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Review

Type 2 diabetes—Therapy with dipeptidyl peptidase IV inhibitors

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Abstract

The sole application of an inhibitor of the dipeptidyl peptidase DP IV (also DP 4, CD26, DPP-IV or DPP-4) to a mammal subsequently leading to improved glucose tolerance marks a major breakthrough in metabolic research bearing the potential of a new revolutionary diabetes therapy. This was demonstrated in rat applying the specific DP IV inhibitor isoleucyl thiazolidine. It was published in 1996 for the first time that a specific DP IV inhibitor in a given dose was able to completely block glucagon-like peptide-1 (GLP-1) degradation in vivo resulting in improved insulin response accompanied, by accelerated peripheral glucose disposal. Later on, these results were confirmed by several research teams applying DP IV inhibitors intravenously or orally. Today, the DP IV inhibition for the treatment of metabolic disorders is a validated principle. Now, more than 10 years after the initial animal experiments, first DP IV inhibitors as investigational drugs are tested in phase 3 clinical trials.

Keywords: Dipeptidyl peptidase; Type 2 diabetes; GLP-1; GIP; Inhibitor; Inhibition; Drug development

1. Introduction

The discovery of the blood glucose lowering potential of the incretins GLP-1 and glucose-dependent insulinotropic peptide (GIP) and their properties as growth hormones opens up a totally new pathway for causal diabetes therapy. Since both hormones are characterized by an acute insulinotropic action and a sustained effect upon insulin resistance and the glucose sensitivity of the cells of the islets of Langerhans a pleiotropic treatment of different diabetic symptoms seems possible for the first time.

Since both hormones are also deactivated simultaneously by the cardiovascular exopeptidase dipeptidylpeptidase IV (DP IV) in contrast to hormone montherapy, a multivalent method of treatment is opened up by the inhibition of this enzyme. Interestingly, other bioactive peptides are also DP IV substrates, for example vasoactive intestinal peptide (VIP), somatostatin, pituitary adenylate cyclase-activating polypeptide (PACAP) and neuropeptide Y (NPY). Consequently, additional synergy potentials may arise by the inhibition of DP IV activity. Unlike hormone therapy, however, which functions with pharmacologically active doses of GLP-1 or its analogues, a DP IV inhibitor modulates "only" the endogenous released hormone concentrations. The following article thus gives a review of the development of DP IV inhibitors as potential antidiabetics.

2. DP IV—a short historical perspective

DP IV (EC 3.4.14.5, also DP 4, CD26, DPP IV, DPP-IV or DPP-4) was discovered in the 1960s as an aminopeptidase [1]. In the 1970s, the enzyme served initially as a model protein for the study of the catalytic mechanism of serine peptidases and for the investigation of the specifics of proline peptide bonds [2]. In the 1980s, the potential of the enzyme to convert bioactive peptides in vitro was discovered, which intensified the search for its function in vivo [3]. The early 1990s is characterized by numerous

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studies on the role of DP IV in immune responses, in particular T cell activation, signal transduction and T cell proliferation [4–6].

DP IV was identified as characteristic antigen marker CD 26 of T cell activation and it was demonstrated that the protein is a component of the T cell receptor complex. Different binding partners were found within this context, for example adenosine deaminase (ADA) [7], the HIV mantle protein gp 120 [8], tyrosine phosphatase CD 45 [9], fibronectin [10] and the renal sodium proton antiporter NHE3 [11].

A controversial debate erupted in this area in 1993 when the hypothesis was put forward that CD26 is co-responsible for the entry of HIV into T cells [12]. New in vivo functions of the enzyme were being debated when in the middle of the 1990s, the involvement of DP IV in metabolism and the regulation of the cytokines, chemokines and different peptide hormones triggered programs for the development of DP IV inhibitors [13].

It was the discovery of the role of the enzyme in energy homeostasis which accelerated the design of potential pharmaceutical agents for the treatment of that most important of metabolic diseases, Type 2 diabetes, and led to the first patent application for the use of DP IV inhibition in the reduction of blood glucose [14].

Further enzymes with post-proline dipeptidyl aminopeptidase activity, which today belong to the DP IV subfamily of the prolyl oligopeptidases (POP), were discovered in the late 1990s [15].

At this time, the crystal structure of POP, a sister enzyme of DP IV was elucidated [16].

At the beginning of the new millennium, far more had become known of the role of cell internalization processes and the compartmentalization of DP IV during the association with binding partners in the expression of the immune response [17,18].

Moreover, specific mechanisms of metastasis which involve DP IV were clarified [19]. The most important milestone in DP IV research is, however, the elucidation of its three-dimensional structure. Seven structures, of which five are human recombinant structures and one a native pig DP IV structure, have been published since January 2003, when resolutions of 1.8–2.6 Å were achieved. A structure determined by electron microscopy based on crystalline mouse DP IV in shown in Fig. 1. All structures exhibit the same principal topology of domain organization [20–26].

The DP IV three-dimensional structures help not only in understanding the unusual protease function of the enzyme, but also explain additional properties which differentiate the enzyme from the "trypsin" serine proteases and characterize it as an important physiological communication molecule.

3. Dipeptidylpeptidase IV—localization, structure and substrates

DP IV was first isolated from rat liver in 1966 [1]. Since then, analogous enzyme activity has been detected in various mammals and also in microorganisms and plants. In humans, the enzyme activity is found ubiquitously in almost all organs and tissues with the highest local concentrations found renally in the proximal tubuli and luminally on the epithelial cells of the small intestine [27].

With the discovery of the role of the enzyme in glucose homeostasis, DP IV research has increased since the middle of the 1990s to such an extent that more than 1700 original papers with the keyword DP IV are registered in the National Library of Medicine, and more than 230 patent applications on protein structure and enzyme inhibition have been submitted. Many of these results have been presented at conferences [28–32] and recently summarized in an excellent review by Ingrid DeMeester [33].

DP IV is a membrane-bound, homodimeric class II protein with a molecular weight of 110–150 kD per subunit. It is bound to the membrane by a transmembrane sequence of ca. 22 amino acids. The 6 cytosolic amino acids play no

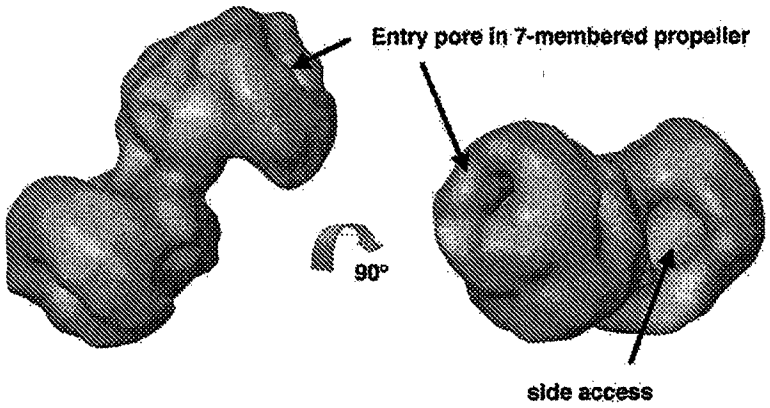

Fig. 1. Surface representation of DP IV obtained by electron microscopy [26]. The propeller domains with an entry pore and a large side opening can be recognized (reproduction with kind permission of W. Reutter).

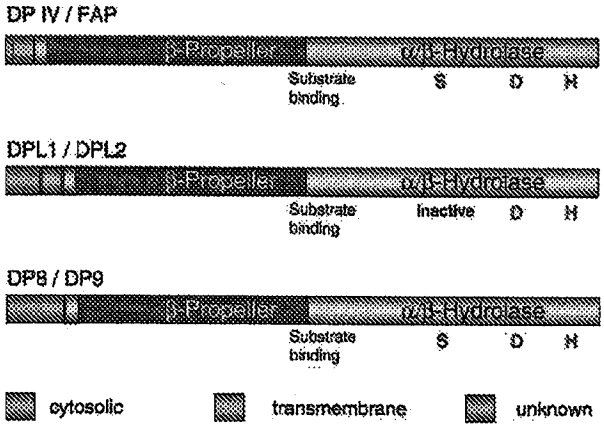

Fig. 2. The human DP IV gene family.

role in the binding functions. In addition to its protease function, DP IV has receptor properties and as extracellular binding protein. Fig. 1 gives an impressive surface profile of DP IV.

The enzymes fibroblast activation protein (FAP or DP 5), DP 8 and DP 9, and the catalytically inactive proteins, DP L1 (DP 6) and DP L2 (DP 10), are structurally related to DP IV [15,34].

Different structural regions of the protein are assigned to the primary sequence in Fig. 2. Whereas the catalytically active residues of the enzyme are localized in the region of the α/β-hydrolase domain, the protein–protein interactions described occur mainly with regions of the so-called β-propeller domain (see also Fig. 1).

Structurally unrelated but furnished with similar substrate specificity are the monomeric enzymes dipeptidyl-peptidase II (DP II, DP 2 or DP 7) and attractin (Atrn). Physiological functions have thus far not been detected for attractin, which, like DP IV, occurs in high concentrations in the blood stream and is also involved functionally in the immune response, nor for DP II.

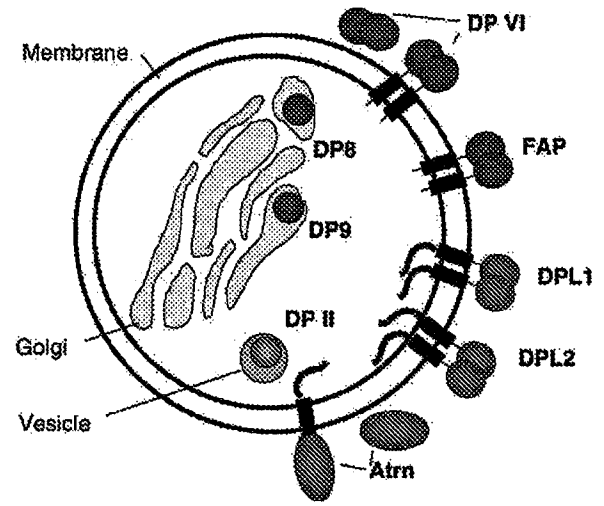

Fig. 3. The cellular localization of DP IV-like enzymes.

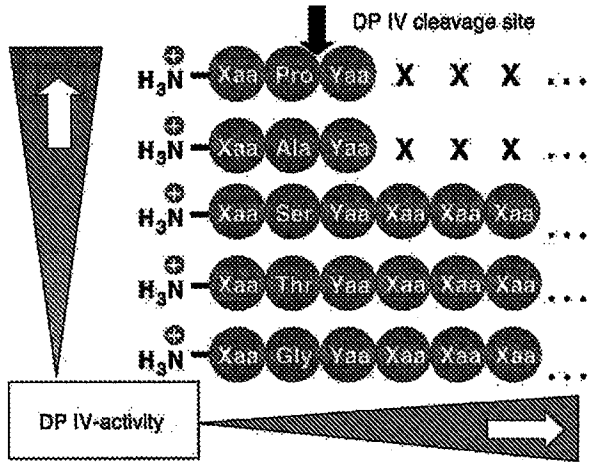

Fig. 4. Characteristics of DP IV specificity: the longer the peptide, the lesser the enzyme is proline specific. Also, the longer the substrate, the faster it is hydrolyzed.

The cellular localization of DP IV-like enzymes is shown schematically in Fig. 3.

The initial work by Hopsu-Havu [1] and the enzymological characterization identify DP IV as a proline-specific peptidase [2,3].

However, the extensive investigation of potential physiological substrates has shown that the preferential proline specificity is lost with increasing substrate size (Fig. 4).

The natural substrates of DP IV include chemokines, cytokines, endomorphines, hormones of the pancreatic polypeptide family and almost all peptides of the PACAP/glucagon peptide family [27,35].

Because of this substrate specificity which allows DP IV-analogous enzymes to be involved in numerous regulation processes, the modulation of DP IV activity by enzyme inhibitors appears to make interesting therapeutic approaches a possibility. For example, the stabilization of neuropeptide Y by DP IV inhibitors in the CNS leads to

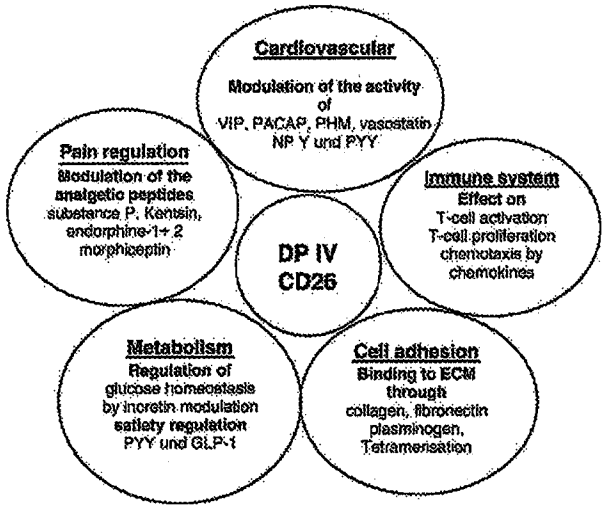

Fig. 5. Physiological processes which are affected by DP IV activity.

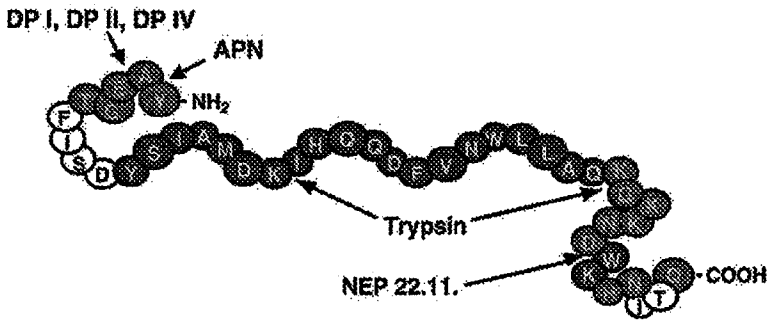

Fig. 6. Protease attack sites at GIP.

significant reduction in the anxiety behavior of experimental animals [34].

Since no tissue-specific activity differences have been found for the enzyme and unlike the enzymes of the coagulation cascade DP IV is controlled neither by limited proteolysis nor by endogenous inhibitors, specific DP IV inhibitors should possess therapeutic potential in different pathophysiological processes (Fig. 5).

One such approach is examined more closely in the following, with the example of the development of DP IV inhibitors for the treatment of Type 2 diabetes.

4. DP IV activity in blood glucose regulation

The discovery of the insulinotropic action of endogenous intestinal factors was a pivotal milestone in the investigation of the regulation of glucose homeostasis.

Its considerable blood glucose-lowering activity led very early to speculation on a therapeutic use of the incretins GIP and GLP-1 as potential antidiabetics. Reviews by Kieffer and Habener [35] and Vilsboll and Holst [36] report in detail on this area of research.

Unfortunately, the short biological half-life of these active peptides impair their sustained therapeutic use. Although the hormones are still detectable in serum 2–4 h after prandial secretion by means of C-terminus specific antibodies their biological activity is of very limited duration. Thus, the half-life of GLP-1 is of the order of 1 min and that of GIP of the order of 10 min [36].

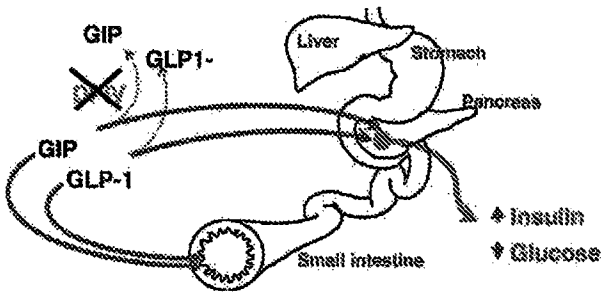

Fig. 7. Schematic representation of DP IV-modulated incretin action.

In a work published in 1993, Mentlein et al. [13] describe the DP IV-catalyzed hydrolysis of a series of neuropeptides and the gastro-intestinal peptide hormones GIP and GLP-1 in vitro. It had been shown previously by Pederson and Brown in 1976 [37] that only the intact N-terminal hormone GIP_{1-42} (or GIP_{1-30}) possesses insulinotropic action and could be deactivated by amino-peptidases or dipeptidyl aminopeptidases (Fig. 6). Exo-peptidases such as inter alia aminopeptidase N and different dipeptidyl peptidases (I, II and IV) come into consideration for the proteolysis of the peptides in vivo as do endopeptidases such as trypsin and neutral endopeptidase (NEP 22.11).

In 1996, the in vivo use of DP IV inhibitors and their potent glucose-lowering activity confirmed the pivotal role of DP IV in the catabolism of incretins [14,38,39] and initiated considerable interest within the pharmaceutical industry for the development of DP IV inhibitors.

Interestingly, it was recently shown that overweight is associated with increased DP IV activity. The authors infer here a connection between adiposity-induced Type 2 diabetes and increase incretin degradation in these patients [40]. Meanwhile, it was demonstrated that DP IV-negative rats and mice have extremely good glucose tolerance and, unlike wild type animals, do not tend towards overweight and diabetic symptoms with a fat-rich diet [41,42]. Thus, as a principle for the therapeutic treatment of hyper-

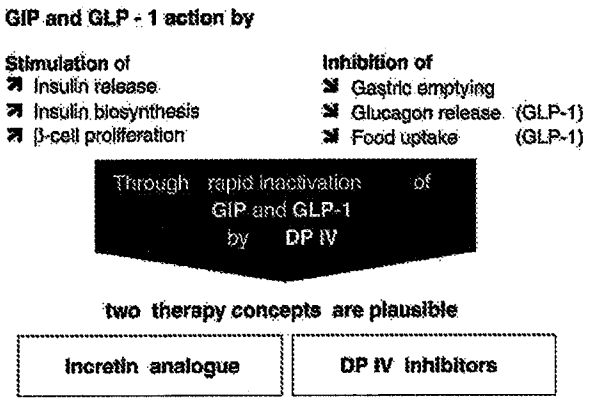

Fig. 8. Incretin-mediated therapy—a multivalent therapeutic approach.

glycaemia and therewith, the associated metabolic disease states and subsequent consequences DP IV inhibition is a recognized current development area [43,44]. The action of DP IV inhibitors is presented in a highly simplified form in Fig. 7.

5. DP IV inhibitors

The possibility of the exploitation of multiple synergetic effects of different peptidic signals in the post-prandial phase appears especially attractive for the potential therapeutic use of DP IV inhibitors. The properties of the incretins are summarized in Fig. 8.

Currently, more than 30 pharmaceutical and biotechnology companies have programs on the development of DP IV inhibitors for the treatment of Type 2 diabetes (Fig. 9).

DP IV inhibitors have been prepared since 1977. Their synthesis and the different structural classes have, in the meantime, been the subject of different reviews [45,46]. Initial reversible and irreversible compounds were chemically modified product analogues whose inhibitory potency extends into the sub-nanomolar region. More recently, compounds of non-peptidic structure have become increasingly known from screening procedures [46]. In addition, the three-dimensional structures of the enzyme published since 2003 have made a rationally motivated inhibitor structure design possible, which, in the case of the aminopyrimidines, has led to increases in activity of about five orders of magnitude [46,47].

In respect of current clinical developments of the thousands of individual compounds prepared in the mean-

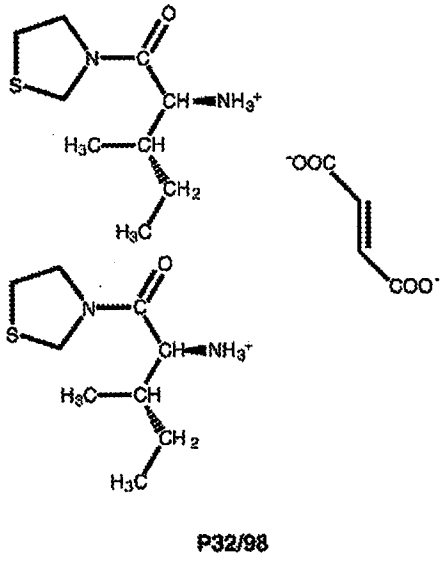

Fig. 10. Reversible product analogue inhibitors (e.g. pyrrolidines and thiazolidines).

time, three substance classes stand out whose representatives are under investigation in man (Figs. 10–12).

- Reversible product analogue inhibitors (e.g. pyrrolidines, thiazolidines).
- Covalently modifying product analogue inhibitors (e.g. cyanopyrrolidines).
- Reversible non-peptidic heterocyclic inhibitors (e.g. xanthines and aminomethylpyrimidines).

Unlike the proteolytic enzymes which occur in the blood stream in only very small amounts, DP IV occurs in very high

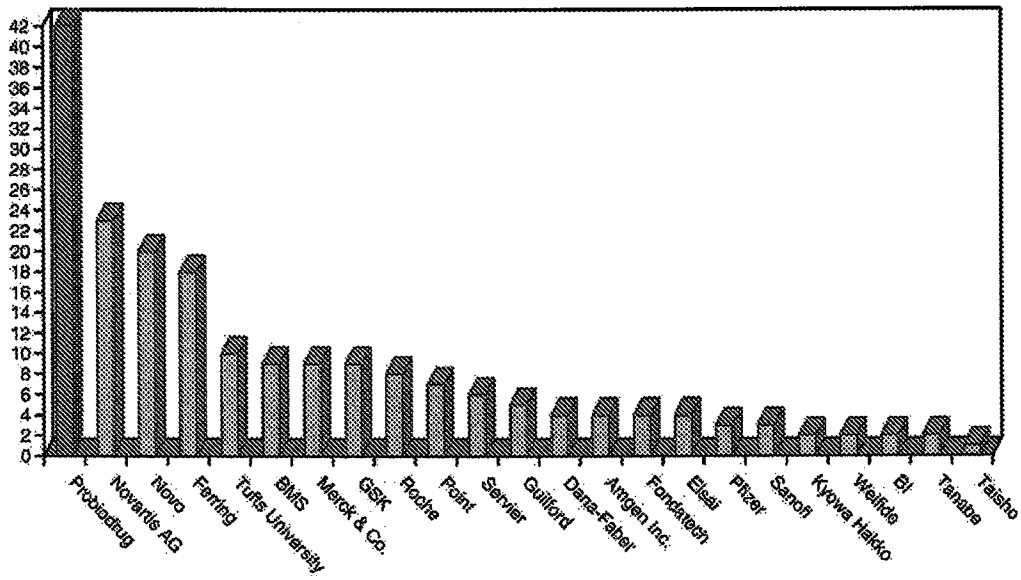

Fig. 9. Estimated number of DP IV inhibitor patents since 1995 (as of 12/2004). (Probiodrug AG sold its DPIV patent estate for metabolic diseases in 2004 to Prosidion Ltd., UK).

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