GLP-1 receptor – Do we really know what we're looking at?

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19   Dear Editors,

20   We read with great interest a recent comprehensive review covering the knowns and the unknowns

21   of glucagon-like peptide-1 (GLP-1) by McLean et al. (McLean et al., 2020). GLP-1 is best known for its

22   incretin effects first described in the 1980s that provided strong foundations for the development of

23   GLP-1 targeting drugs and revolutionized pharmacotherapy of insulin resistance in the 2000s (Drucker

24   et al., 2017). Nevertheless, as evident from (McLean et al., 2020), we can expect that the glory days of

25   GLP-1 are far from over and that this incretin is going to be even more important for the doctors of

26   tomorrow based on many of its relevant actions, especially in the cardiovascular and central nervous

27   system.

28   A review by McLean et al. (McLean et al., 2020) drew our attention not only because it covers a timely

29   and captivating topic, but because it clearly emphasizes serious limitations and methodological

30   caveats regarding GLP-1 receptor (GLP-1R) expression analysis. Failure to acknowledge

31   methodological caveats either willfully or through ignorance far too often leads to misinterpretation

32   of the results and formation of unjustified conclusions challenging to rectify once they pass under the

33   peer-review radar, and unfortunately – they often do. In this letter, we wish to acknowledge the

34   intention of McLean et al. (McLean et al., 2020) to warn the readers about the methodological

35   drawbacks, especially those regarding the specificity and sensitivity of GLP-1R antisera and

36   supplement the „Caveats and limitations" section (McLean et al., 2020) with our own experience and

37   comment regarding bs-1559R anti-GLP-1R rabbit polyclonal antibody.

38   The problem of sensitivity and specificity of GLP-1R antibodies has already been brought up before

39   and is probably best summarized by the statement that „scientists interested in GLP-1R expression

40   face the challenging task of assessing how much, if any, of the data published with these antisera is

41   correct" (Drucker, 2013). Many anti-GLP-1R antibodies are commercially available but data

42   demonstrating their specificity, selectivity, as well as „negative" runs with cells or tissue samples from

43   *Glp1r-/-* animals are usually not available even upon a reasonable request (Drucker, 2013). On the

44 other hand, problems are usually uncovered and reported by thoughtful end-users conducting

45 validation experiments in the pursue of reliable data suitable to provide a meaningful representation

46 of reality(Panjwani et al., 2013; Pyke and Knudsen, 2013). For example, Panjwani et al. demonstrated

47 that three different commercially available antibodies – SantaCruz-sc-66911, LifeSpan-LS-A1205, and

48 Abcam-ab39072 were unable to detect a difference in GLP-1R in lung extracts of *Glp1r+/+* and *Glp1r-*

49 */-* mice using a traditional Westen blot (WB) analysis (Panjwani et al., 2013). We have employed bs-

50 1559R to detect GLP-1R with WB, immunohistochemistry, and catalyzed reported deposition in the

51 rat tissue samples from pharmacological experiments with Exendin9-39. However, a closer look at the

52 antibody specification makes us wonder whether the antibody raised to the keyhole limpet

53 hemocyanine-conjugated synthetic peptide from 101-200/463 derived from the rat GLP-1R can give

54 us the information we are looking for as i) the target epitope covers the orthosteric binding site, and

55 ii) the immunogen sequence is highly dynamic and functionally important receptor domain? The

56 transition of the GLP-1R to the active state requires a major conformational change of its extracellular

57 domain (ECD) that has to reorient from the „closed" transmembrane domain-interacting position to

58 enable peptide binding (Wu et al., 2020). So far, the manufacturer was only able to confirm our

59 suspicion regarding the orthosteric site-bound antibody affecting ligand binding. However, if vice

60 versa is also true, could it be that e.g. ligand binding-induced conformational change of the ECD also

61 affects our anti-GLP-1R-obtained signal? Could „conformational bias" induced by convenient

62 generation of antibodies to the conformationally dynamic „lid" of GPCRs (Wheatley et al., 2012)

63 explain at least some of the discrepant results on the GLP-1Rs, or even GPCRs in general(Pyke and

64 Knudsen, 2013)? Either way, we should think about it and talk about it. We cannot afford to waste a

65 good crisis (Drucker, 2016).

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68 the manuscript. All authors provided final approval of the version to be published.

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