INSULIN AND GLUCAGON SHARE THE SAME MECHANISM OF NEUROPROTECTION IN DIABETES: ROLE OF GLUTAMATE

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In patients with acute ischemic stroke, diabetes and hyperglycemia are associated with increased infarct size, more profound neurologic deficits and higher mortality. Notwithstanding extensive clinical and experimental data, treatment of stroke-associated hyperglycemia with insulin is controversial. Diabetes and even early pre-diabetic insulin resistance are not only characterized by hyperglycemia, but are also associated with increased levels of amino acids in the circulation, including the neurotoxic glutamate. The pleotropic metabolic effects of insulin include a reduction in the concentration of amino acids in the circulation . Here we show that the deleterious effect of stroke-associated diabetes is mediated by increased blood and CNS glutamate and that the reduction of glutamate levels by insulin or other agents within a very brief therapeutic window, significantly improves the neurological outcome after brain injury. Decreasing plasma concentrations of glutamate in diabetic rats with insulin or glucagon after transient middle cerebral artery occlusion (tMCAO) or traumatic brain injury (TBI) lowers blood and CSF glutamate, improves brain histology and preserves neurologic function. The neuroprotective effect of insulin and glucagon was similar, notwithstanding their opposite effects on blood glucose. The therapeutic window of both hormones overlapped with the short duration (~30 min) of elevated brain glutamate post injury in rodents. Similar neuroprotective effects were found after administration of the glutamate scavenger oxaloacetate in TBI model, which has no effect on glucose metabolism. These data indicate that insulin exerts a neuroprotective effect within a very brief therapeutic window that correlates with its capacity to reduce glutamate, rather than with its effect on glucose levels. Other, safer approaches to a reduction of glutamate without affecting glucose levels may be of use in the immediate aftermath of acute ischemic stroke or TBI.

AHNAK IS A NOVEL REGULATOR OF GLUT4 GENE EXPRESSION IN RAT AND HUMAN ADIPOCYTES: ROLE IN OBESITY AND INSULIN RESISTANCE

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High levels of free fatty acids like arachidonic acid (AA) have a major role in the pathogenesis of insulin resistance, obesity and type 2 diabetes (DM2). While elevated levels of AA repressed GLUT4 expression via a specific GLUT4 promoter region (-222/-197 bp), the mediator remained elusive. Using this region as bait for mediators in AA-treated cardiomyocytes, followed by mass spectrometry analysis, we detected the AHNAK/desmoyokin giant protein in association with the GLUT4-promoter (GLUT4-P). This association was confirmed by ChIP assay. In subcutaneous adipose tissue obtained from obese patients undergoing bariatric surgery, AHNAK mRNA levels correlated with the degree of weight loss (R^2=0.943 ; p=0.005). Similarly, AHNAK mRNA levels were ~2 fold increased in adipocytes of aged/obese rats, compared to lean controls. Transient expression of AHNAK in primary rat adipocytes repressed transcription from both GLUT4-P and a synthetic 3xIRS-LUC promoter reporter. This repression was partially curtailed by insulin, acting via nuclear exclusion of AHNAK, as observed by immunofluorescent staining. AHNAK gene silencing by siRNA enhanced Glut4 protein levels by 2-fold and protected GLUT4 expression from AA-induced repression. Thus, AHNAK emerges as a novel regulator of GLUT4 gene expression and as a potential therapeutic target for insulin resistant states like obesity and DM2.

AHNAK GENE EXPRESSION IS INCREASED IN HUMAN OBESITY AND DECREASED AFTER BARIATRIC SURGERY

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Background: AHNAK is a giant phospho-protein that is increased in adipose and muscle tissues in animal models of obesity. Previous results from our group suggest that AHNAK represses the transcription activity of GLUT4 gene leading to insulin resistance. In order to study the relevance of AHNAK to human obesity we studied the expression of AHNAK in obese patients.

Methods: We isolated adipocytes from subcutaneous (SCad) and visceral abdominal (Vad) adipose tissue biopsies obtained from 38 patients undergoing elective surgery (15/23 M/F; age 39.7 13.6 (Mean±SD); BMI 39.4 9.6 kg/m^2 ; and HOMA 3.11 2.52). AHNAK mRNA was quantified using real-time PCR. Adipocyte diameter, as measured by microscopy, was 502 307 l^3x10^6 for Vad and 701 423 l^3x10^6 for SCad. AHNAK mRNA expression was also determined in visceral fat biopsies obtained from 6 patients before and after weight loss after bariatric surgery.

Results: SCad AHNAK mRNA correlated with BMI (R^2=0.38; p=0.029), weight (R^2=0.372; p=0.033), and fasting plasma glucose levels (R^2=0.414; p=0.018). Further, Vad AHNAK mRNA levels positively correlated with adipocyte volume (R^2= 0.493; p=0.004) and AST levels (R^2=0.354; p=0.034). The correlation between AHNAK gene expression and obesity is supported by a good correlation between the extent of weight loss and the reduction of AHNAK mRNA in the 6 patients where biopsies were taken before and after weight loss (R^2=0.943 ; p=0.005). Further, we found a significant correlation between AHNAK mRNA expression in Vad and SCad from the same patient (correlation coefficient 0.555; p=0.001).
AHNAK mRNA level in VAd and SCad was higher in patients with hyperlipidemia (p=0.016 and p=0.047 respectively). However, there was no correlation between AHNAK mRNA expression in a dipocytes and basal insulin levels, HOMA, HbA1c, or a diagnosis of diabetes.

Conclusions: The correlation between the AHNAK levels in human adipocytes, and the degree of obesity and derangement in metabolic parameters, suggests a potential role for AHNAK in the pathogenesis of obesity and the metabolic syndrome.

MORE MUSCLE, LESS FAT, BETTER THAN DIET: THE POSITIVE EFFECTS OF ANG 1-7 TREATMENT IN THE METABOLIC SYNDROME

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The metabolic syndrome (MetSyn) affects over a billion people worldwide and is a leading health concern due to its link to cardiovascular disease. During MetSyn development, adipose tissue expands, but once its capacity to efficiently store energy is exceeded, accumulation of ectopic fat takes place. Skeletal muscles are a major site of insulin-stimulated glucose disposal, and a direct correlation was delineated between intramuscular adipocytes and insulin resistance. Moreover, skeletal muscle loss, sarcopenia, is characteristic of MetSyn and is further enhanced during diet, which is recommended to MetSyn sufferers. We previously showed that rats fed on high-fructose diet (HFrD) and treated with Angiotensin 1-7 (Ang1-7): (a) did not develop MetSyn; (b) had smaller adipocytes with less fat inflammation; and (c) had more myogenic cells without fully differentiated adipocytes in skeletal myofiber culturs. To shed light on the underlying mechanisms, we conducted high throughput real-time PCR analyses. The most significant results are presented. On the HFrD background, Ang1-7 downregulated NOX4 and PKCβ and upregulated PPARα expression in epididymal fat. These results suggest that Ang 1-7 exerted antioxidant effects and a triglyceride lowering effects that may involve fatty acid oxidation through PPARα. In gastrocnemius muscles, HFrD elicited upregulation of leptin, the atrophy associated PAI-1 and the oxidative stress-associated Nox2 and induced downregulation of myosins specific to fast and slow-twitch myofibers. In HFrD-fed, Ang 1-7 treated rats, the expression of these myosins was upregulated and the level of PAI-1 returned to control levels. Hence, Ang 1-7 downregulated pathways involved in deleterious effects of HFrD in skeletal muscle. Further, Ang1-7 enhanced the expression of slow and fast myofibers, possibly indicating new mechanisms by which sarcopenia can be retarded during HFrD. Together, these results point to molecular pathways by which Ang1-7 provides multi-system protection from the metabolic sequels of exposure to high fructose.

VITAMIN D METABOLITES AND VITAMIN D LESS-CALECMIC SYNTHETIC ANALOGS INDUCE REACTIVE OXYGEN SPECIES (ROS) FORMATION AS A SIGNAL TO INHIBIT HUMAN ARTERIAL VASCULAR SMOOTH MUSCLE CELL PROLIFERATION

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Although most vitamin D's actions are traditionally ascribed to 1, 25$(OH)_2D_3$ [1,25D] acting on classical vitamin D receptors, there is now evidence that 24, 25 $(OH)_2D_3$ [24,25D], formerly considered merely an inactivation product, has important independent biological effects as well. Synthetic less-calcemic vitamin D analogs are presumed to act through classical vitamin D receptors but induce lesser rise in serum calcium *in vivo*. Here we examined the effects of these various vitamin D receptor modulators on ROS in arterial vascular smooth muscle (VSMC) harvested from the human umbilical artery, in the context of their known modulatory effects on VSMC proliferation as reported by us in earlier communications (Am. J. Hypertens. 2000; 13:396; J. Steroid Biochem. Mol. Biol. 2004; 89-90:397; Circulation 2005; 111:1666). With the exception of very low concentrations, [1,25D], [24,25D], and 25 (OH)D3 [25D] and the less calcemic synthetic analogs JKF and QW, all decreased VSMC proliferation by 30-60% along with parallel increments in cell metabolic activity as reflected by the ATP-generating system creatine kinase BB (CK). These vitamin-D related agents also increased ROS formation as examined by direct visualization in a fluorescent microscopy system. There were differences in the induction of ROS, which was minimal with [25D] and [1, 25D], potent with [24,25D] and extremely potent with JKF and QW. When the formation of ROS was blocked by diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, the effect of all vitamin D-related compounds on ROS formation was entirely aborted. Likewise, in the presence of DPI, none of the vitamin D-related compounds was able to inhibit VSMC proliferation and CK induction was also attenuated. These results establish a link between the inhibitory effect of vitamin D metabolites and analogs on VSMC growth and ROS formation. ROS formation apparently serves to allow the transduction of vitamin-D induced signals aimed at slowing down VSMC proliferation. This is an energy requiring process which is also blocked when ROS generation is inhibited. These events in the vasculature must be further studied especially in times in which liberal use of mega-doses of vitamin D in clinical medicine is highly fashionable.

THE GLUCOKINASE MUTATION T206P IS COMMON AMONG MODY PATIENTS OF ASHKENAZI JEWISH DESCENT

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Maturity onset diabetes of the young (MODY) is characterized by an autosomal dominant mode of inheritance, a primary defect in insulin secretion with nonketotic hyperglycemia, the age of onset under 25 years and a lack of autoantibodies. Eleven genes were found to be involved in the etiology of the disease, while glucokinase (GCK), Hepatic Nuclear Factor 1 α (HNF1α) and Hepatic Nuclear Factor 4 α (HNF4α) are the most common cause.
The aim of the study was to characterize the genetic basis of MODY in the different ethnic groups of the Israeli population.

The cohort included 151 patients with clinically identified MODY and their first degree family members. The coding regions including the intron–exon boundaries of GCK, TCF1 and HNF4A were examined. Molecular analysis of the three genes was performed on genomic DNA. Exons of the three genes were amplified by PCR with specific primers. All PCR products that showed consistent abnormal migration on DGGE were subjected to sequence analysis and compared to the GeneBank sequence.

Mutations were identified in only 32 families with a distribution of 3% in HNF4alpha, 78% in GCK and 19% in HNF1alpha. All these mutations were family specific, except T206P. This mutation was identified in 6 unrelated families, all from a ethno-origin, thus indicating an ethno-genetic correlation. A simple, fast and relatively cheap restriction-digestion assay was developed to identify this mutation in Jewish- Ashkenazi patients.

We propose that clinically identified GCK-MODY patients of Jewish-Ashkenazi origin be first tested for this mutation.

THE INSULIN-LIKE GROWTH FACTOR I RECEPTOR (IGF-IR) TRANSLOCATES TO THE NUCLEUS AND AUTOREGULATES *IGF-IR* GENE EXPRESSION

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Introduction: The IGF system plays a crucial role in the biology of breast cancer. Most of the biological actions of IGF-I and IGF-II are mediated by the IGF-IR, a membrane-bound heterotetramer with antiapoptotic and cell survival activities. Recent studies have shown that the IGF-IR can be modified by the small ubiquitin-like modifier protein-1, 2, and/or 3 (SUMO), with ensuing translocation to the nucleus. The functional significance of IGF-IR SUMOylation in the specific context of breast cancer has yet to be elucidated.

Aim: To investigate the potential nuclear localization of IGF-IR in both cells and to address the putative autoregulation of *IGF-IR* gene expression.

Material and Methods: Human breast cancer-derived MCF7 cells (ER-positive) and C4.12.5 (ER-depleted) cells were derived by clonal selection of MCF7. A proteomic approach based on genomic *IGF-IR* DNA affinity chromatography followed by Western blot analysis was used to verify association of nuclear IGF-IR with the *IGF-IR* promoter. ChIP analysis was performed to confirm the results. IGF-IR and insulin receptor (IR) subcellular localizations were assessed by confocal microscopy.

Results: Among other proteins found to bind to the *IGF-IR* promoter we identified the IGF-IR in ER-depleted, but not ER positive, breast cancer cells. ChIP analysis confirmed the direct *in vivo* binding of IGF-IR to *IGF-IR* promoter DNA, suggesting that the IGF-IR (or a fragment) may act as transcriptional enhancer. The functional relevance of binding data was assessed by cotransfection experiments with IGF-IR expression vectors along with an *IGF-IR* promoter luciferase reporter. Furthermore, confocal imaging experiments detected IGF-IR and IR staining in the cytoplasm, nuclear and perinuclear areas in both cells.

Conclusions: Our studies demonstrate that IGF-IR is localized in the nuclear areas and that nuclear IGF-IR may act as a modulator of its own promoter. Taken together, we provide evidence that the IGF-IR may act as a transcriptional enhancer.