Role of the Equine Hypothalamic-Pituitary Pars Intermedia Axis in Health and Disease

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Equine pituitary pars intermedia dysfunction (PPID; Equine Cushing's disease) is one of the most common diseases of horses 15 yr and older. It is a naturally occurring, progressive condition characterized by hypertrophy, hyperplasia, and adenoma formation of the pituitary pars intermedia. Despite clinical recognition of this disease for nearly 75 yr, the pathophysiology remains poorly understood. The purpose of this paper is to review recent findings that better define the role of the equine pars intermedia in health and disease. Author's address: Department of Physiological Sciences, Oklahoma State University, 264 McElroy Hall, Stillwater, OK 74078; e-mail: diannem@okstate.edu.

1. Introduction

The equine pituitary has three distinct lobes: pars distalis (anterior lobe), pars nervosa (posterior lobe), and pars intermedia (intermedia lobe). The pars distalis contains five different cell types, each of which is responsible for the release of a unique hormone or set of hormones in response to releasing factors from the hypothalamus. The pars nervosa is a collection of nerve axons and terminals that store and release oxytocin and arginine vasopressin (antidiuretic hormone). The pars intermedia of the horse is comprised of a single cell type—the melanotrope. Regulatory signals to the pars intermedia are delivered by the nerve terminals of the periventricular dopaminergic neurons, by direct systemic arterial supply and from the hypothalamic-hypophyseal portal veins.

The primary product of the melanotrope is the hormone-precursor protein proopiomelanocortin (POMC). POMC is also expressed by the corticotropes of the pars distalis; however, because of differential post-translational processing by proteases called prohormone convertases, each cell type secretes a different complement of POMC-derived peptides (Fig. 1). Because of the action of prohormone convertase I, POMC in corticotropes is primarily processed into adrenocorticotropin (ACTH). ACTH circulates to the adrenal cortex where it stimulates secretion of cortisol. Melanotropes contain active prohormone convertase I and II, and therefore, POMC in the pars intermedia is cleaved into the secretory peptides, α-melanocyte stimulating hormone (α-MSH), β-endorphin (β-end), and corticotrophin-like intermediate lobe peptide (CLIP). A small amount of ACTH may also be produced (Fig. 1). The physiological role of the pars intermedia POMC-derived peptides in the horse has not been extensively studied. α-MSH stimulating hormone is named such because of its ability to induce skin pigmentation in amphibians. α-MSH in other species has a potent anti-inflammatory activity that decreases pro-inflammatory cytokine release after endotoxin administration.[1] α-MSH is also integral in the leptin-melanocortin pathway, functioning in appetite-satiety balance and fat metabolism.[2] β-

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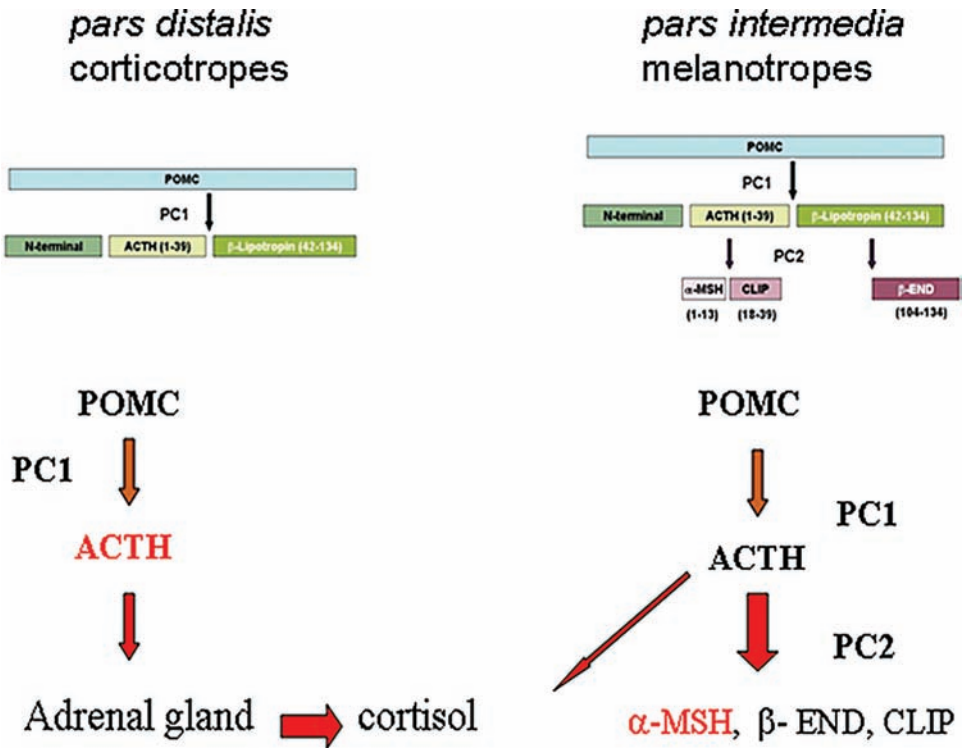

Fig. 1. Physiology of the equine PPID. The melanotropes of the pars intermedia produce the hormone precursor protein POMC. An identical protein is produced by the corticotropes of the pars distalis. The pars intermedia has two enzymes, prohormone convertase I and II, that cleave POMC into α-MSH, β-end, and CLIP. Only a small amount of ACTH is produced. In contrast, the pars intermedia has only one enzyme, prohormone convertase I. Therefore, the final POMC product of the corticotrope is ACTH.

end is an endogenous opioid. Secretion of β-end provides analgesia and behavioral modification. It also suppresses immune responsiveness and has effects on vascular tone.[3] CLIP is a pancreatic beta cell secretagogue that stimulates the release of insulin.[4]

Melanotropes are under inhibitory control by dopamine. Systemic administration of dopamine or dopamine agonists in horses results in decreased plasma concentrations of pars intermedia POMC-derived peptides.[5] Dopamine in the pars intermedia is released directly from nerve terminals. These neurons originate in the periventricular nucleus of the hypothalamus, project through the infundibulum, and terminate in the pars intermedia. Dopamine released from the nerve terminals interacts at dopamine (D2) receptors on the melanotropes to inhibit transcription of POMC and release of POMC-derived peptides. This pathway is known as the periventricular hypophyseal dopaminergic neuronal pathway.

Recent data has shown a distinct seasonal effect on the activity of the pars intermedia in horses and ponies. Plasma α-MSH concentration was considerably higher in horses and ponies in September compared with samples collected in the winter, spring, and early summer.[6] An effect of season on α-MSH concentration has been described for humans, hamsters, sheep, and weasels as well. For Siberian hamsters and short-tailed weasels, α-MSH concentration is reported to increase during the summer at a time when the coat changes color.[7,8] In humans living in Germany, plasma α-MSH concentration peaks in August, and the lowest concentrations are reported in January.[9] In Soay sheep, a feral breed of sheep in Scotland, plasma concentration of α-MSH is greatest in the late summer and fall.[10] The functional importance of the seasonal cycle is unknown, but several physiological events occur in parallel with the α-MSH cycle. In sheep, body weight, voluntary food intake, and condition all peak simultaneously with α-MSH, and the seasonal maximums occur in September. Soay sheep with surgically created hypothalamic-pituitary disconnection have an increase in circulating concentration of α-MSH and chronic increase in body weight.[11] Considered together, these findings suggest that α-MSH or other POMC-derived peptides may play a role in the metabolic preparation of Soay sheep for winter. It is possible that horses and ponies also have a seasonal increase in POMC-derived peptides to metabolically prepare them for a decrease in accessible food observed in the wild in winter. If so, dysregulation of this pathway might be associated with abnormalities in body weight and fat storage. Weight loss and abnormal fat distribution are two clinical signs associated with equine pituitary pars intermedia dysfunction (PPID,

Equine Cushing's disease). Development of a winter coat also begins as length of day decreases in the fall. The development of hirsutism in horses with PPID leads one to speculate that the naturally occurring seasonal increase in POMC-derived peptides contributes to the development of winter-coat growth. This has not been critically assessed in equids.

2. Equine PPID

Equine PPID is one of the most common diseases of horses and ponies 15 yr and older.[12] The pathological hallmarks of PPID are hypertrophy, hyperplasia, and (micro) adenoma formation in the pituitary pars intermedia that results in an increased secretion of POMC peptides. Horses with PPID develop enlarged pituitaries to as much as five times normal weight. As the pars intermedia expands, it compresses the adjacent pituitary lobes and hypothalamus, often resulting in a loss of function of these tissues. In contrast, the pars intermedia remains active in horses with PPID, secreting relatively large quantities of POMC-derived peptides into the peripheral circulation. Horses with disease may have as much as a 40-fold increase in plasma concentration of pars intermedia POMC-derived peptides.[5] Clinical signs of disease likely result from a combination of increased circulating POMC peptides and loss of neuroendocrine function of adjacent tissues.

Evidence indicates that loss of dopamine inhibition is critical in the pathology of PPID. Dopamine and dopamine-metabolite concentrations in the pars intermedia of PPID horses is decreased eight-fold compared with age-matched controls.[13] Systemic supplementation of dopamine or a dopamine agonist to horses with PPID results in a decrease in plasma concentration of POMC peptides.[5] A number of investigators have reported that horses treated with the dopamine agonist pergolide show improvement in both clinical signs and biochemical abnormalities associated with disease.[14–17] Loss of periventricular dopaminergic inhibition of the pars intermedia in other species results in pathologic changes similar to those of PPID. Surgical disruption of the periventricular hypophyseal dopaminergic tracts in rats results in increased expression of pars intermedia melanotropes.[18] In addition, D2 dopamine-receptor knockout mice develop pars intermedia lesions similar to PPID.[19] These data suggest that PPID is primarily a disease of hypothalamic origin rather than the consequence of a spontaneously forming adenoma. Therefore, we hypothesized that equine PPID results from a loss of inhibition of the pars intermedia caused by degeneration of the periventricular hypophyseal dopaminergic neurons.

Using immunohistochemistry, we examined pituitary and hypothalamic formalin-fixed tissue from PPID-affected, young healthy (≤15 yr), and aged healthy (>15 yr) horses.[20] Horses with PPID were selected based on clinical signs; they were confirmed as having the disease by the presence of pars intermedia hyperplasia and micro or macroadenomas at post-mortem examination. Using tyrosine hydroxylase as a marker of dopaminergic neurons, we showed a five-fold decrease in immunoreactivity of the nerve terminals ($p < 0.001$) and a 50% reduction in the number of tyrosine hydroxylase positive cell bodies ($p < 0.01$) in the periventricular nucleus of the hypothalamus in affected animals. We found no difference between young and aged healthy horses, indicating that aging alone has minimal effect on dopaminergic neurodegeneration. This evidence suggests that a loss of functional periventricular dopaminergic neurons or "dopaminergic neurodegeneration" occurs in horses with PPID and is consistent with the decrease in pars intermedia dopamine concentration observed in horses with PPID.

If in fact PPID is a dopaminergic neurodegenerative disease, it raises the questions of (1) what causes the neurons to degenerate and (2) why are only some horses affected? We speculated that PPID may have similar pathologic mechanisms as other dopaminergic neurodegenerative diseases, specifically Parkinson's disease. Although the cause of Parkinson's disease is not well understood, accumulation of oxidative stress damage and misfolded α-synuclein, a nerve terminal protein, has been shown to occur in the affected neurons of patients with Parkinson's disease. Therefore, we investigated the potential role for oxidative stress and protein misfolding in PPID.

Oxidative stress results in modification of cellular components including proteins, DNA, and cell-membrane lipids because of excessive exposure to exogenous or endogenous sources of free radicals. This cellular damage ultimately leads to cell death, or in the case of neurons, neurodegeneration. Chronic exposure to oxidants in excess of an animal's antioxidant capacity results in accumulation of functionally impaired cellular components. Measurement of these altered cellular components is useful as a marker of historical exposure to oxidative stress. Dopaminergic neurons are particularly vulnerable to oxidative damage, because dopamine metabolism itself produces free radicals. Chronic oxidative stress is considered to be an inciting factor in the development of other diseases associated with dopaminergic neurodegeneration, such as Parkinson's disease. Compared with healthy, age-matched individuals, patients with Parkinson's disease have decreased antioxidant capability and increased oxidative damage. Markers of oxidative stress, including 3-nitrotyrosine, have been shown to be increased in patients afflicted with Parkinson's disease.[21] Similar to humans with Parkinson's disease, horses with PPID also have evidence of oxidative stress, including accumulation of 3-nitrotyrosine[20] and decreased plasma thiol.[22]

Immunohistochemical analysis of pituitary and hypothalamic sections showed that horses with PPID had significantly more 3-nitrotyrosine in the

pars intermedia than either young or aged healthy horses ($p < 0.001$).[20] 3-nitrotyrosine is a marker of protein damage that occurs secondary to exposure to peroxynitrite, a potent free radical that is formed when nitric oxide reacts with superoxide radicals. In addition, healthy aged horses had significantly more 3-nitrotyrosine than young horses ($p < 0.05$). This accumulation of oxidative stress markers with age has been shown to occur in other species and may contribute to increased risk of oxidative stress-related disease as animals get older.[23] No difference was found in 3-nitrotyrosine accumulation in the periventricular cell bodies of the hypothalamus. In another study, horses with clinical evidence of PPID had decreased plasma thiol concentration.[22] Depletion of plasma sulfhydryl groups (thiols) has been reported in patients with Parkinson's disease.[24]

An increase in oxidative damage may be caused by an increased exposure to reactive oxygen species or alternatively, a decrease in antioxidant capacity. In horses with PPID, systemic antioxidant capacity seems unchanged. In a study of 31 aged horses, 12 with clinical PPID, glutathione peroxidase was found to be similar in all horses.[21] In a second study, red blood cell total glutathione concentration, antioxidant enzyme activity (glutathione peroxidase and superoxide dismutase), and plasma and tissue 3-nitrotyrosine concentration was measured in 20 pairs of horses. No difference was appreciated between the PPID horses and the age-matched controls in any of the antioxidants measured.[25] These data indicate that oxidative stress in horses with PPID is specifically localized to the pars intermedia and not likely the result of poor systemic antioxidant capacity.

Antioxidant capacity has also been examined in the equine pars intermedia. Total glutathione concentration, total superoxide dismutase, manganese superoxide dismutase, and glutathione peroxidase activities were measured in pars intermedia tissue from 16 horses.[25] Although there was an increase in glutathione peroxidase activity with oxidative stress (an appropriate response), there was no correlation between oxidative stress and decreased antioxidant activity. Antioxidant activity was also evaluated as a function of aging. A significant decrease was identified in pars intermedia manganese superoxide dismutase activity with advancing age. It is possible that the decrease in antioxidant capacity in the pars intermedia contributes to the age-related accumulation of 3-nitrotyrosine we observed.

Oxidative damage and degeneration occurs specifically in the dopaminergic nerve terminals of the pars intermedia in horses with PPID, despite adequate antioxidant capacity. This suggests that these neurons might be more vulnerable to oxidative and neurodegenerative events. In Parkinson's disease, oxidative stress and degeneration occurs preferentially in the dopaminergic neurons of the substania nigra. It is possible that PPID and Parkinson's disease occur because of similar pathologic mechanisms in different anatomical locations.

One important contributing factor in the degeneration of dopaminergic neurons in Parkinson's is the accumulation of misfolded α-synuclein.[26] α-synuclein is a natively unfolded, soluble monomeric nerve-terminal protein. Accumulation of misfolded α-synuclein can occur because of overexpression or modification of the protein. Modifications include primary mutations of the protein or post-translational events, such as oxidative stress damage. Accumulation of misfolded protein is toxic to neurons, and dopaminergic neurons are particularly susceptible. Horses with PPID have been shown to have an increase in α-synuclein expression in the nerve terminals of the pars intermedia.[20] In addition, α-synuclein was found to be nitrated in the pars intermedia of horses with PPID.[20] Nitration of α-synuclein in other species facilitates its tendency to form secondary structures ("misfold"), such protofibrils and fibrils, and is believed to contribute to disease development. Nitration of α-synuclein occurs in the substantia nigra of Parkinson's patients.

3. Discussion

Equine PPID affects many aged equids and results in a constellation of clinical signs that can be life threatening. Understanding what causes certain horses and ponies to develop PPID is important if improved diagnostic tests and preventative strategies are to be developed. Recent data suggest that PPID is a dopaminergic neurodegenerative disease with pathologic mechanisms similar to that of Parkinson's disease. Similar to Parkinson's disease, oxidative stress and α-synuclein protein misfolding seem to have a role in the pathogenesis of PPID.

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