INDUCTION OF RENAL ADENOSINE AND GUANINE DEAMINASES DURING AMMONIUM CHLORIDE ACIDOSIS IN RATS

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Summary - In rats during NH_4Cl acidosis the renal adenosine and guanine deaminases increased by 2 and 2.5-fold respectively. The renal DNA and RNA contents were found to decrease considerably but the protein content of the kidney did not undergo any change in acidotic rats. It is suggested that both adenosine and guanine deaminases might play an important role in the renal regulation of acid-base balance.

INTRODUCTION

During acidosis the increase in the renal ammonia production (2, 5, 6, 7, 15, 19, 21 and 24), and the inductive increase of a number of renal ammonia-producing enzymes (1, 4, 8, 9, 10, 14, 16 and 17) are well established. Increased ammonia formation is accompanied by a decrease in the nucleic acid content of tissues (10, 22 and 23), indicating an increased catabolism of nucleic acids, and consequently, an increase in the activities of the enzymes of nucleic acid catabolism can be expected. Hence, during acidosis when there is a decrease in the renal nucleic acid content (10) as well as an increase in renal ammonia-production, an inductive increase in the activities of renal adenosine and guanine deaminases may be anticipated. The purpose of the present investigation was to study the induction of these two enzymes in rat kidney during acidosis.

MATERIALS AND METHODS

Animals

Eighteen male albino rats weighing 180 to 210 g. were divided into

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three groups of 6 rats each, namely, the normal (N), control (C), and experimental (E) groups. The animals were housed separately in metabolic cages at 25 ± 3°C.

Diets and Feeding of animals

In order to obviate variations in renal acid excretion due to alterations in dietary intake (17) the rats were tube fed constant amounts of liquid diets. Group N received 10 ml. of the normal diet, containing 1.0 g. protein, 0.75 g. fat, 1.0 g. glucose and requisite amounts of essential salts and vitamins, every 24 hours. Group C was fed 10 ml. of a standard electrolyte deficient diet (SEDD), having a composition similar to the normal diet except in that it was deficient in the standard electrolytes, viz., Na^+, K^+ and Cl^-, every 24 hours. Group E received 10 ml. of SEDD every 24 hours for 5 days, i.e., till the urinary ammonia excretion had become and remained constant for two consecutive days. From the 6th day and onwards the diet of group E was supplemented with 4 millimoles of NH_4Cl every 24 hours.

Group E was fed SEDD so as to produce acidosis in the shortest possible time. Group C was kept on SEDD as a control to check whether the changes occurring in group E were merely due to a deficiency of Na^+ and K^+ or due to NH_4Cl acidosis. All animals had access to distilled water ad libitum.

Urinary ammonia estimation

Daily 24-hour urines from each rat were collected under mineral oil using thymol and toluene as preservatives. The urinary ammonia was determined by a modification of the microdiffusion method of Conway (3).

Enzyme assays

On the 15th day, i.e., after 9 days of NH_4Cl administration, when the urinary ammonia excretion of group E had reached a maximum and remained constant for three consecutive days, all animals of the three groups were sacrificed, their kidneys excised, and the whole homogenates and 15,000 x g supernatants of the kidneys from each rat were prepared separately according to Kumar et al. (11).

(i) Adenosine deaminase - Assay of the enzymic activity in whole homogenates was according to Mustafa and Tewari (15).

(ii) Guanine deaminase - The guanine deaminase activity of the rat kidney 15,000 x g supernatant represents the "total" enzymic activity, whereas the actual "effective" enzymic activity, i.e., the amount of enzyme actually effective in the intact kidney is reflected in the activity of the whole homogenate (11). Therefore, guanine deaminase was assayed in both the whole homogenate and 15,000 x g supernatant of the rat kidney by the spectrophotometric method of Roush and Norris (18) as modified by Kumar et al. (11).

DNA and RNA determination

Both DNA and RNA were estimated in the kidney homogenates according to Santen and Agranoff (20).

Protein estimation

The protein in the kidney preparations was estimated according to Lowry et al. (12).

RESULTS

Urinary ammonia

The 24-hour urinary ammonia excretion in animals of group N remained constant throughout the 14-day experimental period. The rats of groups C and E showed a slight increase in the urinary ammonia excretion which became constant on the 4th day and remained at this level on the 5th day also. In group C animals the urinary ammonia excretion remained constant from the 4th day onwards. The group E rats exhibited a further gradual increase in urinary ammonia production from the 6th day onwards (-due to NH_4Cl administration-) till it reached a maximum on the 12th day, (-i.e., after being fed NH_4Cl for 7 days-) and remained constant thereafter till they were sacrificed. The group E rats had developed maximum acidosis possible on the acid load administered which was clear from the increased and constant urinary ammonia excretion (6, 19 and 21). A slight acidosis was also developed by the group C animals.

TABLE 1. Renal adenosine and guanine deaminase activities of the normal, control and experimental rats.

Enzyme	Kidney fraction	Enzymic activity, units/g. fresh weight equivalent (Mean value ± S.D.)		
		Normal	Control	Experimental
Adenosine deaminase	Whole homogenate	18.3 ± 3.1	22.8 ± 3.8	43.9 ± 4.6
Guanine deaminase	Whole homogenate	12.9 ± 2.4	17.1 ± 3.0	32.3 ± 3.9
	15,000 x g supernatant	19.0 ± 3.6	23.9 ± 4.4	37.5 ± 4.5

The results reported are for the separate whole homogenates of kidneys from 6 rats in each group.

Enzymic activities

The standard mean values of the renal adenosine and guanine deaminase activities expressed as units/g. fresh weight equivalent kidney have been reported in table 1.

The renal adenosine deaminase activity of the groups C and E were respectively 1.24 and 2.4-fold higher than that of group N.

The "total" renal guanine deaminase activity of the groups C and E exhibited respectively 1.26 and 1.98-fold increase as compared to group N. The "effective" renal guanine deaminase activity of group C was 1.32-fold higher than that of group N, whereas the increase in the "effective" renal guanine deaminase activity of chronically acidotic rats (group E) as compared to the control and normal animals was 1.89 and 2.50-fold respectively.

DNA and RNA content

The standard mean values of the renal DNA and RNA contents in mg/g. fresh weight equivalent kidney are reported in table 2.

Although the renal nucleic acid content of both the groups C and E was lower than that in group N, this decrease in the nucleic acid content was much marked in the experimental group. The renal DNA content of the

TABLE 2. DNA and RNA content of the kidney of normal, control and experimental rats.

Nucleic acid	Content, mg./g. fresh weight equivalent (Mean value ± S.D.)		
	Normal	Control	Experimental
DNA	2.27 ± 0.28	2.07 ± 0.22	1.88 ± 0.16
RNA	2.72 ± 0.49	2.28 ± 0.18	1.61 ± 0.19

The results are for 6 animals in each group.

groups C and E was respectively 8.8 and 17.2 % lower than that of group N. The renal RNA contents showed greater variations and were 16.2 and 40.8 % lower in the groups C and E respectively as compared to group N.

Protein content

There was no change in the protein content of the kidney in the rats of groups C and E as compared to the normal rats.

DISCUSSION

The authors have demonstrated that there is an inductive increase in the renal adenosine and guanine deaminase activities of acidotic rats receiving a Na^+ and K^+ deficient diet supplemented with NH_4Cl. These increased renal enzymic activities are not solely due to a deficiency of Na^+ and K^+ is clear from the fact that although the group C rats kept on a Na^+ and K^+ deficient diet showed an increase in the urinary ammonia excretion as well as in the adenosine and guanine deaminase activities, they were much less than that in the group E rats.

The renal nucleic acid content of acidotic rats exhibited a significant decrease. The increase in the renal adenosine and guanine deaminase activities together with a decrease of the renal nucleic acid content indicates an increased catabolism of renal nucleic acids in acidotic rats. Jainicki and Argyris (10) reported a decrease in the renal DNA content together with an increase in the renal glutaminase (-an ammonia producing enzyme-) activity.

Vrba and Folbergr (22 and 23) demonstrated that during increased ammonia production in both the guinea pig and rat brain, the nucleic acid content decreased while the glutamine content increased. A similar mechanism may be operative in case of rat kidney during acidosis when renal ammonia production is increased. A tentative pathway for ammonia production by the kidney during acidosis in which adenosine and guanine deaminases are involved is envisaged and has been depicted in the scheme of reactions illustrated in Chart 1. The ammonia produced by the action of the two deaminases on their respective substrates (-available in greater quantities due to an increased catabolism of nucleic acids-) may directly enter the urine, and/or it may be incorporated in the form of amide nitrogen of glutamine and then again relea-

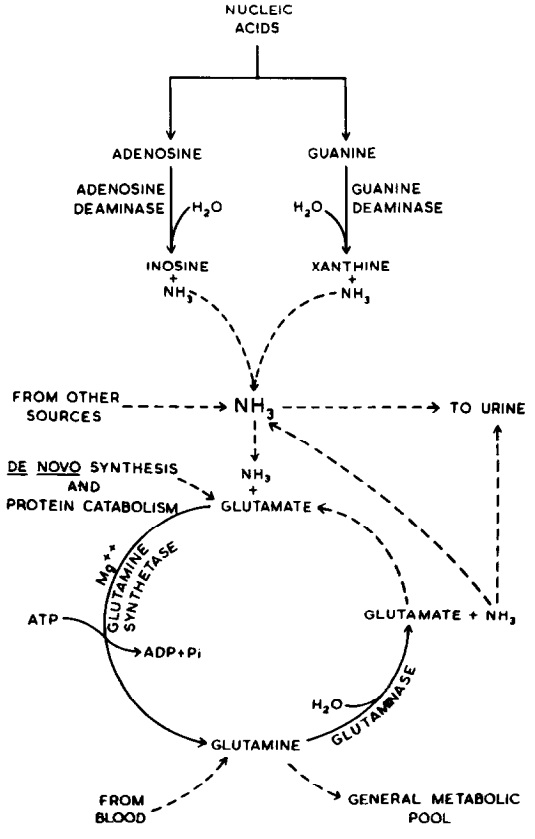

CHART 1. A possible pathway involving renal adenosine and guanine deaminases for the production of urinary ammonia.

sed (-to enter the urine-) by the action of glutaminase, which also increases during acidosis (4, 9, 10 and 17).

The protein content of the kidney exhibited no variations in acidotic rats. Hence, whether the increase in the renal adenosine and guanine deaminases is a result of de novo synthesis or of the modification of existing protein moieties or a combination of the two can not be assumed from the existing data. However, the results strongly suggest that both adenosine and guanine deaminases of the rat kidney are inducible enzymes and are involved in the renal control of acid-base regulation by ammonia production, which is in addition to the roles played by them in nucleic acid catabolism.

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REFERENCES

1. Amos, W.H. and Savage, J.E. Proc. Soc. Exptl. Biol. Med. 118, 102 (1965).
2. Briggs, A.P. J. Lab. & Clin. Invest. 28, 174 (1942).
3. Conway, E.J. Biochem. J. 27, 430 (1933).
4. Davies, B.M.A. and Yudkin, J. Biochem. J. 52, 407 (1952).
5. Ferguson, E.B., Jr. J. Physiol. 112, 420 (1951).
6. Gamble, J.L., Blackfan, K.D. and Hamilton, B.A. J. Clin. Invest. 1, 359 (1925).
7. Gilman, A. and Brazeau, P. Amer. J. Med. 15, 765 (1953).
8. Handler, P., Bernheim, F. and Bernheim, M.L.C. Arch. Biochem. 21, 132 (1949).
9. Ichihara, A., Takahashi, H., Aki, K. and Shirai, A. Biochem. Biophys. Res. Commun. 26, 674 (1967).
10. Jainicki, R.H. and Argyris, T.S. Amer. J. Physiol. 217, 1589 (1969)
11. Kumar, S., Josan, V., Sanger, K.C.S., Tewari, K.K. and Krishnan, P.S. Biochem. J. 102, 691 (1967).
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, A.J. J. Biol. Chem. 193, 265 (1951).
13. Mustafa, S.J. and Tewari, C.P. Biochim. Biophys. Acta 198, 93 (1970).
14. Mylon, E. and Heller, J.H. Amer. J. Physiol. 154, 542 (1948).
15. Pitts, R.F. Fed. Proc. 7, 418 (1948).
16. Pollack, V.E., Mattenheimer, H., DeBruin, H. and Weinman, K.J. J. Clin. Invest. 44, 169 (1965).
17. Rector, F.C., Jr., Seldin, D.W. and Copenhaver, J.H. J. Clin. Invest. 34, 20 (1955).
18. Roush, A.H. and Norris, F.R. Arch. Biochem. 29, 124 (1950).
19. Ryeberg, C. Acta Physiol. Scand. 16, 114 (1949).
20. Santen, R.J. and Agranoff, B.W. Biochim. Biophys. Acta, 72, 251 (1963).

21. Sartorius, O.W., Roemmelt, J.C. and Pitts, R.F. J. Clin. Invest. 28, 423 (1949).
22. Vrba, R. and Folbergr, J. Proc. Intern. Congr. Biochem., 4th Congr. Vienna, 5, 151 (1958).
23. Vrba, R. and Folbergr, J. J. Neurochem. 4, 338 (1959).
24. Wolf, A.V. Amer. J. Physiol. 148, 54 (1947).