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Dear Editors,

We read with great interest a recent comprehensive review covering the knowns and the unknowns of glucagon-like peptide-1 (GLP-1) by McLean et al. (McLean et al., 2020). GLP-1 is best known for its incretin effects first described in the 1980s that provided strong foundations for the development of GLP-1 targeting drugs and revolutionized pharmacotherapy of insulin resistance in the 2000s (Drucker et al., 2017). Nevertheless, as evident from (McLean et al., 2020), we can expect that the glory days of GLP-1 are far from over and that this incretin is going to be even more important for the doctors of tomorrow based on many of its relevant actions, especially in the cardiovascular and central nervous system.

A review by McLean et al. (McLean et al., 2020) drew our attention not only because it covers a timely and captivating topic, but because it clearly emphasizes serious limitations and methodological caveats regarding GLP-1 receptor (GLP-1R) expression analysis. Failure to acknowledge methodological caveats either willfully or through ignorance far too often leads to misinterpretation of the results and formation of unjustified conclusions challenging to rectify once they pass under the peer-review radar, and unfortunately – they often do. In this letter, we wish to acknowledge the intention of McLean et al. (McLean et al., 2020) to warn the readers about the methodological drawbacks, especially those regarding the specificity and sensitivity of GLP-1R antisera and supplement the „Caveats and limitations“ section (McLean et al., 2020) with our own experience and comment regarding bs-1559R anti-GLP-1R rabbit polyclonal antibody.

The problem of sensitivity and specificity of GLP-1R antibodies has already been brought up before and is probably best summarized by the statement that „scientists interested in GLP-1R expression face the challenging task of assessing how much, if any, of the data published with these antisera is correct“ (Drucker, 2013). Many anti-GLP-1R antibodies are commercially available but data demonstrating their specificity, selectivity, as well as „negative“ runs with cells or tissue samples from *Glp1r*-/- animals are usually not available even upon a reasonable request (Drucker, 2013). On the

other hand, problems are usually uncovered and reported by thoughtful end-users conducting validation experiments in the pursue of reliable data suitable to provide a meaningful representation of reality(Panjwani et al., 2013; Pyke and Knudsen, 2013). For example, Panjwani et al. demonstrated that three different commercially available antibodies – SantaCruz-sc-66911, LifeSpan-LS-A1205, and Abcam-ab39072 were unable to detect a difference in GLP-1R in lung extracts of *Glp1r*+/+ and *Glp1r*-/- mice using a traditional Westen blot (WB) analysis (Panjwani et al., 2013). We have employed bs-1559R to detect GLP-1R with WB, immunohistochemistry, and catalyzed reported deposition in the rat tissue samples from pharmacological experiments with Exendin9-39. However, a closer look at the antibody specification makes us wonder whether the antibody raised to the keyhole limpet hemocyanine-conjugated synthetic peptide from 101-200/463 derived from the rat GLP-1R can give us the information we are looking for as i) the target epitope covers the orthosteric binding site, and ii) the immunogen sequence is highly dynamic and functionally important receptor domain? The transition of the GLP-1R to the active state requires a major conformational change of its extracellular domain (ECD) that has to reorient from the „closed“ transmembrane domain-interacting position to enable peptide binding (Wu et al., 2020). So far, the manufacturer was only able to confirm our suspicion regarding the orthosteric site-bound antibody affecting ligand binding. However, if vice versa is also true, could it be that e.g. ligand binding-induced conformational change of the ECD also affects our anti-GLP-1R-obtained signal? Could „conformational bias“ induced by convenient generation of antibodies to the conformationally dynamic „lid“ of GPCRs (Wheatley et al., 2012) explain at least some of the discrepant results on the GLP-1Rs, or even GPCRs in general(Pyke and Knudsen, 2013)? Either way, we should think about it and talk about it. We cannot afford to waste a good crisis (Drucker, 2016).

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