Diabetologia (1986) 29: 122-124

Diabetologia

The effect of insulin and catecholamines on the activities of 3-hydroxy-3-methyl glutaryl coenzyme A reductase and acyl-coenzyme A: cholesterol-o-acyltransferase in isolated rat hepatocytes

R. Devery[1] and G. H. Tomkin[2]

[1]Department of Diabetes and Endocrinology, The Adelaide Hospital, Dublin 8, Ireland
[2]Department of Biochemistry, Trinity College, Dublin 2, Ireland

Summary. This study was concerned with the effect of insulin and catecholamines on the rate limiting enzymes of cholesterol metabolism in rat hepatocytes. Insulin was found to increase the activity of 3-hydroxy-3-methyl glutaryl coenzyme A reductase and to have no effect on the activity of acyl-coenzyme A: cholesterol-o-acyltransferase. Noradrenaline and isoprenaline increased the activities of both 3-hydroxy-3-methyl glutaryl coenzyme A reductase and acyl-coenzyme A: cholesterol-o-acyltransferase. The effect of noradrenaline or isoprenaline in the presence of insulin was that of a lower stimulatory response on 3-hydroxy-3-methyl glutaryl coenzyme A reductase but comparable to that found with either catecholamine alone. The combination of either catecholamine with insulin had no effect on the activity of acyl-coenzyme A: cholesterol-o-acyltransferase. These observations suggest that the activities of 3-hydroxy-3-methyl glutaryl coenzyme A reductase and acyl-coenzyme A: cholesterol-o-acyltransferase are regulated independently by insulin in the presence or absence of catecholamines. By contrast, catecholamines appear to regulate both enzyme activities in a similar fashion.

Key words: Insulin, noradrenaline, isoprenaline, bicyclic cascade system, HMG CoA reductase, ACAT.

Recent evidence has shown that changes in the activity of hepatic 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG CoA reductase) may be required to maintain a constant rate of sterol synthesis in order to compensate for variations in the availability of simple precursors of sterol synthesis [1]. Variations in the availability of such metabolites as acetyl-coenzyme A (acyl-CoA) and NADPH occur as a result of hormonal regulation of fatty acid and cholesterol metabolism. Gross alterations in cholesterol metabolism occur in diabetes mellitus, a condition characterised by severe insulin deficiency and altered plasma catecholamine levels [2]. While it has been shown that activities of hepatic HMG CoA reductase and acyl-CoA: cholesterol-o-acyltransferase (ACAT) are altered in the poorly-controlled diabetic rat compared to the well-controlled diabetic rat [3], the direct effect of hormones such as insulin and noradrenaline on the activity of either enzyme is poorly understood. This study investigates the effects of insulin, noradrenaline, isoprenaline, two adrenergic agonists and insulin in combination with either catecholamine on the activities of HMG CoA reductase and ACAT in isolated rat hepatocytes.

Materials and methods

Hormones and chemicals

(−) Noradrenaline hydrochloride, (−) isoproterenol hydrochloride and collagenase type IV were obtained from Sigma Chemical Company (Poole, Dorset, UK). Ultratard® MC insulin zinc suspension was purchased from Novo (Copenhagen, Denmark). HBSS, Hank's Balanced Salt Solution, Swim's S-77 medium, Bovine albumin Fraction V solution, sodium bicarbonate and Penicillin - streptomycin solution were obtained from Gibco (Paisley, Scotland). 3-hydroxy-3-methyl-[3-^{14}C] glutaryl-coenzyme A (56 mCi/mmol), DL-[2-^{3}H] mevalonic acid lactone (1 Ci/mmol), [1-^{14}C] oleic acid (2 Ci/mmol) and [1α 2α (n)-^{3}H] cholesterol (60 Ci/mmol) were supplied by the Radiochemical Centre (Amersham, UK). All other chemicals were of analytical grade.

Animals

Male Wistar rats weighing from 230-300 g were used in all experiments. Rats had food and water ad libitum and were housed at 21 °C in a cupboard under reversed lighting conditions (lights on 16.00 h to 04.00 h, lights off 04.00 h to 16.00 h). Rats were acclimatised to the lighting conditions for at least 21 days before killing.

Isolation of rat hepatocytes

Rat hepatocytes were prepared by perfusion of liver with collagenase by a modification of the method of Edwards [4]. Rats were killed in the middle of the dark period (10.00 h) under CO_2. Immediately afterwards, an abdominal incision was made extending up to the thoracic region. The stomach and intestine were pulled out to the right hand side to allow for insertion of a cannula (20G) attached to a tube (Pharmaseal, Milton Keynes, UK) into the hepatic portal vein. A second cannula (16G) was inserted into the inferior vena cava to allow for collection of the perfusate. After pre-perfusion of the liver with a non-collagenase-containing modified Hank's buffer pH 7.4, collagenase (25 mg/50 ml) dissolved in bicarbonate-free modified Swim's S-77 medium was then perfused and recirculated 3 times through the liver. The liver was removed, cut into 5 mm size pieces and incubated in fresh collagenase solution (25 mg/50 ml) in a shaking water bath at 37 °C for 15 min. The cells were collected by filtering the suspension through a layer of muslin cloth and washed 3 times as described [4].

This procedure yielded approximately 3.0×10^8 cells/liver with 90% viability as judged by Trypan Blue staining.

Hepatocyte incubations

Cells were suspended in 40 ml modified Swim's S-77 medium, ph 7.4, containing 10 mmol/l bicarbonate, then divided into 4 aliquots. Each 10 ml aliquot of cells was plated at a concentration of 7.7×10^6 cells/ml in plastic micro titre well plates (Nunc, Gibco, Paisley, Scotland) and incubated at 37 °C under 95% O_2:5% CO_2 for 3 h with either buffer, insulin (10 mU), noradrenaline 55 µmol/l and insulin (10 mU) or isoprenaline 55 µmol/l and insulin (10 mU). Aliquots of cells (7.7×10^7 cells/10 ml) obtained from rat liver under identical conditions were also incubated with buffer, noradrenaline or isoprenaline at similar concentration to that used when in the presence of insulin.

Preparation of microsomes from hepatocytes

After incubation, the medium was removed and the cells were suspended in 10 ml homogenization buffer (0.1 mol/l potassium phosphate buffer, pH 7.4, containing 1 mmol/l EDTA, 30 mmol/l nicotinamide). After freezing and thawing, the cells were disrupted with 20 downward passes using a glass hand homogenizer. Cell debris and mitochondria were removed by two successive 10 min centrifugations at 2000 g and 10000 g respectively. The high speed supernatant was centrifuged at 100000 g for 40 min and the microsomal pellet was frozen. Before assay it was suspended (0.25% w/v) in 50 mmol/l Imidazole-HCL buffer, pH 7.4, containing 250 mmol/l NaCl and 10 mmol/l DTT.

Analytical methods

The activities of microsomal HMG CoA reductase and ACAT were assayed as previously described [5]. Protein was measured using the method of Lowry et al. [6].

Results

Figure 1 shows the effect of insulin 1 mU/ml, noradrenaline 55 nmol/ml, isoprenaline 55 nmol/ml, noradrenaline 55 nmol/ml and insulin 1 mU/ml and isoprenaline 55 nmol/ml and insulin 1 mU/ml on the activities of HMG CoA reductase and ACAT. HMG CoA reductase activity was increased twofold by both noradrenaline and isoprenaline (buffer: 0.193 ± 0.03 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$; noradrenaline: 0.355 ± 0.08 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$ and isoprenaline: 0.559 ± 0.24 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$). ACAT activity was also increased by both catecholamines. Noradrenaline produced an approximate twofold increase (32.50 ± 3.41 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$ versus 14.9 ± 3.37 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$), while isoprenaline produced an approximate 3.5-fold increase (48.13 ± 9.31 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$ versus 14.9 ± 3.37 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$). Insulin alone produced a marked stimulatory effect on HMG CoA reductase. The activity of HMG CoA reductase increased approximately fourfold, from 0.193 ± 0.03 nmol·min·$^{-1}$mg·$^{-1}$ ($n=6$) in the absence of insulin to 0.681 ± 0.05 nmol·min·$^{-1}$mg·$^{-1}$ ($n=6$) in the presence of insulin. Incubation

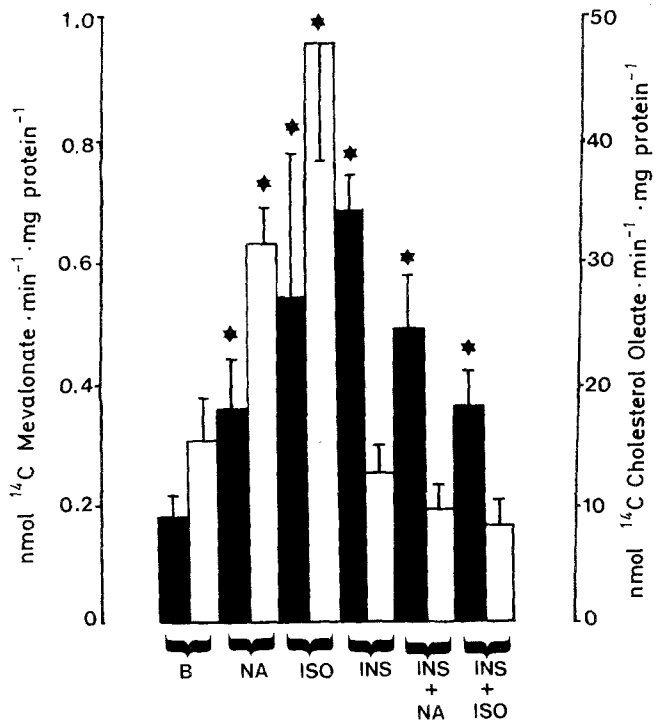

Fig. 1. The specific activities of hepatic microsomal HMG CoA reductase (■) and ACAT (□) from isolated rat hepatocytes incubated with buffer (B), noradrenaline (NA), isoprenaline (ISO), insulin (INS), insulin + noradrenaline (INS + NA) and insulin + isoprenaline (INS + ISO). The concentration of noradrenaline and isoprenaline used 55 nmol/ml, and the concentration of insulin was 1 mU/ml. Specific activity is expressed as mean ± SEM nmol·min^{-1}·mg^{-1} (nmol/min/mg protein) for $n=6$ rats. $p<0.05$ compared to buffer

of cells with insulin and either noradrenaline or isoprenaline produced a two-fold to 2.5-fold increase in the level of HMG CoA reductase (noradrenaline/insulin: 0.459 ± 0.08 nmol/min·$^{-1}$mg·$^{-1}$ ($n=6$); isoprenaline/insulin: 0.366 ± 0.08 nmol·min·$^{-1}$mg·$^{-1}$ ($n=6$)). These alterations in enzyme activity were found to be statistically significant ($p<0.05$). However, no significant difference could be observed between the effect of insulin when in the presence of either catecholamine and the effect of noradrenaline or isoprenaline alone on hepatic HMG CoA reductase activity. Insulin produced no significant change in the level of ACAT activity (11.08 ± 2.24 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$ versus 14.9 ± 3.37 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$). Similarly, the combination of either catecholamine with insulin did not affect the activity of ACAT (noradrenaline/insulin: 9.56 ± 2.02 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$; isoprenaline/insulin: 9.12 ± 2.60 ($n=6$) nmol·min·$^{-1}$mg·$^{-1}$). This is in marked contrast to the effect of either catecholamine alone on ACAT activity.

The microsomal protein concentration (mg/g wet weight) was similar in cells treated with buffer alone (5.02 ± 1.02 mg/g), insulin (6.45 ± 1.75 mg/g), noradrenaline/insulin (5.1 ± 1.1 mg/g), isoprenaline/insulin (6.2 ± 1.5 mg/g), noradrenaline (5.2 ± 1.4 mg/g) and isoprenaline (5.5 ± 1.1 mg/g).

Discussion

Several possible mechanisms must be considered to explain (1) the higher increase in the activity of HMG CoA reductase by insulin than by catecholamines, (2) the relatively similar levels of HMG CoA reductase activity when cells were incubated with catecholamines or catecholamines and insulin (3) the increase in ACAT activity by catecholamines, and (4) the lack of effect of insulin and insulin in combination with either catecholamine on ACAT activity.

Insulin may regulate the activity of HMG CoA reductase by increasing both the total amount of enzyme present in the microsomes and the activation state of the enzyme [7]. Gibbons et al. [1] recently reported that insulin at a concentration of 10^4 μU/ml, while capable of stimulating HMG CoA reductase, did not provoke a similar response in the sterol synthetic rate. It is of interest that in this study insulin at a concentration of 10^3 μU/ml stimulated HMG CoA reductase but had no effect on the rate limiting enzyme of cholesterol esterification. This suggests that, under the experimental conditions described, a decoupling of HMG CoA reductase and cholesterol synthesis may have occurred. It is possible that insulin may decouple the two processes by limiting the supply of pre-reductase substrates in an attempt to stimulate lipogenesis. Such a situation might result in a decreased availability of acetyl CoA and/or NADPH and hence in an increase in the concentration of HMG CoA reductase to provide a constant sterol synthetic rate, as described by Gibbons et al. [1]. By contrast, catecholamines, by stimulating fatty acid oxidation [8], may not limit the supply of pre-reductase substrates and hence lead to an increase in sterol synthesis in the hepatocyte. Such an effect might lead to an increased trend towards cholesterol esterification by ACAT. A possible route for catecholamine regulation of HMG CoA reductase may be through phosphoprotein phosphatase C, which catalyses dephosphorylation (i.e. activation) of existing HMG CoA reductase and reductase kinase.

The mechanism by which both catecholamines and insulin in the incubation medium regulate reductase activity is not known. It has been reported that insulin can lower cAMP levels in the perfused rat liver [9]. It is tempting to speculate that insulin may decrease the catecholamine-mediated increase in intracellular cAMP levels to a level lower than that found when catecholamines alone are present in the medium, and thereby lead to a relative decrease in the rate of lipolysis and fatty acid oxidation to acetyl CoA. Although the cellular demand for acetyl CoA for fatty acid synthesis may not be as high as when insulin alone is present, the concentration of reductase may have to be increased in order to maintain a constant sterol biosynthetic rate. By operating on enzyme synthesis, such a mechanism may explain the relative lower level of HMG CoA reductase compared to that found when insulin alone was present. The lack of effect of insulin either alone or in combination with noradrenaline/isoprenaline on ACAT activity may be explained by an absence of change in the sterol synthetic rate and in the rate of cholesterol esterification.

Thus insulin and catecholamines appear to exert different effects on the mechanisms regulating the total level of HMG CoA reductase and ACAT. Insulin may have increased the concentration of HMG CoA reductase but may have had no effect on ACAT synthesis. Catecholamines may increase the activation state of HMG CoA reductase and thereby increase the rate of sterol synthesis and the supply of ACAT substrate.

This study suggests that the response to insulin of HMG CoA reductase may be antagonised by catecholamines via the ability of catecholamines to decrease the amount of acetyl CoA needed for fatty acid synthesis and to decrease the amount of reductase that has to be synthesised relative to that needed by insulin in order to maintain a constant biosynthetic sterol rate. The effect of insulin and catecholamines on intracellular cholesterol metabolism remains to be investigated.

Acknowledgements. This work was supported by the Medical Research Council of Ireland. The secretarial assistance of Ms. M. Mulligan is gratefully acknowledged.

References

1. Gibbons GF, Bjornsson OG, Pullinger CR (1984) Evidence that changes in hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity are required partly to maintain a constant rate of sterol synthesis. J Biol Chem 259: 14399–14405
2. Christensen NJ (1978) Catecholamines and Diabetes Mellitus. Diabetologia 16: 211–224
3. Scott LM, Tomkin GH (1982) Cholesterol metabolism in diabetes mellitus in the rat. Diabetologia 23: 14 (abstract)
4. Edwards PA (1975) The influence of catecholamines and cyclic AMP on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and lipid biosynthesis in isolated rat hepatocytes. Arch Biochem Biophys 170: 188–203
5. Scott LM, Tomkin GH (1982) Changes in hepatic and intestinal cholesterol regulatory enzymes: the influence of metformin. Biochem Pharmacol 32: 827–830
6. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193: 265–275
7. Ingebritsen TS, Geelen MJH, Parker RA, Everson KJ, Gibson DM (1979) Modulation of hydroxymethylglutaryl-CoA reductase activity, reductase kinase activity, and cholesterol synthesis in rat hepatocytes in response to insulin and glucagon. J Biol Chem 254: 9986–9989
8. Oberhaensli RD, Schwendimann R, Keller U (1985) Effect of norepinephrine on ketogenesis, fatty acid oxidation, and esterification in isolated rat hepatocytes. Diabetes 34: 774–779
9. Exton JH (1980) Mechanisms involved in -adrenergic phenomena: role of calcium ions in actions of catecholamines in liver and other tissues. Am J Physiol 238: E3–E12

Received: 7 November 1985
and in revised form: 19 November 1985

Dr. R. Devery
Department of Biochemistry
Trinity College
Dublin 8, Ireland