Regulation of the glucose transporter GLUT1 in mammalian cells

Stephen A. Baldwin*†, On Kan*†, Anthony D. Whetton†, Sally Martin‡, Hilary A. C. Fawcett*†,
David J. Flint‡ and Colin J. Wilde‡

*Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K., †Department of
Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology,
P.O. Box 88, Manchester M60 1QD, U.K., ‡Hannah Research Institute, Ayr KA6 5HL, U.K. and §Department of
Biochemistry and Chemistry, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, U.K.

Introduction

The uptake of glucose by most mammalian tissues is catalysed by members of the GLUT family of passive sugar transporters. These proteins are characterized by the presence of 12 putative membrane-spanning α-helices, and form a subset of a much larger family of homologous transporters found in a diverse range of organisms including bacteria, plants and fungi [1]. Because of the physiological importance of glucose as a metabolic substrate in mammals, much recent research has focused on the physiological roles and means of regulation of the individual transporter isoforms found in different mammalian tissues. Perhaps the most dramatic example of regulation is seen in GLUT4-containing tissues such as white and brown fat, heart and skeletal muscle, where insulin reversibly brings about a rapid and large increase in the Vmax for glucose uptake. This increase in Vmax stems primarily from the translocation of GLUT4 from an intracellular location to the cell surface.

The GLUT1 glucose transporter isoform is also found in insulin-sensitive cells such as adipocytes, but it is much less abundant than GLUT4 and translocates to the cell surface to a lesser extent than the latter in the presence of insulin. Unlike GLUT4, GLUT1 is not confined to insulin-sensitive tissues, but is in fact the most widely distributed of the sugar transporter isoforms throughout the body. It is particularly abundant in tissues with a barrier function, such as the blood–brain barrier [2,3] and the placental syncytiotrophoblast [4,5], where it brings about the rapid flux of glucose across endothelial and epithelial cell layers. Glucose fluxes across these barriers do not seem to be under physiological regulation by hormones such as insulin. Indeed, GLUT1 is generally considered to be a hormonally insensitive transporter, which is constitutively expressed, is primarily located at the cell surface, and is responsible for the basal uptake of glucose by most cells. It has long been known, however, that in certain circumstances profound changes in glucose transport activity can be induced in cultured mammalian cells that express only GLUT1. For example, exposure of baby hamster kidney cells to a variety of stresses including heat shock, arsenite and viral infection leads to a stimulation of transport that can be accounted for by translocation of GLUT1 from an intracellular pool to the cell surface [6].

Transport regulation in the mammary gland

In the present studies we have pursued the question of whether physiologically significant regulation of GLUT1 also occurs in vivo, in particular via the action of members of the cytokine receptor family. The binding of ligands such as prolactin, growth hormone and interleukin 3 to receptors of this family is known to bring about the tyrosine phosphorylation of cytoplasmic proteins, although the full details of the signal-transduction pathways are not yet clear. The first example of transport regulation by such receptors that we have investigated involves the rat mammary gland. Large amounts of glucose are required by the lactating mammary gland for the synthesis of milk constituents such as fat and lactose, and the rate of glucose supply to the gland is the main factor in determining the rate of milk production [7]. Furthermore, uptake of glucose across the epithelial cell plasma membrane by cytochalasin-B-inhibitable, facilitated diffusion is the rate-limiting step in the utilization of this carbohydrate by the mammary gland [8]. Physiologically relevant regulation of the transport process is suggested by the observation that the capacity of epithelial cells from lactating mouse mammary gland to transport glucose is much greater than that of the corresponding cells from pregnant animals [9].

Western blotting with isoform-specific antibodies revealed that the GLUT1 glucose transporter isoform is abundant in the plasma membranes of secretory epithelial cells from the mammary glands of lactating rats. In contrast, none of the other known transporter isoforms, i.e. GLUT2, 3, 4,
5 or 7, was detectable in these cells. The number of GLUT1 transporters as determined by quantitative Western blotting also corresponded closely to the number of transport sites as determined by cytochalasin B binding measurements [10]. Thus GLUT1 alone appears to be responsible for sugar uptake by the lactating gland. In order to investigate the origin of the changes in transport activity during pregnancy and lactation reported for the mouse mammary gland [9], quantitative Western blotting was next used to assess GLUT1 levels in mammary gland epithelial cells isolated from rats at different stages of pregnancy and lactation. These experiments revealed that levels of GLUT1 remain very low throughout pregnancy, but increase rapidly (≥10-fold) with the onset of lactation [11]. Removal of the litter leads to an equally rapid loss of transporters, with the levels reaching the same low values as found during pregnancy within 24 h. Thus, the mammary gland provides a striking example of the physiologically relevant regulation of GLUT1, which involves changes in the level of protein expression. Preliminary results have indicated that these large changes in transporter expression levels are not associated with similar changes in the levels of GLUT1 mRNA, and so regulation probably occurs at the post-transcriptional level.

In the rat, prolactin is the major lactogenic hormone, although growth hormone is also important, especially during the later stages of lactation [12-14]. Clues about the hormonal basis of transporter regulation were therefore sought by examining the effects of such hormones on the levels of GLUT1 expression in cultured mammary gland explants from mid-pregnant animals. Quantitative Western blotting revealed that the levels of GLUT1 remained essentially unchanged during culture of the explants in cortisol and insulin, but increased 5-fold over a period of 2 days when prolactin was included in the culture medium [11]. This finding suggested that prolactin is involved in bringing about the increase in GLUT1 expression in the mammary gland at lactogenesis. To investigate the role of prolactin and other lactogenic hormones under more physiological conditions, we next examined the effect of inhibiting the action of these hormones in the mid-lactating rat. This was done by giving injections of bromocryptine, which suppresses prolactin release from the anterior pituitary, and of antiserum against growth hormone. Whereas treatment for 2 days with anti-growth hormone produced an insignificant decrease in GLUT1 levels in mammary gland plasma membranes, bromocryptine treatment produced a significant, 40% decrease in GLUT1 levels. In combination, the effects of bromocryptine and anti-growth hormone were synergistic, decreasing GLUT1 levels by >90% [15]. An even larger apparent decrease was seen when the GLUT1 content of isolated epithelial cells was quantified by Western blotting [11]. Taken together, the results of the explant and in vivo experiments suggest that prolactin is required for the initiation of GLUT expression at lactogenesis, and that both prolactin and growth hormone are required for maintenance of GLUT1 levels during lactation. However, although these effects seem to involve post-transcriptional regulation, their precise mechanisms remain unclear.

### Transport regulation in haemopoietic cells

The second example of transport regulation by a member of the cytokine receptor family that we have been investigating is the regulation of glucose uptake by cytokines and oncogenes in interleukin (IL)-3-dependent haemopoietic progenitor cells. It has been known for some years that IL-3 stimulates sugar uptake by such cells in a rapid and reversible fashion, but the mechanism of this phenomenon remains unclear [16]. In the absence of IL-3 these cells undergo the process of programmed cell death known as apoptosis. Regulatory mechanisms in such cells are of interest not only in relation to the physiologically normal processes of cell survival, proliferation and differentiation, but are also of relevance to pathological situations, because suppression of apoptosis by oncogenes is a key facet of leukaemic transformation. To study the mechanisms of these phenomena, we have used an IL-3-dependent haemopoietic cell line (ICDP) that expresses a temperature-sensitive mutant of the v-abl (Abelson) tyrosine kinase (v-ABL), which is active at 32°C but inactive at 39°C [17]. Activation of v-ABL at the permissive temperature (32°C) permits cell survival by suppressing apoptosis in the absence of IL-3, although the latter is still required for proliferation [18,19]. In view of the similarities between the effects of IL-3 and of v-ABL activation on cell survival, in the present study we decided to investigate the effect of kinase activation on sugar transport, and also to assess the possible involvement of transport activation in the suppression of apoptosis.

The rate of 2-deoxy-D-glucose (2dGlc) uptake by cells grown at the restrictive temperature (39°C) in the presence of IL-3 was found to decline rapidly when the cells were deprived of the growth factor, but it rapidly returned (t1/2 ~ 1.5 h) to the original level upon re-addition of IL-3. In contrast, no significant decrease in 2dGlc uptake rates was seen.
when cells were deprived of IL-3 at the permissive temperature (32°C), indicating that activation of the kinase mimicked the effect of the growth factor on hexose transport. Similarly, increasing the incubation temperature to 32°C for cells deprived of IL-3 for a period of 4 h at 39°C mimicked the effect of IL-3 in restoring 2dGlc uptake rates.

Activation of the kinase was found to be associated with an increase in the \( V_{\text{max}} \) for sugar uptake, with no effect on \( K_{\text{m}} \). No significant effect on the levels of GLUT1 in IC.DP membranes was detectable by quantitative Western blotting, indicating that the action of the kinase is not mediated by changes in the total cellular content of transporters. However, confocal immunofluorescence microscopy revealed the presence of an intracellular pool of GLUT1 in the cells. Furthermore, switching IL-3-deprived cells from the restrictive to the permissive temperature, or addition of IL-3, was associated with a significant increase in the level of fluorescence attributable to cell-surface GLUT1. These findings suggest that the effects of both IL-3 and of v-ABL activation on transport are mediated by transporter translocation from an intracellular pool to the cell surface.

The similarity of the effects of IL-3 and v-ABL activation both on the suppression of apoptosis and upon sugar transport suggested that these phenomena might be related. To investigate this hypothesis further, the effects of a potent inhibitor of glucose transport, cytochalasin B, were examined. This compound increased the rate of apoptosis in IL-3-deprived cells at the permissive temperature more than 4-fold, whereas the non-inhibitory disaccharide sucrose had no effect. The rate of apoptosis in IC.DP cells was also found to be increased by maltose, a membrane-impermeant inhibitor of transport, whereas the non-inhibitory disaccharide sucrose had no effect. The effects of the transport inhibitors on the rate of apoptosis could be reversed, at least in part, by the provision of pyruvate or glutamine as alternative energy sources. It is therefore likely that the maintenance of a favourable energy balance is a vital criterion that must be met by haemopoietic cells in order to avoid programmed cell death. Regulation of GLUT1-catalysed glucose transport by cytokines and oncogenes thus forms an important regulatory control point in the suppression of apoptosis.

**Conclusions**

In summary, we have shown that far from being a constitutive, unregulated transporter, GLUT1 is of great physiological importance in at least two instances *in vivo*. In the mammary gland, regulation of transport at the level of protein expression by prolactin and growth hormone ensures the supply of glucose for manufacture of milk constituents. In haemopoietic cells, transport regulation by interleukins suppresses apoptosis, and the circumvention of these regulatory controls by oncogenes such as that encoding v-ABL may contribute to leukaemogenesis in some cases. In contrast to the situation in the mammary gland, in haemopoietic cells regulation of transport appears to be at the level of transporter location within the cell, despite the structural and presumably mechanistic similarities between the prolactin, growth hormone and IL-3 receptors. Experiments aimed at elucidating the origins and mechanisms of these differing types of regulation are now in progress in our laboratories.

*This work was supported by grants from the Science and Engineering Research Council, the Agricultural and Food Research Council, the Medical Research Council, the Leukaemia Research Fund and the Wellcome Trust.*

Membrane Dynamics and Transport

Molecular cloning and functional expression of potassium channels from the adrenal medulla

Miguel Garcia-Guzmán, Francisco Sala, Salvador Sala and Manuel Criado*
Institute of Neurosciences, University of Alicante Ap.374, 03080-Alicante, Spain

Introduction
Potassium channels are molecules involved in the control of the electric potential across the cell membrane, and so they can regulate the flow of information between cells [1]. This is particularly true in the nervous system, where potassium channels are responsible for ionic currents of enormous diversity, underlying a wide variety of neuronal firing patterns [2]. However, a detailed molecular analysis of these proteins is made more difficult in the brain by the ubiquity and diversity of potassium channels and by the cell heterogeneity of neural tissue. For this reason, we chose to study potassium channels in a more accessible and homogeneous system, the adrenal medulla [3]. This tissue includes neuronal, interstitial and vascular components, but the secretory chromaffin cells predominate [4]. Chromaffin cells are of neuronal origin and fire action potentials, which are the result of voltage-dependent Na⁺, Ca²⁺ and K⁺ currents [5,6]. A Ca²⁺-dependent K⁺ current seems to be fundamental to the repolarization of the action potential [7].

Cloning of adrenomedullary potassium channels
cDNA libraries constructed from bovine adrenal medulla RNA [8] were screened at low stringency [9] with a SacI cDNA fragment of the rat brain potassium channel RCK3 [10]. This probe, kindly provided by Dr O. Pongs (University of Hamburg, Germany), codes for the central core of the protein which contains the putative transmembrane segments. The screening of 10⁶ recombinants led to the isolation of 14 cDNA clones. Most of the clones reacted in Southern blots with probes coding for the rat brain potassium channels RCK1, RCK3, RCK4 and RCK5 [10]. Sequence analysis of these clones showed that they were coding for two different potassium channel proteins (BAK4 and BAK5), but none of the clones contained a full protein coding region. Thus, a second adrenal medulla library was screened at high stringency with probes specific for each of the two potassium channels. Several clones were obtained, one of them extended into the 5' untranslated region of the mRNA corresponding to one of the channels (BAK4). Sequence analysis of this clone revealed a long open reading frame of 660 amino acids, which shares high sequence identity (94%) with the designated RCK4 from rat brain [10], the RHK1 from rat heart [11] and other members of the Shaker-related subfamily known as Kv1.4 [12]. For this reason we called this channel BAK4 (bovine adrenal K⁺ channel type 4) [13].

Despite performing repeated screenings of cDNA libraries, we could not obtain full-length cDNA clones for the second potassium channel (BAK5). However, when we screened a bovine genomic library (Clontech) we obtained clones that contained the complete protein coding sequence and the adjacent 5' and 3' untranslated regions. The deduced amino acid sequence contains one long open reading frame of 597 amino acid residues and reveals an overall structure similar to that described for other voltage-gated potassium channels: six putative membrane-spanning segments with the N- and C-terminal domains located in the cytoplasm. It is interesting to note that the N-terminus is shorter than the corresponding segment of the BAK4 channel and is rich in proline residues. This protein shares particular sequence identity with other potassium channels cloned from rat brain (designated Kv1; see [14]) and human insulinoma cells (named hPCN1; see [15]). We have called it BAK5 to indicate that it is assigned to the Shaker-related subfamily Kv1.5, as is the case for its rat and human counterparts [12]. Interestingly, although the overall sequence similarity between the aforementioned Kv1.5 channels is high (85%), only 50% of the first 100 amino acid residues are identical. This suggests that the N-terminal region may determine the func-

---

Received 5 January 1994

---

Abbreviations used: SV40, simian virus 40; TEA, tetraethylammonium.
*To whom correspondence should be addressed.