, after undergoing treatment and rehabilitation for combat trauma, with complaints of ED persisting for more than one month. All patients underwent a thorough medical history, physical, clinical and psychological examination. Baseline laboratory testing included complete blood and urine tests, blood glucose, lipid profile, and hormonal profile analysis. Among the instrumental studies, patients underwent Doppler ultrasonography of the cavernous arteries before and after pharmacological induction of erection.

The research was carried out in compliance with the principles of medical ethics and the protection of patients' rights, human dignity and moral and ethical norms, in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, the laws of Ukraine; permission of the Bioethics Committee of the Danylo Halytsky Lviv National Medical University (protocol No. 7 from 26 June 2023).

According to the form of ED, patients were divided into groups: group 1 consisted of patients with psychogenic ED after a combat injury (n = 84); group 2 consisted of patients with ED of mixed genesis, included patients, participants in hostilities with endothelial dysfunction, metabolic syndrome, dyslipidemia, hypertension, coronary artery disease, late hypogonadism (n = 52).

In turn, each group was divided into two age subgroups. The young age group (subgroup 1.1) consisted of patients with psychogenic ED after a combat injury aged 20–39 years (n = 51). The middle age group (subgroup 1.2) consisted of patients with psychogenic ED after a combat injury aged 40–53 years (n = 33). The young age group (subgroup 2.1) consisted of patients with ED of mixed genesis, included patients, participants in hostilities with endothelial dysfunction, metabolic syndrome, dyslipidemia, hypertension, coronary artery disease, late hypogonadism, aged 20–39 years (n = 32). The middle age group (subgroup 2.2) consisted of patients with ED of mixed genesis and included patients, participants in hostilities with endothelial dysfunction, metabolic syndrome, dyslipidemia, hypertension, coronary artery disease, late hypogonadism, aged 40–53 years (n = 20).

Biochemical assays. Mononuclear lymphocytes isolated from human peripheral blood are the most convenient model, which quickly reacts to harmful endogenous and endogenous factors. Peripheral blood mononuclear lymphocytes were isolated from heparinized fresh blood, diluted 3 times with Hanks' solution without Ca^{2+} and Mg^{2+} in a ficol-urografin gradient (Fafula et al., 2024). Cells were counted in a Goryaev chamber using 0.1% trypan blue as a dye. Viability of lymphocytes was assessed by trypan blue staining and was at least 95% in all experiments. To study the latent ATPase, NO-synthase, and arginase activities, 0.1–0.2% saponin solution was added to the lymphocyte suspension. The protein content was determined by the Lowry et al. method. Enzyme activities were measured by spectrophotometer V-1150 (China, 2023).

Oubain-sensitive Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase activity was determined according to the method described in paper (Fafula et al., 2024). Na^+,K^+-ATPase activity was taken as the activity inhibited by 0.1 mM oubain and expressed in μmol of Pi/min·mg of protein. The activity inhibited by 1 mM EGTA was taken as the activity of Ca^{2+},Mg^{2+}-ATPase and expressed in μmol of Pi/min·mg of protein. Determining the content of inorganic phosphate was carried out according to the Fiske-Subbarow method.

NOS activity was determined in a reaction mixture with the following composition: 2.5 mL of 0.1 M Tris-HCl buffer (pH 7.4), which contained 10 mM $CaCl_2$ and 5 mM $MgCl_2$; 0.3 mL of an aqueous solution of 200 μM L-arg (final concentration in the sample is 20 μM); 0.1 mL of an aqueous solution of 1 mM NADPH (Onufrovych et al., 2024). The reaction was started by introducing a 0.1 mL aliquot of permeabilized lymphocytes. The control sample was prepared similarly to the experimental sample, but without the addition of NADPH. Substrate-free oxidation of NADPH was studied in a reaction mixture containing 0.3 mL of water instead of arginine. To increase the specificity of the calculation of NADPH oxidation, which is associated only with NOS activity, an additional sample was used. A 0.3 mL aqueous solution of the NOS inhibitor (L-NAME) in a concentration similar to the substrate was used instead of L-arg. The difference between the amount of NADPH oxidation with L-arg and with the inhibitor gives the amount of NADPH oxidation dependent on the competitive non-selective inhibitor of all NOS isoforms, i.e. NO-synthase activity. Test samples were spectrophotometer evaluated against control samples at 340 nm, then incubated for 20 min at 37 ºC. The reaction was stopped by adding 0.02 mL of 0.02% NaN_3 and the decrease in extinction was recorded. NOS activity was expressed in nanomoles of NADPH oxidized within 1 min per 1 mg of protein. The method of determining the activity of inducible NO-synthase is similar to the previous one with some differences: to determine the activity of Ca^{2+}-independent iNOS, 2 μmol of EGTA was added to the incubation mixture instead of $CaCl_2$. Constitutive NO synthase (cNOS) activity was calculated by subtracting iNOS activity from total NOS activity.

Arginase activity was determined by measuring levels of urea production in a reaction mixture with the following composition (mmol/mL): L-arginine – 100, $MnCl_2$ – 2, Tris-HCl – 20 (pH 9.5). The reaction was stopped by adding 1 mL 50% trichloroacetic acid. The urea content was determined after centrifugation in the supernatant by means of spectrophotometer at 520 nm according to the assay kit "Simko Ltd".

Statistical analysis. The results are presented as the mean ± standard error (x ± SE). Analysis of variance (ANOVA) was used to compare the difference in the means between studied groups. Differences were considered statistically significant at $P < 0.05$.

Results

Clinical and laboratory indicators show that a normal coefficient of atherogenicity up to 3 units, calculated by the levels of cholesterol, triglycerides, β-lipoproteins, LDL, LDL, and HDL in the blood was observed in 48 of 84 men (57.1%) with combat trauma and psychogenic ED (young age group) and in 14 of 52 men (26.9%) (middle age group) (Table 1). The atherogenicity coefficient was higher than the norm in patients with mixed form of ED. The level of total testosterone was normal in young age patients with psychogenic ED (>3 ng/mL). A normal level of total testosterone was determined in 23 of 32 middle age patients with psychogenic ED (71.9%). The testosterone level was within normal values in young age patients with a mixed form of ED. In 18 (90.0%) out of 20 middle age patients with mixed ED testosterone level was below normal and in 2 out of 20 patients (10.0%) it was within the normal range.

Table 1
Clinical and laboratory indicators of patients with various forms of ED (x ± SE, N = 136)

Groups	Young age group (n = 84)				Middle age group (n = 52)			
Clinical suspicion	number of patients with ED	coefficient of atherogenicity, units	general testosterone, ng/mL	prolactin, ng/mL	number of patients with ED	coefficient of atherogenicity, units	general testosterone, ng/mL	prolactin, ng/mL
Psychogenic ED	52	2.1 ± 0.4 (n = 48) 3.6 ± 0.6 (n = 4)	3.5 ± 0.5 (n = 52)	21.5 ± 7.2 (n = 42) 57.6 ± 9.5 (n = 10)	32	2.2 ± 0.5 (n = 14) 3.7 ± 0.9 (n = 18)	3.3 ± 0.5 (n = 23) 2.2 ± 0.5 (n = 9)	21.3 ± 7.2 (n = 18) 42.6 ± 8.0 (n = 14)
Mixed ED (endothelial dysfunction, metabolic syndrome)	32	4.2 ± 1.1 (n = 32)	3.4 ± 0.6 (n = 32)	24.2 ± 7.2 (n = 24) 45.4 ± 8.6 (n = 8)	20	4.3 ± 1.2 (n = 20)	3.2 ± 0.5 (n = 2) 2.3 ± 0.4 (n = 18)	22.8 ± 7.3 (n = 8) 51.3 ± 8.5 (n = 12)

Normally, prolactin, which increases the number of receptors for LH, is necessary to maintain the maximum steroidogenic activity of Leydig cells in the presence of LH. We determined the level of prolactin up to 25 ng/mL (normal) in 42 of 52 patients (80.8%) of young age patients with psychogenic ED. In 10 patients of this subgroup, the prolactin level exceeded the norm and was 57.6 ± 9.5 ng/mL. In 18 out of 32 middle age patients with psychogenic ED (56.3%), the prolactin level was within the normal range, and in 14 it exceeded the norm. In 24 out of 32 (75.0%) young age patients with mixed ED ptolactin level was within the normal range, and in 8 it exceeded the norm. In 8 out of 20 (40.0%) middle age patients with mixed ED prolactin level was within the normal range, and in 12 patients (60.0%) it exceeded the norm. Increased concentration of prolactin, which is observed as functional hyperprolactinemia, for example, in stressful situations, negatively affects the secretory function of Leydig cells, the secretion of gonadotropins, and erectile function. According to the data in Table 1, it can be concluded that the development of psychogenic and mixed forms of ED is often associated with stress hyperprolactinemia with normal indicators of cavernous dopplerography, lipid metabolism and androgen levels.

When studying different age groups of men with combat trauma, it was found that Na^+,K^+-ATPase activity, Ca^{2+},Mg^{2+}-ATPase of plasma membrane and endoplasmic reticulum in the blood lymphocytes of men of the middle age group was somewhat lower than in the young age group, however these differences are not significant (Table 2). There is no significant difference in enzymes activities between age subgroups both in men psychogenic ED due to combat trauma and healthy men. In the lymphocytes of men with mixed ED the Na^+,K^+-ATPase activity was lower 1.40-fold ($P < 0.05$) in both age groups compared to the control group. The Ca^{2+},Mg^{2+}-ATPase activity of plasma membrane was lower 1.37-fold ($P < 0.05$) in the young age group and 1.29-fold ($P < 0.05$) in the middle age group compared to the control group. Similar changes were observed for Ca^{2+},Mg^{2+}-ATPase activity of the endoplasmic reticulum of peripheral blood lymphocytes in men with a mixed form of ED. It can be seen that Ca^{2+},Mg^{2+}-ATPase activity of both plasma membranes and endoplasmic reticulum decreasesdin men with ED of mixed genesis, but a more pronounced decrease was observed with increasing age of patients.

The change in the activity of ATP-hydrolase systems after the complex treatment of patients, in particular with the help of sildenafil (on demand, but at least 8 times/month), is shown in the Table 3.

Table 2
Activities of ATP-hydrolase systems of peripheral blood lymphocytes in men with ED (x ± SE, N = 184)

Enzyme activity	Control group		Psychogenic ED		Mixed ED	
	young age group (n = 30)	middle age group (n = 18)	young age group (n = 51)	middle age group (n = 33)	young age group (n = 32)	middle age group (n = 20)
Na^+,K^+- ATPase, μmol P_i/min·mg protein	4.21 ± 0.52	4.00 ± 0.48	3.70 ± 0.35	3.50 ± 0.32	3.00 ± 0.30*	2.80 ± 0.29*
Ca^{2+},Mg^{2+}- ATPase of plasma membrane, μmol P_i/min·mg of protein	2.91 ± 0.32	2.71 ± 0.20	2.55 ± 0.22	2.45 ± 0.27	2.13 ± 0.21*	2.10 ± 0.21*
Ca^{2+},Mg^{2+}-ATPase of endoplasmic reticulum, μmol P_i/min·mg of protein	2.76 ± 0.27	2.52 ± 0.24	2.32 ± 0.23	2.15 ± 0.31	2.06 ± 0.20*	1.87 ± 0.20*

Note: * – $P < 0.05$ regarding control group (healthy men).

It can be seen that complex treatment of patients with psychogenic ED (young age group) practically does not lead to a change in the Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}- ATPase activities. However, complex treatment of patients with a mixed form of ED (middle age group) with the use of sildenafil leads to a significant increase in the activity of Na^+,K^+-ATPase and Ca^{2+},Mg^{2+}-ATPase of endoplasmic reticulum. After treatment the enzyme activities approach to control values. A correction of pathogenetic changes in carbohydrate, fat metabolism and late hypogonadism, as well as standardized and rational, clarifying psychotherapy, was carried out simultaneously.

Table 3

Activities of ATP-hydrolase systems of peripheral blood lymphocytes in men with ED after treatment (x ± SE, N = 71)

Enzyme activity	Psychogenic ED young age group (n = 51)		Mixed ED middle age group (n = 20)	
	before treatment	after treatment	before treatment	after treatment
Na^+,K^+- ATPase, μmol P_i/min·mg protein	3.70 ± 0.35	3.81 ± 0.33	2.80 ± 0.29	3.95 ± 0.33*
Ca^{2+},Mg^{2+}- ATPase of plasma membrane, μmol P_i/min·mg of protein	2.55 ± 0.22	2.68 ± 0.33	2.10 ± 0.21	2.64 ± 0.29
Ca^{2+},Mg^{2+}-ATPase of endoplasmic reticulum, μmol P_i/min·mg of protein	2.32 ± 0.23	2.53 ± 0.31	1.87 ± 0.20	2.68 ± 0.21**

Note: * – P < 0.05; ** P < 0.01 regarding values before treatment.

It is known that one of the most widespread mechanisms of ED development is endothelial dysfunction, which is associated with insufficient synthesis of NO by the vascular endothelium in response to adequate stimuli (hemodynamic action, cholinergic stimulation, etc.). It was found that arginase activity in the blood lymphocytes of patients with psychogenic ED was 2.39-fold (P < 0.001) higher in the young age group and 2.53-fold (P < 0.001) higher in the middle age group than in the control group (Table 4). Complex treatment of young age patients with psychogenic ED, in particular with sildenafil 50 mg, with a frequency of at least 8 tablets per month, led to a decrease in the arginase activity in patients with both psychogenic and mixed ED (Table 5). In blood lymphocytes of patients with psychogenic ED the cNOS activity had a tendency to decrease in both age groups, however these changes are not significant compared to the control groups. The iNOS activity in these subgroups was significantly higher than the control values (P < 0.001). In patients with mixed form of ED the cNOS activity was 1.61-fold (P < 0.05) lower in the young age group and 1.31-fold (P < 0.05) lower in the middle age group compated to control groups. A decrease in cNOS activity was accompanied by a corresponding increase in iNOS activity in patients with mixed form of ED. Treatment of patients with the use of sildenafil led to non significant increase in cNOS activity in the lymphocytes of patients of both age groups. Simultaneous determination of iNOS activity showed a significant decreese in enzyme activity after treatment to control values (P < 0.05).

Table 4

Arginase and NO-synthase activity of peripheral blood lymphocytes in men with ED (x ± SE, N = 184)

Enzyme activity	Control group		Psychogenic ED		Mixed ED	
	young age group (n = 30)	middle age group (n = 18)	young age group (n = 51)	middle age group (n = 33)	young age group (n = 32)	middle age group (n = 20)
Arginase, nmol urea /min·mg of protein	58.36 ± 6.22	55.60 ± 5.62	139.24 ± 14.26***	140.62 ± 12.50***	169.12 ± 15.56***	180.45 ± 19.28***
cNOS, pmol NADPH/min·mg of protein	45.82 ± 4.08	38.92 ± 3.80	35.80 ± 3.88	34.51 ± 4.37	28.45 ± 2.35*	29.62 ± 2.48*
iNOS, pmol NADPH/min·mg of protein	1.52 ± 0.02	1.53 ± 0.02	12.25 ± 2.03***	13.08 ± 2.12***	14.26 ± 1.87***	15.55 ± 1.98***

Note: * P < 0.05; *** P < 0.001 regarding control group (healthy men).

Table 5

Arginase and NO-synthase activity of peripheral blood lymphocytes in men with ED after treatment (x ± SE, N = 103)

Enzyme activity	Psychogenic ED young age group (n = 51)		Mixed ED young age group (n = 32)		Mixed ED middle age group (n = 20)	
	before treatment	after treament	before treatment	after treatment	before treatment	after treatment
Arginase, nmol urea /min·mg of protein	139.24 ± 14.26	82.22 ± 9.26***	169.12 ± 15.56	101.50 ± 8.23***	180.45 ± 19.28	120.40 ± 14.37*
cNOS, pmol NADPH/min·mg of protein	35.80 ± 3.88	38.26 ± 2.47	28.45 ± 3.35	35.22 ± 2.14	29.62 ± 3.48	32.26 ± 4.10
iNOS, pmol NADPH/min·mg of protein	12.25 ± 2.03	5.46 ± 0.70***	14.26 ± 1.87	7.14 ± 0.78***	15.55 ± 1.98	8.68 ± 1.05**

Note: * – P < 0.05; ** P < 0.01; *** P < 0.001 regarding values before treatment.

Discussion

It is known that the pathogenesis of ED is closely related to the functioning of the endothelium of cavernous bodies (Ma et al., 2020; Souza et al., 2022). Endothelium is a receptor-effector organ which responds to many physical or chemical stimuli (Alkan et al., 2017; Souza et al., 2022). However, studying endothelial function has huge challenges. Peripheral blood lymphocytes are able to respond quickly to any changes in homeostasis in the organism. Since the modulation of enzyme activity occurs much earlier than change in morphological and biochemical parameters, lymphocytes are a convenient object for carrying out studies related to various pathologies, in particular ED (Fafula et al., 2024; Onufrovych et al., 2024). They express unique antigen-specific receptors on membrane surface, which actually correspond to many environmental antigens. Lymphocytes have similar receptors to nerve or endothelial cells and actively participate in the induction and regulation of the body's stress response. An impaired function and structural disorganization of plasma membranes are universal reactions of cells during pathological processes of various genesis. The impact of various damaging factors on cells, both psychogenic and organic in nature, leads to universal response due to the action of similar molecular mechanisms of damage, regardless of the cause. These include, first of all, the intensification of lipid peroxidation, oxidative modification of protein molecules, activation of endogenous phospholipases and proteases, and a decrease in the activity of the antioxidant defense system (Opelt et al., 2018; Salvagno et al., 2024).

Molecular changes in the plasma membranes of lymphocytes and other blood cells during hyperglycemia are characterized by the disorganization of the phospholipid spectrum, a change in the fatty acid composition of phospholipids, an increase in the cholesterol content, an increase in the microviscosity of the lipid bilayer of the membranes, as well as a change in the activity of cell ion transporting systems. It has also been shown that oxidative stress, which develops in various pathologies, is accompanied by an increase in the content of hydroperoxides of phospholipids in membranes, the level of the oxidized form of cholesterol, a decrease in the content of tocopherol, a change in the fatty acid composition of phospholipids and the activity of ion transporting systems (Salvagno et al., 2024). The patterns of changes in the structure and function of lymphocytes can be extrapolated to other cells with some corrections, due to the specificity of cells. Thus, it is assumed that lymphocytes can be a convenient and adequate model for studying a number of pathobiochemical indicators in the development of ED.

Since testosterone is the main male sex hormone, which regulates many functions of the male body, affects libido, stereotyped sexual behavior and erectile function, as well as the distribution and metabolism of

adipose tissue, its concentration was determined in patients. Biologically active testosterone is about 43% of the total (1–3% free, 40% testosterone bound to albumin). Since the main part of blood testosterone is biologically inactive (about 57%), binding to a specific transport protein as sexsteroid-binding globulin (SSBG), and when the concentration of SSBG increases with age, the effects of estrogens increase, the ratio of free testosterone to free estradiol decreases, even with a normal concentration of total testosterone. A decrease in the secretion of androgens and a decrease in the concentration of their receptors during the development of partial androgenic age-related deficiency in men occurs much earlier than previously thought (7–30% of men after the age of 30). This is closely related to other dysmetabolic changes, and is one of the key points in the development of ED (Zhu et al., 2020; Fan et al., 2021).

Diabetes, arterial hypertension, coronary heart disease are main chronic diseases that affect the age of onset of androgen deficiency (a decrease in testosterone concentration by 10–15% compared to the norm on average). This is due to a decrease in the number of Leydig cells as a result of deterioration of the blood supply to the testicular tissue, as well as a decrease in LH receptors on their surface (Erkan et al., 2019; Souza et al., 2022).

Data are now emerging on the effects of combat wounds on ED (Breyer et al., 2014; Castillo et al., 2022; Onufrovych et al., 2024; Vorobets et al., 2024). Injury is not only physical, but also psychological trauma. In general, war is a survival experience where the adrenaline level increases, and a person in a state of heightened combat readiness often ignores fatigue, hunger, pain and sexual desire. For some, sex may lose its relevance altogether. Post-traumatic stress disorder, depressive states, and post-traumatic chronic pain may develop as a result of combat experience or injury. These conditions also affect sexuality. In addition to ED, men may develop decreased libido, premature ejaculation, and anorgasmia as a result of combat trauma (Castillo et al., 2022).

In recent decades, a sufficient number of scientific facts regarding ED have been accumulated. They confirm the significant influence of age of men not only on the reduction of sexual desire and activity, but also on the development of osteoporosis, anemia, muscle weakness, obesity. Therefore, additional diagnostic methods, such as determination of Ca^{2+},Mg^{2+}- and Na^+,K^+-ATPase activity and nitrate content in peripheral blood lymphocytes, add complexity to the understanding of pathophysiological and pathochemical mechanisms of the development of ED.

The ion homeostasis is an important indicator of the body's functional activity. It is provided by the superposition of various ion transporting systems of the cell. It is now known that changes in the concentration of Na^+ ions in cells reflect their physiological or pathological state (Alevizopoulos et al., 2014; Babsky, 2016). Na^+,K^+-ATPase is main enzyme that regulates the concentration of Na^+ and K^+ ions (Clausen et al., 2017; Contreras et al., 2024). Na^+,K^+-ATPase ensures maintenance of the electrochemical gradient and osmotic potential of monovalent ions in the cell, which is a necessary condition for their functioning. The enzyme plays a key role in the regulation of many cellular functions and processes which depend on the ion concentration and electrical gradients (Kho, 2023; Staehr et al., 2023).

During a comparative analysis of changes in total tissue [Na^+]t and intracellular [Na^+]i in RIF-1 fibrosarcoma tumors, it was found that [Na^+]i level increases as a result of a decrease in the cell's bioenergetic status and Na^+,K^+-ATPase activity (Babsky, 2014). According to modern ideas, changes in ATPase activity and the concentration of cations in cells are mainly associated with the modification of lipid components of biomembranes, disruption of lipid-lipid and lipid-protein interactions, changes in the physicochemical properties of membrane structures, etc. (Clausen, 2017; Staehr, 2023). Inhibition of ion pumps leads to an increase in the concentration of sodium and calcium in cells (Contreras, 2024). This is accompanied by an imbalance of intracellular regulation and activation of phospholipase A_2 and endonuclease. Hydrolysis of membrane phospholipids by phospholipase contributes to further disruption of the barrier properties of the lipid bilayer, which leads to further increase in the Na^+ and Ca^{2+} concentration in the cytoplasm, swelling of mitochondria, their damage and disruption of all cellular functions. In addition, H^+-ATPase also functions as an ATP synthase, ensuring the coupling of ADP phosphorylation with reactions in the respiratory chain. Inhibition of H^+-ATPase leads to energy deficit in the cell. The Na^+,K^+-pump activity changes under the influence of growth factors, hormones, stress factors etc. As a component of the cell's life support system, the Na^+,K^+-pump is under the control of various types of regulatory mechanisms which provide both rapid and long-term changes in ion flows through the plasma membrane (Staehr, 2023). Activation of lipid peroxidation processes under pathological conditions leads to changes in the activities of membrane-bound pumps, in particular Na^+,K^+-ATPase (Onufrovych et al., 2024).

The Ca^{2+},Mg^{2+}-ATPases activities also characterize the cell's functional state. There are two Ca^{2+}-dependent ATPases: Ca^{2+},Mg^{2+}-ATPase of the plasma membrane and Ca^{2+},Mg^{2+}-ATPase of the endoplasmic reticulum (Dyla et al., 2020; Njegic et al., 2021; Xu & Van Remmen, 2021; Kho, 2023). By means of Ca^{2+},Mg^{2+}-ATPases, micromolar concentrateons of Ca^{2+} are maintained in cells, which is an intracellular messenger and regulates almost all cellular functions. In our previous studies we found that Ca^{2+},Mg^{2+}-ATPase activity was inhibited in lymphocytes of men with ED caused by combat trauma (Fafula et al., 2024). This slows down the efflux of Ca^{2+} ions from the cytosol. The accumulation of Ca^{2+} in cells and a decrease in the ATP level leads to a decrease in the activity of ion pumps and overloading of the cytosol with calcium. An increase in [Ca^{2+}]i is a widespread phenomenon in various pathological conditions. These data fully agree with those obtained on platelets (Lopez et al., 2019; Chaudhary et al., 2022). In arterial hypertension, for example, Ca^{2+} channel inhibitors (diltiazem, nifedipine, nicardipine, etc.) are widely used to prevent excessive Ca^{2+} influx into cells (Hao, 2023). Disturbances in the Ca^{2+}-binding and Ca^{2+}-transporting mechanisms leads to a violation of Ca^{2+} homeostasis and multiple pathological changes and metabolic shifts. The above mentioned changes lead to the activation of Ca^{2+}-dependent proteases and the degradation of proteins.

ED of mixed genesis in the presence of dyslipidemia is accompanied by a decrease in total Ca^{2+},Mg^{2+}-ATPase activity, Ca^{2+},Mg^{2+}-ATPase activity of the plasma membrane and endoplasmic reticulum of lymphocytes, which indicates an increase in the concentration of [Ca^{2+}]i and a violation of cellular regulatory mechanisms. The decrease in Ca^{2+},Mg^{2+}-ATPase activities is more expressed with age of patients. Complex treatment of patients with ED against the background of metabolic syndrome leads to an increase in ATPase activities and their approach to control values.

Regarding nitric oxide, a correlation was found between NO and ED (confirmed by pharmaco-penile ultrasonography) in a group with diabetes mellitus (Maniscalco et al., 2023). The effect of NO, which causes the relaxation of smooth muscles, is realized through the activation of sGC (Priviero et al., 2016; Alkan et al., 2017; Banecki et al., 2023). At the same time, the content of cGMP increases, which leads to a decrease in [Ca^{2+}]i and relaxation. The activities of arginase and eNO-synthase are important indicators of a cell's functional state (Fafula et al., 2018; Onufrovych et al., 2024). It is interesting that the difference in the concentration of NO did not depend on the place of blood sampling (peripheral or cavernous), which confirms the systemic nature of the changes, and not the specificity of the cavernous tissue. A comparison of NO concentrations in men with diabetes mellitus and ED versus men with diabetes mellitus alone revealed that NO changes are specific to ED and not to diabetes mellitus. In patients with ED of mixed etiology, the concentration of NO is significantly lower than in healthy subjects (Kedia et al., 2020). The above studies confirm a specific decrease in NO level in men with ED. Several characteristics determine the possibility of using NO as a marker of ED in clinical practice. Correlation between NO concentration and ED according to penile Doppler ultrasonography determines the possibility of distribution of men with ED by severity based on NO level in blood. Thus, in our study we demonstrated that vascular ED as a result of endothelial dysfunction is accompanied by a decrease in the activity of ion-transporting ATPases, which leads to an overload of cells with calcium, a violation of Ca homeostasis and cellular functions, as well as a decrease in the concentration of nitrite anion in peripheral blood lymphocytes, which indicates a decrease in concentration of NO in cells. It was also found that endothelial dysfunction in patients with ED, diabetes, hypertension and partial androgen deficiency is associated with a decrease in the concentration of total and free testosterone, a high coefficient of atherogenicity and an increased concentration of sex steroid-binding globulin in the blood, while psychogenic ED is more likely associated with neurotic symptoms characteristic of hyperactivity of the sympatho-adrenal system and functional hyperprolactinemia. Erection is a neurovascular phenomenon associated with hormonal control, which

includes arterial dilation, relaxation of trabecular muscles, and activation of the corporo-venoocclusive mechanism (Ma et al., 2020; Kim et al., 2021). In recent years, ED has been shown to be largely curable. The targets of action of the most effective modern drugs for the treatment of ED are the vascular endothelium and its dysfunction. Phosphodiesterase type 5 inhibitors, in particular sildenafil, are widely used to normalize endothelial function. Suppression of PDE-5 activity slows down the breakdown and increases the concentration of cGMP in the cavernous tissue and thereby contributes to the induction and maintenance of erection (Priviero et al., 2016; Nunes & Webb, 2021). Thus, sildenafil does not have a direct relaxing effect on the smooth muscle cells of the corpora cavernosa of the penis, but acts by enhancing the physiological erectile response to NO after sexual arousal. Availability and non-invasiveness of sildenafil therapy increases the effectiveness of treatment compared to others with other treatment methods. The development of ED is accompanied by an increase in arginase activity and a decrease in eNOS activity in peripheral blood lymphocytes, which indicates the dominance of the non-oxidative pathway of L-arginine metabolism over the oxidative one. Treatment of patients with sildenafil leads to the approximation of the activities of arginase and NO-synthase in lymphocytes to normal values. Therefore, the obtained experimental data indicate that the treatment of patients with the use of sildenafil significantly stabilizes the activities of arginase and eNOS and inhibits iNOS. Given the small sample of patients, we did not perform gradation based on the difference in enzyme activity depending on the amount of the drug received during the month (from 8 to 12 times).

Conclusion

The development of psychogenic and mixed forms of erectile dysfunction is mostly associated with stress hyperprolactinemia with normal indicators of cavernous dopplerography, lipid metabolism and androgen levels. The Na^+,K^+-ATPase activity of peripheral blood lymphocytes was significantly decreased in men of different age groups with a mixed form of erectile dysfunction. The Ca^{2+},Mg^{2+}-ATPase activity of plasma membrane and endoplasmic reticulum of peripheral blood lymphocytes was significantly decreased in men with erectile dysfunction of both psychogenic and mixed forms, however, a more pronounced decrease was observed with increasing age of patients. Complex treatment of middle age patients with a mixed form of erectile dysfunction with sildenafil leads to a significant increase in Na^+,K^+-ATPase activity and Ca^{2+},Mg^{2+}-ATPase activity. Complex treatment of younger age patients with the psychogenic form of erectile dysfunction with sildenafil leads to a decrease in arginase activity in lymphocytes. The activity of the inducible isoform of NO-synthase increases significantly in patients with psychogenic and mixed forms of erectile dysfunction of both age groups. Treatment of patients with sildenafil leads to a decrease in enzyme activity towards control values.

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