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Breakthroughs and Views

Rebuttal to Deacon and Holst: “Metformin effects on dipeptidyl peptidase IV degradation of glucagon-like peptide-1” versus “Dipeptidyl peptidase inhibition as an approach to the treatment and prevention of type 2 diabetes: a historical perspective”

Hans-Ulrich Demuth,^{a,*} Simon A. Hinke,^b Raymond A. Pederson,^b
and Christopher H.S. McIntosh^b

^a *Probiodrug AG, Biocenter, Weinbergweg 22, D-06120 Halle (Saale), Germany*

^b *Department of Physiology, University of British Columbia, Vancouver, Canada V6T 1Z3*

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It is a concern to us that the recent review by Deacon and Holst [1] may be construed as defamatory, and it is disappointing that this paper was published without an invitation to present a rebuttal in the same issue. We maintain that, considering the topic of our manuscript (i.e., the mode of action of metformin [2]), and the audience of *Biochemical and Biophysical Research Communications*, the scope of our survey of the literature was broad enough, and we defend the accuracy of our statements. Upon reading their “historical review” [1], it was clear that there are important omissions from the document and inaccurate statements included. For example, although the first report of N-terminal degradation of GLP-1 may have been by Buckley and Lundquist in 1992 [3], as reported by Deacon and Holst, the first indication of such degradation of the incretin GIP can be found in two publications in 1981 [4,5] (reviewed in [6]). Brown et al. [5] and Jörnvall et al. [4] described the purification of GIP_{3–42} from intestinal extracts of pigs, and stated that this biologically inactive peptide may result from hydrolysis by “aminopeptidase, elastase, dipeptidyl aminopeptidase, or related enzymes in the intestine.” This finding was independently substantiated in 1987 [7]. It was stated in the review that N-terminally truncated peptides, GIP_{3–42} and GLP-1_[9–36] were inactive at their respective receptors, however, the

definitive paper showing these data for GIP [8] was not cited. Many researchers also consider DP IV-negative rats and CD26 knock-out mice to be models of the effect of chronic DP IV inhibition [9–12], and thus these papers should have been included in a proper historical review. Furthermore, the decades of research that have gone into the development of potent specific inhibitors of DP IV were completely overlooked (reviewed in [13]), including the intellectual source [14,15] of the specific DP IV inhibitor (valine pyrrolidide) used by Deacon et al. [16]. We hope that a public apology and inclusion of these references in the form of an erratum will appear from Drs. Deacon and Holst. Following is a defense to statements of alleged “inaccuracy” [1] contained within our own manuscript [2].

We concur that Mentlein and co-workers [17] first showed that GIP and GLP-1 were substrates for DP IV in vitro. Importantly, our statement in Hinke et al. [2] was: “Mentlein et al. [17] first showed that GIP and GLP-1 were substrates for DP IV in vitro, and shortly thereafter, in vivo degradation was also demonstrated [18].” This statement is entirely accurate; the studies by Deacon et al. only examined degradation of synthetic GLP-1 incubated in plasma in vitro (not by DP IV as reported [1]) and additionally detected truncated metabolites in vivo [19,20], but they failed to look at GIP degradation, whereas Mentlein et al. [17] and Kieffer et al. [18] examined hydrolysis of both incretins by both purified DP IV and serum. Certainly there are many examples of published manuscripts from Deacon or Holst in which they cite their

* Corresponding author. Fax: +49-345-555-9901.

E-mail address: hans-ulrich.demuth@probiodrug.de (H.-U. Demuth).

own papers [19,20], but not Kieffer et al. [18], or even in some cases fail to cite Mentlein et al. [17]; the accuracy of these examples depends on whether the statements made refer to plasma-mediated or DP IV-mediated degradation of the incretins.

It is an important point regarding all of the early work on incretin metabolism that none of the experiments can assign specific peptidases to the inactivation of the incretins in serum samples or in vivo. Additional potential candidates for such in vivo activity are for example: aminopeptidase N, dipeptidyl peptidase β , attractin, dipeptidyl peptidases I and II, prolylendopeptidase, and neutral endopeptidase [13,21–27]. Appropriately, Deacon and colleagues [19] arrived at the conclusion, “The metabolism of GLP-1 (7–36) amide cannot be accounted for entirely by the action of plasma dipeptidyl peptidase IV... Tissues other than the plasma must therefore be important sites of GLP-1 metabolism, but the enzymes involved have not been studied.” The development of specific techniques for the study of N-terminal degradation of the incretins has been valuable “to evaluate the secretory patterns of biologically active GIP and GLP-1 under normal and pathophysiological conditions” [17–20,28–30]. However, none of these techniques can identify DP IV as the principle incretin degrading enzyme at that time, nor do they provide information on the application of DP IV-inhibition to treatment of hyperglycemia.

Deacon and Holst stated “inhibition of dipeptidyl peptidase IV may prove a useful adjunct in the management of type 2 diabetes... inhibition of GLP-1 (7–36) amide degradation would... increase the availability of biologically active peptide” [1,19,31]. This statement is ambiguous at best—the authors have taken it out of context from a paper focused exclusively on the potential therapeutic application of *exogenous* GLP-1 [19]. Indeed, nothing regarding glucose tolerance is stated in [19] or [32]. Pauly et al. were the first to show improvement of glucose tolerance in mammals (rats) with specific DP IV inhibition [33]. However, in our study [2] we used an alternative citation [29] which Deacon and Holst also referred to in their review [1], although they failed to quote the entire statement. In full, the statement was: “... in vivo inhibition of DP IV is predicted to have a profound effect on the enteroinsular axis. An exaggerated incretin response would be expected in response to an increased half life of endogenously released GIP_[1–42] and GLP-1_[7–36].” The definition of an “incretin” hormone is: (1) it must be released by nutrients, particularly carbohydrates, and (2) at physiological levels, it must stimulate insulin secretion in the presence of elevated blood glucose [34–38]. Taken together, it was not inaccurate to credit Pauly et al. [29,33] in our manuscript [2].

With regard to their final point, that we “ignored” their Zander et al. manuscript [39] from our discussion: this paper came to light after the preparation of our manuscript and, indeed, it does examine responses to

GLP-1 infusion combined with metformin treatment. Importantly, it does not directly address Mannucci’s hypothesis [40], but states that data are not consistent with “inhibition of GLP-1 degradation.” As the publication from Zander and associates [39] was an entirely clinical paper, and our manuscript was prepared for *Biochemical and Biophysical Research Communications*, primarily having an audience of biochemists, and for the sake of brevity and pertinence, this citation was not included. As our paper directly questioned the findings of Mannucci and co-workers [40], in a clinical paper, we addressed a letter to the editor of *Diabetes Care* [41] to open a forum for discussion on the topics we addressed in our *BBRC* manuscript [2] with the audience of clinicians in mind. In this letter, we favourably refer to the Zander manuscript [39], thus refuting the claim that we “ignored” their manuscript.

The topic of our paper was not the therapeutic potential of DP IV inhibition, which has already been a broad topic of many recent reviews [31,42–47] but rather, the specific question as to whether metformin acts partially via DP IV-inhibition [2,40]. It would have been impractical to include reference to publications only peripherally connected with this focus. A *thorough* historical review on DP IV inhibition in type 2 diabetes would have been welcomed, however we believe that the unfounded remarks regarding the accuracy of our own manuscript were unwarranted. We believe that the principles of fair scientific comment should have been applied whereby comments on papers and rebuttals are printed in the same issue of the journal. We hope that any defamation arising from the historical review of Deacon and Holst is now rectified, and that the scientific community will judge all parties on the quality and integrity of their respective research, and the value of each of their contributions.

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