Influence of (22R)-22-Aminocholesterol on Cholesterol Metabolism in Rats

A number of sterols in which nitrogen has isosterically replaced one or more side chain carbon atoms have been tested as hypocholestermic agents. The compounds tested were 22, 25-diazacholestanol[1-4], 20, 25-diazacholesterol[5-8], 25-diazacholesterol[9], and 22, 25-diaza-19-norcholesta-1, 3, 5, triene-3-01[10]. All the compounds were found to inhibit the reduction of desmosterol (24-dehydrocholesterol) to cholesterol. The availability of (22R)-22 aminocholesterol[11], has prompted us to investigate the effects of amino side chain substitution of cholesterol upon metabolism of that sterol. Our findings are the subject of this communication.

Materials and methods. Male, Wistar rats (165 g) were maintained for 7 days on a semisynthetic diet containing mixed cereal (70%), skim milk powder (22%), wheat germ (7%), and vitamin mix (1%). This diet is readily accepted by rats[12]. The (22R)-22-aminocholesterol (0.3%) was added at the expense of the cereal. The animals were killed by decapitation. Serum was assayed for total cholesterol by the method of PEARSON et al.[13]. The liver was divided into 4 aliquots which were used for the cholesterol oxidation and hydroxylation studies and the biosynthesis experiment. A fourth aliquot was dissolved in 15% alcoholic KOH, the nonsaponifiable material extracted into petroleum ether and the extract analyzed for total cholesterol[13].

Mitochondrial suspensions were prepared as described previously[14,15]. Incubations were carried out in stoppered 125 ml Erlenmeyer flasks containing center wells. The incubation mixture consisted of 1 ml of mitochondrial preparation (11.1 mg protein); 1 ml of a solution containing adenosine triphosphate (ATP, 25 mg), nicotinamide adenine dinucleotide (NAD, 5 mg), adenosine monophosphate (AMP, 8 mg), reduced glutathione (15 mg), sodium citrate monohydrate (30 mg), magnesium nitrate hexahydrate (10 mg), potassium penicillin G (2000 units) and streptomycin sulfate (1 mg); 5 ml of labeled substrate in 0.25 *M tris* (hydroxymethyl)aminomethane HCl, pH 8.5; and 5 ml of boiled supernatant.

Incubations were carried out at 37 °C for 18 h. At the end of this period 2.5 ml of a 1 *M* methanolic solution of Hyamine 10× (*p*[diisobutyl-cresoxyethoxyethyl] dimethyl-benzylammonium hydroxide) was injected into the center well. The solution was acidified with 1 *N* H_2SO_4 (2.5 ml) and the flasks were shaken for 3 h at 37 °C to displace $^{14}CO_2$. The Hyamine solution was removed from the center well and a sample was taken for radioactive assay by liquid scintillation spectrometry. Previous work has shown[14,15] that the maximum production of $^{14}CO_2$ occurs at between 8 and 18 h of incubation. It has also been demonstrated that at 18 h practically no $^{14}CO_2$ is produced in the absence of the mitochondrial preparation.

Microsomal suspensions were prepared as described by SHEFER, HAUSER and MOSBACH[16]. Incubation was carried out in a 25 ml Erlenmeyer flask containing [1, 2-^{3}H]cholesterol (0.5 μmole, 8.25×10^5 cpm/μmole) solubilized with 6.7 mg Tween 20; potassium phosphate buffer (pH 7.4), 0.167 mmole; $MgCl_2$, 11 μmoles; $NADP^+$, 3.0 μmoles; glucose-6-phosphate, 6.0 μmoles; glucose-6-phosphate dehydrogenase, 1 IU; and 1 ml microsomal suspension (0.9 mg protein). The final volume was 2.3 ml. Incubation

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Influence of (22R)-22-aminocholesterol (0.3) on cholesterol metabolism in Wistar rats[a]

	Group[b]	
	Test	Control
Number (survival)	4/4	4/4
Weight gain (g)	−2 ± 6	47 ± 1[c]
Liver weight (g)	5.3 ± 0.4	8.6 ± 0.7[d]
Liver as percent body weight	3.21 ± 0.02	4.00 ± 0.36
Serum cholesterol (mg/dl)	151 ± 12	122 ± 11
Liver cholesterol (mg/100 g)	259 ± 6	258 ± 17
Oxidation of [26-^{14}C] cholesterol (%/mg N)		
+ cytosol	12.3 ± 0.9	9.8 ± 2.2
− cytosol	3.9 ± 1.4	3.0 ± 01.1
7α hydroxylase (pmoles/mg/min)	24.73 ± 0.96	43.47 ± 3.75[d]
Biosynthesis from [1-^{14}C] acetate (cpm/mg cholesterol)	494 ± 39	677 ± 46[e]

[a] Fed for 7 days. [b] All data ± standard error. [c] $p < 0.001$. [d] $p < 0.01$. [e] $p < 0.05$.

was carried out at 37 °C with shaking. The reaction was stopped by the addition of 7.5 ml methylene dichloride-ethanol (5:1) to a 0.5 ml aliquot of the reaction mixture. Steroids were separated by thin layer chromatography on silica gel G with ethyl acetate-hexane (8:2). The bands were visualized with iodine vapor[17], scraped from the plates, and assayed for radioactivity by liquid scintillation spectrometry.

For lipid biosynthesis experiments, 0.5 g slices of liver were incubated at 37 °C for 3 h in 5 ml phosphate buffer (pH 7) containing 0.0006 *M* $MgCl_2$, 0.03 *M* nicotinamide, and 0.5 μCi (0.24 μ*M*) [1-^{14}C]acetate. The reaction was stopped by addition of 15% alcoholic KOH. Cholesterol was extracted from the saponification mixture and isolated as the digitonide[18]. The aqueous residue was acidified to pH 1 with strong mineral acid and fatty acids were extracted into ether. Cholesterol digitonides were dissolved in methanol[19] and assayed by liquid scintillation spectrometry. The fatty acids were counted directly.

All radioactive substrates were purchased from New England Nuclear Corporation, Boston, MA, and the cholesterol was purified by thin layer chromatography prior to use.

Results and discussion. The data are summarized in the Table. Even at a level of 0.3% of the diet, (22R)-22-aminocholesterol significantly affected weight gain. Although the livers of the rats fed the test diet were smaller than those of the controls, when calculated on the basis of g liver/100 g body weight, the livers were of similar proportionate size. The test compound did not significantly affect serum cholesterol or liver cholesterol levels. Liver cholesterol concentrations were identical and although the difference in serum cholesterol was not statistically significant, the cholesterol level of the test group was 24% higher than that of the control group. The average serum plus liver pool was higher in the control than in the test animals (30.1 vs 21.2 mg). The oxidation of [26-^{14}C]cholesterol to $^{14}CO_2$ by liver mitochondria was about 30% higher in the treated rats, but the difference was not statistically significant. The increased level of oxidation was observed both in the presence or absence of cytosol although oxidation in the absence of cytosol was reduced by almost 70%. This reduction of cholesterol oxidation in the absence of boiled supernatant (cytosol) has been observed consistently in our experiments.

The inital step in bile acid synthesis from cholesterol, 7α hydroxylation by liver microsomal preparations, was reduced by 43% in rats fed (22R)-22-aminocholesterol. We are not aware of any other pharmacologic agents which exert so drastic an effect on 7α hydroxylation of cholesterol. Livers of rats fed (22R)-22-aminocholesterol also showed greatly reduced lipogenesis. Liver slices from test rats converted significantly less [1-^{14}C]acetate to cholesterol than did slices from control rats. Conversion of [1-^{14}]acetate to fatty acids was even more severely restricted. Whereas cholesterol synthesis was inhibited by 27%, fatty acid synthesis was inhibited by 76% (15366 vs 3747 cpm/0.5 g of liver; $p < 0.05$).

The azasterols have all been shown to inhibit cholesterol synthesis at the hydrogenation of desmosterol, thus causing accumulation of desmosterol in blood and liver. Gas liquid chromatographic analysis of serum and liver extracts of rats fed (22R)-22-aminocholesterol showed no desmosterol or other sterols suggesting that inhibition of cholesterogenesis occurs at an early stage of the biosynthetic pathway.

The data indicate that (22R)-22-aminocholesterol fed for 7 days does not affect serum or liver cholesterol levels but significantly inhibits the activity of liver enzymes concerned with lipogenesis and cholesterol hydroxylation.

Zusammenfassung. Nachweis, dass das im Futter zugeführte (22R)-22-Aminocholesterin die Gewichtszunahme der Ratten hemmt, aber keinen Einfluss auf die Cholesterinkonzentration in der Leber und im Serum hat. In Leberpräparationen in vitro wird die Seitenketten-Oxidation von Cholesterin nicht beeinflusst, während die 7α-Hydroxylierung und Syntheserate vermindert wird.

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Carrageenin Hyperthermia in Rats

WINTER et al.[1] demonstrated that a subplantar injection of carrageenin produced edema in the rats hind paw. Many pharmacological laboratories adopted this procedure for screening anti-inflammatory agents. VINEGAR et al.[2] and DI ROSA et al.[3] investigated the phases of carrageenin edema. Carrageenin was also found to induce pleurisy[3,4] and an exudative inflammation of s.c. tissue[5]. Biochemical background of the carrageenin-induced inflammation was studied[3,5,6].

We report here that carrageenin also induces a potent hyperthermic response in rats.

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