Further evidence for a two-step model of glucose-transport regulation

Inositol phosphate-oligosaccharides regulate glucose-carrier activity

Bert OBERMAIER-KUSSER,* Christa MÜHLBACHER,* Joanna MUSHACK,* Eva SEFFER,* Britta ERMEL,* Fausto MACHICAO,† Felix SCHMIDT‡ and Hans-Ulrich HÄRING*§

*Institut für Diabetesforschung, Kölner Platz 1, 8000 München, Federal Republic of Germany,

†Hormon Chemie, Forschungslabor, 8000 München, Federal Republic of Germany,

and ‡Boehringer Mannheim, Stoffwechselabteilung, 6800 Mannheim, Federal Republic of Germany

The insulin effect on glucose uptake is not sufficiently explained by a simple glucose-carrier translocation model. Recent studies rather suggest a two-step model of carrier translocation and carrier activation. We used several pharmacological tools to characterize the proposed model further. We found that inositol phosphate (IP)-oligosaccharides isolated from the drug Actovegin, as well as the alkaloid vinblastine, show a partial insulin-like effect on glucose-transport activity of fat-cells (3-*O*-methylglucose uptake, expressed as % of equilibrium value per 4 s: basal 5.8 %, insulin 59 %, IP-oligosaccharides 30 %, vinblastine 29 %) without inducing carrier translocation. On the other hand, two newly developed anti-diabetic compounds (α -activated carbonic acids, BM 130795 and BM 13907) induced carrier translocation to the same extent as insulin and phorbol esters [cytochalasin-B-binding sites in plasma membranes: basal 5 pmol/mg of protein, insulin 13 pmol/mg of protein, TPA (12-*O*-tetradecanoylphorbol 13-acetate) 11.8 pmol/mg of protein, BM 130795 10.8 pmol/mg of protein], but produce also only 40–50 % of the insulin effect on glucose-transport activity (basal 5.8 %, insulin 59 %, TPA 23 %, BM 130795 35 %). Almost the full insulin effect was mimicked by a combination of phorbol esters and IP-oligosaccharides (basal 7 %, insulin 50 %, IP-oligosaccharides 30 %, TPA 23 %, IP-oligosaccharides + TPA 45 %). None of these substances stimulated insulin-receptor kinase *in vitro* or *in vivo*, suggesting a post-kinase site of action. The data confirm the following aspects of the proposed model: (1) carrier translocation and carrier activation are two independently regulated processes; (2) the full insulin effect is mimicked only by a simultaneous stimulation of carrier translocation and intrinsic carrier activity, suggesting that insulin acts through a synergism of both mechanisms; (3) IP-oligosaccharides might be involved in the transmission of a stimulatory signal on carrier activity.

INTRODUCTION

Insulin stimulates *in vitro* the glucose-transport activity of isolated fat-cells. Cushman and co-workers (Wardzala *et al.*, 1981), using different experimental approaches, have shown that the activation of the glucose-transport activity is paralleled by a translocation of glucose carriers from intracellular storage sites to the plasma membrane, and have proposed that carrier translocation is the molecular basis of glucose-transport activation by insulin (Simpson & Cushman, 1985). This mechanism was widely accepted, but more recently a number of observations in several experimental systems were difficult to reconcile with a pure translocation model. It was shown by several experimental systems were difficult to reconcile with a pure translocation model. It was shown by several investigators (Joost *et al.*, 1986; Kahn & Cushman, 1987; Matthaei *et al.*, 1987; Karnieli *et al.*, 1987; Mühlbacher *et al.*, 1988) that under certain conditions glucose-transport activity and carrier numbers in the plasma membrane are not sufficiently correlated. Furthermore, we have shown (Mühlbacher *et al.*, 1988) that

phorbol esters mimic quantitatively the insulin effect on carrier translocation without mimicking the full insulin effect on transport activity. We concluded that insulin-induced glucose-carrier translocation is followed or paralleled by a second step, apparently an activation of carriers which is necessary to reach the full extent of the insulin response. Subsequently we showed that an activation of carriers without carrier translocation is induced by $AlCl_3$, presumably through activation of a G-protein, and by phospholipase C (Obermaier-Kusser *et al.*, 1988). On the basis of these data, we proposed as a working hypothesis a model of glucose-transport regulation in fat-cells with the following characteristics: (1) carrier translocation and regulation of carrier activity are two independent mechanisms; (2) the insulin effect requires the additive action of both mechanisms; (3) carrier translocation is a protein kinase C-dependent step; carrier activation involves G-proteins and phospholipases.

In the present paper we used a number of pharmacological tools to evaluate this concept. We tested among other substances potential products of cleavage

Abbreviations used: TPA, 12-*O*-tetradecanoylphorbol 13-acetate; IP-oligosaccharides, inositol phosphate-oligosaccharides; CBB, cytochalasin B-binding.

§ To whom all correspondence and reprint requests should be addressed.

by phospholipases. The results are consistent with the proposed model. Furthermore, the data show that glucose-carrier activity can be selectively stimulated by an inositol phosphate (IP)-oligosaccharide fraction which resembles a possible cleavage product of a phospholipase C, supporting the idea that a stimulatory signal on intrinsic carrier activity might involve cleavage of membrane glycolipids by a phospholipase.

MATERIALS AND METHODS

Materials

Pig insulin was purchased from Novo Industrie (Bagsvaerd, Denmark), [^3H]cytochalasin B and [^{14}C]urea were from New England Nuclear (Dreieich, Germany), and TPA (12-*O*-tetradecanoylphorbol 13-acetate), cytochalasins and the 5'-nucleotidase enzyme kit were from Sigma (Munich, Germany). The α -activated carbonic acids BM 130795 [($\pm$)-7-(4-chlorophenyl)-2-(4-methylphenylsulphonyl)heptanoic acid, sodium salt] and BM 13907 [($\pm$)-5-(4-chlorophenyl)-2-(4-methylphenylsulphonyl)pent-4-ynoic acid], acting as anti-diabetic drugs, and the inactive analogue BM 13705 [5-(2-phenylethenyl)isoxazolidin-3-carboxylic acid] were from Boehringer (Mannheim, Germany). Vinblastine was from Sigma, and the commercially available drug Actovegin was from Hormon Chemie (Munich, Germany). All other reagents were of the best grade commercially available.

Cell isolation and determination of 3-*O*-methylglucose transport

Rat adipocytes were prepared as described by Häring *et al.* (1981) from male Sprague-Dawley rats fed *ad libitum* (180–220 g body wt.). Incubation was carried out at 37 °C in the absence (basal) or the presence of insulin, or with other substances mentioned in the Figure legends. The phorbol ester TPA was diluted in pure ethanol, dried with N_2 , taken up in incubation buffer and sonicated. The BM substances were dissolved in 1 M-NaOH (6 mg of BM in 100 μl of 1 M-NaOH), vortex-mixed and adjusted to the final concentrations in incubation buffer. Vinblastine was dissolved in 0.9% NaCl, and IP-oligosaccharides were dissolved in distilled water. Glucose-transport activity was measured as described by Häring *et al.* (1981).

Subcellular fractionation of adipose cells and cytochalasin-B-binding (CBB) assay

Plasma- and microsomal-membrane fractions were prepared as described previously (McKeel & Jarrett, 1970; Karnieli *et al.*, 1981; Mühlbacher *et al.*, 1988). The concentration of D-glucose transporters in the membrane fractions was assessed by using a specific D-glucose-inhibitable CBB assay (Wardzala *et al.*, 1978; Karnieli *et al.*, 1981). The CBB assay was carried out as outlined by Mühlbacher *et al.* (1988), and analysed with a computer program published by McPherson (1985).

Insulin-receptor kinase solubilization and autophosphorylation *in vitro*; determination of kinase activity *in vivo*

Partially purified insulin receptor was prepared and phosphorylated *in vitro* as described by Häring *et al.* (1986). The activation of the receptor kinase *in vivo* was assessed as described by Klein *et al.* (1986). Briefly, fat-

cells were stimulated with insulin, IP-oligosaccharides or BM substances. Insulin receptor was then purified as described by Häring *et al.* (1986), but in the presence of vanadate (1 mM). Kinase activity was then measured with poly(Glu-Tyr) as substrate as described by Häring *et al.* (1986).

Isolation of active IP-oligosaccharide fractions from the drug Actovegin

Actovegin, which is derived from calf blood, was precipitated with ethanol and applied to a TSK-HW 40 column. The eluted 1 ml fractions were assayed for their ability to stimulate lipogenesis from D- ^3H glucose as well as for their anti-lipolytic effect against isoprenaline in adipocytes (F. Machicao & V. Christoffel, unpublished work). The fraction with the highest lipogenic activity, designated P1, was used in our studies. This fraction contained predominantly carbohydrates (F. Machicao & V. Christoffel, unpublished work). To characterize the carbohydrate composition of this fraction, hydrolysis, reduction and derivative formation were performed as follows: (1) hydrolysis to generate monosaccharides with 2 M-HCl at 100 °C for 2–4 h; (2) reduction with 50 mM-NaOH/0.5 M-NaBH $_4$; (3) ion-exchange chromatography with Dowex AG 50W X8; (4) acetylation with acetic anhydride in pyridine; (5) free saccharides were then isolated and identified by g.l.c. (Supelco column; 912 mm $\times$ 2 mm; 180–220 °C, gradient of 2 °C/min).

RESULTS

Description of substances acting like insulin

In order to find pharmacological tools to be used as selective stimulators of glucose-carrier translocation or glucose-carrier activation, we tested agents which were potential candidates as substances acting like insulin.

(1) The phosphatidylinositol cycle is an important signal-transmitting system at the plasma membrane (Berridge, 1984). Activation of the key enzyme of this cycle, the phospholipase C, leads to generation of signal-transmitting compounds. As potential products of cleavage by phospholipase C, we tested diacylglycerol, inositol tris- and mono-phosphate, as well as *myo*-inositol.

(2) It was proposed that phosphatidylinositol-containing glycolipids are the source of second-messenger substances of insulin action (Saltiel & Cuatrecasas, 1986; Fox *et al.*, 1987; Mato *et al.*, 1987; Alemany *et al.*, 1987; for review, see Low & Saltiel, 1988). The compounds tested so far were isolated from liver, and did not stimulate glucose transport (Kelly *et al.*, 1987). We tested an IP-oligosaccharide fraction isolated from the drug Actovegin as described in the Materials and methods section (F. Machicao & V. Christoffel, unpublished work). The contents of neutral sugars and inositol measured as alditol acetates by g.l.c. are described in the legend of Fig. 1. For further chemical characterization of this fraction, particularly to compare its characteristics with those reported by Saltiel & Cuatrecasas (1986) and Low & Saltiel (1988), the following inactivation treatments were applied, and the remaining activity was determined in the lipogenesis assay. The activity after each treatment is expressed as a percentage (numbers in parentheses) of the control activity: no treatment (control, 100% lipogenesis activity), treatment with 2 M-HCl (54%), 1% NH_3 (70%), 10% NH_3 (58%), 0.2 M-

NaNO_2 , pH 3.7 (100 %), methanol/HCl (24 %), NaBH_4 (50 %), HNO_3 (42 %), acetylated (38 %). The lipogenic activity of this fraction could be inhibited by inositol monophosphate (0.3–2.5 mM) as described also by Saltiel & Sorbara-Cazan (1987) for their inositol phosphate-glycan. ^{125}I -insulin binding to fat-cells was not altered by the active fraction. The results of the chemical analysis and the inactivation procedure suggest that the active compound contained in this IP-oligosaccharide fraction has many homologies with, but also some composition differences from, those IP-glycans described as putative second messengers (Saltiel & Cuatrecasas, 1986; Fox *et al.*, 1987; Mato *et al.*, 1987; Alemany *et al.*, 1987; Low & Saltiel, 1988).

(3) The substances BM 13907 and BM 130795 are recently developed anti-diabetic drugs from Boehringer Mannheim. These α -activated carbonic acids were shown to lower blood sugar in animal models resembling type II

diabetes (H.-F. Kühnle, H.-P. Wolf & F. Schmidt, unpublished work). The site of action of these substances is so far unknown. BM 13705 is an inactive analogue.

(4) Furthermore, the effect of the alkaloid vinblastine on glucose-transport activity was investigated: vinblastine binds to the microtubular system and alters its function (Olmsted & Borisy, 1973). The effect of these substances on glucose-transport activity and CBB is described in the following paragraphs.

Stimulation of 3-O-methylglucose transport

There is a stimulatory effect of diacylglycerol (0.25 mM), but no significant effects of inositol 1-phosphate, inositol 1,4,5-trisphosphate and *myo*-inositol (all 0.25 mM; stimulation factors over basal: diacylglycerol 3.3 ± 0.6 , $n = 5$; inositol trisphosphate 1.7 ± 0.7 , $n = 5$; inositol monophosphate 1.7 ± 0.1 , $n = 2$; *myo*-inositol 2.4 ± 1.1 , $n = 4$). In contrast, the IP-oligosaccharide fraction, the BM substances and the alkaloid vinblastine showed significant effects. Fig. 1(a) shows the dose-response effect of the IP-oligosaccharides on glucose transport. Insulin was used as a reference; it stimulates glucose transport in a dose-dependent way up to 10-fold. The IP-oligosaccharide fraction stimulates in a dose-dependent way up to approx. 5-fold. The effect of the oligosaccharide fraction occurs as rapidly as the insulin effect (Fig. 1b). The time course of the effect of the IP-oligosaccharide fraction and, for comparison again, the effect of insulin, which was measured in parallel, are shown in Fig. 1(b). Both agents start to act within 1 min and reach maximal values after 5 min. Acetylation of the IP-oligosaccharide fraction decreased the effect by approx. 50 %. Fig. 2 shows the dose-response effect of the substances BM 13907 and BM 130795, and BM 13705, which was used as inactive analogue. BM 13907 and BM 130795 stimulate in a dose-dependent way the glucose-transport activity of fat-cells. BM 130795 mimics the insulin effect by 40–60 %, i.e. it causes a 5–6-fold stimulation. Again, a similar time course to that with insulin is found (results not shown). Fig. 3(a) shows the

Fig. 1. Dose-response curve and time course of the effect of IP-oligosaccharides on 3-O-methylglucose uptake in isolated fat-cells

(a) Freshly isolated rat adipocytes $[(4.5-5.5) \times 10^6 \text{ cells/ml}]$ were incubated with insulin or IP-oligosaccharides (IP) for 20 min at the concentrations given on the abscissa. The composition of the IP-oligosaccharide fraction was as follows: erythrose 2.54 mM (0.305 mg/ml), ribose 1.49 mM (0.166 mg/ml), arabinose 0.5 mM (0.150 mg/ml), xylose 1.26 mM (0.189 mg/ml), mannose 0.60 mM (0.108 mg/ml), galactose 0.30 mM (0.053 mg/ml), glucose 1.22 mM (0.220 mg/ml), inositol 0.56 mM (0.100 mg/ml). The saccharides are complexed as phosphates or sulphates. The IP-oligosaccharide fraction is acid- and alkali-labile. 3-O-Methylglucose uptake was then determined for 4 s as described in the Materials and methods section and is expressed as % of equilibrium. The curves show mean values $\pm$ S.E.M. for six experiments. Acetylation of the IP-oligosaccharide fraction decreased the effect of 27 or 270 $\mu\text{g/ml}$ by approx. 50 % ($n = 2$). (b) Freshly isolated rat adipocytes $[(4.5-5.5) \times 10^6 \text{ cells/ml}]$ were stimulated with insulin (2 nM) or IP-oligosaccharides (27 $\mu\text{g/ml}$) at zero time (arrow). 3-O-Methylglucose uptake was determined at the time points indicated in the Figure. Mean values of two experiments are shown.

Fig. 2. Effect of BM 13907 and BM 130795 on 3-*O*-methylglucose uptake by isolated fat-cells

Freshly isolated rat adipocytes $[(4.5-5.5) \times 10^6 \text{ cells/ml}]$ were incubated with BM 13907 or BM 130795 for 20 min at the concentrations given on the abscissa. 3-*O*-Methylglucose uptake was then determined as described in the Materials and methods section. Mean values $\pm$ S.E.M. for seven experiments are shown. As a control the inactive analogue BM 13705 was used ($n = 5$).

effect of vinblastine on glucose transport; up to 40% of the insulin effect is reached, and a similar time course to that with insulin was found (Fig. 3b).

Effect on glucose-carrier numbers in the plasma membranes: determination of CBB sites in subcellular membrane fractions

We studied the effect of the active substances on the concentration of glucose carriers in plasma membranes and low-density membranes. Fig. 4 shows the CBB data for plasma-membrane fractions of isolated fat-cells (Fig. 4b) and, in summary also the effect of these substances on glucose-transport activity (Fig. 4a). As a further control substance besides insulin, the phorbol ester TPA was also used. None of these compounds altered the previously described (Mühlbacher *et al.*, 1988) distribution of marker enzymes in the membrane preparation.

The BM substances and TPA were the only compounds acting like insulin which led to glucose-carrier translocation. Insulin increases the glucose-carrier number in the plasma-membrane fraction from 5 to 13 pmol. BM 13907, which is less active in glucose-transport activity stimulation, also has a smaller effect on carrier translocation, whereas TPA and BM 130795 have the same effect as insulin. None of the other substances which stimulated glucose transport in fat-cells caused an increase of CBB sites in the plasma-membrane fraction. The increase of CBB sites leads, in the case of insulin, to an approx. 10-fold stimulation of the glucose-transport activity of the fat-cells, whereas TPA and BM substances cause only an approx. 3-6-fold stimulation of the glucose-

Fig. 3. Dose-response curve and time course of the effect of vinblastine on 3-*O*-methylglucose uptake by fat-cells

(a) Freshly isolated rat fat cells $[(4.5-5.5) \times 10^6 \text{ cells/ml}]$ were incubated for 10 min with vinblastine and insulin at the concentrations given on the abscissa. 3-*O*-Methylglucose uptake was then determined as described in the Materials and methods section. Mean values $\pm$ S.E.M. for seven experiments are shown. (b) Freshly isolated rat fat cells $[(4.5-5.5) \times 10^6 \text{ cells/ml}]$ were incubated with vinblastine and insulin at the concentrations given. 3-*O*-Methylglucose uptake was then determined as described in the Materials and methods section. Mean values of two experiments are shown.

transport activity, even though with BM 130795 and TPA the same numbers of carriers are found as with insulin. The second group of compounds, namely the IP-oligosaccharide fraction and vinblastine, apparently act independently of glucose-carrier translocation; the 5-6-fold stimulation of transport occurs obviously through modulation of the intrinsic glucose-carrier activity.

Fig. 4. Comparison of 3-O-methylglucose uptake rates and numbers of CBB sites in the plasma-membrane fraction of fat-cells

(a) Effects of BM 130795, BM 13907, TPA, insulin, vinblastine (VIN) and IP-oligosaccharides (IP) on 3-O-methylglucose uptake, compared with the basal value (Ba). The data are derived from the experiments shown in Figs. 1–3. (b) Numbers of CBB sites in the plasma membrane, determined in fat-cells treated with the substances shown in the Figure as described in the Materials and methods section. CBB sites were determined by derived Scatchard plots as described in the Materials and methods section and as described in detail by Mühlbacher *et al.* (1988). Mean values of 2–20 experiments are shown.

Effect on tyrosine kinase activity of the insulin receptor

It appears that the substances tested above consist of two sub-groups. In order to localize further the site of action of each group, we tested whether the insulin-like effects of these substances occur through stimulation of the insulin-receptor tyrosine kinase. Insulin stimulates the autophosphorylation of the receptor kinase approx. 10-fold *in vitro*, but there is no stimulation detectable in this assay for IP-oligosaccharides and BM 130795 (^{32}P incorporation, in c.p.m. over background: basal, 97 c.p.m.; 0.1 μM -insulin, 1010 c.p.m.; 27 μg of IP-oligosaccharides/ml, 105 c.p.m.; 10 nM-BM 130795, 93 c.p.m.; means for $n = 2$). Insulin (0.1 μM) given *in vivo* stimulated the ^{32}P incorporation *in vitro* into poly-(Glu-Tyr) from the basal value of 46×10^3 c.p.m. to 147×10^3 c.p.m. No significant stimulatory effect was found after preincubation with 10 nM-BM 130795 (48×10^3 c.p.m.) or 27 μg of IP-oligosaccharides/ml (56×10^3 c.p.m.; mean values for $n = 2$). This means that neither a substance of the translocation-stimulating group (BM) nor of the other group (IP-oligosaccharide fraction) activated the receptor kinase, suggesting that both groups interact with steps in signal transmission beyond the receptor-kinase level.

Combination of substances acting on glucose-carrier translocation with substances acting on glucose-carrier activity

The observation that neither substances of the translocation-stimulating group nor those of the carrier-activating group are able to mimic the full insulin effect suggests the possibility that the insulin effect occurs through an additive action on carrier translocation

and carrier activation. Therefore we tested whether substances acting on translocation and those acting on carrier activity have additive effects on the glucose-transport activity in fat-cells. The combination of TPA and the IP-oligosaccharide fraction showed an additive effect, whereas the combination of BM substances with IP-oligosaccharides was inhibitory. The reason for this inhibitory effect is not known; however, a direct drug interaction seems a good possibility. The additive effect of the combination of TPA and IP-oligosaccharides is shown in Fig. 5. The combination of both substances was significantly more active than either substance alone, and reached approx. 90% of insulin's maximal effect. This suggests that the insulin effect occurs through an additive action on glucose-carrier translocation and glucose-carrier activity.

DISCUSSION

Modified translocation model: two-step model

A modification of the original pure translocation model was previously suggested by several studies which showed discrepancies between glucose-carrier numbers in the plasma membrane and transport activity of the whole cell. Baly & Horuk (1986), using cycloheximide, and Joost *et al.* (1986), using isoprenaline, have shown a dissociation between glucose-transport activity and CBB. Karnieli *et al.* (1987) and Matthaie *et al.* (1987) observed that glucose-transport activity and carrier number do not correlate in type II diabetes, suggesting a modulation of an intrinsic glucose-transporter activity in this situation. Studies by Kahn & Cushman (1987) in fat-cells of streptozotocin-diabetic rats led to the same conclusion.

Fig. 5. Effect of a combination of phorbol ester and IP-oligosaccharides on 3-O-methylglucose uptake by fat-cells

Freshly isolated rat fat-cells $[(4.5-5.5) \times 10^6 \text{ cells/ml}]$ were incubated with $0.1 \mu\text{M}$ -insulin (Ins), $27 \mu\text{g}$ of IP-oligosaccharides/ml (IP), the phorbol ester 1 nM -TPA and the combination of IP-oligosaccharides ($27 \mu\text{g/ml}$) plus TPA (1 nM). For comparison the basal value (Ba) is depicted. Mean values $\pm$ S.E.M. for five experiments are shown.

Furthermore, a direct insulin effect on glucose-transport affinities was shown (Greco-Perotto *et al.*, 1987). Finally, our own results with phorbol esters and insulin were incompatible with an unmodified translocation model (Mühlbacher *et al.*, 1988). Our study had suggested that insulin itself can rapidly up-regulate the intrinsic carrier activity. This conclusion confirms some of the ideas from earlier work by Carter-Su & Czech (1980), who had originally proposed that insulin increases the glucose-transport activity of fat-cells exclusively by activating glucose carriers in the plasma membrane. Such an exclusive action of insulin on carrier activity was more recently newly proposed by Plough *et al.* (1987) for skeletal muscle. Our previous study with AlCl_3 and phospholipase C has shown that transport stimulation can indeed occur without carrier translocation (Obermaier-Kusser *et al.*, 1988); furthermore, that study suggested a possible role of a G-protein-dependent phospholipase for the regulation of carrier activity. The results of the present study are consistent with the previous data (Mühlbacher *et al.*, 1988; Obermaier-Kusser *et al.*, 1988).

A conclusive interpretation of these new data and our earlier results requires the assumption of a two-step model as outlined in the Introduction, consisting of translocation and carrier activation as independent, though synergistic, mechanisms.

Two distinct signal-transmitting chains at the post-kinase level

It is accepted that the first post-binding event in insulin action in fat-cells is the activation of the insulin-receptor tyrosine kinase (Kahn *et al.*, 1985), followed by

phosphorylation of substrates in the cell (White *et al.*, 1985; Häring *et al.*, 1987; Bernier *et al.*, 1987; Perrotti *et al.*, 1987; Machicao *et al.*, 1987). The further signal-transmitting steps linking the insulin-receptor kinase to the glucose-transport system are not understood in detail. The observation that none of the substances tested here activates the insulin-receptor kinase suggests that these substances act at the post-kinase level. As both groups of substances regulate selectively either carrier activity or carrier translocation, it may be concluded that two distinct and independent signal transmitting chains exist at the post-kinase level.

Signal transfer on carrier activity: possible role of IP-oligosaccharides

There is now some evidence that the signal-transmitting chain which regulates carrier activity might involve G-proteins (Obermaier-Kusser *et al.*, 1988; O'Brien *et al.*, 1987; Zick *et al.*, 1986) and activation of phospholipases (Obermaier-Kusser *et al.*, 1988; Fox *et al.*, 1987; Mato *et al.*, 1987; Koepfer-Hobelsberger & Wieland, 1984). Among potential signal-transmitting compounds generated by the activation of phospholipases are the cleavage products of phosphatidylinositol. Consistent with this concept and a recent publication (Standaert *et al.*, 1988), diacylglycerol stimulated glucose transport to the same extent as TPA, and we assume through the same mechanism as TPA. However, in contrast with this concept, the inositol phosphates had no clear stimulating effect. Therefore it is unlikely that phosphatidylinositol is a relevant phospholipase C substrate involved in regulation of carrier activity. On the other hand, the IP-oligosaccharide mixture is a strong stimulator, suggesting the possibility that a glycolipid is the relevant substrate of a phospholipase C involved in insulin action. A role of membrane glycolipids in insulin action was proposed by Saltiel and colleagues (Saltiel *et al.*, 1986; Fox *et al.*, 1987; Low & Saltiel, 1988) and others (Mato *et al.*, 1987; Alemany *et al.*, 1987). Alemany *et al.* (1987) have published that these polar substances can activate lipogenesis in intact cells, and it appears that an uptake mechanism exists for these compounds (Saltiel & Sorbara-Cazan, 1987). However, the putative second-messenger IP-oligosaccharides isolated from liver do not stimulate glucose transport (Kelly *et al.*, 1987). This is in contrast with our findings with the IP-oligosaccharides from Actovegin, and it suggests that different insulin effects involve different IP-glycans. A further separation of the compounds in our IP-oligosaccharide mixture will hopefully allow identification of this particular IP-oligosaccharide, and will then also allow identification of an analogous compound in the intact cell and a study of whether its cleavage indeed correlates with an insulin-induced modulation of the intrinsic carrier activity.

REFERENCES

- Alemany, S., Mato, J. M. & Strålfors, P. (1987) *Nature* (London) **330**, 77-79
- Baly, D. L. & Horuk, R. (1986) *J. Biol. Chem.* **262**, 21-24
- Bernier, M., Laird, D. M. & Lane, M. D. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 1844-1848
- Berridge, M. (1984) *Biochem. J.* **220**, 345-360
- Carter-Su, Ch. & Czech, M. P. (1980) *J. Biol. Chem.* **255**, 10382-10386

- Cushman, S. W. & Wardzala, L. J. (1980) *J. Biol. Chem.* **255**, 4758–4762
- Fox, J. A., Soliz, N. M. & Saltiel, A. R. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2663–2667
- Greco-Perotto, R., Assimacopoulos-Jeannet, F. & Jeanrenaud, B. (1987) *Biochem. J.* **247**, 63–68
- Häring, H. U., Biermann, E. & Kemmler, W. (1981) *Am. J. Physiol.* **240**, E556–E565
- Häring, H. U., Kirsch, D., Obermaier, B., Ermel, B. & Machicao, F. (1986) *J. Biol. Chem.* **261**, 3869–3875
- Häring, H., White, M. F., Machicao, F., Ermel, B., Schleicher, E. & Obermaier, B. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 113–117
- Joost, H. G., Weber, T. M., Cushman, S. W. & Simpson, I. A. (1986) *J. Biol. Chem.* **261**, 10017–10020
- Kahn, B. B. & Cushman, S. W. (1987) *J. Biol. Chem.* **262**, 5118–5124
- Kahn, C. R., White, M. F., Grigorescu, F., Takayama, S., Häring, H. U. & Crettaz, M. (1985) in *Molecular Basis of Insulin Action* (Czech, M., ed.), pp. 67–93, Plenum, New York
- Karnieli, E., Zarnowski, M. J., Hissin, P. J., Simpson, J. A., Salans, L. B. & Cushman, S. W. (1981) *J. Biol. Chem.* **256**, 4772–4777
- Karnieli, E., Armoni, M., Cohen, P., Kanter, Y. & Rafaeloff, R. (1987) *Diabetes* **36**, 925–931
- Kelly, C. L., Mato, J., Merida, I. & Jarrett, L. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6404–6407
- Klein, H., Freidenberg, G. R., Kladde, M. & Olefsky, J. M. (1986) *J. Biol. Chem.* **261**, 4691–4697
- Koepfer-Hobelsberger, B. & Wieland, O. H. (1984) *Mol. Cell. Endocrinol.* **36**, 123–129
- Kono, T., Suzuki, K., Dansey, L. E., Robinson, F. W. & Blewett, T. L. (1981) *J. Biol. Chem.* **256**, 6400–6407
- Low, M. G. & Saltiel, A. R. (1988) *Science* **239**, 268–275
- Machicao, F., Häring, H., White, M. F., Carrascosa, J. M., Obermaier, B. & Wieland, O. H. (1987) *Biochem. J.* **243**, 797–801
- Mato, J. M., Kelly, K. L., Abler, A. & Jarrett, L. (1987) *J. Biol. Chem.* **262**, 2131–2137
- Matthaei, St., Garvey, W. Th., Horuk, R., Hueckstaedt, Th. P. & Olefsky, J. M. (1987) *J. Clin. Invest.* **79**, 703–709
- McKeel, D. W. & Jarrett, L. (1970) *J. Cell Biol.* **44**, 417–432
- McPherson, G. A. (1985) *J. Pharmacol. Methods* **14**, 213–218
- Mühlbacher, C., Karnieli, E., Schaff, P., Obermaier, B., Mushack, J., Rattenhuber, E. & Häring, H. U. (1988) *Biochem. J.* **249**, 865–870
- Obermaier-Kusser, B., Mühlbacher, C., Mushack, J., Rattenhuber, E., Fehlmann, M. & Häring, H. U. (1988) *Biochem. J.* **256**, 515–520
- O'Brien, R. M., Houslay, M. D., Milligan, G. & Siddle, K. (1987) *FEBS Lett.* **212**, 281–288
- Olmsted, J. B. & Borisy, G. G. (1973) *Annu. Rev. Biochem.* **42**, 507–540
- Perrotti, N., Accili, D., Marcus-Samuels, B., Rees-Jones, R. W. & Taylor, S. I. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3137–3140
- Plough, T., Galbo, H. & Vinten, J. (1987) *Diabetologia* **29**, 570A–571A
- Saltiel, A. R. & Cuatrecasas, P. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5793–5797
- Saltiel, A. R. & Sorbara-Cazan, L. R. (1987) *Biochem. Biophys. Res. Commun.* **149**, 1084–1092
- Simpson, J. A. & Cushman, S. W. (1985) in *Molecular Basis of Insulin Action* (Czech, M., ed.), pp. 399–422, Plenum, New York
- Standaert, M. L., Farese, R. V., Cooper, D. R. & Pollet, R. J. (1988) *J. Biol. Chem.* **263**, 8696–8705
- Suzuki, K. & Kono, T. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2542–2545
- Wardzala, L. J., Cushman, S. W. & Salans, L. B. (1978) *J. Biol. Chem.* **253**, 8002–8005
- White, M. F., Maron, R. & Kahn, C. R. (1985) *Nature (London)* **318**, 183–186
- Zick, Y., Sagi-Eisenberg, R., Pines, M., Gierschik, P. & Spiegel, A. M. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 9294–9297

Received 11 August 1988/3 January 1989; accepted 22 March 1989