[Chem. Pharm. Bull. 31(8)2917—2919(1983)]

Effects of Actinomycin D and Cycloheximide on the Activities of Catalase and D-Amino Acid Oxidase in the Rat Kidney Cortex during Sodium Restriction

SHIRO MORIMOTO* and RYOKO ABE

Department of Pharmacology, Osaka College of Pharmacy, 2–10–65 Kawai, Matsubara, Osaka 580, Japan

(Received January 12, 1983)

This study was carried out to determine whether actinomycin D and cycloheximide affect the activities of catalase and D-amino acid oxidase in the rat kidney cortex under conditions of sodium restriction. Animals were maintained on a sodium-deficient diet and injected intraperitoneally with actinomycin D or cycloheximide. Low sodium intake for 7 d resulted in increased activities of catalase and D-amino acid oxidase in the kidney cortex. Daily administration of actinomycin D ($20\,\mu g \cdot kg^{-1} \cdot d$) or cycloheximide ($80\,\mu g \cdot kg^{-1} \cdot d$) with the intake of sodium-deficient diet for 7 d had no influence on the amount of protein in the kidney cortex, but preferentially prevented the elevation of the activities of catalase and D-amino acid oxidase by sodium restriction. These results provide evidence for the induction of peroxisomal enzymes by low sodium intake.

Keywords——catalase; D-amino acid oxidase; actinomycin D; cycloheximide; sodium restriction; enzyme induction; rat kidney cortex

Peroxisomes are cytoplasmic granules surrounded by a single membrane and biochemically characterized by the existence of catalase and oxidative enzymes such as D-amino acid oxidase, L-α-hydroxy acid oxidase and urate oxidase. Although the presence of kidney peroxisomes was demonstrated in the proximal and distal tubules,[1,2] their physiological role remains unclear. We have previously reported[3] that the activities of catalase and D-amino acid oxidase in the rat kidney cortex are increased by the intake of sodium-deficient diet. The present study was undertaken to further determine whether actinomycin D and cycloheximide affect the activities of these peroxisomal enzymes in the kidney cortex under conditions of sodium restriction.

Materials and Methods

Animal Experiments——Male Wistar rats weighing from 180 to 220 g were used. For at least 1 week before the study the rats were fed a standard laboratory diet and provided with tap water *ad libitum*. The animals were divided into control and experimental groups. In the first series of experiments, the experimental animals were maintained on a sodium-deficient diet and on distilled water, while the control animals were given a standard laboratory diet and tap water for 7 d. Seven rats of each group were sacrificed at different times (1, 5 and 7 d) after the start of low-sodium diet intake. In the second series of experiments, the experimental animals were given a sodium-deficient diet with daily intraperitoneal injection of physiological saline ($0.5\,ml \cdot kg^{-1} \cdot d$), actinomycin D ($20\,\mu g \cdot kg^{-1} \cdot d$) or cycloheximide ($80\,\mu g \cdot kg^{-1} \cdot d$) for 7 d. The control animals were given a standard laboratory diet with the same drug treatments as experimental groups for 7 d. All injections were given at 9:00 a.m., and all rats were sacrificed 24 h after the final drug injection. The sodium-deficient diet (3.45 meq of sodium/kg) and standard laboratory diet (172 meq of sodium/kg) consisted of the components described in our previous paper.[4]

Preparation of Kidney Cortex Homogenate and Assays of Enzyme Activities——Under pentobarbital anesthesia (30 mg/kg, *i.p.*), both kidneys were removed and immediately cooled. The cortex was finely sliced with a blade, rinsed thoroughly with ice-cold physiological saline, and then homogenized with cold 0.45 M sucrose (12.5%, w/v). The activities of catalase (EC 1.11.1.6) and D-amino acid oxidase (EC 1.4.3.3) in the homogenate were assayed according

to the method described previously.[5] The unit of catalase activity, which obeys first-order kinetics, was defined as the amount of enzyme causing the logarithm of the hydrogen peroxide concentration to decrease by one unit per min in a volume of 1 ml. One unit of D-amino acid oxidase activity was defined as the amount of enzyme causing the disappearance of 1 μmol of substrate per min. The protein concentration was determined by the method of Lowry *et al.*[6] Statistical significance was determined by means of Student's *t*-test.

Results and Discussion

The experimental animals showed normal body weight gain after being switched to the sodium-deficient diet, and no significant difference could be detected in kidney weight per 100 g of body weight between the control and experimental rats. Our previous study[5] confirmed that L-α-hydroxy acid oxidase shows considerably lower activity than catalase and D-amino acid oxidase and that the activity of urate oxidase is not detectable in the rat kidney cortex, as reported by Ericsson.[7] Accordingly, we followed the changes in the activities of catalase and D-amino acid oxidase as peroxisomal enzymes throughout the experimental periods.

In the first series of experiments, no significant change was observed in protein concentration (17.9 ± 0.42 mg/ml) of the kidney cortex homogenate of control and experimental rats. For 5 d of sodium restriction, the specific activities of catalase and D-amino acid oxidase in the kidney cortex of experimental rats were approximately the same as the control values. However, as shown in Fig. 1, catalase was significantly increased after dietary sodium deprivation for 7 d. Its specific activity was 3.17 ± 0.25 units per mg protein, being approximately twice the control value. In these rats, the specific activity of D-amino acid oxidase was also increased to 3.57 ± 0.39 units per mg protein from the control value of 2.34 ± 0.34 units per mg protein. These findings indicate that the development of peroxisomes occurs in the kidney cortex after dietary sodium deprivation for 7 d.

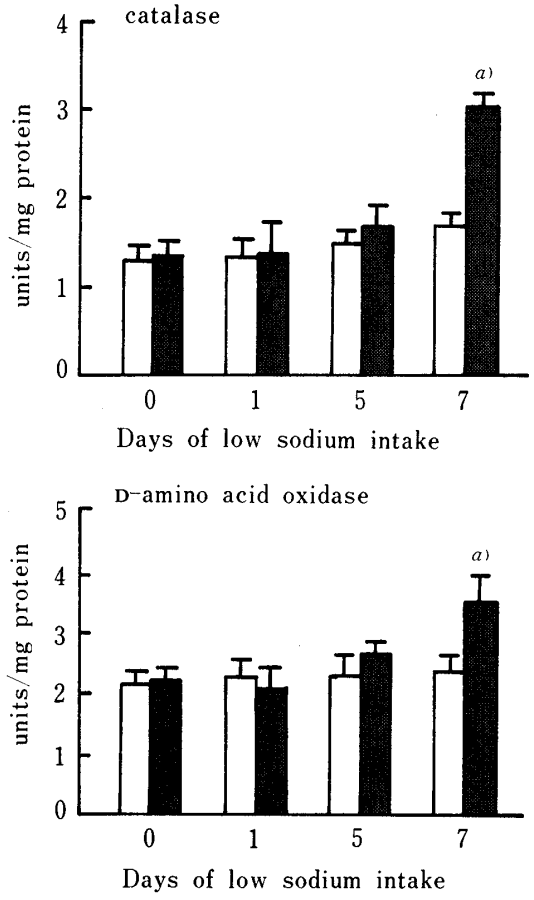

Fig. 1. Effect of Sodium Restriction on Specific Activities of Catalase and D-Amino Acid Oxidase in the Rat Kidney Cortex

Each column represents the mean of 7 animals and vertical bars indicate S.E. of the mean. *a)* Values are significantly different from corresponding control values ($p < 0.01$).

□, control rats; ▩, sodium restricted rats.

TABLE I. Effects of Actinomycin D and Cycloheximide on Protein Concentration and Specific Activities of Catalase and D-Amino Acid Oxidase in the Rat Kidney Cortex during Sodium Restriction

	Protein mg/ml homogenate	Catalase units/mg protein	D-Amino acid oxidase units/mg protein
Control rats			
Saline	17.7±0.45	1.50±0.29	1.96±0.15
Actinomycin D	17.7±0.47	1.96±0.45	1.97±0.16
Cycloheximide	17.3±0.28	1.82±0.28	1.93±0.23
Experimental rats			
Saline	18.7±0.51	2.97±0.41[a)]	3.66±0.27[a)]
Actinomycin D	18.8±0.42	2.01±0.41	2.13±0.14
Cycloheximide	19.0±0.46	1.70±0.22	1.78±0.22

The control and experimental rats were maintained on a standard laboratory diet and a sodium-deficient diet, respectively, for 7 d. All values are means±S.E. of 7 animals.
a) Values are significantly different from the control value ($p<0.01$).

Table I shows the results of the second series of experiments, in which actinomycin D or cycloheximide was injected into rats from the onset of the intake of sodium-deficient diet, and daily administration of these drugs was performed for 7 successive days. Treatments with actinomycin D and cycloheximide had no influence on the specific activities of catalase and D-amino acid oxidase in the control rats. In the experimental animals treated with physiological saline, the specific activities of peroxisomal enzymes were markedly increased after 7 d of sodium restriction, as observed in the first series of experiments. On the other hand, the administration of actinomycin D or cycloheximide preferentially prevented the subsequent increases in the activities of catalase and D-amino acid oxidase by sodium restriction, although no change in the amount of protein was observed in the kidney cortex.

Actinomycin D is proposed to be a potent transcriptional inhibitor which binds to deoxyguanosine groups of deoxyribonucleic acid (DNA),[8)] while cycloheximide is known to inhibit protein synthesis by blocking the initiation of new peptide chains as well as the elongation of nascent peptides on ribosomes.[9,10)] These antibiotics have been widely used for studying enzyme induction by various agents. In the present study, the results of the second series of experiments provide evidence for the induction of peroxisomal enzymes in the kidney cortex during sodium restriction, although morphological examination is required to confirm the increased number of kidney peroxisomes.

References

1) J. Rhodin, *Am. J. Med.*, **24**, 661 (1958).
2) A. B. Novikoff, P. M. Novikoff, C. Davis, and N. Quintana, *J. Histochem. Cytochem.*, **20**, 1006 (1972).
3) R. Abe, A. Fukuhara, and S. Morimoto, *Chem. Pharm. Bull.*, **27**, 2831 (1979).
4) S. Morimoto, R. Abe, A. Fukuhara, K. Tanaka, and K. Yamamoto, *Am. J. Physiol.*, **237**, F367 (1979).
5) R. Kitano and S. Morimoto, *Biochim. Biophys. Acta*, **411**, 113 (1975).
6) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
7) J. Ericsson, *Acta Pathol. Microbiol. Scand. Suppl.*, **168**, 7 (1964).
8) H. M. Sobell, *Progr. Nucleic Acid Res. Mol. Biol.*, **13**, 153 (1973).
9) B. S. Baliga, A. W. Pronczuk, and H. N. Munro, *J. Biol. Chem.*, **244**, 4480 (1969).
10) S. Rajalakshmi, H. Liang, D. S. R. Sarma, R. Kisilevsky, and E. Farber, *Biochem. Biophys. Res. Commun.*, **42**, 259 (1971).