STUDY OF FREE AMINO ACIDS IN WHITE RAT KIDNEYS FOLLOWING TREATMENT WITH MERCURY BICHLORIDE AND PROTEIN HYDROLYSATE "HYDROPROT"

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Mercury as a heavy metal is endowed with expressed cumulative properties. Kidneys represent one of the depots (2, 8, 9, 11).

The toxic action of mercury is linked primarily to the impairment of protein metabolism (5), where an essential role is played by the content of free amino acids in the organism. According to personal experimental data (7), single application of mercury bichloride, at dose 7.5 mg/kg b. w., accounts for the most significant changes in free amino acid concentrations in the kidneys.

Recently protein hydrolysates have been employed very successfully to correct protein metabolism disorders. Their polyvalent properties (1) allow to study the action of the Bulgarian protein hydrolysate "Hydroprot" on the concentration changes of some free amino acids in kidneys of white rats, exposed to subchronic intoxication with mercury bichloride.

Material and methods

The study covers 180 mature male white rats, divided up into 6 groups: 1) controls — treated with physiological saline; 2) controls — treated with hydrolysate; 3) animals treated with 0.25 mg/kg b. w. mercury bichloride; 4) animals treated with mercury bichloride at dose 0.25 mg/kg together with protein hydrolysate; 5) animals treated with 1.00 mg/kg b. w. mercury bichloride, and 6) animals treated with mercury bichloride at dose 1.00 mg/kg together with hydrolysate. Mercury bichloride was introduced subcutaneously every day, while protein hydrolysate — intramuscularly at dose 5 cm^3/kg b. w. Bulgarian protein hydrolysate "Hydroprot", produced after the technology of I. Popdimitrov, was applied.

The 14 free amino acids (Table 1) were determined in 10 per cent renal homogenate after the method of T. S. Pashina (3), at 15-day intervals, over a period of 45-day-long poisoning and 60-day recovery.

Results

The treatment of rats with mercury bichloride (dose 0.25 mg/kg) leads to variable changes in the kidney concentrations of the free amino acids under study. Tryptophan causes the most considerable increase (about six times) on the 15th day. From 112 mcg/g fresh tissue concentration in the controls it reaches 713 mcg/g ($p < 0.001$). Methionine ranks second (by degree and alteration). At 30 and 45 days, its concentration is about 4 times higher compar-

Table 1

Content of Free Amino Acids (mcg/kg fresh tissue) in the Kidneys of White Rats, Treated with $HgCl_2$ at Dose 0.25 and 1 mg/kg

Amino acids	Groups	Duration of the expriment in days			
		15	30	45	75
Cysteine	c	134.44± 6.78	134.75± 5.42	146.20± 4.20	130.06± 6.02
	p_1	175.64± 6.22	170.67± 6.66	152.17± 2.70	162.64± 4.78
	p_2	873.14± 6.48	602.37± 4.74	596.71± 4.08	165.92± 5.27
Lysine	c	252.33± 8.12	263.03± 7.36	237.83± 5.10	227.00± 5.16
	p_1	184.28± 5.32	355.50± 6.34	487.13±10.40	572.10± 5.20
	p_2	684.45± 7.12	1523.60±38.11	469.08± 9.31	747.84±10.29
Histidine	c	134.67± 4.22	157.19± 3.75	136.34± 3.94	169.54± 5.25
	p_1	251.97±11.77	166.39± 4.17	114.72± 5.86	68.84± 3.59
	p_2	339.35±10.19	152.09± 4.25	181.01± 1.32	143.11± 4.70
Arginine	c	228.32± 5.12	261.27± 4.25	218.28± 3.38	181.11± 3.81
	p_1	268.46±11.75	281.11± 7.62	403.13± 8.46	288.95± 5.95
	p_2	449.35± 4.96	253.14± 3.78	156.54± 2.88	163.81± 3.19
Aspartate	c	409.22±20.25	496.29±30.73	498.94±25.14	474.82±23.36
	p_1	446.77±10.91	392.68±20.88	418.20±16.18	360.67±24.04
	p_2	447.78±10.94	325.63±14.39	214.77± 5.69	410.93±13.46
Serine	c	134.77±15.19	165.84±12.78	146.51 ±14.23	142.75±15.20
	p_1	176.11±13.30	204.05±12.30	242.49±10.55	111.86± 7.72
	p_2	860.00±15.63	192.07±11.58	98.28± 3.29	52.53± 3.01
Glutamate	c	1329.67±41.18	1546.13±45.12	1552.11±35.07	1032.56±47.24
	p_1	1337.72±44.31	1808.02±46.94	1673.33±47.87	1686.74±46.33
	p_2	1815.16±43.65	1970.93±62.74	913.20±41.27	604.56± 9.14
Glycine	c	152.10± 3.17	131.53±10.12	163.36± 9.87	174.10±15.11
	p_1	525.37±14.54	224.44±16.91	128.21±11.52	170.10±10.24
	p_2	468.40±13.07	164.04± 7.24	50.43± 3.69	190.43± 8.12
Alanine	c	356.74±12.45	301.37±17.24	305.64±16.28	317.91±15.02
	p_1	656.52±18.40	539.88±17.91	391.80±12.81	276.23±10.43
	p_2	1033.98±58.98	289.23± 8.98	253.97±16.91	285.82±10.09
Proline	c	189.72± 7.12	169.45± 5.83	134.25± 4.56	138.30± 7.01
	p_1	369.72±19.48	268.53±10.86	116.50±11.82	220.57± 0.79
	p_2	367.54±11.73	137.53± 7.77	666.55± 5.04	50.34± 6.36
Methionine	c	200.44± 3.14	189.72±10.21	169.25±11.14	138.30±19.26
	p_1	828.69±41.34	735.48±38.76	808.15±37.76	306.49±20.34
	p_2	1215.51±60.67	474.72±25.02	300.54± 7.75	607.31±25.20
Phenylalanine	c	264.41± 7.12	259.82± 6.39	241.21± 7.77	247.23± 7.13
	p_1	642.51± 7.43	383.38± 7.47	155.40± 8.55	225.16± 7.82
	p_2	425.03± 4.60	223.68± 4.39	154.43± 3.60	248.37± 5.13
Leucine	c	471.18±19.34	461.94±20.35	477.82±18.40	472.03±18.72
	p_1	1139.90±41.98	1199.06±59.57	1744.94±36.52	449.24±14.26
	p_2	1660.60±35.24	342.62±15.83	324.62±13.21	1893.06±29.75
Tryptophan	c	112.08± 6.42	137.07±11.05	153.75±12.13	163.90±11.50
	p_1	713.16±16.36	563.76±14.11	188.44±16.67	136.45±14.72
	p_2	1077.39±39.61	692.38±25.05	563.78±36.66	192.72±12.47

c — Controls treated with physiological saline
p_1 — Poisoned with 0.25 mg$HgCl_2$/kg b.w.
p_2 — Poisoned with 1.00 mg $HgCl_2$/kg b.w.

Table 2

Content of Free Amino Acids (mcg/g fresh tissue) in the Kidneys of White Rats, Treated with $HgCl_2$ at Dose 0.25 and 1 mg/kg × Protein Hydrolysate

Amino acids	Groups	Duration of the experiment in days			
		15	30	45	75
Cysteine	c	219.46±10.26	189.69± 9.19	249.65±11.13	130.99± 9.75
	p_1	282.98±10.28	772.10±30.70	669.08±25.84	158.22± 9.81
	p_2	328.59± 9.71	453.88± 9.27	653.95±16.96	723.58±25.24
Lysine	c	586.84±13.25	383.36±15.75	509.51±11.42	376.96±12.61
	p_1	597.54±11.82	719.82±18.80	857.61±16.83	233.39± 9.08
	p_2	682.36±16.28	736.49±19.21	1190.57±65.35	1772.88±53.17
Histidine	c	178.65± 3.29	178.91± 3.82	1153.83± 6.84	170.00± 7.25
	p_2	675.47±12.77	535.91±19.91	891.64±24.76	329.61± 9.39
	p_2	575.63±12.22	843.33±23.71	1034.83±57.27	582.11±12.42
Arginine	c	415.47±28.86	496.06±28.98	495.63±27.06	175.92±15.62
	d_1	574.49±13.83	562.70±13.43	501.71±21.23	234.29±13.38
	p_2	536.01±12.90	297.36±14.01	218.63±15.58	359.45±10.43
Aspartate	c	404.06±20.12	699.33±24.91	747.09±26.49	323.25±19.82
	p^1	389.24±13.67	760.09±22.92	725.73±24.49	343.73±16.34
	p_2	805.18±19.92	1393.09±49.92	999.24±33.37	774.75±13.96
Serine	c	99.13± 3.12	198.16± 9.49	610.73±14.16	149.71± 9.38
	p_1	403.72±20.37	572.03±18.87	374.28± 6.12	136.50± 8.57
	p_2	668.34±16.68	453.25±12.87	544.29± 8.91	485.86±13.04
Glutamate	c	1667.09±25.38	1756.25±33.59	3011.11±29.52	1309.92±26.86
	p_1	2182.17±30.90	1998.10±23.77	1737.42±23.61	1246.82±34.27
	p_2	1295.32±22.30	1600.04±38.89	1965.72±32.26	1876.15±43.23
Glycine	c	349.24±19.96	374.86±18.50	365.28±12.36	147.74±11.36
	p_1	614.55±28.18	511.94±23.65	351.24±10.38	343.08±13.22
	p_2	399.86±14.49	635.96±24.06	1121.22±40.69	585.13±12.96
Alanine	c	1104.48±60.96	945.05±52.35	763.28±49.73	348.22±29.53
	p_1	1810.23±63.90	1132.07±49.79	1568.29±40.54	1094.72±42.14
	p_2	1951.41±46.73	1693.05±49.15	2535.21±23.21	1209.89±24.74
Proline	c	444.25±13.41	439.38±18.44	345.61±12.56	130.40± 9.73
	p_2	449.47± 9.18	659.32±12.22	350.87±10.24	342.52± 6.26
	p_2	406.45±19.42	354.13± 6.56	334.24± 7.33	262.21±10.08
Methionine	c	1395.02±69.60	977.42±51.19	1126.52±66.56	730.78±15.28
	p_1	1250.07±54.30	1248.50±27.73	820.95±17.28	682.66±16.58
	p_2	637.57±25.70	1381.44±41.34	413.01±16.66	749.65±15.22
Phenylalanine	c	677.52±25.62	558.04±21.47	693.52±28.75	371.07±15.09
	p_1	523.26±20.32	532.77±19.61	558.51±19.47	239.73±11.12
	p_2	454.14±22.97	456.98±20.82	919.51±32.81	744.28±20.58
Leucine	c	555.02±15.91	665.84±14.41	794.23±16.62	251.70±12.00
	p_1	883.15±15.92	1768.90±46.56	1381.54±50.76	294.20±28.30
	p_2	742.14±13.37	624.45±16.22	636.55±19.85	479.44±19.04
Tryptophan	c	324.94±13.03	454.48±13.31	762.59±14.95	456.33±12.78
	p_1	1310.83±40.34	1616.49±35.56	1016.43±33.29	1240.20±27.48
	p_2	378.14±16.70	1838,07±40.39	1894.46±48.45	1757.61±54.74

c — Controls, treated with hydrolysate
p_1 — Treated with hydrolysate and poisoned with 0.25 mg $HgCl_2$/kg b.w.
p_2 — " " " " " " 1.00 mg $HgCl_2$/kg b.w.

ed to controls ($p<0.01$), and maintains its elevated level even after the recovery period. Leicine shows a reliable increase about the 15th day, and keeps its high values by the end of poisoning ($p<0.05$). The concentrations of phenylalanine, glycine, proline and aspartate show a decrease on the 45th day.

The dose 1 mg/kg mercury bichloride causes the earliest and most significant increase in tryptophan concentration, followed by cysteine and methionine ($p<0.001$). The concentrations of serine, proline, phenylalanine and leucine are reliably elevated on the 15th day, and thereafter they show an abrupt fall below the level of the controls. At 45 days the concentrations of cysteine and tryptophan are most appreciably increased. A decrease in the content of methionine, glycone, aspartate, arginine, proline and phenylalanine is established.

Regardless of the additional protein supply in the form of protein hydrolysate, mercury bichloride causes changes in the concentration of free amino acids in the kidneys. Simultaneous treatment of the animals with protein hydrolysate + 0.25 mg mercury bichloride causes a nearly fourfold increase in tryptophan on the 15th day, about 1.6 times of leucine, while methionine concentration remains unaltered (Table 2). About the 45th day the leucine content is substantially elevated, followed by that of lysine and serine. Methionine decreases unreliably ($p>0.05$). The concentrations of glycine, proline, aspartate and arginine are likewise practically unaltered. A decrease is recorded only for the concentrations of phenylalanine and glutamate.

Mercury bichloride (dose 1 mg/kg) causes a weaker effect on the renal amino acids of rats treated simultaneously with protein hydrolysate, as compared to those injected with mercury bichloride only (same dose). Earliest (about the 15th day), and comparatively most expressed (about 4.5 times) is the increase in content of serine, followed by aspartate. At 45 days, the concentrations of cysteine, tryptophan and lysine show a 2—2.5 times increase ($p<0.01$). The concentrations of methionine, arginine and leucine show a decrease, although not as much as that in rats treated with mercury bichloride alone.

Discussion

Mercury bichloride seriously affects the concentrations of free amino acids in the kidneys. The changes observed are multilateral, and depend upon the dose and duration of treatment. It is obvious that more sudden changes are established in the beginning of the experiment (about the 15th day). This feature is particularly clear cut for tryptophan at either of the mercury bichloride doses used, and for methionine, cysteine, lysine and serine at dose 1 mg/kg. According to data submitted by I. M. Trachtenberg (6) there is no definite regularity mechanism of the increase in blood and tissue mercury concentration in the animals treated with mercury compounds, and in all likelihood, this would explain the peculiarities of the changes observed in the free amino acids under study. The described changes reveal the deep mercury induced disorders in amino acid balance within the kidneys. This can be attributed to the formation of mercurochelates, especially at dose 1 mg/kg mercury bichloride. At the cited dose a considerable decrease of methionine, aspartate, glutamate, serine, glycine, leucine and arginine concentrations is established. These amino acids include functional groups with high reaction

capacity for metal chelates formation, which is a satisfactory explanation of their concentration decrease. The considerable increase in some amino acid concentrations — cysteine, lysine, tryptophan — may be interpreted as the result of an overall impairment of the amino acid balance within the organism and re-distribution of free amino acids, or else, as a consequence of renal parenchyma destruction (12).

Hydrolysate administration exerts a positive effect on the degree of changes in the kidneys of animals treated with 1 mg/kg mercury bichloride: at 45 days methionine falls by 78 per cent, whereas in those with extra hydrolysate addition — by 37 per cent. Proline and glutamate remain unchanged, whilst phenylalanine and glycine increase unreliably. According to J. C. Dougherty (10), the addition of proteins exerts a favourable effect on mercury impaired renal functions. The considerably less expressed involvement of free amino acids in the kidneys of poisoned animals treated with hydrolysate may be explained by the additional administration of amino acids with the hydrolysate, since it contains qualitatively all irreplaceable and replaceable amino acids. Amino acids as well as some other by-products of the hydrolysate are endowed with a stimulating action, and thanks to that protein metabolism is improved with parallel activation of the enzyme systems and regenerative processes within the organism (1). Probably the detoxification properties of the hydrolysate (4) have an essential bearing on the rather mild changes observed since they improve the function of the excretory systems, and in turn contribute to the enhanced mercury excretion.

Conclusion

— Subchronic intoxication with mercury bichloride leads to changes (varying in degree and character) in the concentrations of the investigated fourteen free amino acids in the kidneys.

— The most expressed are the changes in tryptophan, methionine, leucine and cysteine, whose concentrations show a 3 to 6-fold increase depending on the dose and duration of mercury bichloride treatment.

— In the kidneys of poisoned rats, treated simultaneously with protein hydrolysate, the concentrations of the investigated free amino acids are affected to a smaller degree. Thus tryptophan increases four times. After the recovery period, the concentrations of all amino acids return to normal values.

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ИССЛЕДОВАНИЕ СВОБОДНЫХ АМИНОКИСЛОТ В ПОЧКАХ БЕЛЫХ КРЫС, ТРЕТИРОВАННЫХ РТУТЬЮ ДВУХЛОРИСТОЙ И БЕЛКОВЫМ ГИДРОЛИЗАТОМ «ХИДРОПРОТ»

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РЕЗЮМЕ

Рассматриваются изменения в концентрациях 14 свободных аминокислот в почках белых крыс, третированных подкожно ртутью двухлористой в дозах 0,25 и 1 мг/кг массы и белковым гидролизатом — по 5 $см^3$ внутримышечно. Свободные аминокислоты определялись в 10% почечном гомогенате по методу Т. С. Пасхиной каждые 15 дней, в течение 45-дневного периода отравления и после 60-го дня восстановления.

Обнаружено, что после 45-дневного третирования $HgCl_2$ в дозе 0,25 мг/кг значительнее всего увеличивается концентрация метионина (приблизительно в 4 раза), следуемая таковой триптофана и лейцина. Прогрессивно и после восстановления уменьшается содержание фенилаланина (на 36%) и аспартата (на 24%). Доза в 1 мг/кг приводит к раннему, сильному увеличению концентрации триптофана (в 9,5 раз) и цистеина (в 6 раз), по сравнению с контролями. Концентрации глицина и глютамата уменьшаются (на 69%, соотв. 41%). Содержание серина, аланина, пролина и лейцина увеличивается на 15-ый день, после чего падает и остается более низкой, в сравнении с контролями. При одновременном третировании гидролизатом и ртутью в дозе 0,25 мг/кг увеличивается только концентрация триптофана. При дозе 1 мг/кг ртути двухлористой и введении белкового гидролизата, изменения более слабые, по сравнению с таковыми у животных, третированных только ртутью двухлористой, в той же дозе. После восстановительного периода все аминокислоты нормализуются.