Species-Dependent Mechanisms Regulating Glucose-Dependent GLP-1 Secretion?

Patricia L. Brubaker

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Since the discovery of glucagon-like peptide $1^{7-36NH2}$ (GLP-1) as an "incretin" hormone (1), numerous GLP-1 receptor agonists and incretin hormone degradation (dipeptidyl peptidase 4) inhibitors have been developed for the treatment of patients with type 2 diabetes (2). The success of these therapeutics in reducing HbA_{1c} levels, as well as decreasing body weight and exerting cardioprotective actions in the case of the agonists, has been well established, and incretin-based drugs are now recommended for dual-therapy management of type 2 diabetes (3). However, considerable interest has been engendered in a third possible approach to GLP-1–based therapy, that being enhancement of endogenous secretion, either alone or with a degradation inhibitor (4). GLP-1 is secreted by the endocrine L cell of the intestinal epithelium in rodents and humans (5). GLP-1 release is stimulated by ingested nutrients, including glucose, although neural and hormonal secretagogues have also been described (4). However, a thorough understanding of the molecular underpinnings of human L-cell secretory responses is critical if GLP-1 secretagogues are to be considered for therapeutic use.

L cells are diffusely scattered through the gut epithelium, providing a major challenge to their study (5). Furthermore, although the advent of techniques to fluorescently label murine L cells has enabled their isolation, purified L cells fail to survive in vitro (6). Consequently, the cellular mechanisms underlying GLP-1 secretion by the primary murine L cell are now commonly studied using heterogeneous cultures derived from isolated crypts (6,7). Such in vitro models have been generated from all regions of the mouse intestine, although most reports have focused on the proximal 50% of the small intestine and the colon. These studies have elucidated the major signaling pathways that underlie the regulation of GLP-1 release by the murine L cell, including, most notably, the response to glucose (Fig. 1) (6,7). Although similar approaches have been used to culture human crypt cells, the majority of such studies have focused on the colon, a relatively accessible site for tissue sampling (8). The article by Sun et al. (9) in this issue of *Diabetes* presents novel insight into the cellular mechanisms underlying glucose-stimulated GLP-1 secretion by the human L cell, using biopsies obtained from not only the colon but also the duodenum and ileum.

In the first part of the study, healthy subjects received an intraduodenal glucose infusion followed by collection of duodenal biopsies after 30 min for immunostaining; plasma was also obtained to determine circulating GLP-1 levels (9). Consistent with findings in rats (10), luminal glucose induced a 70% increase in the number of human duodenal L cells expressing phosphorylated calcium calmodulin–dependent kinase II, an enzyme that links extracellular glucose to insulin secretion in the β-cell (11). A parallel increase was also observed in plasma GLP-1 levels, although the exact source of this GLP-1 cannot be identified, as the rate of infusion and single time point of sampling almost certainly would have permitted glucose activation of the more abundant jejunal L cells (5,12). Consistent with this possibility, GLP-1 release is much greater following intrajejunal as compared with intraduodenal infusion of glucose in normal humans (13).

In the second part of the study, ileal and colonic mucosal biopsies were collected from patients at the time of intestinal surgery (9). These samples, as well as those from the duodenum, were examined acutely for their GLP-1 secretory responses to glucose. Interestingly, the results indicate both similarities to and differences from the murine L cell (Fig. 1). First, as expected based on the mouse studies (6,7), glucose stimulated GLP-1 release from human small intestinal (duodenal and ileal) L cells; however, in contrast to the mouse, human colonic L cells did not respond to glucose. Analysis of the mechanisms underlying ileal L cell glucose sensing demonstrated additional species-dependent intracellular responses. Hence, the human L cell was markedly less sensitive to glucose than that of the mouse (human ileal ≥200 mmol/L vs. mouse proximal ≥1 mmol/L and colonic ≥0.1 mmol/L [Fig. 1]). Both species use the

Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada

Corresponding author: Patricia L. Brubaker, p.brubaker@utoronto.ca.

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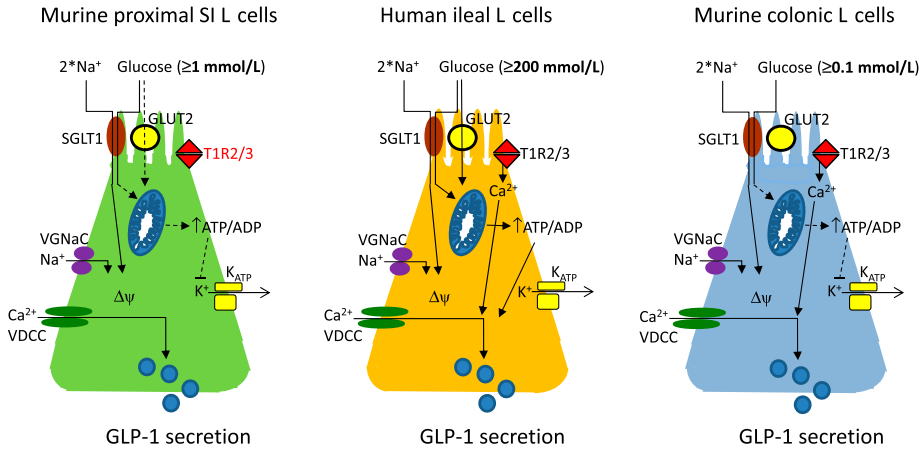

Figure 1—Signaling pathways regulating glucose-stimulated GLP-1 secretion in primary human ileal mucosal biopsy samples as compared with primary murine upper small intestinal (SI; proximal 50%) and colonic crypt cultures. Marked differences in sensitivity to glucose are apparent, as well as in some of the downstream pathways. Solid lines indicate demonstrated significant pathways in the L-cell secretory response to glucose, whereas dashed lines indicate pathways that appear to be of lesser importance. VDCC, voltage-dependent calcium channel; VGNaC, voltage-gated sodium channel.

sodium–glucose transporter 1 (SGLT1) as a key mediator of glucose-induced GLP-1 release, translocating one glucose with two Na^+; the latter causes depolarization (at least in the murine L cell), leading to opening of voltage-dependent calcium channels and activation of calcium-induced exocytosis. As the K_m for Na^+ transport by murine SGLT1 is 2–7 times lower than that for human SGLT1 (14,15), these differences may account, in part, for the differential sensitivities of the small intestinal L cells to glucose. In addition, although human small intestinal L cells are known to express SGLT1 (16), it has not been determined that this is the case for human colonic L cells. Furthermore, recent studies have demonstrated that proximal L cells in mice and humans differ markedly from distal L cells in their polyhormonal expression of several other gut endocrine peptides (17,18). As glucose is normally absorbed by the proximal gut, the physiological relevance of any glucose sensing by the distal L cell remains unclear. Nonetheless, as GLP-1 serves as an "ileal brake" to delay intestinal transit (4), such responses may enable feedback in the setting of an overload of proximal absorptive capacity. Indeed, one possible advantage of this distal sensing capacity is observed following malabsorptive bariatric surgery, which directs unusually large volumes of nutrients into the distal small intestine, thereby increasing GLP-1 secretion in association with improved glycemic tolerance (19). Finally, although other subtle differences found between the human and murine L cells relate to roles for the glucose transporter GLUT2, glucose metabolism, the K_{ATP} channel, and the taste receptors T1R2/R3, none of these appear to be primary regulators of glucose sensing in either species (Fig. 1).

It must be recognized that there are also experimental differences between the study by Sun et al. (9) and those examining the mouse L cell, including the site of origin (ileal vs. proximal and colonic), the method of ex vivo analysis (explants vs. culture), the use of whole mucosal biopsies containing villus and crypt L cells vs. isolated crypts only, and the limited amount of human tissue available, which precluded testing of multiple complementary inhibitors. Nonetheless, the study by Sun et al. represents a first attempt to systematically analyze the intracellular mechanisms underlying glucose sensing by the human L cell. Ultimately, although similarities between the murine and human L cell will suggest potential therapeutic targets for GLP-1 secretagogues, it is the differences between these cells that will be the key to success.

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