ATP IN THE REGULATION OF CHOLESTEROL BIOSYNTHESIS- A SUPRA-ENERGETIC ROLE

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Summary: ATP, given intraperitoneally to starved rats stimulates hepatic biosynthesis of sterols at a pre-mevalonate site.

Hepatic cholesterol biosynthesis is subject to large fluctuations determined by the nutritional state of the animals, being the lowest on complete deprivation of food (1,2,3) and the highest at midnight (4,5) which follows intake of food (5). These changes are demonstrated by incorporation of acetate-1-^{14}C into sterols *in vivo* (5) on giving the tracer intraperitoneally or *in vitro* (4) by incubating liver slices with the tracer. The lack of corresponding changes with mevalonate-2-^{14}C indicated that a pre-mevalonate site is affected (2,4) and it is in most cases, the microsomal enzyme, β-hydroxy-β-methyl glutaryl CoA reductase, the rate-limiting step in the pathway (6,7,8,9,10). Alterations in the activity of this enzyme under starvation-refeeding conditions appear to be obtained by a repression-derepression mechanism and reflect the changes measured by the incorporation of acetate-1-^{14}C into sterols (9).

Refeeding of starved rats with protein, carbohydrate or fat restored the activity (1). Conversely, deficiency of protein, carbohydrate or fat in the diet did not decrease the activity (Subba Rao, G. and Ramasarma, T., unpublished results). It is implicit in these findings that the decrease in biosynthetic activity in starvation is not due to lack of any

particular component in the diet and that caloric intake in any form will suffice to maintain the enzyme activity. It is conjectured that a metabolite modulated parallel with the caloric supply may bring about the changes in the enzyme and, thereby, the biosynthetic rate. Evidence presented here supports that ATP may play this role.

The following set of observations were made on the incorporation of acetate-1-^{14}C and mevalonate-2-^{14}C into hepatic non-saponifiable lipids in starved rats:

1. Refeeding with composite diet increased biosynthetic activity progressively over a period of 6 hr. parallel with increase in food intake.
2. A single oral dose of glucose (2g./rat) gave a similar response in 4 hr.
3. Increased activity was obtained 2 hr. after intraperitoneal injection of a single dose of glucose (400 mg./rat), but not with pyruvate or citrate (20 mg./rat).
4. ATP, 2 hr. after a single intraperitoneal injection (10 mg./rat) significantly stimulated the activity, tested both *in vivo* and *in vitro* (Table 1).
5. ATP treatment did not affect the incorporation of acetate-1-^{14}C into fatty acids or of mevalonate-2-^{14}C into non-saponifiable lipids. If exogenous ATP were merely acting as energy source these also should have changed. These results suggest that the steps of fatty acid synthesis, including acetate activation, and of isoprene synthesis after mevalonate were not stimulated by ATP under these conditions.

The expected stimulation being at a pre-mevalonate site, it is considered appropriate to study the total non-saponifiable lipids which include hydrocarbons, ubiquinone, polyprenols and sterols - all the lipid-products of isoprene synthesis. Fractionation of the non-saponifiable lipids of the above *in vivo* samples on deactivated alumina columns (5) showed that incorporation of acetate-1-^{14}C, and not mevalonate-2-^{14}C, into sterols increased on treatment of starved rats with ATP (fig. 1).

Table 1: Incorporation of acetate-1-^{14}C and mevalonate-2-^{14}C into non-saponifiable lipids

Tracer	Control	ATP	P value
in vivo (6 rats)	CPM/g. liver		
Acetate-1-^{14}C	1530±430	5890±1450	<0.05
Mevalonate-2-^{14}C	7340±670	7480±1390	N.S.
in vitro (4 rats)	CPM/g. liver/hr		
Acetate-1-^{14}C	10770±920	27050±5050	<0.05
Mevalonate-2-^{14}C	4540±570	4430±470	N.S.

Values are mean± S.E.M; N.S. Not Significant

Male albino rats weighing 110 to 140 g., starved for 48 hr. were intraperitoneally administered ATP (10 mg. disodium salt, as a neutral solution in saline 2 hr. before killing). Acetate-1-^{14}C (10 µCi/rat, sp. radioactivity 49 mCi/mmole) or mevalonate-2-^{14}C (0.2 µCi/rat, sp. radioactivity 5.85 mCi/mmole) was intraperitoneally injected 30 min. before killing. The rats were killed by stunning and decapitation, the livers removed immediately, homogenised in 10 volumes of 80% ethanol, saponified using 40% sodium hydroxide (1 ml./g. tissue) for 20 min. and the non-saponifiable lipids were extracted with petroleum ether. Liver slices (about 150 mg., 0.5 mm thick) were incubated in oxygenated Krebs-Ringer phosphate buffer (pH 7.4, 6 ml) at 37° with acetate-1-^{14}C (5 µCi) or mevalonate-2-^{14}C (0.1 µCi) in a metabolic shaker. At the end of the incubation period (2 hr.) 15 ml. of ethanol were added, and the samples were processed as above. The radioactivity was estimated using 0.5% PPO in toluene in a Beckman LS 100 liquid scintillation counter.

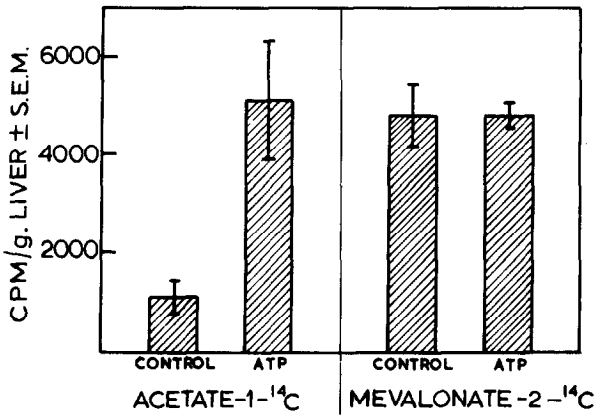

Fig. 1: Stimulation of biosynthesis of sterols on treatment of starved rats with ATP

The stimulatory effect of ATP explains most satisfactorily the need for caloric source to support cholesterol biosynthesis and it should be underscored that a very small quantity (10 mg.) is capable of the effect in contrast to the need of several grams of food or milligrams of glucose. The lack of effect with pyruvate or citrate indicates that they do not have any direct stimulation and the concentrations used may be too low to increase endogenous ATP. Finally, it must be pointed out that exogenous ATP will undergo degradation and may in fact be effective at much lower concentrations in the cell or via another derived metabolite. The most likely target for the observed stimulation by ATP is β-hydroxy-β-methyl glutaryl CoA reductase and ATP may be regulating the synthesis of this enzyme protein, by a supra-energetic mechanism.

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