INVITED COMMENTARY

Glucagon receptor as a drug target: A witches' brew of eye of newt (peptides) and toe of frog (receptors)

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Glucagon has a noble history in the annals of metabolic disease, even though, to a layperson, insulin is its more famous counter-regulatory partner. For decades medical students have been taught that glucagon raises blood glucose by increasing hepatic glucose output and that alleviation of hypoglycaemia is its primary function. Thus, inhibition of glucagon secretion or action are logical approaches to the development of therapeutics that improve glycaemic control in both type 2 and type 1 diabetes mellitus; indeed, this strategy has been pursued for nearly 4 decades.

The situation, however, is complex. The preproglucagon gene product can be cleaved at various points by specific prohormone convertases to yield several bioactive peptides, including glucagon, in a tissue-selective manner. Prohormone convertase 2 is expressed in pancreatic islets to generate mainly glucagon in α cells. By contrast, prohormone convertase 1/3 processes proglucagon in the gut and brain to glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), glicentin and oxyntomodulin. These boundaries are not absolute, however, and the relative abundance of these peptides in a tissue, and therefore its secretory potential, can vary depending on the differential expression of the processing enzymes. There is evidence that GLP-1 is expressed in pancreatic islets, while glucagon may be secreted by enteroendocrine cells in the gut.

Enteroneocrine cells also express other peptides such as peptide-tyrosine-tyrosine (PYY), and it now appears that secretion can be modulated by feedback inhibition by homotypic (GLP-1 reducing GLP-1 secretion) as well as heterotopic mechanisms (GLP-1 reducing PYY secretion).

Glucagon receptor pharmacology is also multifaceted. The classic glucagon receptor is a member of the class B G-protein-coupled receptor (GPCR) family, and its structure has been reported recently. In classic experiments, glucagon was shown to elevate intracellular G-protein-coupled adenyl cyclase activity and increase cAMP levels; however, as with other GPCRs, the intracellular signal cascade can be modulated by other actions of G-proteins. In addition, the glucagon receptor can be desensitized after activation by internalization, and accessory proteins, such as β-arrestin and receptor-activity-modifying proteins (RAMPs), can modulate recycling of receptors to the cell surface. To add complexity to this brew, not only can glucagon signal via the classic glucagon receptor, but it can also interact with the “GLP-1” receptor with an affinity comparable to that of GLP-1 itself. Indeed, oxyntomodulin, essentially a glucagon molecule with a short C-terminal extension, can also signal both via the glucagon and GLP-1 receptors. Recently the oxyntomodulin–GLP-1 receptor interaction has been shown to exhibit biased agonism, and this may also apply to glucagon–GLP-1 receptor signalling.

Thus, glucagon fits into a complex “systems biology” network of ligands and targets in which it can interact with 2 types of receptors with the capacity to generate a range of exquisitely subtle responses via biased signal transduction, as well as having the potential to alter the secretion of other bioactive hormones. This is the broad context within which to consider the report in the current issue of Diabetes, Obesity and Metabolism by Kostic et al. on the results from an elegant first-time-in-human (FTIH) study with the glucagon receptor-blocking monoclonal antibody REGN1193. More specifically, previous studies with small molecule glucagon receptor antagonists and antisense molecules have uncovered good, bad and ugly aspects in humans; therefore, the essential question for drug development is whether these features can be separated sufficiently to open a wide therapeutic index, and allow safe long-term use. Small molecules targeting the glucagon receptor have both common structural elements and molecule-specific motifs that can confound the interpretation of adverse event profiles. Comparing the results from totally different molecular classes, small molecule-, antibody- and antisense-based approaches, therefore, allows some parsing of effects that can reasonably be attributed to mechanism-of-action vs those that may be related to molecule-specific “toxicity.” Moreover, preclinical models such as glucagon
receptor knockout mice are also helpful, as are humans with loss-of-function glucagon receptor mutations, especially when the biology and pharmacology are consistent and receptor mutants recapitulate the effects of glucagon receptor blockade in humans.

REGN1193 is a monoclonal antibody that blocks the glucagon receptor with a half maximal inhibitory concentration (IC₅₀) of ≈68 pM, as estimated from the inhibition of glucagon signalling in a cell-based reporter assay. This antibody binds with high affinity to mouse, rat, monkey and human glucagon receptors, allowing translation of pharmacodynamics across species. REGN1193 has been administered to diabetic and obese mice and diabetic monkeys and data from these studies were used to select doses for evaluation in the present FTIH study. In this randomized, placebo-controlled FTIH study, REGN1193 was administered by intravenous (i.v.) infusion to healthy subjects as single doses ranging from 0.05 to 0.6 mg/kg. In addition to safety and pharmacokinetic measurements, the authors

| TABLE 1 | Summary of glucagon receptor blocking drugs evaluated in humans |
| --- | --- | --- | --- |
| **Structure** | **Status** | **Reference** |
| BAY27-9955 3,5 Diisopropyl-2-(1-hydroxyethyl)-6-propyl-4'- fluoro-1,1-biphenyl | Discontinued | Phase I study reported¹⁰ |
| MK-0893 | Discontinued in Phase II | ³¹-³³ |
| MK-3577 | Discontinued in Phase II | ³⁴,³⁵ |
| LY2409021 | Discontinued in Phase II | ³⁶-³⁹, ⁵⁴ |
| PF-06291874 | Non-peptide small molecule | Discontinued in Phase II | ⁴⁰,⁴¹ |
| LGD-6972 | Non-peptide small molecule | Phase II | ⁴² |
| Ionis GCGRx antisense | Phase II | ⁴³-⁴⁶a |
| REGN1193 | Human monoclonal IgG4 antibody | Phase I | This issue²⁴ |

⁻ Data from a Phase I study with a different antisense molecule, ISIS 325568, were reported by van Dongen et al.⁴⁷

| TABLE 2 | Comparison of adverse effects reported in human studies with glucagon receptor-blocking drugs |
| --- | --- | --- | --- |
| **Adverse events reported in human studies** | **REGN1193** | **Glucagon receptor antagonists** | **Glucagon receptor antisense** |
| ALT/AST elevation | † | † | † |
| LDL cholesterol | No change | † | No increase |
| Hypoglycaemia (≤70 mg/dL) | † | † | No change |
| Blood pressure | No increase | † | No increase |
| Body weight | No change | † | No change |
| Hepatic steatosis | ? | † | ? |
| Circulating gluconeogenic amino acids⁴⁸ | † | † | ? |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL, low density lipoprotein.

⁻ In rodent studies, REGN1193, glucagon receptor antagonists,⁴⁹,⁵⁰ and glucagon receptor knockout²⁶ increase circulating amino acid levels, resulting in pancreatic islet α-cell hyperplasia.⁵¹,⁵²
tracked pharmacodynamics directly using the inhibition of the hyperglycaemic response to a standardized glucagon challenge (0.8 mg i.v.). Onset-of-action was investigated using a pharmacologically active 0.3 mg/kg dose and the standardized glucagon stimulation test. Development of anti-drug antibodies was monitored over the 15-week observation period.

REGN1193 exhibited markedly non-linear clearance, resulting in half-life estimates of 0.5 days for the 0.05 mg/kg dose to 4 days for the 0.6 mg/kg dose. Anti-drug antibody formation was unremarkable and did not contribute to the variable pharmacokinetics. The pharmacodynamic profile observed is summarized in Table 3. As expected from the non-clinical data, the higher doses of REGN1193 blocked the elevation of plasma glucose and C-peptide seen with the glucagon challenge, effects consistent with the reported actions of the non-peptide small molecule antagonists31,32,34, and antisense molecules.43 The data relating to reversible increases in ALT and AST (without elevation of bilirubin) appear to be consistent across all glucagon receptor blocking modalities and there is now a report that LY240921 increases liver fat measured by MRL.37

The data relating to reversible increases in ALT and AST (without elevation of bilirubin) appear to be consistent across all glucagon receptor blocking modalities and there is now a report that LY240921 increases liver fat measured by MRL.37 As regards the elevation of LDL cholesterol initially observed with MK-0893 and MK-3577,32,34 this appears to correlate with the magnitude of glucose-lowering in people with type 2 diabetes, suggesting a mechanism-based association, perhaps through altered absorption of cholesterol.50 Initially, variable data were presented for LY240921 (no significant increase in LDL cholesterol after 12 or 24 weeks of treatment),36 elevation of total cholesterol54, but a more recent full publication clearly shows that 6 months of treatment with the compound increases circulating total cholesterol accompanied by a non-significant increase in LDL cholesterol compared with placebo.37 This is consistent with results using a different molecule, PF-06291874, in which the highest dose

| TABLE 3 Summary of the pharmacodynamic effects of REGN1193 compared with those observed with other methods of modulating the glucagon and GLP-1 systems |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Glycaemic response to glucagon challenge | REGN1193 | Glucagon receptor antagonists | Glucagon receptor antisense | Glucagon receptor knockout | Glucagon receptor agonist | GLP-1 receptor agonist |
| HbA1c | No data | ↓ | ↓ | ↓ | ↑ | ↑ |
| Circulating glucagon concentration | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| Circulating insulin concentration | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| Circulating C-peptide concentration | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| Circulating total GLP-1 concentration | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| Circulating GLP-2 concentration | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| Glucose-dependent insulinotropic peptide (GIP) | No change | ↑ | ↑ | ↑ | ↑ | ↑ |
| Circulating PYY concentration | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| Circulating bile acid metabolites | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |

Abbreviation: NA, not applicable.

a No consistent change in active GLP-1.
(150 mg) administered for 4 weeks significantly increased LDL cholesterol.\textsuperscript{30} No significant lipid effects have been described with the small molecule LGD-6972\textsuperscript{22} or the antibody REGN1193, but it is not yet clear whether these are true differences from the other molecules or simply a reflection of shorter duration of dosing or the populations studied. LY240291 and MK-0893 increased body weight and blood pressure,\textsuperscript{31,32,37} and there were trends observed with PF-06291874,\textsuperscript{30} but again these have not been observed with LGD-6972 or REGN1193. Interestingly, preliminary communications indicate that up to 14 weeks of dosing with the antisense ion AGGRX was not associated with increases in LDL cholesterol, body weight or blood pressure,\textsuperscript{44} raising mechanistic questions about these phenomena.

Although the development of glucagon receptor antagonists is proving a thorny issue, it is yielding insights into the witches’ brew of their action at the hepatic glucagon receptor, and these are compared across classes of drugs in Table 3. For example, the universal observation of elevated glucagon secretion when the receptor is blocked raises the possibility that there may be significant signalling through the GLP-1 receptor,\textsuperscript{18} as suggested by receptor knockout studies.\textsuperscript{19} Overlying this is the interesting possibility that there is differential feedforward stimulation (insulin/C-peptide) and feedback inhibition (oxyntomodulin, glicentin, PYY, and even GLP-1 and GLP-2) of the secretion of bioactive peptides that may vary greatly depending on how much the receptor signalling is disturbed and whether this is being done in a “biased” manner. Some caution is prudent, therefore, when attributing the pharmacological and safety profiles of glucagon receptor blockers solely to their action at the hepatic glucagon receptor, and constant comparison across molecules is justified. As an example, circulating GLP-2, a peptide that stimulates growth of the gastrointestinal tract,\textsuperscript{59,60} is elevated when the glucagon receptor is blocked, indicating that long-term safety will require monitoring not only for the potential effects of absence of glucagon action, but also for actions attributable to accompanying changes in related bioactive peptides.

In conclusion, the road to a useful medicine based on blocking glucagon receptor antagonists is proving a thorny issue, it is yielding insights into the witches’ brew of their action at the hepatic glucagon receptor, and these are compared across classes of drugs in Table 3. For example, the universal observation of elevated glucagon secretion when the receptor is blocked raises the possibility that there may be significant signalling through the GLP-1 receptor,\textsuperscript{18} as suggested by receptor knockout studies.\textsuperscript{19} Overlying this is the interesting possibility that there is differential feedforward stimulation (insulin/C-peptide) and feedback inhibition (oxyntomodulin, glicentin, PYY, and even GLP-1 and GLP-2) of the secretion of bioactive peptides that may vary greatly depending on how much the receptor signalling is disturbed and whether this is being done in a “biased” manner. Some caution is prudent, therefore, when attributing the pharmacological and safety profiles of glucagon receptor blockers solely to their action at the hepatic glucagon receptor, and constant comparison across molecules is justified. As an example, circulating GLP-2, a peptide that stimulates growth of the gastrointestinal tract,\textsuperscript{59,60} is elevated when the glucagon receptor is blocked, indicating that long-term safety will require monitoring not only for the potential effects of absence of glucagon action, but also for actions attributable to accompanying changes in related bioactive peptides.

In conclusion, the road to a useful medicine based on blocking glucagon signalling appears steep and sticky, but the subtlety of the system may provide some hope that a golden key can be found if investment in drug discovery is maintained.

Conflict of interest

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Author contributions

D.J.N. wrote the first draft. D.J.N. and D.D’A. reviewed the drafts and final submitted version.

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