犬におけるインクレチン作用と
インクレチン製剤の糖代謝へおよぼす影響

(Incretin action and effect of the incretin preparation for glucose metabolism in dogs)

学位論文の内容の要旨

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インクレチンには GIP と GLP-1 の 2 種類がある。これらは摂食に伴い小腸から分泌され、血糖依存的にインスリン分泌を促進させる働きを持つ消化管ホルモンである。本研究では犬におけるインクレチンの糖代謝へおよぼす影響について検討した。

始めに、ヒトでいわれるインクレチン作用が犬においても存在するのかを検討するため、同等の血糖変動条件下にて OGTT と IVGTT のインスリン分泌量を比較した所、OGTT の方がインスリン分泌量が多くなった。また、両試験下でのインクレチン濃度を測定した所、OGTT では糖負荷後有意な上昇を認めた。以上より、犬においてもインクレチン作用が存在することが確認された。次に、インクレチン分泌細胞の RNA 発現解析を行った所、犬において、GIP は小腸上部で最も発現しており、次いで中部、そして小腸下部ではほとんど発現していないことがわかった。GLP-1 は上部ではほとんど発現していないが、中部、下部となるにつれて発現が高くなっており、他動物種の報告と異なることがわかった。

次に、栄養組成の違いがインクレチン分泌におよぼす影響を検討するため、5 種の異なる栄養組成のフードを給与してインクレチン濃度を測定した。犬の GLP-1 分泌には栄養組成の違いというより、むしろ繊維を添加し、吸収を緩やかにすることが重要であることがわかった。これは犬の GLP-1 分泌細胞が小腸の下部において高発現していることと関連があると考えられた。また、GIP 分泌は他動物種の報告と同様脂質で上昇し、繊維を添加すると低下した。

最後に、現在ヒトの糖尿病患者に使用されているインクレチン製剤であるリラグルチドを用いて犬での効果を検討した。健常犬におけるリラグルチド投与によって血糖降下作用が認められたが、この作用は GLP-1 のインスリン分泌促進に加えて消化管運動抑制やグルカゴン分泌抑制作用が寄与したことが考えられた。そこでインスリン自己分泌がない糖尿病犬に対して検討した所、インスリンとリラグルチドの併用療法によって、食後高血糖抑制や血糖変動幅の顕著な減少が認められた。

現在、ヒトの糖尿病患者に対する薬剤治療や食事療法にて、インクレチンに焦点を当てた研究が数多く行われている。インクレチンは血糖正常化のみならず肥満、生活習慣病予防や糖代謝改善の重要な因子とされており、今後、小動物臨床においてもその有用性は大きく期待できる。

Incretin action and effect of the incretin preparation for glucose metabolism in dogs.

Hitomi Oda

Incretin was shown to exert their insulinotropic effects through a variety of mechanisms, including increasing the rates of insulin synthesis, granule docking, and exocytosis. In the presence of matched glucose concentrations, insulin secretion is greater following ingestion of glucose than following infusion of glucose. This was referred to as "the incretin effect" and is believed to be modulated at least in part by intestinally secreted hormones such as glucose-dependent insulinotropic peptide (GIP) and glucagon like peptide-1 (GLP-1). Therefore, the aims of this study were to investigate the effect of incretin for glucose and insulin metabolism in dogs.

In order to confirm whether dog also have “the incretin effect”, we performed the oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT) under similar blood glucose variation using artificial pancreas apparatus. No significant difference was observed in temporal serum glucose concentrations between OGTT and IVGTT, since we adjust the intravenous glucose infusion to reproduce similar blood glucose variation as the OGTT. However, insulin, GIP and GLP-1 concentration in OGTT were significantly higher than those in IVGTT.

GIP produced by K-cells have commonly identified in the upper small intestine, whereas GLP-1 is produced by the L-cells primarily located in the distal small intestine and colon in human study. Therefore, the aim of this study was to examine distribution of tissue genetic expressions of GIP and GLP-1 in dogs. GIP genetic expression was highest in the duodenum as compared to other small intestine (jejunum, ileum). However, genetic expression of GLP-1 was the highest in ileum as compared to duodenum and jejunum. This result was not consistent with other mammals.

In order to determine changes in incretin secretion with different nutritional composition, different diets were fed in 5 healthy dogs. GLP-1 concentration was significantly increased in high-fiber diet. Meanwhile, GIP secretion was increased in high-fat diet and decreased in high-fiber diet.

In order to determine the effect of incretin preparations on serum glucose and insulin concentrations, dipeptidyl peptidase-4 (DPP-4) resistant GLP-1 analog, liraglutide was administered in 5 healthy dogs. Liraglutide was dispensed medication before starting OGTT. Significant decrease was observed in temporal serum glucose concentrations in liraglutide group as compared to control group. Since these dogs are healthy, insulin regulation is properly functioning and regulated. Therefore only a minimal amount of additional insulin was likely secreted, since it was not required. Insulin stimulation and glucagon inhibition contribute equally for the effect of GLP-1 in human study. Furthermore, it is known that liraglutide is capable of increasing insulin secretion and suppressing prandial glucagon secretion in a glucose-dependent manner. Hence, we speculate that stabilized glucose concentrations, after the OGTT, due to liraglutide treatment, may have been principally attributed to reduced glucagon secretion, instead of insulin secretion in healthy animals. Liraglutide's prandial glucagon suppressive ability appears to play a key role in its glucose-lowering effect. Since a liraglutide associated glucose lowering effect was observed in healthy dogs, and we suspect that this was principally resulting from liraglutide’s ability to suppress prandial glucagon release into the blood, we tested whether T1DM dogs would respond favorably to

liraglutide treatment. T1DM dogs responded favorably to liraglutide treatment, which lead to a significant reduction of 46.0% in glucose AUC0-12h (total area under the curve for 0-12h), and a significant reduction of 66.5% in serum glucose as compared to baseline controls (insulin treatment only).

This study demonstrated that healthy dogs have the incretin effect and the incretin secretion was affected by different nutritional composition. Moreover, incretin preparations affect glucose and insulin metabolism in healthy and diabetes dogs.