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Direct interaction of phenylarsine oxide with hexose transporters in isolated rat adipocytes

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It has previously been shown that phenylarsine oxide (PhAsO), an inhibitor of protein internalization, also inhibits stereospecific uptake of D-glucose and 2-deoxyglucose in both basal and insulin-stimulated rat adipocytes. This inhibition of hexose uptake was found to be dose-dependent. PhAsO rapidly inhibited sugar transport into insulin-stimulated adipocytes, but at low concentrations inhibition was transient. Low doses of PhAsO (1 μM) transiently inhibit stereospecific hexose uptake and near total (approx. 90%) recovery of transport activity occurs within 20 min. Interestingly, once recovered, the adipocytes can again undergo rapid inhibition and recovery of transport activity upon further treatment with PhAsO (1 μM). In addition, PhAsO is shown to inhibit cytochalasin B binding to plasma membranes from insulin-stimulated adipocytes in a concentration-dependent manner which parallels the dose-response inhibition of hexose transport by PhAsO. The data presented suggest a direct interaction between the D-glucose transporter and PhAsO, resulting in inhibition of transport. The results are consistent with the current recruitment hypothesis of insulin activation of sugar transport and indicate that a considerable reserve of intracellular glucose carriers exists within fat cells.

Introduction

Sulphydryl groups have structural and functional importance both in the insulin receptor [1–4] and the D-glucose transporter [5–7]. Furthermore, it has been proposed that thiol/disulphide groups on the cell surface and those immediately inside the cell membrane may be critical in the mediation of insulin action [8].

Phenylarsine oxide (PhAsO), an agent which reacts with vicinal disulphide groups to form stable cyclic complexes [9] and is known to be a potent inhibitor of protein internalization [10] has been found to inhibit hexose transport in both 3T3-L1 cultured adipocytes [11,12] and isolated rat adipocytes [13–15]. However, despite the fact that PhAsO is capable of reacting with sulphydryl groups, 100 μM PhAsO, a concentration 10-times higher than that thought to effectively inhibit all protein incorporation [10], did not affect the cellular ATP content in alveolar macrophages [16] and human astrocytoma cells [17], while only a small (10%) decrease in cellular ATP was observed in 3T3-L1 adipocytes using 18 μM PhAsO [11].

Abbreviation: PhAsO, phenylarsine oxide.

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Previously, we observed that the effects of PhAsO on D-glucose and 2-deoxyglucose uptake in rat adipocytes were analogous and that the arsenical did not affect hexokinase action [15]. Hence, the effect of PhAsO seems unlikely to be explained on the basis of inhibition of energy metabolism. Indeed, it has been suggested that the plasma membrane which is exposed first to the highest concentration of the arsenical is probably the main site of its action [10].

The realization that hexose transport inhibition by PhAsO can in part be reversed by bifunctional sulphydryl reducing agents but not monofunctional sulphydryl agents [11,13], indicating that vicinal sulphydryl groups are critical in the hexose transport process. Although PhAsO acts rapidly (within seconds) to prevent insulin-stimulation of transport, this compound does not inhibit insulin binding to its receptor at concentrations where significant inhibition of hexose transport occurs [18,15]. Additionally, PhAsO does not impair insulin-induced autophosphorylation of the receptor β-subunit, but appears to induce accumulation of a phosphorylated 15 kDa cytosolic protein. Bernier et al. [12] have suggested that accumulation of this protein by PhAsO may account for hexose uptake inhibition in 3T3-L1 adipocytes.

Although PhAsO did not inhibit basal transport activity in 3T3-L1 adipocytes, our previous studies demonstrated PhAsO-induced inhibition of sugar transport in basal state isolated rat adipocytes [13–15] and indicated that PhAsO may be acting directly on the hexose carrier to impair its function. We attempt here to characterise further this inhibitory effect of PhAsO on glucose uptake and demonstrate that PhAsO inhibits cytochalasin B binding to isolated rat adipocyte plasma membranes in a concentration-dependent fashion which closely parallels the dose-response inhibition of sugar transport.

Materials and Methods

Materials. Labelled L-[U-^{14}C]glucose and [4(n)-^{3}H] cytochalasin B were obtained from Amersham International (Amersham, Buckinghamshire, U.K.); L-[1-^{3}H]glucose was obtained from New England Nuclear (Dreieich, F.R.G.). Bovine insulin, Hepes, PhAsO and cytochalasin B were obtained from Sigma (Poole, Dorset, U.K.). Crude bacterial collagenase (batch nos. 14443357 and 12O4458) was obtained from Boehringer (Lewes, East Sussex, U.K.). Fatty acid-free bovine serum albumin (fraction V) was obtained from both Sigma and Boehringer. All other chemicals were obtained from B.D.H. Chemicals (Poole, U.K.) and were of analytical grade.

Preparation of adipocytes. Adipocytes were prepared by collagenase digestion of epididymal and perirenal fat pads as described by Rodbell [19]. All steps were carried out at 37°C and plastic vessels were used throughout. Routinely, 3 or 4 male Sprague-Dawley rats of approx. 150–180 g were used. The fat was excised, chopped with scissors and put into a freshly made Krebs-Henseleit buffer (pH 7.4) (120 mM NaCl/4.8 mM KCl/1.2 mM KH_2PO_4/1.2 mM $MgSO_4$/24 mM $NaHCO_3$/2.5 mM $CaCl_2$) which contained 1% bovine serum albumin and glucose (2 mM) [20].

D-Glucose uptake measurements. Stereospecific carrier-mediated uptake of D-glucose was taken to be the difference in the amounts of D- and L-glucose taken up by adipocytes as previously described [15]. All assays were performed in Eppendorf tubes preincubated at 37°C. Adipocytes were maintained throughout all incubations and assays at the required temperature. In experiments using PhAsO inhibitor, the stock PhAsO solution (1 mM) contained 1.7% (v/v) ethanol.

Adipocytes were separated from the medium by an oil-flotation technique as described by Gliemann et al. [21]. Dinonyl phthalate (0.3 ml) was layered over the stopping buffer and the tube was centrifuged in an Eppendorf microfuge (12000 × *g*) for 40 s. Radioactivity was determined using a Beckman LS9800 liquid scintillation counter.

Preparation of plasma membranes. The method employed was a modification of that used by Simpson et al. [22]. Adipocytes were prepared from 20–25 male Sprague-Dawley rats (200–250 g) as described above. The adipocytes were divided equally into two portions and one of these was stimulated with insulin (100 nM for 20 min at 37°C). The cells from both basal and insulin-stimulated states were then subjected to the following steps. Adipocytes were washed twice with homogenizing buffer at 17°C (10 mM Hepes/NaOH; 2 mM EDTA; 250 mM sucrose

(pH 7.4)), resuspended in approx. 20 ml of this same buffer and homogenized at room temperature in a glass homogenzing tube fitted with a Teflon pestle (seven strokes). The homogenate was cooled on ice and all subsequent operations carried out at 4°C. Homogenization buffer was used throughout the preparation.

The original homogenate was spun at 16000 × g_{max} for 15 min using a Beckman J2-21 centrifuge. The solid fat cake and supernatant were carefully removed. The initial pellet was resuspended in 5 ml of buffer, applied to a 1.12 M sucrose cushion (20 mM Tris-HCl/1.12 M sucrose/1 mM EDTA) and centrifuged at 101000 × g_{ave} for 70 min using a Prepspin 65 ultracentrifuge. The plasma membranes were collected at the interface and resuspended in approx. 35 ml of buffer and centrifuged at 48000 × g_{max} for 45 min. The pellet was resuspended in 10 ml buffer, repelleted, and finally resuspended to approx. 0.5 mg protein/ml. The membranes were stored as follows: 100 μl aliquots from the suspension were pipetted into separate Eppendorf tubes and each vial was flushed with a stream of nitrogen. Samples were stored at −20°C. Once thawed, the samples were not reused.

This method yielded membranes with an approx. 3-fold higher 5′-nucleotidase activity than the microsomal membranes, as determined by the liberation of inorganic phosphate from adenosine 5′-monophosphate [23].

Cytochalasin B binding. Portions (70 μl) of the plasma membrane suspensions prepared from either basal or insulin-stimulated adipocytes were incubated in glass centrifuge tubes containing 25 μM cytochalasin B and [^{3}H]cytochalasin B to give a final radioactivity of 0.03125 μCi/ml, for 10 min at 4°C in the presence of 500 mM L-glucose [24] and 0–20 μM PhAsO. [U-^{14}C]Sucrose was added to aid in determining trapped, unbound [^{3}H]cytochalasin B in the membrane pellets obtained during the binding assay.

Buffer solution (Hepes, pH 7.4) was added to adjust the volume to 1 ml; after equilibration, the tubes were centrifuged for 20 min at 12000 rpm in a Beckman Model J2-21 centrifuge, and then 50 μl aliquots of the supernatant were removed for measurement of the free [^{3}H]cytochalasin concentration. The remaining supernatant was aspirated and discarded; the pellet was dissolved in LKB Optiphase Safe scintillation fluid (10 μl) and transferred to a 5 ml polyethylene scintillation vial together with washings to remove any remaining radioactivity and the [^{3}H]cytochalasin concentration was counted to give the cytochalasin bound.

Protein concentration and cell counts. The protein content of the adipocytes washed free of bovine serum albumin and sonicated was determined by Coomassie blue dye binding as described by Bradford [25] using a bovine serum albumin standard. The adipocytes were also counted using a Coulter counter model Z_B with a 100 μm orifice.

Results

The inhibitory effect of PhAsO (10 μM) on basal and insulin-stimulated D-glucose transport is shown in Fig. 1. Concentrations of 5 and 10 μM PhAsO completely inhibited insulin stimulation of D-glucose uptake. Furthermore, at these PhAsO concentrations, uptake fell below that of the basal state, thus demonstrating basal state inhibition. Since PhAsO acts covalently, it is not surprising that, whether PhAsO is added prior to or after insulin treatment, the extent of inhibition is comparable. Although PhAsO (10 μM) caused substantial inhibition, glucose transport remained

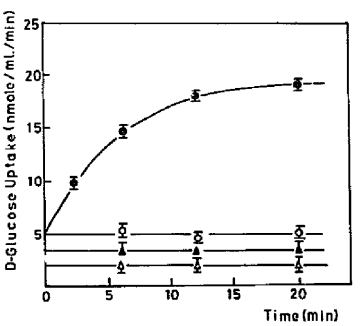

Fig. 1. The effect of PhAsO on stereospecific D-glucose uptake in insulin-stimulated rat adipocytes at 37°C. Rat adipocytes were treated with insulin (100 nM) (●), insulin plus 5 μM PhAsO (▲) and insulin plus 10 μM PhAsO (△). D-Glucose uptake in 60 s was measured after the indicated times of incubation. D-Glucose uptake by basal state cells is also shown (○). The cell concentration was approx. $3 \cdot 10^7$ cells/ml and the points are the means ± S.E. of three independent experiments.

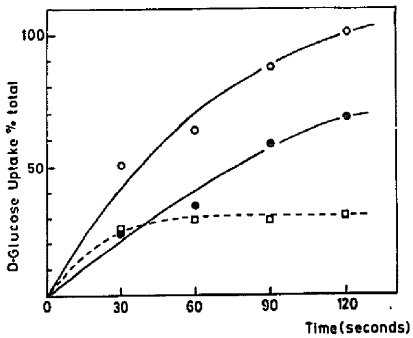

Fig. 2. Time-course for the effect of PhAsO on stereospecific D-glucose uptake by rat adipocytes at 37 °C. D- (○) and L- (□) glucose uptake after incubation with insulin (50 nM) plus PhAsO (10 μM) for 20 min. ●, Stereospecific D-glucose uptake ($D_{total} - L_{total}$).

stereospecific (Fig. 2), indicating that membrane integrity was not a factor in transport inhibition.

Although high concentrations of PhAsO (10 μM) permanently inhibit D-glucose uptake, at relatively low concentrations (1 μM), the initial rapid inhibition occurring after 2 min is followed by a slow time-dependent recovery of transport, such

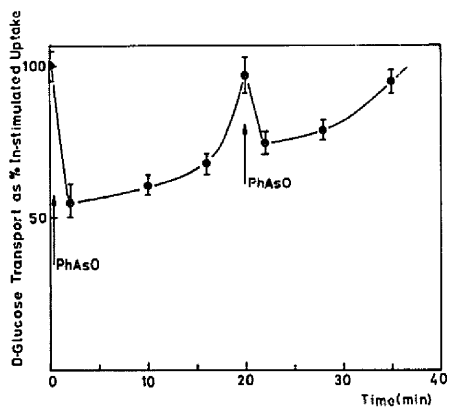

Fig. 3. The effect of PhAsO on stereospecific D-glucose uptake by insulin-stimulated rat adipocytes at 37 °C. Adipocytes were treated with insulin (100 nM for 20 min) and D-glucose uptake (in 60 s) was measured. Insulin-stimulated cells were then treated with PhAsO (1 μM) and D-glucose uptake (in 60 s) was monitored over 20 min. After this time, PhAsO (1 μM) was again added and D-glucose uptake (in 60 s) was measured over a further 16 min.

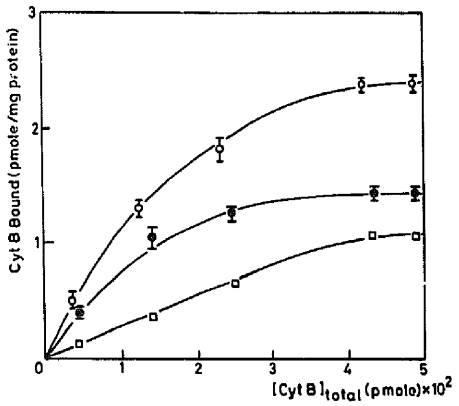

Fig. 4. The effect of PhAsO on the binding of cytochalasin B to adipocyte plasma membranes isolated from insulin-stimulated cells. ○, cytochalasin B binding in the absence of PhAsO; ●, cytochalasin B binding in the presence of 10 μM PhAsO; □, PhAsO-inhibitable cytochalasin B binding. The points are the means ± S.E. of three independent experiments.

that after 20 min, approx. 90% of the original transport activity is recovered (Fig. 3). The percentage recovery in five independent experiments after treatment of cells with 1 μM PhAsO was 86 ± 9%. Once recovered, the adipocytes are again susceptible to transport inhibition and recovery upon further treatment with 1 μM PhAsO (Fig. 3).

Cytochalasin B, a potent inhibitor of carrier mediated D-glucose transport, binds to the inner face of the hexose carrier and in so doing alters the carrier conformation, such that the transport sites are all recruited to the intracellular surface [26]. Fig. 4 shows the effect of 10 μM PhAsO on

TABLE I

COMPARISON OF THE EFFECTS OF PhAsO AND CYTOCHALASIN B ON STEREOSPECIFIC CARRIER-MEDIATED D-GLUCOSE UPTAKE IN RAT ADIPOCYTES AT 37 °C

Results are means ± S.D. of quadruplicate determination.

Inhibitor	Inhibition of stereospecific D-glucose uptake (in 120 s) (%)	
	basal	insulin
Cytochalasin B (25 μM)	96.3 ± 0.6	99.0 ± 0.25
PhAsO (10 μM)	70.5 ± 3.5	87.33 ± 6.5

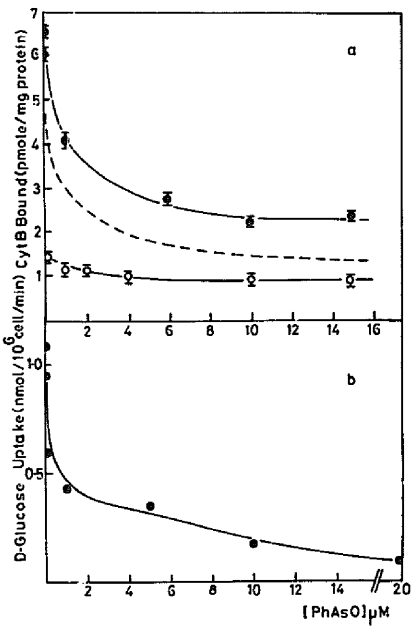

Fig. 5. (a) The effect of PhAsO concentration on the binding of cytochalasin B to adipocyte plasma membranes isolated from basal state (○) and insulin-stimulated (●) cells. The dashed line was obtained as described in the text and represents the effect of phenylarsine oxide on *specific* cytochalasin B binding. (b) The effect of PhAsO concentration on the stereospecific uptake of D-glucose (in 60 s) by insulin-stimulated rat adipocytes at 37 °C.

the concentration dependence of cytochalasin B binding to plasma membranes prepared from insulin-stimulated rat adipocytes and Fig. 5a compares the effect of PhAsO concentration on cytochalasin B binding to plasma membranes from basal and insulin-stimulated cells. Whereas PhAsO causes only a small inhibition of cytochalasin B binding to basal state plasma membranes, there is a dramatic inhibition of cytochalasin B binding to plasma membranes prepared from insulin-stimulated cells in a manner which bears resemblence to the concentration dependence of the inhibition of insulin-stimulated hexose uptake by PhAsO in intact cells (Fig. 5b).

Table I compares the inhibitory effect of cytochalasin B (25 μM) and PhAsO (10 μM) on D-glucose transport and demonstrates that PhAsO inhibits both basal and insulin-stimulated D-glucose uptake to comparable extents. There is only a small probability (0.17) that PhAsO inhibits insulin-stimulated cells more than basal state cells (*t*-test).

Discussion

In recent years, a number of major advances have been made in understanding the mechanism of insulin activation of the glucose transport system. The realization that insulin can recruit intracellular hexose carriers [27–29] and the elucidation of the amino-acid sequence of both the insulin receptor [2,3] and the D-glucose transporter [5] are significant contributions to the understanding of insulin action. However, many aspects of insulin enhancement of sugar transport remain unsolved and no suitable mediator for this process has been identified.

There are a number of similarities between our observations [13–15] and those of others [11,12] on the action of PhAsO. These include the ability of PhAsO to inhibit insulin-stimulated hexose uptake, the partial reversibility of PhAsO's action with dithiols and the inability of PhAsO to impair insulin binding to its receptor at concentrations where effective inhibition of sugar transport occurred.

However, two fundamental differences were observed. Firstly, the inhibition of basal uptake by PhAsO in isolated rat adipocytes was observed by us and was not observed in 3T3-L1 adipocytes [11]. Secondly, whereas we reported a rapid marked decrease in glucose transport apparent within 1 min of addition of PhAsO (1–10 μM) to insulin-stimulated rat adipocytes, Frost and Lane [11] observed a slow time-dependent decrease in transport activity, which seemed to parallel the withdrawal of insulin from the medium. The reasons for these differences are presently unclear, but could be due to tissue specificity.

Phosphorylation of the insulin receptor β-subunit is believed to play a critical role in signal transduction [30,31]. Recently, Bernier et al. [12] showed that PhAsO does not inhibit insulin-induced receptor phosphorylation, but evidently, the arsenical allows the accumulation of a phosphorylated 15 kDa protein only in the presence of insulin, thus implying a possible role for this pro-

tein in insulin receptor signalling. Inhibition of insulin-stimulation of serine-specific phosphorylation of two endogenous phosphoproteins (24 and 240 kDa) by PhAsO has also been found [32]. However, Figs. 4 and 5a show that PhAsO is capable of significantly inhibiting cytochalasin B binding to insulin-stimulated plasma membranes. Wardzala et al. [24] have shown that *non-specific* binding of cytochalasin B to both basal and insulin-stimulated membranes are identical: hence, subtraction of the curve for cytochalasin B binding to plasma membranes from basal state cells from that for plasma membranes from insulin-stimulated cells gives a curve (Fig. 5a) which shows PhAsO inhibition of *specific* cytochalasin B binding to insulin-stimulated membranes. Interestingly, the concentration dependence of PhAsO-induced inhibition of specific cytochalasin B binding bears a striking resemblence to the inhibition of D-glucose transport in insulin-stimulated cells (Fig. 5b) and strongly suggests that the inhibition of hexose uptake is due largely to the direct interaction of PhAsO with the sugar transporter. PhAsO may either interact with the transporter at the cytochalasin B binding site or it may interact with vicinal sulphydryl groups on the carrier to such an extent as to alter its conformational structure, thereby preventing cytochalasin B binding. It might also be postulated that the insulin-recruited transporters are more sensitive to PhAsO inhibition of cytochalasin B binding than basal state transporters, although further studies using non-specific cytochalasin B binding inhibitors would be required to test this postulate.

Should the focus of PhAsO inhibition of glucose uptake be through direct interaction with the transporters then one would anticipate that even in the basal state, where only a small proportion of the carriers are membrane-bound, this agent should exert some degree of transport inhibition. The ability of PhAsO to inhibit stereospecific D-glucose uptake in both the basal and insulin-stimulated states is shown in Table I and supports the proposal that some direct interaction between the arsenical and the transporters occurs. PhAsO, though a potent inhibitor of hexose uptake, appears to be less efficient than cytochalasin B.

In our previous studies [15], we demonstrated that when the concentration of PhAsO was sufficiently low (i.e. 1 μM), transport inhibition of the insulin-stimulated state was transient and transport activity was almost totally restored after 20 min. Since PhAsO binds covalently to inhibit protein internalization, we speculated then that if PhAsO directly inhibited the transporter function, then the subsequent transport recovery observed must be due to recruitment of cytoplasmic transporters in response to insulin-initiated signal transduction.

Fig. 3 demonstrates that once the transport activity has recovered from the initial interaction with PhAsO (1 μM), it can again undergo further rapid inhibition (within 2 min) and recovery. Such an effect is consistent with a direct inhibition of hexose transport by PhAsO. Recently, there has been conflicting reports as to whether recruitment of intracellular transporters is in fact responsible for enhancement of hexose transport. Whitesell and Abumrad [33] suggested that the principle effect of insulin on adipocytes was an approx. 9-fold decrease in the K_m of the glucose transporter, while Martz et al. [34] have opposed this view. If, as the data suggest, PhAsO interacts directly with and inhibits transporter function, then the observation that fat cells can undergo successive transient transport inhibition by PhAsO (1 μM) is not only consistent with the recruitment hypothesis but also further demonstrates the existence of a large reserve of transport activity present within adipocytes.

In conclusion, it would appear that at least in this cell type, transport inhibition by PhAsO is in part due to the interaction between PhAsO and the D-glucose transporters, but still does not preclude the possibility that PhAsO might also act in some way to interupt signal transmission from the insulin receptor. Insulin exhibits a wide range of biological responses, and PhAsO could clearly affect other systems as well as the hexose transporters.

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References

1 Massague, J., Pilch, P.F. and Czech, M.P. (1980) Proc. Natl. Acad. Sci. USA 77, 7137–7141.
2 Ullrich, A., Bell, J.R., Chen, E.Y., Herrera, R., Petruzzelli, L.M., Dull, T.J., Gray, A., Coussens, L., Liao, Y-C., Tsubokawa, M., Mason, A., Seeburg, P.H., Grunfeld, C., Rosen, O.M. and Ramachandran, J. (1985) Nature 313, 756–761.
3 Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J., Masairz, F., Kan, Y.W., Goldfine, I.D., Roth, R.A. and Rutter, W.J. (1985) Cell 40, 747–758.
4 Wilden, P.A., Boyle, T.R., Swanson, M.L., Sweet, L.J. and Pessin, J.E. (1986) Biochemistry 25, 4381–4388.
5 Mueckler, M., Caruso, C., Baldwin, S.A., Panico, M., Blench, I., Morris, H.R., Allard, W.J., Lienhard, G.E. and Lodish, H.F. (1985) Science 229, 941–945.
6 Lowe, A.G. and Walmsley, A.R. (1986) Biochim. Biophys. Acta 857, 146–154.
7 May, J.M. (1985) J. Biol. Chem. 260, 462–467.
8 Morgan, M.S., Darrow, R.M., Nafz, M.A. and Varandani, P.T. (1985) Biochem. J. 225, 349–356.
9 Hannestad, U. and Sorbo, B. (1980) J. Chromatogr. 200, 171–177.
10 Wallace, R.A. and Ho, T. (1972) J. Exp. Zool. 181, 303–317.
11 Frost, S.C. and Lane, M.D. (1985) J. Biol. Chem. 260, 2646–2652.
12 Bernier, M., Laird, D.M. and Lane, M.D. (1987) Proc. Natl. Acad. Sci. USA 84, 1844–1949.
13 Douen, A. and Jones, M.N. (1986) Biochem. Soc. Trans. 14, 313–314.
14 Douen, A. and Jones, M.N. (1986) Biochem. Soc. Trans. 14, 1023–1024.
15 Douen, A. and Jones, M.N. (1988) Biochim. Biophys. Acta 968, 109–118.
16 Kaplan, J., McVeyward, D. and Wiley, H.S. (1985) J. Cell Biol. 101, 121–129.
17 Hertel, C., Coulter, S.J. and Perkins, J.P. (1985) J. Biol. Chem. 260, 12547–12553.
18 Jones, M.N., More, J.E. and Riley, D.J. (1986) J. Recept. Res. 6, 361–380.
19 Rodbell, M. (1964) J. Biol. Chem. 239, 375–380.
20 Krebs, H.A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–36.
21 Gliemann, J., Osterlind, K., Vinten, J. and Gammeltoft, S. (1972) Biochim. Biophys. Acta 286, 1–9.
22 Simpson, I.A., Yver, D.R., Hissin, P.J., Wardzala, L.J., Karnieli, E., Salans, L.B. and Cushman, S.W. (1983) Biochim. Biophys. Acta 763, 393–407.
23 Atkinson, A., Gatenby, A.D. and Lowe, A.G. (1973) Biochim. Biophys. Acta 320, 195–204.
24 Wardzala, L.J., Cushman, S.W. and Salans, L.B. (1978) J. Biol. Chem. 253, 8002–8005.
25 Bradford, M. (1976) Anal. Biochem. 72, 248–254.
26 Wang, J.F., Falke, J.J. and Chan, S.I. (1986) Proc. Natl. Acad. Sci. USA 83, 3277–3281.
27 Karnieli, E., Zarnowski, M.J., Hissin, P.J., Simpson, I.A., Salans, L.B. and Cushman, S.W. (1981) J. Biol. Chem. 256, 4772–4777.
28 Kono, T., Suzuki, K., Dansey, L.E., Robinson, F.W. and Blevins, T.L. (1981) J. Biol. Chem. 256, 6400–6407.
29 Wardzala, L.J. and Jeanrenaud, B. (1981) J. Biol. Chem. 256, 7090–7093.
30 Grumberger, G., Zick, Y. and Gorden, F. (1984) Science 223, 932–934.
31 Chou, C.K., Dull, T.J., Russell, D.S., Gherzi, R., Lebwohl, D., Ullrich, A. and Rosen, O.M. (1987) J. Biol. Chem. 262, 1842–1847.
32 Frost, S.C., Kohanski, R.A. and Lane, M.D. (1987) J. Biol. Chem. 262, 9872–9876.
33 Whitesell, R.R. and Abumrad, N.A. (1985) J. Biol. Chem. 260, 2894–2899.
34 Martz, A., Mookerjee, B.K. and Jung, C.Y. (1986) J. Biol. Chem. 261, 13606–13607.