0.25 mM, and the lower value in Table 1 applying at AMP concentrations above 0.25 mM. For the cytoplasmic enzyme a linear plot was obtained over the same range of AMP concentration.

Isoenzyme differences were also found for ADP, where linear plots were obtained for the cytoplasmic form when reciprocal velocity was plotted as a function of the reciprocal of the square of ADP concentration. For the mitochondrial enzyme a simple linear Lineweaver–Burk plot was obtained.

Both isoenzymes of cardiac adenylate kinase are inhibited by diadenosine pentaphosphate, the cytoplasmic form being markedly more susceptible to inhibition than the mitochondrial form, for both directions of the reaction.

Normally the heart readily oxidizes both carbohydrate and fatty acids, preferring fatty acids under aerobic conditions (Shipp et al., 1961). The fatty acyl-CoA ligases use ATP and release AMP, which inhibits the ligase. We have established the cardiac location of this enzyme as close to that of mitochondrial adenylate kinase. Mitochondrial AK is therefore well placed to prevent the accumulation of AMP. However, in hypoxic myocytes ADP concentration increases, stimulating adenylate kinase to produce more AMP, thereby inhibiting fatty acid activation and conserving ATP when oxygen deprivation blocks the oxidation of fatty acids.

The catalytic data suggest that cytoplasmic adenylate kinase has a relatively minor role under aerobic conditions. But, in hypoxic or anoxic myocytes with high concentrations of ADP and little ATP, the cytoplasmic enzyme has an essential role. The adenylate kinase equilibrium directs both increased production of ATP, a direct source of energy for contraction, and simultaneously increased production of AMP. The latter, by stimulating both glycolysis (Regen et al., 1964; Lorensen & Mansour, 1969) and glycogenolysis (Morgan & Parmeggiani, 1964), will augment the rate of metabolic production of ATP.

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The effects on lipoprotein metabolism of rats of glycerolipids substituted with 4-benzyloxybenzoic acid (BRL 14280)

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We described previously (Fears et al., 1978; Fears & Richards, 1981) how lipid-lowering activity for BRL 14280 and various other aryl-containing carboxylic acids is associated with their ability to form CoA esters as measured by the accumulation of substituted glycerolipids. To determine whether the presence of these novel lipids affects lipoprotein turnover we examined the metabolism in vitro of lipoprotein fractions isolated from rats receiving a dietary supplement (0.25%) of BRL 14280 for 7 days.

In the first experiment, lymph was collected (Bollman et al., 1948) for 4 h after a meal of corn oil (5 ml/kg body wt.) containing [1-14C]glycerol (10 μCi/kg body wt.) and BRL 14280 (250 mg/kg body wt.). Chylomicrons, containing radiolabelled triacylglycerol and the substituted glycerolipids, were isolated and used as substrate for lipoprotein lipase (EC 3.1.1.34) prepared from epididymal adipose tissue (Fears et al., 1978) and aorta taken from control rats. Chylomicrons obtained from rats receiving BRL 14280 supported increased adipose tissue lipase activity (2.15 ± 0.63 compared with a control value of 1.33 ± 0.50 μmol of fatty acid released/h per mg of protein, n = 4) but decreased aortic lipase activity (0.06 ± 0.04 compared with 0.11 ± 0.01 μmol of fatty acid released/h per mg of protein, n = 4).

To obtain further information concerning changes in reactivity, individual serum lipoproteins were isolated from rats maintained either on the stock diet or on a cholesterol-rich (1%) diet. In normolipidaemic rats, BRL 14280 decreased the concentration of cholesterol and triacylglycerol of each fraction, in agreement with the decrease in total serum lipids observed previously (Baggaley et al., 1977), the effects being least for high-density lipoprotein (−20%) and greatest for very-low-density lipoprotein (−60%). Phospholipid concentrations were unaffected. BRL 14280 did not alter the concentration of lipoprotein lipid in rats receiving dietary cholesterol, although substituted triacylglycerol accumulated in the very-low-density lipoprotein and chylomicron fractions to a value approx. 20% of the total glyceride content.

The lipoproteins isolated from this experiment were used as substrate for the measurement of cholesterol uptake in vitro by aortic rings taken from control rats (Fig. 1). Lipoproteins

Fig. 1. Lipoprotein metabolism by aortic rings

The number of individual experiments is given in parentheses. Each experiment involved the incubation of a lipoprotein fraction, isolated from serum pooled from eight rats, with approx. 50 mg of aortic rings pooled from 12 control rats (each analysis performed in triplicate). The mass of lipoprotein cholesterol added is given for each assay (total volume, 3.5 ml). (a) Chylomicrons; (b) very-low-density lipoproteins; (c) combined lipoprotein fractions (d < 1.006–1.21).
prepared from cholesterol-fed control animals induced greater cholesterol uptake than did the equivalent fractions from normolipidaemic rats in proportion to the increased cholesterol content of the fractions. Lipoproteins isolated from rats receiving the stock diet plus BRL 14280 supported less uptake than did control samples and this can also be explained by their differing cholesterol concentration. However, although sup-
plementation of the cholesterol-containing diet with BRL 14280 did not lower the cholesterol content of chylomicrons or very-low-density lipoproteins there were marked decreases in cholesterol uptake by the aortic rings and we attribute this response to the presence of the substituted triacylglycerol.

Cholesterol turnover was also measured in vivo in groups of eight rats 24 h after an intraperitoneal dose of [G-3H]cholesterol (10 μCi/100 g body wt.). Adding BRL 14280 to the stock diet decreased lipoprotein cholesterol uptake into the aorta (~40%) and kidneys (~20%) and dietary cholesterol promoted uptake into aorta (+85%) and kidneys (+90%) in support of the results in vitro, but addition of BRL 14280 to the cholesterol-supplemented diet did not influence cholesterol turnover in vivo. This lack of effect is explained by the rapid hepatic clearance of remnant particles after triacylglycerol hydrolysis by adipose tissue lipoprotein lipase. Thus although the hepatic and intestinal production of a substituted glycerolipid may be a useful mechanism for the efficient delivery of an agent to adipose tissue, the potential as a novel approach to the treatment of disorders in lipid metabolism and atherosclerosis remains to be established.


Association between lipid-lowering activity of aryl-substituted carboxylic acids and formation of substituted glycerolipids in rats

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We have shown previously (Fears et al., 1978) that the novel hypolipidaemic agent BRL 14280 (4-benzoxozybenzoic acid) and a number of other pharmacologically important acids can produce unusual glycerolipids wherein one of the fatty acid moieties is replaced by the exogenous compound. Other hypolipidaemic compounds have been identified as 'fatty acid-like' (Parker et al., 1977; Takashima et al., 1979) and it was proposed more recently (Harris & McCune, 1980) that the lipid-lowering effects of several carboxylic acids can be explained by their conversion into CoA esters, which then decrease lipogenesis by inhibiting acetyl-CoA carboxylase (EC 6.4.1.2) or by limiting the availability of CoA.

It was the purpose of the present experiments to examine in more detail the relationship between lipid-lowering activity and CoA esterification when the latter was reflected in the subsequent formation of a substituted triacylglycerol. By using methods previously described (Fears et al., 1978) we investigated various compounds, described in the literature as hypolipidaemic agents or discovered by us to be so, for their ability to form a substituted glycerolipid from [1-14C]glycerol in vivo. When compared with the control incorporation of approx. 100 nmol of glycerol/h per g of liver into phospholipid and triacylglycerol and 25 nmol of glycerol/h per g of liver into diacylglycerol, substantial quantities of novel glycerolipids were produced by the following compounds at a concentration of 0.5 mM (results are expressed as nmol of glycerol incorporated/h per g of liver, being the mean of two experiments; each analysis was performed in triplicate): BRL 14611 (ethyl (p-chlorophenoxy)methylxylbonylbenzoate), 45 nmol; BRL 16700 (ethyl 3-(4-crotyloxyphenyl)propionate), 40 nmol; cetabem (p-hexadecylaminobenzoic acid), 65 nmol; RMI 14514 (5-tetra-
decyloxy-2-furoic acid), 20 nmol. Benzalacene and phenyl-
butyric acid were inactive. The results for cetabem and RMI 14514 are consistent with the claims for an effect on CoA metabolism (Largia et al., 1976; Kariya & Wille, 1978). When cetabem, BRL 14611 or BRL 16700 were included as a supplement to the stock diet (Oxoid breeding diet) fed to groups of eight rats for 7 days the characteristic metabolites accumulated in adipose tissue and the serum concentrations of cholesterol and triacylglycerol were lowered.

In addition to the known hypolipidaemic agents we have also identified several anti-inflammatory compounds, e.g. fenoprofen, ibuprofen, as able to form substituted glycerolipids (Fears et al., 1978), although they were not known to exert other effects on lipid metabolism. We have now obtained some correlation between their capacity to form an abnormal triacylglycerol and inhibition of synthesis of sterols and fatty acid from [1-14C]acetate in liver slices in vivo as measured by the method of Fears & Morgan (1976). At 0.1 mm, fenoprofen and ibuprofen supported an incorporation of approx. 80 nmol of glycerol/h per g of liver into the substituted triacylglycerol, a rate equivalent to the synthesis of conventional triacylglycerol. Ketoprofen formed less metabolite (approx. 10 nmol/h per g) and flurbiprofen and indomethacin appeared inactive. Feno-
profen inhibited cholesterogenesis from acetate by 86% and fatty acid synthesis by 66%, ibuprofen inhibited by 60% and 53% respectively. Ketoprofen and flurbiprofen did not affect cholesterogenesis and only weakly inhibited fatty acid synthesis, whereas indomethacin was inactive.

To obtain further information, fenoprofen was included as a supplement (0.1%) to the stock diet fed to a group of six rats for 7 days. In comparison with the control group, serum cholesterol was lowered from 68 ± 2.1 to 56 ± 4.3 mg/100 ml (P < 0.05) and serum triacylglycerol from 140 ± 11 to 82 ± 6.4 mg/100 ml (P < 0.01). Hepatic cholesterogenesis, measured in vitro 1 h after an intraperitoneal injection of [1-15O]H2O (1.5 mCi/100 g body wt.) in 0.9% NaCl (0.1 ml/100 g body wt.), was decreased by fenoprofen from 400 ± 40 to 230 ± 30 μg/h per liver (P < 0.01).

The results described in the present communication support the suggestion that lipid-lowering activity for a variety of carboxylic acids can be explained by depletion of CoA pools due to the production of novel CoA esters, which subsequently form glycerolipid metabolites. However, this esterification of an exogenous compound will not necessarily lead to hypolipidaemia if circulating lipid concentrations are maintained from the diet rather than by endogenous synthesis. Thus we have observed that BRL 14280 and BRL 16700 are not hypolipidaemic when included in diets supplemented with cholesterol (1%) or containing a high proportion of fat (40% of