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## Role of peptide tyrosine tyrosine (PYY) in the regulation of gastrointestinal motility and pancreatic exocrine secretion in sheep.

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The present study was performed to evaluate physiological roles of PYY in the regulatory mechanisms in the ovine alimentary tract. Immunohistochemistry revealed that PYY localized only in mucosal endocrine cells in the ileum and large intestine in sheep. In HPLC analysis, identical PYY-immunoreactivity with pure porcine PYY was detected from the mucosa of the ovine large intestine. Specific radioimmunoassay using scraped intestinal mucosa revealed that tissue contents of PYY in mucosa are higher in the colon and rectum in the ovine intestine, whereas they are approximately ten-times smaller than that in the rat intestine. Plasma concentration of PYY before feeding in sheep was as low as that in interdigestive period in rats and humans, however, it was hardly altered over 48 hours in sheep, even after feeding with lucerne pellets and hay or concentrate. Intravenous infusion of CCK, that has been shown to stimulate PYY release in non-ruminant species, did not alter plasma concentration of PYY in sheep. No intraluminal infusions of oleic acid, butyric acid, casein, and maltose in the ileum and colon at supra-physiological concentrations increased plasma concentration of PYY. Moreover, no infusions of the four nutrients in the ileum exerted inhibitory effect on duodenal contractile activity in sheep. Finally, effects of PYY administration on the gastroduodenal motility and pancreatic exocrine secretion was examined in healthy mature sheep. Exocrine secre-

tion of the ovine pancreas fluctuated in association with phases of spiking activity on a duodenal electromyogram and the exocrine secretion remained constantly at a high rate during phase II that took more than 80% of MMC. Administrations of atropine and L-364,718 revealed that the exocrine secretion of the ovine pancreas during phase II highly depends on both cholinergic nerve activity and endogenous CCK, which resemble regulatory system during postprandial period in non-ruminant species. Intravenous continuous infusion of porcine PYY during phase II at a dose, which produces twice higher levels of postprandial plasma concentration of PYY in dogs, shortened duodenal MMC cycle in sheep with delay after the end of infusion. However, it did not alter regular ruminal contractions and pancreatic exocrine secretion. Likewise, intravenous bolus injection of PYY at the supra-physiological range of doses scarcely altered ruminal contractions and pancreatic exocrine secretion at even supra-physiological doses.

In conclusion, PYY is localized in the mucosa of the lower intestine, whereas it is barely released into blood by ingestion of feed, the luminal nutrients and circulating CCK in mature sheep. Therefore, endogenous PYY in the lower intestine is not probably involved in feedback regulatory mechanisms in the ovine digestive tract. Humoral kinetics and physiological role of PYY in the ovine digestive tract definitely differ from those evaluated in

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**non-ruminant species.**

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**On-line measurement of released catecholamine and ATP :  
mechanisms of their release and uptake**

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Adenosine-5'-triphosphate (ATP) coexists with catecholamine (CA) in the secretory vesicles of sympathetic nerve terminals and adrenal chromaffin cells. We developed an on-line system for continuous measurement of CA and ATP released from cultured porcine adrenal chromaffin cells and PC 12 cells.

CA and ATP were continuously measured with an electrochemical detector and ATP photometer with luciferin-luciferase, respectively. Acetylcholine (ACh, 0.1 mM) or high K<sup>+</sup> (60 mM) was applied to perfused porcine adrenal chromaffin cells and CA and adenine nucleotides in the collected effluent were analysed with HPLC. The most of CA in the effluent was noradrenarine (81%) and the other was adrenaline (19%). The relative amounts of ATP, ADP and AMP in the effluent were 1, 1.3 and 1, respectively. These ratios were almost the same throughout the period of stimulation.

Using the on-line system, CA and ATP released from cultured porcine adrenal chromaffin cells in response to ACh (0.1 mM) or high K<sup>+</sup> (60 mM) were measured. Increases of CA and ATP in perfused effluent were transient in nature regardless of continuous pres-

ence of the secretagogues and their time courses were almost coincident. The molar ratios of CA to ATP (CA/ATP) in the effluent were  $10 \pm 1$  and  $12 \pm 1$  with ACh and high K<sup>+</sup> stimulation, respectively. In response to repetitive high K<sup>+</sup> stimulation (60 mM), CA and ATP were also released into perfused effluent with the same time courses. Although the amounts of released CA and ATP decreased with repetition, their time courses appearing in the effluent were almost the same and the CA/ATP was  $12 \pm 1$ . Ba<sup>2+</sup> (5 mM) produced rapid increases in CA and ATP, and then the secretory responses declined to about 50% of the peak and sustained during the presence of Ba<sup>2+</sup>. Even in this case, the time courses of CA and ATP appearing in the effluent were also almost the same and the CA/ATP was  $12 \pm 2$ .

The HPLC analysis of CA revealed that the most CA released from pheochromocytoma (PC 12) cells in response to stimulus was dopamine (90%). On-line measurement revealed that high K<sup>+</sup> (60 mM), ACh (0.1 mM) and Ba<sup>2+</sup> (5 mM) caused dopamine and ATP release with almost the same time courses. These secretory responses of dopamine and ATP were quite similar to those of cultured