Insulin-stimulated glucose transport in muscle

Evidence for a protein-kinase-C-dependent component which is unaltered in insulin-resistant mice

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The aim of our work was to investigate a possible role of protein kinase C (PKC) in insulin-stimulated glucose uptake in mouse skeletal muscle, and to search for a defect in PKC activation in insulin resistance found in obesity. In isolated soleus muscle of lean mice, insulin (100 nM) and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (1 μ M) acutely stimulated glucose uptake 3- and 2-fold respectively. The effects of insulin and TPA were not additive. When PKC activity was down-regulated by long-term (24 h) TPA pretreatment, before measurement of glucose transport, the TPA effect was abolished, but in addition insulin-stimulated glucose transport returned to basal values. Furthermore, polymyxin B, which inhibits PKC in muscle extracts, prevented insulin-stimulated glucose uptake in muscle. In muscle of obese insulin-resistant mice, glucose uptake evoked by insulin was decreased, whereas the TPA effect, expressed as a fold increase, was unaltered. Thus both agents stimulated glucose transport to the same extent. Furthermore, no difference was observed when PKC activation by TPA was measured in muscle from lean and obese mice. These results suggest that : (1) PKC is involved in the insulin effect on glucose transport in muscle; (2) PKC activation explains only part of the insulin stimulation of glucose transport; (3) the defect in insulin response in obese mice does not appear to be due to an alteration in the PKC-dependent component of glucose transport. We propose that insulin stimulation of glucose uptake occurs by a sequential two-step mechanism, with first translocation of transporters to the plasma membrane, which is PKC dependent, and second, activation of the glucose transporters. In obesity only the activation step was decreased, whereas the translocation step was unaltered.

INTRODUCTION

Insulin binding to its receptor initiates a large array of cellular events, among them stimulation of glucose uptake in adipocytes and muscle. Currently, the molecular steps between the hormone binding and the increase in glucose uptake are not totally elucidated (Rosen, 1987; Gammeltoft & Van Obberghen, 1986). Activation of the receptor tyrosine kinase activity plays a key role in this process, since cells transfected with insulin receptors, lacking kinase activity owing to mutagenesis of the ATP-binding site, are unable to respond to insulin (Chou *et al.*, 1987; Ebina *et al.*, 1987). Likewise, modifications of insulin-stimulated glucose transport are associated with parallel alterations in insulin-receptor tyrosine kinase activity (Debant *et al.*, 1987; Freidenberg *et al.*, 1987; Le Marchand-Brustel *et al.*, 1985). However, the intermediary steps between activation of insulin-receptor tyrosine kinase activity and stimulation of glucose transport are entirely unknown.

Phorbol esters, which bind to and activate a Ca^{2+} - and phospholipid-dependent kinase (protein kinase C, PKC), exert insulin-like effects on glucose and amino acid transport, lipogenesis from glucose, and activation of pyruvate dehydrogenase (Cherqui *et al.*, 1986; Farese *et al.*, 1985; Kirsch *et al.*, 1985; Van de Werve *et al.*, 1985). PKC activation could thus be an important step in transducing part of the insulin signal. Several lines of

evidence have suggested that the phosphatidylinositol cycle could be the link between insulin-receptor kinase and activation of glucose uptake (Kirsch *et al.*, 1985; Van de Werve *et al.*, 1985). For instance, insulin is able to activate phospholipase C in isolated rat adipocytes (Köpfer-Hobelsberger & Wieland, 1984). In BC3H-1 myocytes, insulin increases both diacylglycerol content, via synthesis *de novo* of phosphatidic acid and phosphoinositides, and PKC activity (Farese *et al.*, 1985; Cooper *et al.*, 1987). Hearts of obese *fa/fa* rats are both insulin- and TPA-resistant for glucose uptake, and exhibit a total unresponsiveness to TPA-induced protein kinase C translocation (Van de Werve *et al.*, 1987). By contrast, some reports have shown that PKC depletion induced by long-term exposure to TPA had no effect on insulin-stimulated glucose uptake (Klip & Ramlal, 1987).

In the present work we have looked for the involvement of PKC in the effect of insulin on glucose transport in skeletal muscle, a major site of glucose utilization. We then investigated whether an alteration in this pathway could explain, in part, the insulin-resistance of glucose uptake in skeletal muscle of obese mice. To answer these questions, we studied TPA-stimulated glucose uptake and PKC activation in soleus muscle of mice rendered obese and insulin-resistant by gold–thioglucose injection and which display decreased insulin-receptor kinase activity and glucose transport (Le Marchand *et al.*, 1978; Le Marchand-Brustel *et al.*, 1978, 1985).

MATERIALS AND METHODS

Materials

Radioactive substances were from Amersham International, Amersham, Bucks., U.K. DEAE-cellulose DE52 was from Whatman. Phospholipids, histones, TPA, polymyxins, phorbol esters and other chemicals were from Serva or Sigma (St. Louis, MO, U.S.A.). TPA was dissolved in ethanol, and equal volumes of solvent were added to control and insulin-treated muscles.

Animals

Male Swiss albino mice (7–8 weeks old) were given laboratory chow *ad libitum* (Usine d'Alimentation Rationnelle, Villemoisson, Epinay/Orge, France) and maintained at 23 °C on a 12 h-light cycle until the time of experiment. Mice were rendered obese by gold-thiogluco- injection at 3 weeks of age and were used at 25–30 weeks, when obesity had reached a plateau (Le Marchand *et al.*, 1978). In experiments with obese mice, age-matched lean control animals were used.

Soleus-muscle incubation and measurement of 2-deoxyglucose uptake

Soleus muscles from lean and obese mice were isolated as previously described (Le Marchand-Brustel *et al.*, 1978), and preincubated for 15 min at 37 °C in Krebs–Ringer bicarbonate buffer, pH 7.4, containing defatted bovine serum albumin (10 mg/ml) and 2 mM-sodium pyruvate. Muscles were then incubated for 30 min in the absence or the presence of insulin (0.5–100 nM), TPA (1 μ M) or polymyxins. 2-Deoxy-D-[1-¹⁴C]-glucose (0.5 mM, 0.1 μ Ci) was added for 15 min in the same medium. Uptake was terminated by washing the muscles for 30 min in ice-cold iso-osmotic NaCl solution, and the muscles were dissolved in 1 M-NaOH. Radioactivity of samples was counted and protein concentration was determined (Lowry *et al.*, 1951). Results are expressed as means $\pm$ S.E.M. for the numbers of muscles indicated in legends to Figures or Tables.

Down-regulation of PKC

Isolated soleus muscles of lean mice were incubated for 24 h at 37 °C in Krebs–Ringer bicarbonate buffer containing defatted serum albumin (10 mg/ml), 15 mM-glucose, bacitracin (1 mg/ml) and gentamycin (50 μ g/ml) with or without 1 μ M-TPA. After this incubation, to remove phorbol ester, muscles were washed for 3 $\times$ 20 min with Krebs–Ringer bicarbonate buffer containing 1% defatted bovine serum albumin and 2 mM-pyruvate before measurement of 2-deoxyglucose transport as described above.

Determination of PKC activity

To measure PKC activation by TPA in obese or lean mice, soleus muscles were incubated in Krebs–Ringer bicarbonate buffer without or with TPA (1 μ M) as described above. Thereafter, each muscle was homogenized by brief sonication in 500 μ l of ice-cold 20 mM-Tris/HCl buffer (pH 7.4) containing 0.33 M-sucrose, 0.5 mM-EGTA, 2 mM-EDTA and leupeptin (25 μ g/ml). The homogenate was centrifuged for 20 min at 15000 g. The supernatant will be termed 'cytosolic fraction'. In this fraction, protein kinase activity was measured as described by Klip & Ramlal (1987) with minor modifications. Briefly, subcellular fractions were passed through

a 0.5 ml DE52 (DEAE-cellulose) column to remove endogenous lipids, diacylglycerol and kinase inhibitors. Columns were washed with 5 vol. of 20 mM Tris/HCl buffer (pH 7.4) / 2 mM-EDTA / 0.5 mM-EGTA / 2 mM-phenylmethanesulphonyl fluoride and eluted with 1.5 ml of the same buffer supplemented with 100 mM-NaCl.

PKC activity was measured with histone type IIIS (Sigma) as substrate. Assays were performed for 30 min at 20 °C with 100 μ l of each fraction in a final volume of 250 μ l of 20 mM-Tris/HCl buffer (pH 7.5) containing [γ -³²P]ATP (5 μ Ci; 50 μ M), 10 mM-magnesium acetate, 0.75 mM-CaCl₂ and 25 μ g of histone IIIS. At the end of the incubation, samples were adsorbed on phosphocellulose P81 paper, and extensively washed in 1% H₃PO₄ solution. PKC activity was measured in the absence (basal kinase activity) or the presence of 24 μ g of phosphatidylserine and 1.6 μ g of 1,2-diolein. PKC activity was calculated by subtracting basal activity from phosphatidylserine/diolein-stimulated activity (basal activity was 10–20% of stimulated activity and was not changed when muscles were incubated without or with TPA).

RESULTS

Effect of insulin and TPA on 2-deoxyglucose transport in soleus muscle of lean mice

To search for a role of PKC in stimulation of glucose transport, soleus muscles were incubated with insulin and TPA for 30 min before measurement of deoxyglucose uptake. As shown in Fig. 1, insulin (100 nM) stimulated deoxyglucose uptake by 3-fold, half-maximal effect being obtained at 0.5 nM. The phorbol ester TPA (1 μ M) increased this uptake by 1.7-fold, and was thus as efficient as a half-maximal insulin concentration. For the concentrations of insulin used (0.5 or 100 nM), the effects of insulin and TPA were not additive.

Fig. 1. Insulin and TPA stimulation of glucose transport in soleus muscles of lean mice

Soleus muscles were incubated for 30 min in Krebs–Ringer bicarbonate buffer, containing 1% albumin and 2 mM-pyruvate, in the presence of insulin or TPA as indicated. Deoxyglucose uptake was then measured during a 15 min incubation period. Values are means $\pm$ S.E.M. for eight muscles.

Table 1. Effect of PKC down-regulation on insulin and TPA stimulation of glucose transport

Soleus muscles were preincubated for 24 h in Krebs–Ringer bicarbonate buffer, containing 1 % bovine serum albumin, 15 mM-glucose, bacitracin (1 mg/ml) and gentamycin (50 µg/ml), without or with TPA (1 µM). After washes, deoxyglucose uptake was measured without (basal) or with insulin or TPA as indicated. Values are means $\pm$ S.E.M. of 8–11 different muscles. Values statistically different from basal are shown by * $P < 0.05$ or ** $P < 0.005$

Preincubation	Deoxyglucose uptake (nmol/15 min per mg of protein)	
	Without TPA	With TPA
Incubation		
Basal	2.85 $\pm$ 0.20	2.70 $\pm$ 0.20
Insulin (50 nM)	4.85 $\pm$ 0.47**	2.95 $\pm$ 0.22
TPA (1 µM)	3.71 $\pm$ 0.30*	2.95 $\pm$ 0.17

Effect of PKC depletion on glucose uptake

To look more directly for a role of PKC in glucose transport, muscles were preincubated for 24 h with TPA (1 µM). As described in different cell types, this treatment induced down-regulation of PKC in muscles also. Indeed, PKC activity was 23.1 $\pm$ 2.8 and 2.8 $\pm$ 1.0 pmol/assay (means $\pm$ S.E.M. for five muscles preincubated without or with TPA respectively). We then measured 2-deoxyglucose uptake in the presence of insulin and TPA, after extensive washes to remove the phorbol ester. As shown in Table 1, basal deoxyglucose uptake was similar whether the 24 h preincubation was performed in the absence or the presence of TPA. Consistent with a down-regulation of PKC, preincubation with the TPA rendered the muscle unresponsive to this agent. Moreover, stimulation of glucose uptake by insulin was also abolished after this PKC down-regulation.

Effect of polymyxin B on deoxyglucose uptake in soleus muscles

To substantiate further a role for PKC in insulin-stimulated glucose uptake, we studied the effect of polymyxin B, an inhibitor of PKC (Mazzei *et al.*, 1982; Wise *et al.*, 1982). As shown in Table 2, polymyxin B abolished PKC activity when added to DEAE-cellulose eluates obtained from soleus-muscle homogenates, whereas polymyxin E, which differs from polymyxin B by only one amino acid residue, was completely ineffective. Furthermore, when polymyxin B was added to isolated soleus muscles, it caused a concentration-dependent inhibition of insulin-stimulated glucose uptake, whereas basal uptake was unchanged (Fig. 2). By contrast, polymyxin E did not affect this insulin effect (Grémeaux *et al.*, 1987). Higher concentrations of polymyxin B were required in intact muscle compared with acellular systems, a phenomenon which could be accounted for by the necessity for polymyxin to diffuse in the intact tissue.

Effect of insulin and TPA on glucose uptake in soleus muscles of obese mice

To investigate whether a PKC defect is implicated in insulin resistance associated with obesity, we have

Table 2. Inhibitory effect of polymyxin B on PKC activity

Soleus muscles were solubilized and extracts were used to measure PKC activity as described in the Materials and methods section. Results are expressed as the phospholipid–Ca²⁺-dependent kinase activity towards histones. Polymyxins were added during the assay at the indicated concentrations. Values are means of duplicate determinations.

Addition (µg/ml)	PKC activity (pmol of ³² P incorporated)	
	Polymyxin B	Polymyxin E
0	10.2	10.2
25	7.9	12.7
50	0.6	12.3
100	0.3	14.6
200	0.2	11.8

Fig. 2. Concentration-dependence of polymyxin B effect on glucose transport in soleus muscles of lean mice

Soleus muscles were incubated as described in Fig. 1, without (□) or with (▨) insulin and polymyxin B (PMB) at the concentration indicated. Deoxyglucose uptake was measured at the end of a 15 min incubation period. Values are means $\pm$ S.E.M. for four muscles.

analysed the effects of TPA in muscle of mice rendered obese by gold–thioglucose injection. These mice are markedly obese and insulin-resistant (Le Marchand *et al.*, 1978). As shown in Fig. 3 and as previously documented (Le Marchand-Brustel *et al.*, 1978; Cuendet *et al.*, 1976), in muscle of obese mice basal deoxyglucose uptake was slightly, but significantly, decreased compared with control mice ($P < 0.025$). This alteration of glucose uptake in basal conditions makes the interpretation of stimulatory effects of insulin or TPA more complex. Thus muscles from obese mice were markedly resistant to insulin whether results of insulin stimulation were expressed as a fold increase (1.8-fold, compared with 2.7-fold in lean mice) or in absolute values (Fig. 3). This is different from what was observed with TPA: in absolute terms, TPA-stimulated uptake was lower in obese than in lean mice, but, when expressed as a fold stimulation, TPA stimulation was as efficient in obese- as in lean-mouse muscles (1.7-fold compared with 1.9-fold). Furthermore, in obese-mouse muscles the TPA

Fig. 3. Insulin and TPA stimulation of glucose transport in muscles of lean and obese mice

Muscles from lean mice or mice rendered obese by gold-thioglucose injection were incubated as described in Fig. 1, in the absence (□) or presence of insulin (50 nM; ▨) or TPA (1 μ M; ■), before the measurement of deoxyglucose uptake. Values are means $\pm$ S.E.M. for 26 (lean) or 18 (obese) muscles. Differences between basal values in lean and obese muscles are significant at $P < 0.025$.

Table 3. TPA-induced disappearance of cytosolic PKC activity in soleus muscles from lean and obese mice

Muscles from lean mice or mice rendered obese by gold-thioglucose injection were incubated as described in Fig. 1, in the absence or presence of TPA (1 μ M). Muscles were then homogenized and the cytosolic fraction was used to measure PKC activity as described in the Materials and methods section. Values are means $\pm$ S.E.M. for 16 lean or obese muscles.

Mice ...	PKC activity (pmol of 32 P incorporated/assay)	
	Lean	Obese
Without TPA	22.8 $\pm$ 0.9	23.2 $\pm$ 1.4
With TPA	13.8 $\pm$ 0.8	14.2 $\pm$ 0.5

effect was similar to the stimulation obtained with a maximally effective insulin concentration, whereas in lean mice the TPA effect was only 50 % of the maximal insulin effect.

TPA activation of PKC in lean and obese mice

In basal conditions, PKC is located mostly in the cytosolic fraction. Under TPA stimulation, the enzyme is transferred to the plasma membrane, and the activity disappears from the cytosol. This translocation process has been shown to be linked to activation of the enzyme (Nishizuka, 1986). As shown in Table 3, in basal conditions, in the absence of TPA, PKC activity was identical in the cytosolic fraction of lean- and obese-mouse muscles (no differences were observed in the Ca^{2+} /phospholipid-

independent kinase activities; results not shown). After TPA treatment, PKC activity decreased by 40 % in the cytosolic fraction, in both lean and obese mice. Those results show that in skeletal muscles of obese mice TPA-induced PKC translocation was unaltered.

DISCUSSION

Previous studies have suggested that the phosphatidylinositol cycle might participate in insulin action through activation of PKC. This conclusion was supported by the observation that phorbol esters, which activate PKC, exert insulin-like effects in adipocytes, myocytes and heart muscle (Farese *et al.*, 1985; Kirsch *et al.*, 1985; Van de Werve *et al.*, 1985). In the present work, we have studied TPA effects on glucose transport in isolated soleus muscle. Indeed, skeletal muscle is the major site of glucose utilization *in vivo*, and an important target tissue for insulin action. The phorbol ester TPA was able to increase glucose transport by 1.5-fold in soleus muscle, an effect that was 50 % of the maximal insulin effect. This result differs from those reported by Sowell *et al.* (1988), who did not observe an effect of phorbol esters in rat skeletal muscle, the reason for this discrepancy being at present unclear. Our data suggest that activation of PKC could be a regulator of glucose uptake in skeletal muscle. To search for a role of this enzyme in insulin stimulation of glucose uptake in muscle, the hormone effect has been studied under two conditions: (1) after down-regulation of PKC by long-term preincubation with TPA; (2) in the presence of polymyxin B, which inhibits PKC activity (Mazzei *et al.*, 1982; Wise *et al.*, 1982; Table 2). In both cases, insulin-stimulated deoxyglucose uptake was markedly decreased, but basal uptake was unaffected. Although polymyxin B or TPA might have multiple actions, these results point to a role of PKC in the effect of insulin on glucose transport in muscle.

In muscle, as in most previously studied systems (Kirsch *et al.*, 1985; Van de Werve *et al.*, 1985; Mühlbacher *et al.*, 1988), TPA, which maximally activates PKC, is only able to elicit part of the full insulin response. This suggests that the stimulatory action of insulin on glucose uptake involved not only a PKC-dependent mechanism, but also an additional component which cannot be mimicked by PKC activators. Stimulation of glucose transport by both insulin and TPA is thought to be due to a recruitment of glucose carriers from an intracellular pool to the plasma membrane (Mühlbacher *et al.*, 1988; Kitagawa *et al.*, 1985; Wardzala & Jeanrenaud, 1981; Cushman & Wardzala, 1980). However, although these compounds produce the same increase in plasma-membrane carrier sites, the magnitude of the insulin effect on glucose-transport activity surpasses the effect of TPA. Furthermore, the effect of insulin on the translocation of glucose transporters cannot account quantitatively for the complete insulin-induced glucose transport. To explain the discrepancy between the magnitude of the insulin effect on the increase in glucose transporters in the plasma membrane and on glucose uptake, it has been proposed that in adipocytes or in heart muscle insulin activates glucose carriers after their translocation (Baly & Horuk, 1987; Greco-Perotto *et al.*, 1987; Mühlbacher *et al.*, 1988; Zaninetti *et al.*, 1988).

On the basis of this concept, we speculate that insulin stimulation of glucose transport in skeletal muscle occurs

by a sequential two-step mechanism. In a first step, glucose transporters are translocated from the intracellular pool by a process which appears to be PKC-dependent, and is therefore mimicked by TPA and abolished by PKC inhibitors. After this translocation, glucose transporters would be activated by insulin. Blockade of the initial translocation step, for instance by down-regulation of PKC or by addition of PKC inhibitor (i.e. polymyxin B), would lead to suppression of insulin stimulation of glucose transport. The involvement of PKC would be restricted to some effects of insulin such as glucose uptake, since this enzyme does not seem to be involved in long-term responses such as stimulation of ornithine decarboxylase mRNA amounts (Blackshear *et al.*, 1987). Likewise, polymyxin B blocks the insulin action solely on membrane transport without affecting hormonal activation of glycogen synthase or insulin's antilipolytic effects (Grémeaux *et al.*, 1987; Amir *et al.*, 1987).

We then looked for the PKC functioning in muscles of obese insulin-resistant mice. As previously reported, basal glucose uptake was slightly but significantly decreased in muscle from obese mice compared with lean animals, a decrease which can be accounted for by a lower number of glucose transporters, as described in heart muscle of obese Zucker rats (Zaninetti *et al.*, 1987) or in adipocytes of obese patients (Garvey *et al.*, 1988). Insulin stimulation was also markedly altered in muscle of obese mice. On the contrary, there was no alteration in the TPA response, when expressed as a fold increase over basal. Furthermore, in contrast with what we found in lean mice, TPA was able to mimic fully the insulin effect in obese mice, suggesting that the muscles are not as resistant to TPA as they are to insulin. The diminution of the total number of glucose transporters could totally explain the lower absolute value of TPA-stimulated glucose uptake in muscles of obese animals compared with lean animals. This is consistent with a normal PKC activation by TPA in muscles of obese animals. (It should be noted that we could not detect any effect of insulin on PKC translocation in muscles of lean or obese mice.) Our results are at variance with those reported in the heart muscle of obese Zucker rats, where tumour-promoting phorbol esters were unable to stimulate glucose transport and/or translocate PKC (Van de Werve *et al.*, 1987). Whether this discrepancy is due to the different obesity model (genetically obese hyperlipidaemic rats, compared with normal mice rendered obese by hyperphagia) or to a difference in tissue (heart compared with skeletal muscle) is not clear at the present.

Taken together, our results suggest that the defect in insulin-stimulated glucose transport in obese mice is not due to an alteration in the PKC-dependent component. We propose that in muscles from obese mice the glucose-transporter translocation induced by insulin or TPA is normal, but that the subsequent activation step is defective. Thus the regulation of these two processes appears to be different. The decrease in insulin-stimulated glucose transport would result first, from a decreased number of glucose transporters, and second, from a defect in their activation once translocated to the plasma membrane. It is tempting to speculate that the activation of glucose transporters by insulin is a consequence of the insulin-receptor kinase action, a process which is also altered in obese insulin-resistant states (Freidenberg *et al.*, 1987; Le Marchand-Brustel *et al.*, 1985). Although it

is clear that this receptor enzymic function is crucial for insulin-stimulated glucose transport (Chou *et al.*, 1987; Ebina *et al.*, 1987), the cascade of events leading to the increase in glucose transport is still a matter of speculation. So far, it has not been possible to correlate a change in the phosphorylation state of the glucose transporter and a modification of its activity (Gibbs *et al.*, 1986; Kohanski *et al.*, 1986).

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REFERENCES

- Amir, S., Sasson, S., Kaiser, N., Meyerovitch, J. & Shechter, Y. (1987) *J. Biol. Chem.* **262**, 6663–6667
- Baly, D. & Horuk, H. (1987) *J. Biol. Chem.* **262**, 21–24
- Blackshear, P. J., Nemenoff, R. A., Hovis, J. G., Halsey, D. L., Stumpo, D. J. & Huang, J. K. (1987) *Mol. Endocrinol.* **1**, 44–52
- Cherqui, G., Caron, M., Wicek, D., Lascols, O., Capeau, J. & Picard, J. (1986) *Endocrinology (Baltimore)* **118**, 1759–1769
- Chou, C. K., Dull, T. J., Russell, D. S., Gherzi, R., Lebwohl, D., Ullrich, A. & Rosen, O. M. (1987) *J. Biol. Chem.* **262**, 1842–1847
- Cooper, D. R., Konda, T. S., Standaert, M. L., Davis, J. S., Pollet, R. J. & Farese, R. V. (1987) *J. Biol. Chem.* **262**, 3633–3639
- Cuendet, G. S., Loten, E. G., Jeanrenaud, B. & Renold, A. E. (1976) *J. Clin. Invest.* **58**, 1078–1088
- Cushman, S. W. & Wardzala, L. J. (1980) *J. Biol. Chem.* **255**, 4758–4762
- Debant, A., Guerre-Millo, M., Le Marchand-Brustel, Y., Freychet, P., Lavau, M. & Van Obberghen, E. (1987) *Am. J. Physiol.* **252**, E273–E278
- Ebina, Y., Araki, E., Taira, M., Shimada, F., Masataka, M., Craik, C. S., Siddle, K., Pierce, S. B., Roth, R. A. & Rutter, W. J. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 704–708
- Farese, R. V., Davis, J. S., Barnes, D. E., Standaert, M. L., Babischkin, J. S., Hock, R., Rosic, N. K. & Pollet, R. J. (1985) *Biochem. J.* **231**, 269–278
- Freidenberg, G. R., Henry, R. R., Klein, H. H., Reichart, D. R. & Olefsky, J. M. (1987) *J. Clin. Invest.* **79**, 240–250
- Gammeltoft, S. & Van Obberghen, E. (1986) *Biochem. J.* **235**, 1–11
- Garvey, W. T., Huecksteadt, T. P., Matthaei, S. & Olefsky, J. M. (1988) *J. Clin. Invest.* **81**, 1528–1536
- Gibbs, E. M., Allard, W. J. & Lienhard, G. E. (1986) *J. Biol. Chem.* **261**, 16597–16603
- Greco-Perotto, R., Assimacopoulos-Jeannet, F. & Jeanrenaud, B. (1987) *Biochem. J.* **247**, 63–68
- Grémeaux, T., Tanti, J. F., Van Obberghen, E. & Le Marchand-Brustel, Y. (1987) *Am. J. Physiol.* **252**, E248–E254
- Kirsch, D., Obermaier, B. & Häring, H. U. (1985) *Biochem. Biophys. Res. Commun.* **128**, 824–832
- Kitagawa, K., Nishino, H. & Iwashima, A. (1985) *Biochem. Biophys. Res. Commun.* **128**, 1303–1309
- Klip, A. & Ramlal, T. (1987) *Biochem. J.* **242**, 131–136
- Kohanski, R. A., Frost, C. & Lane, M. D. (1986) *J. Biol. Chem.* **261**, 12272–12281
- Köpfer-Hobelsberger, B. & Wieland, O. H. (1984) *Mol. Cell. Endocrinol.* **36**, 123–129

- Le Marchand, Y., Freychet, P. & Jeanrenaud, B. (1978) *Endocrinology* (Baltimore) **102**, 74–85
- Le Marchand-Brustel, Y., Jeanrenaud, B. & Freychet, P. (1978) *Am. J. Physiol.* **234**, E348–E358
- Le Marchand-Brustel, Y., Grémeaux, T., Ballotti, R. & Van Obberghen, E. (1985) *Nature* (London) **315**, 676–679
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mazzei, G. J., Katoh, N. & Kuo, J. F. (1982) *Biochem. Biophys. Res. Commun.* **109**, 1129–1133
- Mühlbacher, C., Karnieli, E., Schaff, P., Obermaier, B., Mushack, J., Rattenhuber, E. & Häring, H. U. (1988) *Biochem. J.* **249**, 865–870
- Nishizuka, Y. (1986) *Science* **233**, 305–312
- Rosen, O. M. (1987) *Science* **237**, 1452–1458
- Sowell, M. O., Treutelaar, M. K., Burant, C. F. & Buse, M. G. (1988) *Diabetes* **37**, 499–506
- Van de Werve, G., Proietto, J. & Jeanrenaud, B. (1985) *Biochem. J.* **225**, 523–527
- Van de Werve, G., Zaninetti, D., Lang, U., Vallotton, M. B. & Jeanrenaud, B. (1987) *Diabetes* **36**, 310–314
- Wardzala, L. J. & Jeanrenaud, B. (1981) *J. Biol. Chem.* **256**, 7090–7093
- Wise, B. C., Glass, D. B., Chou, C. H. J., Raynor, R. L., Katoh, N., Schatzman, R. C., Turner, R. S., Kibler, R. F. & Kuo, J. F. (1982) *J. Biol. Chem.* **257**, 8489–8495
- Zaninetti, D., Greco-Perotto, R., Assimacopoulos-Jeannet, F. & Jeanrenaud, B. (1987) *Lessons from Animal Diabetes* **2**, 247 (abstr.)
- Zaninetti, D., Greco-Perotto, R., Assimacopoulos-Jeannet, F. & Jeanrenaud, B. (1988) *Biochem. J.* **250**, 277–283

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