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Source / Izvornik: **Acta Histochemica, 2021, 123**

Journal article, Accepted version

Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

<https://doi.org/10.1016/j.acthis.2021.151732>

Permanent link / Trajna poveznica: <https://urn.nsk.hr/urn:nbn:hr:105:200103>

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Download date / Datum preuzimanja: **2024-11-02**



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1 **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).

66 **Conflict of interest:** None.

67 **Author's contributions:** JH wrote the letter. ABP, AK, JOB, and MSP provided a critical feedback on
68 the manuscript. All authors provided final approval of the version to be published.

69 **Funding source:** This work was funded by the Croatian Science Foundation (IP-2018-01-8938). The
70 research was co-financed by the Scientific Centre of Excellence for Basic, Clinical, and Translational
71 Neuroscience (project “Experimental and clinical research of hypoxic-ischemic damage in perinatal
72 and adult brain”; GA KK01.1.1.01.0007 funded by the European Union through the European Regional
73 Development Fund).

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