REVIEW

Translational Medicine in Fish-derived Peptides: From Fish Endocrinology to Human Physiology and Diseases

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Abstract. Recent studies have revealed the importance of fish-derived peptide hormones to human endocrinology. These peptides include melanin-concentrating hormone (MCH), urocortins (human urotensin-I), and urotensin-II. MCH, a hypothalamic peptide, is a potent stimulator on appetite. Urocortins, e.g. urocortin 1 and urocortin 3 (stresscopin), are endogenous ligands for the corticotropin-releasing factor (CRF) receptors, particularly CRF type 2 receptor, that mediates a vasodilator action, a positive inotropic action and a central appetite-inhibiting action. These actions mediated by CRF type 2 receptor may ameliorate the stress response. Human urotensin-II is a potent vasoconstrictor peptide, while it acts as a vasodilator on some arteries. Human urotensin-II is expressed in various types of cells and tissues, including cardiovascular tissues, as well as many types of tumor cells. Thus, these fish-derived peptides appear to play important roles in human physiology, such as appetite regulation, stress response and cardiovascular regulation, and also in diseases, for example, obesity, cardiovascular diseases and tumors. Development of antagonists/agonists against the receptors for these peptides may open new strategies for the treatment of various diseases, including obesity-related diseases, hypertension, heart failure and malignant tumors.

Key words: Melanin-concentrating hormone, Urotensin, Urocortin, Neuropeptide, Cardiovascular

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IN the last century, a large number of peptide hormones were discovered. Some of the novel peptides are produced and secreted by classically non-endocrine organs, such as brain, heart and vascular vessels [1], while some were originally discovered in non-mammalian species including fishes.

The peptides produced by classically non-endocrine organs include hypothalamic hormones and various neuropeptides secreted by neurons [2–5], atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) by heart [6] and endothelins by vascular endothelium [7]. Some peptides appear to be ubiquitously expressed. For example, adrenomedullin, a vasodilator peptide discovered from pheochromocytoma tissue, is produced and secreted by various types of

normal cells [1, 8, 9, 10], such as fibroblasts [10], macrophages [10, 11], glial cells [12] and retinal pigment epithelial cells [13, 14], and by various tumor cells [1, 15–19]. Endothelin-1 is also ubiquitously expressed like adrenomedullin although it was originally isolated from aortic endothelial cells [7, 20, 21]. Thus, a large number of studies on hormones now aim not only at classical endocrine organs, but also at non-endocrine organs, such as cardiovascular organs.

Another important aspect of the recent advances in peptide endocrinology is recognition of the importance of fish-derived hormones in human physiology and diseases. In this review, I will discuss three bioactive peptides: melanin-concentrating hormone (MCH) [22], urotensin-I [23, 24] and urotensin-II [25, 26]. These three peptides were originally discovered in fishes, and are now recognized to have very important physiological roles in mammals including human. Furthermore, receptors for these peptides are targets for the discovery of novel drugs for human diseases, such as cardio-

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vascular disease and obesity.

Melanin-concentrating hormone (MCH)

A dual hormonal control of color change by two antagonistic pituitary melanophorotropic hormones was first postulated in amphibia by Hogben and Slome in 1931 [27]. α -Melanocyte-stimulating hormone (α -MSH) and β -MSH are responsible for pigment dispersion in the integumentary melanopore of lower vertebrates. MCH is a 17 amino acid peptide originally isolated from chum salmon pituitaries by Kawauchi *et al.* [22] (Table 1). In contrast to α - and β -MSH, MCH induces melanosome aggregation within melanophores of teleost fish, and makes the skin color of fishes pallor.

MCH is present in the neurons of lateral basal hypothalamus of fishes such as the teleost fishes, trout and carp [28, 29] and MCH immunoreactive axons projected into the brain and pituitary. MCH is a circulating hormone in fishes and plasma MCH concentrations were increased by stress and when placed on a white background [30–33]. Salmon MCH is a potent inhibitor of CRF-induced secretion of α -MSH and ACTH *in vitro* by teleost pituitary glands [34, 35]. MCH may play important roles in skin pigmentation and in certain aspects of the stress response in fishes.

Subsequent studies have shown the presence of MCH in mammals including human. Rat MCH was isolated from rat hypothalamus [36] and the cDNAs encoding MCH precursor have been cloned from a rat hypothalamic cDNA library [37]. The amino acid sequence of human MCH was predicted by the cDNA cloning using a human hypothalamic cDNA library [38]. Human MCH, a 19 amino acid peptide, is identical to rat MCH, but differs from salmon MCH by an N-terminal extension of two amino acids and four additional substitutions (Table 1). Immunocytochemistry and *in situ* hybridization in the rat brain showed that MCH neurons were mostly present in the lateral hypo-

thalamic area, with smaller cell groups found in the olfactory tubercle and pontine tegmentum [39–41]. MCH is also expressed in human hypothalamus, mostly posterior and lateral hypothalamic areas (Fig. 1), including perifornical nucleus, tuberomammillary nucleus, and posterior nucleus of hypothalamus [42–44]. The distribution of MCH immunoreactive cell bodies is similar to that in other mammals. Immunoreactive-MCH was detected in the extrahypothalamic brain regions [44, 45], but MCH mRNA was detected only in the hypothalamus by Northern blot analysis [46] (Fig. 1, 2), suggesting that immunoreactive-MCH in the extrahypothalamic brain regions may be due to the immunoreactive-MCH in the nerve fibers originated from the hypothalamic nuclei as mentioned above.

MCH has been shown to have important physiological roles in the central appetite regulation in mammals [47, 48]. MCH is one of the most potent stimulators of feeding and may be a target molecule for leptin, that is secreted by adipocytes and suppresses appetite. Differential display polymerase chain reaction showed overexpression of MCH in the hypothalamus of *ob/ob* obese mice, that genetically lack bioactive leptin [47]. Fasting further increased expression of MCH mRNA in both normal and obese animals. Intracerebroventricular injection of MCH increased food consumption. These findings suggest that MCH participates in the hypothalamic regulation of body weight. Moreover, MCH-deficient mice have reduced body weight and leanness due to hypophagia (reduced feeding) and an inappropriately increased metabolic rate, whereas plasma leptin levels were decreased and pro-opiomelanocortin mRNA expression levels in arcuate nucleus were suppressed [48].

In addition to appetite-stimulating effects, various biological actions of MCH in the brain and pituitary have been demonstrated in mammals. MCH inhibited pentylentetrazole-induced seizure [49, 50]. MCH modulated ACTH secretion, probably via corticotropin-releasing factor (CRF) release [51]. On the other hand, Navarra *et al.* reported that rat MCH did not

Table 1. Amino acid sequences of salmon melanin-concentrating hormone (MCH) and human/rat MCH

Salmon MCH	Asp	Thr	Met	Arg	Cys	Met	Val	Gly	Arg	Val	Tyr	Arg	Pro	Cys	Trp	Glu	Val		
Human/rat MCH	Asp	Phe	Asp	Met	Leu	Arg	Cys	Met	Leu	Gly	Arg	Val	Tyr	Arg	Pro	Cys	Trp	Gln	Val

Amino acids commonly found in salmon and human/rat MCH are shown by square.

Fig. 1. Immunocytochemistry of melanin-concentrating hormone (MCH) in the human hypothalamus. (A) Perifornical nucleus ($\times 100$). F; fornix. (B) Tuberomammillary nucleus ($\times 100$). (C) MCH positive nerve fiber in the periventricular area ($\times 250$). Reproduced from ref [44] Mouri *et al.* Peptides 14: 643–646; 1993 with kind permission from Elsevier Science.

Fig. 2. Northern blot analysis of melanin-concentrating hormone (MCH) mRNA in human brain. Each lane contained 15 μ g total RNA. The bottom panel shows the expression of β -actin mRNA. Cerebellum, h = cerebellar hemisphere; cerebellum, v = cerebellar vermis. Reproduced from ref [46] Takahashi *et al.* Neuroendocrinology 61: 493–498; 1995 with kind permission from S. Karger AG.

affect the release of CRF from the rat hypothalamus or ACTH from the anterior pituitary *in vitro* [52]. MCH stimulated release of luteinizing hormone-releasing hormone (LH-RH) and gonadotropins from the hypothalamo-pituitary axis [53, 54]. Furthermore, MCH stimulated sexual behavior, when injected into the medial preoptic area and the ventromedial nucleus [55]. On the other hand, it was reported that intracerebro-ventricular administration of MCH suppressed pulsatile LH release in the female rat [56]. This inconsistency may be due to the difference of experimental conditions, such as injection sites in the brain, presence or absence of estrogen treatment and sampling conditions. MCH inhibited the thyroid axis via the

suppression of TSH release [57]. Central administration of MCH also had effects on behavior in rats. For example, MCH had an anxiogenic effect when injected into the medial preoptic area [55]. MCH modulated memory retention by acting on the amygdala and hippocampus [58]. Most of these biological actions of MCH are mediated by an opposite mechanism of action compared with those of α -MSH.

Thus, MCH acts primarily as a neurotransmitter or a neuromodulator in mammals, whereas MCH is a circulating hormone in fishes. It has recently been shown, however, that MCH has biological effects on the peripheral tissues, and that MCH-like immunoreactivity is present in rat plasma. Bradley *et al.* reported that MCH stimulated leptin mRNA expression and leptin secretion in isolated rat adipocytes [59]. MCH activated signaling pathways, such as p44/42 MAPK and pp70 S6 kinase activities, in 3T3-L1 adipocytes [60]. In our previous studies, immunoreactive-MCH was not detected in human plasma (<0.25 fmol/ml) [46] or in rat plasma (our unpublished observations), whereas Bradley *et al.* [59] and Stricker-Krongrad *et al.* [61] have suggested the presence of MCH-like immunoreactivity in rat plasma. Bradley *et al.* reported that rat plasma levels of MCH or MCH-like peptide, measured by a commercially available RIA kit (Phoenix Pharmaceuticals, Mountain View, CA), ranged between 54 and 397 pg/ml [59]. Stricker-Krongrad *et al.* reported that plasma concentrations of MCH-like immunoreactivity, measured by a commercially available competitive enzyme immunoassay kit (Phoenix Pharmaceuticals, Mountain View, CA), were 65.9 ± 3.2 ng/ml (mean $\pm$ SEM) in obese fa/fa Zucker rats and 1.7 ± 0.1 ng/ml in lean homozygous Zucker rats [61]. Surprisingly, these levels were about 1000 times higher than the detection limit of our plasma MCH assay. In the study by Stricker-Krongrad *et al.* [61], plasma samples were measured without extraction using the commercially available kit. Therefore there is the possibility that proteins in unextracted plasma had non-specific effects on the assays. Chromatographic characterization of MCH-like immunoreactivity in plasma has not been presented by these groups or by others.

There are at least two isoforms of MCH receptors, MCH receptor 1 and MCH receptor 2, which are distinct from melanocortin receptors. Five independent groups reported that the orphan G-protein-coupled receptor, SLC-1, is the MCH-specific receptor almost at the same time [62–66]. SLC-1 is now called MCH

receptor 1. The pathway mediated by this MCH receptor 1 was activated by MCH at nM concentrations, but not by α -MSH. Consistent with these findings, MCH did not block α -MSH binding or α -MSH-induced cAMP accumulation in cells expressing either the melanocortin 3 or melanocortin 4 receptor [67].

MCH receptor 1 mRNA is expressed not only in brain tissues, but also in peripheral tissues, such as adrenal glands, tumor tissues of pheochromocytomas and neuroblastomas, and various human tumor cell lines [68]. Subsequently, another isoform of the MCH receptor, MCH receptor 2, has been identified by several groups almost at the same time [69–74]. MCH receptor 2 is also a G-protein-coupled receptor with 7 transmembrane domains, and shows 38% identity to MCH receptor 1. Both MCH receptor 1 and 2 are expressed predominantly in the brain, consistent with the presence of MCH binding sites in the brain tissues [75]. Although the distribution of these two receptors are similar, higher relative levels of MCH receptor 1 were found in the pituitary, hypothalamus, locus ceruleus, medulla oblongata and cerebellum. MCH receptor 2 is also expressed in the peripheral tissues, such as the intestines, lymphocytes, adipose tissue and prostate gland, at lower levels than the brain [70]. Targeted disruption of the MCH receptor 1 results in hyperphagia and resistance to diet-induced obesity [76, 77]. This may suggest that the MCH signaling in the brain is related not only to appetite stimulation, but also to the central control of energy metabolism. Further studies are also required to clarify whether MCH receptor 2 signalling is increased in the MCH receptor 1 gene knockout mice.

Obesity is related to higher incidence of diabetes, hyperlipidemia and cardiovascular disease, such as hypertension and coronary artery diseases, and is therefore one of the greatest problems in health and medical care in developed countries. The MCH receptors are now regarded as an important target for novel drugs against obesity. Several types of MCH receptor 1 antagonists have been reported [78–80]. Interestingly, one antagonist for the MCH receptor 1, SNAP-7941, had antidepressant and anxiolytic effects, in addition to anorectic effects, when administered intraperitoneally [80]. This observation suggests that an MCH receptor 1 antagonist may be useful not only in the management of obesity but also as a treatment for depression and/or anxiety. Alternatively, an MCH receptor agonist [81] may be useful for the treatment

of anorexia, for example in cachectic patients with an advanced stage of cancer.

Urocortins (human homologues of fish urotensin-I)

Urotensins are peptide hormones that are secreted by the fish neuroendocrine organ located in the caudal spinal cord, urophysis [82–84]. Urotensin-I is a CRF-like peptide hormone [85] and urotensin-II is a somatostatin-like peptide in fishes [86]. Urocortin (now called urocortin 1) is a human homologue of urotensin-I and belongs to the CRF family [24, 87]. Human urocortin 1 has 63% identity with fish urotensin-I and 43% identity with rat/human CRF at the amino acid level (Table 2). Urotensin-II is described in latter section.

Urocortin 1 is a 40-amino acid peptide discovered originally in the rat midbrain. Human urocortin 1 is expressed in the brain and pituitary [87–90] as well as various peripheral tissues, such as gastrointestinal tract [91, 92], placenta, fetal membranes [93], ovaries [94], uterus [95], synovial tissue [96], and lymphocytes [97]. CRF receptors are classified into CRF type 1 receptor (CRF₁ receptor) [98] and CRF type 2 receptor (CRF₂ receptor) [99]. CRF₁ receptor is highly expressed in the brain and pituitary, and mediates the stress response and anxiety-related behavior, as well as ACTH release from the pituitary [98, 100]. CRF₂ receptor is expressed not only in the brain but also in the peripheral tissues, including heart and skeletal muscle tissues [99]. CRF₂ receptor mediates stress-coping responses including anxiolysis, anorexia, vasodilatation, cardioprotection and dearousal [101–103]. Urocortin 1 binds with high affinity to both CRF₁ receptor and CRF₂ receptor, but this ligand has a much higher affinity for CRF₂ receptor than CRF [24, 87]. The distribution of urocortin 1 nerve fibers in the brain correlates well with that of CRF₂ receptor but not with that of CRF₁ receptor [24]. Urocortin 1 is therefore considered to be a physiological ligand for CRF₂ receptor.

There is accumulating evidence that indicates urocortin 1 has important pathophysiological roles in the cardiovascular regulation. Urocortin 1 has been demonstrated to have potent coronary vasodilatory and cardiac inotropic effects, and these effects have been shown to be more potent than CRF [104–106]. Urocortin 1 has protective effects on cardiac myocytes

from ischemic or reperfusion injury [107–113]. These protective effects of urocortin were mediated by up-regulation of p42/p44 MAPK signaling pathway, activation of protein kinase B/Akt, and induction of K(ATP) channel gene expression. Moreover, urocortin 1 stimulates ANP and BNP secretions from neonatal rat cardiomyocytes [114].

CRF receptors, particularly CRF₂ receptor, are expressed in the heart [99, 115–117]. CRF_{2(a)} receptor mRNA was predominantly expressed in the four chambers of human heart [117] (Fig. 3). CRF₁ receptor mRNA was only weakly expressed in some of these samples, and CRF_{2(b)} receptor mRNA was expressed in the left atrium in all four cases and in the right atrium of only one out of four cases studied [117] (Fig. 3). Actually, CRF acted directly on cardiac myocytes, presumably through CRF₂ receptor, to elevate intracellular cAMP levels [115]. Physiological ligands for CRF₂ receptor in the cardiac myocardium, however, had long been unknown, because plasma levels of CRF are very low and CRF expression in the heart was very weak or undetectable [115]. Very recently, several investigators, including our group, have shown that both urocortin 1 mRNA and peptide are expressed in the heart [107, 108, 117, 118]. Urocortin 1 mRNA was expressed in all four chambers of human heart [117] (Fig. 3). Immunoreactive-urocortin 1 was present in the human heart at levels comparable to those found in the brain [117] (Fig. 4). Urocortin 1 was expressed in rat cardiomyocytes, and its expression levels increased by heat shock and ischemia [107, 108]. Nishikimi *et al.* [118] have reported that the human myocardium was immunohistochemically positive for urocortin 1, the staining of which was more intense in the failing heart. Actually in sheep with experimental heart failure, intravenous infusion of urocortin showed beneficial hemodynamic, endocrine and renal effects, such as increased cardiac output, decreased peripheral resistance, decreased plasma concentrations of renin, aldosterone, and endothelin-1, and increased urine volume and sodium excretion [119].

Another important aspect of urocortin is its relation to inflammation [120]. Urocortin 1 is expressed in immune tissues, such as thymus and spleen [121–123], and immune cells, such as lymphocytes [96, 97] and macrophages [92]. Urocortin 1 mRNA expression increased in thymus after immune activation by lipopolysaccharide in a corticosterone-dependent manner

Table 2. Amino acid sequences of human corticotropin-releasing factor (CRF), fish urotensin-I (85), urocortin 1, urocortin 2, stresscopin-related peptide (SRP), urocortin 3 and stresscopin

CRF	Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg
Urotensin-I	Asn-Asp-Asp-Pro-Pro-Ile-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Asn-Met-Ile-Glu-Met-Ala-Arg
Urocortin 1	Asp-Asn-Pro-Ser-Leu-Ser-Ile-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Thr-Leu-Leu-Glu-Leu-Ala-Arg
SRP	His-Pro-Gly-Ser-Arg-Ile-Val-Leu-Ser-Leu-Asp-Val-Pro-Ile-Gly-Leu-Leu-Gln-Ile-Leu-Leu-Glu-Gln-Ala-Arg
Urocortin 2	Ile-Val-Leu-Ser-Leu-Asp-Val-Pro-Ile-Gly-Leu-Leu-Gln-Ile-Leu-Leu-Glu-Gln-Ala-Arg
Stresscopin	Thr-Lys-Phe-Thr-Leu-Ser-Leu-Asp-Val-Pro-Thr-Asn-Ile-Met-Asn-Leu-Leu-Phe-Asn-Ile-Ala-Lys-
Urocortin 3	Phe-Thr-Leu-Ser-Leu-Asp-Val-Pro-Thr-Asn-Ile-Met-Asn-Leu-Leu-Phe-Asn-Ile-Ala-Lys-

Amino acids commonly found among CRF, fish urotensin-I, urocortin 1, urocortin 2 (SRP) and urocortin 3 (stresscopin) are shown by squares.

Fig. 3. RT-PCR analysis for urocortin 1 (Ucn), CRF, CRF type 1 receptor (CRF-R1), CRF type 2(a) receptor (CRF-R2α), and CRF type 2(b) receptor (CRF-R2β) in four human hearts (patients 1–4) in the four constituent chambers (RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle). Total RNA from placenta, pituitary gland, hypothalamus, and left atrium were used as positive controls for urocortin and CRF, CRF-R1, -R2α, and -R2β, respectively. The bottom panel shows GAPDH employed as an internal control. P, Positive control. Reproduced from ref [117] Kimura *et al.* *J Clin Endocrinol Metab* 87: 340–346; 2002 with kind permission from the Endocrine Society.

[121]. Furthermore, urocortin 1 expression in the synovial was increased in rheumatoid arthritis [96]. Urocortin 1 expression was observed in synovial cells, lymphocytes, macrophages, and fibroblasts in the synovial tissue obtained from patients with rheumatoid arthritis [96]. Urocortin 1 was present in the gastric mucosa, and the level of immunoreactive-urocortin 1 was higher in gastric biopsies from the group of patients with active *Helicobacter pylori* gastritis than in normal controls [122]. It was reported that urocortin 1, as well as CRF, suppressed experimental auto-

immune encephalomyelitis [124]. Intracerebroventricular injection of urocortin 1 produced a marked decrease in the proliferative response of splenocytes to a mitogen, suggesting that urocortin 1 is involved in the brain control of peripheral immune functions, such as stress-induced immunosuppression, via the sympathetic nervous system [125]. On the other hand, urocortin 1 has been shown to be one of the most potent triggers of mast cell degradation and skin vascular permeability [126]. Thus, urocortin 1 expression appears to be increased in inflammatory conditions of various

Table 2. (continued)

-Ala-	Glu-	Gln-Leu-Ala-Gln-	Gln-Ala-His-Ser-Asn-	Arg-Lys-Leu-Met-Glu-Ile-Ile-NH2
-Ile-	Glu-Asn-Glu-Arg-	Glu-Gln-Ala-Gly-Leu-Asn-	Arg-Lys-Tyr-Leu-Asp-Glu-Val-NH2	
-Thr-Gln-Ser-Gln-Arg-	Glu-Arg-Ala-Glu-Gln-Asn-	Arg-Ile-Ile-Phe-Asp-Ser-Val-NH2		
-Ala-Arg-Ala-Ala-Arg-	Glu-Gln-Ala-Thr-Thr-Asn-	Ala-Arg-Ile-Leu-Ala-Arg-Val-NH2		
-Ala-Arg-Ala-Ala-Arg-	Glu-Gln-Ala-Thr-Thr-Asn-	Ala-Arg-Ile-Leu-Ala-Arg-Val-NH2		
-Ala-Lys-Asn-Leu-Arg-Ala-	Gln-Ala-Ala-Ala-Asn-	Ala-His-Leu-Met-Ala-Gln-Ile-NH2		
-Ala-Lys-Asn-Leu-Arg-Ala-	Gln-Ala-Ala-Ala-Asn-	Ala-His-Leu-Met-Ala-Gln-Ile-NH2

Fig. 4. Urocortin 1-like immunoreactivity in the human heart (mean $\pm$ SEM, $n = 4$). RA, right atrium; RV, right ventricle; LA, left atrium; LV, left ventricle. Reproduced from ref [117] Kimura *et al.* J Clin Endocrinol Metab 87: 340–346; 2002 with kind permission from the Endocrine Society.

diseases, and urocortin may modulate local inflammatory states.

Urocortin 1-deficient mice displayed an impaired acoustic startle response, suggesting that urocortin 1 modulates the acoustic startle response through the urocortin 1-expressing neuron projections from the region of the Edinger-Westphal nucleus [127, 128]. On the other hand, there was inconsistency in the results of stress-induced anxiety behavior between these two reports: Vetter *et al.* reported that these mice showed increased anxiety-like behavior [127], whereas Wang *et al.* reported that the mice showed normal stress-

induced anxiety behavior and autonomic control [128]. It is not known whether these urocortin-deficient mice show altered cardiovascular and/or inflammatory responses to various stressful stimuli.

Recent studies have shown the presence of two other isoforms of urocortin 1; urocortin 2 (stresscopin-related peptide) [129, 130] and urocortin 3 (stresscopin) (Table 3) [130–132], both of which are specific ligands for CRF₂ receptor and do not bind to CRF₁ receptor. Two research groups identified two cDNAs encoding peptides similar to urocortin 1 and CRF. Different interpretations of post-translational processing sites resulted in slightly different amino acid sequences of the peptides: Human urocortin 3 is a 38 amino acid peptide that corresponds to the sequence 3–40 of human stresscopin, a 40 amino acid peptide, while human urocortin 2 (urocortin-related peptide sequence) is a 38 amino acid that corresponds to the sequence of 6–43 of stresscopin-related peptide, a 43 amino acid. Urocortin 2 and urocortin 3 have about 20–40% homology with CRF and urocortin 1 (Table 3). The homology between urocortin 2 and urocortin 3 was about 40%. Urocortin 2 and urocortin 3 are both widely expressed in the brain and the peripheral tissues. The presence of urocortin 2, however, has not been clarified in the human tissues, because the human sequence lacks a consensus proteolytic cleavage site that would allow for C-terminal processing of the peptide. Further studies are therefore required to clarify whether urocortin 2 is a real peptide or merely an artifact in human.

Urocortin 3-positive neurons were found predomi-

Table 3. Amino acid sequences of human urotensin-II, goby urotensin-II (86) and human somatostatin-14

Human urotensin-II	Glu-Thr-Pro-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val
Goby urotensin-II	Ala-Gly-Thr-Ala-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val
Human somatostatin	Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys

Amino acids commonly found in human and goby urotensin-II are shown by squares.

Amino acids commonly found in somatostatin and urotensin-II are underlined.

nantly within the hypothalamus and medial amygdala of rat brain. The urocortin 3 fibers were distributed mainly in the hypothalamus and limbic structures [133]. Although further studies are required to clarify the physiological significance of these two novel urocortin isopeptides, at least urocortin 3 may act as an important mediator working against stress through their actions on CRF₂ receptor, such as anxiolysis, anorexia, vasodilatation, cardioprotection and dearousal.

Urotensin-II

Urotensin-II is a “somatostatin-like” cyclic peptide which was originally isolated from fish urophysis [86, 134]. Human urotensin-II is an 11-amino-acid peptide [25, 26] (Table 3). The reverse pharmacological approach has shown that human urotensin-II is an agonist for the orphan receptor GPR14 [26]. Urotensin-II is a potent vasoconstrictor peptide and its vasoconstrictive potency is one order of magnitude greater than endothelin-1 [26, 135]. On the other hand, urotensin-II elic-

ited vasodilatation in some arteries, possibly through the release of endothelium-derived hyperpolarizing factor and nitric oxide [136, 137]. In addition to actions on vascular vessels, this peptide has a positive inotropic action [138], stimulates proliferation of vascular smooth muscle cells [139–141], and induces hypertrophic responses in cultured cardiomyocytes [142]. Furthermore, it inhibited insulin release from the perfused rat pancreas [143].

Urotensin-II and urotensin-II receptor are expressed in various human tissues including brain, heart and kidney [26, 144] (Fig. 5). We have shown for the first time the presence of urotensin-II-like immunoreactivity in human plasma [144] (Fig. 6). The levels of plasma urotensin-II-like immunoreactivity were elevated in patients with chronic renal failure. Plasma concentrations of urotensin-II-like immunoreactivity were 2-fold higher in patients not on dialysis and 3-fold higher in those on hemodialysis than in healthy individuals (Fig. 6). Kidneys play important roles in the metabolism and the elimination of many peptide hormones. Decreased elimination of urotensin-II from

Fig. 5. Urotensin II and urotensin II receptor messenger RNA expression in various tissues. Reproduced from ref [144] Totsune *et al.* Lancet 358: 810–811; 2001 with kind permission from the Lancet.

Fig. 6. Plasma concentrations of urotensin II in patients with chronic renal failure. Open circles and bars show mean and SD, respectively. Reproduced from ref [144] Totsune *et al.* Lancet 358: 810–811; 2001 with kind permission from the Lancet.

blood may explain increased plasma levels of urotensin-II-like immunoreactivity in patients with chronic renal failure. Urotensin-II-like immunoreactivity was present in the human urine [145]. In the kidney, urotensin-II was expressed in the epithelial cells of tubules and ducts and in the endothelial cells of renal capillaries [146]. Urotensin-II secreted by renal tubular cells into urine may account for urotensin-II-like immunoreactivity in urine. Urotensin-II produced by kidneys may also have roles in the renal circulation and the water-electrolyte transport in the kidney.

Subsequent studies have shown that plasma levels of urotensin-II-like immunoreactivity were also increased in patients with heart failure [147, 148], liver cirrhosis and portal hypertension [149], or diabetes mellitus [150]. Urotensin-II was strongly expressed in the cardiomyocytes in patients with end stage congestive heart failure, suggesting that cardiomyocytes are one of the major source of elevated plasma urotensin-

II in these patients [151]. Plasma levels of urotensin-II-like immunoreactivity were elevated in patients with diabetes mellitus without renal failure (with normal serum creatinine) regardless of the presence or absence of proteinuria [150]. Because urotensin-II was expressed in cultured vascular endothelial cells, vascular endothelial damage accompanied with diabetes mellitus may account for the elevation of plasma urotensin-II-like immunoreactivity. Interestingly, the association of certain polymorphism in the urotensin-II gene with type 2 diabetes mellitus has been reported in the Chinese population [152] and the Japanese population [153]. Urotensin-II may be related not only to the diabetic vascular complications, but also to the control of carbohydrate metabolism.

On the other hand, it should be noted that plasma concentrations of urotensin-II-like immunoreactivity measured by enzyme-linked immunosorbent assay were extremely high [149, 154], when compared with the concentrations measured by radioimmunoassay after the extraction [144, 147, 148, 150]. In control subjects, plasma concentrations of urotensin-II-like immunoreactivity measured by enzyme-linked immunosorbent assay were about 150–600 times higher [149, 154] than those measured by radioimmunoassay after the extraction [144, 147, 148, 150]. This may suggest that the measurement of unextracted plasma by enzyme-linked immunosorbent assay may be affected by non-specific effects of proteins in plasma, and that the extraction procedure of plasma samples is essential for the precise measurement of plasma urotensin-II-like immunoreactivity.

Another possible pathophysiological role of urotensin-II may be a growth stimulatory effect, particularly in tumors. Urotensin-II and its receptor are expressed in various tumor cell lines, such as T98G glioblastoma cells, SW-13 adrenocortical carcinoma cells, DLD-1 colorectal adenocarcinoma cells and HeLa cervical cancer cells [155, 156] (Fig. 7). Reverse-transcriptase polymerase chain reaction revealed expression of urotensin-II and its receptor in the normal adrenal glands and tumor tissues of adrenocortical tumors and pheochromocytomas [157]. The growth stimulatory effects were found not only in vascular smooth muscle cells [139–141], but also in SW-13 adrenocortical carcinoma cells and VMRC-RCW renal cell carcinoma cells [157]. Ziltener *et al.* reported that the proliferative action of urotensin-II is mediated by the long-lasting activation of extracellular signal-

Fig. 7. Reverse transcriptase-polymerase chain reaction of urotensin II (U-II) mRNA and urotensin II receptor (U-II receptor) mRNA in various human tumor cell lines; T98G glioblastoma cells, IMR-32 neuroblastoma cells, NB69 neuroblastoma cells, BeWo choriocarcinoma cells, SW-13 adrenocortical carcinoma cells, DLD-1 colorectal adenocarcinoma cells and HeLa cervical cancer cells. The lower panel indicates expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as an internal control. MM, molecular weight markers expressed in base pairs (bp). Reproduced from ref [155] Takahashi *et al.* Peptides 22: 1175–1179; 2001 with kind permission from Elsevier Science.

Fig. 8. Possible relations of fish-derived peptides to human physiology and diseases. MCH, melanin-concentrating hormone. U-II, urotensin II. (+), positive regulation. (-), negative regulation.

regulated kinase 1/2 (ERK1/2) [158]. Thus, urotensin-II may act as an autocrine growth stimulator in various types of tumors, as well as in other benign disorders, such as atherosclerosis, cardiac hypertrophy and proliferative glomerulonephritis.

Possible translational medicine related to fish-derived peptides

The impact of fish endocrinology on human physiology and diseases has long been ignored [159]. It is evident, however, that fish-derived peptides are involved in the control and regulation of the fundamental mammalian physiology, such as appetite regulation,

stress response, cardiovascular regulation and cell proliferation (Fig. 8). These peptides may therefore be related to various diseases, such as obesity-related diseases, cardiovascular diseases, diabetes mellitus, and neoplastic diseases as well as mental diseases. Development of antagonists or agonists for the receptors of these peptides may open new strategy of treatment of many diseases, such as obesity, anorexia, cardiovascular diseases such as hypertension and coronary artery diseases, and various types of tumors. Actually, one recently developed antagonist for the MCH receptor 1, SNAP-7941, was reported to have appetite-suppressing effects, as well as antidepressant and anxiolytic effects [80].

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