# REGULATION OF THE SYNTHESIS OF RAT LIVER SERINE DEHYDRATASE BY ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE

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Summary: The formation of rat liver serine dehydratase was stimulated by the injection of animals with adenosine 3',5'-cyclic monophosphate, epinephrine, or glucagon. The enhanced formation of serine dehydratase was sensitive to actinomycin D. An inhibition of the formation of serine dehydratase by glucose could be overcome by adenosine 3',5'-cyclic monophosphate or, more effectively, by its dibutyryl derivative.

The formation of the enzyme serine dehydratase can be induced in the livers of intact or adrenalectomized rats by the oral intubation of a mixture of amino acids or by the injection of glucagon (1,2,3). Administration of glucose to animals fed amino acids blocked the increased rate of formation of serine dehydratase (3). Actinomycin D and puromycin also inhibited the induced formation of serine dehydratase. This suggested that the induction of serine dehydratase activity represented de novo synthesis of the enzyme. Recent investigations in which quantitative immunochemical techniques were used (4) confirmed this suggestion and also showed that the feeding of glucose both decreased the rate of synthesis and increased the rate of degradation of serine dehydratase.

A series of studies (5,6,7,8,9) showed that one of the metabolic effects of glucagon on liver is to increase the intracellular steady state concentration of adenosine 3',5'-cyclic monophosphate (cyclic AMP). It is also well established that epinephrine increases the intracellular concentration of cyclic AMP by stimulating adenyl cyclase (10). For this reason we tested the effect of both epinephrine and cyclic AMP on the stimulation of the formation of serine dehydratase.

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Our findings show that the activity of serine dehydratase in rat liver is controlled by cyclic AMP. The inhibition of serine dehydratase formation by glucose in particular can be overcome by the administration of cyclic AMP (as well as by epinephrine or glucagon).

We used Wistar (Don B Lab Animals, Chatsworth, California 91311) rats weighing 100-115 grams. The animals were kept on an eight percent protein diet for five days prior to the experiment, starved overnight, and injected intraperitoneally at six hour intervals. The livers were homogenized and high speed supernates prepared as described previously (4). Serine dehydratase was determined according to Goldstein et al. (11), glucokinase according to Walker and Parry (12), tyrosine aminotransferase according to Pitot and Pries (13). Protein was measured by the Biuret reaction (15).

The stimulation of the synthesis of serine dehydratase by amino acids, glucagon, epinephrine, cyclic AMP, $N^6,O^{2'}$-dibutyryl adenosine 3',5'-cyclic monophosphate (dibutyryl cyclic AMP) and a combination of cyclic AMP and theophylline, respectively, is shown in Table 1. For comparative purposes the glucokinase and tyrosine aminotransferase activities of the extracts were also measured. All treatments led to a significant increase in the specific activity of serine dehydratase over that of the untreated animals. Glucokinase activity was not affected whereas tyrosine aminotransferase activity was enhanced in the rats injected with cyclic AMP, dibutyryl cyclic AMP, and the combination of cyclic AMP and theophylline but not by the other treatments. Similar results were obtained by Wicks using fetal rat liver maintained in organ culture (16). The dibutyryl derivative of cyclic AMP was more effective than cyclic AMP in the stimulation of serine dehydratase formation. At concentrations between $5 \times 10^{-6}$ and $5 \times 10^{-4}$ M cyclic AMP had no effect on the activity of serine dehydratase *in vitro*.

Earlier work (1) showed that actinomycin D inhibited the induction of serine dehydratase by amino acids suggesting that the induction represented the *de novo* synthesis of protein coded by newly synthesized messenger RNA. The results presented in Table 2 demonstrate that actinomycin D, at the concentration employed,

Table 1

Stimulation of the Synthesis of Serine Dehydratase

| | Dosage per 100 g body weight | | Serine dehydratase, μmoles pyruvate/h/mg of protein | Glucokinase, μmoles NADPH/h/mg of protein | Tyrosine aminotransferase, μmoles hydroxyphenylpyruvate/h/mg of protein |
|---|---|---|---|---|---|
| | mg | μmoles | | | |
| Control | - | - | 0.11 ± 0.05 | 0.17 ± 0.001 | 157 ± 42 |
| Amino acids | 200 | 1280 | 2.86 ± 0.40 | 0.18 ± 0.001 | |
| Glucagon | 0.2 | 0.05 | 2.22 ± 0.50 | 0.19 ± 0.001 | 95 ± 40 |
| Epinephrine | 0.01 | 0.05 | 1.19 ± 0.20 | 0.17 ± 0.002 | 149 ± 51 |
| 3',5' cyclic AMP | 5.0 | 15.0 | 0.77 ± 0.20 | 0.21 ± 0.001 | 220 ± 50 |
| $N^6$-$O^{2'}$-dibutyryl cyclic AMP | 5.0 | 8.1 | 1.47 ± 0.60 | 0.21 ± 0.001 | 350 ± 33 |
| 3',5' cyclic AMP | 5.0 | 15.0 | | | |
| plus theophylline | 5.0 | 11.0 | 0.83 ± 0.40 | | 574 ± 350 |

Each experimental condition was tested in groups of 3-4 rats. The equimolar mixture of the 10 essential amino acids was given by oral intubation (4). The other compounds were injected intraperitoneally. The intubation and injections were performed at 0 time and at 6 hours; the rats were killed after 12 hours. Enzyme activity is expressed as micromoles of reaction product formed per hour per mg of protein in liver extract.

Table 2

Effect of Actinomycin D on the Synthesis of Serine Dehydratase

| | Serine dehydratase, μmoles pyruvate/h/mg protein | |
|---|---|---|
| | minus actinomycin D | plus actinomycin D |
| Control | 0.23 ± 0.11 | |
| $N^6,O^{2'}$-dibutyryl cyclic AMP | 0.88 ± 0.21 | 0.51 ± 0.01 |
| Glucagon | 2.09 ± 0.72 | 0.59 ± 0.11 |

The experimental design was as described in Table 1. 0.1 mg of actinomycin D per 100 g of body weight was injected 30 minutes prior to the injection of the stimulating agent. The serine dehydratase values represent the average of 3 animals.

also inhibited, albeit partially, the induction of serine dehydratase by dibutyryl cyclic AMP as well as by glucagon. This suggests that induction by cyclic AMP also reflects the de novo synthesis of protein.

A number of adenosine monophosphates were compared for their ability to stimulate the formation of serine dehydratase; in addition to 3',5' cyclic AMP, only the 2',3' analogue stimulated significantly.

Glucose blocks the induction of serine dehydratase by amino acids. Peraino and Pitot (3) showed that when glucagon was employed as inducer, the inhibition by glucose could be overcome by increasing the concentration of glucagon. It was therefore of interest to see, whether epinephrine or dibutyryl cyclic AMP would also derepress the formation of serine dehydratase in the presence of glucose. The results presented in Table 3 show that repeated delayed injections of glucagon, epinephrine, or dibutyryl cyclic AMP overcame the repression by glucose.

It is well known that cyclic AMP affects the activity of a number of enzymes (5); these include muscle phosphorylase (17,18) and epididymal fat pad lipase (19). Both of these enzymes catalyze reactions that make available to the cell sources of energy additional to glucose.

Table 3

The Glucose Effect and its Reversal

| | Serine dehydratase μmoles pyruvate/h/mg protein |
|---|---|
| 1. Untreated, animals killed at 0 time | 0.28 ± 0.0 |
| 2. Amino acids at 0 time and 6 hrs | |
| Animals killed at 6 hrs | 1.61 ± 0.65 |
| Animals killed at 12 hrs | 5.65 ± 0.42 |
| 3. Amino acids and glucose at 0 hrs and 6 hrs | |
| Animals killed at 6 hrs | 0.51 ± 0.20 |
| Animals killed at 12 hrs | 0.47 ± 0.30 |
| 4. Amino acids and glucose at 0 hrs and 6 hrs | |
| Animals killed at 12 hrs | |
| a) Glucagon 6 hrs + 9 hrs | 1.44 ± 0.30 |
| b) Glucagon + caffeine 6 hrs + 9 hrs | 1.79 ± 0.28 |
| c) Epinephrine + caffeine 6 hrs + 9 hrs | 1.94 ± 0.37 |
| d) $N^6,O^{2'}$-dibutyryl cyclic AMP 6 hrs + 9 hrs | 1.11 ± 0.08 |

There were three groups of "control rats" and four groups of rats treated to reverse the inhibition by glucose. (1) Untreated rats (3 animals) killed at 0 time. (2) Rats "induced" by the mixture of amino acids. Six rats were intubated with 200 mg (per 100 g of body weight) of the mixture of amino acids at 0 time. Three of the rats were killed after 6 hours. The remaining three animals were given a second dose of amino acids and killed at 12 hours. (3) Rats in which "induction" inhibited by glucose. Six rats were intubated with the amino acids and 1 g of glucose (per 100 g of body weight) at 0 time. Three of the rats were killed after 6 hours. The remaining animals were given a second dose of amino acids and glucose and killed at 12 hours. (4) Rats in which inhibition by glucose reversed. Four groups of three rats each were treated with the mixture of amino acids and glucose at 0 time and at 6 hours. At 6 hours and at 9 hours the same groups of three rats received respectively (per 100 g of body weight): (a) 0.1 micromole of glucagon, (b) 0.1 micromole of glucagon and 15 micromoles of caffeine, (c) 0.2 micromoles of epinephrine and 15 micromoles of caffeine, and (d) 6 micromoles of dibutyryl cyclic AMP (injected via tail vein). The 12 animals were killed at 12 hours; i.e. 6 hours after reversal of inhibition by glucose.

There are, to our knowledge, only a few reported cases of the stimulation of the synthesis of mammalian enzymes by cyclic AMP per se. Yeung and Oliver (20)

found that cyclic AMP induced the precocious formation of phosphoenolpyruvate carboxykinase[3] when injected into fetuses *in utero*. Wicks (21) observed that dibutyryl cyclic AMP induced tyrosine-α-ketoglutarate transaminase in explants of fetal rat liver.

The stimulation of the synthesis of a bacterial enzyme by cyclic AMP has been reported recently. Perlman and Pastan (22) found that in *Escherichia coli* the addition of cyclic AMP to the medium partially overcame the inhibition of β-galactosidase formation by glucose. Janeček et al. (23) observed a positive correlation between the endogenous steady state concentration of cyclic AMP and the inducibility of β-galactosidase in *E. coli*. Taken together these findings suggest that in *E. coli* cyclic AMP may be a key intermediate that regulates the availability of sources of energy to the cell: the higher the cellular concentration of cyclic AMP, the greater the inducibility of those enzymes which make available to the bacterium sources of energy additional to glucose.

The present experiments show that cyclic AMP stimulated and glucose repressed the activity of serine dehydratase in rat liver. Serine dehydratase is a catabolic enzyme and its function is the introduction of the amino acids serine and threonine into energy metabolism. There may be an analogy between the presumptive regulatory role of cyclic AMP in the mammalian cell and in *E. coli*. Our experiments in which actinomycin D partially inhibited the increase in serine dehydratase activity suggest, but do not prove, that cyclic AMP stimulated the *de novo* synthesis of the enzyme. However earlier experiments (4) demonstrated conclusively that glucagon stimulated the *de novo* synthesis of serine dehydratase. In view of recent findings (5,6,7,8,9,14) showing that glucagon exerts its effect by increasing the cellular concentration of cyclic AMP, it appears likely that the enhancement of serine dehydratase activity noted in the present work also reflects the *de novo* synthesis of the enzyme. Furthermore our findings suggest that the term "induction" may be inappropriate when applied to the stimulation of the formation of

[3]The authors refer to the enzyme as "phosphopyruvate carboxylase"; however the proper nomenclature for the enzymic activity measured is phosphoenolpyruvate carboxykinase.

serine dehydratase. "Induction" in the case of the induced formation of enzymes in microorganisms implies a steric relationship between inducer and the enzyme induced (24). Usually the inducer is a substrate or a product (or an analogue of either) of the reaction catalyzed by the induced enzyme. No such relationship exists between serine dehydratase and the substances which stimulate its formation; the substrates serine and threonine do not induce significantly (2). On the basis of these considerations and the finding that glucose represses the formation of serine dehydratase we should like to suggest that the regulation of serine dehydratase formation may be analogous to "catabolite repression" (25) and its reversal (22) and not to induction, sensu stricto.

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