Shimane J. Med. Sci. Vol. 2, pp. 20—24, 1978

Induction of Tyrosine Aminotransferase by Cholate

(induction/cyclic AMP/cAMP phosphodiesterase)

YOSHINORI TANIGAWA, MASAO KAWAMURA, AKIHIDE KITAMURA, YOSHIYUKI MIYAKE, and MAKOTO SHIMOYAMA

Department of Biochemistry, Shimane Medical University, Izumo 693, Japan

(Received August 8, 1978)

The intraperitoneal injection of cholate (10 mg per 100 g body weight) to rats elevated the hepatic cyclic AMP levels by 1.5 to 2.0-fold. Furthermore, the hepatic tyrosine aminotransferase was induced by this agent in the both intact and adrenalectomized rats. These data together with previous findings (ref. 4) contribute to an understanding of so-called "permissive effect" produced by glucocorticoids on cyclic AMP mediated process.

Considerable evidence has accumulated which indicates that glucagon, epinephrine, adrenocorticotropic hormone, and other hormones biologically stimulate adenyl cyclase and thereby increase the intracellular cyclic AMP levels in the respective target tissues (1). Investigations concerning the mechanisms of hormone action have focussed on the adenyl cyclase system ; however, as proposed in enzyme regulation, the behavior of the degradation of cyclic AMP might be equally as important as that of its formation.

Schmidtke *et al.* (2) and Senft *et al.* (3) independently reported that rat testicular and beef heart cyclic AMP phosphodiesterases were inhibited by such steroid hormones as predonisolone succinate, 6-α-methylpredonisolone, and cortisol. Recently, we observed that the other steroid compounds such as bile acids also inhibit cyclic AMP phosphodiesterase *in vitro* (4). Therefore, it was of interest to determine whether or not cyclic AMP accumulates *in vivo*, following the administration of bile acid. In this case, tyrosine aminotransferase induction will be expected, since this is one of the enzymes induced by cyclic AMP (5). When we injected cholate directly into the abdominal cavity of adrenalectomized rats, hepatic tyrosine aminotransferase was induced through cholate inhibition of cyclic AMP phosphodiesterase.

MATERIALS AND METHODS

White male Wistar rats, weighing about 200 g and on an Oriental Yeast solid diet provided *ad libitum* were allowed free access to drinking water. Bilateral adrenalectomy under ether anesthesia was performed within one week following their purchase ; 1% NaCl was substituted for the drinking water thereafter. Dehydrocholate (sodium salt) was purchased from Sigma. All other chemicals were of reagent grade and were obtained from Miyata Chemicals, Shimane.

Assay for Cyclic AMP

Immediately after decapitation, the trunk blood was collected for assay of corticosterone, into an iced tube for 15 sec. The liver (3g) was then removed and placed in 10 ml of ice-cold 10% trichloroacetic acid. These operations were completed within 40−50 sec. The liver was homogenized at full speed for 2 min in a Teflon homogenizer in ice. The homogenate was then centrifuged at $8,500 \times g$ for 30 min. The supernatants were extracted 3 times with 8 volumes of ether. The extracts were then dried and redissolved in 0.02 M phosphate buffer, pH 7.5. Cyclic AMP was then measured by using the protein binding assay of Gilman (6).

Assay for Plasma Corticosterone

The plasma was separated from the blood by clinical centrifuge and the corticosterone was measured by a fluorometric method (7) ; in this procedure, 1 μg corticosterone per ml was used as standard solution.

Assay for Tyrosine Aminotransferase

Tyrosine aminotransferase was measured by a modification (8) of Diamondstone's method (9). The reaction mixture contained 80 nmoles of pyridoxal-5′-phosphate, 10 μmoles of α-ketoglutarate, 30 nmoles of diethyldithiocarbamate, 100 nmoles of Tris-Cl^- buffer and appropriate amounts of $105,000 \times g$ supernatant of the liver homogenate as enzyme in a total volume of 1.0 ml. The reaction was started by heating at 37°C. After incubation for 10 min, the reaction was stopped by the addition of 4 ml of 1 M KOH. The KOH-treated samples were incubated for 30 min at 37°C. The absorbance of the samples was read at 331 nm in a Hitachi spectrophotometer (Type 100−60). To calculate the amount of *p*-hydroxyphenylpyruvate formed, an effective extinction coefficient of 19,900 M^{-1} was used (10). One unit of enzyme was defined as the amount of enzyme producing 1 nmol of *p*-hydroxyphenylpyruvate in 10 min. Protein was determined by the method of Lowry *et al.*(11).

RESULTS AND DISCUSSION

Elevation of Cyclic AMP in Liver after Cholate Administration to Rats

Previously, we observed that compounds such as predonisolone, hydrocortisone, cholate and deoxycholate were potent inhibitors of partially purified cyclic AMP phosphodiesterase prepared from rat liver. Therefore, the investigation concerning the *in vivo* significance of the *in vitro* inhibition of phosphodiesterase by steroid compounds was of special interest. We studied this factor using cholate, as this agent has no effects as mineralocorticoids and glucocorticoids. As shown in Fig. 1, the hepatic cyclic AMP content rose to a peak at 15 min, and then gradually reverted to normal levels. To confirm further the increase in cyclic AMP levels after cholate injection, other experiments were carried out to yield the following results : (a) no influence of cholate on the cyclic AMP assay system was observed, (b) no cyclic AMP could be detected after the samples, the cyclic AMP levels of which were elevated by

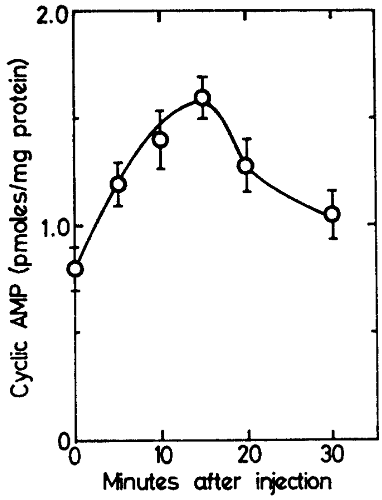

Fig. 1. Changes in cyclic AMP concentrations in rats liver following the administration of cholate. The agents were injected intraperitoneally in a dose of 10 mg per 100 g body weight, and rats were sacrificed at the time indicated after injection. The results are the mean ± S. D. of five separate determinations.

cholate injection, were treated with cyclic AMP phosphodiesterase. These data strongly suggest that injection of cholate elevates cyclic AMP contents in the liver.

Effect of Cholate Administration on Tyrosine Aminotransferase and Plasma Corticosterone

As cyclic AMP has been shown to increase the activity of a number of hepatic enzymes involving tyrosine aminotransferase *in vivo*, tyrosine amino-

TABLE I. *The Effect of Cholate Administration on Liver Tyrosine Aminotransferase Activity and Plasma Corticosterone Levels on Adrenalectomized Rats*

Injection	Intact		Adrenalectomized	
	Enzyme activity units/mg protein	Corticosterone γ/dl	Enzyme activity units/mg protein	Corticosterone γ/dl
Saline	18.98±2.11	26.96±4.25	7.27±1.67	2.10±0.22
Cholate	112.89±15.18*	70.72±9.81**	25.34±9.69***	2.12±0.55****

Cholate was given intraperitoneally, 10 mg per 100 g body weight and rats were sacrificed 5 hr after the administration. Enzyme activity and plasma corticosterone were determined as described in the assay method. Rats were used 1 week after adrenalectomy. The values represent the mean ± S. D. of 5 rats. The statistical significance of the results was determined by the *t* test. The increases of *, **, and *** were significant at 0.5, 1, and 5%, respectively ; that of **** was insignificant.

transferase activity following cholate injection was assayed. As shown in Table I, intraperitoneally administered cholate increased hepatic tyrosine aminotransferase activity in normal and adrenalectomized rats by approximately 6 and 3.5 times, respectively. We used adrenalectomized rats for the present experiment in order to avoid the effect of corticosterone increase on the enzyme activity. Changes in plasma corticosterone levels in both intact and adrenalectomized rats after cholate administration are shown in Table I. Cholate seems to produce a high plasma corticosterone in intact rats. In preliminary experiments, we observed that bile acid inhibits both hydroxylation and dehydroxylation of corticosterone by rat liver homogenate in the presence of NAD and $NADH_2$ at 37°C.

It has been reported such compounds as methylxanthines (12, 13) puromycine (14), 4-benzyl-2-imidazolidinoes (15, 16, 17), papaverine (18, 19), and others (20, 21, 22) act as inhibitors of cyclic nucleotide phosphodiesterase. Furthermore, multiple forms of cyclic nucleotide phosphodiesterase were reported independently from several laboratories (23, 24, 25). Although, it is unknown what form of the enzymes can be influenced mainly by steroids including cholate, other steroids including corticosterone may participate in induction of certain enzymes by inhibiting cyclic AMP phosphodiesterase *in vivo*. Therefore, our observation contributes to an understanding of so-called "permissive effect" produced by glucocorticoids on cyclic AMP mediated process (2, 4).

REFERENCES

1) Perkins, J. P. (1973) In : Advances in cyclic nucleotide research, 3 (Greengard, P., and Robison, G. A., ed.) pp. 1–64, Raven Pr., New York
2) Schmidtke, J., Wienker, Th., Flügel, M., and Engel, W. (1976) *In vitro* inhibition of cyclic AMP phosphodiesterase by cortisol. *Nature* **262**, 593–594
3) Senft, G., Schutz, G., Munske, K., and Hoffman, M. (1968) Influence of insulin on cyclic 3′, 5′-AMP phosphodiesterase activity in liver, skeletal music, adipose tissue and kidney. *Diabetologia* **4**, 330–335
4) Tanigawa, Y., Kitamura, A., Kawamura, M., and Shimoyama, M. (1978) The possible participation of glucocorticoid in the elevation of 3′, 5′-cyclic AMP levels through inhibition of cyclic AMP phosphodiesterase. *Physiol. Chem. Phys.* (*in press*)
5) Holten, D., Wicks, W. D., and Kenney, F. (1967) Studies on the role of vitamin B_6 derivatives in regulating tyrosine alpha-ketoglutarate transaminase activity *in vitro* and *in vivo*. *J. Biol. Chem.* **242**, 1053–1056
6) Gilman, A. G. (1970) A protein binding assay for adenosine 3′, 5′-cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.* **67**, 305–312
7) Mattingly, D. (1962) A simple fluorimetric method of the estimation of free 11-hydroxycorticoids in human plasma. *J. Clin. Path.* **15**, 374–379
8) Shioji, K., Imai, H., Tai, J., Ueda, I., Tanigawa, Y. , and Shimoyama, M. (1978) Tyrosine aminotransferase from chick liver : heat activation and cold inactivation of the enzyme. *Biochem. Biophys. Acta* **522**, 96–103
9) Diamondstone, T. I. (1966) Assay of tyrosine transaminase activity by conversion of *p*-hydroxyphenylpyruvate to *p*-hydroxybenzaldehyde. *Anal. Biochem.* **16**, 395–401
10) Hayashi,S., Granner, D. K., and Tomkins, G. M. (1967) Tyrosine aminotransferase : purification and characterization. *J. Biol. Chem.* **242**, 3998–4006
11) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
12) Sutherland, E. W., and Rall, T. R. (1958) Fractionization and characterization of a cyclic adenosine ribonucleotide formed by tissue particles. *J. Biol. Chem.* **232**, 1077–1091
13) Butcher, R. W., and Sutherland, E. W. (1962) Adenosine 3′, 5′-phosphate in biological materials. *J. Biol. Chem.* **237**, 1244–1250

14) Appleman, M. M., and Kemp, G. (1966) Puromycin : A potent metabolic effect independent of protein synthesis. *Biochem. Biophys. Res. Commun.* **24**, 564–568

15) Dalton, C., Quinn, J. B., Burghardt, C. R., and Sheppard H. (1970) Investigation of the mechanism of action of the lipolytic agent 4-(3, 4-dimethoxybenzyl)-2-imidazolidinone. *J. Pharmacol. Exp. Ther.* **173**, 270–276

16) Sheppard, H, and Wiggan, G. (1970) Analogues of 4-(3, 4-dimethoxybenzyl)-2-imidazolidinone as a potent inhibitor of rat erythrocyte adenosine cyclic 3′, 5′-phosphate phosphodiesterase. *Mol. Pharmacol.* **7**, 111–115

17) Sheppard, H., and Wiggan, G. (1971) Different sensitivities of the phosphodiesterase (adenosine-3′, 5′-cyclic phosphate 3′-phosphohydrolase) of dog cerebral cortex and erythrocytes to inhibition by synthetic agents and cold. *Biochem. Pharmacol.* **20**, 2128–2130

18) Kukovetz, W. R., and Pöch, G. (1970) Inhibition of cyclic 3′, 5′-nucleotide phosphodiesterase as a possible mode of action of papaverine and similarly acting drugs. *Naunyn Schmiedebergs Arch. Pharmacol.* **267**, 189–194

19) Jriner, L., Villiemoz, Y., Schwartz, I., and Nahas, G. G. (1970) Cyclic phosphodiesterase activity and the action of papaverine. *Biochem. Biophys. Res. Commun.* **40**, 64–69

20) Shimoyama, M., Kawai, M., Tanigawa, Y., Ueda, I., Sakamoto, M., and Hagiwara, K., Yamashita, Y., and Sakakibara, E. (1972) Evidence for and some properties of a 3′, 5′-cyclic AMP phosphodiesterase inhibitor in potato. *Biochem. Biophys. Res. Commun.* **47**, 59–65

21) Shimoyama, M., Sakamoto, M., Nasu, S., Shigehisa, S., and Ueda, I. (1972) Identification of the 3′, 5′-cyclic AMP phosphodiesterase inhibitor in potato : Feed-back control by inorganic phosphate. *Biochem. Biophys. Res. Commun.* **48**, 235–241

22) Shimoyama, M., Kawai, M., Hoshi, Y., and Ueda, I. (1972) Nicotinamide inhibition of 3′, 5′-cyclic AMP phosphodiesterase *in vitro*. *Biochem. Biophys. Res. Commun.* **49**, 1137–1141

23) Nasu, S., Shioji, K., Ueda, I., Tanigawa, Y., and Shimoyama M. (1978) Ca^{2+}/protein modulator-dependent and -independent cyclic GMP phosphodiesterase from hog heart. *J. Biochem.* **83**, 1449–1458

24) Tanigawa, Y., Shimoyama, M., Tai, J., Fujii, K., and Ueda, I. (1976) Reciprocal changes in Ca^{2+}/protein activator-dependent and -independent cyclic AMP phosphodiesterase during the development of chick embryos. *Biochem. Biophys. Res. Commun.* **73**, 19–24

25) Russel, T. R., Terasaki, W. L., Appleman, M. M. (1973) Separate phosphodiesterase for the hydrolysis of cyclic adenosine 3′, 5′-monophosphate in rat liver. *J. Biol. Chem.* **248**, 1334–1340