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Title:

Bioactive peptides derived from natural proteins with respect to diversity of their receptors and physiological effects-

Running title: Bioactive peptides derived from natural proteins

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Abstract

We have found various bioactive peptides derived from animal and plant proteins, which interact with receptors for endogenous bioactive peptides such as opioids, neurotensin, complements C3a and C5a, oxytocin, and formyl peptides etc. Among them, rubiscolin, a δ opioid peptide derived from plant RuBisCO, showed memory-consolidating, anxiolytic-like, and food intake-modulating effects. Soymorphin, a μ opioid peptide derived from β -conglycinin showed anxiolytic-like, anorexigenic, hypoglycemic, and hypotriglyceridemic effects. β -Lactotensin derived from β -lactoglobulin, the first natural ligand for the NTS2 receptor, showed memory-consolidating, anxiolytic-like, and hypocholesterolemic effects. Weak agonist peptides for the complements C3a and C5a receptors were encrypted in many proteins and exerted various central effects. Peptides showing antihypertensive, anxiolytic-like and antialopecia effects via different types of receptor were also obtained. Based on these study, new functions and post-receptor mechanisms of receptor common to endogenous and exogenous bioactive peptides have been clarified.

Key words:

Angiotensin

Bradykinin

Complement C3a

Complement C5a

Formyl peptide

Neurotensin

Opioid peptide

Oxytocin

Milk

RuBisCO

Soy

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1. Introduction

A concept that endogenous bioactive peptides are released by limited proteolysis of their specific precursor proteins was established in 1970s. As another route for the release of bioactive peptides, opioid peptides derived from casein and gluten, which has not been regarded as precursors of bioactive protein, were reported in 1979 [1,2]. The term exorphin was given for those opioid peptides of exogenous origin by Zioudou [2,3]. Since then, a lot of examples in which bioactive peptides are released from natural proteins have been reported. It should be noted that peptides acting on animals are released not only of animal but also of plant origin. They are classified as follows: 1) ligands for receptors [1-3], 2) inhibitors for enzymes [4], 3) peptides modulating transport [5], 4) anti-microbial peptides [6], 5) anti-oxidative peptides [7].

In this article, peptides interacting with receptors for endogenous bioactive peptides and inhibitory peptides for some enzymes derived from animal and plant proteins are reviewed. Then, central and peripheral effects of selected bioactive peptides we found will be described with special reference to their receptors and post-receptor mechanisms.

The affinities of those peptides for receptors are much smaller than those of endogenous ones since they have only a few homologous amino acid residues with endogenous ligands. However, they exhibited central and peripheral effects after oral administration, a route that renders most endogenous bioactive peptides ineffective.

2. Natural proteins as precursors of bioactive peptides.

Various kinds of bioactive peptides have been isolated from enzymatic digests of natural

proteins of animal and plant origin. In tables 1-A and -B, those peptides are classified according to the receptors or enzymes they interact.

2-1. ANIMAL proteins

a) Milk proteins

i) β -casein: β -casomorphin (β -CM-7: YPFPGPI) has been isolated from a commercial peptone as an opioid peptide in an assay system using isolated guinea pig ileum [1]. This is the first example that bioactive peptide could be released from not only specific precursor proteins but natural proteins. This is also the first example of the opioid peptide in which Gly-Gly sequence found in enkephalin and other endogenous opioid peptides are replaced with Pro residue giving resistance for gastrointestinal proteases. Although β -casein is ubiquitously found in mammals, β -CM sequence is not found in β -casein of mouse and rat. We demonstrated that β -CM-7 is selectively released by the action of pancreatic elastase and leucine-aminopeptidase from specific genetic variants A¹, B and C, in which Pro⁶⁷ in variants A² and A³ is replaced with His [8]. From variants A² and A³, β -CM-9, β -CM-13 and β -CM-21 which have weaker activities than β -CM-7 are released.

β -CM 5 (YPFPG), which was obtained by treating β -CM with carboxypeptidase Y, was more potent by 5 times than β -CM [9]. β -CM 4 amide (YPFP-amide), which was obtained initially by a chemical synthesis and later found also in the gastrointestinal digest, was the first potent peptidic ligand selective for the μ opioid receptor [10]. It was named morphiceptin. Endomorphins I and II (YPWF-amide and YPFF-amide, respectively) isolated from the brain as potent ligands selective for μ receptor could be regarded as analogues of morphiceptin [11,12]. However, precursor proteins of

endomorphin couldn't be found so far.

We isolated an opioid peptide YPVEPF corresponding to the β -casein(114-119) from an enzymatic digest of bovine β -casein and named it neocasomorphin [8]. Neocasomorphin upregulated expression of the transmembrane-associated mucin MUC4 in intestinal goblet cells [13]. Synthetic fragment peptides of human β -casein, YGFLP and YPSF-amide, corresponding to the residue No.41-44 and 59-63, respectively, also showed opioid activities [14].

Immunostimulating peptides PGIPN and LLY have been found in bovine β -casein. Similar peptides VEPIPY, GFL were also found in human β -casein [15]. An octadeca peptide derived from the carboxyl terminus of bovine β -casein has a mitogenic activity for lymphocytes [16].

ii) α_{s1} -casein: Phosphopeptide derived from α_{s1} - as well as β -casein have been reported to facilitate intestinal absorption of calcium by preventing precipitation of calcium phosphate [4]. They are also reported to stimulate production of Ig-G in lymphocytes [17]. Islacidin, the bovine α_{s1} -casein(1-23) stimulates phagocytes and proliferation of T-lymphocytes [18].

Loukas et al. isolated weak opioid peptides RYLGYE from a pepsin digest of bovine casein using an opioid assay system of isolated mouse vas deferens [19]. The peptide was named α -casein exorphin since it corresponds to the α_{s1} -casein(90-96). Its fragments peptides RYLGY and YLGYE also showed weaker activity than the hexapeptide.

YLGYLEQLLR, a peptide extended at the carboxyl terminus of α -casein exorphin, has been reported to show anxiolytic-like activity and named casozepein based on a

homology with diazepam binding inhibitor (DBI) [20]. However, its anxiolytic-like activity might be different from that of diazepam since its affinity to the diazepam binding site is about 1/10,000 that of diazepam. Furthermore, Ohinata et al. found that YL and YLG corresponding to the di- and tri-peptide sequence at the aminoterminal of caseozepin also show anxiolytic-like activity in diazepam binding site-independent manner [21,22].

α_{s1} -Casein has been thought to be specific component of luminant milk until we found it in human milk as a minor component [23]. Casoxin D (YVPFPPF) corresponding to the human α_{s1} -casein(143-149) was isolated from the pepsin-chymotrypsin digest of human casein as an opioid antagonist peptide counteracting with morphiceptin in a field-stimulated guinea pig ileum preparation [24]. It showed affinities for the μ receptor and bradykinin B₁ receptor. In canine mesenteric artery, casoxin D showed a vasorelaxing activity which was blocked by des-[Arg¹][Leu⁸]-bradykinin, an antagonist of the B₁ receptor [25]. While the Tyr residue at the amino terminus casoxin D was essential for the opioid antagonist activity, des-[Tyr¹]-casoxin D retained a vasorelaxing activity and an affinity for the B₁ receptor. Furthermore, casoxin D also showed an ileum-contracting activity in the absence of field stimulation. While des-[Tyr¹]-casoxin D did not show an ileum-contracting activity, [Phe¹]-casoxin D retained it. The ileum-contracting activity of casoxin D was not blocked by either naloxone or des-[Arg¹][Leu⁸]-bradykinin suggesting that it was mediated by unidentified receptor other than μ or B₁ receptor. Thus, casoxin D is a multifunctional peptide acting through three types of receptors.

Kampa et al. reported that α_{s1} -casomorphin (YVPFPP), which corresponds to casoxin D(1-6), is an opioid agonist selective for the κ -receptor [26]. They reported that the

peptide suppressed growth of cells derived from cancer.

TTMPLW derived from the carboxyl terminus of α_{s1} -casein has been reported to show immunostimulating activity and ACE-inhibitory activity [15].

iii) κ -casein: Casoxins A and B (YPSYGLN and YPPY) were synthesized as fragment peptides of bovine κ -casein containing Tyr residues at their amino termini [27]. These peptides are opioid antagonists since they have affinities for the μ receptor and suppress analgesic activity of morphine.

We obtained an opioid antagonist peptide SRYPST-OCH₃ from a pepsin digest of bovine κ -casein, and named it casoxin-6 [28]. The peptide was selective for the μ and κ receptors. The esterification of Tyr residue at the carboxyl terminus of the peptide has occurred during the extraction of the lyophilized digest with anhydrous chloroform/methanol. Tyr methylester moiety was essential for the antagonist activity since non-esterified peptide was inactive. Among derivative peptides truncated at the amino terminus, RYPST-OCH₃ (casoxin-5) was selective for the μ and κ receptors while YPSY-OCH₃ (casoxin-4) was selective for the μ receptor. Considering the fact that basic amino acid residues at the carboxyl terminus of dynorphin A gave the peptide selectivity toward the κ receptor, we assumed that casoxins-6, -5 and -4 might bind retro-wise to the agonists on the surface of the receptors. Then, we synthesized retro-[Leu]-enkephalin methyl ester (LFGGY-OCH₃) and retro-dynorphin A8 methyl ester (IRRLFGGY-OCH₃). The former was selective for the μ receptor while the latter was selective for both μ and κ receptors coinciding with the retro-wise binding mode of those opioid antagonist peptides on the surface of the receptors [29].

We also tried to obtain similar opioid antagonist peptides from a pepsin digest of

human κ -casein and obtained three opioid antagonists YLGSGY-OCH₃, RYYGY-OCH₃ and KYLGPQY-OCH₃ [30]. Esterification of the peptides might occur during the preparation as described above. Corresponding peptides sequences, however, were found in the primary structure of not κ -casein but lactoferrin since the κ -casein preparation used was contaminated with human lactoferrin. We named them lactoferroxins-A, -B and -C, respectively. Lactoferroxin-A was selective for the μ receptor while lactoferroxins-B and -C were selective for the μ and κ receptors. Matching with the retro-wise binding mode of the antagonists on the receptor as described above. Based on these results we found that the retro-nociceptin methyl ester was an antagonist of the nociceptin receptor [31]. The antagonist stimulated memory consolidation after icv administration in mice. We also found the retro-nociceptin methyl ester made of enantiomeric amino acid was an agonist of the nociceptin receptor (unpublished results).

Casoxin C (YIPIQYVLSR) was isolated from the trypsin digest of bovine κ -casein based on an apparent anti-opioid activity in the field-stimulated guinea pig ileum preparation [27]. In the absence of the field stimulation, casoxin C also showed ileum-contracting activity. Finally, apparent anti-opioid activity of casoxin C was due to its ileum-contacting activity which is mediated by the complement C3a receptor. [32] The complement C3a is an ileum-contracting peptide, which is released from the amino terminus region of Complement C3. Casoxin C(6-10), YVLSR, also showed the ileum-contracting activity. This is because a homology is observed between YVLSR and LGLAR, a pentapeptide sequence at the carboxyl terminus of C3a, which is the minimum essential structure for the C3a agonist activity [33]. These could be expressed as hydrophobic residue-X₁-Leu-X₂-Arg. Rather unexpectedly, both casoxin C and

casoxin C(6-10) behaved as weak agonists for the μ receptor in the isolated guinea pig ileum strip which showed tachyphylaxis for C3a by preincubation with it. This suggests that antiopioid nature of casoxin C is due its C3a agonist activity not by an opioid antagonist activity. As well as C3a casoxin C stimulated phagocytosis by polymorphonuclear leukocyte [32].

LSR corresponding to the casoxin C(8-10) showed hypocholesterolemic effect by stimulating bile acid secretion in a C3a receptor-independent manner [34]. The LSR sequence is also found in soy glycinin and bioactive peptides such as calcitonin gene related peptide, secretin, leptin and PAMP etc.

IPI, which corresponds to the casoxin C(2-4), has been reported as a DPP-IV inhibitor (diprotin A), which might have a hypoglycemic effect by elevating the serum level of GLP-1, an incretin [35]. On the other hand, IPIGY, corresponding to the casoxinC(2-6) was reported to be a substrate for the enzyme [36].

PHLSF and MAIPPKKNQDK derived from the carboxyl terminus region of para- κ -casein and the amino terminus region of κ -casein glycomacropeptide, respectively, have been reported to inhibit platelet aggregation [37]. The latter was named casoplatelin. A long fragment peptide corresponding to the human κ -casein(57-134) has been reported to suppress growth of cancer cells [38]. It was named lactaptin and has also antimicrobial activity.

Glycomacropeptide, a large fragment released by the action of chymosin from the carboxyl terminus region of κ -casein, has been reported to show various effects such as suppression of gastric acid secretion, immunosuppression and promotion of Bifidobacterial growth [39].

iv) Lactoferrin: Lactoferrin is an iron-binding protein found not only in milk but also in body fluids such as tear, saliva, and bile. Lactoferricin, which is released from the amino terminus region of bovine and human lactoferrin has been reported as a microbial peptides [5]. Anti-coagulant peptides KRDS is released from lactoferrin [37].

We isolated 2 ileum-contracting peptides, FKDCHLAR and CFQWQR, from a trypsin digest of human lactoferrin, and named them lactomedin-1, and -2, respectively [40]. The former proved to be a weak agonist for the complement C5a receptor (C5a-R: $K_i = 800 \mu\text{M}$). On the other hand, a small homology was observed between lactomedin 2 and peptides belonging to the oxytocin-vasopressin family. In fact, lactomedin 2 proved to be an agonist of the oxytocin receptor (OT-R: $K_i = 62 \mu\text{M}$) as the first example of that derived from natural proteins [41]. Lactomedin 2 showed a weak affinity for the vasopressin V_1 receptor. Ileum-contracting peptides corresponding to the lactomedin 1 and 2 are not released from ruminant or murine lactoferrin.

Inhibitory peptides for the angiotensin I-converting enzyme have been reported to be released from various natural proteins. Among them, LRP isolated from enzymatic digest of zein is the most potent ACEI derived from food proteins [4]. There are three LRP sequences in lactoferrin. LRAVA, which is released from bovine and human lactoferrin by digestion with trypsin and pancreatic elastase is an inhibitor of ACE ($\text{IC}_{50} = 0.7 \mu\text{M}$) [42]. It is converted to more active LRP ($\text{IC}_{50} = 0.21 \mu\text{M}$) by ACE itself. We have found previously that an ACE-inhibitory peptide LKPNM (bonitopril: $\text{IC}_{50} = 2.4 \mu\text{M}$) derived from bonito was converted to more active LKP ($\text{IC}_{50} = 0.3 \mu\text{M}$) by ACE and defined it as a prodrug-type ACE-inhibitory peptide [43,44]. Thus, LRPVA is another example of the prodrug-type ACE-inhibitory peptide. As well as bonitopri, LRPVA showed its maximum antihypertensive effect 4 hrs after oral administration in

SHR while LRP showed maximum effect 2 hrs after the administration. We named it lactopril.

v) α -lactalbumin: A synthetic YGLF-NH₂ corresponding to the α -lactalbumin(50-53) showed opioid activity in an assay system using isolated guinea-pig ileum [14]. We named it α -lactorphin. Interestingly, α -lactorphin as well as β -casomorphin stimulates secretion of mucin in gastrointestinal tracts [45]. It has been also reported that non-amidated α -lactorphin has vasorelaxing and anti-hypertensive activity in spontaneously hypertensive rats (SHR) [46].

A tripeptide GLF corresponding to the [desTyr¹]- α -lactorphin was isolated from human casein digest as a peptide stimulating phagocytosis by macrophages [15]. However, GLF might be released from contaminating α -lactalbumin since GLF sequence is not found in the primary structure of human casein components. It has been suggested that receptor of GLF might be common to that of complement C1q [47]. Peptides stimulating the release of cholecystokinin have been reported to be released from α -lactalbumin [48].

vi) β -lactoglobulin: YLLF corresponding to the β -lactoglobulin also showed an opioid-like activity in the guinea pig ileum assay system [14]. We named it β -lactorphin though its activity was not blocked by naloxone, an opioid antagonist.

An ileum-contracting peptide HIRL was isolated from a chymotrypsin digest of β -lactoglobulin and named β -lactotensin [49]. Although its homology with neurotensin (PyrLYENKPRRYIL) was small it showed affinity for the NTS2 receptor ($K_i = 7.7 \mu\text{M}$). β -lactotensin was 50 times more selective for the NTS2 receptor while neurotensin

being 50 times more selective for the NTS1 receptor. In this sense, β -lactotensin is the first natural ligand selective for the NTS2 receptor.

Recently, we found that β -lactotensin has a very weak affinity for the MC₄ receptor (IC₅₀ = 800 μ M) probably because of its homology with HFRW, which is the essential structure for the binding to the receptor (unpublished results). Although β -lactotensin has an apparent inhibitory activity for ACE it is not an inhibitor but a substrate since it is cleaved by the enzyme.

Yamada et al. found that MH corresponding to the β -lactoglobulin(145-146) showed anxiolytic-like activity in prostaglandin D₂-dependent manner [50].

IIAEK (lactostatin) showed hypocholesterolemic effect by disrupting the stability of cholesterol micelles. It also upregulated expression of the *CYP7A1* gene [51,52].

IPA and IPAVF derived from β -lactoglobulin are inhibitors of DPP-4 which cleaves GLP-1, an incretin [53,54].

b) Blood proteins

i) *Hemoglobin*: Hemorphin-4 (YPWT) and hemorphin-5 (YPWTQ) are μ -selective opioid peptides found in serum, which are derived from hemoglobin β -chain [55]. We found that hemorphin-5 was released by the action of pancreatic elastase from human hemoglobin [56]. V-hemorphin-5, which could be converted to hemorphin-5 by leucine aminopeptidase, was released in larger amount at the same time. LVV-hemorphin-7 as well as angiotensin IV are agonists of the angiotensin AT₄ receptor that are inhibitors of the insulin-regulated aminopeptidase (IRAP) [57]. We found that V-hemorphin-3 (VYPW) is the minimum peptide to show an affinity for the AT₄-receptor while hemorphin-3 is the minimum peptide to show a μ opioid activity [58]. It also showed

hypertensive activity which is mediated by the AT₁ receptor after *iv* administration in Wister Rats. Hazato et al. isolated LVV-hemorphin-4 from spinal cord and named it spinorphin [59]. It potentiated antinociceptive activity of [Leu]-enkephalin by inhibiting various enkephalin-degrading enzymes. They also found VVYPW is an inhibitor selective for dipeptidyl peptidase III [60]. Spinorphin has an antagonist activity for the formyl peptide receptor FPR [61].

Kagawa et al. reported that VVYP derived from the hemoglobin β -chain show hypolipidemic effect by inhibiting lipases [62]. They also found LSEL derived from globin digest has a hypoglycemic effect [63].

We isolated an ileum-contracting peptide LLGNVLVVVLAR from a trypsin digest of bovine hemoglobin. It is an agonist of the C3a receptor since it satisfies the minimal essential structure for the C3a agonist activity as described above [64]. The peptide corresponds to hemoglobin β -chain(105-116) and was named hemotensin.

Rioli *et al* identified PVNFKFLSH (hemopressin) showing a potent hypotensive activity at a dose of $\mu\text{g/kg}$ *ip* in the hemoglobin α -chain [65]. Interestingly, hemopressin proved to be an inverseagonist of cannabinoid CB1 receptor [66]. These peptide might work as endogenous peptides in vivo.

Many kinds of antimicrobial peptides were reported to be released from hemoglobin [67].

ii) Serum albumin: Serum albumin, the major component of serum, is also found in milk of various mammals. We isolated an opioid peptide YGFQNA corresponding to the bovine serum albumin(399-404) from a pepsin digest of the protein and named it serorphin [68]. This peptide was rather selective for the δ receptor.

We found ileum-contracting activity in a trypsin digest of bovine serum albumin and identified 4 peptides AWSVAR, ALKAWSVAR, RHPEYAVSVLLR, and HPEYAVSVLLR, and named them albutensins-A', -A, -C, and C', respectively [69,70]. Compared with the minimally essential structure for the C3a activity as described previously, the 3rd residue from the carboxyl terminus of bovine albutensin A is not Leu but Val. The sequence corresponding to albutensin A in human and porcine serum albumin is AFKAWAVAR and AFKAWSLAR, respectively [71]. These are the agonist of the C3a receptor though the third residue from the carboxyl termini are Val not Leu. Among them porcine albutensin A showed the most potent ileum-contracting activity. Human and porcine albutensins A also exerted C5a agonist activities as will be described later since they are also homologous to the sequence at the carboxyl terminus of C5a *ie.* -SHKDMQLGR, which is essential for the C5a activity.

The minimally essential structure of albutensin C for the C3a activity was the octapeptide sequence at the carboxyl terminus [71]. Although Ser residue situated at the 5th position from the carboxyl terminus of albutensin C is a hydrophobic residue, which is essential for the C3a activity, the Tyr residue at 8th position from the carboxyl terminus might substitute the requirement. These peptides proved to be agonists for the C3a receptor since their ileum-contracting activities were lost in the ileum preparation pretreated with C3a. This phenomenon called tachyphylaxis is an evidence to demonstrate that two peptides act via common receptor. Another ileum contracting peptide YLYEIAR was obtained from a trypsin digest of bovine serum albumin and named albutensin B [72]. The peptide was not an agonist of the C3a receptor.

A fragment peptide of bovine serum albumin YLSLILNR was synthesized since it

This peptide proved to be an agonist for the C3a receptor since its ileum-contracting activity was lost in the ileum preparation which showed tachyphylaxis for the receptor. We named it albutensin D (unpublished results).

c) Egg proteins

i) Ovalbumin: Ovokinin (FRADHPFL) was isolated from a pepsin digest of ovalbumin based on relaxing activity in isolated canine mesenteric artery [73]. Its vasorelaxing activity was mediated by the B₁ receptor. On the other hand, Ovokinin(2-7) (RADHPF) was isolated from a trypsin digest of ovalbumin based on vasorelaxing activity in mesenteric artery isolated from SHR [74]. It showed a weak affinity for the AT₂ receptor.

YPILPEY corresponding to the ovalbumin(111-117), which was synthesized as a homologue of a δ opioid peptide rubiscolin (YPLDLF) having the Tyr-Pro-Aliphatic amino acid residue as will be described later, showed a weak opioid activity in the mouse vas deferens assay system and was named ovalulin [75].

We isolated an ileum-contracting peptide VTEQESKPVQMMYQIGLFR from a trypsin digest of ovalbumin and named ovotensin [64]. It proved to be an agonist of C3a since it was inactive in an ileum preparation pretreated with C3a. The pentapeptide sequence IGLFR at its carboxyl terminus was minimally essential for the ileum-contracting activity. On the other hand, MMYQIGLFR, a nonapeptide sequence at the carboxyl terminus of ovopressin, proved to be an agonist of the formyl peptide receptor FPR1 (unpublished results). Interestingly, a phagocytosis-stimulating peptide sequence GLF derived from α -lactalbumin is also found in this region. In this sense, the ovopressin region look like a functional domain in ovalubumin. GLW,

analogue of GLF is also found in the ovalbumin [76].

Oda et al. isolated VYLPR from the trypsin digest of ovalbumin as a peptide showing anxiolytic-like activity [77].

Biological roles of these bioactive sequence during embryonic development is an interesting problem to be solved.

ii) Ovotransferrin and lysozyme: Anti-microbial peptides are released from ovotransferrin and lysozyme although these proteins themselves also have anti-microbial function [78,79].

d) Other proteins

A peptide sequence YPIEHG, which is found ubiquitously in animal and plant actin, was synthesized as a homologue of rubisco. This peptide showed weak opioid activity in an opioid assay system using mouse vas deferens and was named actinolin [80].

Khachenko et al. found that YGFGG, YGFIL and YSFGG, synthetic fragment peptides of histone H4, carboxypeptidases and immunoglobulin κ -chain, had opioid activities and named them historphin, valentorphin and kapporphin, respectively [3].

We found VTIMPKDIQLAR, a synthetic fragment peptide of histone H3, showed an ileum-contracting activity since it satisfies the essential condition for the C3a receptor agonist [64].

A peptide sequence YSTEVALSR having a weak agonist activities for the C3a receptor was found in leptin(140-149). This peptide exhibited anorexigenic activity at 100 mg/kg, *ip* in mice [81].

Another C3a agonist peptide sequence VLLQQLLLPR was found in

prepro-corticotrophin-releasing factor(114-123). This peptide showed an anorexigenic activity at 3 mg/kg, *ip* in mice [82].

2-2. Plant proteins

An anti-cancer peptide Lunacin isolated from soy is a typical example of plant-derived peptides acting on animals [83]. Peptides acting on animals are also released from plant proteins after enzymatic digestion.

a) Leaf protein

Ribulose-bisphosphate carboxylase/oxygenase (RuBisCO) is found ubiquitously in photosynthetic organisms as an enzyme catalyzing carbon fixation reaction. RuBisCO is the most abundant protein on the earth since it occupies 10-30% of total leaf proteins. The enzyme consists of two types of subunits A and B with a structure A₈B₈. The primary structure of subunit A, which is encoded in chromosomal DNA, is highly conserved among species while those of subunit B, which is encoded in chloroplast DNA, is variable.

We isolated an opioid peptides YPLDL and YPLDLF from a pepsin digest of spinach RuBisCO by using an opioid assay system in isolated mouse vas deferens [84]. We named them rubiscolin-5, and-6, respectively. These peptides were selective for the δ receptor. The rubiscolin sequence is conserved in the large subunit of Rubisco from most plant species. YPLDLF found in Rubisco from some primitive algae showed 4 times more potent opioid activity than YPLDL [85,86]. Thus, rubiscolin might be the first opioid ligand appeared on the earth, and that it has existed in plants far much earlier than the appearance of its receptor in the animal kingdom. Rubiscolin is reported to

suppress escape reaction of cockroach [87].

Based on inhibitory activity for the angiotensin I-converting enzyme (ACE), we isolated four peptides MRW, MRWRD, VW, and VWIS from a pepsin-pancreatin digest of spinach RuBisCO [88]. MRWRD is a prodrug type peptide since it was converted to MRW by the action of ACE. While antihypertensive effect of most ACE inhibitors are weaker in old SHR, those of MRW were not weakened in old SHR of 30 weeks after birth. After all, MRW proved to have vasorelaxing activity which is mediated by prostaglandin D₂ as an endothelium-derived vasorelaxing factor [89]. It was also found that MRW binds formyl peptide receptor FPR2, which was previously designated FPRL1, though the peptide is not formylated at the methionine residue [90]. We named the peptide rubimetide. It has antioxidative activity to scavenge DPPH radical. The rubimetide sequence is conserved in the large subunit of RuBisCO of all photosynthetic organisms.

b) Seed proteins

i) Wheat proteins

Zioudrou reported that enzymatic digest of wheat gluten showed opioid activity in the assay system using isolated mouse vas deferens [2]. We isolated three opioid peptides GYYPT, YGGWL, and YPISL from thermolysin digest of wheat gluten [91-93]. We named them gluten exorphins A, B and C. Among them, gluten exorphin B corresponding to the [Trp⁴]Leu-enkephalin showed most potent activity in vitro assay system. These are selective for the δ opioid receptor.

Another peptide YPLGQ, which was synthesized according to the primary structure of gliadin showed μ opioid activity in the assay system using guinea pig ileum [94]. We named this peptide gluten exorphin D. Gluten exorphin D could be regarded as a

homologue of YPLG-amide (Tyr-MIF 1) which showed opioid activity in the GPI system [95].

ii) Rice proteins

Based on an anti-opioid activity in the isolated guinea pig ileum preparation, we isolated GYPMYPLPR from a trypsin digest of rice protein [96]. The peptide might derived from rice albumin since homologous sequence was found in it. Although the peptide showed weak affinity for the μ opioid receptor, its apparent anti-opioid activity in the ileum preparation was mediated by C3a receptor not μ receptor. We named the peptide oryzatensin [97]. Oryzatensin(5-9), YPLPR, also showed essentially the same character because both peptides fit the essential structure for the C3a agonists, that is hydrophobic residue-X₁-Leu-X₂-Arg. As well as casoxin C, both oryzatensin and oryzatensin(5-9) behaved as weak agonists rather than antagonists for the μ opioid receptor in the ileum preparation which show tachyphylaxis for C3a.

RGDL found in rice albumin showed more potent in preventing platelet aggregation than RGDS, which is a typical antiaggregating peptide derived from fibronectin. It also inhibited metastasis of melanoma cells after intravenous administration (unpublished results).

Kotani et al. reported that IHRF derived from glutelin exert vasorelaxing and anorexigenic activities by stimulating CCK release [98].

iii) Soy proteins

YPFVV sequence found at the carboxyl terminus region of β -conglycinin β subunit could be regarded as a homologue of human β -casomorphin-5 (YPFVE) [99]. In the

guinea pig ileum assay system, synthetic YPFVV showed higher opioid activity than that of human β -casomorphin-5 (YPFVE), though it was weaker than the bovine β -casomorphin-5 (YPFPG). We named the peptide soymorphin-5. Soymorphin-4 and -5 were released from the protein by the actions of pancreatic elastase and leucine aminopeptidase, while soymorphin-6 (YPFVVA) was released by the action of pepsin and pancreatic elastase.

We found that a trypsin digest of soy protein isolate stimulated phagocytosis by polymorphonuclear leukocyte in vitro, and isolated a tridecapeptide MITLAIPVНКPGR as an active component [100]. We named the peptide soymetide since the methionine residue at the amino terminus was essential for the activity. Residues at its carboxy terminus could be deleted and soymetide-9 (MITLAIPVN) showed the highest activity. Soymetide-4 (MITL) was the minimally essential structure. Soymetides exhibited affinity for the formyl peptide receptor FPR1 inspite that Met at the amino terminus is not formylated. MIII, which was synthesized as a fragment peptide corresponding to the glycinin_{A5-A4}(71-74), also exhibited an affinity for the FPR1. We named it soymetide B-4. Interestingly, MITL promoted extension of pollen tube and roots while a typical formyl peptide fMLP didn't [101].

We also found that HCQRPR, a fragment peptide of glycinin synthesized as an analogue of rigin (GRPR) and tuftsin (TKPR) stimulated phagocytosis [102].

A peptide LPYPR was initially synthesized as homologue of a rice-derived peptide oryzarensin(5-9) (YPLPR), which is an agonist of both complement C3a-R and μ opioid receptor. It showed neither C3a nor μ opioid activity as expected. In stead, its hypocholesterolemic effect was higher than that of YPLPR [103]. Furthermore, it showed various effects such as hypotriglyceridemic, anorexigenic and analgesic and

memory-enhancing effects [104,105]. The LPYPR sequence was found in the primary structure of glycinin A5-A4 cloned by Momma *et al* [106]. However, in the primary structure published later, corresponding sequence it is SPYPR [107]. In the glycinin A3 subunit, corresponding sequence is LPYPQ [107]. Therefore, whether LPYPR sequence exist in genetic variants of glycinin is uncertain. On the other hand, LPYP was reported to inhibit HMG-CoA reductase, a rate-limiting enzyme in the cholesterol biosynthesis pathway [108].

Nishi et al. identified VRIRLLQRFNKRS derived from soy β -conglycinin β subunit as a anorexigenic peptide acting by stimulating CCK release in the calcium sensing receptor (CaSR)-dependent manner [109,110]. Interestingly, the dipeptide RF sequence, which was reported to stimulate CCK release is found in this peptide [111].

Fragment peptides of soy proteins, VAWWMY, IAVPGEVA, WGAPSL and FVVNATSN have been reported to have hypocholesterolemic effects by disrupting the stability of cholesterol micelles [112-114]. Among them, FVVNATSN stimulates expression of the gene encoding *LDLR* in HepG2 cells [115].

Three peptides, ILL, LLL and VHVV derived from soy proteins have been reported to stimulate lipolysis [116].

Inoue et al. reported that dipeptides, KA, VK and SY, which are found several times in many soy proteins including glycinin, β -conglycinin, Kunitz-type trypsin inhibitor and lipoxygenase, inhibited synthesis of triglyceride in HepG2 cells [117].

Three fragment peptides of β -conglycinin, KNPQLR, EITPEKNPQLR, and RKQEEDDEEEQQRE have been reported to inhibit fatty acid synthase by binding to its thioesterase domain [118].

iv) Rapeseed proteins

Based on inhibitory activity for ACE, we isolated four peptides VW, VWIS, IY and RIY from a subtilisin digest of rape seed protein [119]. Although the ACE-inhibitory activity of RIY was smaller (about 1/8) than that of IY, it showed almost the same antihypertensive effect after oral administration in SHR. On the other hand, RIY but not IY showed an endothelium-dependent vasorelaxing activity in small mesenteric artery isolated from SHR, which might contribute to its antihypertensive effect. We named the peptide rapakinin [120].

3. Potent ligands and hybrid ligands obtained by replacing amino acid residues in bioactive peptides derived from natural proteins

In order to determine the amino acid residue essential for the activity, we synthesized a lot of derivatives of bioactive peptides derived from natural proteins. In the course of this approach we found activity of peptides were elevated more than one hundred times by the replacement of a single L-amino acid residue. Such a case is never encountered in the structure-activity studies of endogenous bioactive peptide since individual amino acids might be already optimized during the evolution. Thus, we got highly active derivatives as shown in Table II.

Among various rubiscolin-6 derivatives synthesized, δ opioid activities of [Ile³]-rubiscolin-6 and [Met³]-rubiscolin-6 were 4 times higher than that of rubiscolin. The most potent was [Met³,Val⁶]-rubiscolin-6 with 20 times higher activity [122].

Oryzatensin(5-9), YPLPR, is a minimally essential peptide having agonist activity for the complement C3a receptor. It is resistant against gastrointestinal proteases. [Trp⁵]-oryzatensin(5-9) which was obtained by replacing the Tyr residue with Trp

residue, showed higher affinity for the C3a receptor than original peptide [123]. At the same time, a weak affinity of oryzatensin(5-9) for the μ receptor was lost by the substitution. Thus, a protease-resistant agonist peptide with higher affinity and selectivity for the C3a receptor was obtained. It exerted anorexigenic effect after oral administration at a dose of 300 mg/kg *po* in mice [124].

The phagocytosis-stimulating activity of an FPR1 ligand [Trp³]-soymetide-4, MIWL, was 180 times higher than that of soymetide-4, MITL [125]. This derivative was produced in genetically modified soy [126].

We obtained a potent anti-hypertensive peptide RPLKPW by substituting 4 amino acid residues in RADHPF *ie.* ovokinin(2-7) [127]. Pro residues at the 2nd and 5th position of the peptide might contribute to make it resistant against gastrointestinal proteases. This peptide lowered blood pressure of SHR after oral administration at a dose of 0.1 mg/kg, which is 1/100 that of ovokinin(2-7). This is the first example of peptide selective for the AT₂ receptor and was named it novokinin [128]. Novokinin has been produced in genetically modified soy and rice showing antihypertensive effect after oral ingestion [129-132].

In contrast to the opioid antagonist activity of casoxin D, des-[Val²]-casoxin D (YPFPPF) showed a potent μ opioid activity [133]. As well as casoxin D, this peptide retained vasorelaxing activity mediated by B₁ receptor [24]. Thus, a hybrid agonist ligand for the μ and B₁ receptors was obtained and named casokinin F. By replacing Phe residue at the carboxyl terminus of casomokinin F with Leu, another hybrid agonist ligand for the μ and NK₁ receptors was obtained and named casomokinin L [134]. Vasorelaxing activity of casomokinin L was mediated by nitric oxide.

3. Central and peripheral effects exerted by bioactive peptides derived from natural proteins

Bioactive peptides derived from natural proteins have only a few homologous amino acid residues with endogenous ligands. Therefore, their affinities for receptors are much smaller than those of endogenous peptides. However, they exhibited central and peripheral effects after oral administration, a route that renders most endogenous bioactive peptides ineffective. This situation is caused partly because some of them are more resistant against gastrointestinal proteases than exogenous ones as typically shown in opioid peptides derived from natural proteins.

In this chapter central and peripheral effects exerted by bioactive peptides we found in natural proteins are described with special reference to the receptors they bind and their post-receptor mechanisms (Tables III-A and -B).

4-1. Rubiscolin and gluten exorphins as δ opioid receptor agonist peptides.

Rubiscolin and gluten exorphins-A, -B, and -C are δ selective opioid peptides derived from plant proteins [84,91,92]. They exerted central effects such as memory consolidation and anxiolytic-like activity. They also affected food intake and endocrine function.

i) Memory consolidation: Rubiscolin, an opioid peptide selective for δ receptor, stimulated memory consolidation in passive avoidance experiment using step-through cages at a dose of 100 mg /kg *po* in mice [135]. Gluten exorphin A also showed similar effect [136]. The effect was blocked by naltrindole, and raclopride, antagonists of the δ opioid receptor and dopamine D₂-receptor, respectively. The effect was also blocked by

BMY14802 and WAY100135, antagonists of the σ_1 receptor and serotonin HT_{1A} receptor, respectively (unpublished results). Considering the mechanism for the anxiolytic activity of rubiscolin as described later, rubiscolin might stimulate memory consolidation by elevating the level of endogenous σ ligands such as DHEA downstream of the δ opioid receptor and then followed successively by the serotonin-HT_{1A} receptor and dopamine-D₁ receptor systems (Table 3-A).

ii) Anxiolytic-like effect: Rubiscolin showed anxiolytic-like effect at a dose of 100/kg *po* in mice in tests using the elevated-plus maze [137]. The effect was also blocked by naltrindole, and BMY14802 and WAY100135, The anxiolytic activity of rubiscolin was also blocked by SCH23390 and bicuculline, antagonists of dopamine D₁-R, and GABA_A-R, respectively. Therefore, rubiscolin might exert anxiolytic-like effect by successively stimulating the σ_1 -R, HT_{1A}-R, dopamine D₁-R, and GABA_A-R systems downstream of the δ receptor (Table 3-A). Actinolin (YPIEHG) derived from animal and plant actin also showed similar anxiolytic-like effect to that of rubiscolin [80].

iii) Effect on food intake: Rubiscolin showed orexigenic effect for normal diet which was mediated successively by the PGD₂-DP₁ receptor and the neuropeptide Y-Y₁ receptor systems downstream of the δ receptor (Table 3-A) [138]. We found previously that prostaglandin D₂ has an orexigenic effect via DP₁-R followed by the NPY-Y₁-R receptor system [139]. In this sense, the δ opioid system is a typical example of the receptor situated upstream of the orexigenic DP₁-Y₁-receptor pathway [140]. The orexigenic effect of rubiscolin was observed even in old mice which has become resistant to ghrelin [141].

On the other hand, rubiscolin exerted an anorexigenic effect for high fat diet [142]. The anorexigenic effect was mediated by the α -MSH-MC4-R and the CRF-CRF-R systems downstream of the δ receptor since it was blocked by HS024 and astressin, selective antagonists of individual receptors, respectively (Table 3-A).

A μ opioid peptide β -casomorphin has been reported to stimulate intake of high fat diet [143]. On the other hand, another μ opioid peptide soymorphin suppressed intake of normal diet as will be described below. This means that δ and μ opioid peptides derived from natural proteins have opposite effects each other on intake of normal and high fat diets.

iv) Effect on the endocrine system: Gluten exorphins A-5 and B-5 elevated postprandial insulin level in serum after *po* administration at doses of 30 mg/kg and 300 mg/kg, respectively. [144]. On the other hand, gluten exorphin B-5 elevated serum prolactin level after *iv* administration at a dose of 3 mg/kg [145].

v) Effect on skin: Rubiscolin has been reported to reduce the skin inflammation leading to improvement in dermis differentiation and skin barrier properties [146].

4-2. Soymorphin as μ opioid receptor agonists

As for μ opioid peptide derived from natural proteins, β -casomorphin, hemorphin and some others have been reported. We found that soymorphin, which exist at the carboxy terminus region of β -conglycinin β subunit had an opioid activity selective for the μ receptor [99]. As will be described below, soymorphin reduced anxiety and food intake. It also showed hypoglycemic and hypotriglyceridemic effects.

i) Anxiolytic-like effect: Soymorphins-5, and -6 exhibited anxiolytic like activities in the elevated-plus maze experiment in mice after oral administration at a dose of 100 mg/kg [99]. Soymorphin might elevate GABA level in the brain since the effect was blocked by bicuculline, an antagonists of the GABA_A receptor. It has been reported that mental stress was reduced by ingestion of soy proteins [147]. Soymorphin released in vivo might be an active component for the stress reduction

ii) Effect on food intake: Soymorphin-5 suppressed food intake by slowing gastric emptying in a manner which involves HT_{1A}-R, dopamine D₁-R and GABA_B-R (Table 3-A) [148].

iii) Hypoglycemic and hypotriglyceridemic effects: Interestingly, soymorphin-5 orally given at a dose of 10 mg/kg for 5 weeks showed hypoglycemic and hypotriglyceridemic effect via elevating serum adiponectin level and activating the PPAR- α system (Table 3-B) [149]. β -Conglycinin has been reported to show similar effects by elevating serum adiponectin level [150]. Soymorphin might be one of the active component responsible for the effect.

4-3. β -lactotensin as a neurotensin NTS2 receptor agonist

Neurotensin exerts various physiological effect such as analgesic, anxiolytic-like, memory-enhancing, anorexigenic, and hypotensive effects [151]. Though some of its effects such as anorexigenic and hypotensive ones have been ascribed to NTS1-R, the major receptor, function of NTS2-R and NTS3-R, the minor receptors, remained unclear.

Function of the NTS2 receptor was investigated by using β -lactotensin, the first natural ligand for the NTS2 receptor. It stimulated memory consolidation and reduced anxiety. It also showed hypocholesterolemic activity by stimulating bile acid secretion.

i) Analgesic effect: We found that β -lactotensin showed antinociceptive effect at doses of 200 nmol/mice *icv* or 300 mg/kg *sc* [152]. Tolerance against β -lactotensin was not developed after repeated treatment for 5 days. The antinociceptive effect was not observed in mice treated with antisense ODN for the NTS2-R. The antinociceptive effect of β -lactotensin was blocked by SCH23390, an antagonist of the dopamine D₂ receptor suggesting that it was mediated by the D₂ receptor downstream of the NTS2 receptor (Table 3-A). β -Lactotensin has been reported to suppress neuropathic pain [153].

ii) Memory consolidation: β -lactotensin also stimulated memory consolidation at doses of 300 mg/kg *po* or 60 nmol/mice *icv* [154,155]. The effect was blocked by levocabastine and raclopride, antagonists of NTS2 and D₂ receptors, respectively. We also demonstrated by the *in vivo* microdialysis experiment that dopamine level in hippocampus was elevated by the administration of β -lactotensin. These suggest that β -lactotensin stimulated memory consolidation in the dopamine-D₁ receptor-dependent manner downstream of the NTS2 receptor (Table 3-A).

iii) Anxiolytic effect: β -Lactotensin showed an anxiolytic-like effect in mice at a dose of 10 mg/kg *po* [156]. The effect was blocked by levocabastine, SCH23390 and bicuculine, antagonists of NTS2-R, D₁-R and GABA_A-R, respectively. The anxiolytic-like activity of the natural NTS2 agonist β -lactotensin was mediated successively by the

dopamine-D₁-R, and GABA-GABA_A-R systems downstream of the NTS2 receptor (Table 3-A).

i) Effect on food intake: β -lactotensin exerted an anorexigenic effect after the oral administration at a dose of 300 mg/kg [157]. The effect was not blocked by either SR48692, or levocabastine, antagonists of the NTS1-R and NTS2-R, respectively. On the other hand, it was blocked by astressin and calcitonin gene-related peptide(8-37) (CGRP(8-37)), antagonists of the corticotrophin releasing factor receptor (CRF-R) and CGRP-R, respectively. Since β -lactotensin shows no affinities for CRF-R and CGRP-R, it might exert anorexigenic effect by indirectly activating CRF-R and CGRP-R systems downstream of an unidentified receptor for the peptide (Table 3-A).

v) Hypocholesterolemic effect: We found that β -lactotensin given for 2 days at a dose of 100 mg/kg *po* or 30 mg/kg *ip* reduce serum cholesterol level of mice fed hypercholesterolemic diet by increasing faecal excretion of bile acids [158]. The hypocholesterolemic affect was observed 90 min after the single administration of the peptide. The hypocholesterolemic effect was blocked by levocabastine and raclopride, suggesting that it was mediated by the NTS2 and D₁ receptors (Table 3-B). We also found the peptide stimulate bile acid secretion in NTS2 receptor and D₁ receptor-dependent manner in rats [159]. We observed that orally given β -lactotensin elevated mRNA level encoding CYP7A, which is involved in conversion of cholesterol to bile acid, in mice fed normal diet. However, similar effect couldn't be observed in mice fed with hypercholesterolemic diet probably because cholic acid added to the diet to facilitate emulsification of cholesterol inhibited the elevation of the mRNA concoding

CYP7A. However, it is still possible under usual dietary conditions in which cholic acid is not added that β -lactotensin stimulate bile acid secretion by elevating gene expression of CYP7A.

4-4. Casoxin C, albutensin A and oryzatensin as agonists of the complement C3a receptor, and lactomedin 2 as an agonist of the complement C5a receptor,

Complement C3a and C5a are released from the amino terminal domains of C3 and C5, respectively, on the activation of the complement system [160]. While C3b and C5b, corresponding to the carboxyl terminus of the proteins act as opsonins, C3a and C5a stimulate phagocytosis and chemotaxis of neutrophils. Depending on contracting activity for the isolated guinea pig ileum, we isolated weak agonist peptides of C3a and C5a from the enzymatic digests of natural proteins (Table 1). They also stimulated phagocytotic activities of neutrophils though far much higher concentration than those for C3a and C5a are required, complements C3a and C5a have been called anaphylatoxins since they induce inflammatory responses and anaphylaxis. They were regarded as rather toxic peptides and their antagonists have been the target of drug research. However, weak agonists for the C3a-R and C5a-R widely encrypted in natural proteins showed central effects such as anti-analgesia, anti-amnesia and regulation of food intake etc. as will be described below. It was also demonstrated for the first time by our study that C3a and C5a themselves could exert central effects.

C3a-des-Arg, of which Arg at the aminoterminal were removed by carboxypeptidase N, have been called acylation-stimulating protein (ASP) since it stimulate triglyde synthesis which might lead to obesity [161]. However, short C3a agonist peptides we found in natural proteins contain minimal structure essential for

the C3a activity at their carboxy termini and lack ASP portion at the amino termini, which bind the C5L2 receptor. Therefore, peptides stimulating triglyceride synthesis will be not formed from these weak C3a agonist peptides derived from natural proteins even if their Arg residues at the carboxyl termini were removed by carboxypeptidases.

i) Anti-analgesic effect: Casoxin C showed an apparent anti-opioid activity in the guinea-pig ileum as an agonist of the C3a receptor, not as an antagonist of the opioid receptor. Then, we tested whether casoxin C could suppress analgesic activity of the μ opioids. After the *icv* administration at a dose of 1 nmol/mice, casoxin C blocked analgesic activity of morphine. C3a itself also showed similar effect [162].

Endogenous peptides such as CCK-8, thyrotrophin releasing hormone, neuropeptide FF and nociceptin *etc.* have been reported to have anti-opioid or anti-analgesic effects in mechanisms independent of the opioid receptors themselves [163]. These peptide might interact with the system upstream or downstream of the opioid receptor. This is the first demonstration that C3a-R agonists could work as anti-opioid peptides.

ii) Anti-amnesic effect: In model amnesic mice which have been treated with scopolamine or brain ischemia operation, casoxin C exerted an anti-amnesic effect after *icv* administration at a dose of 10 nmol/mice. C3a also showed essentially the same effect. However, both casoxin C and C3a did not stimulate memory consolidation in normal mice [164].

iii) Anxiolytic like effect: Casoxin C exerted anxiolytic-like effect at a dose of 10 mg/kg *ip* in mice [165]. WPLPR corresponding to [Trp⁵]-oryzatesin(5-9) exerted anxiolytic-like

effect at a dose of 30 mg/kg *po* in mice [166]. The Anxiolytic-like activities of the peptide was blocked by ONO-AE3-208, WAY100135, SCH23390 and bicuculine, antagonists of the EP₄-R, 5HT_{1A}-R, D₁-R, and GABA_A-R, respectively, suggesting that they are mediated by the PGE₂-EP₄ receptor system downstream of the C3a receptor (Table 3-A). It was also found for the first time that C3a itself also showed similar effect.

Lactomedin 1, an agonist of complement C5a receptor derived from human lactoferrin, showed anxiolytic-like effect in mice at a dose of 30 mg/kg *po* [167]. We also found for the first time that C5a itself has a similar anxiolytic-like effect [168]. The anxiolytic-like effect of lactomedin-1 was blocked by BWA868C, SCH58262, and bicuculine, antagonists of the DP₁-R, A_{2A}-R and GABA_A-R, respectively, These suggest that the anxiolytic-like effect was mediated by the complement C5a receptor followed successively by the by the prostaglandin D₂-DP₁ receptor, the adenosine-A_{2A} receptor, and the GABA-GABA_A receptor systems. Thus, anxiolytic-like mechanism of the complement C5a receptor agonist is quite distinct from those of the C3a agonists [166].

iv) Effect on food intake: We found that albutensin A, an agonist of the C3a receptor, exert anorexigenic effect after oral administration at a dose of 0.3 mg/kg *ip* in mice [169]. [Trp⁵]-oryzatensin(5-9) exerted similar effect after oral administration at a dose of mg/kg [170]. The effect was blocked by indomethacin and ONO-AE3-208 suggesting that it was mediated by the PGE₂-EP₄-R system (Table 3-A). Based on this results, we found C3a itself exert anorexigenic effect. On the other hand, C5a showed an orexigenic effect in PGD₂- and neuropeptide Y-dependent manner (Table 3-A) [171]. These are the first evidence that C3a and C5a, immunostimulating peptides of the complement system, could affect food intake.

4-5. Lactomedin 2 as an oxytocin OT receptor agonist

Besides controlling contraction of uterus and ejection of milk, oxytocin proved to be involved in social interaction between animals and regulation of stress. Lactomedin 2, the first ligand for oxytocin receptor derived from human protein lactoferrin exerted anxiolytic activity.

i) Anxiolytic-like effect: Lactomedin 2, an agonist of the oxytocin OT-receptor derived from human lactoferrin, showed anxiolytic-like effect in mice at a dose of 30 mg/kg *po* [40]. The effect was blocked by L-371,257, SCH58267, and bicuculine, antagonists of the oxytocin OT receptor, the adenosine A_{2A} receptor, and the GABA_A receptor, respectively. However, the anxiolytic-like effect of lactomedin 2 was not blocked by BWA868C, an antagonist of the DP₁ receptor. Thus, the anxiolytic-like effect of lactomedin 2 is mediated by the OT receptor followed successively by the adenosine-A_{2A} receptor and the GABA-GABA_A receptor systems (Table 3-A).

4-6. Soymetide as a formyl peptide receptor FPR1 agonist.

Formyl peptide receptors (FPR) mediate stimulation of phagocytosis and chemotaxis of neutrophils by formyl peptides such as Formyl-MLF (fMLP) carrying formylated-Met residue at the amino terminus. There are subtypes such as FPR1 and FPR2 etc. in formyl peptide receptors (FPRs) [172]. Soymetide initially isolated as a phagocytosis-stimulating peptide showed affinity for the FPR1 receptor though it was not formylated at the amino terminus. In fact, its phagocytosis-stimulating activity was blocked by Boc-MLF, an antagonist of the FPR1. However, soymetide didn't show

any anti-infective effect against *C. albicans*, after ip administration in mice.

Anti-alopecia effect: Soymetide-4 given to newborn rats at a dose of 300 mg/kg *po* or 100 mg/kg *ip* for 4 days prevented alopecia induced by etoposide, a chemotherapy agent [173]. Soymetide B-4 showed similar effect at a dose of 30 mg/kg *ip*. The anti-alopecia effect of soymetide-4 given by oral or intraperitoneal route was not blocked by either Boc-FLFLF or WRWWWW-NH₂ (WRW₄), antagonists of the FPR1 and FPR2, respectively. However, the anti-alopecia effect of soymetide-4 was blocked by indomethacin, AH23848B and pyrrolidine dithiocarbamate (PDTC), an inhibitor of cyclooxygenase, an antagonists of the EP₄ receptor and an inhibitor of NF-κB. This means that it was mediated by the PGE₂-EP₄-R system and NF-κB system downstream of an unidentified receptor other than FPR1 or FPR2 (Table 3-B) [174].

4-7. Rubimetide as a formyl peptide receptor FPR2 agonist

Rubimetide (MRW) initially isolated as an ACE inhibitor from a pepsin-pancreatin digest of spinach rubisco proved to be an agonist of the FPR2 receptor [88,89]. It showed vasorelaxing, and anxiolytic-like effect. Furthermore, it also showed anti-alopecia effect.

i) Vasorelaxing and anti-hypertensive effects: Although rubimetide was isolated as an inhibitor of ACE [88], it exerted anti-hypertensive effect even in old SHR above 30 weeks after birth, in which most of other ACE inhibitors derived from natural proteins are inactive. It showed vasorelaxing activity in small mesenteric artery isolated from SHR, which was blocked by BWA868C, an antagonist of the DP₁-R [89]. Its antihypertensive effect of in SHR was also blocked by the DP₁ antagonist. These

suggest that the antihypertensive effect of rubimetide was due to its vasorelaxing activity, which is mediated by prostaglandin D₂ as an endothelium-dependent relaxing factor (Table 3-B).

ii) Anxiolytic-like effect: Rubimetide showed an anxiolytic-like effect in mice at a dose of 1 mg/kg *po* [175]. The effect was blocked by WRW₄, an antagonist of the FPR2 receptor. In fact, rubimetide showed affinity for the FPR2 receptor (K_i = 266 µM) [90]. Humanin, an endogenous agonist of the FPR2 also exerted anxiolytic-like effects. These are the first evidence that the FPR2 agonists show anxiolytic-like effect. The anxiolytic effect of rubimetide was also blocked by BWA868C, SCH58261, and bicuculine, suggesting that the effect was mediated successively by the prostaglandin D₂-DP₁ receptor, the adenosine-A_{2A} receptor, and the GABA-GABA_A receptor systems downstream of the FPR2 receptor (Table-3A). Based on these results we have also demonstrated that PGD₂ exert anxiolytic-like effect for the first time [176].

iii) Anti-alopecia effect: We found that typical agonist peptides of the FPR2 receptor such as MMK-1 and mitochondrial peptide exhibited anti-alopecia effect at a dose of 10 mg/kg *ip* [177]. However, rubimetide showed a marginal anti-alopecia effect at a dose of 300 mg/kg *ip*. (data not shown). The antialopecia effects of orally given MMK-1 was blocked by indomethacin, AH23848B and PDTC, similarly to that of soymetide-4 (Table-B) [178]. However, the antialopecia effects of MMK-1 given *ip* was blocked by pyrilamine, K(D)PT, and PDTC, an antagonist of the H₁-receptor, inhibitors of IL-1, and NF-κB, respectively, suggesting the involvement of histamine, IL-1 and NF-κB systems downstream of the FPR2 receptor (Table 3-B) [179]. Interestingly, the antialopecia

effect of intraperitoneously given fMLP, a typical agonist of the FPR1, was not blocked by Boc-FLFLF, an antagonist of the FPR1. It was partially blocked by WRW₄, an antagonist of the FPR2 suggesting that fMLP acted as an agonist of the FPR2 in this system.

4-8. GLF and GLW as phagocytosis-stimulating and anti-alopecia peptides.

An immunostimulating peptide GLF has been suggested to bind to the complement C1q receptor. We found GLF derived from α -lactalbumin and ovalbumin prevent alopecia induced by chemotherapy agent etoposide at a dose of 30 mg/kg *po* in newborn rats [180]. The antialopecia effects of GLF was blocked by pyrilamine and PDTC, suggesting the involvement of histamine and NF- κ B (Table 3-B). Furthermore, GLW derived from ovalbumin exerted more potent antialopecia effect than GLF at 10 mg/kg *po* [76]. GLW also promoted hair growth in shaved mice.

4-9. Ovokinin and ovokinin(2-7) as vasorelaxing and antihypertensive peptides derived from ovalbumin.

Ovokinin (FRADHPFL), which was released from ovalbumin by pepsin digestion, showed endothelium-dependent vasorelaxing activity in an isolated canine mesenteric artery. Ovokinin showed affinity for the bradykinin B₁ receptor, and its vasorelaxing activity was blocked by des-Arg¹-[Leu⁸]bradykinin, an antagonist of the bradykinin B₁ receptor [73]. Its vasorelaxing activity was mediated by PG-I₂ not by nitric oxide (Table 3-B). Ovokinin exerted antihypertensive effect at a dose of 100 mg/kg *po* in SHR. When given orally as a emulsion in 30% egg yolk, however, it lowered blood pressure of SHR at a dose of 25 mg/kg [181].

On the other hand, ovokinin(2-7) (RADHPF) is a vasorelaxing peptide released from ovalbumin by chymotrypsin digestion [74]. Although it showed weak affinity for the angiotensin AT₂ receptor, its vasorelaxing effect was not blocked by PD123319, an antagonist of the AT₂ receptor. Its vasorelaxing activity in isolated mesenteric artery of SHR was blocked by L-NAME suggesting it was mediated by nitric oxide downstream of an unidentified receptor (Table 3-B). Ovokinin(2-7) exerted antihypertensive effect at a dose of 10 mg/kg *po* in SHR when given orally as a emulsion in 30% egg yolk. Hypotensive activity of ovokinin(2-7) after *iv* administration has been reported to be blocked by HOE-140, an antagonist of the bradykinin B₂ receptor [182]. However, ovokinin(2-7) and their degradation products have no affinity for the B₂-R in our study. For explanation of this discrepancy, ACE-inhibitory activity of degradation product of ovokinin(2-7) produced *in vivo* might working by elevating bradykinin level in this system after the *iv* administration.

4-10. Novokinin as a designed agonist of the angiotensin AT₂ receptor.

There are many subtypes in angiotensin receptors such as AT₁, AT₂, AT₄, AT(1-7). While the AT₁ receptor mediates vasoconstricting and hypertensive activities, the AT₂ receptor mediates vasorelaxating and hypotensive ones [182].

A potent vasorelaxing peptide novokinin (RPLKPW) obtained by replacing 4 amino acid residues in ovokinin(2-7) proved to be an agonist peptide of the AT₂ receptor as described above [126]. It showed potent antihypertensive effect after oral administration in spontaneously hypertensive rats (SHR) in the AT₂ receptor dependent manner. Genetically modified soy and rice containing novokinin also showed antihypertensive effect in SHR. Novokinin also showed anti-analgesic and

anorexigenic effects after icv administration in mice indicating new functions of the AT₂ receptor. Furthermore, novokinin also showed anti-alopecia effect in an AT₂ receptor-dependent manner. On the other hand, novokinin exerted anxiolytic-like and anti-diabetic effects though involvement of the AT₂ receptor is not clear.

i) Vasorelaxing and anti-hypertensive effects: Novokinin showed affinity for the angiotensin AT₂ receptor ($K_i = 10^{-5}$ M), and its vasorelaxing activity was blocked by PD123319, an antagonist of the AT₂ receptor [183]. The vasorelaxing activity of novokinin was also blocked by indomethacin and CAY-10441, an inhibitor of cyclooxygenase and IP receptor, respectively, suggesting the involvement of PGI₂ as an endothelium-dependent relaxing factor downstream of the AT₂ receptor (Table 3-B) [184]. Nitric oxide-dependent process has been proposed for a mechanism downstream the AT₂ receptor [185]. However, as shown here, the vasorelaxation downstream the AT₂ receptor is mediated by the PGI₂-IP-R system at least in a small mesenteric artery of SHR.

When emulsified in 30% egg yolk, novokinin reduced blood pressure of SHR at a dose of 0.1 mg/kg *po.*, which is 1/100 of the minimum effective dose of ovokinin(2-7) under the same conditions [184]. Novokinin has been produced in genetically modified soy and rice [128-132]. Those genetically modified crops containing novokinin as seed proteins reduced blood pressure of SHR at a dose corresponding to 0.1 mg/kg of the peptide even without the emulsification in 30% egg yolk. Some components in the crops such as phospholipids might facilitate emulsification of the peptide to promote the intestinal absorption of the hexapeptide in stead of the egg lethicine,.

ii) Anti-analgesic effect: Novokinin suppressed analgesic activity of morphine after icv administration at a dose of 20 nmol/mice [186]. The anti-opioid activity was blocked by PD-123319 and ONO-AE-240, an antagonist of EP₃-receptor for PGE₂. The anti-opioid effect of angiotensin II was also blocked by the same antagonists, suggesting that anti-analgesic effects of the AT₂ agonists are mediated by EP₃ receptor downstream of the AT₂ receptor (Table 3-A).

iii) Effect on food intake: Novokinin suppressed food intake after oral administration at a dose of 100 mg/kg in mice though it showed no effect on water intake [187]. The anorexigenic effect was blocked by PD123319 and was not observed in the AT₂ receptor-knockout mice. The anorexigenic activity of novokinin was also blocked by ONO-AE3-208, an antagonist of the EP₄ receptor for PGE₂. The anorexigenic activity of angiotensin II was also blocked by the same antagonists. These suggests that anorexigenic activities of AT₂ agonists are mediated by the EP₄-receptor downstream of the AT₂ receptor (Table 3-A).

iv) Anti-alopecia effect: Novokinin exhibited an alopecia effect after oral administration at a dose of 100 mg/kg, which was blocked by PD123319, AH23848B and PDTC suggesting the involvement of the PGE₂-EP₄R and NF- κ B systems downstream of the AT₂ receptor. (Table 3-B) [188]. Novokinin also stimulated hair growth after oral administration in shaved mice.

v) Anxiolytic-like effect: Novokinin exerted anxiolytic effect in mice at a dose of 10 mg/kg ip (unpublished results). Interestingly, its anxiolytic effect was blocked not by

PD123319, but by an enantiomeric substance P(1-7), an antagonist of substance P(1-7) receptor.

vi) Anti-diabetic effect: Novokinin given to KKA^y mice at a dose of 100 mg/kg for 5 weeks suppressed elevation of plasma glucose and insulin levels with concomitant increase of plasma adiponectin level [189]. In mesenteric fat, mRNA levels encoding adiponectin, PPAR γ , and glucose transporter 4 (GLUT4) was also upregulated (Table 3-B). In 3T3-L1 cells, mRNA levels encoding the same proteins were also upregulated by novokinin. However, the upregulation of the genes were not blocked by PD123319 suggesting that the anti-diabetic effect of novokinin might be mediated by a receptor other than the AT₂ receptor.

4-11. rapakinin as a CCK-releasing peptide.

Rapakinin (RIY) is an example of vasorelaxing peptide isolated initially as an inhibitor of ACE from subtilisin digest of rape seed protein [119, 120]. It also showed central effects such as anti-analgesic, anorexigenic, and memory-stimulating effects in the CCK-dependent manner as described below.

i) Vasorelaxing and antihypertensive effects: Both vasorelaxing and antihypertensive activities of rapakinin were blocked by lorglumide, indomethacin, and CAY10441, an antagonist of cholecystokinin_A receptor (CCK_A-R), an inhibitor of cyclooxygenase, and antagonist of prostaglandin I₂ receptor IP-R [120]. Rapakinin might stimulate CCK release from the artery in the PGI₂-IP receptor system-dependent manner, since it had no affinity for the CCK_A receptor and an IP receptor agonist also showed CCK_A

receptor-dependent vasorelaxation. While the vasorelaxing activity of PGI₂ was blocked by the CCK_A antagonist, that of cholecystokinin-8 was not blocked by the IP-R antagonist. From these results, rapakinin might stimulate CCK release in PGI₂-dependent manner downstream of unidentified receptor of the peptide. ACEI could elevate CCK level by inhibiting its degradation by ACE as will be described later. However, this reaction is independent of the IP-receptor. Therefore, the antihypertensive activity of rapakinin is mediated not by ACEI but by its vaso-relaxing activity which is dependent on both IP-R and CCK_A-R (Table 3-B).

ii) Anti-analgesic activity: Rapakinin at a dose of 10 nmol/mice *icv* counteracted with the analgesic effect of morphine in mice [190]. The anti-analgesic effect of rapakinin was blocked by indomethacin and CAY10441 suggesting the involvement of the PG-I₂-IP-R system. The anti-analgesic effect of rapakinin was also blocked by LY225910, an antagonist of CCK_B receptor, though it no affinity for the receptor. CCK released downstream of an unidentified receptor for rapakinin might bind the CCK_B-R to induce anti-analgesic effect.

iii) Memory consolidation: Rapakinin stimulated memory consolidation in passive avoidance experiment using step-through cages at a dose of 1 mg/kg *ip* in mice [42]. As well as anti-analgesic activity of rapakinin, its memory consolidating effect was also blocked by LY225910. Rapakinin might stimulate memory consolidation in CCK_B receptor-dependent manner by potentiating the release of CCK (Table 3-A).

iv) Effect on food intake: Rapakinin showed anxiogenic effect at a dose of 150 mg/kg *po*

in mice, which was blocked by lorglumide, an antagonist of CCK_A receptor [191]. Since rapakinin has no affinity for the CCK_A-R, CCK released downstream of an unidentified receptor of the peptide might bind CCK_A-R to induce anorexigenic activity (Table 3-A),

4-12. Lactopril (LRPVA) as a pro-drug type ACEI derived from lactoferrin.

We have previously shown that most inhibitory peptides of Angiotensin I-converting enzyme (ACEI) derived from food proteins larger than tripeptides are hydrolysed by ACE releasing dipeptide from their carboxyl termini [42]. After all, peptides made of even numbers of amino acid residues are hydrolysed to dipeptides, while those made of odd numbers of amino acids finally leave tripeptides. As well as bonitopri (LKPNM), lactopril is a typical example of the produg-type ACEI, of which ACE-inhibitory activities are elevated by conversion to the tripeptide by ACE itself [42,43]. Interestingly, lactopril also stimulated memory-consolidation after oral administration in mice in a CCK-dependent manner.

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i) Antihypertensive effect: Duration of antihypertensive effect of lactopril was longer than that of LRP after oral administration in SHR. This is probably because the tripeptide might be degraded quicker than the pentapeptide in vivo [42]. On the other hand, a hexapeptide LRPVAA reported by Lee et al. as an ACEI derived from lactoferrin [192] did not show any antihypertensive effect in our system after the oral administration.

ii) Memory consolidation: Interestingly, lactopril stimulated memory retention in the passive avoidance experiment after post-training administration at a dose of 15 mg/kg

po in mice. The memory consolidation by lactopril was blocked by LY225910, an antagonist of the CCK_B receptor (Table 3-A) [42]. It has been reported that ACE hydrolyses CCK [193]. These suggest that ACE inhibitors might stimulate memory-consolidation by elevating CCK level by way of the enzyme inhibition. Interestingly, LRP, an activated form of lactopril with higher ACE inhibitory activity, did not stimulate memory consolidation. Duration of the antihypertensive effect of LRP was shorter than that of lactopril as described previously. These suggest that a long-lasting ACE inhibition is required for the memory consolidation.

5. Discussion

We have screened bioactive peptides in enzymatic digests of various proteins. We found various peptides which bind to the common receptors to those of endogenous bioactive peptides such as opioid, neurotensin, bradykinin, angiotensin, complement C3a and C5a, formyl peptides, and oxytocin etc from enzymatic digests of natural proteins not only of animal but also of plant origin. Especially, guinea pig ileum preparation, which we initially used for opioid assay, proved to be effective in screening many kinds of peptides since various types of receptors coupled to its contraction are expressed in there.

Peptides derived from blood and muscle protein might act as endogenous regulators and those derived from milk protein might act as endogenous ones at least for infant animals. Some of peptides derived from plant proteins might beneficial effect for plant since opioid peptide rubiscolin suppressed escape reaction of insects [87].

The fact that the rubiscolin sequence is conserved in Rubisco of primitive plant species implies that the opioid ligands has existed on the earth far much earlier before

the appearance of its receptor in animals. Amino acid score of the large subunit of rubisco, which exist in large amounts in plants, is exceptionally good among plant proteins. If we interpret this as a result that primitive animals adapted their essential amino acid composition to that of rubisco large subunit, it might be also possible that they adapted themselves to peptides released by its digestion.

It has been also reported that opioid peptides rubiscolin-5, gluten exorphin C and hemorphin-6 have shaperone-like activity to preventing aggregation of denatured proteins and promote refolding reaction [194]. They might be working to protect organisms from various stress by preventing denaturation of proteins.

However, most of bioactive peptide sequence encrypted in protein might exist independent of teleological function. In this sense, fragment peptides of natural proteins obtained by enzymatic digestion or chemical synthesis could be regarded as a sort of random peptide library reflecting the variety of protein sequences. Some of bioactive peptides might be evolved from such bioactive sequences found in proteins. If so, bioactive peptides encrypted in proteins might be a prototype of endogenous ones.

It should be noted that most of peptide derived from natural proteins bind to minor subtypes of receptor of endogenous bioactive peptides *ie.* NTS₂, B₁, AT₂, and FPR₂, of which structural requirements are looser than those of the major receptors having higher potency and selectivity. Furthermore, some of them show affinities for more than two receptors. It is an attractive idea to speculate that potent endogenous bioactive peptides having high affinities and selectivities for the major receptors might evolved from those having weak affinity for the minor receptor, and that highly potent major receptors for endogenous peptides might also evolved from minor receptors.

Physiological effect of bioactive peptides are not necessarily parallel to that of *in*

vitro potency [195]. Those peptides encrypted in natural proteins are generally smaller in the size and weaker in the specific activities than those of endogenous bioactive peptides. However, probably because of their smallness in size and resistance against gastrointestinal proteases, some of them exert various central and peripheral effect even after oral administration, by which only a few endogenous bioactive peptides are effective.

From the point of view of intestinal absorption, di- or tri-peptide which could be transported by PepT1 might sound more effective than longer peptides. However, as typically shown in the prodrug-type ACE inhibitors derived from natural proteins, antihypertensive effect of di- or tri-peptides last for shorter time than pentapeptides. For this reason, di- or tri-peptides might be hydrolysed more quickly by peptidases than longer peptides in *vivo*. Pharmacokinetic studies of individual bioactive peptides will be required to predict their efficacy after oral administration.

Large amounts of samples were required to detect physiological effects of those weak agonist peptides derived from natural proteins after the single administration. However, the mode of action of those long-acting weak agonists given in large amounts might be different from those of endogenous bioactive peptides, which are potent and usually short-acting. Bioactive peptides derived from natural protein could exert long-term effect by modifying basic level of receptor occupancy probably in a similar manner to traditional oriental medicines. In this sense, proteins containing bioactive peptide sequence themselves could be regarded as a sort of prodrug.

6. Conclusion

Various bioactive peptides are released from animal and plant proteins which have not assumed to have precursor roles. Most of these peptide acted through receptors for endogenous bioactive peptides. In spite of their lower affinity for the receptor than those of endogenous peptide, some of them exerted physiological effect even after oral administration in animals. More extensive study will be required to clarify the effect of these peptides on human health.

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TABLE I-A. RECEPTORS FOR BIOACTIVE PEPTIDES DERIVED FROM NATURAL PROTEINS

Receptors	Peptides	Origin
μ -R	YFPFGPI (β -casomorphin-7)	β -casein
	YPVEPF (neocasomorphin)	β -casein
	YPWTQ (hemorphin-6)	hemoglobin β -chain
	YPFVV (soymorphin-5)	β -conglycinin β
δ -R	RYLGYLE (α -casein exorphin)	α_{s1} -casein
	YPLDLF (rubiscolin-6)	RuBisCO
	GYYPYPT (gluten exorphin A)	gluten
	YGGLW (gluten exorphin B)	gluten
	YPISL (gluten exorphin C)	gluten
	YGFQN (serorphin)	bovine serum albumin
	YPILPEY (ovalulin)	ovalbumin
	YPIEHG (actinolin)	actin
κ -R	YVPFPP (α_{s1} -casomorphin)	human α_{s1} -casein
NTS2-R	HIRL (β -lactotensin)	β -lactoglobulin
B ₁ -R	YVPFPPF (casoxin D)	human α_{s1} -casein
	FRADHPFL (ovokinin)	ovalbumin
C3a-R	YIPIQYVLSR (casoxin C)	κ -casein
	GYPMYPLPR (oryzatensin)	rice albumin
	ALKAWAVAR (albutensin A)	serum albumin
	RHPEYAVSVLLR (albutensin C)	serum albumin
	YLSLILNR (albutensin D)	serum albumin
	LLGNVLVVVLAR (hemotensin)	hemoglobin β -chain
	VTEQESKPVNQMMTQIGLFR (ovotensin)	ovalbumin
	VTIMPKDIQLAR	histone H3
	YSTEVVALSR	leptin
C5a-R	FKDCHLAR (lactomedin 1)	human lactoferrin
OT-R	CFQWQR (lactomedin 2)	human lactoferrin
FPR ₁₁ -R	MITLAIPVНКPGR (soymetide)	β -conglycinin α'
FPR ₁ -R	MIII (soymetide B-4)	glycinin _{A5-A4}
FPR ₂ -R	MRW (rubimetide)	RuBisCO
AT ₂ -R	RPLKPW (novokinin)	synthetic
CB ₁ -R	PVNFKFLSH (hemopressin)	hemoglobin α -chain
(continued to next page)		

?	RIY (rapakinin)	napin
?	LPYPR	glycinin _{A5-A4} ?

TABLE I-B. ENZYME INHIBITOR PEPTIDES DERIVED FROM NATURAL PROTEINS

Peptide	Origin	Target Enzyme	Reference
LKP	Bonito muscle	ACE	43
LKPNM (bonitopril)	Bonito Muscle	ACE	43
LRP	lactoferrin, zein	ACE	4
LRPVA (lactopril)	lactoferrin	ACE	42
IPI (diprotin A)	κ -casein	DPP-IV	35
IPA	β -lactoglobulin	DPP-IV	53
LVV-hemorphin-7	hemoglobin β chain	AT ₄ /insulin-regulated Aminopeptidase	57
LVVYPWT (spinorphin)	hemoglobin β chain	Enkephalin-degrading enzymes	59
VVYPW (tynorphin)	hemoglobin β chain	DPP-III	60
VVYP	hemoglobin β chain	Lipase	62
KLPQLR	β -conglycinin	Fatty acid synthase	118
EITPEKNPQLR	β -conglycinin	Fatty acid synthase	118
RKQEEDEDEEQQRE	β -conglycinin	Fatty acid synthase	118
LPYP	glycinin	HMG-CoA reductase	108
LLEYSL	oyster	HIV-1 protease	121
LLEYSI	oyster	HIV-1 protease	121

TABLE II. POTENT DERIVATIVES OF BIOACTIVE PEPTIDES DERIVED FROM NATURAL PROTEINS

Original peptide	potent derivative	receptors	potency ratio	Ref.
YPLDLF (rubiscolin-6)	YPIDLF	δ	4	85
	YPMDLF	δ	4	85
	YPMDLV	δ	20	85
YPLPR (oryzatensin(5-9))	WPLPR	C3a-R	5	122
MITL (soymetide-4)	MIWL	FPR1	180	124
RADHPF (ovokinin(2-7))	RPLKPW (novokinin)	AT ₂ -R	100	126
YVPFPPF (casoxin D)	YPFPPF (casomokinin F)	μ ant./B ₁ $\rightarrow$ μ /B ₁		133
	YFPPL (casomokinin L)	μ ant./B ₁ $\rightarrow$ μ /NK ₁		134

TABLE III-A. MODE OF ACTIONS OF BIOACTIVE PEPTIDES DERIVED FROM NATURAL PROTEINS: Central effects

Anti-analgesia

Casoxin C $\rightarrow$ C3a-R $\rightarrow$ (PGE₂) $\rightarrow$ EP₃-R $\rightarrow$ Anti-analgesia

Novokinin $\rightarrow$ AT₂ $\rightarrow$ (PGE₂) $\rightarrow$ EP₃-R $\rightarrow$ Anti-analgesia

Rapakinin $\rightarrow$? $\rightarrow$ (PGI₂) $\rightarrow$ IP-R $\rightarrow$ (CCK) $\rightarrow$ CCK_B-R $\rightarrow$ Anti-analgesia

Memory consolidation

YPLDLF $\rightarrow$ δ -R $\rightarrow$ (σ ligand) $\rightarrow$ σ_1 -R $\rightarrow$ (5-HT) $\rightarrow$ 5HT_{1A}-R $\rightarrow$ (dopamine) $\rightarrow$ D₂-R $\rightarrow$

Memory consolidation

HIRL $\rightarrow$ (dopamine) $\rightarrow$ D₂-R $\rightarrow$ Memory consolidation

Rapakinin $\rightarrow$? $\rightarrow$ (CCK) $\rightarrow$ CCK_B-R $\rightarrow$ Memory consolidation

Lactopril $\rightarrow$ ACE inhibition $\rightarrow$ (CCK) $\rightarrow$ CCK_B-R $\rightarrow$ Memory consolidation

Anxiolytic-like

YPLDLF $\rightarrow$ δ -R $\rightarrow$ (σ ligand) $\rightarrow$ σ_1 -R $\rightarrow$ (5-HT) $\rightarrow$ 5HT_{1A}-R $\rightarrow$ (dopamine) $\rightarrow$ D₁-R $\rightarrow$ (GABA) $\rightarrow$ GABA_A-R $\rightarrow$ Anxiolytic-like effect

HIRL $\rightarrow$ NTS2-R $\rightarrow$ (dopamine) $\rightarrow$ D₁-R $\rightarrow$ (GABA) $\rightarrow$ GABA_A-R $\rightarrow$ Anxiolytic-like effect

WPLPR $\rightarrow$ C3a-R $\rightarrow$ (PGE₂) $\rightarrow$ EP₄-R $\rightarrow$ (5-HT) $\rightarrow$ 5HT_{1A}-R $\rightarrow$ (dopamine) $\rightarrow$ D₁-R $\rightarrow$ (GABA) $\rightarrow$ GABA_A-R $\rightarrow$ Anxiolytic-like effect

FKDCHLAR $\rightarrow$ C5a-R $\rightarrow$ (PGD₂) $\rightarrow$ DPR₁-R $\rightarrow$ (adenosine) $\rightarrow$ A_{2A}-R $\rightarrow$ (GABA) $\rightarrow$ GABA_A-R $\rightarrow$ Anxiolytic-like effect

MRW $\rightarrow$ FPR2 $\rightarrow$ (PGD₂) $\rightarrow$ DPR₁-R $\rightarrow$ (adenosine) $\rightarrow$ A_{2A}-R $\rightarrow$ (GABA) $\rightarrow$ GABA_A-R $\rightarrow$ Anxiolytic-like effect

CFQWQR $\rightarrow$ OT-R $\rightarrow$ (adenosine) $\rightarrow$ A_{2A}-R $\rightarrow$ (GABA) $\rightarrow$ GABA_A-R $\rightarrow$ Anxiolytic-like effect

Food intake

YPFVV $\rightarrow$ μ -R $\rightarrow$ (5-HT) $\rightarrow$ HT_{1A}-R $\rightarrow$ (dopamine) $\rightarrow$ D₁-R $\rightarrow$ (GABA) $\rightarrow$ (GABA_B-R) $\rightarrow$ suppression of gastrointestinal motility $\rightarrow$ anorexigenic (normal diet)

YPLDLF $\rightarrow$ δ -R $\rightarrow$ (PGD₂) $\rightarrow$ DP₁-R $\rightarrow$ (NPY) $\rightarrow$ Y₁-R $\rightarrow$ orexygenic (normal diet)

YPLDLF $\rightarrow$ δ -R $\rightarrow$ (α -MSH) $\rightarrow$ MC₄-R $\rightarrow$ (CRF) $\rightarrow$ CRF-R $\rightarrow$ anorexigenic (high fat diet)

HIRL $\rightarrow$? $\rightarrow$ (CRF) $\rightarrow$ CRF-R $\rightarrow$ (CGRP) $\rightarrow$ CGRP-R $\rightarrow$ anorexigenic

Novokinin $\rightarrow$ AT₂-R $\rightarrow$ (PGE₂) $\rightarrow$ EP₄-R $\rightarrow$ anorexigenic

Rapakinin $\rightarrow$? $\rightarrow$ (CCK) $\rightarrow$ CCK_A-R $\rightarrow$ anorexigenic

(continued to next page)

$C3a \rightarrow C3a\text{-}R \rightarrow (PGE_2) \rightarrow EP_4\text{-}R \rightarrow \text{anorexigenic}$

$C5a \rightarrow C5a\text{-}R \rightarrow (PGD_2) \rightarrow DP_1\text{-}R \rightarrow (NPY) \rightarrow Y_1\text{-}R \rightarrow \text{orexygenic}$

Table III-B. MODE OF ACTIONS OF BIOACTIVE PEPTIDES DERIVED FROM NATURAL PROTEINS: Peripheral effects

Vaso-relaxation

Ovokinin $\rightarrow$ B₁-R $\rightarrow$ (PGI₂) $\rightarrow$ IP-R $\rightarrow$ Vaso-relaxation

Ovokinin(2-7) $\rightarrow$? $\rightarrow$ (NO) $\rightarrow$ Vaso-relaxation

Novokinin $\rightarrow$ AT₂-R $\rightarrow$ (PGI₂) $\rightarrow$ IP-R $\rightarrow$ Vaso-relaxation

Rubimetide $\rightarrow$ FPR2 $\rightarrow$ (PGD₂) $\rightarrow$ DP-R $\rightarrow$ Vaso-relaxation

Rapakinin $\rightarrow$? $\rightarrow$ PGI₂ $\rightarrow$ (CCK) $\rightarrow$ CCK_A-R $\rightarrow$ Vaso-relaxation

Anti-alopecia

Soymetide (*po/ip*) $\rightarrow$? $\rightarrow$ (PGE₂) $\rightarrow$ EP₄-R $\rightarrow$ NF- κ B $\rightarrow$ anti-alopecia

MMK-1(*po*) $\rightarrow$ FPR2 $\rightarrow$ (PGE₂) $\rightarrow$ EP₄-R $\rightarrow$ NF- κ B $\rightarrow$ anti-alopecia

MMK-1(*ip*) $\rightarrow$ FPR2 $\rightarrow$ (Histamine) $\rightarrow$ H₁-R $\rightarrow$ IL-1 $\rightarrow$ NF- κ B $\rightarrow$ anti-alopecia

fMLP(*ip*) $\rightarrow$ FPR2 $\rightarrow$ (Histamine) $\rightarrow$ H₁-R $\rightarrow$ IL-1 $\rightarrow$ NF- κ B $\rightarrow$ anti-alopecia

GLF(*ip*) $\rightarrow$ C1q-R? $\rightarrow$ (Histamine) $\rightarrow$ H₁-R $\rightarrow$ NF- κ B $\rightarrow$ anti-alopecia

Novokinin (*po*) $\rightarrow$ AT₂-R $\rightarrow$ (PGE₂) $\rightarrow$ EP₄-R $\rightarrow$ NF- κ B $\rightarrow$ anti-alopecia

Hypocholesterolemic

β -lactotensin $\rightarrow$ NTS2 $\rightarrow$ (dopamine) $\rightarrow$ D₂-R $\rightarrow$ Bile acid secretion $\rightarrow$ Hypocholesterolemic effect

LPYPR $\rightarrow$? $\rightarrow$ Bile acid secretion $\rightarrow$ Hypocholesterolemic effect

Hypoglycemic/Hypotriglyceridemic

Soymorphin-5 $\rightarrow$ μ -R? $\rightarrow$ adiponectin $\rightarrow$ AdipoR2 $\rightarrow$ PPAR α $\rightarrow$ Lipolytic Enzymes *etc.*

Novokinin $\rightarrow$? $\rightarrow$ adiponectin $\rightarrow$ $\rightarrow$ PPAR γ $\rightarrow$ GLUT4 *etc.*

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