# STIMULATION OF HEPATIC 3-HYDROXY-3-METHYLGLUTARYL CoA REDUCTASE ON ADMINISTRATION OF ATP

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**Summary:** The depressed activity of hepatic 3-hydroxy-3-methylglutaryl CoA reductase in starved or cholesterol fed rats was stimulated on intraperitoneally administering small quantities of ATP.

Biogenesis of hepatic cholesterol as tested by the incorporation of [1-$^{14}$C]acetate is depressed under conditions of starvation (1,2) and cholesterol feeding (2,3) and exhibits circadian variation with peak activity at midnight (4,5). The regulation of cholesterol biogenesis occurs at a pre-mevalonate site and the changes under the above conditions were shown to be due to alterations in the rate-limiting microsomal enzyme 3-hydroxy-3-methlglutaryl CoA (HMG CoA) reductase (6,8).

It was reported earlier from this laboratory (9) that ATP, administered intraperitoneally to starved rats, was able to stimulate incorporation of [1-$^{14}$C] acetate but not [2-$^{14}$C] mevalonate into hepatic sterols suggesting a regulatory role for ATP at a pre-mevalonate site. In the present communication, it is shown that administration of ATP stimulated HMG CoA reductase, the regulatory enzyme, in both starved and cholesterol-fed rats - the first report of a small, naturally occurring metabolite stimulating the activity of this enzyme.

Groups (at least 6 each) of albino rats were starved for 2 days or fed cholesterol at 0.1% (wt/wt) in the diet for 5 days whereby the concerned biogenetic and enzyme activities are known to be depressed. Exogenous ATP, 10 mg/rat, Sigma Grade II, disodium salt, as a neutral solution in saline

Table 1. Effect of administration of ATP on the activity of HMG CoA reductase and cholesterol biogenesis

| | Treatment | | |
|---|---|---|---|
| | Saline | ATP | P value |
| HMG CoA reductase | nmoles/hr/mg protein | | |
| Starved | 0.20 ± 0.04 | 0.65 ± 0.03 | < 0.01 |
| Cholesterol fed | 0.31 ± 0.03 | 0.77 ± 0.13 | <0.02 |
| [1-$^{14}$C] acetate →Sterols | cpm/g liver | | |
| Starved | 1120 ± 320 | 5200 ± 1200 | <0.02 |
| Cholesterol fed | 1260 ± 120 | 4450 ± 490 | <0.01 |

Groups of rats were starved for 48 hr or fed cholesterol at 1% (wt/wt) level in the diet for 5 days. ATP (18 μmoles/rat) was administered as a neutral solution in saline 2 hr before killing. Assay of HMG CoA reductase: 300 nmoles of [3-$^{14}$C] HMG CoA (specific radioactivity 360 cpm/nmole) was incubated with 2 nmoles of $NADP^+$, 20 nmoles of glucose-6-phosphate, I unit of glucose-6-phosphate dehydrogenase and microsomal fraction containing 4-6 mg of protein in a final volume of 1 ml. Mevalonic acid was separated (3) and the radioactivity incorporated into it estimated. Incorporation of [1-$^{14}$C] acetate into sterols: [1-$^{14}$C] acetate (10μCi/rat) (specific radioactivity 49 mCi/nmole) was administered intraperitoneally to each rat 30 min. before killing. The radioactivity incorporated into hepatic sterols was estimated. Values are mean ± S.E.M. from at least 6 animals.

(0.2 ml) was administered to one set of animals in the starved and cholesterol-fed groups. The controls were given 0.2 ml saline. The animals were killed after 2 hr by cervical dislocation and the livers processed. Two measurements were carried out in separate sets of animals; the microsomes were prepared and HMG CoA reductase activity was assayed as described by Shapiro and Rodwell (8); the incorporation of

[1-$^{14}$C]acetate into hepatic sterols *in vivo* was measured as described by Subba Rao and Ramasarma (5).

The results in Table I show that treatment with small amounts (10 mg/rat) of ATP showed significant increase both in enzyme activity and biogenetic activity from [1-$^{14}$C]acetate. This effect is found in the two classical cases of starvation and cholesterol feeding, wherein the depression of enzyme and biogenetic activities to 10-20% of initial levels occurs.

The effect observed with ATP most satisfactorily explains the well-known need for any caloric source to maintain cholesterol biogenesis (7). It appears that the effect of small concentrations of ATP is not merely as a supply of energy source (9) and is probably an effect on the activity or the amount of the enzyme. Addition of ATP *in vitro* in the assay medium of the enzyme had no effect.

Preliminary studies showed that the stimulatory effect was obtained on administration of ADP, AMP, cyclic AMP and also adenosine but not by adenine, guanosine or GTP. Administration of ATP or adenosine is known to increase ATP concentration in the liver (10). Nevertheless, the possibility remains that a metabolite derived from ATP is the true active agent for the stimulation. Alteration of this component or ATP itself by a number of hormones, drugs and compounds (Triton, ethionine) may explain the effectiveness of diverse class of compounds in altering cholesterol biogenesis.

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