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HORMONAL REGULATION OF SERINE DEHYDRATASE ACTIVITY IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES[1]

Chiseko Noda, Toshikazu Nakamura and Akira Ichihara

Institute for Enzyme Research, School of Medicine,
University of Tokushima, Tokushima 770, Japan

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SUMMARY: The basal activity of serine dehydratase in primary cultured rat hepatocytes decreased during culture. Addition of dexamethasone(1×10^{-5}M) plus glucagon(2.5×10^{-7}M) not only prevented the decrease, but also caused 3-8 fold increase in the activity. Glucagon could be replaced by dibutyryl cyclic AMP(1×10^{-4}M). Neither dexamethasone alone nor glucagon alone had any effect. Insulin(1×10^{-7}M) strongly inhibited the induction by glucagon plus dexamethasone. The induction was completely blocked by cycloheximide(5×10^{-6}M) or puromycin(1×10^{-6}M). Of the other hormones tested, testosterone(1×10^{-7}M) enhanced the induction by dexamethasone plus glucagon, whereas epinephrine(1×10^{-5}M) and dibutyryl cyclic GMP(1×10^{-4}M) inhibited the induction. Epinephrine seems to cause inhibition via α-adrenergic receptor, because its effect was completely blocked by simultaneous addition of phenoxybenzamine(1×10^{-5}M), an α-adrenergic blocker. These results suggest that this enzyme activity is regulated by several hormones, such as pancreatic, adrenal, sexual and adrenergic hormones.

Metabolism of amino acids is enhanced by feeding a high protein diet or in catablic states, such as during starvation or the diabetic conditions. It is known that these conditions cause an increase in the activity of serine dehydratase(EC 4.2.1.13, SDH) [1-4]. This enzyme catalyzes the formation of pyruvate from serine and is assumed to play a key role in gluconeogenesis[3,4], although an alternative pathway may be involved in gluconeogenesis from serine[5,6]. These and further results[7] suggested that the activity of this enzyme is regulated by adrenal and pancreatic hormones, and Ishikawa and Nakajima[8] reported the antagonistic effects

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of insulin and glucagon and permissive action of glucocorticoid on the induction of this enzyme in adrenalectomized and pancreatectomized rats. However, in studies *in vivo* it is difficult to exclude possible secondary effects after injection of hormones or excision of endocrine organs. Recently we showed that in primary cultures of adult rat hepatocytes, insulin induced ornithine decarboxylase, glucokinase and glucose-6-phosphate dehydrogenase, while glucagon enhanced urea formation, amino acid release and the activities of some key enzymes in amino acid metabolism[9-14]. Therefore, studies with these cells are simpler and give clear results than those *in vivo*. This paper reports studies using these cells on the hormonal regulation of SDH.

MATERIALS and METHODS

Materials --- Insulin, glucagon, estradiol, dibutyryl cyclic AMP(bt_2cAMP), dibutyryl cyclic GMP(bt_2cGMP) and DL-propranolol were obtained from Sigma Chemical Co., St. Louis. Testosterone, triiodo-DL-thyronine and phenoxybenzamine were from Nakarai Chemicals, Kyoto. Dexamethasone was from Schering AG, Berlin. l-Epinephrine was from Merck, Darmstadt. Cycloheximide was from P-L Biochemicals, Milwaukee. Puromycin was from Makor Chemicals Ltd., Jerusalem.

Primary cultures of adult rat hepatocytes --- Adult male Wistar strain rats, weighing about 200 g, were given laboratory chow *ad libitum*. Parenchymal hepatocytes were obtained by perfusion of the liver with collagenase *in situ* as reported previously[15] and isolated hepatocytes(2×10^6 cells) were cultured on 60 mm Corning dishes as monolayers in Williams medium E with 5% calf serum and 1×10^{-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 various hormones were added, and the cells were cultured for a further 24h.

Assay of SDH --- The cells were washed with ice-cold phosphate buffered saline, harvested with a rubber policeman, and homogenized in 0.5 ml of SDH assay mixture in the absence of serine in a Polytron homogenizer for 1.5 min. SDH activity was measured by the method of Suda and Nakagawa[16] except that the final volume was 0.3 ml after addition of 0.03 ml of 1 M serine. One unit of activity is defined as the amount forming 1 μmole of pyruvate per min at 37°C. Protein was measured by the method of Lowry et al.[17].

RESULTS

In previous studies we showed that freshly isolated hepatocytes have impaired functions and show low responsiveness to hormones,

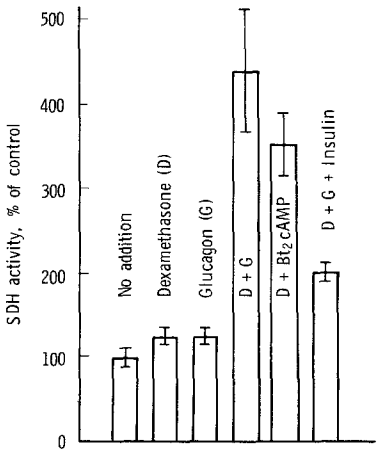

Fig.1. Induction of SDH in cultured hepatocytes. Rat hepatocytes were cultured for 24 h and various hormones were added. SDH activity was assayed 24 h after addition of hormones. Hormone concentrations used:dexamethasone(D), 1 x 10^{-5}M;glucagon(G), 2.5 x 10^{-7}M;bt_2cAMP, 1 x 10^{-4}M;insulin, 1 x 10^{-7}M. SDH activities were expressed as percentages of the control value ± S.E. of means for 6 experiments. The specific activity of the control was 1.39 ± 0.42 mU/mg protein.

but that these characters are restored during culture of the cells for 1 day[14,15,18]. In this experiment, isolated hepatocytes were cultured for the first 6 h with insulin, which is necessary for attachment of the cells to the culture dishes and their spreading. After further culture without hormone for 18 h, various hormones were added and SDH activity was measured 24 h after their addition. As shown in Fig.1. neither dexamethasone(1 x 10^{-5}M) alone nor glucagon(2.5 x 10^{-7}M) alone had any effect on the enzyme activity. However, the activity was increased by the simultaneous additions of dexamethasone and glucagon. Bt_2cAMP(1 x 10^{-4}M) was as effective as glucagon for the induction of SDH activity in the presence of dexamethasone. On the contrary, insulin strongly suppressed the increase of SDH activity by dexamethasone plus glucagon. The stimulatory effects of these hormones were reproducible, but the extents of stimulation were variable(3-8 fold) and the specific activity of untreated control cells also varied in different pre-

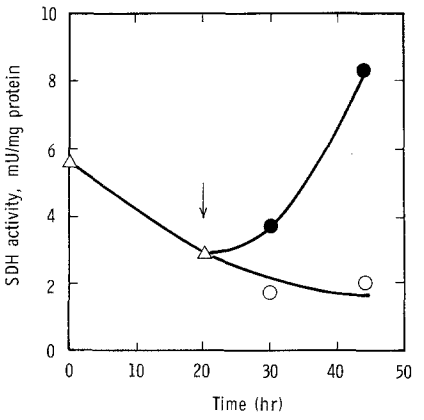

Fig.2. Time course of SDH induction by dexamethasone and glucagon in cultured hepatocytes. Hepatocytes were cultured with insulin (1×10^{-9}M) for 6 h and then without hormone for 14 h, Δ–Δ and various hormones were added (arrow); dexamethasone (1×10^{-5}M), O - O; dexamethasone (1×10^{-5}M) and glucagon (2.5×10^{-7}M), ● - ●.

parations of hepatocytes, probably owing to the difference between individual rats that is observed in studies in vivo[8].

The time course of induction of SDH activity is shown in Fig.2. Half the initial activity was lost during the first day of culture. Similar decreases in activity have been observed in lysine-2-oxoglutarate reductase[13] and tryptophan 2,3-dioxygenase[12], but not in tyrosine transaminase[19]. The decrease in SDH activity during culture was not prevented by either dexamethasone or glucagon alone, but dexamethasone plus glucagon led to increase in SDH activity after a lag of a few hours and 24 h after hormone addition the activity was about 4-fold that with dexamethasone alone. The inductive effect of dexamethasone and glucagon was completely blocked by cycloheximide (5×10^{-6}M) or puromycin (1×10^{-6}M), suggesting that the enzyme induction was dependent on the synthesis of new protein (data not shown).

It seemed interesting to determine whether any other hormones affected SDH activity. Therefore, the effects of various hormones were tested in the presence of dexamethasone with or without glu-

Table 1. Effects of various hormones on SDH in cultured hepatocytes. Culture conditions were as described in Fig.1. Although 6 experiments were carried out and hormonal effects were reproducible, absolute values in each experiment varied. Results of a typical experiment are shown.

Addition	Concentration	SDH activity (mU/mg protein)
None		1.56
Dexamethasone(D)	1×10^{-5}M	2.13
Glucagon(G)	2.5×10^{-7}M	2.24
D + G		7.21
" + " + human growth hormone	0.5 µg/ml	6.97
" + " + estradiol	1×10^{-7}M	7.23
" + " + testosterone	"	8.93
" + " + triiodothyronine	"	6.24
" + " + epinephrine	1×10^{-5}M	2.86
" + " + bt_2cGMP	1×10^{-4}M	4.24
" + " + insulin	1×10^{-7}M	2.88
None		2.87
D		2.94
D + G		21.2
" + " + epinephrine		8.81
" + " + " + propranolol	1×10^{-5}M	8.59
" + " + " + phenoxybenzamine	"	20.2

cagon. None of these hormones had any effect on SDH activity when added in combination of dexamethasone(data not shown). On the other hand, in the presence of glucagon, testosterone slightly enhanced induction, while bt_2cGMP was inhibitory(Table I). An interesting observation was that epinephrine(1×10^{-5}M) prevented the induction and this effect was completely abolished by the α-adrenergic antagonist phenoxybenzamine(1×10^{-5}M) but not by the β-antagonist propranolol.

DISCUSSION

It is well known that glucagon and glucocorticoid induce many key enzymes for amino acid metabolism and thus enhance gluconeogenesis and ureogenesis from amino acids. Insulin is known to have the opposite effect on these metabolisms. Primary cultured hepatocytes are a very useful system for study of these hormonal regulations and we showed that dexamethasone and glucagon induced the activity of some key enzymes in amino acid metabolism,while insulin strongly inhibited these inductions. However, many studies have shown that insulin induces tyrosine transaminase[see Refs in 19]. We recently found that insulin regulates the activity of this enzyme in a two-phase fashion: it induces enzyme activity in a few hours after its addition, while it prevents the induction of this enzyme by dexamethasone and dexamethasone plus glucagon during further culture(24 h after its addition)[19].

In the present report we characterized the hormonal control of SDH activity. The induction of this enzyme was absolutely dependent upon the presence of both dexamethasone and glucagon or bt_2cAMP. This is generally consistent with reports of *in vivo* studies[3,4, 7,8], except for one report of SDH induction by glucagon alone in adrenalectomized-pancreatectomized rats [8]. Both hormones are also necessary for induction of lysine-2-oxoglutarate reductase[13], while dexamethasone alone induced tyrosine transaminase and tryptophan 2,3-dioxygenase, although simultaneous addition of glucagon resulted in much greater induction[12,13,19]. The mechanisms of enzyme induction by these hormones are still unclear, although dexamethasone and cAMP seem to increase mRNA for enzyme [20]. The mechanism of action of insulin in enzyme induction is also unknown. In this report bt_2cGMP inhibited SDH induction. Glucokinase[21] and ornithine decarboxylase[22] are induced by both

insulin and cGMP. Insulin has been shown to induce a rise in cGMP in liver, but it is still uncertain whether cGMP is involved in mediating the action of insulin[23].

It is interesting that SDH activity is regulated by epinephrine via an α-adrenergic receptor. Catecholamines transferred to the liver are either secreted by the adrenal medulla or released from nerve endings, both sources being controlled by the sympathetic nerve system. Shimazu reported that hypothalamus-autonomic nerves are involved in the induction of tryptophan 2,3-dioxygenase and in the metabolism of glycogen[24]. More detailed hormonal and neurochemical studies are necessary to understand the regulatory mechanism of SDH activity.

REFERENCES

1. Pitot,H.C.,Potter,V.R. and Morris,H.P.(1961) Cancer Res. 21, 1001-1008
2. Goldstein,L.,Knox,W.E. and Behrman,E.J.(1962) J. Biol. Chem. 237, 2855-2860
3. Freedland,R.A. and Avery,E.H.(1964) J. Biol. Chem. 239, 3357-3360
4. Ishikawa,E.,Ninagawa,T. and Suda,M.(1965) J. Biochem. 57, 506-513
5. Veneziale,C.M.,Gabrielli,F. and Lardy,H.A.(1970) Biochemistry 9, 3960-3970
6. Rowsell,E.V.,Al-Tai,A.H.,Carnie,J.A. and Rowsell,K.V.(1973) Biochem. J. 134, 349-351
7. Jost,J.-P.,Khairallah,E.A. and Pitot,H.C.(1968) J. Biol. Chem. 243, 3057-3066
8. Ishikawa,E. and Nakajima,K.(1970) J. Biochem. 67, 685-692
9. Nakamura,T.,Aoyama,K.,Kato,S.,Tomita,Y.,Tanaka,K. and Ichihara, A.(1979) in Carcino-Embryonic Proteins(Lehmann,F.-G.,ed.),Vol. II,pp.723-728, Elsevier/North Holland, Amsterdam
10. Nakamura,T.,Aoyama,K. and Ichihara,A.(1979) Biochem. Biophys. Res. Commun. 91, 515-520
11. Tanaka,K.,Kishi,K. and Ichihara,A.(1979) J. Biochem. 86, 863-870
12. Nakamura,T.,Shinno,H. and Ichihara,A.(1980) J. Biol. Chem. 255, 7533-7535
13. Shinno,H.,Noda,C.,Tanaka,K. and Ichihara,A.(1980) Biochim. Biophys. Acta 633, 310-316
14. Ichihara,A.,Nakamura,T.,Tanaka,K.,Tomita,Y.,Aoyama,K.,Kato,S. and Shinno,H.(1980) Ann. N.Y. Acad. Sci. 349, 77-84
15. Tanaka,K.,Sato,M.,Tomita,Y. and Ichihara,A.(1978) J. Biochem. 84, 937-946
16. Suda,M. and Nakagawa,H.(1970) Methods in Enzymol. 17, 346-351

17. Lowry,O.H.,Rosebrough,N.J.,Farr,A.L. and Randall,R.J.(1957) J. Biol. Chem. 193, 265-275
18. Kato,S.,Aoyama,K.,Nakamura,T. and Ichihara,A.(1979) J. Biochem. 86, 1419-1425
19. Nakamura,T.,Noda,C. and Ichihara,A.(1981) Biochem. Biophys. Res. Commun. in press
20. Granner,D.,Olson,P.,Seifert,S.,Block,C.,Diesterhaft,M.,Hargrove, J. and Noguchi,T.(1980) Ann. N.Y. Acad. Sci. 349, 183-194
21. Spence,J.T.,Haars,L.,Edwards,A.,Bosch,A. and Pitot,H.C.(1980) Ann. N.Y. Acad. Sci. 349, 99-110
22. Tomita,Y.,Nakamura,T. and Ichihara,A.(1981) Exp. Cell Res. submitted
23. Czech,M.P.(1977) Ann. Rev. Biochem. 46, 359-384
24. Shimazu,T.(1979) Acta Physiol. Pol. Suppl. 18, 1-18