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Influence of derivates of phenylanthranilic acid on a spontaneous diuresis and water-electrolyte balance

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Experimental research of influence of 8 first synthesized compounds in a row of phenylanthranilic acid derivates on a spontaneous diuresis and water-electrolyte balance has been conducted. Among the studied sodium salts of 5-N,N- diethyl sulfamoil -N- phenylanthranilic acid the most active compound proved to be the 2nd one, namely 4- chlorine -5-N,N- diethyl sulfamoyl - N -(4'- methyl) of phenylanthranilic acids, which increased diuresis by 121.7% and its action exceeds the effect of hydrochlorothiazide by 45.6%.

***Key words*:** derivates of phenylanthranilic acid, hydrochlorothiazide, spontaneous diuresis, water-electrolyte balance.

INTRODUCTION.

Along with the recently growing occurrence of cardiovascular pathology, increase in life expectancy of the patients with cardiologic problems and applying interventional methods of medical examination and treatment, the occurrence of acute renal insufficiency is also very frequent. So it is necessary to detect kidneys affection at an early stage for the patients with cardiovascular pathology and diabetes mellitus to assess the risk, to work out a strategy and treatment regiment that lead to forming the definition of cardio-renal syndrome (CRS) [2, 7].

Diuretics are often used when treating the patients with acute chronic heart failure. Although their efficiency when treating the patients with CDS is somewhat restricted by the developed resistance and sometimes unstable hemodynamics[10]. In some cases loop diuretic can increase the risk of sudden cardiac death фтв inopectic complications [13, 17].

The regulation of sodium-water balance is one of the most important homeostatic functions of an organism [3]. The balance of the composition of intracellular and

extracellular fluid within an organism plays an important role in vital functions of an organism. The function of kidneys and the regulating mechanisms are aimed at correcting the changes of -electrolyte balance within an organism. Knowing the mechanisms which regulate sodium-water balance in physiological and pathologic situations is extremely important for developing the methods of rational pharmacotherapy applying diuretics [4, 5].

The derangements of sodium metabolism reveal primiraly in changes of body's volume and weigh. The increase in extracellular fluid volume is characterized by excess fluid as well as appearing of edemata. When suffering from heart failure the antracele is usually to take place in interstitial space of feet legs [2, 13].

If an elderly patient with severe pathology suffers from severe or chronic diseases with misbalanced metabolism and diminished function of some organs and systems, then the following diuretics are used for treatment: Hydrochlorothiazidum, Furosemide, Buphenox, Clopamide, ethacrynic acid and others [9, 11, 15]. Along with evident diuretic action diuretics can cause a number of undesirable side effects: hypokaliemia, hypochloremic alkalosis, metabolic acidosis, hypercalcinemia, hyperlipemia , hyperglycemia, azotemia, protein metabolism misbalance and others [12, 16].

Thereby the search for diuretics is an urgent task of modern experimental pharmacology. The derivatives of 5-N,N- diethyl sulfamoil -N- phenylanthranilic acid play a great role in the processes of vital functions regulation [14].

The aim of this research is studying the diuretic action of 5-N,N- diethyl sulfamoil -N- phenylanthranilic acid derivatives by carrying out experiments on rats and determining some sides of diuretic action mechanism of the most active substances.

MATERIALS AND METHODS.

The object of research are 8 synthesized compounds in a row of 5-N,N-diethylsulfamoil -N- phenylanthranilic acid derivatives. The studying of diuretic action of the above mentioned compounds was carried out on white Wistar rats,

weighing 190-210 g according to Berkhin's method [1]. The target animals were given standard food allowance in vivarium conditions in accordance with sanitary and hygienic norms.

The content of electrolytes in the urine was determined by the method of flame photometry using flame photometer Page 2, and the quantity of the excreted creatinine - by Folin's method [1]. The experimental animals were divided into 10 groups. The rats in the first eight groups were injected with the 2, 5, 7, 9, 38, 40 and 42 compounds at a dose of 0.05 LD_{50}, in the ninth group - hydrochlorothiazide at a dose of 25 mg/kg, and the tenth group was a control group. The rats were kept in individual cages with free access to food and water. The tested substances and the comparator hydrochlorothiazide were injected intraperitoneally with a metal probe. The light conditions corresponded to the natural ones in accordance with the provisions and requirements of the "European Convention for the protection of vertebrate animals that are used for experimental and scientific purposes" (Strasbourg, 1986) and "General ethical principles of animal experimentation" (Kyiv, 2001) [5] .

The data obtained was processed according to the standard methods of variation statistics using the Student's t-test [12] and such software as «Windows-2003", Excel spreadsheets and mathematical processing package Mathcad-5.0. The differences were considered as reliable compared to the inaccuracy index $p < 0,05$.

THE RESULTS.

The analysis of data (Table 1) shows that the sodium salts of 4-chlorine-5-N,N- diethyl sulfamoil -N- phenylanthranilic acid (compounds 1, 2, 5, 7, 9, 10 and 12) increase the spontaneous diuresis of rats. Thus the sodium acid of 4-chlorine-5-N,N- diethyl sulfamoil -N- phenylanthranilic acid (compound 1) increases the spontaneous urine output by 60.9% ($p < 0.05$) and creatinine excretion by 4.2%, sodium ions by 27.9% ($p < 0.05$) and potassium by 6.7% . The replacement in the phenyl fragment of 5-N,N-dietilsulfamoil-N-phenylanthranilic acid molecule of hydrogen atom (comp.1) with 4-methyl radical one (comp. 2) leads to an increase in urine output by 121.7% ($p < 0.01$), creatinine excretion by 14.8%, sodium ions

by 51.9% ($p < 0.05$) and potassium by 13.1%. Introducing the second methyl radical (comp. 5) into the 2'-position of the phenyl fragment of 4-chlorine-5-N,N-diethylsulfamoil-N- phenylanthranilic acid molecule led to increase in creatinine excretion by 3.4%, sodium by 6.4%, potassium by 4.1% and renal excretory function by 21.1% ($p < 0.05$). By transferring of the two methyl radicals from position 2 ', 4' (comp. 5) into the 3 ', 5'-position (comp. 7) within the molecules of 4-chlorine-5-N,N- diethylsulfamoil-N- phenylanthranilic acid leads to an increase in urine output by 60.7% ($p < 0.05$) and creatinine excretion by 3.4%, sodium – by 14% and potassium – by 7.3%. The replacement of chlorine atom in a fragment of anthranilic acid molecule with hydrogen atom , and 3 ', 5'-dimethyl radical (comp. 7) with the 3-methoxy substitute (comp. 9) increases urine output by 93.5% ($p < 0, 01$), creatinine excretion by 7.4%, sodium - by19.9%, potassium – by 2.7%. The introduction of 2'4'-dimethyl radical (comp. 10) instead of the 3'-methoxy substitute (comp. 9) into the molecule of 4-chlorine-5-N,N-diethylsulfamoil-N-phenylanthranilic acid resulted in a reduction of spontaneous diuresis of rats by 23.9%, creatinine excretion - by 5.2%, sodium – by 3.8% and potassium - by 5.5% in comparison with the activity of compound 9. Transferring two methyl radicals from 2'4'- into 3'5'-position in the phenyl fragment of 4-chlorine-5-N,N-diethylsulfamoil-N-phenylanthranilic acid leads to a further reduction of renal excretory function.

Under the action of the standard comparator hydrochlorothiazide in doses of 25 mg/kg, an increase in urinary excretion of rats by 76.1%, in urinary creatinine -by 4.6%, sodium - by 29.8% and potassium - 20.3 % was observed. The advantage of compound 2 is the ability to provide half the potassium output compared to hydrochlorothiazide.

According to research results by Lebedev A.A. [8] the mechanism of action of the active sodium transport in the area of the basement membrane goes on without any significant effect on the permeability of both the individual cell membranes and intercellular spaces. The diuretic effect of the compound number 2 was 45.6% higher compared with hydrochlorothiazide and, apparently, is connectted with

inhibition of active sodium transport in the tubules of the nephron and increasing its excretion in the urine.

Thus, the most evident diuretic effect was found in compound 2 which increases the spontaneous diuresis by 121.7% on average. The increase in creatinine excretion proves the improved filtration function of the kidneys. The diuretic effect of the studied compounds is also connected with a decrease in sodium ions reabsorption in the tubules of the nephrons and an increase of their excretion, as well as improving the filtration function of the kidneys.

CONCLUSIONS.

1. Among the studied sodium salts of 5-N,N-diethylsulfamoil-N- phenylanthranilic acid the most active proved to be compound 2 (4-chlorine-5-N,N-diethylsulfamoil-N-phenylanthranilic acid) which increases urine output by 121.7% and it exceeds the action of hydrochlorothiazide by 45.6%.
2. The mechanism of diuretic activity of the compound number 2 is a consequence of an increase in filtration function of the kidneys, a reduction of the re-absorption of sodium ions in the tubules of the nephron and an increase in their excretion with urine.

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Table 1

THE INFLUENCE OF SINGLE INJECTIONS OF 5-N,N-DIETHYLSULFAMOIL-N-PHENYLANTHRANILIC ACID DERIVATIVES ON WATER CONSUMPTION, SPONTANEOUS DIURESIS AND ELECTROLYTE EXCRETION ON THE EXAMPLE OF WHITE WISTAR RATS

(n=7)

№ of compound	Dose mg/kg	Indices				
		Water consumption, ml	Urine output, ml	Creatinine excretion, mg	Sodium excretion, micromole /l.	Potassium excretion, micromole / l
1	3.75	14.1±0.61	7.4±0.23	2.96±0.11	233.4±22.42	117.6±10.8
2	6.5	17.2±0.61	10.2±0.32*	3.26±0.13*	267.5±27.18*	124.8±11.3
4	4.1	13.5±0.32	6.5±0.21*	2.91±0.16	208.6±16.73	115.9±9.17
5	2.38	16.5±0.32	5.7±0.18	2.89±0.12	194.6±26.27	114.5±11.8
7	2.75	17.6±0.74	9.4±0.27*	3.12±0.14*	238.217.19*	118.7±11.4
9	2.7	16.8±0.51+	8.9±0.22*	3.050.12	227.1923.14	116.3±13.7
10	11.25	15.5±0.73+	7.8±0.21*	2.90±0.15	218.8±18.19	113.210.6
12	3.55	14.9±0.73+	7.2±0.19*	2.88±0.09	213.5±21.19	111.49.19
hydrochlorothiazide	25.0	16.2±0.53	8.1±0.41*	2.97±0.14	236.8±23.28	132.6±11.7
Control	–	12.3±0.65	4.6±0.31	2.84±0.13	182.5±21.13	110.2±12.4

«»the reliability with control index (p<0,05).*