Review Article

Frogs as a Rich Source for New Treatment Principles: A Review of Bio-Active Peptides in Phyllomedusae (*Phyllomedusa Bicolor*) and Bombinae (*Bombina Variegate*)

Jan M. Keppel Hesselink[1*] and Paola Sacerdote[2]

[1]Department of Health, University of Witten/Herdecke, Germany and Institute Neuropathic Pain, The Netherlands

[2]University of Milan, Italy

ARTICLE INFO

Received Date: April 12, 2019
Accepted Date: June 06, 2019
Published Date: June 13, 2019

KEYWORDS

Dermorphin
Cerulein
Cerulitide
Bombesin
Prokineticin
Bombina variegate
Phyllomedusa bicolor

Citation for this article: Jan M. Keppel Hesselink and Paola Sacerdote. Frogs as a Rich Source for New Treatment Principles: A Review of Bio-Active Peptides in Phyllomedusae *(Phyllomedusa Bicolor)* and Bombinae *(Bombina Variegate)*. SL Pharmacology And Toxicology. 2019; 2(1):116

Corresponding author:
Keppel Hesselink JM,
Department of health, University of Witten/Herdecke, Germany and Institute Neuropathic Pain, Netherlands,
Tel: +316517005427;
Email: jan@neuropathie.nutel

ABSTRACT

Since some decade's frog's secretion are also recognized as an important source of new drugs, such as analgesics and antibiotics. We will review a series of bioactive peptides from two different frog species, with a special focus on the potential use of these peptides for the treatment of chronic pain. Kambo is the Amerindian name of a skin secretion of the tropical frog *Phyllomedusa bicolor* from the Amazon basin, which has been used for centuries by local tribes to enhance their hunter skills. *Bombina variegate* is a European frog. Both frogs are characterized by the secretion of peptides containing analgesic compounds.

Since the end of last century Kambo is increasingly used in the West outside of medical context as a 'healing' concoction. Kambo consists of a great number of bioactive peptides, with high affinity for a number of receptors. Some have specific bradykinin-like actions, others have analgesic or antibiotic actions. Initial data on dermorphin and cerulean clearly indicated potential medical benefits in the field of the treatment of chronic cancer and postoperative pain. The same holds true for the newly isolated peptides influencing the prokineticin system from *Bombina variegate*.

INTRODUCTION

The Number Needed to Treat (NNT) of pregabalin for 50% pain reduction is only 7.7 [1]. This is not unique, but rather the general rule: many of recommended analgesics in neuropathic pain are ineffective in most of the patients. So, it's a challenge to find new molecules or reactivate old drug for the treatment of neuropathic pain. We have to look further into innovative and promising molecules in natural world, which did not yet reach the patients, although clinical data were supportive.

Dermorphin is such a promising compound, a small neuropeptide, sourced via the skin secretion of an Amazonian frog, *Phyllomedusa bicolor*, tested in the clinic in the middles 80s and with a superior profile compared to morphine [2]. *Bombina variegate* is a second example of a frog as a source for new powerful analgesics. In this article, we present a number of such bioactive peptides found in the secretion of two different frogs that can represent a potentially promising in pain treatment. One secretion is known as Kambo (from the *Phyllomedusa bicolor*) is a source of many bioactive peptides and at least 3 neuroactive peptides with analgesic potential, the other is from the European frog *Bombina variegate*, that contain a promising an 8 kD protein.

Kambo is increasingly used outside of a medical context and within a shamanistic context to enhance 'healing' [3]. The potential health benefits of bio-active peptides

derived from natural sources such as Amazonian frogs are increasingly attracting interest from scientists [4]. These peptides are characterized by low-molecular-weight, high activity, easy absorption, with reduced negative side effects because of fast tissue and blood clearance [4]. This might be of great therapeutic use, for instance in the prevention of neurodegeneration [4].

Recently a Kambo sample was analyzed, and sixteen bio-active peptides were detected via ultra-high resolution Electro-Spray Ionization-Mass Spectrometry (ESI-MS) fingerprinting: adenoregulin, bombesin, bombesinnona peptide, a bradykinin derivate, caerulein, deltorphin, neurokinin B, phyllomedusin, phyllocaerulein, phyllokinin, phyllolitorin, preprotachykinin B, ranatachykinin A, sauvagine, T-kinin and urechistachykinin II [5]. Kambo pharmacology started arround 1992 [6]. Insights in the pharmacology of the *Bombina variegate* peptide started recently and suggest similar bio-active principles than Kambo.

KAMBO RITUAL: A 'MEDICINE' AND ITS EFFECTS

Since the beginning of this century a special Kambo ritual is gaining interest, a ritual which sounds strange to medical professionals [3]. The ultimate goal of this ritual is to achieve 'healing', however, Kambo contains many compounds which can be of great interest in the field of 'curing' () These compounds are pharmacological active and some proved already to be valuable in a great number of animal models and some, such as dermorphin and cerulean have been tested in the clinic as analgesics quite successfully in the last century, sadly enough without any follow-up.

A Kambo ritual in general starts with drinking around 1 liter of water or cassava soup, and subsequently the therapist, mostly called a Kambo practitioner, creates a number of small burns via a glowing timber, often around 5, traditionally on the ankle or upper arm [3]. After scraping away the blister skin, the secretion is applied on the wound, reaches the lymphatic system and quickly the bloodstream. The first symptoms start within minutes, with a suddenly feeling of warmth, palpitations and rapid pulse, followed by flushed red skin, or paleness, feeling like a lump in the throat or difficulty swallowing, abdominal pain, nausea, vomiting (yellow-greenish due to bile), diarrhea, runny nose and tears, swollen lips, eyelids or face, and rarely a swollen tongue. In general, the duration of the entire symptom-complex is mostly 5-30 minutes, rarely longer 30-60 minutes, and very rarely it lasts for several hours. Clearly, from a clinical point of view one could define this symptom-complex as an anaphylactic shock. However, as we will see in this paper, most of these symptoms, if not all, can be attributed to the pharmacological activity of the peptides of Kambo, and thus the symptom-complex seems not to be anaphylactic of nature, but directly related to its pharmacological effects.

PEPTIDE RESEARCH FROM THE SCHOOL OF VITTORIO ERSPAMER

Start of the peptide research in skin secretion of the frogs belonging to the Phyllomedusae, such as the *Phyllomedusa bicolor*, was situated in the early 60`s and was conducted by the research group of the famous professor Vittorio Erspamer (1909-1999) [7-9]. In the first 4 years of research, the group could isolate at least five new pharmacologically active polypeptides [9-11]. In 1966 they reported that the methanol extract of skins from frogs from the family of Phyllomedusae all contain large amounts of powerful bradykinin-like and physalaemin-like polypeptides [11]. This family of frogs subsequently was found to be a rich source of bio-active peptides, which at the time raised much interest in the pharmaceutical world [10].

One of the first isolated bio-active peptides was named physalaemin [11]. It was a decapeptide with powerful vasodilator and hypotensive mode of action. Many of the bioactive bradykinin-like peptides isolated subsequently had powerful hypotensive actions [12-15]. Currently the various peptides of the Phyllomedusa belong to different classes: bombesin-like peptides, bradykinin-related peptides, caeruleins, sauvagines, tachykinins; tryptophyllins, opioids, and a number of antimicrobial peptides. Basically, these peptides have either a function as defensive peptides against predators, or help the animal to combat micro-organisms, based on broad-spectrum antimicrobial peptides.

PEPTIDES EFFECTING SMOOTH MUSCLES

A number of Kambo peptides (phyllomedusin, phyllokinin, sauvagine and a number of bombesin-like compounds) act on smooth muscles, giving rise to bradykinin-like effects on the cardiovascular and intestinal systems: hypotension, flushing, palpitations, nausea, vomiting, bile secretion and the

Frogs as a Rich Source for New Treatment Principles: A Review of Bio-Active Peptides in Phyllomedusae (Phyllomedusa Bicolor) and Bombinae (Bombina Variegate). SL Pharmacology And Toxicology. 2019; 2(1):116.

angioedema and explain the clinical symptomatology after administration.

Phyllomedusin

Phyllomedusin is a decapeptide isolated in 1970 [13]. Its amino acid composition is Pyr-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-NH2, and it was classified as a tachykinin, just as physalaemin, eledoisin and substance P (SP), all containing the same Gly-Leu-Met-NH2 peptide-end. Frog skin secretioncontained 1100 ug of the pure peptide per g fresh tissue. Tachykinins are peptides that are involved in the nervous systems, the cardiovascular and the immune system; SP, Neurokinin A (NKA) and Neurokinin B (NKB) are examples. Tachykinins interact the three Neurokinin receptor subtype NK1, NK2 and NKB or NK3 [14]. Phyllomedusin appeared to be such a NK1 agonist, with high affinity. Its biological effects are related to smooth muscle contraction affecting the salivary glands, tear ducts, tractus intestinalis and glands, and dilation of blood vessels, leading to blood pressure effects [15]. This compound did not raise much interest, perhaps as its affinity for the NK1 receptor is lower compared to SP. We detected only 17 paper on this topic were identified in PubMed, 15 from the last century, so the interest in this compound has waned considerably.

Phyllokinin

Phyllokinin, a natural bradykinin analogue, also did not arouse particularly much interest, only 13 papers were identified in PubMed [16]. The group of Erspamer characterized its pharmacological pattern, and stated that it greatly resembles bradykinin, but stronger acting; related to lowering blood pressure effects phyllokinin seems three times more active than bradykinin; after trypsin digestion phyllokinin is transformed into bradykinin [17]. The contraction of the ileum and colon was also more powerful compared to bradykinin. Its biological effects are related to…

Sauvagine

Sauvagine was first isolated in 1979, and behaves as a corticotropin releasing factor [18]. It consistently mimics the functional consequences of exposure to stress. It has not been found in other frog families, only in those belonging to the Phyllomedusinae, and amounts ranging from a few micrograms to 240 µg per g fresh skin [19]. In animal models, Its biological effects are related to induces intense, long-lasting hypotensive action accompanied by tachycardia, antidiuresis, decreasing GFR and an increasing in tubular Na+ reabsorption [20-22]. Sauvagine binds with two subtypes of CRF receptor (CRFR), CRFR1 and CRFR2. Via its CRFR2 agonism it mediates anxiety [23]. Interestingly, it is more potent than CRF at the CFR2 receptor. Some effects are quite different from CRF, such as the fact that sauvagine produced long-term increases in mesenteric blood flow in a dog model [18]. Furthermore, it decreases body temperature, the latter being a D2 dopamine receptor-mediated mechanism.

Analgesic neuropeptides

A number of neuropeptides in the skin secretion of the frog have been identified, and characterized in animal models, as well as some in the clinic. Comparable analgesic peptides have been found in frogs of different families. We will present 3 peptides, present in Kambo: Caerulein, Dermophin and Deltorphin. We will also discuss in some detail the value of a new peptide, belonging to the secretion of *Bombina variegate*, Bv8, as an additional example how such bioactive peptides can help us finding new leads in the field of analgesics.

Caerulein

Caerulein, also known under the name ceruletide, is a decapeptide, stimulating smooth muscle, and increasing digestive secretions from stomach, gall bladder and pancreas [24]. The compound is a Cholecystokinin (CCK) analogue, has been introduced in the clinic as a diagnostic tool for gallbladder dyskinesia and pancreas insufficiency. It is used in pharmacological experiments to induce pancreatitis [19]. Initial studies focused on indications such as cognition and tardive dyskinesia [25,26]. In the late 80s its analgesic effects were documented. In a pilot placebo-controlled study the compound could reduce colic pain and in a follow-up study it could reduce the intensity of experimental pain [27,28].

In 1988 German investigators published effects of the Intramuscular (IM) administration of 5 microgram cearulein and compared it to 10 microgram morphine [29]. Although the results were promising, the findings did not lead to follow up studies. In the German study caerulein and morphine were compared in 36 patients suffering from cancer pain. Patients included had a VAS pain score of at least 50, and did not receive any analgesics for at least 6 hours before the experimental treatment. Both drugs were comparable

analgesic. Within 30 minutes after injection pain reduction started, and analgesia lasted between 4-5 hours. However, much more patients treated with morphine reported side effects: only 11% of all patients treated with caerulein suffered from side effects, compared to 56% of patients on morphine. A further study into its analgesic effects documented its effect on a number of specific pain qualities [30]. Taking these data together, four clinical studies supported its analgesic effects, but since the last century no further explorations are known in this field.

Dermorphin

Dermorphin is a heptapeptide. In preclinical models after intracerebroventricular application its analgesic activity compared to morphine was longer and it was more potent [31]. Dermorphin appeared also to be more than 1000 times more potent that morphine when given intrathecally in the rat, and led to a dose-dependent increase in analgesia in the picomolar range [32]. The compound is a selective agonist at Mu-Opioid Receptor (MOR), without any relevant affinity for the Kappa-Opioid Receptor (KOR), suggesting potentially a more favorable adverse event profile compared to morphine [33]. Preclinical data also indicated that dermorphin, in a broad concentration-range when intrathecally administered, might lead to clinical relevant effects, as increasing the dose did not lead to decreased analgesic effects as seen in morphine [34].

In 1985 the results of a double-blind RCT in 150 patients was published, evaluating the effects of intrathecal administered dermorphin 20 microgram versus 500 microgram morphine versus a control arm, pentazocine IM plus puncture of the skin in the lumbar region, in postoperative analgesia [35]. All patients received prophylactic domperidone (10 mg IM). Primary endpoint was a visual analogue scale of 10 cm, range from 'no pain' to maximum pain'. Pain was scored every 4th hour during 5 days after the operation. On all moments after the first measurement at 4 hours, dermorphin was significantly superior to morphine, and both were significantly better than the control arm. During the 5-day post-operative period 88% of the control patients, 58% of the morphine patients and only 22% of the dermorphin patients required additional analgesics. The mean analgesic consumption for the patients in the control arm was nearly 60 mg of pentazocine, 40 mg in the morphine group and only 9 mg in the dermorphin group. The mean duration of postoperative analgesia, defined as the interval between administration of the study drug and patient's request for additional analgesics, was significantly longer in the dermorphin group when compared to both the morphine and the placebo group. The mean postoperative hospital stay in the dermorphin group was significantly shorter than in the control group. The authors concluded that a single intrathecal administration of dermorphin adequately relieved pain in 4 out of 5 patients for the entire 5-day postoperative period, and dermorphin thus has a very potent and long lasting analgesic activity which compared favorably to intrathecal morphine.

Deltorphin

Deltorphin is a selectve DOR agonist, an heptapeptide [36]. Analgesia of deltorphin or its derivates can be blocked by selective DOR receptor antagonist such as naltrindole, indicating that the analgesic is indeed mediated by the δ-opioid receptor [37]. DOR analgesia seems to be different from analgesia induced by MOR receptors as it does not dependent on induced microglial activation [38]. Deltorphin has never been evaluated in a clinical study for its analgesic effects.

***Bombina variegate* and the prokineticin system**

More recently peptides involved in nociception are identified in other frog species, such as the Bombina variegata protein 8 kDa (Bv8), a peptide secreted by the skin of the frog *Bombina variegate* [39]. Mollay (1999) Initially described the peptide for its ability to induce gastrointestinal motility in rodents, and soon two mammal homologs of Bv8 were found: the prokineticin 1 (PK1 or endocrine gland derived vascular endothelial growth factor, EG-VEGF) and PK2 (mammalian Bv8) [40]. All members of the PK family weigh approximately 8 kDa and have a structural conserved motif characterized by an N-terminal AVITGA sequence, a Trp residue in position 24 and the presence of five disulfide bridges. These peptides activate two closely related G-protein coupled receptors: prokineticin receptor 1 and 2 (PKR1 and PKR2) that belong to the family of neuropeptide Y receptor and have an amino acid identity of 85% [41]. In contrast to the other peptides described above, this family of peptides has been described as pronociceptive and hyperalgesic andthe possibility to identify its role as a druggable target for paintherapy has been actively pursued in

the last years [42,43]. Bv8/prokineticins have been involved in many biological activities and coordinate complex behaviors like feeding, drinking, circadian rhythm, neurogenesis, angiogenesis and haematopoiesis. Moreover, the presences of PK members (PK and PKRs) in immune cells and in the main stations involved in pain transmission suggest PK as important players in inflammation and pain pathophysiology. The first evidence of their hyperalgesic effects came from the observation that systemic administration of the amphibian Bv8 protein induced a characteristic biphasic hyperalgesia to tactile and thermal stimuli in rats [40]. The initial phase of hyperalgesia is caused by a local action on nociceptors; the secondary phase of hyperalgesia is attributable to a central action.

The amphibian protein Bv8 was a useful research tool to characterize prokineticin pharmacology [43]. It induces hyperalgesia by activating the PKRs that are expressed in regions of the nervous system associated with pain. At peripheral level PKR1 is mainly expressed on small nociceptors together with the transient potential receptor vanilloid 1 (TRPV1), while PKR2 is expressed on medium/large sized neurons expressing TRPA1 besides TRPV1. These co-localizations provide the anatomical basis for a cooperative interaction in nociceptor sensitization through activation of PKCe. Regarding neuropathic pain, the involvement of prokineticins was investigated in different experimental animal models of neuropathic pain: derived from an injury of sciatic nerve (chronic constriction injury model (CCI), spared nerve injury), diabetesand cancer-induced.In all these models, the presence of aberrant pain correlates well with an increase of the levels of PK2 in the spinal cord, especially in activated astrocytes. The treatment with a PK system antagonist like PC1 or with PK neutralizing antibody was able to counteract thermal hyperalgesia and allodynia, reduce the injury-induced overexpression of PK2 and restore the physiological levels of proinflammatory and anti-inflammatory cytokines both in periphery and spinal cord [44]. The Bv8/prokineticin story is an example how frog derived bioactive peptides can help us finding new leads in the field of analgesics.

OTHER PEPTIDES IN KAMBO

In order to complete this review, we will briefly discuss some other peptides from Kambo, as these are also contributing to its pharmacological effects, often disguised for people as toxic effects.

Adrenoregulin has the interesting properties that it enhances binding of ligands to certain receptors, such as the Al-adenosine receptor (60%), the alpha-2-adrenergic receptor and the 5HT1a-receptor (around 25%). This might thus contribute and enhance indirectly the adrenergic effects of other bioactive peptides in Kambo, and even enhance the analgesic effects of the opioid-ligands such as dermorphin [45].

Some of the non-main peptides, such as Dermaseptins most probably do not contribute to the direct symptomatology of Kambo. However, these peptides have interesting antimicrobial activity, while being devoid of lytic effect on mammalian cells [46,47]. Dermaseptin inhibits cell proliferation in a dose-dependent manner in certain tumor lines, such as cell prostate carcinomas, albeit in a micromolar range [47].

Urechistachykinin II is an exotic decapetide, found in worms, where this peptide induces contractile actions on the inner circular body-wall muscle of the worm. It was not previously described in Kambo, but was recently described as a result of the analysis of Kambo used during a ritual [5,48].

Then there are a number of tachykinins, such as preprotachykinin B and ranatachykinin A, the latter a tachykinin with potent stimulant effects on smooth muscle [49]. Phyllolitorin and related Bombesins are also vasoactive, but give rise to slight increases in blood pressure in some animals, while in others decreases blood pressure, for instance monkeys [50]. T-kinin is a undecapeptide, and is also a bradykinin derivate, (isoleucyl-seryl-bradykinin [51].

CONCLUSION

Frogs skin secretions are rich sources of bioactive peptides, and some of these peptides play a role in the development of future treatments for chronic pain. Kambo is increasingly used in the West as a shamanistic 'healing' ritual, and many anecdotal cases can be found in the net, claiming reduction of chronic pain and improvement of symptoms in a great many disorders. The administration of Kambo results in a symptom-complex resembling a transient anaphylactic shock. However, it seems that this symptom-complex is not caused by an overreaction of the immune system to an allergen, but it is a pharmacological effect of a number of bioactive

neuropeptides, which most probably act synergistically. In Kambo there are many peptides, which can have important contributions in the clinic, such as the analgesic effects of dermorphin or cerulein and the antibiotic effects is some other bioactive peptides. Bv8 is a small protein, and seems to act via the prokineticin system. All these polypeptides may play a role in the search for new analgesics, especially in the treatment of neuropathic pain and cancer pain.

REFERENCES

1. Finnerup NB, Attal N, Haroutounian S, McNicol E, Baron R, et al. (2015). Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. The Lancet Neurology. 14: 162-173.
2. Keppel Hesselink JM, Schatman M. (2018). Rediscovery of old drugs: the forgotten case of dermorphin for postoperative pain and palliation. J Pain Res. 11: 2991-2995.
3. Keppel Hesselink JM. (2018). Kambo: A Shamanic Medicine - Personal Testimonies. JOJ Case Stud. 8: 555739.
4. Barbosa EA, Oliveira A, Placido A, Socodato R, Portugal CC, et al. (2018). Structure and function of a novel antioxidant peptide from the skin of tropical frogs. Free Radic Biol Med. 115: 68-79.
5. de Morais DR, Lanaro R, Barbosa IL, Santos JM, Cunha KF, et al. (2018). Ayahuasca and Kambo intoxication after alternative natural therapy for depression, confirmed by mass spectrometry. Forensic Toxicology. 36: 212-221.
6. Daly JW, Caceres J, Moni RW, Gusovsky F, Moos M, et al. (1992). Frog secretions and hunting magic in the upper Amazon: identification of a peptide that interacts with an adenosine receptor. Proceedings of the National Academy of Sciences. 89: 10960-10963.
7. Erspamer V, Anastasi A. (1962). Structure and pharmacological actions of eledoisin, the active endecapeptide of the posterior salivary glands of Eledone. Experientia. 18: 58-59.
8. Cei JM. (1963). Some precipitin tests and preliminary remarks on the systematic relationships of four South American families of frogs. Serol Mus Bull. 30: 4-6.
9. Cei JM, Erspamer V. (1966). Biochemical Taxonomy of South American Amphibians by Means of Skin Amines and Polypeptides. Copeia. 1: 74-78.
10. Arcamone, F. M. (2009). Fifty years of chemical research at farmitalia. Chemistry–A European Journal. 15: 7774-7791.
11. Erspamer V, Anastasi A, Bertaccini G, Cei JM. (1964). Structure and pharmacological actions of physalaemin, the main active polypeptide of the skin of Physalaemusfuscumaculatus. Experientia. 20:489-490.
12. Erdös EG. (1966). Hypotensive peptides: bradykinin, kallidin, and eledoisin. Advances in pharmacology. 4: 1-90.
13. Falconieri GE, Anastasi A, Cei JM. (1970). Pharmacological observations on phyllomedusin. Journal of Pharmacy and Pharmacology. 22: 466-467.
14. Ganjiwale AD, Cowsik SM. (2009). Three-dimensional structure of Phyllomedusin, a NK1 receptor agonist bound to dodecylphosphocholine micelles. Journal of structural biology. 167: 176-184.
15. FalconieriErspamer G, Mazzanti G, Yasuhara T, Nakajima T. (1984). Parallel bioassay of physalaemin and hylambatin on smooth muscle preparations and blood pressure. J. Pharm. Pharmacol. 36: 284-286.
16. Bernardi L, Bosisio G, De Castiglione R, Goffredo O. (1966). Synthesis of phyllokinin, a natural bradykinin analogue. Experientia. 22: 425-427.
17. Anastasi A, Bertaccini G, Erspamer V. (1966). Pharmacological data on phyllokinin (bradykinyl-isoleucyl-tyrosine o-sulphate) and bradykinyl-isoleucyl-tyrosine. British journal of pharmacology and chemotherapy. 27: 479-485.
18. Lovejoy DA, Balment R J. (1999). Evolution and physiology of the corticotropin-releasing factor (CRF) family of neuropeptides in vertebrates. General and comparative endocrinology. 11: 1-22.
19. Gullo L. (1988). Maximal effective dose of cerulein in the secretin-cerulein test. Digestion. 39: 225-229.
20. Montecucchi PC, Henschen A, Erspamer V. (1979). Structure of sauvagine, a vasoactive peptide from the skin of a frog. Hoppe-Seyler's Z. Physiol. Chem. 360: 1178.

21. Erspamer V, Erspamer GF, Improta G, Negri L, Castiglione R. Sauvagine, a new polypeptide from Phyllomedusasauvagei skin. Naunyn-Schmiedeberg's archives of pharmacology. 12: 265-270.
22. Heinrichs SC, Taché Y. (2001). Therapeutic potential of CRF receptor antagonists: a gut-brain perspective. Expert Opin Investig Drugs. 10: 647-659.
23. Eckart K, RadulovicJ, Radulovic M, Jahn O, Blank T. et al. (1999). Actions of CRF and its analogs. Curr Med Chem. 6: 1035-1053.
24. Anastasi A, Erspamer V, Endean R. (1967). Isolation and structure of caerulein, an active decapeptide from the skin of Hylacaerulea. Experientia. 23: 699-700.
25. Dodt C, Sarnighausen HE, Pietrowsky R, Fehm HL, Born J. (1996). Ceruletide improves event-related potential indicators of cognitive processing in young but not in elderly humans. J Clin Psychopharmacol. 16: 440-445.
26. Kojima T, Yamauchi T, Miyasaka M, Koshino Y, Nakane Y, et al. (1992). Treatment of tardive dyskinesia with ceruletide: a double-blind, placebo-controlled study. Psychiatry Res. 42: 129-136.
27. Basso N, Bagarani M, Materia A, Gizzonio D, De Paolis C, et al. (1985). Effect of caerulein in patients with biliary colic pain. Gastroenterology. 89: 605-609.
28. Stacher G, Steinringer H, Schmierer G, Schneider C, Winklehner S, et al. (1984). Ceruletide increases dose dependently both jejunal motor activity and threshold and tolerance to experimentally induced pain in healthy man. Gut. 25: 513-519.
29. Meyer-Lindau F, Pfister E, Gyr N, Obrecht JP. (1988). Randomized double-blind study of the analgesic effect of caerulein and morphine in chronic tumor pain. Onkologie. 11: 77-80.
30. Pause BM, Drews C, Scherhag C, Pohl J, Pietrowsky R, et al. (1996). Analgesic effect of ceruletide in men is limited to specific pain qualities. Physiol Behav. 59: 1025-1031.
31. Broccardo M, Erspamer V, Falconieri Erspamer G, Improta G, Linari G, et al. (1981). Pharmacological data on dermorphins, a new class of potent opioid peptides from amphibian skin. Br J Pharmaco. l73: 625-631.
32. Westphal M, Harmnonds RG, Li CH. (1985). Binding characteristics of dermorphin, and dermorphin 1-7-beta-endorphine in rat brain membranes. Peptides. 6: 149-152.
33. Stevens CW, Yaksh TL. (1986). Spinal action of dermorphin, an extremely potent opioid peptide from frog skin. Brain Res. 385: 300-304.
34. Puglisi-Allegra S, Castellano C, Filibeck U, Oliverio A. Melchiorri P. (1982). Behavioral data on dermorphins in mice, Eur J Pharmacol. 82: 223-227.
35. Basso N, Marcelli M, Ginaldi A, De Marco M. (1985). Intrathecal dermorphine in postoperative analgesia. Peptides. 3: 177-179.
36. Kreil G, Barra D, Simmaco M, Erspamer V, Erspamer GF, et al. (1989). Deltorphin, a novel amphibian skin peptide with high selectivity and affinity for delta opioid receptors. Eur J Pharmacol. 162: 123-128.
37. Pescatore R, Marrone GF, Sedberry S, Vinton D, Finkelstein N, et al. (2015). Synthesis and pharmacology of halogenated δ-opioid-selective [d-Ala(2)]deltorphin II peptide analogues. ACS Chem Neurosci. 17: 905-910.
38. Mika J, Popiolek-Barczyk K, Rojewska E, Makuch W, Starowicz K, et al. (2014). Delta-opioid receptor analgesia is independent of microglial activation in a rat model of neuropathic pain. PLoS One. 8: e104420.
39. Lattanzi R, Sacerdote P, Franchi S, Canestrelli M, Miele R, et al. (2012). Pharmacological activity of a Bv8 analogue modified in position 24. Br J Pharmacol. 166: 950-963.
40. Mollay C, Wechselberger C, Mignogna G, Negri L, Melchiorri P, et al. (1999). Bv8, a small protein from frog skin andits homologue from snake venom induce hyperalgesia in rats. EurJ Pharmacol. 374: 189-196.
41. Negri L, Maftei D. (2018). Targeting the prokineticin system to control chronic pain and inflammation. Current medicinal chemistry. 25: 3883-3894.
42. Franchi S, Sacerdote P, Panerai AE. (2017). The prokineticin system: an interface between neural inflammation and pain. Neurol Sci. 38: 27-30.
43. Negri L, Lattanzi R, Giannini E, Metere A, Colucci M, et al. (2002). Nociceptive sensitization by thesecreted protein Bv8. Br J Pharmacol. 137: 1147-1154.
44. Castelli M, Amodeo G, Negri L, Lattanzi R, Maftei D, et al. (2016). Antagonism of the prokineticin system prevents

and reverses allodynia and inflammation in a mouse model of diabetes. PLoS One. 11: e0146259.

45. Moni RW, Romero FS, Daly JW. (1995). The amphiphilic peptide adenoregulin enhances agonist binding to A1-adenosine receptors and [35S] GTP gamma S to brain membranes. Cell Mol Neurobiol. 15: 465-493.
46. Mor A, Amiche M, Nicolas P. (1994). Structure, synthesis, and activity of dermaseptin b, a novel vertebrate defensive peptide from frog skin: relationship with adenoregulin. Biochemistry. 21: 6642-6650.
47. Dos Santos C, Hamadat S, Le Saux K, Newton C, Mazouni M, et al. (2017). Studies of the antitumor mechanism of action of dermaseptin B2, a multifunctional cationic antimicrobial peptide, reveal a partial implication of cell surface glycosaminoglycans. PLoSOne. 12: e0182926.
48. Ikeda T, Minakata H, Nomoto K, Kubota I, Muneoka Y. (1993). Two novel tachykinin-related neuropeptides in the echiuroid worm, Urechisunicinctus. Biochemical and biophysical research communications. 192: 1-6.
49. Kozawa H, Hino J, Minamino N, Kangawa, K, Matsuo H. (1991). Isolation of four novel tachykinins from frog (Rana catesbeiana) brain and intestine. Biochemical and biophysical research communications. 177: 588-595.
50. Erspamer V, Melchiorri P, Sopranzi N. (1972). The action of bombesin on the systemic arterial blood pressure of some experimental animals. British journal of pharmacology. 45: 442-450.
51. Okamoto H, Greenbaum LM. (1983). Isolation and structure of T-kinin. Biochemical and biophysical research communications. 112: 701-708.