Effects of insulin-like growth factor I on basal and stimulated glucose fluxes in rat liver

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Effects of insulin-like growth factor I (IGF-I) and insulin on glucose and potassium fluxes were examined by measuring transhepatic glucose and potassium balance in isolated perfused rat livers. At 1 nM, both IGF-I and insulin decreased basal glucose release by $\approx 64\,\%$ ($P < 0.05$). Adrenaline (epinephrine)-stimulated glucose release ($42.6 \pm 4.5\,\mu$mol/g of liver within 30 min) was inhibited ($P < 0.05$) by ≈ 32 and $\approx 52\,\%$ during IGF-I and insulin exposure, which was accompanied by reduced cAMP release (-71 and $-80\,\%$, $P < 0.05$). IGF-I- and insulin-induced reduction of glucose release only decreased during calcium-free perfusion, but not during inhibition of phosphoinositide 3-kinase by wortmannin. Both IGF-I and insulin induced net potassium uptake, while insulin also attenuated the response to adrenaline. In conclusion, IGF-I causes (i) insulin-like inhibition of hepatic glycogenolysis, even at low, nanomolar concentrations, which is associated with decreased cAMP release, reduced in the absence of Ca^{2+}, but not mediated by phosphoinositide 3-kinase, (ii) reduction of adrenaline-induced glycogenolysis and (iii) net potassium uptake under basal conditions.

Key words: glycogenolysis, insulin-like growth factor I, potassium, wortmannin.

INTRODUCTION

Insulin-like growth factor I (IGF-I) is synthesized mainly by the liver and circulates in plasma bound to IGF-binding proteins (IGFBPs) [1,2]. IGF-I participates in the regulation of tissue proliferation by stimulating DNA synthesis, cell replication and cell-cycle progression as well as cell differentiation and function via specific type-1 IGF receptors [2,3]. But aside from its function as a growth factor, IGF-I may also play a role in the regulation of glucose metabolism.

Intravenous IGF-I administration enhances whole-body glucose uptake [4,5] and can induce hypoglycaemia [6] in healthy adults. Moreover, IGF-I stimulates peripheral glucose clearance in diabetes mellitus types 1 and 2, and syndromes of severe insulin resistance [1]. In the latter group, IGF-I proved to be even more efficient than insulin, suggesting that IGF-I may be complementary to insulin in the regulation of carbohydrate metabolism [2]. These effects of IGF-I have been studied extensively and attributed primarily to its action on skeletal muscle, since IGF-I stimulates transport of glucose and amino acids, glycogen synthesis, glycolysis and lactate production in isolated rat skeletal muscle [7,8].

In contrast, the action of IGF-I on the liver is still unclear. Suppression by IGF-I of endogenous glucose production in humans *in vivo* was observed in some [4,5] but not in all studies [9]. During euglycaemic clamp studies, IGF-I decreased endogenous glucose production in spontaneously diabetic BB/w rats [10], but not in pancreatectomized diabetic [11] or non-diabetic rats [12]. Although IGF-I might exert its effect through binding to insulin receptors [13], the mechanism of hepatic IGF-I action has not yet been elucidated.

The aims of this study were (i) to examine the effect of physiological IGF-I concentrations on basal and stimulated glucose production, (ii) to compare these effects with that of insulin at equimolar concentrations and (iii) to evaluate the respective roles of second-messenger systems that could be involved in the metabolic IGF-I action in isolated perfused rat liver. This experimental model allows the study of hepatic glucose production independently of counter-regulatory hormones and influences due to peripheral metabolic effects of IGF-I and insulin.

EXPERIMENTAL

Animals

Livers were obtained from fed, male rats, which were kept on a constant 12-h:12-h day/night cycle with free access to standard laboratory rat chow and water. Sprague–Dawley rats (210–290 g; Him:OFA/SPF, Versuchstieranstalt, Himberg, Austria) were used for studies on glucose and lactate fluxes. Mutant TR^- Wistar rats (180–230 g; rats that are devoid of the canalicular anion-transport system; provided kindly by P. L. M. Jansen, University of Groningen, Groningen, The Netherlands, and supplied by Versuchstieranstalt) were used for studies on potassium fluxes [14]. All experimental protocols were performed according to local law and to the principles of laboratory animal care.

Materials

The perfusion medium consisted of Krebs–Henseleit buffer, 5 mM D(+)-glucose and 0.2 % (w/v) BSA (fraction V; Boehringer Mannheim, Mannheim, Germany). Human insulin (Actrapid, Novo-Nordisk 40U, Copenhagen, Denmark),

Abbreviations used: IGF-I, insulin-like growth factor I; IGFBP, IGF-binding protein; PI 3-kinase, phosphoinositide 3-kinase.

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adrenaline (epinephrine; Suprarenin, provided kindly by Hoechst, Frankfurt, Germany), glucagon (Lilly, Indianapolis, IN, U.S.A.) and recombinant human IGF-I (provided kindly by Lilly) were diluted in perfusion medium containing 3 % BSA (vehicle). To achieve Ca^{2+}-free conditions EGTA was added to the perfusion medium giving a final concentration of 10 mM. Wortmannin was dissolved in DMSO (0.05 %, v/v) and added to yield a final concentration of 100 nM.

Isolated liver perfusion

The preparation and perfusion were performed according to techniques reported previously [15–17] and was identical in both studies. Livers (11.8–14.3 g, wet weight) were perfused at 37 °C with oxygenated (95 % O_2/5 % CO_2) perfusion medium in a non-recirculating system (constant flow rate, 3–3.5 ml·min^{-1}·g of liver^{-1}). For continuous monitoring of bile flow, a small cannula was inserted into the bile duct [17]. Portal pressure was measured by using a T-tube located 15 cm before the liver and is expressed in terms of buffer height (cm). Bile flow was calculated from single drop weight (8 mg/drop), liver wet weight and from drop frequency and is given as mg·min^{-1}·g of liver^{-1}. During the equilibration period (time, $t = -40$–0 min), livers were checked for uniform colour of the blood-free liver, constant lactate release, stable portal pressure ($< +5$ cm from hydrostatic buffer height) and bile flow (> 0.5 mg·min^{-1}·g of liver^{-1}).

From $t = 0$ to 60 min, IGF-I or insulin (calculated final concentrations, 0.1 nM and 1 nM, respectively) or vehicle (control) were infused continuously via the portal tubing (0.5 ml/min; Perfusor V, Braun, Melsungen, Germany). An additional infusion of adrenaline (final concentration, 0.05 μM) was commenced from $t = 30$ to 60 min. This resulted in the following experimental groups ($n = 6$ for all): (i) control; (ii) IGF-I, 1 nM; (iii) IGF-I, 0.1 nM; (iv) insulin, 1 nM; (v) insulin, 0.1 nM; and (vi) IGF-I, 1 nM, and insulin, 1 nM. From $t = 60$ to 80 min, glucagon (final concentration, 1.15 nM) was administered to test the liver's hormonal responsiveness at the end of the experiments [15,16].

In addition, experiments were performed to test the effect of Ca^{2+}-free conditions and of wortmannin on IGF-I action. To obtain Ca^{2+}-free conditions, EGTA was infused from $t = 0$ to 25 min and IGF-I (final concentration, 1 nM) was infused from $t = 5$ to 12 min ($n = 4$). Wortmannin (final concentration, 100 nM) was infused from $t = 0$ to 27 min and IGF-I (final concentration, 1 nM) was infused from $t = 15$ to 27 min ($n = 4$).

Analytical procedures

Glucose and lactate concentrations were determined enzymically by the hexokinase method (Glucose liquiUV; Human, Taunusstein, Germany) and the lactate dehydrogenase method (ACA, Du Pont Company, Wilmington, DE, U.S.A.) respectively. Perfusate samples for the determination of cAMP, cGMP (Amersham, Little Chalfont, Bucks., U.K.), IGF-I and IGFBP-3 (Mediagnost, Frankfurt, Germany) were immediately frozen and stored at −80 °C. Concentrations of cAMP [15], cGMP, IGF-I (^{125}I-IGF-I tracer, Amersham) and IGFBP-3 were measured by radioimmunoassays. K^+ was measured in the effluent venous perfusate using the NOVA 9 electrolyte analyser (Nova Biomedical, Newton, MA, U.S.A.). These data are presented as changes in K^+ balance with correction for continuous background efflux (≈ 3 nmol·s^{-1}·g of liver^{-1}) measured prior to the beginning of experimental procedures.

Data analyses

Transhepatic balances of glucose and lactate (production rates [15,16]) or of K^+ were calculated as (portal to hepatic venous concentration difference) × (perfusate flow rate)/liver wet weight and are expressed as μmol·min^{-1}·g of liver^{-1} or nmol·s^{-1}·g of liver^{-1} (for K^+). Cumulative glucose release [15,16] in μmol/g of liver was assessed by the trapezoidal rule from the area under the concentration/time curves corrected for glucose production rates at $t = 0$ for basal, and at $t = 30$ min for adrenaline-stimulated glucose production. Data are presented as means ±S.E.M. Differences between groups and controls were compared by using the Dunnet test and differences among groups by analysis of variance (ANOVA) followed by the Scheffe test or by paired two-tailed t test as appropriate (SAS, version 1994).

RESULTS

IGF-I and IGFBP-3 concentrations

Total (free + bound) IGF-I concentrations in the effluent perfusate were ≈ 1.7 nM at baseline. During infusion of IGF-I at a rate to achieve a final concentration of 1 nM, effluent perfusate IGF-I concentrations rose by 1.08 ± 0.35 nM ($P < 0.05$; IGF-I, 1 nM) and by 1.26 ± 0.04 nM ($P < 0.001$; IGF-I and insulin) at $t = 55$ min, respectively. Perfusate IGF-I concentrations were not affected during insulin and control experiments ($t = 55$ min, insulin, 1 nM, 1.69 ± 0.02 nM; control, 1.60 ± 0.11 nM). Perfusate IGFBP-3 concentrations remained unchanged throughout the experiments and were not different between groups ($t = 55$ min, control, 182.4 ± 7.6 μg/l; IGF-I, 1 nM, 178.7 ± 4.6 μg/l; insulin, 1 nM, 180.1 ± 9.2 μg/l; IGF-I + insulin, 180.5 ± 4.4 μg/l).

Effects of IGF-I and insulin on basal and adrenaline-stimulated glucose release

During control experiments, basal hepatic glucose production rates declined slightly, but not significantly within 30 min (Figure 1A). At that time, glucose production rate was markedly decreased by 1 nM IGF-I ($t = 30$ min, 0.34 ± 0.14 μmol·min^{-1}·g of liver^{-1}) as compared with $t = 0$ min (1.52 ± 0.22 μmol·min^{-1}·g of liver^{-1}; $P < 0.001$) or with control ($t = 30$ min, 0.81 ± 0.14 μmol·min^{-1}·g of liver^{-1}, $P < 0.05$). At equimolar concentrations insulin induced a comparable decrease in glucose production rates.

Cumulative glucose release within 30 min was lower than during the preceding equilibration period (control, −10.3 ± 1.2 μmol/g of liver defined as 100 % in Figure 2A). Both IGF-I and insulin inhibited cumulative glucose release with no difference between IGF-I and insulin at 1 nM each (both $P <$ 0.05 versus control). Simultaneous infusion of IGF-I (1 nM) + insulin (1 nM) had no additive effect on glucose release (45 ± 7 %).

During control experiments, adrenaline infusion from $t = 30$ to 60 min resulted in a rapid, maximally 3-fold stimulation of hepatic glucose production rates ($t = 36$ min versus $t = 30$ min; $P < 0.001$; Figure 1A). IGF-I or insulin (1 nM) markedly reduced peak glucose production rates in response to adrenaline.

Cumulative adrenaline-stimulated glucose release (control, 42.6 ± 4.5 μmol/g of liver defined as 100 % in Figure 2B) was reduced by ≈ 32 % and by ≈ 52 % in the presence of 1 nM IGF-I and 1 nM insulin, respectively. At 0.1 nM, insulin ($P < 0.05$ versus control) but not IGF-I inhibited cumulative glucose release. Simultaneous infusion of IGF-I (1 nM) + insulin (1 nM)

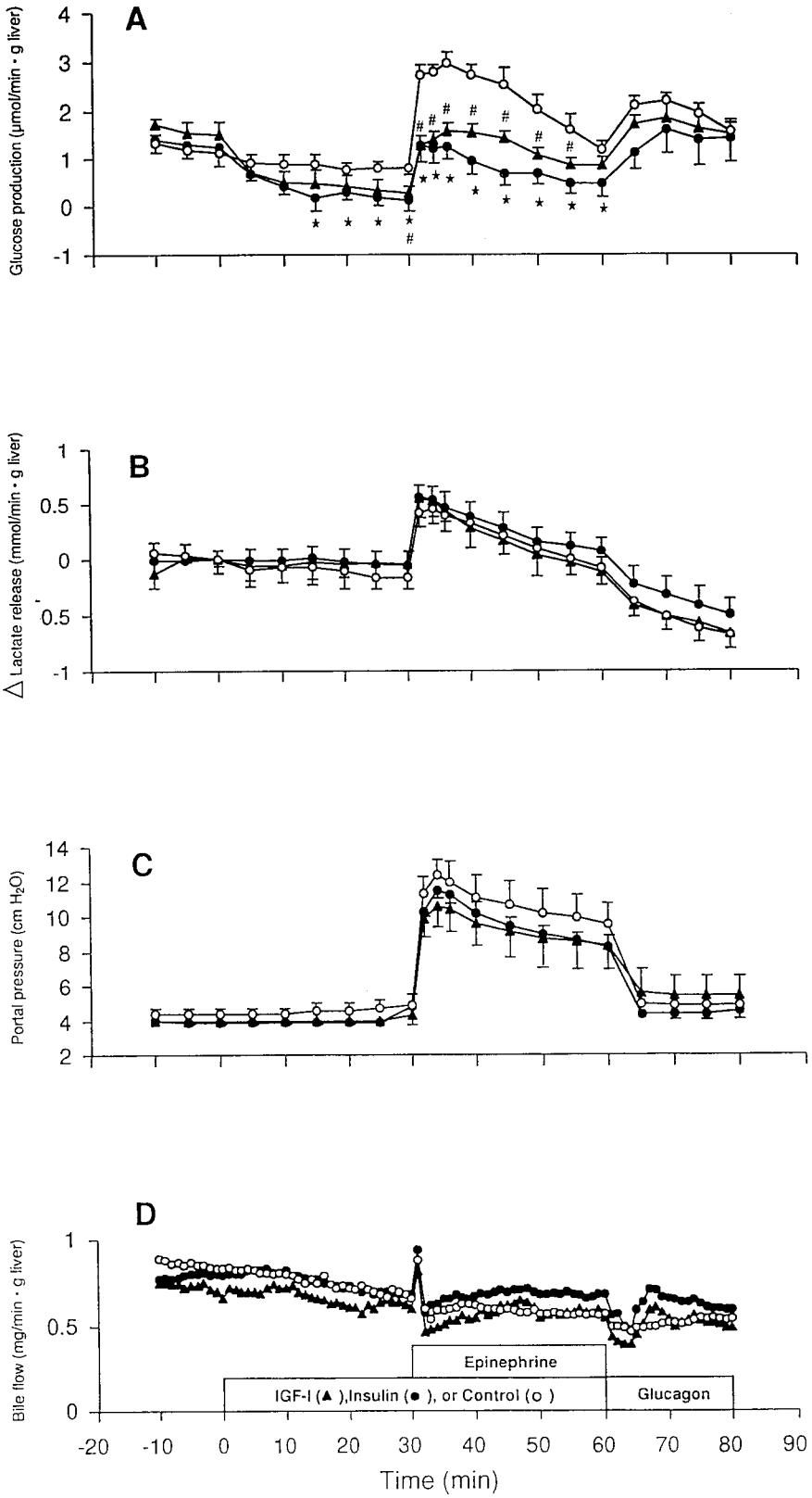

Figure 1 Effect of IGF-I and insulin on time courses of glucose production rates (A), Δlactate release (B), portal pressure (C) and bile flow (D) in isolated perfused livers of Sprague–Dawley rats

IGF-I (1 nM, ▲), insulin (1 nM, ●) or Krebs–Henseleit buffer (control, ○) were infused into the portal vein from 0 to 60 min during the basal period (0–30 min) and adrenaline (epinephrine, 50 nM) stimulation (30–60 min). Glucagon (1.15 nM) alone was administered from 60 to 80 min. Means ± S.E.M., $n = 6$, in each group are shown. $^*P < 0.05$, insulin versus control; $\#P < 0.05$, IGF-I versus control.

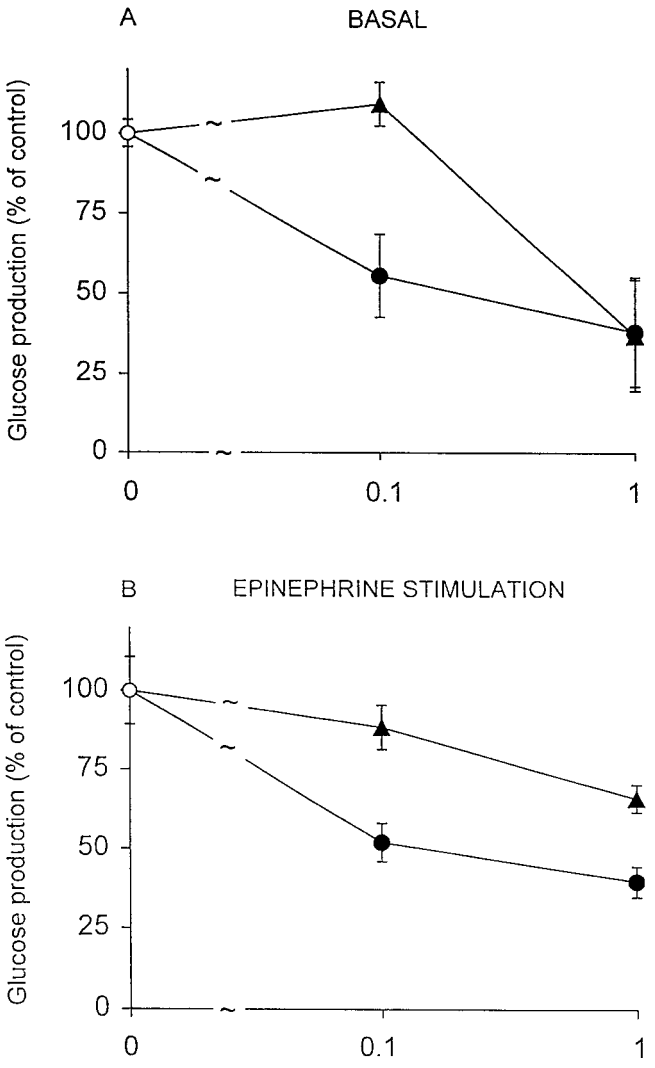

Figure 2 Dose-dependent inhibition by IGF-I and insulin of basal (A) and adrenaline (epinephrine)-stimulated (B) cumulative glucose production

IGF-I (0.1 and 1 nM, ▲), insulin (0.1 and 1 nM, ●) or Krebs–Henseleit buffer (control, ○) was infused for 60 min into isolated perfused livers of Sprague–Dawley rats. Cumulative glucose release was assessed from the area under the respective time curve corrected for baseline values. $^{*}P < 0.05$ versus control.

had no additive effect on suppression of adrenaline-stimulated glucose production ($45 \pm 5\%$).

Liver viability: lactate release, portal pressure and bile flow

During the basal period, lactate production (Figure 1B), portal pressure (Figure 1C) and bile flow (Figure 1D) were not different between groups. Adrenaline induced a rapid and long-lasting elevation of lactate release to a maximum of 0.45 ± 0.08 mmol·min^{-1}·g of liver^{-1} and of portal pressure by 7.2 ± 0.7 cm buffer height in all groups. Bile flow exhibited an immediate peak followed by a slow biphasic response during adrenaline exposure. Neither IGF-I nor insulin affected the dynamic changes of lactate production, portal pressure and bile flow induced by adrenaline.

Table 1 Concentrations of cAMP in the effluent perfusate of isolated perfused rat livers corrected for cAMP concentration during the equilibration period

Data from the experiments described in Figure 1 are given as means ± S.E.M. for five experiments in each group. $^{*}P < 0.005$ and $\#P < 0.001$, versus control; ¶$P < 0.05$, adrenaline versus basal; †$P < 0.05$ and §$P < 0.005$, versus control; ‡$P < 0.001$, glucagon versus adrenaline.

	ΔcAMP (pM)		
	Basal (0–30 min)	Adrenaline (30–60 min)	Glucagon (60–90 min)
Control	+73 ± 33	+309 ± 95¶	+2022 ± 478‡
IGF-I			
0.1 nM	−60 ± 17*	+356 ± 88¶	+1061 ± 137‡
1.0 nM	−118 ± 44*	+90 ± 40†	+2053 ± 276‡
Insulin			
0.1 nM	−208 ± 28#	+59 ± 30†	+1667 ± 310‡
1.0 nM	−181 ± 21#	−63 ± 31§	+1290 ± 520‡

Effects of IGF-I and insulin on hepatic release of cAMP and cGMP

During the equilibration period, concentrations of cAMP in the effluent perfusate were ≈ 360 pM. Both IGF-I and insulin decreased perfusate cAMP during the basal period ($P < 0.005$ versus control, $P < 0.025$ versus respective equilibration period; Table 1). Adrenaline elicited hepatic cAMP release in all groups with the increase being lower in the presence of 1 nM IGF-I or 0.1 nM and 1 nM insulin. At the end of IGF-I or insulin infusions, glucagon caused a further increase of perfusate cAMP concentrations in all groups.

Perfusate cGMP concentrations were ≈ 190 pM during the equilibration period and did not change during basal and adrenaline-stimulated conditions. Subsequent glucagon infusion induced a rise ($P < 0.01$) in perfusate cGMP following exposure to IGF-I (+115 ± 11 pM) or insulin (+83 ± 22 pM).

Effects of IGF-1 and insulin on net K^+ balance

During adrenaline stimulation, K^+ balance was transiently shifted from positive to negative values, indicating K^+ uptake (Figure 3). Baseline positive values indicating K^+ release were reached again within ≈ 4 min. Then K^+ balance exhibited a biphasic response characterized by a small decrease followed by an increase in K^+ release. Insulin markedly decreased both baseline K^+ release ($P < 0.05$) and the adrenaline-dependent transient K^+ uptake (Figures 3A and 3B). Administration of IGF-I resulted in a small decrease ($P < 0.05$) of baseline K^+, which was, however, abolished after exposure to adrenaline (Figures 3C and 3D). IGF-I did not affect the fast transient K^+ uptake induced by adrenaline, but slightly enhanced ($P < 0.05$) the subsequent decrease in K^+ release (Figures 3C and 3D).

The patterns of glucose release and bile flow in livers from TR$^-$ rats were comparable with those of normal Wistar and Sprague–Dawley rats (results not shown).

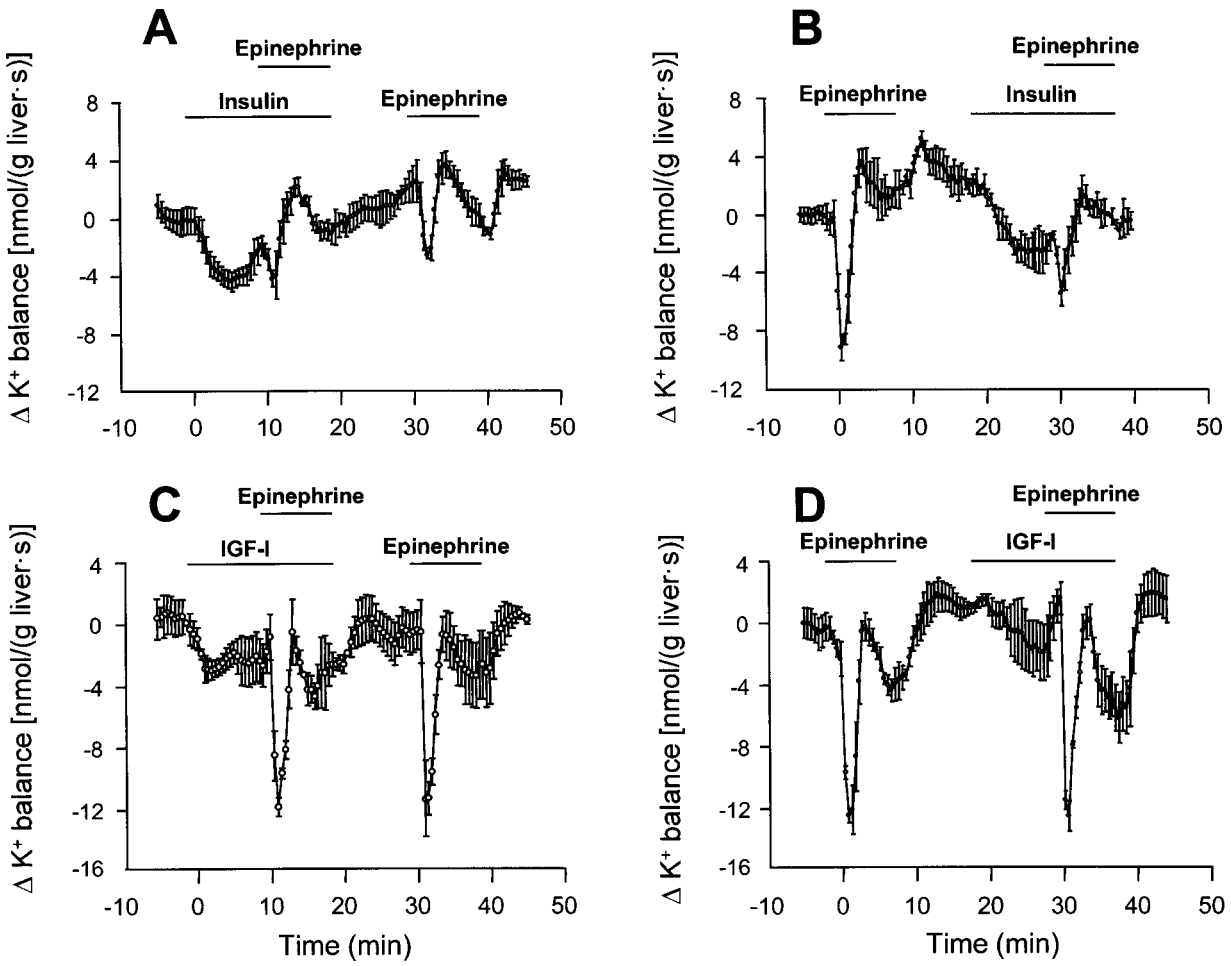

Figure 3 Effects of insulin (A, B) and IGF-I (C, D) on basal and adrenaline (epinephrine)-stimulated net transhepatic K^+ balance in isolated perfused livers of TR^- rats

K^+ fluxes are presented as Δtranshepatic K^+ balance recorded during exposure to adrenaline (50 nM) and/or IGF-I (1 nM).

Effects of Ca^{2+}-free perfusion and wortmannin on hepatic IGF-I action

During Ca^{2+}-free perfusion, inhibition by IGF-I of basal glucose production was reduced (Figure 4A), while the effect on net K^+ balance was not affected under these conditions (Figure 4B).

In the presence of 100 nM wortmannin, IGF-I was still able to decrease basal glucose release (Figure 4C). Wortmannin *per se* caused a gradual decline of net K^+ balance, but attenuated the IGF-I-dependent decrease of the K^+ balance (Figure 4D).

DISCUSSION

During IGF-I infusion, the measured increase in total perfusate IGF-I concentration by ≈ 1.1 nM was found to be similar to the calculated final concentration of 1 nM. Since IGF-I or insulin did not affect perfusate concentrations of IGFBP-3, the rise of total IGF-I concentration was likely to be due to an increase in unbound IGF-I. The IGF-I concentration employed is in good agreement with reported free plasma concentrations of ≈ 2.5 nM [6] and substantially lower than total IGF-I concentrations of 16–94 nM in rat and human plasma [1,10,11]. At 1 nM, IGF-I was as effective as insulin in suppressing glucose and cAMP release, suggesting that IGF-I could indeed take part in the regulation of hepatic glucose metabolism under certain physiological conditions. Neither IGF-I nor insulin affected lactate release, portal pressure and bile flow under basal and adrenaline-stimulated conditions, thereby excluding non-specific effects of IGF-I and insulin.

Whereas IGF-I receptors are widely distributed in various tissues including rat and human skeletal muscle [1,2], there is less evidence for the presence of IGF-I receptors in adult rat liver and human hepatocytes [18]. In isolated hepatocytes IGF-I rather binds to insulin receptors with a half-maximally effective concentration (K_m) of ≈ 30 nM compared with ≈ 0.8 nM for insulin [13], which is similar to the affinity of IGF-I for binding to the insulin receptor in other tissues [19]. In agreement with these findings, Hartmann et al. [13] reported ≈ 60 times and ≈ 50 times higher half-maximally effective concentrations of IGF-I for stimulation of glycolysis and inhibition of glucagon-activated glycogenolysis, respectively, compared with insulin. However, from the data of the present study the half-maximally effective concentrations of IGF-I for inhibition of basal and adrenaline-stimulated glucose production and cAMP release can be assumed to be ≈ 1 nM, suggesting 50-fold higher potency of IGF-I than previously indicated. This is in keeping with the comparable

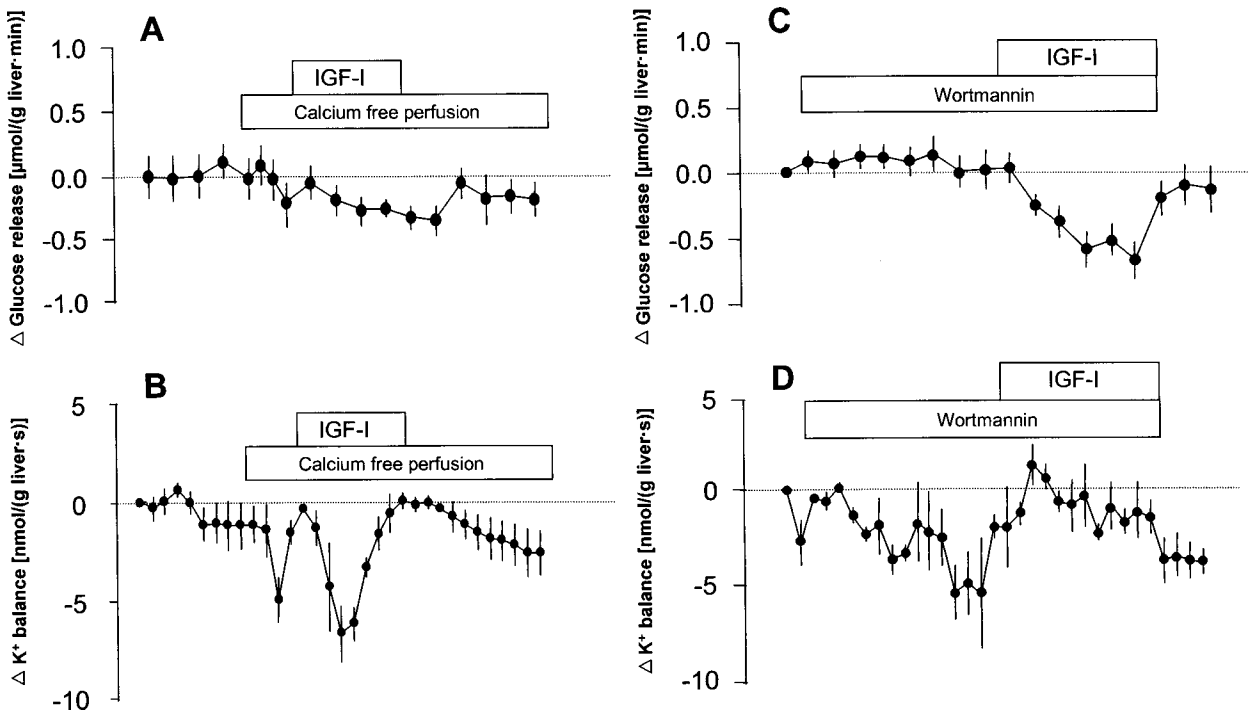

Figure 4 Effect of Ca^{2+}-free perfusion (A, B) and phosphoinositide 3-kinase inhibitor wortmannin (C, D) on IGF-I-dependent changes of glucose production rates (A, C) and K^+ balance (B, D)

In the presence of 10 mM EGTA (**A**, **B**) or 100 nM wortmannin (**C**, **D**), changes of glucose and K^+ fluxes presented as Δtranshepatic balances were monitored before and during IGF-1 (1 nM) infusion into the portal vein of livers from TR^- rats.

efficacy of IGF-I, IGF-II and insulin at 5 nM each in stimulating [U-^{14}C]glucose incorporation into glycogen in cultured chick embryo hepatocytes [20]. Comparison of the half-maximally effective concentrations of IGF-I and insulin for glycogen synthesis indicates an even higher glycogenic potency of IGF-I [18]. The present study suggests that IGF-I receptors are also present in adult rat liver. Alternatively, hepatocytes might express insulin/IGF hybrid receptors that have a high affinity of 0.2–1 nM for binding of IGF-I, but 15–50-fold lower affinity for insulin [2,3,21]. Under conditions *in vivo*, Petersen et al. [22] recently showed that IGF-I is even more efficient than insulin in decreasing hepatic glucose production in a rodent model of insulin resistance. Similarly, we previously found mean liver glycogen concentrations to be ≈ 1.7-fold higher during intravenous IGF-I infusion, even in non-diabetic rats [23].

Autophosphorylation, tyrosine kinase activity and subsequent insulin-receptor substrate-1 phosphorylation are essential for both insulin and IGF-I receptor signalling [2], although it is not yet clear whether the metabolic IGF-I action in particular could be mediated by signalling pathways not requiring tyrosine phosphorylation [24]. Activation of phosphoinositide 3-kinase (PI 3-kinase) is held responsible for stimulation of glycogen synthesis, while phospholipase C generates inositol phosphates and Ca^{2+} release which serve as second messengers for hepatic glycogenolysis.

In the present study, the IGF-I-dependent reduction of basal glucose production was almost completely inhibited during Ca^{2+}-free perfusion suggesting that the metabolic effect of IGF-I is at least partly mediated by Ca^{2+}. Calcium is also essential for insulin-induced stimulation of cAMP phosphodiesterase and protein metabolism in rat hepatocytes [25]. Of note is that the present study shows that IGF-I decreases hepatic cAMP release in the basal period. Taken together, these results indicate that IGF-I exerts an insulin-like effect on basal hepatic glucose production that is mediated by cAMP and requires the presence of Ca^{2+}. Other growth factors, such as epidermal growth factor [26] and hepatocyte growth factor [27], stimulate phospholipase C and release phosphoinositides and Ca^{2+}. In contrast, insulin, IGF-I and IGF-II were reported not to stimulate phospholipase C and generate inositol trisphosphate in membrane preparations of human hepatocytes [28].

On the other hand, IGF-I-dependent reduction of basal glucose production was not affected by wortmannin, which inhibits PI 3-kinase [29] and PI 4-kinase [30]. This suggests that the metabolic action of IGF-I rather involves a Ca^{2+}-dependent and PI 3-kinase-independent pathway. Interestingly, certain IGF-I-dependent effects, such as mitogenesis and tumourigenesis, also require mechanisms other than mitogen-activated protein kinase and PI 3-kinase activation [31].

Stimulation of hepatic α_1-adrenergic receptors activates glycogen phosphorylase [32] which is due to mobilization of intracellular Ca^{2+} and opening of Ca^{2+} channels resulting in influx of extracellular Ca^{2+} [33]. Suppression of adrenaline-induced glucose production by IGF-I predominantly results from glycogenolysis in the fed state [32]. IGF-I might therefore act as an inhibitor of α_1-adrenergic Ca^{2+} mobilization. Since pre-exposure to wortmannin reduced glucose responses to adrenaline in rat liver, the PI 3-kinase pathway could be involved in the inhibitory IGF-I action on adrenaline-induced glycogenolysis (results not shown). Adrenaline also increases hepatic cAMP via β_2- and to less extent via α_1-adrenergic receptors [34], which is required for maximal glycogen degradation [34]. IGF-I inhibited

the adrenaline-induced increase in cAMP release, but was less effective than insulin. Thus metabolic IGF-I action exhibits a different pattern of effects compared with other growth factors such as epidermal growth factor [34].

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