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CHANGES IN HEPATIC AND INTESTINAL CHOLESTEROL REGULATORY ENZYMES

THE INFLUENCE OF METFORMIN

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Abstract—The effect of metformin (*N,N*-dimethylbiguanide) on the rate-limiting enzymes of cholesterol metabolism was observed. 3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) and acyl-CoA–cholesterol acyltransferase (ACAT) activities were estimated in hepatic microsomal and intestinal cell preparations from normal and alloxan-diabetic rats. Metformin administration had no effect on either hepatic enzyme. Intestinal ACAT activity was significantly decreased in the metformin-treated rats when compared with controls ($P < 0.001$). Intestinal HMG-CoA reductase activity was not significantly affected. Diabetic rats showed a significantly higher intestinal HMG-CoA reductase activity when compared with controls ($P < 0.001$). Intestinal ACAT activity in the diabetic group was similar to control values. Metformin administration to diabetic rats caused a marked decrease in both intestinal HMG-CoA reductase activity ($P < 0.001$) and ACAT activity ($P < 0.002$). It is concluded that the effect of metformin on the intestinal cholesterol enzyme system may be responsible for its cholesterol-lowering action.

In conditions where there are increased levels of circulating lipids, such as diabetes, the risk of vascular disease due to atherosclerosis is increased, and this constitutes the chief risk to the diabetic today. It is, therefore, important to know what effect the various treatments used in controlling diabetes have on lipid metabolism and on the subsequent development of vascular complications. Biguanides have long been used in the treatment of maturity-onset diabetes [1], especially metformin which has been reported to exert its action not only on carbohydrate metabolism but also to have a normalising effect on lipid disturbances [1, 2]. Metformin has been used in various studies on experimental atherosclerosis with promising results [3–7]. It has been shown that metformin exerts a preventive effect against the development of dietary atherosclerosis in rabbits [3, 4]. Metformin also causes the inhibition of labelled cholesterol incorporation into rabbit aorta [3, 5] and inhibition of intestinal lipid absorption in rabbits [6]. An altered lipoprotein composition together with decreased atheromatosis was also shown after metformin treatment [7]. This laboratory has demonstrated a cholesterol-lowering action in guinea pigs where metformin administration resulted in a decrease in bile-salt absorption together with increased bile-salt excretion [8]. Clinical studies with metformin also indicate a lipid-lowering effect of the drug [9, 10]. We have previously shown that bile-salt pool sizes which were expanded in diabetic patients in comparison with controls were brought back to normal levels after metformin treatment [11]. This cholesterol-lowering action of metformin may in theory be due to a number of effects—decreased absorption of cholesterol or bile salts, increased excretion of bile salts, increased synthesis of bile salts or decreased synthesis of cholesterol or cholesterol esters. Although metformin is known to inhibit the intestinal absorption of a number of substances [12–14], including cholesterol and bile salts [7, 15, 16], this effect is not believed to be sufficient to account for the total cholesterol-lowering action observed [6]. Increased excretion of bile salts, although demonstrated in the guinea pig [8], has not been confirmed in the rat or in man [14]. Thus, it appears probable that metformin may exert an effect on cholesterol, cholesterol ester or bile-acid synthesis. This study is concerned with the action of metformin on cholesterol and cholesterol ester synthesis. 3-Hydroxy-3-methylglutaryl-CoA reductase [HMG-CoA reductase (EC.1.1.1.34)] catalyses the rate-determining step of cholesterol biosynthesis, both in the liver and in the intestine—the two main sites of cholesterol production in the body. Acyl-CoA–cholesterol acyltransferase [ACAT (EC.2.3.1.26)] functions in the formation of intracellular cholesterol esters for storage purposes. We examined the activities of the hepatic and intestinal forms of these enzymes, initially in normal rats and then in alloxan-diabetic rats which are believed to be more responsive to biguanides [17], in an effort to discover if metformin exerts its cholesterol-lowering action at the enzymatic level.

MATERIALS AND METHODS

Materials. 3-Hydroxy-3-methyl[3-^{14}C]glutaryl-CoA (56.6 mCi/mmole), DL-[2-^{3}H]mevalonic acid

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Table 1. Hepatic enzyme activities in normal and diabetic rats after acute metformin administration

	Normal rats	Normal rats +250 mg/kg metformin	Diabetic rats	Diabetic rats +250 mg/kg metformin
HMG-CoA reductase	0.356 ± 0.009	0.365 ± 0.014	0.143 ± 0.010*	0.149 ± 0.027*
ACAT	0.134 ± 0.003	0.135 ± 0.004	0.115 ± 0.002†	0.121 ± 0.007

Results are in nmoles/min/mg protein.
Each value is the mean ± S.E.M. of six rats.
* $P < 0.01$.
† $P < 0.05$.

lactone (629 mCi/mmole), [1-^{14}C]oleic acid (57.4 mCi/mmole) and [1α,2α(n)-^{3}H]cholesterol (58 Ci/mmole) were obtained from the Radiochemical Centre (Amersham, U.K.). β-Nicotinamide–adenine dinucleotide phosphate, glucose 6-phosphate, glucose-6-phosphate dehydrogenase, dithiothreitol (DTT), glutathione, human serum albumin (fatty acid free), oleoyl chloride, sodium sulphate, nicotinamide and magnesium chloride were all obtained from Sigma-London Chemical Co. Ltd (London, U.K.). Kieselgel 60G was from Merck (Darmstadt, F.R.G.). Solvents used were all BDH laboratory reagent grade.

Housing and feeding of rats. Adult male Wistar rats weighing 250–300 g were used for all experiments. The rats were housed at a controlled temp of 27° under a reversed dark/light cycle, lights being on from 4.00 pm to 4.00 am, for at least 3 weeks before the experiment, and were given a standard rat diet and water *ad lib.* Metformin hydrochloride (250 mg/kg) was either given orally as an acute dose 2 hr before death or else chronically in dosages of 150–500 mg/kg/day for a period of 14 days. Diabetes was induced by intraperitoneal injection of 100 mg/kg alloxan in citrate–phosphate buffer (pH 4.0). Only those rats with > 2% glucosuria were used. All rats were killed at 10.00 am, i.e. the peak of the dark phase on the day of the experiment. Livers and intestines were removed immediately and placed on ice.

Preparation of liver microsomes and intestinal cells. The livers were chilled on ice, carefully minced and then homogenised in 4 vols of ice-cold 0.1 M potassium phosphate buffer containing 1 mM EDTA and 30 mM nicotinamide (pH 7.4). The homogenate was centrifuged at 800 *g* for 10 min, and the resulting supernatant was centrifuged at 1500 *g* for 20 min. This supernatant was then centrifuged at 104,000 *g* for 60 min to obtain the microsomal pellet. The microsomes were suspended in 0.25 vol. of 5 mM imidazole/HCl buffer containing 150 mM NaCl and 5 mM DTT (pH 7.4).

Intestinal cells were obtained by the dual-buffer technique [18], as modified by Merchant and Heller [19], and essentially as described in Ref. 20 except that the entire intestine minus 4 cm at the proximal (duodenal) end was used, and the enriched villous and crypt cells were combined before the final suspension.

Protein content of liver and intestinal preparations was determined by the Lowry method [21].

Enzyme assays. The activity of HMG-CoA reductase was measured by the method of Mitropoulos and Balasubramaniam [22], using 0.5–0.6 mg of microsomal protein in the presence of 90 mM DL-hydroxymethyl[3-^{14}C]glutaryl-CoA (specific radioactivity 6 Ci/mole) in a total incubation vol. of 0.5 ml. The activity of ACAT was measured by the incorporation of [1-^{14}C]oleic acid into cholesterol ester as described in Ref. 23. In the reaction mixture, of total vol. 0.2 ml, 0.1–0.2 mg of microsomal protein was incubated with 0.1 mM [1-^{14}C]oleic acid (specific radioactivity 1 Ci/mole).

RESULTS

The activities of HMG-CoA reductase and ACAT in hepatic microsomal preparations from control and metformin-treated rats (250 mg/kg for 2 hr) are shown in Table 1. No significant difference between the two groups was observed.

Even when metformin was administered at different dose levels (150–500 mg/kg) daily for 14 days, no change in either HMG-CoA reductase or ACAT activities was observed. Diabetic rats (Table 1) showed significantly lower activity for both hepatic HMG-CoA reductase ($P < 0.01$) and ACAT ($P < 0.05$), when compared with normal controls. However, diabetic rats who had been given metformin showed no further change in activity (Table 1). In

Table 2. Intestinal enzyme activities in normal and diabetic rats after metformin treatment

	Normal rats	Normal rats +250 mg/kg metformin	Diabetic rats	Diabetic rats +250 mg/kg metformin
HMG-CoA reductase	0.026 ± 0.017	0.030 ± 0.015	0.057 ± 0.009*	0.021 ± 0.005*
ACAT	0.119 ± 0.005	0.054 ± 0.009*	0.115 ± 0.007	0.072 ± 0.006†

Results are in nmoles/min/mg protein.
Each value is the mean ± S.E.M. of six rats.
* $P < 0.001$.
† $P < 0.002$.

contrast, when intestinal enzyme activities were measured in normal and diabetic rats (Table 2) ACAT activity was markedly reduced in both metformin-treated normal rats when compared with controls ($P < 0.001$) and in metformin-treated diabetic rats when compared with untreated diabetic rats ($P < 0.002$). Intestinal HMG-CoA reductase was not significantly changed in normal rats after metformin administration (Table 2). However, HMG-CoA reductase activity was significantly increased in the intestine of diabetic rats ($P < 0.001$), whereas metformin-treated diabetic rats showed activity similar to normal values.

DISCUSSION

Although metformin has been shown to effect lipid metabolism in a number of species, including man, the rabbit, guinea-pig and rat [8, 11, 24], the mechanism of its action is as yet unclear. This study was designed to examine the effect of metformin on cholesterol metabolism at the enzymatic level, particularly in relation to diabetes. Initial studies were performed on hepatic enzyme levels, since the liver usually accounts for approximately 80% of the entire cholesterol production in the body [25]. No effect was observed in normal rats 2 hr after the administration of 250 mg/kg metformin (Table 1). The half-life of this drug is very short and, therefore, any acute effect would have been observed at this stage. Even when rats were chronically treated with metformin for 2 weeks, at different dose levels (150–500 mg/kg), no long-term effect could be seen. In diabetic rats, both hepatic enzymes were significantly reduced when compared to controls (Table 1). Diabetic animals are reported to be more responsive than normal rats to biguanides [17]. However, when diabetic rats were treated with metformin (250 mg/kg) they showed no further alteration in enzyme activities (Table 1). Our finding of reduced hepatic HMG-CoA reductase activity in diabetic rats is in agreement with a previous report [26]. Reduced hepatic ACAT activity in diabetic rats may be consistent with the belief that, if intracellular cholesterol esters serve a protective function by trapping excess cholesterol inside the cell [27], then a decrease in ACAT activity might reduce this action, and consequently lead to increased serum cholesterol levels. The inability of metformin to modify either enzyme activity in normal or diabetic rats leads one to propose that the cholesterol-lowering action of metformin is not caused by interaction at the hepatic level.

The intestine is the most important extra-hepatic site of cholesterol production in the body [25] and, as metformin accumulates in the intestine [28], the effect of metformin on intestinal HMG-CoA reductase and ACAT seemed worthy of study. In normal rats it was observed that intestinal HMG-CoA reductase remained unchanged after metformin treatment, but there was a significant decrease in ACAT activity (Table 2). Diabetic rats had normal intestinal ACAT and significantly increased intestinal HMG-CoA reductase activity ($P < 0.001$) when compared with controls (Table 2). This finding of increased HMG-CoA reductase activity in the intestine of diabetic rats, supported by a previous observation [29], may be significant in view of the high cholesterol levels frequently associated with diabetes, especially as HMG-CoA reductase activity in the liver has been shown to be reduced. When diabetic rats were given metformin (Table 2), intestinal HMG-CoA reductase activity was reduced to normal values and ACAT activity was markedly reduced ($P < 0.002$). The reduction of both these enzyme activities could make a major contribution to the overall cholesterol-lowering action of metformin. Since the general mechanism of action of biguanides is believed to occur through membrane interaction [30], and as both HMG-CoA reductase and ACAT are membrane-bound enzymes [31], then it is not unreasonable to suggest that their activities could be altered by membrane fluidity changes caused by the binding of metformin. The finding that both enzymes are not similarly altered may be explained by the fact that they are situated on different parts of the endoplasmic reticulum [32], and thus could be independently regulated. In conclusion, this study suggests that metformin exerts its effect on cholesterol metabolism at the intestinal level, resulting in a reduced ACAT activity in normal rats and reduced HMG-CoA reductase and ACAT activities in alloxan-diabetic rats.

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