Casomorphin

The □-casomorphins are fragments of the bovine □-casein sequence 60–68 and in sheep, water buffalo, and human whey, they are found in analogous positions.

From: Handbook of Biologically Active Peptides (Second Edition), 2013

Related terms:

Eicosanoid Receptor, Endorphins, Pervasive Developmental Disorders, Opioid, Peptide, Amino Terminal Sequence, Tripeptide, Beta Casein, Casein

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Learn more about Casomorphin

Treatment and Management

ROSE ANN (ROZ) PARRISH, ... EUGENIA CHAN, in Developmental-Behavioral Pediatrics, 2008

The Gluten-Free/Casein-Free Diet

One of the most commonly used dietary treatments for autism, this protocol is based on the "opioid-excess" theory that autism results from a metabolic disorder in which a "leaky gut," unable to break down proteins such as gluten and casein, allows the systemic absorption of peptide fragments (gliadinomorphins and casomorphins) that then act as endogenous opioids in the central nervous system.[43] This theory remains speculative.[44] Results of one small single-blind study of the gluten-free/casein-free diet in 20 children with autism and abnormal urinary peptides suggested improvements in behavior and cognition.[45] However, results of a preliminary double-blind, placebo-controlled crossover trial in 15 children with autism revealed no significant differences on the Childhood Autism Rating Scale, on the Ecological Communications Orientation Language Sampling Summary, or in frequencies of behavior such as child initiation and child response.[46] These con-

tradictory results reflect the lack of evidence supporting or refuting the effectiveness of the gluten-free/casein-free diet.

Disadvantages of the gluten-free/casein-free diet include the potentially higher cost of gluten-free/casein-free foods, the responsibility of parents to be vigilant in reading food labels, and the nutritional implications of eliminating milk products rich in calcium, vitamin D, and protein from the diet. In addition, maintaining adequate nutrition with this dietary intervention may be especially difficult for children who have unusual or restricted food preferences.

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Aminopeptidase P1

Graeme S. Cottrell, Anthony J. Turner, in Handbook of Proteolytic Enzymes (Third Edition), 2013

Activity and Specificity

APP1 is an aminopeptidase, which cleaves N-terminal residues from peptides with a penultimate Pro residue. Peptides with Pro→Ala substitution are also cleaved, but at much slower rates. The enzyme exhibits little specificity for residues in the P1′ and P2 positions. APP1 hydrolyzes a number of physiological substrates including bradykinin (Arg↓Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-NH_2), substance P (Arg↓Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2), corticortropin-like intermediate lobe peptide, casomorphin and [Tyr]melanostatin. Synthetic substrates include the tripeptides, Gly↓Pro-HydroxyPro and Arg↓Pro-Pro [1–3]. The enzyme's optimum pH appears to be between 7.8 and 8.2 but is dependent upon species and substrate. Activity is promoted by Mn^{2+} and Co^{2+} ions, but inhibited by Zn^{2+}, Cu^{2+} and Ca^{2+} ions. Addition of the reducing agent glutathione further promotes the activity of APP1 in the presence of Mn^{2+} and Co^{2+} ions [4]. The metallo nature of APP1 is confirmed by its sensitivity to the chelating agents, EDTA and 1,10-phenanthroline. Apstatin, a specific inhibitor of APP2 [5], is also effective at suppressing the activity of APP1 [4]. APP1 is insensitive to the general aminopeptidase inhibitor, bestatin and the serine protease inhibitor, PMSF. Amastatin was effective against the rat protein [6] but not the recombinant human enzyme [4].

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Immunologic Reactions to Wheat

Autism and the Gluten-Free Casein-Free Diet

There are many hypotheses regarding the correct etiology and treatments for autism, one of which is the opioid hypothesis. This hypothesis suggests that autism results from excessive brain opioid activity during the neonatal period. Excessive opioid activity leads to inhibition of social motivation, resulting in aloofness and isolation. Arguments supporting this hypothesis include similar behaviors in animals after injections of exogenous opioids with decreased vocalization and increased aloofness; biochemical evidence of abnormal peripheral endogenous opioids in autistic patients; and case reports showing naltrexone (an opioid receptor blocking agent) to be therapeutic in autistic patients.[54,55] Reichelt theorized in 1991 that gluten and casein peptides, which have similar chemical structures, play a role in the pathogenesis of autism. A variety of disorders, including autism, schizophrenia, and postpartum psychosis, were thought to be due to an inability to process gluten and casein adequately.[56] These products of inadequate digestion, "gliadorphin" and "casomorphin", can be measured in the urine and cerebrospinal fluid of autistic patients. They theoretically cross both the gut and brain barriers, and then bind with endogenous opioid receptors, causing "interference of signal transmission". It has been proposed that "gliadorphin" and "casomorphin" have negative pharmacological effects on attention, learning, social interactions, and brain maturation.[57]

Complementary and alternative medicines are often used by the parents of children with autism spectrum disorders. These include high-dose dietary supplements, and many different types of restrictive diets. Supplements and diets are thought to be more acceptable and to have fewer issues with safety and side effects than prescribed pharmaceuticals for autism. However, even though diets and supplements are considered "food", there should still be equal concern to scrutinize the available evidence for their efficacy and effectiveness, as well as any associated risks. Cochrane Reviews published in 2004 and 2008, examined the evidence of the effect of diets on children, adolescents, and adults clinically diagnosed with autism spectrum disorder. Publications included trials in which gluten-free diet was compared to placebo (or no treatment); casein-free diet was compared to placebo (or no treatment), gluten-free casein-free diet was compared to placebo (or not treatment), and gluten-free diet was compared directly to casein-free diet. Measured outcomes included standardized autistic behavioral assessments, communication and linguistic abilities, cognitive functioning, motor abilities, urine peptide concentrations, and disbenefits (harms, costs, and impact on quality of life).[58,59]

Between 1965 and 2007, 61 studies were identified; of these, only 3 were considered to be of a high enough quality to be included in the analysis.[57,60,61] The studies

excluded had significant bias, or were not randomized or blinded and consisted mostly of case reports. The three publications consisted of two small trials: the first with 10 participants in each arm; the second with 15 participants in total. In the first trial, a gluten-free casein-free diet reduced the autistic traits of "social isolation" and "bizarre behavior" at only the age of 12 months. In the second trial, there was no significant difference in outcome measures between the diet group and the control group in regards to cognitive skills at 12 months, motor ability at 12 months, communication and language sampling at week 6 of the diet, or Childhood Autism Rating Scale at week 6 of the diet. Surprisingly, there were no reported adverse outcomes or potential disbenefits in regard to cost of the diet or further social isolation. The conclusion of these two meta-analyses was that this is an important area of investigation, and large-scale, good quality randomized control trials are needed.[58,59]

What might be an adverse outcome to using the gluten-free casein-free diet in autism? First, it costs significantly more than a standard diet to purchase gluten-free substitutes for bread, pasta, and other staples in the diet. Secondly, it involves extra effort in providing special meals for the child with autism and normal meals for the rest of the family. In autism, many children have well-established particular dietary preferences which are difficult to change with regard to texture and taste. It is also a challenge with the gluten-free diet to source food products that are guaranteed not to contain gluten or casein. While gluten is not an essential nutrient in the human diet, the loss of casein and its nutrients found in cow's milk products, such as calcium, protein, magnesium, potassium, and other vitamins and minerals, must be supplemented to the child in another way. Finally, the autistic patient is already perceived as "different", and further social restrictions via diet may place additional burdens on the family unit.[59]

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Endomorphins

Hirokazu Mizoguchi, ... Shinobu Sakurada, in Handbook of Biologically Active Peptides (Second Edition), 2013

Discovery of Endomorphins

Since the discovery of opioid receptors, neuroscientists have searched for their endogenous ligands. The search led to the discovery of enkephalins, endorphins, and dynorphins in the 1970s. The three families of opioid peptides contain a common N-terminal amino acid sequence, Tyr-Gly-Gly-Phe. The enkepkalins ([Met-

^{5}]enkephalin and [Leu^{5}]enkephalin) are the endogenous ligands for δ-opioid receptors and dynorphins (dynorphin A, dynorphin B, α-neoendorphin, and β-neoendorphin) are the endogenous ligands for κ-opioid receptors. β-Endorphin binds μ-opioid receptors and δ-opioid receptors equally. These compounds can also bind μ-opioid receptors, but they display relatively low selectivity and efficacy at the μ-opioid receptors. Before 1997, no mammalian opioid peptide that exhibited both high affinity and selectivity for μ-opioid receptors was identified. As a peptide with opioid-related activity in the central nervous system, Tyr-Pro-Trp-Gly-NH_2 (Tyr-W-MIF-1), which was isolated from bovine and human brain and contains the same structure at the N-terminus (Tyr-Pro-aromatic amino acid) with casomorphin, shows high selectivity for μ-opioid receptors, but its affinity for μ-opioid receptors is relatively low.[34] Based on this information, the peptide with the highest affinity and selectivity for μ-opioid receptors was searched from the 20 peptides that have a common N-terminal sequence Tyr-Pro-Trp-X and whose amino acid at the fourth position was substituted by the possible natural amino acids. Among the 20 peptides tested (Tyr-Pro-Trp-X-NH_2, X: Phe, Leu, Ile, Met, Val, Pro, Gln, Trp, Cys, Thr, Tyr, Asn, Ser, Ala, Gly, Arg, Lys, His, Asp, Glu), Tyr-Pro-Trp-Phe-NH_2 showed a highest affinity and selectivity for μ-opioid receptors. This peptide showed very potent analgesia after intracerebroventricular and intrathecal administration.[33] Moreover, specific μ-opioid receptor antagonists reversed the pharmacological effects of this peptide in both *in vitro* and *in vivo* experiments. Zadina and colleagues generated a specific antibody against this peptide that showed no crossreactivity for >40 opioid or nonopioid peptides and used it in combination with HPLC to screen extracts of bovine brain for immunoreactivity. After multiple purification steps, the immunoreactive fraction was applied to automated Edman degradation, and this yielded two peptide sequences, Tyr-Pro-Trp-Phe-NH_2 and Tyr-Pro-Phe-Phe-NH_2. Both peptides showed a high affinity and selectivity for μ-opioid receptors and pharmacological effects as μ-opioid receptor agonists. Because these peptides are the first endogenous mammalian peptides to have a high affinity and a clear specificity for μ-opioid receptors (see below), they were named endomorphin-1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2).[33] Interestingly, the structure of endomorphins is different from those of traditional endogenous opioid peptides, which have a common N-terminal sequence, Tyr-Gly-Gly-Phe. Unlike other endogenous opioid peptides, however, the precursors or its cording DNAs for endomorphins have not yet been identified, although it has been already been 15 years since their discovery. Therefore, the biosynthesis for endomorphins is still unknown, although Ronai and colleagues have provided evidence of de novo biosynthesis of endomorphin.[10,11,19]

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Dipeptidyl-Peptidase II

Ingrid De Meester, in Handbook of Proteolytic Enzymes (Third Edition), 2013

Activity and Specificity

Substrates

Substrate preferences of DPP II have been summarized and compared elsewhere [12]. DPP II releases N-terminal dipeptides from oligopeptides, particularly tripeptides, and from 2-naphthylamides, amides and methyl esters of dipeptides, provided their N-termini are unsubstituted [2,3]. Virtually any residue may reside at the terminal (P2) position, although acidic ones are the least favorable. In general, Ala and Pro are the preferred residues in the P1 position.

In the literature, relative rates and specific activities of DPP II are often mentioned and compared instead of the catalytic parameters k_{cat} and K_m. Interpretation of such data is difficult as differences in methodology and purity of the enzyme preparation may account for part of the variation seen in substrate specificity profiles between the various enzyme preparations. Among the dipeptide-derived chromogenic and fluorogenic substrates, hydrolysis was observed of derivatives Lys-Pro, Gly-Pro, Arg-Pro, Phe-Pro, Lys-Ala, Ala-Ala, Arg-Ala, and Leu-Ala.

No cleavage of DPPI (see Chapter 447) selective substrates was seen, nor did DPP II hydrolyze substrates with a protected N-terminus. Among the dipeptide-derived chromogenic and fluorogenic substrates, the leaving group on the P1′ position has a great impact on substrate selectivity. Virtually any residue may reside at the N-terminal P2 position, provided that the amino function is free and protonated. Basic residues are preferred over neutral and acidic ones. Some authors suggest that the P2 preference is influenced by size rather than the acidity/basicity. Leiting *et al.* [13] examined human DPP II's substrate specificity by using a positional scanning synthetic combinatorial dipeptide substrate library (Xaa-Xaa-MCA). Also here, proline was preferred P1, but the next most preferred P1 residue was norleucine. At the P2 position, DPP II preferred lysine, norleucine, methionine and alanine.

Action on peptides by DPP II from most sources is virtually limited to tripeptides, with rates usually highest on Ala-Ala-Ala. Relative rates on a wide range of tripeptides have been summarized and compared elsewhere [12]. By way of contrast, a nonlysosomal, membrane-associated species of DPP II in the rat brain ('DPP II-M') is capable of releasing Xaa-Pro dipeptides from a range of oligopeptides at pH 5.5 [14]. Examples include Arg-Pro and Lys-Pro from substance P (an 11 residue peptide) and Tyr-Pro and Phe-Pro from casomorphin (a pentapeptide). Tripeptides such as Pro-Pro-Gly, His-Pro-Val and Gly-Hyp-Ala are also cleaved. In contrast to DPP IV

(Chapter 745), a membrane-bound peptidase that is unable to cleave the Pro-Pro bond [15,16], DPP II-M from the rat brain cleaves this bond. Xaa-Pro sequences at the N-termini of larger molecules, *e.g.* prolactin and aprotinin, are resistant to the action of DPP II-M [14]. A species of DPP II ('DPP II-S') present in a soluble fraction of the rat brain has a substrate specificity that is similar to that of DPP II-M and also to that reported for a dipeptidyl-peptidase purified from the rat brain by Imai *et al*. [17]. Two dipeptidyl-peptidase species displaying postproline-cleaving activity at acidic pH are present in the human cerebral cortex [18]. The catalytic activity of one species ('DPP-A') is characteristic of cattle pituitary DPP II, while that of the other ('DPP-B') is anomalous. The latter is capable of liberating Arg-Pro and Lys-Pro sequentially, at pH 5.2, from substance P – a characteristic that is the same as that of DPP II-M of the rat brain.

Assays

Assays are usually performed at pH 5.5–6.0 with fluorogenic or chromogenic substrates such as Lys-Ala, Lys-Pro or Gly-Pro as the dipeptide moiety [2,10,14,17,19,20]. Also at neutral pH, the enzyme is able to hydrolyze the above mentioned substrates, albeit with a reduced rate [7,10]. When DPP II is assayed in impure biological fluids, 1,10-phenanthroline (1 mM) can be employed to block aminopeptidase action without affecting DPP II activity. Alternatively, a specific DPP II inhibitor can be used to determine the specific DPP II activity [21]. Assays may also be performed on a tripeptide substrate such Ala-Pro-Ala, Lys-Pro-Ala or Ala-Ala-Ala (pH 4.5).

Inhibitors

The influence of general and specific peptidase inhibitors on DPP II activity has been reviewed [12]. DPP II from many sources is sensitive to classical inhibitors of the serine catalytic class of peptidases, *e.g.* DFP, and to a lesser extent PMSF. DPP II was not inhibited by thiol reagents (E-64, *N*-ethylmaleimide, *p*-chloromercuribenzoate, iodoacetic acid, iodoacetamide) or reducing agents (mercaptoethanol, cysteine, DTT) nor by EDTA, EGTA or 1,10-phenanthroline. Candidate metal cofactors Co^{2+}, Mn^{2+}, Mg^{2+} also did not significantly affect the DPP II activity [22]. $HgCl_2$, however, appeared to be a very effective inhibitor [4,8,23–25].

DPP II from different sources is very sensitive to cations. The degree of inhibition is directly proportional to the size of the cation: Li^+<Na^+<K^+<Tris pH 5.5<puromycin. The presence of cationic detergents like Hyamine 10-X [3], cetyltrimethylammonium bromide [26] and benzethonium chloride [27] or of ammonium sulfate [4] in the reaction mixture also appears to inhibit DPP II activity. To date there are no known natural activators or inhibitors of DPP II.

Most of the DPP II inhibitors reported in the literature were initially designed as DPP IV inhibitors and thus lacked selectivity. Examples are found among the aminoacyl pyrrolidides and thiazolidides (micromolar K_i), the thioxo aminoacyl pyrrolidides and thiazolidides (high nanomolar K_i), the boronic acid dipeptide analogs (nanomolar IC_{50}) [51], and the dipeptide diphenyl phosphonates [28–31]. From 1990 onwards, several series of new, potent, and selective DPP II inhibitors were developed [32]. Lysyl-piperidide, and especially diaminobutyric acid-piperidide (Dab-Pip) were selected as lead compounds [31,33]. The latter has an IC_{50} of 0.13 μM for DPP II and a more than 7500-fold selectivity over DPP IV. A number of □-aminosubstituted analogs of diaminobutanoyl-piperidine are highly potent (subnanomolar potency) and highly selective (more than million-fold selectivity *versus* DPP IV) inhibitors of DPP II [34]. The compound *N*-(4-chlorobenzyl)-4-oxo-4-(1-piperidinyl)-1,3-(*S*)-butanediamine dihydrochloride (UAMC00039) has been used in an *in vitro* and in an *in vivo* study on the function of DPP II [35,36].

A structure activity relationship study on a series of *N*-alkyl Gly-boro-Pro inhibitors demonstrated nanomolar DPP II inhibitors, but with less selectivity towards DPP IV and FAP than the above mentioned inhibitors [37]. The best DPP II inhibitors from this study were the *N*-cyclodecane (IC_{50} 2 nM, 30-fold selectivity over DPP IV) and the 4-pentyl-bicyclo[2.2.2]octane derivatives (IC_{50} 1 nM, 60-fold selectivity over DPP IV) [37]. The use of boro-Nle over boro-Pro as P1 residue greatly increased DPP II selectivity (over DPP IV, FAP, DPP8 and DPP9) in a series of dipeptide-based inhibitors in which Dab-boro-norleucine proved to be the most selective and most potent DPP II inhibitor (IC_{50} 0.5 nM, > 68 000-fold selectivity *versus* DPP IV, 9000 *versus* DPP8 and 1700 *versus* DPP9) [38]. Lastly, an azabicyclo(3.3.0)octane-based compound named AX8819 proved to be a potent and selective DPP II inhibitor that, like UAMC00039, can be used for further functional studies of DPP II [35,39].

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