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Professional paper

THE IMPACT OF PERINDOPRIL ON SERUM SEROTONIN LEVELS IN A WHITE LABORATORY RAT WITH STREPTOZOTOCIN INDUCED DIABETIC NEPHROPATHY

Majlinda Ademi[1]

[1*]*Faculty of Medical Sciences, University of Tetova, Republic of N. of North Macedonia*
Corresponding author e-mail: majlinda.ademi@unite.edu.mk

Abstract

Extensive clinical trials of angiotensin converting enzyme inhibitors (ACEi) as antihypertensive drugs have provided numerous examples of enigmatic experiences in improving hypertension in diabetic nephropathy (DN).This study aimed to determine the effect of ACEi, perindopril on serum serotonin levels in Wistar rats with streptozotocin (STZ)-inducedDN. In this study, 50 normotensive white laboratory rats of both sexes were selected, aged 9 to 11 weeks with an approximately equal body weight of 200 to 300 grams. The rats were kept in 2 cages, and were fed standard laboratory rat food and water ad libitum. Serum serotonin levels were measured by the enzyme-linked immunosorbent assay (ELISA) method in 2 experimental groups: a DN control group and a DN + perindopril group. Perindopril (6 mg / kg / day) was administered orally daily for 8 weeks, starting after 4 weeks of STZ administration, while the control group received saline only. After the treatment, all rats were anesthetized deeply with sodium thiopenthal (50 mg/kg) and their whole brains were immediately removed from the skulls (usually within 1,5 min) on an ice chilled glass plate and stored at -80°C until further analysis. At the end of treatment, the rats were sacrificed and serotonin levels were taken. The results show that in both groups, blockade of the renin-angiotensin system (RAS) with perindopril significantly reduced serum serotonin levels. The results were processed using Two-Way Factorial ANOVA for Independent Samples. The statistical package SPSS 11 was used.
Keywords: perindopril, diabetic nephropathy, serotonin, streptozotocin

1. Introduction

Serotonin or 5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter important in transmitting nerve impulses. It has also been described as a vasoconstrictor, found in blood serum important in transmitting nerve impulses. Serotonin also has some cognitive functions, including in memory and learning. Modulation of serotonin at synapses is considered to be one of the major activities of several classes of pharmacological antidepressants (King, 2009; Berger *et al*, 2009). Perindopril is in a group of drugs called ACEi. It is used in the treatment of high blood pressure (essential hypertension), heart failure or stable coronary artery disease. Inhibits the angiotensin-converting enzyme (Myers, 1996; the EUROPA study, 2007).RAS has been identified as a complex enzyme pathway that produces several active peptides that control water homeostasis, blood pressure, hormonal secretion, behavior, and cognitive responses (Baltatu *et al*, 2003). ACEi are a group of drugs that work by inhibiting the angiotensin converting enzyme. At first they were introduced in clinical practice in the treatment of arterial hypertension, but later the indicative area of this group of drugs is significantly expanded and used in the treatment of various other diseases such as: congestive heart failure, myocardial infarction, diabetic nephropathy, XB atherosclerotic cardiovascular disease. The mechanism of action of angiotensin converting enzyme inhibitors (ACEi) is explained by preventing the conversion of inactive Ang I to a highly active Ang II (potent vasoconstrictor) by altering the activity of the renin-angiotensin-aldosterone system (RAAS) and thus the regulation of blood pressure. A series of experimental studies performed on various animal models of streptozotocin-induced diabetes have shown the renoprotective effects of ACEi. In these studies, the preventive use of ACEi, although not completely preventing DNA, results

in a significant reduction in microalbuminuria, improvement in renal function, and a significant improvement in renal morphology (relative to glomerular and tubular basement membrane, maxillary membrane) (Gross, 2003; Kalender, 2002; Fabris, 2001). Diabetes mellitus (DM) is a metabolic disorder that results in a defect of insulin secretion, insulin activity, or both (Bastaki, 2005). According to the current classification, there are two basic types: type 1 diabetes (T1DM) and type 2 diabetes (T2DM). The difference between the two types is historically based on age of onset, degree of loss of β-cell function, degree of insulin resistance, presence of autoantibodies associated with diabetes, and the requirement for survival insulin treatment (Leslie *et al,* 2016).Chronic renal failure (HBI) is a syndrome resulting from a gradual, progressive, and irreversible decrease in glomerular filtration rate (GFR) to the final stage of uraemia. It is characterized by retention of uremic toxins (urea, creatinine, uric acid, etc.) occurring mainly during protein metabolism, changes in the volume and composition of body fluids and electrolytes, and an imbalance of many hormones. Diabetes mellitus is one of the most important risk factors for the development of HBI, about 30% -50% of patients with end-stage renal disease (ESRD) worldwide are of diabetic origin (Ruiz-Ortega *et al,* 2020).DN is becoming a leading cause of HBI and the need for hemodialysis in developed countries. Renal complications occur in 5% of patients with DM Type I and in 40-50% of patients with DM Type II. In patients with DM, importance is given to heredity, hyperglycemia and hypertension and in connection with this to the sodium-lithium (Na-Li) pump. Hyperglycaemia is thought to cause an increased conversion of glucose to sorbitol, leading to an increase in its level in the cell. At the time of detection of DM, about 12% of patients have developed some form of diabetic neuropathy, and according to some late epidemiological studies, they are present in about 80% of respondents. In the pathology of these patients, demyelination is present, as well as the narrowing of small blood vessels, and the reasons are in the thickening of the basement of the membrane and in the hyperplasia and hypertrophy of the endothelial cells. Neurons of the peripheral nerve pathways, sympathetic innervation, and often the central nervous system are involved (Vrhovac *et al,* 2003). Streptozotocin (STZ) was first identified in 1950 as an antibiotic (Vavra *et al*, 1959). The drug was discovered by a strain of the bacterium Streptomyces achromogenes by a group of scientists at the pharmaceutical company Upjohn (modern-day Pfizer) in Michigan, USA. Towards the middle of the 60s STZ has been found to be highly toxic to pancreatic β-cells, which secrete insulin. It was then suggested that this drug can be used for an animal model of DM (Mansford and Opie, 1968; Rerup, 1970), and for medical treatment (chemotherapy) of pancreatic β-cell tumors (Murray-Lion *et al,* 1968). Due to its high toxicity to pancreatic β-cells, STZ has been used in research to induce induced DM in experimental animals (Rossini *et al*, 1977).The aim of our study was based on our hypothesis that serotonin levels may be elevated in white laboratory rats with STZ-induced DNA that were affected by perindopril therapy.

2. Material and methods

1. Laboratory animals

To perform the experiments provided in this study, 75 normotensive white laboratory rats of the Wistar breed of both sexes were selected. To minimize the effects of interindividual differences, all animals were aged 9 to 11 weeks, with an approximately equal body weight of 200 to 300 grams. The influence of external factors on renal function was minimized by standardized animal care and injection of equivalent amounts of fluid for administration. The rats were kept in 3 cages, and were fed standard laboratory rat food and water ad libitum.

2. Experimental model

Previous research has shown that rats are best suited for experimental DN modeling as an animal species because of the similarity of their interrenal enzyme distribution to that of humans. On the other hand, in the experimental scientific research work, the most commonly used model of induced diabetes in rats, and consequently DN, is the induction of diabetes with single administration of STZ. Therefore, for the performance of this study, it was planned to set up an experimental model of DN in white rats with single intraperitoneal administration of STZ.

3. Method for determination of serum serotonin in white laboratory rat

The enzyme-linked immunosorbent assay (ELISA) method was used to determine serotonin in serum and brain tissue in a white laboratory rat.

4. Experimental protocols

To respond to the selected rats, they were divided into 2 groups, each with 25 animals

- **Experimental protocol No 1**

For the realization of the predicted experiments, in rats from the other 2 groups (n = 50), DN was experimentally induced.
The experimental model of induced DN in rats was set by single intraperitoneal administration of STZ at a dose of 60 mg / Kg / TT. Considering the criteria for inclusion of rats in the study, in the further course of the study only rats were included in which the blood glucose level was higher than 11 mmol / L, on an empty stomach (in the morning). For the next 4 weeks to develop DN, the animals were left in a diabetic state without any treatment. To meet the set goals these 50 rats were divided into 2 groups of 25 animals.

- **Experimental protocol No 2**

The animals in this group were a positive (diabetic) control group. In this group of rats, in order to assess the symptoms and signs of DN, in the next 8 weeks after the administration of STZ they did not receive an active drug but only saline in the same amount as the animals in which the therapeutically active drug was used.

- **Experimental protocol No 3**

To evaluate the therapeutic effect of ACEi perindopril monotherapy in the treatment of experimentally induced DN, in this group of rats after 4 weeks of STZ administration, oral perindopril was started by intragastric tube, dissolved in 5% glucose at a dose of 6 mg / Kg / TT / day for 8 weeks.
After the treatment, all rats were anesthetized deeply with sodium thiopenthal (50 mg/kg) and their whole brains were immediately removed from the skulls (usually within 1,5 min) on an ice chilled glass plate and stored at -80°C until further analysis.

3. Results

The effect of peridopril on serum serotonin levels was examined in the experiment.
The results were processed using Two-Way Factorial ANOVA for Independent Samples. The statistical package SPSS 11 was used.
The graph below shows the mean values for serum serotonin concentration in both experimental groups.

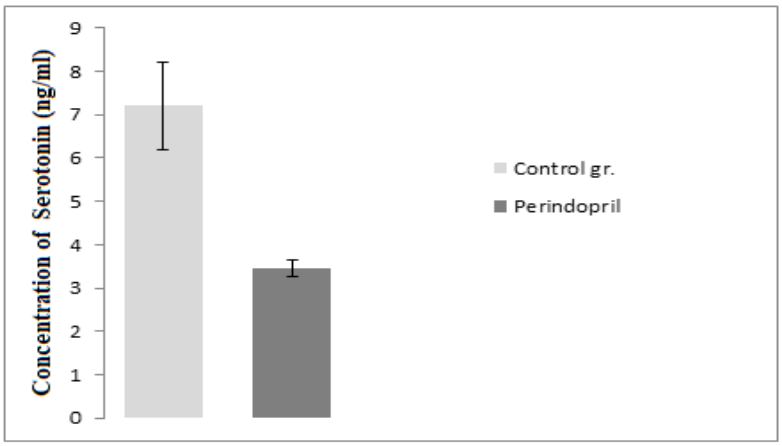

Figure 1. Serum serotonin concentration (mean ± standard error). The legend is shown on the graph itself

From the graph shown, we can receive the general impression that drug treatment leads to a decrease in serum serotonin levels.

Dose: 0 = the drug is not given 1 = the animals were treated with the drug	0	1	Average in rows:
	7,209±1,03	3,881±0,26	5,545±0,16

For a clearer view, the following graph was constructed for the averages of the experimental groups:

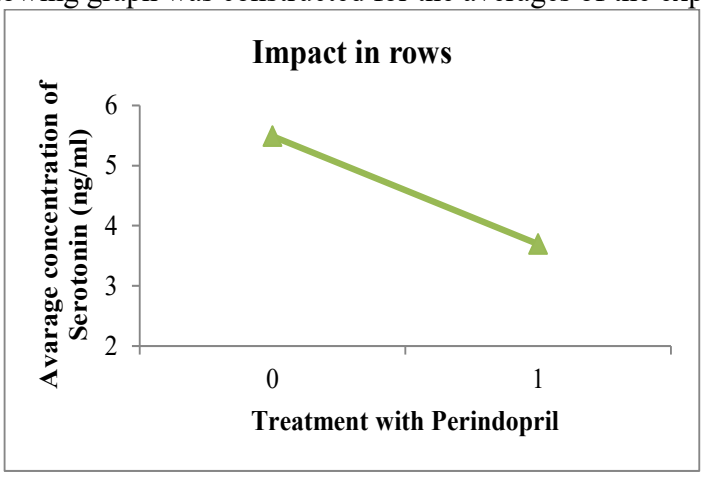

Figure 2. Effect of perindopril treatment (0 = level in control animals; 1 = level in treated animals)

The impact of a certain degree of drug interaction when no treatment was given 0 and when the animals were treated 1 is confirmed by the results of the ANOVA analysis, given in the following table:

Table 1. Results from Two-Way Anova analysis of serum serotonin concentration data

ANOVA Summary					
Source	**SS**	**df**	**MS**	**F**	**P**
Rows	12.12	1	12.12	8.64	0.0135
Columns	11.62	1	11.62	8.28	0.015
r x c	11.83	1	11.83	8.43	0.0144
Error	15.43	11	1.4		
Total	51	14			

The statistics (p) obtained for the effect in the columns actually represent the effect of perindopril treatment on the level of the examined parameter. It can be concluded that treatment with perindopril leads to a significant reduction in serum serotonin levels ($p < 0.05$).
The results of the Two-Way ANOVA analysis also show a significant effect on drug interaction ($p < 0.05$. Treatment with perindopril reduces serotonin levels to an average of 3,881 ng / ml.

4. Discussion

Our research was aimed on determining whether the effect of perindopril and candesartan alone and in combination would cause significant changes in serum white serum levels in DN-induced laboratory rats. There is partial and / or very little confirmed information in the literature on changes in serotonin levels in white laboratory rats with induced DN under the influence of perindopril. Since the first identification of renin by Tigersteed and Bergman in 1898, RAS has been extensively studied. The current review of the system is characterized by increasing complexity, confirmed by the discovery of new functional components and pathways of RAS. In recent years, the pathophysiological implications of the system have been the focus of attention, and RAS inhibitors such as ACEi and ARB have become important clinical tools in the treatment of cardiovascular and renal diseases such as hypertension, heart failure, and DN. However, tissue RAS also plays an important role in mediating various physiological functions. This, not only focuses Angiotensin classical actions on the cardiovascular system, namely the maintenance of cardiovascular homeostasis, but also on other functions (Paul *et al*, 2006). RAS is a peptidergic system with endocrine characteristics. The substrate of this system, angiotensinogen, α-glycoprotein, is released by the liver (Hall, 2003), and cleaves in the circulation under the influence of renin secreted by the juxtaglomerular apparatus of the kidney (Hall, 2003; Persson *et al*, 2004) for to form the decapeptide Ang I. Ang I is then activated in the octapeptide Ang II by ACEi, which is a membrane-bound metalloproteinase that is predominantly expressed in high concentrations on the surface of the endothelial cells of the pulmonary circulation (Hall, 2003). Ang II is thought to be the major effective RAS peptide that acts on specific receptors, such as causing vasoconstriction by interacting with angiotensin receptors in vascular smooth muscle cells or by stimulating the release of aldosterone from the adrenal cortex (Hall, 2003). The vascular wall is the major (effector) organ for hormonal or plasma RAS where AT1 receptors located in vascular smooth muscle cells mediate vasoconstriction. The concept of vascular RAS was generated when it became clear that Ang II could affect the large properties of vascular cells differently so that RAS components could form intracellularly in blood vessels. In addition to local synthesis, renin uptake via nonspecific binding sites in endothelial cells or prorenin/renin receptors has been proposed as a relevant mechanism (Catanzaro, 2005). Our results showed that Perindopril has a significant effect on reducing serum serotonin levels.

5. Conclusion

The results obtained during the experimental work enable the following conclusions to be drawn:

- We can conclude that the blockade of serum RAS has an effect on the level of serotonin, namely a significant reduction in the level of serotonin in the serum.
- Blockade of ACEi in our study caused a significant reduction in serum serotonin levels in animals receiving perindopril monotherapy.

References

[1]. Baltatu O. and Bader M. 2003. Brain renin-angiotensin system. Lessons from functional genomics. *Neuroendocrynology* 78: 253-259.

[2]. Bastaki S. 2005. "Diabetes mellitus and its treatment". *Int J Diabetes & Metabolism* 13:111-134

[3]. Berger M., Gray J.A. and Roth B.L. 2009. The expended biology of serotonin. *Annu. Rev. Med.* 60: 355-366.

[4]. Catanzaro D.F. 2005. Physiological relevance of renin/prorenin binding and uptake. *Hypertens Res* 28: 97-105.

[5]. Fabris B., Candido R., Carraro M., Fior F., Artero M., Zennaro C., Cattin M.R., Fiorotto A., Bortoletto M., Millevoi, C., Bardelli M., Faccini L. and Carreta R. 2001. Modulation of incipient glomerular lesions in experimental diabetic nephropthy by hypotensive and subhypotensive dosages of an ACE inhibitor. *R. Diabetes* 50 (11): 2619-24.

[6]. Gross M.L., El-Shakmak A., Szabo A., Kosch A., Kuhlmann A., Munter K., Ritz E. and Amann K. 2003. ACE-inhibitors but not endothelin receptor blockers prevent podocyte loss in early diabetic nephropathy. *Diabetologia* 46 (6): 856-868.

[7]. Hall J.E. 2003. Hystorical perspective of the rennin angiotensin system. *Mol Biotechnol* 24: 27-39.

[8]. Kalender B., Ozturk M., Tuncdemir M., Uysal O., Daginstanli F.K., Yegenaga I. and Erek E. 2002. Renoprotective effects of valsartan and enalapril in STZ-induced diabetes in rats. *Acta Histochem.* 104 (2): 123-30.

[9]. King M.W. 2009. Serotonin. *The Medical Biochemistry Page. Indiana University School of Medicine. Retrivied* 2009-12-01.

[10]. Leslie R. D., Palmer, J., Schloot, N. C., Lernmark, A. 2016. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. *Diabetologia.* 59:13–20.

[11] .Mansford K.R. and Opie L. 1968. Comparison of metabolic abnormalities in diabetes mellitus induced by streptozotocin or by alloxan. *Lancet 1* (7544): 670–1.

[12]. Murray-Lyon I.M., Eddleston A.L., Williams R., Brown M., Hogbin B.M., Bennett A., Edwards J.C. and Taylor K.W. 1968. Treatment of multiple-hormone-producing malignant islet-cell tumour with streptozotocin. *Lancet 2* (7574): 895–8.

[13]. Myers M.G. 1996. (on behalf of the perindopril multicentre dose-response study group) A dose-response study of perindopril in hypertension: effects on blood pressure 6 and 24h after dosing. *Can J Cardiol*. 12:1191-1196.

[14]. Paul M., Mehr A.P., Kreutz R. 2006. Physiology of Local Renin-Angiotensin Systems. *Physiol Rev* 86: 747-803.

[15]. Persson P.B., Skalweit A. and Thiele B.J. 2004. Controlling the release and production of rennin. *Acta Physiol Scand* 181: 375-381.

[16]. Rerup C.C. 1970. Drugs producing diabetes through damage of the insulin secreting cells. *Pharmacol Rev* 22 (4): 485–518.

[17]. Rossini A. A., Like A. A., Chick W. L., Appel M. C., Cahill Jr G. F. 1977. Studies of streptozotocin-induced insulitis and diabetes. *Proceedings of the National Academy of Sciences of the United States of America* 74 (6): 2485–2489.

[18]. Ruiz-Ortega M., Rodrigues-Diez R. R., Lavoz C. and Rayego-Mateos S. (2020). Special Issue "Diabetic Nephropathy: Diagnosis, Prevention and Treatment". *J Clin. Med*. 9: 813;

[19]. The European trial On reduction of cardiac events with Perindopril in stable coronary Artery disease Investigators. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomized, double-blind, placebo controlled, multicentre trial (the EUROPA study).2003. *The Lancet*362:782-788.

[20]. Vavra J.J., Deboer C., Dietz A., Hanka L.J. and Sokolski W.T. 1959. Streptozotocin, a new antibacterial antibiotic. *Antibiot Annu* 7: 230–5.

[21]. Vrhovac B. et al. 2003. *Interna Medicina*; 1176-1202.