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INDUCTION OF CYTOSOLIC ASPARTATE AMINOTRANSFERASE BY GLUCAGON IN PRIMARY CULTURED RAT HEPATOCYTES

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The activity and the mRNA content of cytosolic aspartate aminotransferase (EC 2.6.1.1) were examined in cultured rat hepatocytes. Addition of glucagon (1×10^{-7}M) in the presence of dexamethasone (1×10^{-7}M) caused about 2-fold increase in the activity and mRNA content. Dibutyryl cAMP (1×10^{-4}M) could replace glucagon for this effect. Maximal induction of cytosolic aspartate aminotransferase mRNA was observed 8 h after their additions. Insulin (1×10^{-7}M) did not inbibit the enzyme induction by glucagon or dibutyryl cAMP. These results suggest that the cytosolic aspartate aminotransferase gene is regulated by cAMP, and not by insulin.

Recently we found that cytosolic aspartate aminotransferase (cAspAT) of rat liver was induced in the gluconeogenic state *in vivo*, that is, a high-protein diet, starvation or administration of glucagon increased the cAspAT activity and cAspAT mRNA about 2-fold *in vivo* (1,2). The physiological significance of induction of cAspAT is considered to be to increase the supply of oxaloacetate as a substrate for cytosolic phosphoenolpyruvate carboxykinase (cPEPCK; EC 4.1.1.32), which is also induced 2- to 3-fold by starvation or injection of glucagon (3,4). In this work, we studied the effects of various hormones and inducers on the cAspAT activity and the content of cAspAT mRNA in primary cultures of adult rat hepatocytes.

Abbreviations used : AspAT, aspartate aminotransferase (EC 2.6.1.1); cAspAT and mAspAT, cytosolic and mitochondrial isozyme, respectively; cPEPCK, cytosolic phosphoenolpyruvate carboxykinase (EC 4.1.1.32); Bt_2cAMP, dibutyryl cyclic AMP.

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Results showed that cAspAT was induced by glucagon and dibutyryl cAMP and that this induction was not suppressed by insulin.

MATERIALS AND METHODS

Cell Culture and Assay of Enzyme Activity --- Adult Male Wistar rats weighing around 200 g were used. Parenchymal hepatocytes were obtained by perfusion of the liver with collagenase, and the cells were cultured as monolayers as reported by Tanaka *et al.* (5). Hepatocytoes were seeded at a density of 1×10^5 cells/cm^2 cultured in Williams medium E with 10% fetal bovine serum, 1×10^{-6}M dexamethasone, and 1×10^{-7}M insulin. After 8 h, the medium was replaced by hormone-free medium. Inducers were added 14 h later and the cells were cultured further for 24 h. In time-course experiments, the cells were harvested at the indicated times. The cells were washed with ice-cold phosphate-buffered saline, and harvested. Samples of 5×10^5 cells in 0.2 ml of 50 mM potassium phosphate, pH 6.0, containing 50 mM potassium succinate, 0.5 mM 2-oxoglutarate, 0.2 mM EDTA, 0.1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 0.1% Triton X-100 and 0.05 mM pyridoxal 5'-phosphate were sonicated for three 30 sec periods at 0°C. The sonicate was centrifuged at 10,000 x g for 10 min and AspAT activity in the supernatant was assayed. AspAT isozymes were assayed separately with their antisera as described previously (6). Protein was determined by the method of Lowry *et al.* (7).

Isolation of RNA and quantification of cAspAT mRNA --- Total RNA was isolated by the method of Chirgwin *et al.* (8). Samples (5 µg) of total RNA were subjected to dot blot analysis using rat cAspAT cDNA (9) labelled with [α-^{32}P] dCTP as described previously (2). The specific activity of the probe was about 1×10^8 cpm/µg DNA. Quantification of cAspAT mRNA was performed by densitometry after autoradiography.

Materials --- All materials used were standard commercial products.

RESULTS

Isolated hepatocytes were first cultured for 8 h with insulin and dexamethasone, which are necessary for attachment of the cells to the culture dishes and their spreading (5). After further culture without hormones for 14 h, various hormones were added, and cAspAT activity was determined 24 h later. As shown in Table 1, neither dexamethasone (1×10^{-6}M) nor insulin (1×10^{-7}M) had any effect on the enzyme activity but glucagon (1×10^{-7}M) increased the activity. In the presence of dexamethasone, glucagon increased the activity to about twice that of control cells. Dibutyryl cyclic AMP (Bt_2cAMP, 1×10^{-4}M) was as effective as glucagon in the presence of dexamethasone. On the other hand, glucagon and Bt_2cAMP did not affect the activ-

Table 1. Effects of various hormones on cAspAT activity in cultured hepatocytes. The cells were incubated for 24 h with various inducers. Values are mean activities (mU/10^5 cells) ± S.D. for those in 4 experiments. *Significant difference from value for control ($p<0.005$) by Student's t-test.

Addition	Concentration	cAspAT activity (mU/10^5 cells)	Relative activity
None	-	5.0 ± 0.5	1.0
Dexamethasone (D)	1×10^{-6}M	5.5 ± 0.4	1.1
Insulin (I)	1×10^{-7}M	6.1 ± 0.6	1.2
Glucagon (G)	1×10^{-7}M	6.9 ± 0.5*	1.4
(G)+(I)		6.9 ± 1.2	1.4
(G)+(D)		9.3 ± 1.3*	1.9
(G)+(D)+(I)		9.7 ± 1.7*	1.9
Bt_2cAMP (B)	1×10^{-4}M	6.8 ± 0.7	1.4
(B)+(D)		9.0 ± 1.1*	1.8
(B)+(D)+(I)		10.0 ± 2.9	2.0
(B)+(D)+(G)		10.5 ± 2.1*	2.1
(B)+(D)+(G)+(I)		13.4 ± 2.5*	2.7
8-Bromo-cGMP	1×10^{-3}M	4.3 ± 0.3	0.9
TPA	1×10^{-7}M	4.0 ± 0.7	0.8
A-23187	2×10^{-6}M	2.2 ± 0.6	0.4

ity of mitochondrial aspartate aminotransferase (mAspAT) (data not shown). Insulin did not suppress the increase of cAspAT activity by glucagon and Bt_2cAMP. 8-Bromo-cGMP (1×10^{-3}M), 12-O-tetradecanoyl phorbol-13 acetate (TPA, 1×10^{-7}M) and A23187 (calcium ionophore, 2×10^{-6}M) did not increase cAspAT activity. Fig. 1 shows the dose dependences of the increases of cAspAT activity induced by glucagon and Bt_2cAMP. Maximal effects were observed with 10^{-7}M glucagon and 10^{-5}M Bt_2cAMP. Fig. 2 shows that cycloheximide (10 μg/ml) prevented the inductions of cAspAT by glucagon and Bt_2cAMP, suggesting that the enzyme induction was dependent on the synthesis of new protein. Fig. 3 shows the time-course of enzyme induction by Bt_2cAMP. In the presence of 1×10^{-4}M Bt_2cAMP and 1×10^{-6}M dexamethasone,

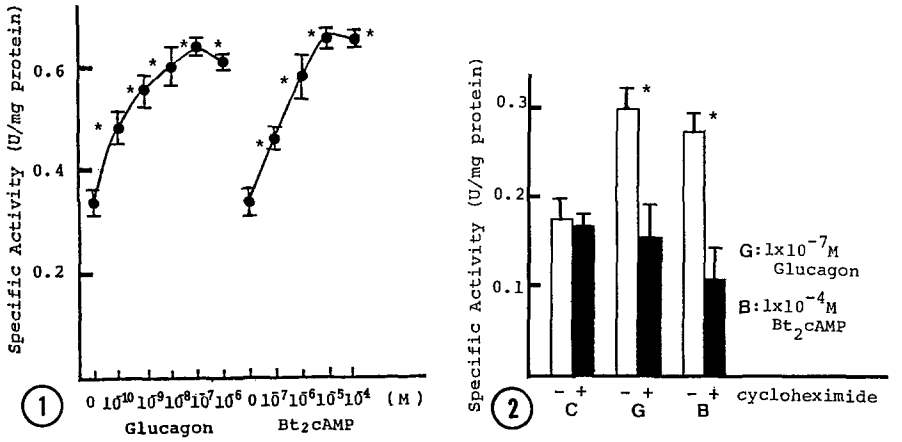

Fig. 1 Dose-response curves of cAspAT activity to glucagon and Bt_2cAMP in the presence of dexamethasone. The cells were incubated for 24 h with 1 x 10^{-6}M dexamethasone and various concentrations of glucagon or Bt_2cAMP. Values are means (U/mg protein) ± S.D. for 6 experiments. *Significant difference from value for control ($p<0.005$) by Student's t-test.

Fig.2 Effect of cycloheximide on cAspAT activity in cultured hepatocytes. Cells were incubated for 24 h with 1 x 10^{-7}M glucagon or 1 x 10^{-4}M Bt_2cAMP in the presence of 1 x 10^{-6}M dexamethasone with (+) or without (-) cycloheximide (10 µg/ml). Values are means (U/mg protein) ± S.D. for 4 samples. *Significant difference from value for control (without cycloheximide)($p< 0.005$) by Student's t-test.

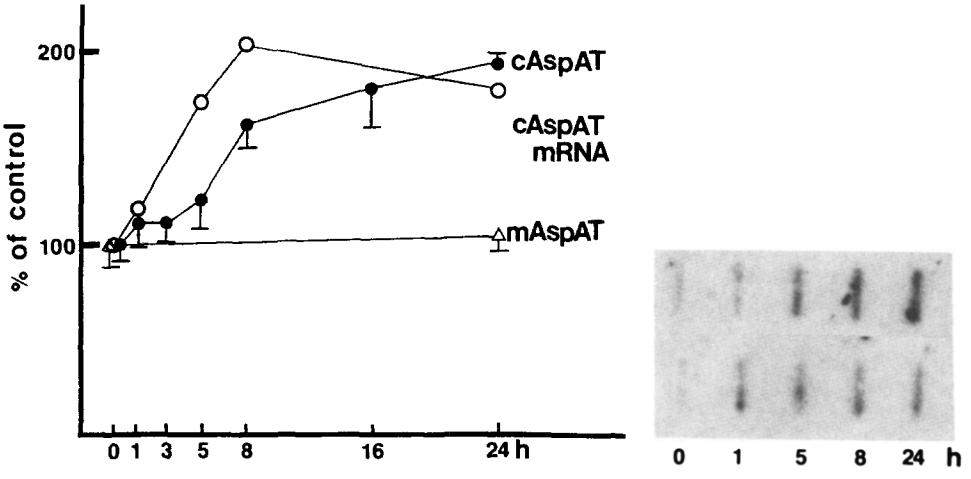

Fig. 3 Time-courses of change in cAspAT activity, cAspAT mRNA, and mAspAT activity in response to Bt_2cAMP (1 x 10^{-4}M)and dexamethasone (1 x 10^{-6}M). cAspAT and mAspAT activity were assayed at the indicated times. Activities are expressed as percentages of the control value ± S.D. of means in six separate experiments. The specific activites of cAspAT and mAspAT of the control were 0.338 ± 0.042 and 1.327 ± 0.115 U/mg protein, respectively. Total RNA was also extracted at the indicated times and cAspAT mRNA was analyzed by dot blot analysis using nick-translated rat cAspAT cDNA with [α-^{32}P]dCTP. Relative amounts of cAspAT mRNA are indicated as percentages of the control value of means in two separate experiments.

the increase of cAspAT activity was evident after 3 h and reached a plateau after 8 h. On the other hand, the cAspAT mRNA level was significantly higher than that in uninduced control cells, as early as 1 h after the addition of Bt_2cAMP, and reached a maximum of about 2.2-fold the uninduced control level after 8 h. The time-course of the induction of cAspAT activity by glucagon was similar to that by Bt_2cAMP (data not shown). Addition of 1×10^{-7}M insulin did not affect the time-course of cAspAT induction by glucagon or Bt_2cAMP (data not shown). The mAspAT activity was not changed 24 h after the addition of Bt_2cAMP, indicating that the viability of the cells was not changed.

DISCUSSION

In the present study we showed that glucagon and Bt_2cAMP induced cAspAT activity with elevation of its mRNA. These data are consistent with our previous *in vivo* findings (1,2).

Bt_2cAMP was as effective as glucagon in inducing cAspAT. The response was slower than that of cPEPCK (10) or tyrosine aminotransferase (11), the mRNA levels of which reached maxima within 2 or 3 h. The induction of ornithine aminotransferase by Bt_2cAMP was also slow (12). This slow induction suggests that Bt_2cAMP regulates the expression of an intermediate gene product(s) required for cAMP stimulation of expression of the cAspAT gene.

Glucagon and insulin are known to have opposite effects on metabolism and to affect the expressions of the genes of many enzymes. Transcription of the cPEPCK gene is increased by cAMP, and insulin inhibits its induction (10). However, the induction of cAspAT by glucagon and Bt_2cAMP was not affected by insulin. This result supports previous findings on cPEPCK, which showed

that the mechanism of action of insulin was independent of that of glucagon (13).

Although dexamethasone did not induce cAspAT activity, 1 x 10^{-6}M dexamethasone greatly enhanced the inductions by glucagon and Bt_2cAMP, so the effect of dexamethasone on cAspAT induction cannot be neglected. Glucagon is reported to induce serine dehydratase(EC 4,2,1,13) and tryptophan oxygenase(EC 1,13,11,11) in cultured rat hepatocytes only in the presence of dexamethasone (14,15). But dexamethasone is thought to be essential for maintenance of hepatocytes (5), so, the low inducibility of cAspAT activity by glucagon in the absence of dexamethasone may be attributed to the low responsiveness of the cells to the hormone.

The precise role of regulation of the cAspAT gene is not known, but primary cultures of hepatocytes are useful for exploring the mode of action of factors affecting the expression of liver genes. These cultures will be of great value for further studies on expression of the cAspAT gene.

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REFERENCES

1. Horio,Y., Nishida,Y., Sakakibara,R., Inagaki,S., Kamisaki,Y., and Wada,H. (1988) Biochem Int. 16, 579-586.
2. Horio,Y., Tanaka,T., Taketoshi,M., Uno,T., and Wada,H. (1988) J. Biochem. (in press)
3. Shargo,E., Lardy,H.A., Nordlie,R.C., and Foster,D.O. (1963) J. Biol. Chem. 238, 3188-3192.
4. Nordlie,R.C., Varricchio,F.E., and Holten,D.D.(1965) Biochim. Biophys. Acta 97, 214-221.
5. Tanaka,K., Sato,M., Tomita,K., Sato,M., Tomita,Y., and Ichihara,A. (1978) J. Biochem. 84, 937-946.
6. Horio,Y., Sakakibara,R., Tanaka,T., Taketoshi,M., Obaru,K., Shimada,K., Morino,Y., and Wada,H. (1986) Biochem. Biophys. Res. Commun. 134, 803-811.
7. Lowry,O.H., Rosebrough,N.J., Farr,A.L., and Randall,R.J. (1951) J. Biol. Chem. 193, 265-275.
8. Chirgwin,J.M., Przydyla,A.E., MacDonald,R.J., and Rutter,W.J. (1979) Biochemistry 18, 5294-5299.

9. Horio,Y., Tanaka,T., Taketoshi,M., Nagashima,F., Tanase,S., Morino,Y., and Wada,H. (1988) J. Biochem. (in press)
10. Sasaki,K., Cripe,T.P., Koch,S.R., Andreone,T.L., Peterson,D.D., Beale,E.G., and Granner,D.K. (1984) J. Biol. Chem. 259, 15242-15251.
11. Schmid,E., Schmid,W., Jantzen,M., Mayer,D., Jastorff,B., and Schütz,G. (1987) Eur. J. Biochem. 165, 499-506.
12. Merrill,M.J., and Pitot,H.C. (1985) Arch. Biochem. Biophys. 237, 375-385.
13. Beale,E.G., Koch,S.R., Brotherton,A.F.A., Sheorain,V.S., and Granner,D.K. (1986) Diabetes 35, 546-549.
14. Noda,C., Nakamura,T., and Ichihara,A. (1981) Biochem. Biophys. Res. Commun. 100, 65-72.
15. Nakamura,T., Shinno,H., and Ichihara,A. (1980) J. Biol. Chem. 255, 7533-7535.