INCRETIN-SECRETING K AND L CELLS IN THE EQUINE SMALL INTESTINE

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INTRODUCTION

Laminitis is a common and debilitating disease of horses, resulting in significant suffering and economic loss. 1,2 Research has identified hyperinsulinemia as a risk factor for laminitis. 1 The exact reason for hyperinsulinemia in susceptible horses is unknown, but one hypothesis pertains to the enteroendocrine axis and, specifically, the incretin hormones known as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GIP and GLP-1 are secreted by intestinal cells called K and L cells, respectively, and one of their chief functions is to stimulate insulin release from pancreatic β cells in response to ingesta. 3 It has been proposed that the intestinal lining of EMS-affected horses may be populated by an exceptionally high number of K and L cells, and hyperinsulinemia results from excessive incretin responses to feeding. Therefore, the objective of this study was to first assess the quantity and distribution of K and L cells in the small intestine of healthy horses.

METHODS

Three horses (two mares and one gelding, ranging in age from 5-19 years) without gross evidence of endocrine or gastrointestinal pathology were used in this study. Immediately following euthanasia (to preclude autolysis), two samples of the mucosa (one from the mesenteric and one from the antimesenteric region) were collected from the duodenum, jejunum, and ileum. These samples were fixed in formalin, embedded in paraffin, sectioned to 5 μm thickness, and mounted on glass slides. The sections were then stained with anti-GIP or anti-GLP-1 antibodies to identify K cells or L cells (Fig. 3). Briefly, the prepared sections were pretreated by a heat-induced antigen retrieval method using citrate buffer (0.01M, pH 6.0). The primary antibodies were rabbit anti-human GIP polyclonal antibody (1:300, Bioss, Woburn, MA) or mouse anti-

\[ \text{GLP-1 monoclonal antibody (1:500, Abcam, Cambridge, MA).} \]

EnVision™ system (Dako, Carpinteria, CA) was used for antigen detection and the immunoreactivity was visualized using AEC Chromogen (Biocare Medical, Concord, CA). Hematoxylin was used for counterstain. The slides were systematically viewed on a microscope at 200X magnification so the populations of positively-stained cells in crypts and villi could be identified and counted by a trained observer (Fig. 4). The length of sample in each case was measured along the mucosal border for comparative purposes by digitally scanning the slides, then tracing the outline in an image editing program. Results were expressed as mean number of positive cells [(mesenteric + antimesenteric)/2] per unit length of intestinal segment.

RESULTS and DISCUSSION


REFERENCES

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