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TWO PHASE REGULATION OF TYROSINE AMINOTRANSFERASE ACTIVITY BY INSULIN IN PRIMARY CULTURED HEPATOCYTES OF ADULT RATS[1]

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SUMMARY: In primary cultures of adult rat hepatocytes, dexamethasone(10^{-5}M) induced tyrosine aminotransferase(TAT) 24 h after its addition. Glucagon(10^{-7}M) alone had no effect, but strongly enhanced the induction by dexamethasone. Glucagon could be replaced by butyryl cyclic-AMP(10^{-4}M), which caused about 20-fold increase in activity. In contrast to many previous reports that insulin induced TAT activity *in vivo* and *in vitro*, it inhibited the inductions of TAT by dexamethasone and dexamethasone plus glucagon 24 h after its addition. However, insulin significantly induced TAT activity in the early pahse, 4 h after its addition. Dose-response curves of the effect of insulin on TAT activity showed reverse relations to activity in early and late phase. These results show that TAT activity is regulated by insulin in a two phase fashion.

It is generally accepted that insulin and glucagon have opposite effects in regulation of metabolism in the liver:glucagon induces many key enzymes for amino acid metabolism and thus enhances gluconeogenesis and ureogenesis from amino acids, while insulin inhibits these activities increased by glucagon, but stimulates glycolysis and lipogenesis. We recently found that in primary cultures of adult rat hepatocytes glucagon or dibutyryl cyclic AMP(bt_2c-AMP) induced the activities of some key enzymes for amino acid metabolism, such as tryptophan oxygenase, lysine 2-oxoglutarate reductase and serine dehydratase, while insulin strongly inhibited the inductions of all these activities(1-3). However, it is well known that tyrosine aminotransferase(EC 2.6.1.6, TAT) can be induced by insulin as well as glucagon in rat liver *in vivo*(4), in isolated

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perfused liver(5), and in a hepatoma-derived cell line(6-8). Thus, the effect of insulin on synthesis of TAT seems to be an exceptional for this hormone.

This paper reports the two phase regulation of TAT by insulin in primary cultures of adult rat hepatocytes: insulin induces TAT activity in the early phase, but inhibits the activity in the late phase.

MATERIALS and METHODS

Materials --- The rats and materials used for cell isolation and culture were as reported previously(9). Insulin, glucagon and bt_2c-AMP were obtained from Sigma Chemical Co.,St. Louis;dexamethasone was from Schering AG.,Berlin and other chemicals were from Wako Pure Chemicals, Osaka.

Primary Cultures of Adult Rat Hepatocytes --- Parenchymal hepatocytes were isolated from adult Wistar rats(150 to 200 g) by perfusion of the liver in situ with collagenase, and the cells were cultured as monolayers as reported previously(9). Inocula of 2 x 10^6 cells were plated in 60 mm Corning plastic dishes in 4 ml of Williams medium E supplemented with 5% calf serum and 1 x 10^{-9}M insulin under 5% CO_2 and 30% O_2 in air. After 6 h, the medium was replaced by hormone-free medium, and 18 h later, hormones were added and the cells were cultured further for 24 h.

Assay of TAT --- The cells were washed with ice-cold phosphate buffered-saline, harvested with a rubber policeman, and homogenized in a Polytron homogenizer for 1.5 min in 0.5 ml of 20 mM potassium phosphate buffer(pH 7.0) containing 0.2 mM pyridoxal phosphate. The homogenate was centrifuged and the supernatant was used as the enzyme sample. TAT was measured by the method of Granner and Tomkins(10). One unit of activity is defined as the amount forming 1 μmole of p-hydroxyphenylpyruvate per min at 37°C. Protein was measured by the method of Lowry et al.(11).

RESULTS and DISCUSSION

In previous work we showed that freshly isolated hepatocytes have greatly impaired functions and reduced responsiveness to hormones(9,12,13), and that these characters are restored during culture for 1 day. However, it is necessary to add insulin or dexamethasone to maintain functionally active cells in culture(9). Therefore , insulin was added first 6 h after the start of culture. When cells became well attached and spread, they were cultured without hormone for 18 h and then the effects of various hormones on TAT

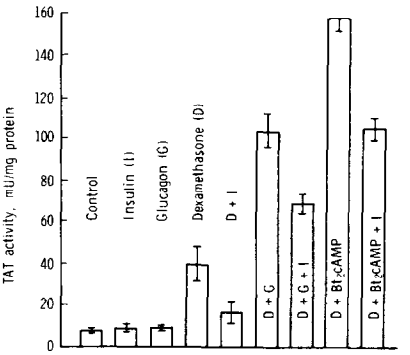
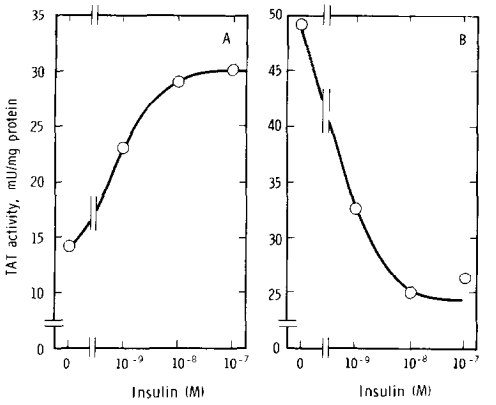


Fig. 1. Effect of various hormones on induction of TAT in primary cultures of adult rat hepatocytes. Hormone concentrations were: insulin, 1×10^{-7}M; glucagon, 1×10^{-7}M; dexamethasone, 1×10^{-5}M; Bt_2c-AMP, 1×10^{-4}M. Values are means ± S.D. for 4 experiments.

were examined during 2nd 24 h. As shown in Fig. 1, TAT activity increased to about 5 times the control value in 24 h in medium containing 10^{-5}M dexamethasone. Glucagon(10^{-7}M) alone did not induce TAT, but it markedly enhanced the induction by dexamethasone. Glucagon could be replaced by bt_2c-AMP(10^{-4}M) and the highest induction could be achieved by addition of dexamethasone and bt_2c-AMP. These findings are consistent with previous findings *in vivo* (4,14) and *in vitro*(8,15,16). In cultured adult rat hepatocytes dexamethasone and glucagon or bt_2c-AMP have been found to have synergistic effects on the inductions of other liver enzymes; tryptophan oxygenase(1), lysine-2-oxoglutarate reductase(2) and serine dehydratase(3). Thus, glucagon under the permissive effect of glucocorticoid enhances catabolism of amino acids by inducing many amino acid-catabolizing enzymes in liver cells.

On the other hand, as shown in Fig. 1, insulin strongly inhibited induction of TAT by dexamethasone and dexamethasone plus glucagon or bt_2c-AMP. Its effect was seen at a physiological conc-



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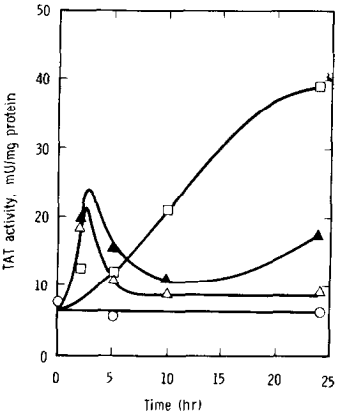

(3)

Fig. 2. Dose-response curves for the effects of insulin on TAT activities in the early and late phases. A:early phase, 4 h after addition of insulin plus dexamethasone(10^{-5}M). B:late phase, 24 h after addition of insulin plus dexamethasone(10^{-5}M). Values are means for duplicate experiments.

Fig. 3. Two-phase regulation of TAT activity by insulin. ○:no addition, □:dexamethasone(10^{-5}M), △:insulin(10^{-7}M), ▲:dexamethasone(10^{-5}M) plus insulin(10^{-7}M). Values are means for duplicate experiments.

entration and was dose-dependent(Fig.2B). Surprisingly, many other reports on the effect of insulin on TAT activity are contradictory to our findings. These apparently discrepant results may be due to the time when TAT activity was measured after addition of hormones, since in a short period TAT is induced by glucocorticoid and pancreatic hormones, but there have been no studies on the long term effects of insulin on TAT induction by dexamethasone and glucagon. The present results were obtained by measuring TAT activity at 24 h after addition of insulin with dexamethasone or dexamethasone plus glucagon(or bt_2c-AMP).

To demonstrate induction of TAT by insulin in a short period,we examined the time course of the response of TAT to insulin alone or with dexamethasone(Fig.3). When cultured hepatocytes were incubated with 10^{-5}M dexamethasone, TAT activity began to increase after 5 h and reached a plateau of about 5 times the initial value

after 20 h. In contrast, when the cells were incubated with 10^{-7}M insulin alone, the enzyme activity rapidly increased, reaching a maximum of about twice the basal value in 4 h and then decreased toward the initial level. Simultaneous additions of insulin and dexamethasone resulted in two phase regulation of TAT activity by insulin; in the early phase TAT activity increased rapidly as that by insulin alone, but somewhat higher rate and then the activity induced by dexamethasone was inhibited. Fig.2 shows the dose-dependent curves of the effect of insulin on TAT activity in the early(A) and late phase(B), respectively. The curves for increase in TAT activity in the early phase and inhibition of the activity induced by dexamethasone in the late phase were reverse and both effects of insulin were maximum at a concentration of 10^{-8}M. It can be concluded from these findings that TAT activity is regulated by insulin in a two-phase fashion in liver cells.

Stimulation of TAT synthesis by insulin is known to be resistant to treatment with actinomycin D, suggesting that insulin acts on the translational step in TAT synthesis(6,7). Furthermore, insulin also stimulates hepatic uptake of amino acids and polysome aggregation,and hence general protein synthesis. Thus, the early effect of insulin on TAT synthesis, which is short-lived, appears to be secondary to an effect on uptake of amino acids and general protein synthesis. On the contrary, the late effect of insulin on TAT synthesis seems to be the true action, like its inhibition of induction of other amino acid-degrading enzymes. Therefore, present findings that glucocorticoid and glucagon induced TAT and insulin inhibited it are physiologically reasonable. Thus, the effect of insulin on TAT synthesis can now be considered as not to be exceptional.

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