Potential Role of SHP-1 in Angiotensin II-induced Insulin Resistance

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To:
The Honors College
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In partial fulfillment of the
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To: Honors College, Oakland University

It has been a pleasure serving as a Thesis Mentor for Antonio Barbat.

His research into the expression and activity of the phosphatase SHP-1 in insulin resistance has resulted in surprising results. We anticipated that canine tissue would respond similarly to rodent tissues that others have published in the literature. What Antonio's study has demonstrated is that not only is SHP-1 not involved in the Angiotensin II-induced insulin resistance but that there are significant differences between rodent and canine responses. This is very important for future pre-clinical studies. Rodents are the primary pre-clinical model but these data suggest that they might not be the most accurate predictors of higher mammalian responses. These data are therefore very important clinically and we will be following up on his results. Antonio has worked very hard on this project, and while aided in some of his studies by other members of my laboratory, he has clearly driven this project. Antonio is leaving soon for Medical school and they are very lucky to be able to recruit him.

Sincerely,

Amy K. L. Banes-Berceli

Amy Banes-Berceli, PhD
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Acknowledgements

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Additionally, I would like to thank my peers in Dr. Banes-Berceli's laboratory over the past few years, for helping me complete the experiments and gather the data involved in this project. Namely, I would especially like to thank Anthony Duncan, Jonathan Bucan, Bernard Le Page, Tudor Moldovan, Marissa Cervantes and Zachary Walker for all their help. I would also like to thank our collaborator, Dr. Michael Brands, at the Medical College of Georgia, for providing the tissue samples used in our experiments.

Lastly, I would like to thank the Honors College at Oakland University for their hard work and for the opportunity given to me, to work on this project. This research has immersed me in the scientific process and has enriched my undergraduate learning experience.

Abstract

There are many contributing factors to the development of Type II diabetes mellitus (DM2), including both genetics and lifestyle factors, such as poor diet and lack of exercise. On the molecular level Diabetes Mellitus Type 2 (DM2) is characterized by the development of insulin resistance, in which cells do not correctly respond to the effects of insulin. The molecular mechanisms that are responsible for the development of insulin resistance are not well understood and are of significant importance, considering the high prevalence of DM2 in our population. It is known from the current literature that the proteins AKT, PI3Kinase, JAK2, SHP-1 and PTEN are involved in the pathology of DM2, but there is much to be learned yet. This thesis investigated the role of the intracellular protein SHP-1 to determine if Angiotensin II utilized it in the molecular mechanisms of insulin resistance. I used sample tissues from canine kidney cortexes that were treated with various concentrations of insulin and Angiotensin II. These samples were generated by our collaborator at the Medical College of Georgia. We hypothesized that an increase in SHP-1 activity would result in decreased AKT activation, leading to insulin resistance and eventually DM2.

Abbreviation-Expansion Reference Table

Abbreviation	Expansion
SHP-1	Tyrosine-protein phosphatase non-receptor type 6
SHP-2	Tyrosine-protein phosphatase non-receptor type 11
AKT	Protein-serine/threonine kinase- also known as Protein Kinase B
PKB	Protein Kinase B
JAK	Janus Kinase
JAK2	Janus Kinase 2
PTEN	Phosphatase and Tensin Homolog
AG490	Tyrphostin (inhibitor of JAK2)
GLUT4	Glucose Transporter Type 4
IR	Insulin Receptor
IRS-1	Insulin Receptor Substrate-1
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PDK-1	Pyruvate Dehydrogenase Lipoamide Kinase Isozyme 1
PI3K	Phosphoinositide 3-kinase
AdV	Adenovirus
(DN)SHP-1	Dominant Negative SHP-1
mRNA	Messenger Ribonucleic Acid
ERK	Extracellular Signal-Related Protein Kinase
HepG2cells	Human Liver Carcinoma Cells
ANG II	Angiotensin II
RAS	Renin-Angiotensin System

ARBs	Angiotensin Receptor Blockers
ACE	Angiotensin Converting Enzyme Inhibitors
STAT	Signal Transducer and Activator of Transcription
DMEM	Dulbecos Modified Media
dH_2O	Distilled Water
INS	Insulin
PBS	Phosphate Buffered Saline
PMSF	Phenylmethylsulfonyl Fluoride
EGTA	Ethylene Glycol Tetraacetic Acid
SDS-polyacrylamide gels	Sodium Dodecyl Sulfate- Polyacrylamide Gels
BSA	Bovine Serum Albumin
TBS-T	Tris Buffered Saline-Tween 20
TBS	Tris Buffered Saline
ECL	Enhanced Chemiluminescence
SEM	Standard Error of the Mean

Introduction

The molecular mechanism for insulin resistance has proven to be quite complex due to the sheer number of proteins involved, and the many different pathways each is involved in. Before exploring the pathophysiology, it is important to understand the physiology of insulin and molecular mechanism in which it exerts its effects, under normal conditions. Insulin is a peptide hormone secreted by pancreatic beta cells, such as when stimulated by chemoreceptors after plasma blood glucose rises after a meal [1]. Insulin mainly functions to regulate carbohydrate, protein and lipid metabolism, but also has mitogenic effects [1,2]. For the purposes of this thesis, we will focus on the relation of insulin and carbohydrate metabolism. Insulin exerts its effects *via* binding to the insulin receptor (IR), a transmembrane tyrosine kinase receptor [2,5]. Activation of this receptor enables tyrosine mediated phosphorylation of the insulin responsive substrates (IRS), which are a family of intracellular substrate proteins [2]. This group of proteins perpetuate the effects of insulin, through downstream binding and activation of other signaling molecules [1,2]. One of these signaling molecules being the enzymatic protein, Phosphoinositide 3-kinase (PI3K), which is one of the main mediators of the metabolic effects of insulin [1,2]. PI3K then phosphorylates PIP2 into PIP3, which activates AKT (or PKB) [1]. In turn, PDK-1 is recruited and phosphorylates AKT [2]. AKT activates several proteins, which mediate the main effects of insulin [1]. These effects include the translocation of glucose transporter proteins to the plasma membrane, and synthesis of proteins, lipids and glycogen [1,2,5]. Additional effects include anti-lipolysis and promotion gluconeogenesis [1,2,5].

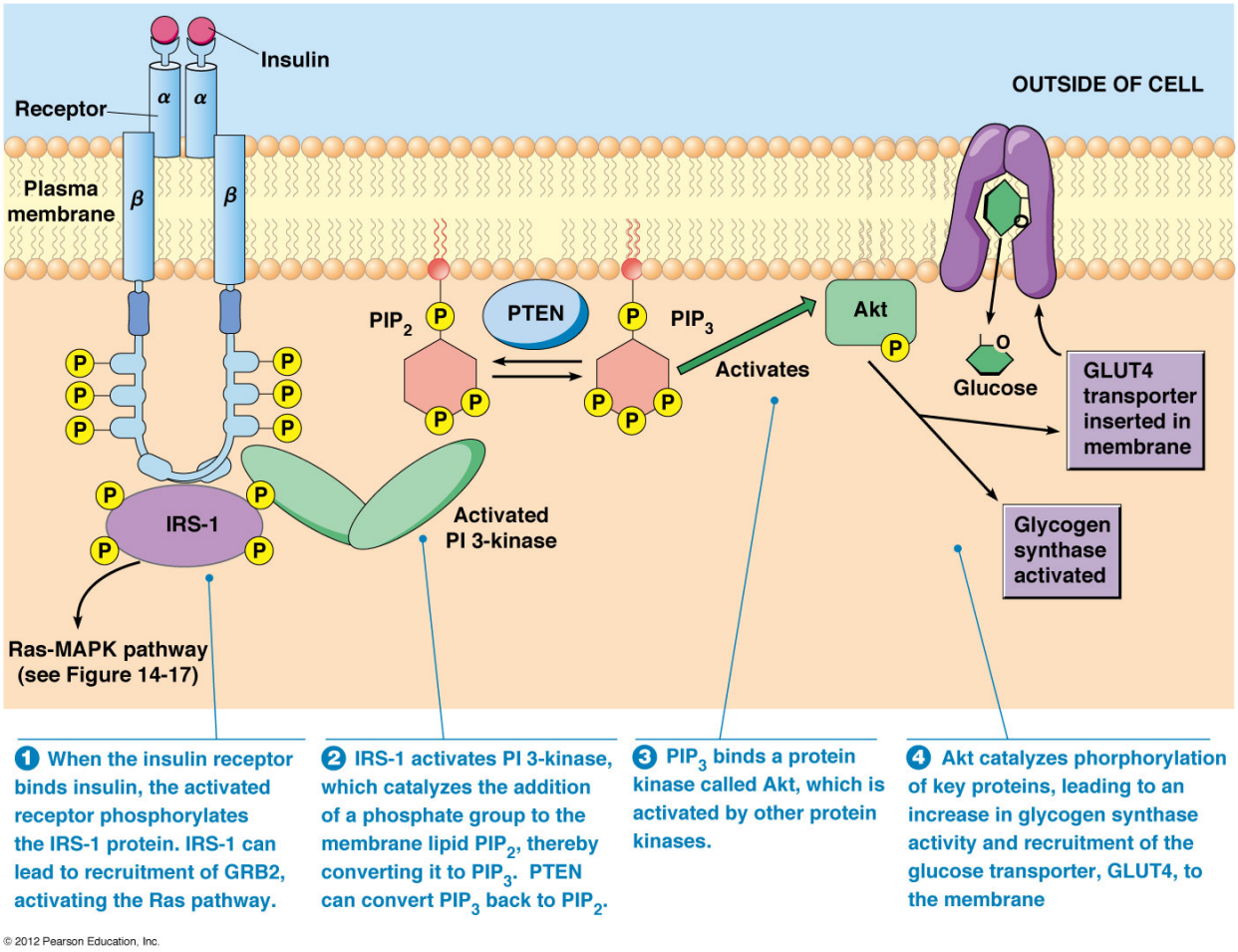
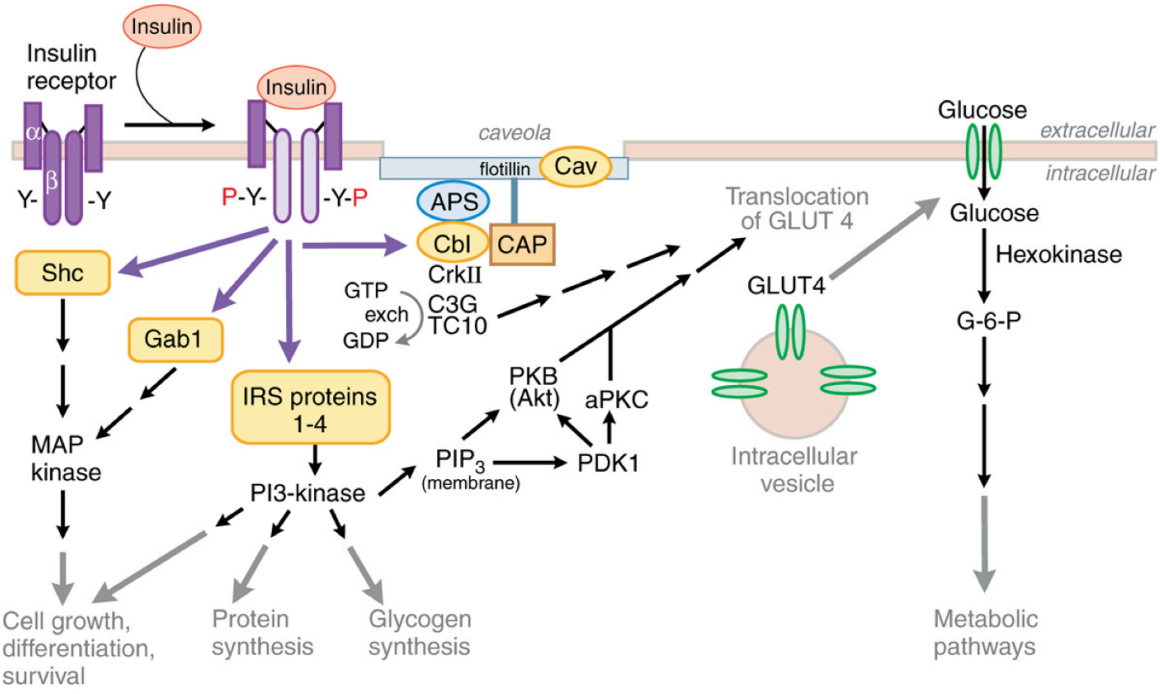


Figure 1. Insulin receptor signaling cascade: PI3K mediated induction of GLUT4. Copyright 2012 by Pearson Education Inc.

There are five identified subtypes of the glucose transporter proteins (GLUT1 through GLUT5), each of which have different characteristics and differential tissue expression [2,5]. These transporters are what allows glucose to enter the cell. In turn, the glucose is either used for energy by the cells immediately or converted to glycogen and stored until glucose is needed for energy once again [2]. GLUT4 will specifically be pertinent to this study and is primarily found in skeletal and cardiac myocytes, liver and adipose cells [2]. GLUT4 is stored intracellularly and expressed on the plasma membrane *via* exocytosis, as a result of the intracellular pathway described above [2,5].

Insulin resistance is characterized by a defect in the molecular mechanism allowing insulin to activate translocation of the GLUT4 transporter by the liver, muscle, and adipose tissue cells [2]. GLUT4 must be translocated to the cell surface to transport glucose into the cells. This signaling defect renders the body less sensitive, and eventually incapable of responding to insulin's stimulation of glucose uptake [1]. To compensate for this, the beta cells will then secrete additional insulin resulting in hyperinsulinaemia or excessively high plasma levels of insulin [3]. Eventually, this lack of response to insulin will cause hyperglycemia or elevated blood glucose levels



http://basicmedicalkey.com/endocrine-pancreas-and-pharmacotherapy-of-diabetes-mellitus-and-hypoglycemia-2/

Figure 2. Insulin receptor signaling cascade: Schematic view of main pathways involved. Source above.

Due to the overstimulation to compensate for the resistance, pancreatic beta cells will eventually fail and die [3]. Insulin resistance, and the compensatory hyperinsulinaemia lead to

various metabolic and cardiovascular complications [2]. These complications can include DM2, obesity, dyslipidaemia, coronary artery disease, kidney disease and hypertension [4]. Sleep apnea, and ovarian dysfunction have also been known to occur [3]. It is important to note that insulin inhibits gluconeogenesis (synthesis of glucose from other substrates) and glycogenolysis (breakdown of glycogen, which is the polysaccharide glucose is stored as), in the liver [1]. In the insulin resistant state, these two processes will not be inhibited and the liver will exacerbate hyperglycemia, by releasing more glucose into the bloodstream [2,4].

The consequences of insulin resistance are extremely concerning when you consider what exactly is happening. As stated, when the affected cells are no longer sensitive to insulin and the pancreas tries to compensate, hyperinsulinaemia will occur [2]. When hyperinsulinaemia and hyperglycemia are simultaneously occurring, the body is going to be under twice as much stress [4]. Each condition comes with somewhat different symptoms, and each condition renders the body less able to provide its cells with the adequate energy need to maintain homeostasis [3]. Eventually, pancreatic beta cells will die from exhaustion and the liver is no longer inhibiting gluconeogenesis or glycogenolysis [4]. At this point, the body has no insulin available and further development of pre-diabetes and eventual DM2 occur [4].

Three of the most common signs of uncontrolled DM2 include polyuria (excessive urination), polydipsia (excessive thirst) and polyphagia (excessive hunger) [21]. To understand the causes of these symptoms, we must understand the consequences of hyperglycemia. In a normal healthy patient, there should be little to no glucose in the urine. This is because the kidney nephrons reabsorb almost all the glucose passing through, back into the bloodstream. In a patient with hyperglycemia, the transporters in the nephron become overloaded with glucose and are unable to reabsorb all the excess glucose. This results in glucose remaining in

the filtrate (which is what eventually becomes urine), resulting in a hyperosmotic environment inside the nephron tubules. Using the basic biochemical principle of osmosis, more water is excreted with this highly concentrated filtrate. This is the basis for the polyuria observed in DM2 and it results in hypotension, dehydration and electrolyte imbalances [21]. These patients reciprocally experience polydipsia, as a means to rehydrate their body and increase blood pressure. Given that patients with uncontrolled DM2 are not efficiently utilizing, or eventually discontinue producing insulin, there is no means for glucose to enter their cells. The body interprets this as a deficiency of glucose in general and stimulates the patient to eat, which is the cause of polyphagia [21]. This is a vicious cycle, because the patient is further exacerbating their hyperglycemia by eating more. Other adverse events secondary to hyperglycemia include improper glycosylation of proteins (rendering them nonfunctional), formation of reactive oxygen species (which alter intracellular signaling, among other things) and alternation in other intracellular or growth responses [21].

Long-term complications of DM2 are even more worrisome and mainly effect the cardiac and renal systems. These complications can either effect the larger vessels of the body (macrovascular complications) or the smaller vessels of the body (microvasculature complications). Macrovascular complications include congestive heart failure, cerebrovascular accidents and peripheral vascular disease (eventually leading to gangrene and possible amputation of the lower extremity). Given these risk, diabetics are often initiated on anti-dyslipidemia therapy (most commonly a statin agent). Microvascular complications include retinopathy (decrease in eyesight, leading to eventual blindness and other ocular issues), neuropathy (degradation of peripheral and autonomic nerve function, manifesting as nerve pain, paresthesia and gangrene) and nephropathy (initially presents as microalbuminuria and

eventually leads to end stage renal disease). [22]

Current treatment and management of DM2 include diet/lifestyle changes, oral pharmacological agents and subcutaneous insulin injections [2,23]. This is not including treatment for the comorbidities of DM2, which as mentioned above include dyslipidemia, hypertension, other cardiovascular diseases and obesity. Initial therapy usually consists of diet/lifestyle changes and educational changes, with an oral agent [23]. This oral agent is usually Metformin, a drug in the biguanide class [2,23]. Biguanides work to increase cell sensitivity of insulin (hence, these drugs require the presence of insulin and functioning beta cells), and are also thought to block gluconeogenesis [2,23]. Further progression of DM2 requires additional oral agents to be added to the treatment regimen and eventually subcutaneous insulin injections may be needed [23]. These therapies are not perfect and often carry the risk of causing hypoglycemic events [23]. Patients are usually required to monitor their blood glucose when on insulin, to prevent these hypoglycemic events. Additionally, patients can be on regimens requiring several injections of insulin throughout the day, to efficiently mimic the normal post-prandial insulin secretions of the body [23]. Given the inconvenience of the aforementioned methods, there is often low patient compliance with these therapies.

According to the American Diabetes Association (ADA), 9.3% of the American population suffers from DM. Of that 9.3%, about 95.7% of those cases are DM2 and about 27.8% of those cases are undiagnosed. Furthermore, 1.4 million Americans are diagnosed with DM each year. In 2010, DM was the 7th leading cause of death in the United States. It was estimated that in 2012, the total medical costs of DM in the United states was $176 billion and the total cost in reduced productivity was $69 billion. [20]

This is clearly a serious problem for our individual patients and for our population health. To develop better treatments for DM2 and other complications that stem from insulin resistance, it is most efficient and beneficial to understand the root of the problem- the initiating mechanisms of insulin resistance. Revealing this molecular mechanism may allow us to develop drugs that treat insulin resistance before pancreatic beta cell death and development of DM2. New treatment methods may also help avoid the low patient compliance associated with current insulin injections. Determining the causes of insulin resistance, as well as methods to reverse insulin resistance is a very active area of research. As mentioned, there are many proteins regulating and contributing to the insulin-signaling pathway, and research can involve experimenting what the up regulation or down regulation of a protein would result in.

It is known that the protein PTEN reverses the phosphorylation of PIP2 by PI3K [6], and other portions of the insulin-signaling pathway. In a study done by Rosivatz, it was shown that inhibition of PTEN resulted in an increase of AKT phosphorylation [10]. This coincides with the notion that PTEN is inhibiting PI3K, and suggests that PTEN could possibly be a new drug target. The tyrosine phosphatase, SHP-1, has also been shown to prevent insulin's actions by inhibiting PI3K directly and activating PTEN [7].

In a study done in 2011, researchers inhibited SHP-1 and observed the results [18]. The researchers used an adenovirus (AdV)-mediated gene transfer process to express an interfering mutant of the SHP-1 gene in the skeletal muscle of mice [18]. The mutation produced-(DN)SHP-1 which was specifically expressed in L6 myocytes [18]. The expression of this mutant gene increased glucose uptake and glycogen synthesis, by increased insulin-induced phosphorylation of the AKT pathway [18]. Additionally, they found that GLUT4 mRNA, and protein expression were also being enhanced due to expression of (DN)SHP-1 [18]. The results

of this study indicate that SHP-1 not only down-regulates AKT expression, but also GLUT4 translocation and expression [18]. The mechanisms by which a phosphatase exerts these effects are not yet understood. However, these actions of SHP-1 clearly promote insulin resistance.

Interestingly, in a very recent study done, the extract of the Perilla plant stem was shown to inhibit the effects of SHP-1 pertaining to insulin resistance [19]. The researchers used an *in vitro* based experiment, and found that the extract inhibited the catalytic domain of SHP-1 [19]. Furthermore, their research showed that the extract was more sensitive to SHP-1 versus other protein tyrosine phosphatases, possibly meaning SHP-1 may be the target of the Perilla stem extract [19]. Specifically, there was increased phosphorylation of IR, and extracellular signal-related protein kinase (ERK) in HepG2cells and an increase of activation in the insulin signaling mechanism [19]. This study has paved the way for further studies regarding the extract of the Perilla plant, and shows a promising future.

Angiotensin II, which has an important role in renal and vascular homeostasis, has also been shown to be involved in insulin resistance [9]. Angiotensin II was shown to effect liver, muscle, and adipose tissue where it exerts its effects on IR, IRS, PI3K, AKT, and GLUT 4 [9]. The molecular mechanism by which angiotensin II (ANG II) utilizes is not yet fully understood, and is highly debated. In a study done by the Center for Research and Advanced Studies of the National Polytechnic Institute, it was shown that ANG II promotes insulin resistance [9]. In the clinical trials preformed, after inhibition of the renin-angiotensin system (RAS), development of type 2 diabetes-induced complications were prevented and ANG II inhibiting drugs improved insulin sensitivity [9]. Specifically, when hypertensive patients were treated with angiotensin receptor blockers (ARBs), and angiotensin converting enzyme inhibitors (ACE) insulin sensitivity was improved [9].

An interesting study done at the Medical College of Georgia researched the roles of the proteins SHP-1 and SHP-2 in angiotensin II induced JAK2 phosphorylation [16]. The researchers found that SHP-1 and SHP-2 have opposite effects in the regulation of the ANG II induced JAK/STAT pathway [16]. It was founded that SHP-1 was involved in dephosphorylation of JAK2 and therefore termination of the pathway, while SHP-2 was involved with phosphorylation of JAK2, and initiation of the pathway [16]. This pathway is important and plays a role in transcriptional cell growth [16]. Although SHP-1 is involved in downregulation of JAK2 in this model and in others, this correlation has not yet been shown in insulin resistance. When SHP-1 is expressed in high concentrations, as it is in insulin resistance, one would expect that JAK2 activation would be decreased. However, this has not yet been demonstrated in insulin resistance. In overt diabetes there is an increase in JAK2 which is associated with the development of complications [16]. ANG II has also been shown to activate JAK2 in pathological conditions to mediate its effects [16]. Whether this is occurring in insulin resistance induced by ANG II is as yet unknown.

Another pathway that is regulated by SHP-1 is the PI3-kinase pathway and its negative regulator PTEN. Inhibiting PI3 kinase is not the only function of PTEN. PTEN is also known to be a tumor suppressor [11]. According to a study done at the University of Geneva, dysregulated PTEN is correlated to the development of liver disease [11]. According to the study, dysregulated PTEN is associated with diabetes, obesity, insulin resistance, hepatitis B and hepatitis C virus infections, and abusive alcohol intake [11]. Mutations and deletions have also been associated with cancer of the liver [11]. Therefore, it can be said that PTEN contributes more to just insulin resistance, and is a complex molecule in itself [11]. Further research may help us gain a better understanding of how the different pathologies correlated with PTEN can

affect each other, making the production of future medication more conceivable.

Additional research has further linked type II diabetes, obesity, and cancer to PTEN. A study conducted at the University of Oxford measured insulin resistance and beta cell function in 30 different patients [12]. 15 of those patients were carriers for PTEN haploinsufficiency (one copy of the PTEN gene was inactivated by a mutation leaving the patient with only one functional copy [13]), and the other 15 patients were matched controls [12]. As expected insulin resistance was less prevalent in the patients with the PTEN mutation because as stated earlier, PTEN suppresses the mechanism in which insulin acts on [12]. Additionally, the patients with the PTEN mutation were obese compared to the controls [12]. Furthermore, the patients with the PTEN mutations also had a high risk of cancer-predisposition syndrome [12]. Cancer predisposition syndrome is hereditary predisposition to cancer due to genetic mutations [14]. This does not mean the patient will always develop cancer, but the likelihood is high [14]. This information coincides with PTEN being a tumor suppressor. This study has exemplified that PTEN mutations lead to not only a decreased risk of type II diabetes, but also an increased risk of cancer and obesity. This makes the mechanism of insulin resistance more problematic because suppression of PTEN to reduce insulin resistance may cause other pathologies to occur.

The mechanisms of insulin resistance are intricate, and still somewhat unclear. There have been many new discoveries, but there are also just as many questions to be answered. It is important to gain a deeper understanding of this topic due to its increasing prevalence in our society, and the threats it poses. The purpose of this study is to get a better understanding of the initiating molecular mechanisms by which insulin resistance results from and how SHP-1 is involved. This information can help isolate important proteins, and eventually may pave the way for new medication to treat the complications associated with insulin resistance. We hypothesize

that ANG II causes an increase in SHP-1 activation which results in decreased AKT activation, leading to insulin resistance and eventually DM2.

Methodology

Rationale for Study Design:

This experiment was conducted *in vitro* versus *in vivo* to isolate the initial molecular steps and to minimize confounding variables. With an *in vitro* approach, it was possible to control the glucose levels within the cell culture to the needed specifications. The samples were prepared using normal glucose levels. The samples were also treated with different concentrations of insulin. For example, the samples were treated with insulin levels consistent with what is maintained in homeostasis, and insulin concentration levels consistent with insulin resistance. Hyperinsulinemia (high levels of insulin) are common in the early stages of insulin resistance and we mimicked this in culture conditions to isolate the effects of high insulin from high glucose.

Sample Preparation:

These methods have been previously published by my mentor [17]. A collaborator at the Medical College of Georgia provided all the tissues that were used. Dog kidneys were removed and placed into physiological buffered saline solution. The samples were dissected and placed into warmed Dulbecos Modified Media (DMEM). They were maintained at 37°C throughout the experiment. Tissues were incubated with the appropriate treatments: control (dH_2O), Angiotensin II (ANG II; 100 nM 60 min) Insulin (INS; 100 nM, 5, 10 or 30 min) alone or in combination where ANG II was added 60 minutes prior to the addition of INS. Tissues were then removed,

washed with ice cold PBS 3 times and then homogenized with a tissue lysis buffer Tissues in a 255 mM sucrose/10mM Tris buffer (pH 7.4) with protease (0.5 mM PMSF, 2mM EGTA, 10 μg/ul aprotinin and 10 μg/ml leupeptin) and phosphatase inhibitors. Homogenates were centrifuged (14,000g for 10 minutes, 4°C) and supernatant total protein (Bio-Rad) were measured.

Western Blotting:

As previously published by my mentor's laboratory [17], the supernatant from the tissue samples and the cell lysates were briefly boiled for 5 minutes and separated on SDS-polyacrylamide gels (SDS-PAGE; 7.5% polyacrylamide) and transferred onto nitrocellulose membrane. Membranes were blocked for 1 hour in 5% BSA tris buffered saline with tween (0.01%; TBS-T) solution and were then washed (twice with TBS-T rinse, 5 minute TBS). Incubation was done overnight at 4°C using appropriate antibodies for either with phospho-specific or total forms of the proteins being investigated. Blots were then washed once with TBS-T for 20 minutes and twice with TBS for 10 minutes. Membranes were incubated for 1 hour at room temperature using either mouse or rabbit anti-IgG horseradish peroxidase as the secondary antibody. Membranes were then developed with enhanced chemiluminescence (ECL) in a darkroom using autoradiography film to visualize labeled bands. After developing with phospho-specific antibodies, membranes were incubated in stripping reagent and analyzed to compare the total protein and phosphorylated protein levels. Blots were also probed for β-actin to ensure equal protein loading in all wells. Data was analyzed with Image J software to determine the pixel density of the bands.

Data analysis and statistics:

Data has been presented as means with standard error of the mean (SEM) for the number of trials in parentheses. Statistical analysis for the Western protein blot data was carried out with the Graph Pad Prism program (GraphPad Software, Inc. San Diego, CA). The appropriate Student's t test was used when comparing two groups. One-way ANOVA followed by a Student-Newman-Keuls post hoc test was performed when comparing three or more groups to determine significance. Band density was quantified using the program NIH Image.

IACUC

No IACUC approval was required for this project. All the tissues were already provided from a collaborator at the Medical College of Georgia. No animals were used in the studies proposed here.

Results

As a first step we first incubated the tissues with ANG II and Insulin for multiple time points to determine the responses. As we can see in Figure 3 treatment with ANG II alone decreased Akt activation levels as measured by phosphorylation. Treatment with Insulin increased Akt activation only at 10 minutes. This is consistent with the known time to activation published by others. Treatment with ANG II did prevent the Insulin-induced increase in Akt activation levels.

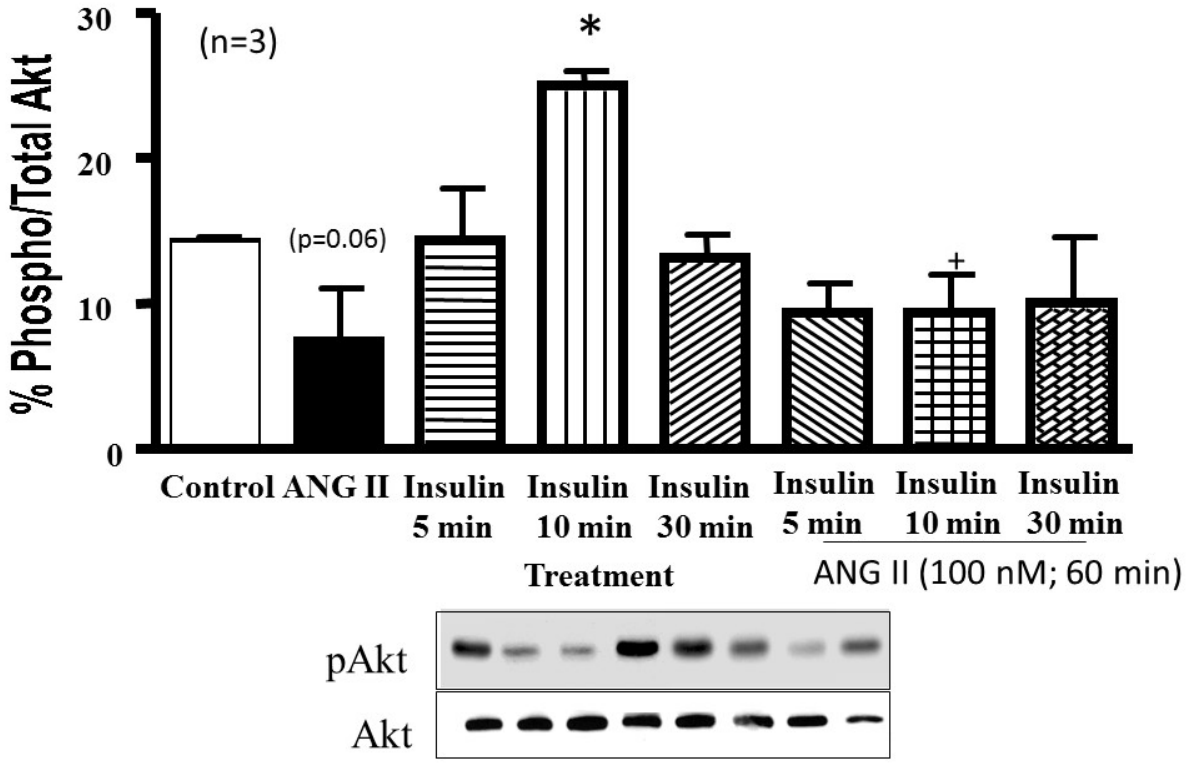


Figure 3. Levels of phosphorylated AKT (pAKT) in canine kidney cortexes, treated with ANG II, Insulin or ANG II + Insulin for varying time points. *Top:* Densitometric analysis of western blots utilizing treated tissues. *Bottom:* Representative western blots.

These data indicated that the optimal incubation time for these experiments was 10 minutes with Insulin so all the rest of the studies used this time. In Figure 4 we examined the immediate upstream regulatory kinase, PDK-1. These data showed that ANG II treatment resulted in a decrease in PDK-1 activation levels but Insulin alone increased the activation. These data were consistent with the Akt data. We expected Insulin to increase levels of PDK-1 as this is a critical step in the pathway to allow for activation of the GLUT-4 transporter.

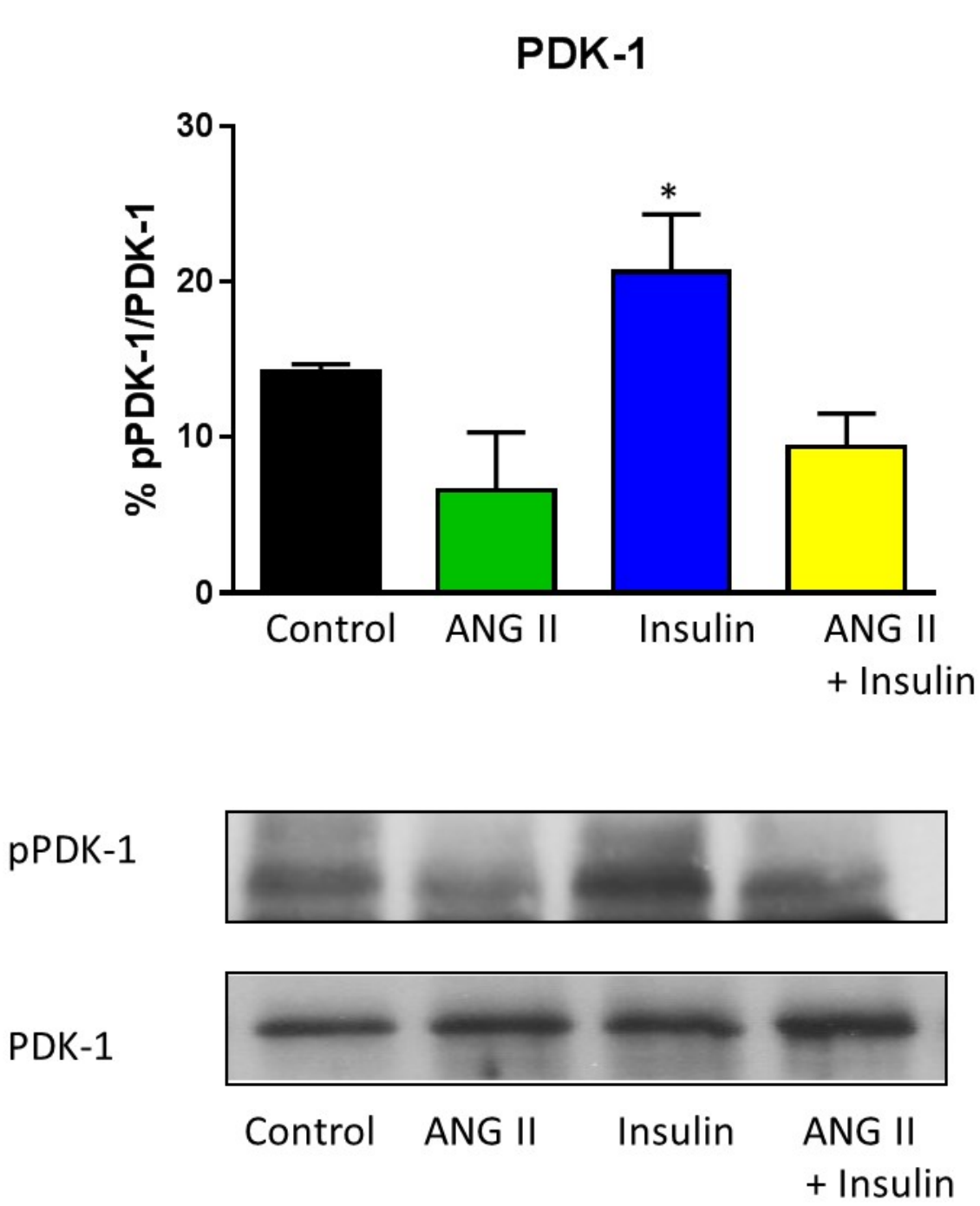


Figure 4. Levels of phosphorylated PDK-1 (pPDK-1) in canine kidney cortexes, treated with ANG II, Insulin or ANG II + Insulin for 10 minutes. *Top:* Densitometric analysis of western blots utilizing treated tissues. *Bottom:* Representative western blots.

We next examined the activation levels of SHP-1. SHP-1 is a phosphatase that must be phosphorylated on the tyrosine 11 residue to become activated. When we examine the phosphorylation levels we did not observe any differences between the treatment groups. The data presented in Figure 5 clearly show no differences in activation of the SHP-1 phosphatase.

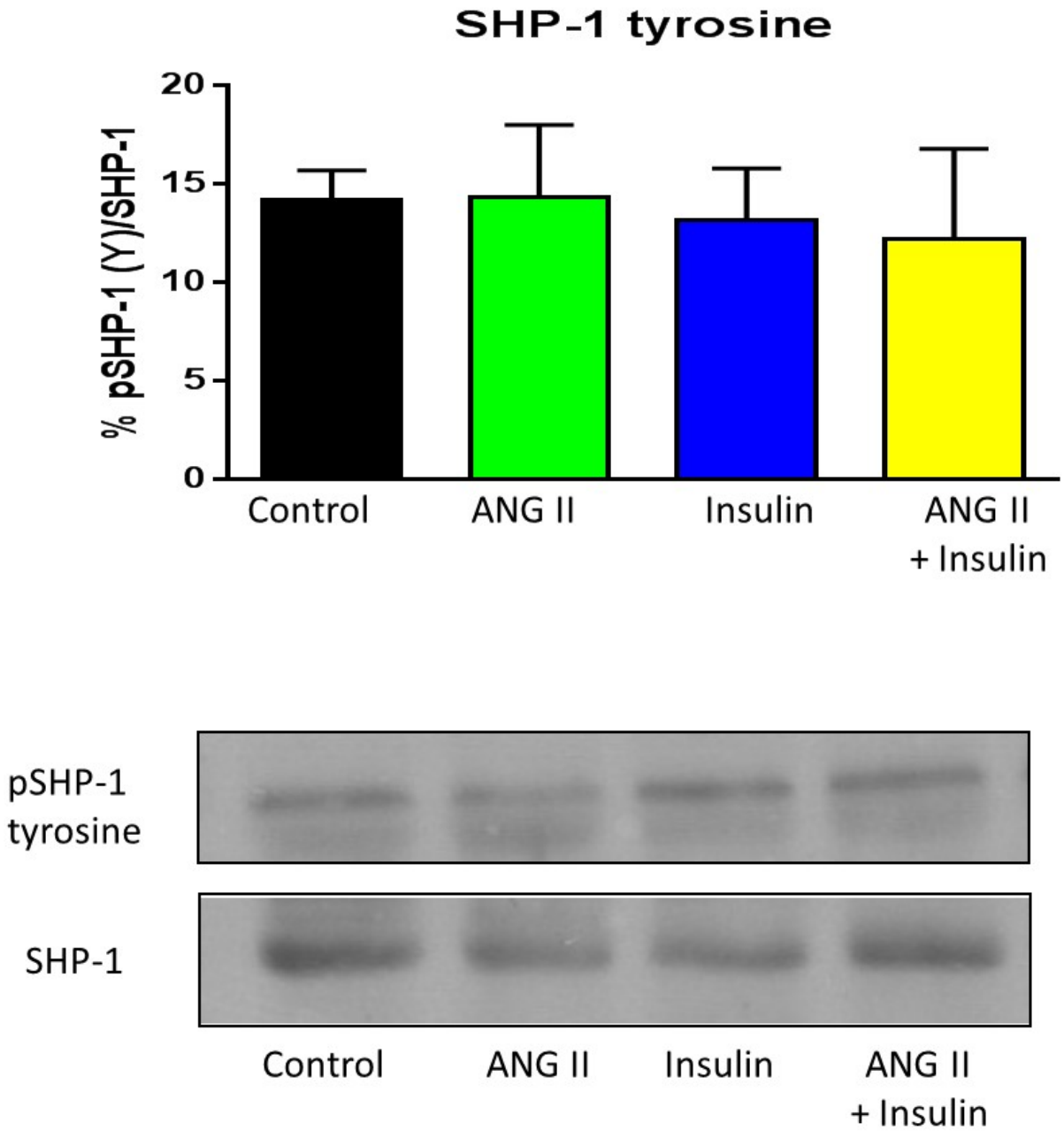


Figure 5. Levels of phosphorylated SHP-1 tyrosine (pSHP-1 tyrosine) in canine kidney cortexes, treated with ANG II, Insulin or ANG II + Insulin for 10 minutes. *Top:* Densitometric analysis of western blots utilizing treated tissues. *Bottom:* Representative western blots.

SHP-1 also has an inhibitory phosphorylation site on Serine 51. To determine if the treatment with ANG II and Insulin had any effect on this inhibitory site we next examined its phosphorylation levels. We found (Figure 6) that there were significant increases in the inhibitory phosphorylation in all the treatment groups when compared to the control group. These data were surprising and clearly indicate that SHP-1 is not responsible for the ANG II-induced signaling changes in the AKT pathway that we observed.

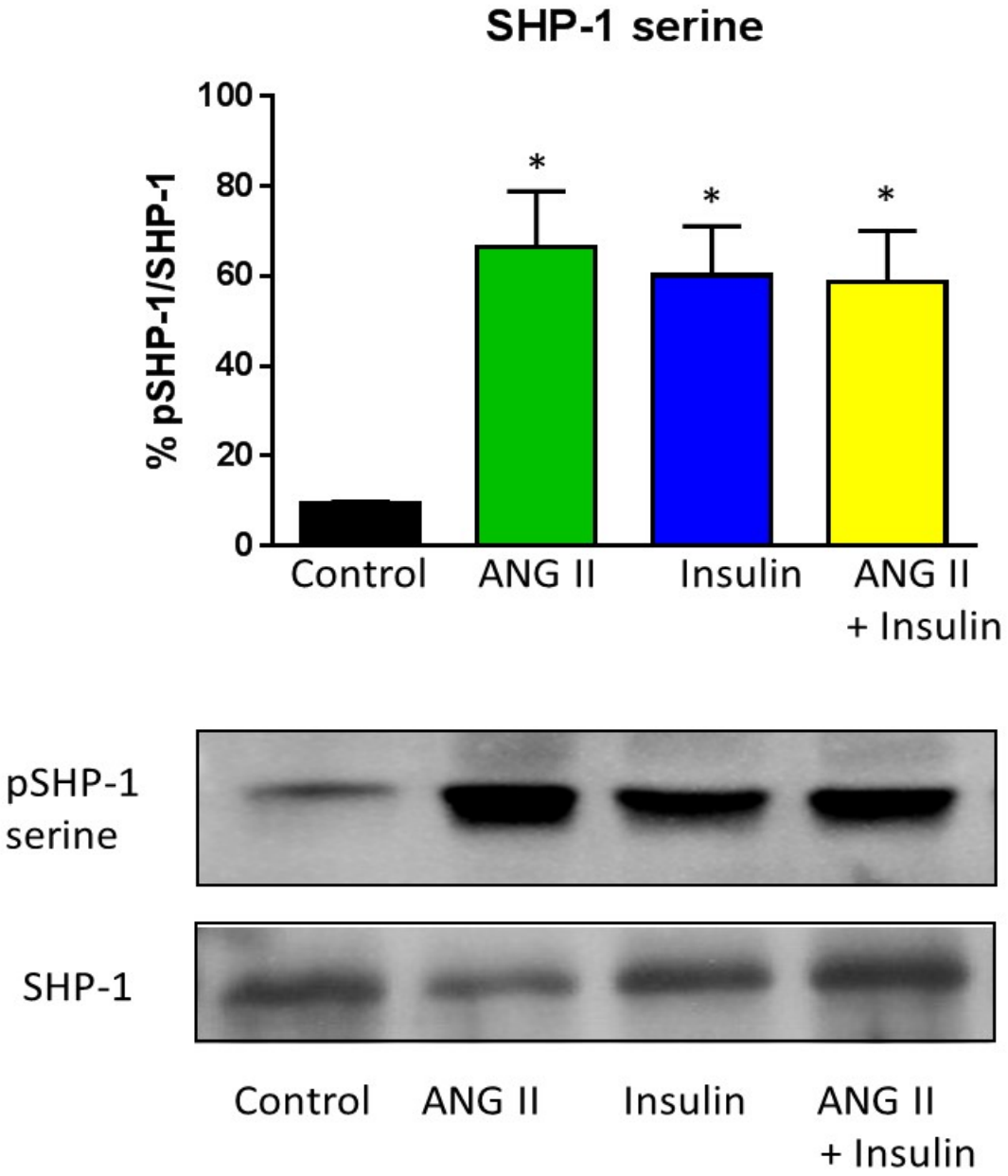


Figure 6. Levels of phosphorylated SHP-1 serine (pSHP-1 serine) in kidney cortexes, treated with ANG II, Insulin or ANG II + Insulin for 10 minutes. *Top:* Densitometric analysis of western blots utilizing treated tissues. *Bottom:* Representative western blots.

Conclusion

Based on our proposed hypothesis in Figure 7 we thought that ANG II induced SHP-1 to directly and indirectly (via activation of PTEN) inhibit PI3K, decreasing AKT activation and downregulating GLUT 4 expression. The results from Figures 3 and 4 show that ANG II inhibits AKT and PDK-1 activation, respectively. This is in-line with the notion that ANG II is a promoter of insulin resistance. What is interesting however, is that SHP-1 does not seem to be a mediator in these described effects of ANG II. Given the results in Figure 5, we know that there is no significant difference in the activation of the SHP-1 phosphatase from ANG II, insulin or ANG II + insulin. Furthermore, the results in Figure 6 show that all three treatments showed a significant increase in phosphorylation of the inhibitory Serine 51 site on the SHP-1 protein. Given this data, our original hypothesis is invalid, as it seems that both ANG II and Insulin inhibit SHP-1. These results suggest that ANG II is exerting its effects through a different mechanism of action. Future research on this pathway should focus on what could be mediating the insulin resistance promoting effects of ANG II, such as the PTEN protein.

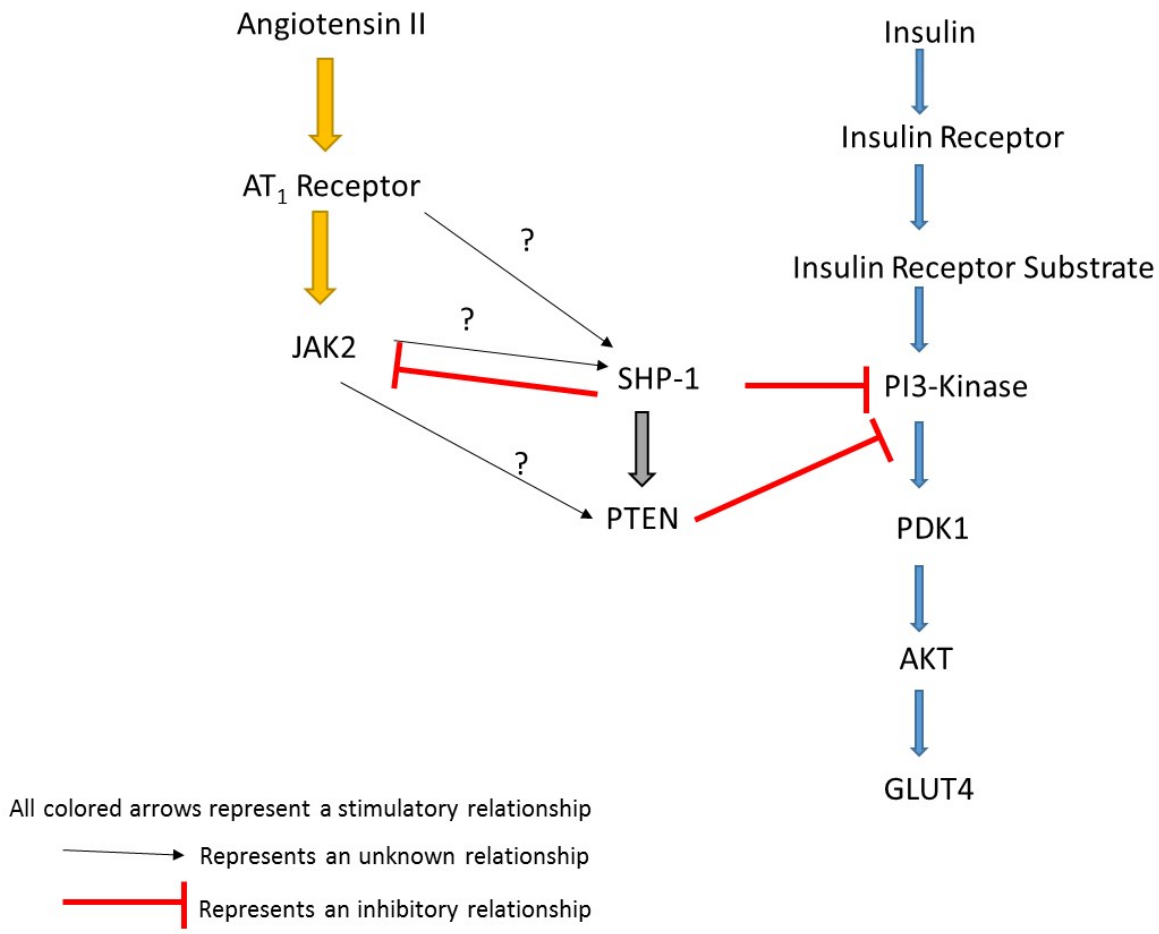


Figure 7. Proposed pathway for the ANG II induced SHP-1 mediated changes on the PI3K/AKT pathway.

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