Metabolic Aspects of 4-Aminopyrazolopyrimidine-Induced Fatty Liver in the Rat (33588)

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The development of fatty livers in mice treated with the adenine analogue, 4-aminopyrazolo (3, 4-D) pyrimidine (APP), is associated with a profound inhibition of liver RNA synthesis (1). Henderson and Junga (2) also obtained evidence that radio active APP was incorporated into liver nucleic acids and that the principal metabolites in the liver were the mono-, di-, and triphosphates of APP-riboside. These findings showed that there were some changes in nucleotide metabolism associated with the APP-induced fatty infiltration of the liver which lead to the hypothesis that this type of fatty liver may be due to alterations of nucleotide control mechanisms of lipid metabolism (1). It has been suggested that the adenine nucleotides, notably ATP, which plays a part in lipoprotein synthesis, may also participate in the release of hepatic triglycerides into the plasma (3). Moreover, it has been shown that a variety of agents (ethionine, orotic acid, ethanol, CCl_4 and azaserine) which produce a fatty liver, lead to a rapid decrease in the hepatic concentration of ATP, and that the development of fatty liver is prevented by the administration of ATP or adenine (4–9).

In the present paper the effect of the administration of APP in the rat on liver lipid composition, on liver lipogenesis *in vitro*, on the serum lipoproteins and on the liver content of ATP is reported. The effects of administration of ATP and of adenine to APP-treated rats were also investigated.

Materials and Methods. 4-Aminopyrazolo(3, 4-D)pyrimidine (APP), adenine sulfate, adenosine-5′-triphosphate (ATP) disodium, $3H_2O$, crystalline 99–100%, α-ketoglutarate, NADPH, 3-phosphoglycerate, glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase were obtained from the Sigma Chemical Co. St. Louis, Missouri. Sodium acetate-1-^{14}C (10.5 mCi/mmole) was obtained from the Radiochemical Centre, Amersham, England. All other chemicals used were of the highest purity available commercially.

Female weanling albino rats of the Wistar strain were fed *ad libitum* for 60 days on a purified diet composed of sucrose (59%), vitamin-free casein (20%), autoclaved egg white (11%), peanut oil (5%) and mineral and vitamin supplements. At the end of this period the animals were divided into 5 groups and were fasted just before the following treatments: the rats of group 1 were used as controls and were injected with 0.9% NaCl; the animals of group 2 were injected intraperitoneally with 8 mg of APP/100 gm of body wt in two equal doses of 4 mg at intervals of 24 hr; the rats of group 3 were treated with APP plus 150 mg of ATP subcutaneously in three equal doses of 50 mg at intervals of 8 and 16 hr; the animals of group 4 received APP plus 300 mg of ATP in three equal doses of 100 mg at intervals of 8 and 16 hr; the rats of group 5 were treated with APP plus 120 mg of adenine sulfate (equimolecular to 300 mg of ATP) subcutaneously in three equal doses of 40 mg at intervals of 8 and 16 hr. Three hr after the last injection of APP venous blood of the rats of the five groups was collected. Then, the rats were sacrificed and the livers were removed for analysis.

Blood was allowed to clot at 37° for 30 min and serum was separated by centrifuging the blood at 300 rpm for 15 min. Paper electrophoresis of serum proteins was carried out in Veronal buffer, pH 8.6, and I 0.1 at 7° for 12 hr. After the run, the strips were dried and stained with Sudan black in 55% (v/v) ethanol (10).

Total liver lipids were measured gravime-

TABLE I. Effect of APP, ATP, and Adenine on the Composition of Liver Lipids and on the Incorporation of Acetate-^{14}C into Liver Lipids of the Rat.[a]

Group	Animals in expt.	Liver lipids (mg/g of tissue)				Incorporation of acetate-^{14}C (cpm/g of tissue)
		Cholesterol	Phospho-lipids	Neutral fats	Total lipids	
1	Control	2.68 ± 0.07	32.2 ± 1.7	21.2 ± 2.0	56 ± 2	14,840 ± 780
2	Treated with APP	3.02 ± 0.24[b]	34.7 ± 1.0[b]	83.8 ± 7.0[c]	121 ± 7[c]	8600 ± 110[c]
3	Treated with APP and 150 mg ATP	2.63 ± 0.08[b]	33.9 ± 1.2[b]	82.2 ± 4.5[c]	117 ± 5[c]	7950 ± 230[c]
4	Treated with APP and 300 mg ATP	2.52 ± 0.11[b]	35.7 ± 1.3[b]	99.3 ± 5.9[c]	127 ± 7[c]	9654 ± 680[c]
5	Treated with APP and 120 mg adenine	2.23 ± 0.08[b]	28.8 ± 1.1[b]	54.0 ± 2.4[d]	85 ± 3[d]	16,050 ±1660[b]

[a] Values represent mean of eight determinations on different animals ± SE of the mean.
[b] Nonsignificant ($p > .05$).
[c] Significant difference ($p < .001$).
[d] Significant difference ($p < .01$).

trically after extraction by a method previously described (7); cholesterol was determined by the method of Sperry and Webb (11); phospholipids by phosphorus analysis in total lipids (12).

In order to determine ATP content, the livers were frozen *in situ* with metal blocks which have been cooled to a low temperature. Then the livers were homogenized with 4 vol of 1 *N* $HClO_4$ and centrifuged. The supernatants were neutralized (pH 6.5) with 0.5 *N* KOH and the precipitates $KClO_4$ removed. Aliquots of the extracts were mixed, in 1-cm glass cuvettes, with 250 μmoles of triethanolamine buffer, pH 7.6, 10 μmoles of $MgSO_4$, 20 μmoles of 3-phosphoglycerate, and 0.6 μmole of NADH. The decrease in E_{340} was measured 8 min after the addition to the reaction mixture of 0.03 ml of a suspension containing glyceraldehyde-3-phosphate dehydrogenase (4 mg of protein/ml) and phosphoglycerate kinase (1 mg of protein/ml) (13).

Liver slices (500–600 mg) were incubated at 37° for 1 hr with 5 ml of calcium-free Krebs–Ringer phosphate medium (pH 7.4), 5 μmoles of α-ketoglutarate, and 0.5 μmole of sodium acetate-1-^{14}C. The reaction was stopped by placing the flask on ice. After removal of the medium, the slices were rinsed three times in ice-cold buffer, and were homogenized in 20 vol of 2:1 chloroform–methanol (v/v). Total lipid extracts were purified according to Folch *et al.* (14) and evaporated to dryness. The residues were dissolved first in light petroleum (bp 40–60°), then in chloroform. Aliquots of this solution were taken for radioactivity measurement in a windowless gas-flow counter.

Results. Paper electrophoresis of serum lipoproteins showed a marked decrease of α- and β-lipoproteins in APP-treated rats (group 2) when compared with controls (group 1). A remarkable decrease of serum lipoproteins was also observed in APP-treated rats and injected either with 150 mg of ATP (group 3) or with 300 mg of ATP (group 4). No relevant decrease of serum lipoproteins was noted in APP and adenine-treated rats (group 5).

As shown in Table I the total lipid content in liver of APP-treated rats increased when compared with the control rats ($p < .001$); the increase was entirely due to neutral fats ($p < .001$). A higher content of lipids and in particular of neutral fats was also observed in the liver of APP-treated rats and injected either with 150 mg ($p < .001$) or with 300 mg of ATP ($p < .001$). Adenine administration in APP-treated rats (group 5) caused a de-

TABLE II. Effects of APP, ATP, and Adenine on Hepatic Levels of ATP in the Rat.[a]

Group	Animals in expt.	Liver ATP (mμmole/g of tissue)
1	Control	890 ± 16
2	Treated with APP	1910 ± 25[b]
3	Treated with APP and ATP (150 mg)	2480 ± 100[b]
4	Treated with APP and ATP (300 mg)	2700 ± 120[b]
5	Treated with APP and adenine (120 mg)	1930 ± 120[b]

[a] Values represent mean of eight determinations on different animals ± SE of the mean.
[b] Significant difference ($p < .001$).

crease in the neutral fat content of the liver when compared either with APP-treated rats (group 2) ($p < .001$) or with both APP- and ATP-treated animals (group 3 and 4) ($p < .001$ and $p < .001$, respectively).

The data concerning lipid synthesis *in vitro* show that the incorporation rate of acetate-^{14}C into total lipids was decreased in the slices from liver of APP-treated rats when compared with the control animals ($p < .001$). A decreased incorporation was also found in APP-treated rats and injected either with 150 mg of ATP ($p < .001$) or 300 mg of ATP ($p < .001$). No significant decrease of acetate-^{14}C incorporation into liver lipids was observed in slices from rats treated with APP together with adenine (group 5) when compared with control rats (group 1). As shown in Table II the cellular ATP content in the liver of APP-treated rats (group 2) increased when compared with control animals (group 1) ($p < .001$). An increased ATP content was also observed in livers of APP-treated rats and injected with ATP (group 3 and 4) ($p < .001$ and $p < .001$, respectively) or with adenine (group 5) ($p < .001$).

Discussion. Administration of APP to rats induces a fatty liver, which is characterized by the increase of neutral fat, whereas cholesterol and phospholipids show no significant change. The development of APP-induced fatty liver could be related to an inhibition of secretion of lipids from the liver, since it has been shown that in APP-treated rats the concentration of serum lipoproteins markedly decreases. The action of APP does not appear to be due to a decrease in liver protein synthesis and hence in lipoprotein synthesis (1).

These data could suggest, as shown by Roheim *et al.* (15) to be the case in the orotic acid-induced fatty liver, that there may be one or more steps in lipoprotein formation subsequent to the synthesis of the protein that may be inhibited during the development of APP-induced fatty liver. It has been demonstrated that the fatty liver induced by orotic acid is associated with a fall in the level of adenine nucleotides, particularly ATP (16, 8) and that is completely prevented by adenine administration (4, 7). In APP-treated animals the fatty liver is associated with an increased liver ATP concentration. This could be a consequence of the decreased ATP utilization. In fact, it is possible that APP ribonucleotides, largely formed in the liver, (2) compete with purine nucleotides in ATP-dependent reactions and that they are directly related to the development of fatty liver (1). The administration of high doses of adenine to APP-treated rats might prevent the formation of APP ribonucleotides and therefore the development of fatty liver. On the contrary, the administration of ATP to APP-treated rats is unable to reverse the effects of APP, probably because it is more slowly utilized by the cell than adenine, as it was previously shown in some strains of *Neurospora crassa* (17, 18).

Summary. The effect of the adenine analogue, 4-aminopyrazolo pyrimidine, (APP) on liver lipid composition, on liver content of ATP, on lipogenesis *in vitro* in liver slices and on serum lipoproteins in the rat is reported. The effect of ATP and of adenine in

APP-treated rats is also presented. The neutral fats in the liver of APP-treated rats increased significantly, while the incorporation *in vitro* of acetate-^{14}C into total lipids decreased when compared with the controls. Under the same experimental conditions the concentration of serum lipoproteins fell and the liver content of ATP increased. The administration of ATP to APP-treated rats did not prevent either the development of fatty liver, or the decrease of serum lipoprotein concentration, or the fall in incorporation of acetate-^{14}C into liver lipids. The administration of adenine to APP-treated rats partially reversed the effects of APP on the lipid content of liver, on the levels of serum lipoproteins and on the incorporation of acetate into lipids.

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