Biochemical Pharmacology, Vol. 40, No. 10, pp. 2285–2289, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

THE INTERACTION OF ORAL HYPOGLYCAEMIC DRUGS WITH INSULIN ON STEROID METABOLISM IN HEPATOCYTES ISOLATED FROM CONTROL AND DIABETIC MALE RATS

ABAS HJ. HUSSIN* and PAUL SKETT†

Molecular Pharmacology Laboratory, Department of Pharmacology, The University, Glasgow G12 8QQ, U.K.

(*Received* 3 *May* 1990; *accepted* 17 *July* 1990)

Abstract—The effects of the oral hypoglycaemic drugs, phenformin and tolbutamide, and insulin, alone and in combination, on steroid metabolism in hepatocytes isolated from control and streptozotocin-diabetic male rats has been studied. Both phenformin and tolbutamide mimic the action of insulin in stimulating hepatic steroid metabolism in a dose-dependent manner in control cells. Unlike insulin, however, both drugs give a similar effect in cells derived from diabetic animals although to a lesser extent. Both drugs can partially restore the effect of insulin in cells derived from diabetic animals. Biguanides and sulphonylureas, therefore, have a direct effect on liver cells to mimic insulin action and can still have an effect under conditions where insulin is inactive. Both types of oral hypoglycaemics can also affect insulin-insensitive cells isolated from diabetic rat liver to restore to a certain extent their response to insulin.

Diabetes mellitus is a complex disease state caused by the lack of insulin (type 1) or lack of effect of insulin (type 2). Two major groups of drugs are used in the treatment of diabetes, the biguanides and the sulphonylureas. Although these drugs unquestionably reduce the elevated blood glucose levels seen in diabetes mellitus, it is unclear how they act. There are reports of pancreatic [1] and extrapancreatic actions [2, 3] with reports indicating effects on insulin receptors in target tissues [3, 4] and others indicating post-receptor actions [5]. One target site for insulin is the hepatocyte and oral hypoglycaemics have been shown to have direct effects on this cell type [6, 7]. It has also been indicated that oral hypoglycaemics can enhance the action of insulin on the liver in normal and diabetic animals [4, 8].

One hepatic function that is known to be insulin-dependent in the rat is the metabolism of steroids [9]. We have shown that hepatocytes cultured in hormone- and serum-free medium can respond to insulin by a marked stimulation of enzyme activity [9] but that hepatocytes isolated from streptozotocin-induced diabetic male rats are refractory to these effects of insulin [10].

This study was undertaken, therefore, to examine the direct effects of biguanides (phenformin was used in this study) and sulphonyureas (tolbutamide) on steroid metabolism in hepatocytes isolated from control and streptozotocin-induced diabetic male rats and to examine the interaction of the oral hypoglycaemic drugs with insulin in the control of this hepatic function.

* Current address: School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.

† To whom correspondence should be addressed.

MATERIALS AND METHODS

Chemicals. Streptozotocin, androst-4-ene-3,17-dione, tolbutamide and bovine serum albumin were obtained from the Sigma Chemical Co. (Poole, U.K.), Ham's F-10 culture medium and penicillin/streptomycin solution from Gibco BRL Ltd (Paisley, U.K.) and collagenase from BRL Ltd (Lewes, U.K.) [4-^{14}C]Androst-4-ene-3,17-dione was purchased from Amersham International plc (Aylesbury, U.K.) and insulin (porcine) from Novo Research Institute (Copenhagen, Denmark). Phenformin was the kind gift of Sterling Winthrop (Surrey, U.K.) All other chemicals were of, at least, reagent grade.

Animals. Mature, male Wistar rats, bred in the Department, were used throughout the study. The animals were kept, maximum five per cage, in light- and temperature-controlled conditions (light on 7:00 a.m.–7:00 p.m.; temperature 19 ± 1°) and given food (CRM Nuts, Labsure, Surrey, U.K.) and water *ad lib*. The animals weighed 300–350 g at the start of the study. Animals were made diabetic by a single intravenous injection of streptozotocin (60 mg/kg) in distilled water given under halothane/nitrous oxide anaesthesia. Control animals were treated similarly except that drug vehicle only was given. Treated animals were tested for induction of diabetes mellitus by measurement of blood glucose concentration before preparation of hepatocytes by removal of a drop of blood from the tail vein after induction of anaesthesia. The blood was analysed using a Corning Glucometer and only those animals with a glucose level above 30 mM were considered to be diabetic (control animals were typically below 10 mM glucose concentration).

Preparation of hepatocytes. Animals (control or 3 days after induction of diabetes mellitus) were anaesthesized using halothane/nitrous oxide and a

cannula placed in the hepatic portal vein. Hepatocytes were isolated by the double perfusion technique of Seglen [11] as modified by Hussin and Skett [12]. The cells, as isolated, were tested for viability by Trypan blue exclusion. Typically 1×10^8 cells/g wet wt liver were obtained with a viability of greater than 95%. Cells were plated onto 9-cm plastic dishes (NUNCLON, Denmark) at a density of 3×10^5 cells/cm^2 in Ham's F-10 medium supplemented with 0.1% bovine serum albumin as described by Hussin and Skett [12].

Preincubation of hepatocytes. Hormones and drugs were added to the incubation medium after a resting period of 24 hr. Insulin was added as a solution in 0.1 M hydrochloric acid and tolbutamide and phenformin were dissolved in 0.1 M sodium hydroxide solution. The compounds were added in the smallest volume possible and in no case did the addition alter the pH of the medium. In the case of the addition of the oral hypoglycaemic drugs, the cells were used 24 hr following addition of the drug, whereas for insulin the preincubation time was 30 min. If both oral hypoglycaemic drug and insulin was used, then the 24 hr pretreatment with oral hypoglycaemic was followed by 30 min with insulin. These were the preincubation times found to give the optimum effect. The cells were kept in an incubator during the preincubation period at a temperature of 37 ± 0.1°, 98% humidity and 5% carbon dioxide/95% air. After the preincubation time, the cells were assayed for steroid metabolizing capacity.

Assay of steroid metabolism. After treatment with the hormone or drugs for the required period, the cells were carefully scraped from the culture plate and lightly centrifuged (200 *g* for 2 min at 4°). The cells were washed with incubation medium (Hank's Balanced Salt Solution supplemented with 1 g/L glucose, 0.1 g/L of each of magnesium chloride and sulphate and 0.185 g/L calcium chloride) and resuspended in the same solution. The cells were assessed again for number and viability and adjusted to 3×10^6 cells/mL. The cell viability was at least 90% in all cases. To triplicate 1 mL aliquots of this suspension was added the substrate, androst-4-ene-3,17-dione (500 μg; 0.1 μCi) dissolved in 60 μL acetone, and 1.94 mL incubation medium. The mixture was incubated in a shaking waterbath at 37° for 30 min and the metabolites extracted, separated and quantitated according to the method of Gustafsson and Stenberg [13]. Actual enzyme activity expressed as pmoles product formed per minute per 10^6 cells was calculated using a custom-made computer program.

Statistics. Results were calculated as mean ± 1 standard deviation of the values obtained from similarly treated cell suspensions. Statistical significance was assessed using Student's *t*-test and the level of significance set at $P < 0.05$ in all cases.

RESULTS

The assay of steroid metabolism as performed allows the measurement of five separate enzyme activities, namely; 7α-, 6β and 16α-hydroxylases, 17(α,β)-oxosteroid oxidoreductase and the 5α-reductase. The two isomers of the 17-reduced metabolite were not separated in this assay. The 5α-reductase measurement represents a composite figure for the production of 5α-androstane-3,17-dione, 5α-androstane-3α-ol-17-one and 5α-androstane-3β-ol-17-one, the latter being secondary metabolites formed from the first.

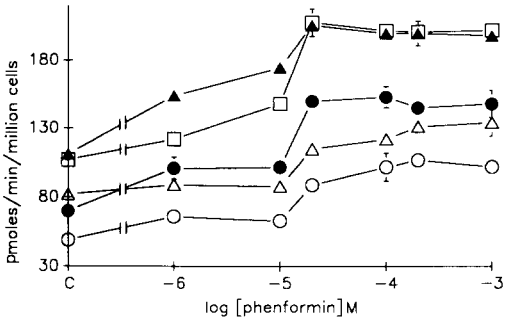

Fig. 1. The effect of pretreatment with phenformin (10^{-6}–10^{-3} M) for 24 hr on the metabolism of androst-4-ene-3,17-dione by hepatocytes isolated from the control male rat. The various enzyme activities are denoted by —○— (7α-hydroxylase), —●— (6β-hydroxylase), —△— (16α-hydroxylase), —▲— (17-oxosteroid oxidoreductase) and —□— (5α-reductase). Results are expressed as mean ± 1 SD of six independent samples from the same batch of cells and where no error bars are shown, these were less than the size of the symbol. All treated values were significantly different from their respective controls ($P < 0.05$).

Figure 1 shows the dose–response curve for phenformin for its action on the five steroid metabolizing enzymes noted above in control male rat hepatocytes. It is seen that there is a marked increase in all of the enzyme activities with increasing concentrations of phenformin with significant elevations in enzyme activity seen at 1×10^{-6} M (14–44% above control; $P < 0.05$) and a maximum being reached at about 1×10^{-4} M (60–119% above control; $P < 0.05$). The 16α-hydroxylase reacted least and the 6β-hydroxylase the most.

A similar dose–response curve for tolbutamide is shown in Fig. 2. Again it is seen that all enzyme activities increased with increasing concentrations of tolbutamide. There was, as with phenformin, a significant effect at 1×10^{-6} M (9–27% above control; $P < 0.05$) and a maximum effect was seen at 1×10^{-4} M (52–102% above control; $P < 0.05$). The 6β- and 7α-hydroxylase activities responded the least whereas the 17-oxosteroid oxidoreductase gave the largest rise. Concentrations of tolbutamide above 5×10^{-4} M appeared to cause a diminution of the effect.

If similar dose–response curves are constructed for the effect of phenformin and tolbutamide on steroid metabolism in hepatocytes isolated from diabetic male rats (3 days after treatment with streptozotocin), then it is seen (Fig. 3 for phenformin and Fig. 4 for tolbutamide) that both drugs still give a significant increase in metabolic activity with increasing concentrations of drug. The apparent sensitivity of the cells to the drugs and the maximum effect is, however, diminished. There is no significant effect ($P > 0.05$) with 1×10^{-6} M phenformin and the

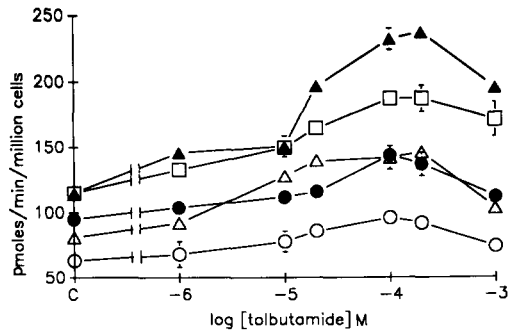

Fig. 2. The effect of pretreatment with tolbutamide (10^{-6}–10^{-3} M) for 24 hr on the metabolism of androst-4-ene-3,17-dione by hepatocytes isolated from the control male rat. The various enzyme activities are denoted by —○— (7α-hydroxylase), —●— (6β-hydroxylase), —△— (16α-hydroxylase), —▲— (17-oxosteroid oxidoreductase) and —□— (5α-reductase). Results are expressed as mean ± 1 SD of six independent samples from the same batch of cells and where no error bars are shown, these were less than the size of the symbol. All treated values were significantly different from their respective controls ($P < 0.05$) except the 7α-hydroxylase activity at 10^{-6} M.

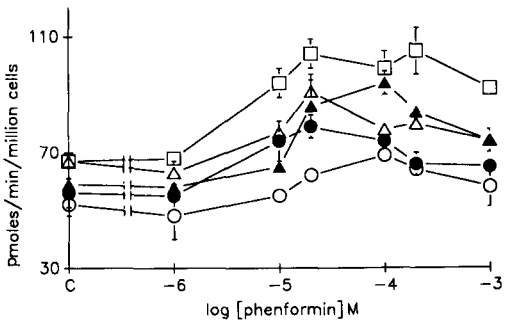

Fig. 3. The effect of pretreatment with phenformin (10^{-6}–10^{-3} M) for 24 hr on the metabolism of androst-4-ene-3,17-dione by hepatocytes isolated from the diabetic male rat. The various enzyme activities are denoted by —○— (7α-hydroxylase), —●— (6β-hydroxylase), —△— (16α-hydroxylase), —▲— (17-oxosteroid oxidoreductase) and —□— (5α-reductase). Results are expressed as mean ± 1 SD of six independent samples from the same batch of cells and where no error bars are shown, these were less than the size of the symbol. All treated values were significantly different from their respective controls ($P < 0.05$) except those at 10^{-6} M.

maximum effect is an increase to 23–59% above control (compared to 63–119% above control for control animals). For tolbutamide, only the 6β-hydroxylase and 5α-reductase are significantly raised ($P < 0.05$) by the 1×10^{-6} M concentration (cf. control animal, Fig. 2) and the maximum increase is 13–73% above control (compared to 52–106% above control for the control animal).

An extension of this experiment was also performed, whereby hepatocytes were pretreated with phenformin or tolbutamide (at the concentration giving the maximum effect, i.e.

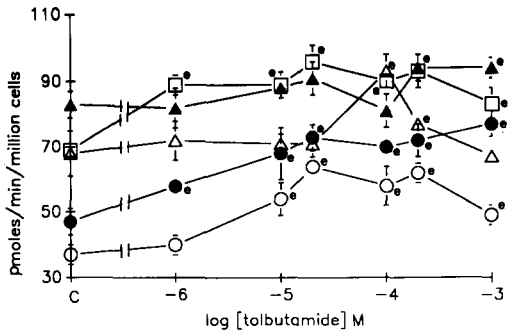

Fig. 4. The effect of pretreatment with tolbutamide (10^{-6}–10^{-3} M) for 24 hr on the metabolism of androst-4-ene-3,17-dione by hepatocyte isolated from the diabetic male rat. The various enzyme activities are denoted by —○— (7α-hydroxylase), —●— (6β-hydroxylase), —△— (16α-hydroxylase), —▲— (17-oxosteroid oxidoreductase) and —□— (5α-reductase). Results are expressed as mean ± 1 SD of six independent samples from the same batch of cells and where no error bars are shown, these were less than the size of the symbol. e = significantly different from respective control ($P < 0.05$).

5×10^{-4} M for phenformin and 1×10^{-4} M for tolbutamide) and subsequently treated with insulin (1×10^{-9} M). The results of this experiment are given in Table 1.

It is seen in the control animal that both phenformin and tolbutamide increase all enzyme activities significantly ($P < 0.05$) as seen in the previous experiment. It is also seen that insulin (1×10^{-9} M) alone increases all enzyme activities to a similar extent. A combination of insulin and either phenformin or tolbutamide further increases all enzyme activities over that seen for insulin or the drug alone. In the hepatocytes isolated from the 3 day diabetic rat, a slightly different effect is seen. Firstly, the enzyme activities measured in the cells were markedly lower than those seen in the cells from control animals. Both phenformin and tolbutamide give a significant increase in most enzyme activities ($P < 0.05$), as noted above, but insulin is ineffective in these cells. Combined treatment with phenformin and insulin gave a significant increase ($P < 0.05$) in all activities except the 5α-reductase as compared to phenformin alone and tolbutamide and insulin gave significant increases ($P < 0.05$) in 6β-hydroxylase, 17-oxosteroid oxidoreductase and 5α-reductase activities when compared to the effects of tolbutamide alone.

DISCUSSION

Sulphonylureas and biguanides are widely used as hypoglycaemics in the treatment of type 2 (insulin-independent diabetes mellitus) but the exact mechanism of action of these drugs is unclear. Using our model of insulin-sensitive and -insensitive hepatocytes [9, 10], we have investigated the actions of phenformin (a biguanide) and tolbutamide (a sulphonylurea) on a known insulin-sensitive hepatic function, the metabolism of steroids [9]. It was found that both phenformin and tolbutamide mimicked the action of insulin on this liver function in insulin-

Table 1. The effects of insulin (10^{-9} M), phenformin (5×10^{-4} M) and tolbutamide (1×10^{-4} M) alone and in combination on the metabolism of androst-4-ene-3,17-dione by hepatocytes isolated from control and streptozotocin-diabetic male rats

Treatment	7α-OHase	6β-OHase	16α-OHase	17-OHSD	5α-Red
Control male					
Control	56 ± 3	79 ± 7	53 ± 7	77 ± 4	81 ± 3
Phenformin	91 ± 7*	113 ± 5*	73 ± 3*	103 ± 5*	112 ± 5*
Insulin	85 ± 6*	104 ± 7*	72 ± 3*	94 ± 2*	94 ± 4*
Phen. + insulin	112 ± 7†	180 ± 6†	86 ± 4†	120 ± 2†	121 ± 4†
Tolbutamide	74 ± 1*	103 ± 1*	80 ± 2*	98 ± 3*	93 ± 3*
Tol. + insulin	85 ± 2‡	113 ± 5‡	104 ± 3‡	116 ± 4‡	135 ± 6‡
Diabetic male					
Control	21 ± 3	28 ± 1	45 ± 4	45 ± 2	49 ± 6
Phenformin	26 ± 1*	33 ± 2*	54 ± 2*	64 ± 2*	77 ± 5*
Insulin	25 ± 3	31 ± 1	50 ± 4	45 ± 3	52 ± 2
Phen. + insulin	30 ± 2†	40 ± 4†	76 ± 6†	71 ± 5†	83 ± 5
Tolbutamide	33 ± 1	42 ± 1*	62 ± 3*	54 ± 2*	64 ± 2*
Tol. + insulin	36 ± 3	52 ± 5‡	74 ± 9	69 ± 2‡	86 ± 7‡

Results are expressed as pmoles product formed per minute per 10^6 cells and as mean ± 1 SD of at least six independent samples.

Phen. = phenformin; Tol. = tolbutamide.

* $P < 0.05$ compared to respective control.

† $P < 0.05$ compared to phenformin treated cells.

‡ $P < 0.05$ compared to tolbutamide treated cells.

sensitive cells isolated from control male rats, giving a significant increase in all enzyme activities assayed (Figs 1 and 2). The mechanism of action of phenformin in giving this effect is unknown. There are a number of reports, as detailed below, concerning the enhancement of insulin action by biguanides but few dealing with the insulin-like activity of these drugs. One such report [14], however, suggests that phenformin stimulates a high affinity cAMP phosphodiesterase as does insulin [15]. This would have the effect of reducing intracellular cAMP levels. As cAMP is known to reduce the metabolism of steroids by the liver [16], a reduction in cAMP may be expected to increase steroid metabolism as seen in this study. It has also been reported that metformin (another biguanide) can enhance hexose transport in the absence of insulin [8]. For tolbutamide there have also been reports of direct effects on liver cells related to cAMP. Luly *et al.* [14] reported a similar activation of high affinity cAMP phosphodiesterase as seen with phenformin above. More recently, tolbutamide has been shown to inhibit protein kinase A [17], the kinase activated by cAMP and, thus, further diminishing the effects of cAMP in the cell. Tolbutamide also activates hepatic guanylate cyclase (therefore increasing intracellular cGMP) but the significance of this is not known as no role for this cyclic nucleotide in the control of hepatic steroid metabolism has been postulated.

Phenformin and tolbutamide also increase all enzyme activities measured in insulin-insensitive hepatocytes isolated from male rats made diabetic by treatment with streptozotocin. Streptozotocin is a pancreatic B-cell toxin and, as such, would be expected to give a type 1 (insulin-dependent) model. The hepatocytes isolated from these animals, however, have been shown to be refractory to the effects of insulin [10], indicating a type 2 model. This was confirmed in this study (Table 1). The oral hypoglycaemics can, therefore, give an insulin-like action in conditions where insulin itself cannot. There is no insulin present in this system and, therefore, the drugs cannot be enhancing insulin action. Few reports have addressed the question of the direct effects of oral hypoglycaemics on liver from diabetic animals but one [18] has suggested that the reduction in hepatic calmodulin levels noted in streptozotocin-induced diabetic rats can be reversed by glyburide (a second generation sulphonylurea).

It is clear, therefore, that both phenformin and tolbutamide can mimic the action of insulin on hepatic steroid metabolism by a direct effect on the hepatocyte and that this effect is still seen in hepatocytes isolated from a diabetic animal, unlike the effect of insulin which is not seen in such cells. Both drugs can work, at least on this insulin-sensitive function, in the complete absence of insulin and even on cells that are refractory to insulin.

If consideration is given to the interaction of phenformin and tolbutamide with insulin, it is seen (Table 1) that there is an additive effect of both drugs with insulin on all enzyme activities studied in the control animal. The concentrations of hormone and drugs used were similar to those found *in vivo* during treatment [14] and, thus, represent potential clinical interactions. There does not seem to be any enhancement/synergism although many reports have suggested such enhancement. For instance, phenformin has been reported to increase insulin binding to target cells [3] and metformin has been shown to enhance insulin-stimulated incorporation of glucose into glycogen and tyrosine aminotransferase activity [19]. Likewise, tolbutamide has been shown to increase insulin binding to target cells [20] but this is disputed [21]. The apparent discrepancy between this and the present study may reflect the design of

the experiment which was specifically to investigate the effect of the drug *alone* and in combination with insulin.

In the insulin-insensitive hepatocyte model, it is confirmed in this study that insulin has no action on steroid metabolism in cells isolated from streptozotocin-diabetic male rats (Table 1) as reported earlier [10]. As seen above, both phenformin and tolbutamide still give their actions in these cells. What is of great interest, however, is that although insulin appears to have no action of its own, it can enhance the action of the oral hypoglycaemics in this test system (Table 1). The effect is not as marked as in the cells isolated from the control animal but there is a significant increase in a majority of the enzymes studied after treatment with phenformin/insulin or tolbutamide/insulin than with the oral hypoglycaemic alone. This effect could be interpreted as the oral hypoglycaemic drug restoring the ability of the cell to respond to insulin as well as mimicking insulin action. This effect has been reported previously by a number of workers. Gawler *et al.* [22] showed that metformin could restore the ability of insulin to inhibit glucagon-stimulated adenylate cyclase activity and Lord *et al.* [5, 23] have suggested that biguanides can restore insulin effects by a post-receptor action. No such reports have appeared related to sulphonyureas.

Oral hypoglycaemics and insulin, therefore, interact in an additive manner to increase hepatic steroid metabolism in control animals but in the hepatocytes isolated from diabetic male animals (which are insulin-insensitive), oral hypoglycaemics of both classes can restore insulin action to a certain extent.

Acknowledgements—This work was supported by the Scottish Home and Health Department (Grant No. K/MRS/50/C864), Scottish Hospitals' Endowment Research Trust (Grant No. SHERT 766) and the University of Glasgow Medical Research Funds. A.H.H. is grateful to the Government of Malaysia and the Universiti Sains Malaysia for joint sponsorship.

REFERENCES

1. Lebovitz HE, Cellular loci of sulphonylurea actions. *Diabetes Care* **7**: 67–71, 1984.
2. Lockwood DH, Maloff BL, Nowak SM and McCaleb ML, Extrapancreatic effects of sulphonylureas. Potentiation of insulin action through post-binding mechanisms. *Am J Med* **74**: 102–108, 1983.
3. Cohen D, Pezzino V, Vigneri R, Avola R, D'Agata R and Polosa P, Phenformin increases insulin binding to human cultured breast cancer cells. *Diabetes* **29**: 329–331, 1980.
4. Salhanick AI, Konowitz P and Amatruda JM, Potentiation of insulin action by a sulphonylurea in primary cultures of hepatocytes from normal and diabetic rats. *Diabetes* **32**: 206–212, 1983.
5. Lord JM, Atkins TW and Bailey CJ, Effect of metformin on hepatocyte insulin receptor binding in normal, streptozotocin diabetic and genetically obese diabetic (ob/ob) mice. *Diabetologia* **25**: 108–113, 1983.
6. Vigneri R, Pezzino V, Wong KJ and Goldfine ID, Comparison of the *in vitro* effects of biguanides and sulphonylureas on insulin binding to its receptor in target cells. *J Clin Endocrinol Metab* **54**: 95–100, 1982.
7. Chaujar M, Chaudhuri BN, Yadav HS and Chauhan UPS, Effect of alloxan diabetes and phenformin on insulin binding with liver plasma membrane receptors. *Jap J Exptl Med* **54**: 189–193, 1984.
8. Jacobs DB, Hayes GR, Truglia JA and Lockwood DH, Effects of metformin on insulin receptor tyrosine kinase activity in rat adipocytes. *Diabetologia* **29**: 798–801, 1986.
9. Hussin AHj and Skett P, The effect of insulin on steroid metabolism in isolated rat hepatocytes. *Biochem Pharmacol* **36**: 3155–3159, 1987.
10. Hussin AHj and Skett P, Lack of effect of insulin in hepatocytes isolated from streptozotocin-diabetic male rats. *Biochem Pharmacol* **37**: 1683–1686, 1988.
11. Seglen PO, Preparation of rat liver cells. III. Enzymatic requirements for tissue dispersion. *Exptl Cell Res* **82**: 391–398, 1973.
12. Hussin AHj and Skett P, Maintenance of steroid metabolism in primary cultures of adult rat hepatocytes in serum-free medium *Biochem Soc Trans* **14**: 914–915, 1986.
13. Gustafsson JÅ and Stenberg Å, Irreversible androgenic programming at birth of microsomal and soluble rat liver enzymes active on 4-androstene-3,17-dione and 5α-androstane-3α,17β-diol. *J Biol Chem* **249**: 711–718, 1974.
14. Luly P, Baldini P, Cocco C, Incerpi S and Tria E, Effect of chlorpropamide and phenformin on rat liver: the effect on plasma membrane-bound enzymes and cyclic AMP content of hepatocytes *in vitro*. *Eur J Pharmacol* **46**: 153–164, 1977.
15. Pyne NJ, Cooper ME and Houslay MD, The insulin- and glucagon-stimulated 'dense-vesicle' high affinity cyclic AMP phosphodiesterase from rat liver. *Biochem J* **242**: 33–42, 1987.
16. Berry LA and Skett P, The role of cyclic AMP in the regulation of steroid metabolism in isolated rat hepatocytes. *Biochem Pharmacol* **37**: 2411–2416, 1988.
17. Okuno S, Inaba M, Nishizawa Y, Inoue A and Morii H, Effect of tolbutamide and glyburide on cyclic-AMP-dependent protein kinase activity in rat liver cytosol. *Diabetes* **37**: 857–861, 1988.
18. Solomon SS, Deaton J, Shankar TP and Palazzolo M, Cyclic-AMP phosphodiesterases in diabetes: effect of glyburide. *Diabetes* **35**: 1233–1236, 1986.
19. Purrello F, Gullo D, Buscema M, Pezzino V, Vigneri R and Goldfine ID, Metformin enhances certain insulin actions in cultured rat hepatoma cells. *Diabetologia* **31**: 385–389, 1988.
20. Prince MJ and Olefsky JM, Direct *in vitro* effect of a sulphonylurea to increase human fibroblast insulin receptors. *J Clin Invest* **66**: 608–611, 1980.
21. Dolais-Kitabgi J, Alengrin F and Freychet P, Sulphonylureas *in vitro* do not alter insulin binding or insulin effect on amino acid transport in rat hepatocytes. *Diabetologia* **24**: 441–444, 1983.
22. Gawler D, Milligan G, Spiegel AM, Unson CG and Houslay MD, Abolition of the expression of inhibitory guanine nucleotide binding protein (Gi) activity in diabetes. *Nature* **327**: 229–232, 1987.
23. Lord JM, Puah JA, Atkins TW and Bailey CJ, Post-receptor effect of metformin on insulin action in mice. *J Pharm Pharmacol* **37**: 821–823, 1985.