THE EFFECT OF CARBON DIOXIDE GAS ON THE CONTENT OF AMMONIA, GLUTAMINE, AND UREA IN THE BLOOD OF ANIMALS AFTER INJECTION OF SOLUTIONS OF AMMONIUM CHLORIDE

N. B. Kozlov

Department of Biochemistry (Head-Prof. V. I. Panisyak),
Smolensk Medical Institute
(Presented by Active Member AMN SSSR V. V. Parin)

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It was shown in an earlier study that in ammonia intoxication which developed in warming animals, carbon dioxide gas has a good therapeutic effect. In the present work we give results of experiments on the study of the effect of carbon dioxide gas on the concentration of ammonia, glutamine, and urea in the blood in animals with ammonia intoxication developed after injection of a solution of ammonium chloride.

EXPERIMENTAL METHOD

The experiments were carried out on 29 rabbits. The solution of ammonium chloride was injected subcutaneously in doses of 0.1, 0.25, 0.4, and 0.5 g per kg. In order to determine the effect of carbon dioxide gas on the blood content of the nitrogenous components studied, the rabbits were placed for one hour in a special glass chamber with capacity of 500 liters. The concentration of carbon dioxide gas in the chamber was 7-8%; the oxygen concentration was 18.5-19.5%.

Blood was taken for study from the marginal vein of the ear. In the blood we determined: ammonia according to Conway, glutamine by the method of Harris, urea by the urease method, and pH by the electrometric method.

RESULTS

In the first series of experiments we studied the effect of carbon dioxide gas on the content of ammonia, glutamine and urea in the blood of intact animals. The blood in the experimental animals in this series was taken before placing them in the chamber with the carbon dioxide gas and immediately after taking them out of it. The results are given in Table 1.

As Table 1 shows, an hour's exposure of rabbits in an atmosphere with 7-8% carbon dioxide gas does not cause a change in the concentration of blood ammonia, glutamine, or urea.

TABLE 1. Effect of Carbon Dioxide Gas on Content of Ammonia, Glutamine, and Urea in Blood of Intact Animals (Duration of Keeping in the Chamber with the Carbon Dioxide Gas, One Hour)

Rabbit No.	Ammonia nitrogen (in mg%)		Amide N of glutamine (in mg%)		Urea nitrogen (in mg%)	
	Before keeping in chamber	After keeping in chamber	Before keeping in chamber	After keeping in chamber	Before keeping in chamber	After keeping in chamber
1	0.20	0.19	0.75	0.76	15.0	15.0
2	0.23	0.24	0.52	0.51	17.0	20.0
3	0.17	0.16	0.57	0.61	17.0	17.0
4	0.06	0.08	—	—	22.0	23.0
5	0.15	0.18	0.90	0.87	15.0	14.0
Average	0.16	0.17	0.69	0.69	17.2	17.8

In the next series of experiments the rabbits were injected subcutaneously with ammonium chloride in the doses mentioned earlier. Each rabbit was studied twice. In one case, after injection of ammonium chloride, the rabbit remained under ordinary atmospheric conditions (such experiments we called the controls), and in another, after injection of the same dose of ammonium chloride, the rabbit was at once placed for one hour in the chamber with the carbon dioxide gas. The blood in the animals of this series was taken before injection of ammonium chloride, and one hour and two hours after injection of the solution. In the experiments using carbon dioxide, the second sample was taken at the moment of removing the animal from the gas chamber. The results of the experiments are given in Tables 2 and 3.

TABLE 2. Effect of Carbon Dioxide Gas on Concentration in the Blood of Ammonia, pH of the Blood, and on the State of the Animal after Injection of Solutions of Ammonium Chloride

Animal No.	Wt, kg	Dose ammonium chloride, g/kg	Control experiments						Experiments using carbon dioxide gas					
			ammonia nitrogen, mg %			blood pH		State of animal and result	Ammonia nitrogen (in mg %)			Blood pH		State of animal and result
			before injection	aft. injection after 1 hr	aft. injection after 2 hr	before injection	after 1 hr		before injection	aft. injection after 1 hr	aft. injection after 2 hr	before injection	after 1 hr	
6	2.6	0.1	0.20	0.43	0.10	—	—	Good	0.20	0.33	0.22	—	—	Good
7	2.7	0.1	0.27	0.37	0.24	—	—	»	0.26	0.30	0.23	—	—	»
8	2.3	0.1	0.14	0.50	0.26	—	—	»	0.10	0.13	0.06	—	—	»
9	2.6	0.25	0.21	1.88	0.81	—	—	Convulsions	0.28	1.10	0.45	—	—	»
10	2.7	0.25	0.36	1.34	0.73	—	—	Ataxia	0.38	0.93	0.49	—	—	»
11	2.6	0.25	0.28	1.83	0.90	—	—	Convulsions	0.32	1.51	0.73	—	—	Ataxia
12	2.1	0.25	0.13	0.88	0.18	—	—	Good	0.08	0.36	0.09	—	—	Good
13	2.2	0.25	0.08	0.96	0.34	—	—	Ataxia	—	—	—	—	—	—
14	2.0	0.25	0.12	0.91	0.18	—	—	»	0.08	0.38	0.18	—	—	Good
15	2.1	0.25	0.09	1.70	0.23	7.56	7.32	Convulsions	0.15	1.36	—	7.66	7.41	Ataxia
16	2.7	0.25	0.12	1.55	—	7.53	7.01	»	0.20	0.85	—	7.53	7.40	Good
17	2.3	0.25	0.19	0.60	—	7.49	7.45	Good	0.08	0.79	—	7.49	7.26	Satisfactory
18	2.1	0.25	0.18	1.04	—	7.53	7.27	Ataxia	0.19	0.69	—	7.44	7.32	Good
19	2.0	0.4	—	—	—	—	—	Convulsion, death	—	—	—	—	—	—
20	1.8	0.4	0.17	2.55	2.89	—	—	Convulsion	—	—	—	—	—	—
21	1.9	0.4	—	—	—	—	—	—	0.13	1.42	0.51	—	—	Satisfactory
22	3.1	0.4	—	—	—	—	—	—	0.23	1.10	0.69	—	—	Good
23	2.3	0.5	0.18	2.86	3.27	—	—	Convulsion, death	—	—	—	—	—	—
24	2.0	0.5	0.10	2.28	2.60	—	—	» »	—	—	—	—	—	—
25	2.3	0.5	—	—	—	—	—	—	0.23	1.45	0.70	—	—	Good
Average	. . .		0,18	1,36	0,98	—	—	—	0,19	0,85	0,40			

TABLE 3. Effect of Carbon Dioxide Gas on Concentration of Glutamine and Urea in the Blood and Animal after Injection of Ammonium Chloride Solution

Rabbit No.	Dose ammonium chloride g/kg	Control experiments						Experiments using carbon dioxide gas					
		Amide N of glutamine, mg %			Urea nitrogen, mg %			Amide N of glutamine, mg %			Urea nitrogen, mg %		
		before injection	after 1 hr	after 2 hr	before injection	after 1 hr	after 2 hr	before injection	after 1 hr	after 2 hr	before injection	after 1 hr	after 2 hr
6	0.1	0.70	0.85	1.03	13.0	15.5	16.6	0.75	0.90	0.91	13.0	17.0	17.0
7	0.1	0.26	0.33	0.31	9.5	10.0	12.5	0.24	0.58	0.37	9.0	9.0	12.0
8	0.1	—	—	—	13.0	12.0	13.5	—	—	—	12.0	14.0	14.0
9	0.25	0.49	1.18	0.19	20.0	24.0	25.5	0.65	1.38	0.70	15.0	22.5	21.0
10	0.25	0.73	0.99	0.16	9.0	13.0	14.0	0.62	1.37	0.51	12.5	19.5	17.5
12	0.25	0.25	0.20	0.27	9.5	8.1	7.3	0.77	1.09	0.66	9.1	13.2	11.5
14	0.25	0.73	1.57	0.97	7.7	11.5	11.1	0.65	1.49	1.14	5.8	9.3	11.2
15	0.25	0.68	1.55	0.70	10.0	12.7	12.9	0.43	0.99	—	13.9	14.6	—
16	0.25	0.67	1.84	—	14.7	23.2	—	1.05	1.93	—	15.7	20.8	—
17	0.25	0.50	0.97	—	—	—	—	0.45	1.11	—	—	—	—
18	0.25	0.62	0.81	—	8.5	19.9	—	0.69	1.11	—	11.5	13.5	—
20	0.4	—	—	—	10.0	16.0	—	—	—	—	—	—	—
21	0.4	—	—	—	—	—	—	0.85	1.41	—	16.5	20.5	—
22	0.4	—	—	—	—	—	—	0.52	0.83	0.36	13.5	18.5	20.5
23	0.5	0.57	1.34	—	14.5	19.5	—	—	—	—	—	—	—
24	0.5	0.88	1.90	—	12.0	19.0	—	—	—	—	—	—	—
25	0.5	—	—	—	—	—	—	0.90	2.05	—	16.5	25.0	—
Average . . .		0.59	1.13	0.52	11.6	15.7	14.2	0.65	1.25	0.66	12.6	16.7	15.6
Ratio to ammonia in blood		3.3	0.8	0.5	64	12	14	3.4	1.5	1.7	66	20	39

As Table 2 shows, injecting the rabbits with ammonium chloride leads to a distinct rise in the concentration of ammonia in the blood. The greater the dose of ammonium chloride, the greater the increase in the level of ammonia in the blood. If, for example, injection of 0.1 g/kg of ammonium chloride raises the level of ammonia in the blood on the average by 0.23 mg%, then on injecting 0.25 g/kg, the ammonia level in the blood rises on the average by 1.09 mg%. Injection of doses of 0.4-0.5 g/kg lead to an increase in ammonia concentration in the blood of 2-3 mg%, that is, 15-20 fold.

Corresponding to this, the condition of the experimental animals is also changed. Small doses of ammonium chloride do not cause much change in the condition of the rabbits. When higher doses of ammonium chloride (0.25 g/kg) are injected, the rabbits show an increased irritability, ataxia, convulsions, opistotonos. Injection of ammonium chloride in doses of 0.4-0.5 g/kg usually leads to death of the rabbits.

Table 2 also shows that if the rabbit, after injection of ammonium chloride, is placed for one hour in the chamber with an increased carbon dioxide content, the raised concentration of ammonia in the blood is less apparent than in the control experiments. Thus, with injection of 0.1 g/kg ammonium chloride the ammonia level in the blood under conditions of breathing carbon dioxide gas is increased on the average by 0.07 mg% (in the control experiments, as already stated, the ammonia concentration in the blood with this dosage of ammonium chloride increases by 0.23 mg%); with injection of 0.25 g/kg the ammonia concentration in the blood of the experimental animals increases by 0.69 mg% (in the controls, by 1.09 mg%). The ammonia concentration in the blood is considerably less as compared to the controls in the case of injecting fatal doses of ammonium chloride.

It must be emphasized that breathing air with an increased content of carbon dioxide gas prevents convulsions and death of the rabbits after injection of large doses of ammonium chloride. The condition of the rabbits in such a case does not as a rule differ much from the normal.

It is known that injection of ammonium chloride into an organism leads to the development of a state of acidosis. Therefore it can be assumed that the anticonvulsive effect of carbon dioxide gas in our experiments is connected with a normalization of the pH. The test experiments (Table 2) showed, however, that removal of the acidosis which develops under the influence of ammonium chloride does not occur when carbon dioxide is breathed. It seems to us that the effect can be explained by the neutralization of ammonia. In confirmation of this we also ran control experiments with injection into four rabbits of 0.25 g/kg of ammonium lactate, a salt which does not cause acidosis. Without carbon dioxide gas, the level of ammonia in the blood then rose from 0.17 to 0.97 mg% while with carbon dioxide breathing the ammonia concentration in the blood of the experimental animals rose only to 0.58 mg%.

The binding of ammonia in the animal organism occurs basically either by urea formation, or by amidation of glutamic acid. Therefore in this work along with the study of the variation of ammonia in the experimental animals we also determined the content of glutamine and urea in the blood. The results are given in Table 3, from which it is evident that the level of glutamine in the blood after injection of ammonium chloride is increased, and this effect is more marked in the experiments with use of carbon dioxide gas. When the animals are injected with 0.25 g/kg, the glutamine concentration in the blood increases on the average from 0.6 to 1.05 mg%. If, after the injection of ammonium chloride, the rabbits are placed in the chamber with the carbon dioxide gas the glutamine concentration in the blood rises on the average to 1.31 mg%.

The content of urea in the blood after ammonium chloride injection is also increased. Analysis of the data obtained on the same rabbit shows that breathing carbon dioxide gas permits a more rapid elevation of urea concentration in the blood even in the first hour after injecting the solution.

Thus, on the basis of our results we can conclude that breathing carbon dioxide gas after injection of ammonium chloride hastens the binding of the ammonia, increases the glutamine and urea formation, and prevents death of the animals after injection of large doses of ammonium chloride (0.25, 0.4 g/kg).

SUMMARY

The author studied the influence of carbon dioxide on the concentration of ammonium, glutamine, and urea in rabbit's blood. He showed that administration of ammonium chloride to rabbits in doses of 0.1 g/kg of body weight, 0.25 g/kg, 0.4 g/kg and 0.5 g/kg leads to a corresponding rise of ammonium, glutamine and urea in the blood. When rabbits are placed into a chamber containing 7-8% carbon dioxide, ammonium is neutralized with simultaneous increase in the formation of glutamine and urea. Inspiration of carbon dioxide prevents cramps and death in rabbits after administration of large doses of ammonium chloride.

LITERATURE CITED

1. E. A. Vladimirova, Byull Éksp. Biol. Med. 30, 11, 345 (1950).
2. D. L. Ferdman, in: Progress of Biological Chemistry [in Russian] (Moscow, 1950) Vol. 1, p. 216.
3. P. P. Cohen and M. Hayano, J. Biol. Chem. 166, 239 (1946).
4. P. P. Cohen and M. Hayano, J. Biol. Chem. 166, 261 (1946).
5. P. P. Cohen and M. Hayano, J. Biol. Chem. 170, 687 (1947).

6. P. P. Cohen and M. Hayano, J. Biol. Chem. 172, 405 (1948).
7. E. A. Evans and L. Slotin, J. Biol. Chem. 136, 805 (1940
8. S. Grisolia, R. H. Burris, and P. P. Cohen 191, 203 (1951).
9. H. A. Krebs and K. Henseleit, Z. physiol. Chemie 210, 33 (1932).
10. H. A. Krebs, Biochem. J. 29, 1951 (1935).
11. D. Rittenberg and H. Waelsch, J. Biol. Chem. 136, 799 (1940).