Incretins are hormones released by gut enteroendocrine cells in response to nutrient ingestion that regulate circulating glucose levels. One member of the incretin family is the glucagon-like peptide-1 (GLP-1), which is secreted by intestinal L cells located mainly in the mucosa of ileum and colon. It is produced by posttranslational processing of the glucagon gene product. GLP-1 binds to a specific receptor (GLP-1R), a seven-transmembrane G protein-coupled receptor originally identified in islet \( \beta \)-cells (1) with a broad range of tissue-specific functions (1–8). The binding of GLP-1 to the GLP-1R on pancreatic \( \beta \)-cells augments glucose-stimulated insulin secretion, increases transcription of the insulin gene, and enhances both the stability of insulin mRNA and biosynthesis of insulin (reviewed in References 9 and 10). In addition to the glucose-regulatory effects, GLP-1 has been reported to protect \( \beta \)-cells from apoptosis (11) and to stimulate their neogenesis and proliferation (12, 13).

After secretion, GLP-1 is rapidly degraded into GLP-1 (9–36) by the enzyme dipeptidyl-peptidase IV (DPP-4) (14). As a result, the biological half-life of GLP-1 is less than 5 minutes (15). DPP-4 enzymatic activity can be inhibited by DPP-4 inhibitors (DPP-4-i), which prevents the cleavage of GLP-1 and other DPP-4 targets. In doing so, the inhibitor maintains the intact levels of the molecules, improving glucose tolerance (reviewed in Reference 16). Because of their beneficial effect in controlling blood glucose levels, DPP-4is and GLP-1 agonists are current therapies for type 2 diabetes. Recently, however, the use of GLP-1-based therapies has been deemed dangerous by a publication indicating that they increase the risk of pancreatic cancer (17).

One of the leading arguments that underpinned the proposed relationship between incretins and cancers of the pancreas and thyroid was the reported localization of the GLP-1R to the pancreatic duct and acinar tissue (19) of rodents and to the human thyroid gland (18). The hypothesis raised by the authors of those studies proposed that long-term stimulation of cells expressing the GLP-1R resulted in cell hyperplasia and tumor formation. In the human thyroid, neoplastic and hyperplastic lesions of thyroid \( C \) cells were reported to contain GLP-1R-immunopositive cells (18). The experiments analyzing the effect of GLP-1 stimulation in the pancreas were performed in rats receiving daily injections of the GLP-1 analog exendin-4 and in a mouse model of pancreatic cancer. These studies revealed the presence of immune-positive GLP-1R cells in lesions of the pancreatic duct [termed pancreatic duct glands (PDGs)] that appear during chronic injury and in pancreatic intraepithelial neoplasia (PanIN) (19). Because PDGs and PanIN were also found in the pancreas of diabetic patients who received DPP-4is, the conclusion was reached that the inhibitors caused tumor development (17). This report, suggesting a deleterious effect of DPP-4is in the pancreas of human diabetic patient, was highly controversial, mainly because the diabetic groups receiving either incretin therapy or other insulin secretagogues were not comparable with regard to age and duration of the disease. Several publications have already raised severe concerns regarding the clinical aspects of this publication (16, 20–23).

A critical issue in the studies reporting a correlation between incretin therapy and cancer development is the
specificity of the antibodies used to localize the GLP-1R to the rat pancreas and human thyroid gland. These were commercial antisera from Novus and Abcam, respectively, which were used to precipitate GLP-1R protein produced by cell lines (18). Western blot analysis of that GLP-1R protein indicated the presence of two bands, but it was not tested whether these bands were removed by binding to GLP-1R protein present in homogenates of high expressing tissues. An additional missing control in those studies was whether the immunohistochemical localization in pancreas and thyroid was eliminated by immunoadsorption of the antisera with the protein produced by the cell lines. The presence of immunostaining indicates only that the antibody recognizes an antigen in the tissue sections but not the identity of that antigen. No other test was performed by Butler et al (17) to confirm the expression of the receptor in immunoreactive positive tissues. Other laboratories (16), including my own (24), tested the specificity of commercial antisera used by Gier et al (19) using extracts of islets and kidney by Western blot; this analysis revealed the presence of multiple nonspecific bands, questioning whether the antigen labeled in pancreatic lesions in a published report (19) was indeed the GLP-1R. Taken together, these observations and those discussed in previous publications (15, 19–22) raise serious doubts regarding the possible causal relationship between the expression of GLP-1R and the development of PDG and PanIN.

The article of Charles Pyke and collaborators from Novo-Nordisk and Rigshospitalet (Copenhagen, Denmark), which appears in this issue of Endocrinology (30), provides much needed clarification of the pancreatic cell types expressing the GLP-1R in humans. This group examined the localization of GLP-1R in tissues samples from two species of monkeys and in human tissue specimens. The monoclonal antibody to the GLP-1R selected for this study detected the GLP-1R in Western blotting using lysates from cells transfected with human GLP-1R but not in lysates from cells transfected with rabbit or mouse GLP-1R, indicating that this antibody was primate specific. The epitope reactivity of the monoclonal antibody used was determined and blast analysis indicated that that particular GLP-1R sequence corresponded to primates, in agreement with the results obtained by Western blot. In the pancreas, receptor expression was highly localized to β-cells and, to a lesser extent, to the acinar tissue. The pancreatic duct and the thyroid gland, two of the sites that were believed to be induced by GLP-1 to become tumorigenic (17–19), were found by Pyke and collaborators to lack GLP-1R immunoreactivity. GLP-1R-positive cells were found in the kidney, lung, and intestine but not in liver or thyroid. Importantly, the results of receptor expression obtained by immunohistochemistry were validated by in situ ligand binding of 125I-GLP-1. Thus, 125I-GLP-1 was localized to the same tissue sites containing immunopositive GLP-1R cells. These results confirm that the development of the PanIN lesions is unrelated to the direct activity of GLP-1.

Low level of expression of the GLP-1R was found by Pyke and collaborators in pancreatic exocrine tissue by immunohistochemistry and in situ ligand binding. This finding could raise concern regarding the clinical use of this GLP-1 analog (25) because a relationship was reported between long-term administration of exenatide and pancreatic acinar inflammation in rats (26). However, it should be noted that the disparity in dose usage in rats (10 μg/kg) and in diabetic patients (approximately 0.05 μg/kg) in that study might explain the development of inflammation of the pancreas of rodents. Moreover, administration of a very high dose of liraglutide, a GLP-1 analog, for 2 years did not result in the appearance of lesions in the pancreas of mice, rats, or monkeys (27). Other studies in rodents confirmed the absence of pancreatic inflammation with exenatide administration (25, 28, 29).

Another point of concern raised regarding the use of DPP-4is in the clinic was that most of the studies showing the absence of the dangerous effects of the drug were funded by pharmaceutical companies, as is the work of Pyke and collaborators. However, the data presented should be judged objectively. In this particular case, it is difficult, notwithstanding current technological developments, to manipulate the addition of 125I-GLP-1 label or immunohistochemical staining on tissue sections. The results obtained by these two complementary approaches validate the results described in this study. The report therefore indicates that the pancreatic ducral cells lack the GLP-1R and makes a strong case against a role of GLP-1 in the initiation of pancreatic inflammation and cancer. As with any other drug used in the clinic, a watchful eye needs to be kept regarding adverse effects of incretin therapy. It is hoped that future analysis of human pancreatic samples from comparable groups of diabetic patients who received either incretin therapy or other treatment to control glucose levels will help settle remaining questions on this issue.

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