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Critical Review

Prolactin and Vasoinhibins: Endogenous Players in Diabetic Retinopathy

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Summary

Diabetic retinopathy is a disease of the retinal microvasculature that develops as a complication of diabetes mellitus and constitutes a major cause of blindness in adults of all ages. Diabetic retinopathy is characterized by the loss of capillary cells leading to increased vasopermeability, ischemia, and hypoxia that trigger the excessive formation of new blood vessels in the retina. The influence of the pituitary gland in the pathophysiology of diabetic retinopathy was recognized nearly six decades ago, but the contribution of pituitary hormones to this disease remains unclear. Recent studies have shown that the pituitary hormone prolactin is proteolytically cleaved to vasoinhibins, a family of peptides with potent antivasopermeability, vasoconstrictive, and antiangiogenic actions that can protect the eye against the deleterious effects of the diabetic state. In this review, we summarize what is known about the changes in the circulating levels of prolactin and vasoinhibins during diabetes and diabetic retinopathy as well as the implications of these changes for the development and progression of the disease with particular attention to hyperprolactinemia in pregnancy and postpartum. We discuss the effects of prolactin and vasoinhibins that may impact diabetic retinopathy and suggest these hormones as important targets for therapeutic interventions.

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Keywords diabetic retinopathy; angiogenesis inhibitors; hormones; prolactin; vasoinhibins; 16 kDa prolactin; diabetic complications; retina; pregnancy.

INTRODUCTION

The global prevalence of diabetes mellitus for all age groups was estimated to be 2.8% in the year 2000 and is projected to rise to 4.4% in 2030, corresponding to 366 million people with diabetes. Countries with a particularly high prevalence of diabetes mellitus in 2000 and a strong increase projected for the year 2030 include the United States of America (8.8% in 2000, 11.2% in 2030), Spain (8.7%/12.1%), and Indonesia (6.7%/10.6%) (*1*). Inevitably, the prevalence of diabetic retinopathy, a retinal microangiopathy and one of the earliest complications of the disease, will increase in parallel and will continue to contribute significantly to the burden of diabetes in term of years lived with disability due to loss of vision and blindness. Diabetic retinopathy constitutes the leading cause of blindness in working-age individuals and affects a majority of diabetic patients by 20 years after disease onset (*2*).

The role of hormones in the progression of diabetic retinopathy is indicated by their actions on glucose metabolism, blood pressure, and blood vessel growth, which are direct determinants of the disease. Loss of insulin receptor action and metabolic dysregulation on the retina lead to the progression of diabetic retinopathy (*3*), and clinical trials have shown the benefits of blocking the action of angiotensin (*4*) and the secretion of growth hormone (GH) (*5*). Also, the recognition of pregnancy as a risk factor for the progression of diabetic retinopathy (*6*) points to an influence of reproductive hormones in the pathophysiology of this disease.

In this review, we focus on the pituitary hormone prolactin (PRL), which is able to protect against diabetic retinopathy by its proteolytic conversion to vasoinhibins, a family of PRL fragments that inhibit retinal vasopermeability, vasodilation, and angiogenesis. We discuss the involvement of the pituitary gland in the pathophysiology of diabetic retinopathy reported in early work that may implicate PRL. Attention is given to the changes in the endogenous levels of PRL and vasoinhibins in diabetic retinopathy and to PRL and vasoinhibin actions that may affect the progression and treatment of the disease. Finally, the increased risk for progression of diabetic retinopathy during pregnancy is discussed in the context of a putative interplay between PRL and vasoinhibins.

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DIABETIC RETINOPATHY

Among the risk factors in diabetic retinopathy is chronic hyperglycemia (*7*) which, by producing reactive oxygen species, activates multiple biochemical pathways that lead to retinal microvascular dysfunction (*8*). An early event of vascular damage is the loss of pericytes and endothelial cells resulting in acellular and ischemic capillaries. Reduced perfusion increases vasopermeability and the accumulation of extracellular fluid and hard exudates that impair vision when the macula is affected. Over time, intraretinal hemorrhages and capillary occlusion create areas of ischemia, and the resulting hypoxia upregulates the production of proangiogenic factors, such as vascular endothelial growth factor (VEGF). In the more severe stages, the new blood vessels invade and bleed into the vitreous, producing a fibrovascular tissue that may result in retinal detachment and blindness [for reviews on the pathogenesis of diabetic retinopathy, the reader is referred to references (*9, 10*)].

Among the current treatments for diabetic retinopathy, laser photocoagulation remains the most effective for preventing visual loss. However, despite of its efficacy, the destructive nature of the laser damages neural tissue and has other significant side effects (*9*). The search for effective strategies to prevent both excessive retinal vasopermeability and angiogenic responses has become a major research focus. In this regard, the intravitreal delivery of inhibitors of VEGF, a prominent vasopermeability and proangiogenic factor in diabetic retinopathy, has demonstrated positive outcomes in clinical trials albeit with some long-term complications (*9, 11, 12*).

In contrast to the tremendous efforts concentrated on investigating the effect of factors thought to be specific for vascular function, the role of "broadly acting agents" such as hormones in diabetic retinopathy remains obscure (*13*). Of interest, pituitary hormones were among the first substances implicated in the progression of diabetic retinopathy.

DIABETIC RETINOPATHY AND THE PITUITARY GLAND

Regression of diabetic retinopathy was observed nearly six decades ago after postpartum insufficiency of the pituitary gland (Sheehan's syndrome) (*14*). Based on this observation, therapies against diabetic retinopathy targeting the pituitary gland by surgical ablation or stalk section, today considered unethical, were developed (*15*). Beneficial effects included the cessation of hemorrhagic activity, loosening and reabsorption of blood in preretinal spaces or vitreous, self-occlusion of new vessels, decrease in size and number of exudates, spontaneous reattachment of the retina and improvement of vision (*16*). Nevertheless, the regression of diabetic retinopathy did not occur in all patients, and visual acuity deteriorated, remain unchanged, or improved after hypophysectomy (*16*).

The beneficial effects of damaging or removing the pituitary gland were attributed to a reduction of systemic GH and IGF-I levels following the intervention, and subsequent studies produced clear evidence that the GH/insulin-like growth factor-1 (IGF-1) axis was involved in the progression of diabetic retinopathy (*17*). Yet data emerged that other players besides GH and IGF-1 were also involved. Indeed, the improvement in diabetic retinopathy after hypophysectomy can occur independently of GH-suppression (*18*), clinical trials with the GH antagonist pegvisomant yield negative results (*19*), and circulating IGF-I levels do not predict the incidence and progression of diabetic retinopathy (*20*). Moreover, severe cases of diabetic retinopathy are rare in patients with acromegaly (*21*), and diabetic retinopathy occurs in patients with congenital IGF-I deficiency (Laron syndrome; Ref. *22*). In the absence of more direct evidence, it was proposed that IGF-I has a permissive rather than a causal role in the development of diabetic retinopathy (*22*).

The influence of other pituitary hormones such as PRL on diabetic retinopathy has not been studied in such detail. Of note, hyperprolactinemia may arise from the remaining pituitary tissue after surgical stalk section or after hypophysectomy (*23*). Also, in Sheehan syndrome, hyperprolactinemia can occur independently of thyroid-stimulating hormone levels (*24*). Therefore, an increase in circulating PRL may have occurred in association with the observed regression of diabetic retinopathy following hypophysectomy and stalk section treatments. If so, it is possible that PRL played a protective role in those enigmatic cases in which the improvement of diabetic retinopathy after pituitary gland lesions could not be attributed to a GH/IGF-1 deficiency.

PRL AND VASOINHIBIN ACTIONS AGAINST DIABETIC RETINOPATHY

PRL, the hormone fundamental for lactation, is known to exert a wide variety of actions in reproduction, osmoregulation, immune response, brain function, behavior, energy metabolism, and angiogenesis (*13, 23*). Among these effects, the last two could have protective value against diabetes and diabetic retinopathy. PRL acts on pancreatic β-cells to stimulate proliferation, survival, and synthesis and secretion of insulin (*25*). These effects occur at least in part through the classic janus kinase (JAK)/signal transducers and activators of transcription (STAT) pathway that leads to the activation of cyclin D2 (*26*) and the upregulation of glucose transporter 2, glucokinase (*27, 28*), and pyruvate dehydrogenase activities (*29*). PRL also triggers β-cell expansion by upregulating the expression of tryptophan hydroxylase-1, which increases serotonin levels (*30*).

These actions on β-cells are essential for maintaining glucose homeostasis during pregnancy (*31*), when the PRL receptor serves both PRL and placental lactogens, but they are not restricted to pregnancy, as both male and female PRL receptor-null mice have reduced islet density, β-cell mass, and insulin synthesis and are glucose intolerant (*32*). Of relevance, by increasing islet number and reducing mononuclear cell infiltration, PRL is able to protect against the development of hyperglycemia in diabetic rats (*33*). Although protecting β-cells from dysfunction could lower diabetes progression and the appear-

ance of long-term complications such as retinopathy, there is another potent mechanism by which PRL could prevent progression and promote regression of diabetic retinopathy, namely by its proteolytic conversion to vasoinhibins.

PRL is cleaved by cathepsin D, matrix metalloproteases (MMP), and bone morphogenetic protein-1 to yield vasoinhibins (*13*). Cathepsin D appears to be the primary protease cleaving PRL in the pituitary gland during the secretory process (*34*), whereas MMP generate vasoinhibins in the extracellular space and at the target tissue level (*35*). The vascular effects and signaling mechanisms of vasoinhibins have been recently reviewed (*13*). The following summarizes some of the relevant findings. Vasoinhibins act directly on endothelial cells to inhibit vasopermeability, vasodilation, and angiogenesis induced by several vasoactive substances, including: VEGF, basic fibroblast growth factor (bFGF), interleukin 1-β, bradykinin, and acetylcholine. Also, vasoinhibins promote the apoptosis-mediated regression of blood vessels. Vasoinhibins signal through a still-unidentified receptor distinct from the PRL receptor to block activation of the Ras-Raf-MAPK pathway, the Ras-Tiam1-Rac1-Pak1 pathway, and the Ca^{2+}/calmodulin-activation of endothelial nitric oxide synthase (eNOS). They also promote protein phosphatase 2A-induced dephosphorylation/inactivation of eNOS, the activation of proapoptotic proteins of the Bcl-2 family, and the NFκB-mediated activation of caspases. Vasoinhibins decrease angiogenesis in the chick embryo chorioallantoic membrane, in coronary vessels, and in several tumor models.

Vasoinhibins are potent inhibitors of blood vessels in the eye. The local administration of vasoinhibins reduces the stimulation of corneal angiogenesis induced by bFGF (*36*), and gene transfer of vasoinhibins via an adenoviral vector inhibits ischemia-induced retinal angiogenesis (*37*). Furthermore, vasoinhibins block increased retinal vasopermeability in diabetic rats and in response to intravitreous injection of VEGF or of vitreous from patients with diabetic retinopathy (*38*).

Besides having therapeutic potential for controlling blood vessel dysfunction, vasoinhibins have emerged as endogenous inhibitors of ocular blood vessels. PRL and vasoinhibins are present in the retina (*39*) and may derive from the local synthesis of PRL by different glial and neuronal cell types (*40*). In addition, retinal PRL and vasoinhibins may also originate from systemic PRL. Radioactive PRL injected intracardially is incorporated into the retina, the choroids, and the ciliary body (*41*). Also, hyperprolactinemia leads to the accumulation of vasoinhibins in the retina, and lowering PRL levels with the dopamine D2 receptor agonist bromocriptine, an inhibitor of pituitary PRL secretion, blocks this effect (*42*). In this regard, the ciliary body, which is responsible for the transport of plasma proteins to ocular fluids (*43*), expresses the PRL receptor, and the genetic deletion of the PRL receptor prevents the hyperprolactinemia-induced accumulation of retinal vasoinhibins (*42*). Therefore, it is concluded that the PRL receptor mediates the incorporation into the eye of systemic PRL, which can then be cleaved to vasoinhibins.

In support of a functional role for endogenous vasoinhibins, antibodies sequestering these peptides stimulate angiogenesis in the cornea (*36*) and in the retina (*39*), and the intraocular transfection of small interfering RNA to block the local expression of PRL stimulates retinal angiogenesis and vasodilation (*39*). Moreover, immunodepletion of vasoinhibins in neonatal rats reduces the apoptosis of the hyaloid vasculature, suggesting that vasoinhibins stimulate the physiological regression of intraocular blood vessels after birth (*44*). These findings implicate vasoinhibins as major inhibitors of ocular blood vessels and raise the possibility that altering their levels could influence the progression of diabetic retinopathy.

CIRCULATING PRL AND VASOINHIBIN LEVELS IN DIABETIC RETINOPATHY

In 1985, Mooradian et al. reported that male patients with diabetes mellitus had elevated levels of mean fasting serum PRL which could not be attributed to diseases or medications known to elevate circulating PRL levels (*45*). These patients had increased PRL concentrations compared with healthy controls without diabetes. A subgroup analysis of the diabetic patients did not reveal a significant difference in systemic PRL levels between patients with or without clinical signs of diabetic retinopathy. However, other studies found circulating PRL levels to be significantly higher in male diabetic patients without severe diabetic retinopathy, compared with those with severe deteriorating hemorrhagic diabetic retinopathy (*46*). Recently, these findings were confirmed in a larger group of male diabetics who were classified as either having no diabetic retinopathy, nonproliferative diabetic retinopathy, or proliferative diabetic retinopathy (*42*). All patients with diabetes had higher serum PRL levels than healthy, nondiabetic males. Remarkably, patients with proliferative diabetic retinopathy had a significantly less elevated PRL concentration than diabetic patients without diabetic retinopathy. Considering that vasoinhibins derive from PRL and can increase in the retina as a result of hyperprolactinemia (*42*), these observations suggest that patients with higher PRL levels might have a lower risk for development and progression of diabetic retinopathy due to the protective effects of vasoinhibins. This possibility is supported by experimental work showing that hyperprolactinemia mitigates excessive retinal vasopermeability in diabetic rats and in response to the intravitreal injection of VEGF and that both effects are prevented by treatment with bromocriptine, which lowers the levels of systemic PRL and retinal vasoinhibins (*42*). Furthermore, consistent with a protective role of vasoinhibins against diabetic retinopathy, systemic vasoinhibin levels were found to be reduced in male patients with diabetic retinopathy compared with healthy male subjects without diabetes (*47*).

DIABETIC RETINOPATHY AND PREGNANCY: A PRL/VASOINHIBIN INTERPLAY?

Pregnancy and lactation represent ideal physiological states to study how PRL and vasoinhibins influence the progression of diabetic retinopathy. The pregnancy state itself is a well-accepted, independent risk factor for the progression of the disease. Early reports that diabetic retinopathy is exacerbated during pregnancy date back to the mid-20th century (*48*). One of the most recognized recent studies on this subject is a longitudinal analysis of the Diabetes Control and Complications Trial (DCCT), in which pregnant and nonpregnant women where studied during an average of 6.5 years of follow-up. The subjects were randomly assigned to conventional therapy or to an intensive treatment regimen. Pregnant women in the intensive treatment group had a 1.63-fold greater risk than before pregnancy of worsening retinopathy, and the risk was 2.48-fold in the conventional therapy group. Of note, the increased risk was transient and an analysis of the end-of-study data suggested that the worsening of retinopathy during pregnancy had no long-term consequences (*6*).

During pregnancy, there is a proangiogenic environment essential for organ growth that very likely worsens diabetic retinopathy. For example, it has been shown that the pregnancy-induced increase in IGF-1 is associated with the progression of diabetic retinopathy in pregnant women with type 1 diabetes (*49*). The pregnant/proangiogenic environment involves not only the increased production of proangiogenic factors but also the reduction of angiogenesis inhibitors. Along this line, the conversion of PRL to vasoinhibins may be reduced during pregnancy, particularly when considering the large amounts of PRL being produced. Before pregnancy, PRL circulating levels do not exceed an upper limit of 25 ng/mL. During pregnancy however, maternal PRL serum levels progressively increase and reach 200–300 ng/mL at term (*50*). Without breastfeeding, the PRL serum levels remain high during the first 2–3 weeks postpartum and then decline to pre-pregnancy levels (*50*). Despite these major changes in serum patterns, PRL has not become a major focus of research in regard to diabetic retinopathy. One study published in 1982 demonstrated that serum PRL levels were significantly lower in female subjects with diabetes throughout pregnancy compared with healthy pregnant subjects (*51*). However, no data exist about the course of diabetic retinopathy in patients with physiological hyperprolactinemia during breastfeeding and those with normalized PRL levels who do not breastfeed after parturition. Also, there is no information concerning vasoinhibin levels in pregnancy or postpartum. Considering the protective effects of PRL/vasoinhibins against diabetic retinopathy, a transient reduction in the generation of vasoinhibins during pregnancy might play a role in the deterioration of preexisting diabetic retinopathy, and that after pregnancy, the recovered production of vasoinhibins, further promoted by the hyperprolactinemia of the lactation state, would help reduce the progression of the disease.

CONCLUSIONS

The fact that some patients with diabetes under good metabolic control develop diabetic retinopathy while others with poorly controlled diabetes remain free of this complication has not been explained and is still a matter of scientific and clinical debate. An analysis of material from the DCCT database showed that more than 40% of the patients with poor metabolic control remained free of diabetic retinopathy (*52*). Hence, the existence of undisclosed, protective factors explaining such paradoxical clinical situations must be acknowledged. We suggest that such factors include PRL and vasoinhibins given their influence on the pathophysiology of diabetic retinopathy, and that they merit further investigation as important targets for therapeutic interventions.

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