Is non-insulin dependent glucose uptake a therapeutic alternative?
Part 2: Do such mechanisms fulfil the required combination of power and tolerability?

NF Wiernsperger

SUMMARY

The worldwide burden of diabetes, the unavoidable worsening which is observed in long-term clinical trials despite treatment and the close link between glycaemia and microangiopathy appeal for much stronger treatment strategies. This, in turn, either requires polypharmacy (with new risks) or new, more powerful drugs to be invented. The first part of this review dealt with a thorough analysis of pros and cons for some selected pathways which could potentially increase glucose uptake without necessitating insulin. The choice of such targets for developing completely new drugs, however, requires a favourable background from existing tentatives with either drugs or cell biology approaches. Moreover, because vascular complications are what must ultimately be avoided when treating diabetic patients, we must be sure that increasing glucose uptake in a fashion which is no more controlled by normal physiology is compatible with the physiology of vascular cells (long-term tolerance). The aspect of drug side-effects must therefore be considered systematically. For reasons which are individually developed, it appears that each of the potential pathways analyzed either lacks sufficient power and/or is likely to induce side effects which are not acceptable for long-term application. The fact that GLUT-1 transporters are ubiquitously distributed even extends this cardinal question to the general principle of increasing glucose uptake. In conclusion a precise evaluation suggests that, although non-insulin dependent glucose uptake represents ¾ of whole body glucose transport, it is difficult to consider such mechanisms able to generate a new treatment fulfilling the unavoidable request of combined efficacy and tolerability.

Key-words: Type 2 diabetes · Glucose transport · Non-insulin dependent glucose transport · Vascular complications · Drug tolerance.

RéSUMÉ

Le captage non-insulinodépendant du glucose représente-t-il une nouvelle voie thérapeutique ? 2e partie

L'explosion du diabète de type 2 dans le monde, son évolution clinique inéluctable récemment démontrée et le lien étroit entre glycéémie et microangiopathie justifient l'urgence de nouveaux traitements plus agressifs. Ceci signifie soit une polythérapie, avec ses risques inhérents et souvent mal connus, soit des molécules nouvelles et puissantes. Dans la première partie de cette revue ont été analysés précisément plusieurs mécanismes ne nécessitant pas l'insuline et susceptibles d'augmenter fortement le transport du glucose dans les cellules. Le choix d'une telle cible thérapeutique requiert cependant 1) un contexte favorable en provenance d'expériences récentes de type pharmacologique ou de biologie cellulaire et 2) l'assurance que le système vasculaire est apte à tolérer une stimulation supraphysiologique et relativement incontrôlée du transport de glucose ; Pour des raisons exposées pour chaque hypothèse sélectionnée, il apparaît qu'aucune de ces cibles potentielles ne satisfait à l'exigence indispensable d'une combinaison de puissance d'action et de tolérabilité à long terme. La distribution ubiquiste des transporteurs GLUT-1 sur les parois vasculaires étend même cette question au principe même d'une augmentation permanente du transport de glucose. En conclusion, une analyse approfondie laisse peu d'espoirs quant à une nouvelle entité pharmacologique issue de mécanismes originaux impliqués dans le transport du glucose non dépendant de l'insuline, qui représente pourtant ¾ du transport total de glucose dans l'organisme.

Mots-clés : Diabète de type 2 · Transport du glucose · Complications vasculaires · Tolérance médicamenteuse.
Insulin is the ideal substance because it is the physiological regulator, exerting various simultaneous effects on interconnected metabolic pathways, stimulating some, inhibiting others in a reversible, timely regulated manner. Thereby glucose homeostasis is normally tightly controlled. In type 2 diabetes, resistance to the hormone develops and worsens with time, making the efficacy and therapeutic use of insulin increasingly complicated.

On the other hand, this unique property of insulin cannot be mimicked by therapies bypassing the insulin receptor or the early post receptor signalling cascade. Such molecules would then likely need to stimulate alternative pathways to supraphysiological extents if they are expected to match the clinical requirements. This in turn means that these processes will not be regulated anymore as would be the case if insulin-dependent pathways would be at work. The counterpart of this parameter is its consequence on various aspects of glucose handling by cells, in particular cell types known to be sensitive to sugars. Prominent among all tissues are blood vessels, which lead to macro- and microangiopathy after long-term exposure to high glucose levels.

Non-insulin dependent glucose uptake (GU) is almost entirely ensured by the ubiquitous GLUT-1 subtype in both hormone and insensitive cells. For that reason GLUT-1 acquires a cardinal position in the analysis of such an alternative approach. In human diabetes the kidneys exhibit an over expression of GLUT-1 and increased GU [1,2]. In fibroblasts and smooth muscle cells, GLUT-1 was increased in the basal state, due to transporters translocation without change in their total number [3]. Increased GLUT-1 was also seen in erythrocytes [4] and in some studies [acute] in diabetic retinal endothelial cells [5,6]. By contrast, usually no changes in GLUT-1 expression were found in skeletal muscle, which suggests that rather vascular tissues (i.e. those prone to lesions!) may be concerned by GLUT-1 modifications. It is therefore important to analyze the known behaviour of GLUT-1 in cellular glucose transport, in particular in situations of engineered GLUT-1 expression modifications.

Metabolic consequences and limits of sustained glucose transport

The first question which must be addressed is whether higher GU via GLUT-1 has beneficial effects on the metabolic part of diabetes. In fact, rather deceiving-or even negative-effects have resulted from such manipulations; while GLUT-4 over expression is accompanied by improved sensitivity to insulin, over expression of GLUT-1 can lead to insulin resistance [7]. It was also shown that whereas basal GU increased, insulin-dependent GU in turn was strongly reduced [8]. Glycogen synthesis was increased but also free glucose accumulated, a very unsuitable effect [9]. Activation of glucokinase is potentially an interesting alternative [10]. Using glucokinase instead of GLUT-1 overexpression to increase glucose transfer reduced glycaemia but, as expected, it also increased hepatic and plasma lipid levels [11,12]. Increased lipid synthesis is a highly unsuitable manifestation [13] and, for example, non-alcoholic steatotic hepatitis is increasingly considered as an important player in diabetes pathophysiology. Recently, prolonged over expression of glucokinase in the liver was also shown to lead to insulin resistance in vivo [12]. Moreover reduction of glycaemia is not necessarily correlated with complete metabolic improvement. It was even hypothesized that glycaemia normalization might improve acquired (such as in STZ rats) but not inherited insulin resistance. Control of glycaemia by the renal glucose reuptake inhibitor T-1095 while almost normalizing liver and fat GU, also did not improve GU in skeletal muscle [14].

Vascular consequences of sustained glucose transport

The second cardinal question is the consequence of elevated glucose entry on long-term behaviour of vascular cells, since hyperglycaemia is tightly linked with microvascular lesions [15] and aggravates large vessel disease. Higher glucose flux via GLUT-1 overexpression is known to lead to increased matrix deposition in renal mesangial cells, with all the excess glucose going to lactate instead of being channelled into the mitochondrial respiratory chain [16,17]. In an interesting hypothesis, Ebeling [18] proposed that glucose might be metabolised differently according to which transporters would convey the sugar into the cell. Thus GLUT-1 carried glucose would be channelled into all metabolic pathways, including the hexosamine pathway, while glucose carried by GLUT-4 would go towards glycolysis and glycogen synthesis. This would strongly suggest that GLUT-1 activation would direct glucose towards harmful directions. In view of the potential causal importance of the hexosamine pathway [19] this hypothesis bears great attractiveness. High intracellular glucose concentrations also stimulate oxidative stress, particularly the formation of super oxide [20,21] which induces stress fiber and cytoskeleton reorganization [22]. High glucose-related oxidative stress is linked with a variety of cellular derangements [23] and is now a major concern in both metabolic and vascular worsening of type 2 diabetes [24]. Elevated intracellular glucose, directly or via its metabolism to methylglyoxal [25], can also promote glycation of proteins (AGEs) [26] and thereby chronically exert harmful effects on vessel wall function and structure [27] as well as lead to cell death by apoptosis [28]. Glucose also drives GU in capillaries, resulting into elevated formation of lactate and sorbitol [29].
The main question about increasing cellular glucose availability concerns the vascular bed, since microangiopathy is almost linearly related to ambient glucose levels. If, as shown above, GLUT-1 expression and activity are increased, the flux through cells or their intracellular sugar content will increase. It is of high interest to note here that this can occur without prevailing hyperglycaemia: cytokines as in inflammatory states [1,30], cell stretching [17], osmotic shock [31] or simply GLUT-1 over expression all increase the flux of glucose across the cell membrane. This suggests that rather than glucose concentration, it might be glucose flux which could be deleterious. At least some capillaries exhibit a glucokinase-like enzyme [32], an easy possibility to increase glucose uptake without substrate inhibition and therefore dangerous for vascular cells. Recent observations suggest the presence of various types of glucose transporters in endothelium, which raises further caution about the suitability of their activation [33,34].

A brief review on data originating from tissues typically lesioned in diabetes, i.e. retina and kidney, should help gain insight into this question. In 50% of retinal microvascular cells GLUT-1 expression was increased 18 fold on both luminal and abluminal sides of endothelial cells [5,34]. Acute hyperglycaemia increased GLUT-1 mRNA in human retinal endothelial cells [6]. By contrast, other reports revealed reduced expression of GLUT-1 [35,36] but one study found elevated free glucose in retinal endothelial cells [36]. A biochemical analysis showed that, while not increasing GLUT-1 expression, hyperglycaemia increased Vmax 2.5 fold in retinal endothelium [37]. Also absence of changes in GLUT-1 expression was reported in retina [37,38]. Thus there is no compensatory decrease in GLUT-1 expression in retinal vascular cells for limiting the entry of glucose [38,39], whereas other tissues protect themselves by down regulating GLUT-1 in the presence of hyperglycaemia [40]. Indeed capillaries normally form a barrier to glucose by maintaining a glucose gradient between blood and tissue [41]. Here again, the discrepancy between reports are likely due to very different lengths of cell exposure to high glucose. In renal mesangial cells, tolazamide increased GLUT-1 protein expression and GU was increased by 184%; this was accompanied by increased TGFβ1 levels as well as by accumulation of collagen and fibronectin in the extra cellular matrix [42,43]. High glucose also activates matrix metalloproteinases 1, 2 and 9, making vessels prone to atherosclerosis [43]. During the final elaboration of this manuscript, a very interesting publication showed that, while over expressing semicarbazide sensitive aminooxidase (SSAO) in mice enhanced glucose uptake and improved glucose tolerance, it led concomitantly to obesity and vascular damage through AGE formation [44]. This new finding lends support to the risk linked to generalized increases in glucose uptake to lower hyperglycaemia.

Both the data on metabolic and vascular consequences of increased glucose flux through cells clearly provide strong warnings for closely integrating these aspects into the development of treatments based on a stimulation of glucose uptake.

**Conclusions**

The rapidly growing knowledge about defective mechanisms in diabetes leads to an impressive list of potential therapeutic targets, some of which are analysed in this review. However there is a dramatic confusion between both concepts, i.e. a defect is far from being a therapeutic target. Indeed not only must the latter play a cardinal causative role either in the initiation or in the aggravation of the disease, it also must be reversed by treatment in order to obtain a clear-cut improvement in the patient’s clinical state. Moreover it should exhibit a certain degree of selectivity. Most importantly, however, the therapy must fit with chronic administration for a disease which will last increasingly longer. Finally it must be not only well tolerated per se, it also must be relatively harmless in the context of polypharmaceutical treatment.

With no doubt the list of criteria to be matched sets severe requirements to the development of new therapies. However the linear relationship between glycaemia and microangiopathy as well as our present experience clearly call for powerful answers in order to reach the ambitious but necessary goal. The question then is whether non-insulin dependent glucose metabolism is an area where to find such corresponding therapeutic targets.

One key concern is whether we have to deal with correction of a defect in T2DM or to activate supraphysiologically mechanisms which are not clearly defective. A first cardinal question is conceptual: if defects exist in these mechanisms, are they reversible and do they effectively respond by reversal? The next main question is: while it is probably suitable to normalize glucose uptake when it is subnormal, is activation of glucose uptake still meaningful if this parameter is normal or only slightly subnormal? If glucose uptake is normal in the real in vivo situation (thanks to prevailing hyperglycaemia), then we must indeed carefully appreciate the meaning and the limits of any supraphysiological glucose uptake stimulation. In fact it is presently not sufficiently established whether glucose effectiveness is really defective in T2DM. The absence of a clear-cut defect in glucose effectiveness suggests that the “reserve” capacity for additional glucose uptake is probably rather low and its correction therefore unlikely to have a major quantitative impact on hyperglycaemia. If stimulation occurs at supraphysiological levels, the main questions will be: a) will cells be able to manage the additional (excessive?) glucose supply or its flux and b) how will blood vessels react? Although the same question also applies to pathways linked to insulin, increasing non-insulin dependent glucose uptake represents a particularly
important problem because of its ubiquitous and non-regulated distribution throughout the body tissues. Based on our present knowledge, this approach appears eventually incompatible with long-term safety/tolerability concerns [44]. Possibly cell engineering in the future might provide more promising tools by successful over expression of selected proteins in specific tissues.

The long-lasting clinical and pharmacological experience with metformin provides an interesting basis for insights into the present question. Many pharmacological investigations have demonstrated that metformin is more efficacious when hyperglycaemia is present [45]. In fact this compound clearly exerts its therapeutic metabolic effect to a large extent via several of the non-insulin dependent pathways described above (stimulation of transporters intrinsic activity, stimulation of glycogen synthase activity/glycogen storage, partial inhibition of G-6-phosphatase, activation of glycogen synthase with basal insulin, possibly stimulation of AMPK). Nevertheless, as is the case for most oral antidiabetic drugs, its impact is limited to around 20% reduction in fasting glycaemia and its superiority on the cardiovascular patient’s outcome in the UKPDS [46] is largely attributable to glycaemia-independent effects of the drug [47]. Conversely the limits of a strong stimulation of such processes are illustrated by the problems of tolerability observed when more potent biguanides were used in the past.

Taken together, this analysis suggests that there are a number of potential therapeutic targets in the non-insulin dependent pathways of glucose handling which could be subjected to pharmacological development. However, if the goal is to prevent diabetic vasculopathy by a drastic reduction in glycaemia, it is unlikely that any of these mechanisms will be of sufficient strength to generate drugs fulfilling these requirements as monotherapy. The development of new antidiabetic compounds will increasingly require awareness of the problems of tolerability/side effects for growing long-term therapy durations. Considering the risk of promoting vascular lesions and/or lipid synthesis/deposition linked with powerful and/or permanent stimulations of glucose transport, it appears that non-insulin dependent glucose uptake can hardly fulfil both these requirements simultaneously. However new therapeutic principles based on such mechanisms may eventually become interesting drugs to be integrated into therapeutic combination schemes.

References


