Invest Clin 47(2): 133 - 141, 2006

Cardiovascular excitatory effect on rats of a fraction isolated from the eyestalk of shrimp: *Peneaus vanameii*.

Francisco Rosa[1], Jesús Cedeño[1], Luis León[2], Omar Estrada[3], Eduardo Romero-Vecchione[3], José Vásquez[3] y Rafael Antequera[3].

[1]Departamento de Ciencias Fisiológicas, Escuela de Ciencias de la Salud, Núcleo Bolívar, [2]Escuela de Ciencias Aplicadas del Mar, Núcleo Nueva Esparta. Universidad de Oriente y [3]Laboratorio de Estudios Cardiovasculares, Escuela de Medicina "José María Vargas", Universidad Central de Venezuela. Caracas, Venezuela. E-mail: frankrosa@cantv.net y frankrosa_69 @hotmail.com y garciel@ucv.ve.

Key words: *Peneaus vanameii*, peptide fraction, blood pressure, heart rate.

Abstract. The crustacean nervous system is an important source of substances with diverse biological activities, particularly affecting invertebrate cardiocirculatory physiology. However, the effects of these substances on the cardiovascular system of higher vertebrates are not very well documented. The purpose of this study was to evaluate the effects of a cardioexcitatory substance (CES) isolated from the eyestalk of the shrimp *Peneaus vanameii* on rat cardiovascular function. The administration of a purified fraction of this substance raised mean arterial pressure by 37.33 ± 5.00 mm Hg, pulse pressure 35.00 ± 4.93 mm Hg and heart rate 80.00 ± 12.83 beats/min over basal values ($p < 0.01$). Evaluation of the possible underlying mechanisms of this hypertensive and tachycardic effect reveled that dihydroergotamine pretreatment (20 μg/0.2 mL) reduced the effect of CES on mean blood pressure, but not on heart rate. Propranolol pretreatment (4 μg/0.2 mL) reduced the tachycardia, but not the hypertensive response. Enalapril pretreatment (5 μg/0.2 mL) did not modify the effects induced by CES on heart rate or blood pressure, and the verapamil pretreatment (1 μg/0.2 mL) reduced both cardiovascular changes by 85% ($p < 0.01$). These results indicate that CES isolated from the shrimp eyestalk produces hypertension and tachycardia mediated by adrenergic receptors in association to calcium channels activation.

Autor de correspondencia: Francisco Javier Rosa Alemán. Urbanización Santa Mónica, Avenida Francisco Pimentel, Quinta Cachita. Caracas, Venezuela. Teléfonos (0212) 6615353) (Hab.) (0212) 6053524 6053715 (Ofic.) Fax (0212) 6053265. E-mail: frankrosa@cantv.net y frankrosa_69 @hotmail.com y garciel@ucv.ve.

Efecto cardiovascular excitatorio en la rata, de una fracción aislada del tallo óptico del camarón: *Peneaus vanameii*.
Invest Clín 2006: 47(2): 133 - 141

Palabras clave: *Peneaus vanameii*; fracción peptídica, presión arterial, frecuencia cardiaca.

Resumen. El sistema nervioso de crustáceos es una fuente importante de sustancias con actividad biológica diversa, particularmente la que afecta la fisiología cardiocirculatoria de los invertebrados. Sin embargo, los efectos de estas sustancias sobre el sistema cardiovascular de mamíferos no están bien documentados. El objetivo de este estudio, fue evaluar los efectos de una sustancia cardioexcitatoria (SCE) aislada del tallo óptico del camarón *Peneaus vanameii* sobre la función cardiovascular de la rata. La administración de una fracción purificada de esta sustancia incrementó la presión arterial media en 37,33 ± 5,0 mm de Hg, la presión arterial diferencial en 35,00 ± 4,93 mm de Hg, así como la frecuencia cardiaca 80,00 ± 12,83 lat/min sobre los valores basales ($p < 0,01$). La evaluación del mecanismo por el cual este efecto hipertensor y taquicardizante se produjo indicó que el tratamiento con dihidroergotamina (20 μg/0,2 mL) redujo los efectos del SCE sobre la presión arterial media, pero no sobre la frecuencia cardiaca. El pretratamiento con propranolol (4 μg/0,2 mL) redujo la taquicardia pero no la respuesta hipertensiva. El pretratamiento con enalapril (5 μg/0,2 mL) no modificó los efectos inducidos por SCE sobre el corazón o los vasos sanguíneos, el pretratamiento con verapamil (1 μg/0,2 mL) redujo ambos cambios cardiovasculares en un 85% ($p < 0,01$). Estos resultados indican que el SCE aislado del tallo óptico del camarón produce hipertensión y taquicardia mediada a través de receptores adrenérgicos, en asociación con una activación de los canales de calcio.

Recceived: 31-03-2005. Accepted: 12-01-2006.

INTRODUCTION

The crustacean nervous system is an important source of neurohormones, neurotransmitters and neuromodulators with diverse biological activities, exhibiting important effects on the cardiocirculatory physiology of both vertebrates and invertebrates. The neuronal groups and neurosecretory cells responsive to these substances have been identified in vertebrates and invertebrates by autoradiographic, immunocytochemical and histochemical techniques, which have allowed to establish their biochemical homology in different animal species (1-7).

Extensive research on crustacean neuropeptides confirmed the physiological role of these substances; however their effects as neurohormones or neurotransmitters in vertebrate and invertebrate species raised the possibility of some phylogenetic relationship between related species, and their role in neuroendocrine integration (8-14).

Among the peptides identified in invertebrates, particularly in crustaceans, are worthy of mention: the hyperglycemic hor-

mone, some peptides associated to circadian rhythm control, several hormones related to water-mineral balance, and a group of substances with important stimulating actions on the cardiovascular system (15-21).

These cardioexcitatory substances exert important effects on the heart function of several animal species, particularly related to environmental adaptation and the circadian rhythm. Preliminary studies carried out with substances isolated from mollusks and crustaceans suggest their coexistence with other biogenic amines such as catecholamines, serotonin and octopamin, in the same nerve terminal. Biochemical studies and purification techniques allowed the isolation and characterization of different peptidic compounds with specific biological activities, such as the peptide FMRFamide, which has the property of stimulating cardiac activity in mollusks and crustaceans (22-24). Furthermore, an additional group of low molecular weight peptides were identified in crustaceans, exhibiting remarkable chemical homology, and physiological effects similar to the ones found in mammalian neuropeptides. Autoradiographic and immunocytochemical studies have shown the presence of specific receptors for these peptides in the rat dorsal root and primary afferent fibers of the spinal cord. The presence of these peptides was also established in autonomic fibers innervating cardiac muscle, pericardium and ganglionic tissue in crustaceans, gastropods and higher vertebrates (25-32).

Experimental evidence indicates that these neuropeptide families are associated to cardiocirculatory control in invertebrates; and their chemical resemblance to the ones present in vertebrate cardiac and neural tissues suggests that they represent an evolutionary step in vertebrate cardiovascular regulation. Because the cardiovascular activity of neuropeptides present in the eyestalk of the shrimp *Peneaus vanameii* has not been explored, the objective of this study was to evaluate the cardiovascular activity of a purified peptide fraction from the eyestalk of *Peneaus vanameii* on blood pressure and heart rate of anesthetized rats.

MATERIALS AND METHODS

Penaeus vanameii shrimps (n = 1900) 100 g average weight were provided by Siembramar, C.A., Barcelona, Anzoátegui State, Venezuela.

Cardiovascular tests were performed on 60 male Sprague-Dawley rats, 300 g average weight, from the Venezuelan Institute of Scientific Research (IVIC). These animals were kept in the animal room of the Health Sciences School of the Universidad de Oriente Venezuela, maintained with *ad libitum* access to water and food pellets (Ratarina®, Purina C.A.).

Preparation and fractionation of crude eyestalk fraction

Out from 1900 crude shrimps, 300 g of crustacean eyestalks were obtained by a cut at the point of attachment to the shrimp cephalothorax. The eyestalks were maintained in reagent grade acetone at 12°C and later homogenized in a Sorvall® homogenizer at 3500 rpm for 15 min; afterwards, acetone was evaporated at room temperature. The resulting 300 mg powder was treated with chloroform under the same condition as above, and dried. The eyestalk powder obtained was then extracted with 400 mL of distilled water. The yellow supernatant was decanted after centrifuging at 4°C at 12000 rpm for 10 min, and dried under reduced pressure at 40°C, yielding a 180 mg residue and was termed "crude extract", which was then fractionated on Sephadex G-25 (Pharmacia Fine Chemicals) column, using methanol as eluent to give five fractions termed I-V.

Bioassay with the purified fractions

Bioassays on motor neurons from the third abdominal ganglion of *Penaeus vanameii* were performed to each of the five obtained fractions, in order to establish its biological activity, according to methods published elsewhere which briefly, consists of recording the spontaneous activity of the superficial branch oh the third root of this ganglion with a suction electrode. The measurement of firing rate corresponds to the activity of motoneuron f-5) (32). The highest activity level was observed in fraction V. All cardiovascular assays, made on rats, were performed with this particular fraction. The purified fraction V corresponds to extracted peptidic compound (CES) according to already published procedures carried out in samples of invertebrate nervous tissues The percentage of recovery at the end of this procedure was (w/w = 0.06%) (1, 2, 11, 34).

Evaluation of Fraction V Cardioexcitatory substance (CES) on blood pressure and heart rate

In order to test the cardiovascular activity of CES, 50 rats were anesthetized with sodium pentobarbital (35 mg/Kg i.p.) and its femoral vein catheterized with an Intramedic® PE 50 polyethylene catheter; this venous line was used to administer CES or its vehicle; the femoral artery was catheterized, for blood pressure recording with a Statham® Strain-Gauge transducer, connected to a Grass® 7E Polygraph via a $7DA_1$ preamplifier. Heart rate was monitored from the electrocardiography recordings obtained from four subcutaneous platinum electrodes placed on the animal's limbs, and connected to the Polygraph EKG leads I and II preamplifier.

Bolues of 0.2 mL intravenous injections containing increasing doses of CES, with a range of 10-800 μg were administered to rats in order to establish the dose evoking the maximal response. Blood pressure and heart rate were continuously recorded; the maximal effect was achieved at the 800 μg dose. This dose was used in all subsequent experiments before and after pharmacological blockade. The control group consisted of 10 rats intravenously injected with a 0.2 mL bolus of 0.9% NaCl, under the same experimental conditions. Mean arterial pressure was calculated as diastolic blood pressure + 1/3 [systolic (SBP)-diastolic blood pressure (DBP)].

Evaluation of pharmacological blockade on CES cardiovascular responses

In order to establish the role of α-adrenergic receptors on the response, a group of 10 rats received 20 μg/0.2 mL dose of intravenous dihydroergotamine. β-adrenoceptor cardiovascular stimulation was evaluated through 4 μg/0.2 mL propranolol pretreatment. Pretreatment with enalapril (5 μg/0.2 mL) and verapamil (1 μg/0.2 mL) was also evaluated to rule out the role of the renin-angiotensin system and calcium channels, respectively, on CES cardiovascular induced changes. In all cases, after 15 minutes of drug pretreatment, 800 μg of CES was injected, with continuous blood pressure and heart rate recordings.

Statistical analysis

All results are expressed as mean ± standard error of the mean. Statistical significance was tested by non-paired t tests for intergroup mean comparisons. All other comparisons were made with two-way ANOVA. Statistical significance was considered when $p < 0.05$. Data was computed with SPSS program version 11.0.

RESULTS

Intravenous administration of 800μg of CES increased mean arterial pressure by

37.33 ± 5.00 mmHg (41%), and pulse pressure (SBP minus DBP) by 35.00 ± 4.93 mmHg (113%). Values were significantly higher than the ones observed after vehicle injection. $F(19) = 38.30$, $p < 0.0001$ for mean arterial pressure, $F(13) = 23.46$, $p = 0.0004$ for pulse pressure (Figs. 1 and 2).

CES increased heart rate by 80.00 ± 12.83 beats/min, representing an increase of 27. 46% over basal values $F(19) = 21.97$, $p = 0.0002$ (Fig. 3). Effects on blood pressure began approximately 10 seconds after CES injection and lasted for 15-20 minutes, after which all parameters returned to their original levels. The injection of 0.2 mL vehicle did not change heart rate.

Effect of pharmacological pretreatment on CES effect

Dihydroergotamine (DHE) pretreatment produced a mild 10.0 mm Hg initial reduction of both SBP and DBP basal blood pressure. After injection of DHE, there was a 26.13 ± 1.26 mmHg reduction of control response induced by CES on blood pressure (Fig. 4). However, DHE did not significantly modify the CES- induced tachycardia by CES. $F(14) = 40.52$, $p < 0.0001$ (Fig. 5).

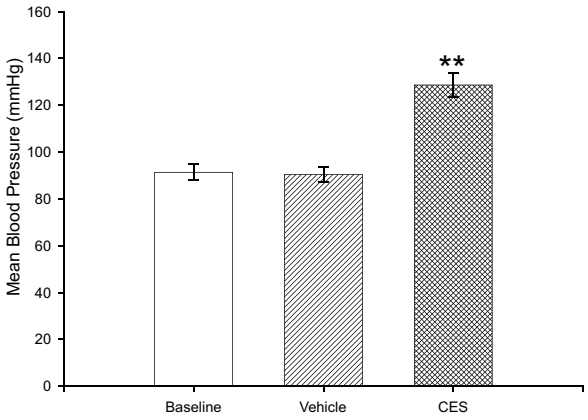

Fig. 1. Effect of eyestalk CES on Mean Blood Pressure. Bars represent the mean of 10 experiments and vertical lines the standard error of the mean. ** = $p < 0.01$ *vs* Vehicle.

Intravenous pretreatment with propranolol produced a 60 beats/min (–17%) heart rate decrease below baseline, which was considered evidence of β-adrenergic blockade. Propranolol did not significantly reduce the changes in blood pressure induced by CES ($p > 0.05$) (Fig. 4). However, propranolol reduced the CES induced tachycardia by 46.25 ± 3.39 beats/min (–66%) $F(13) = 79.89$, $p < 0.0001$ (Fig. 5).

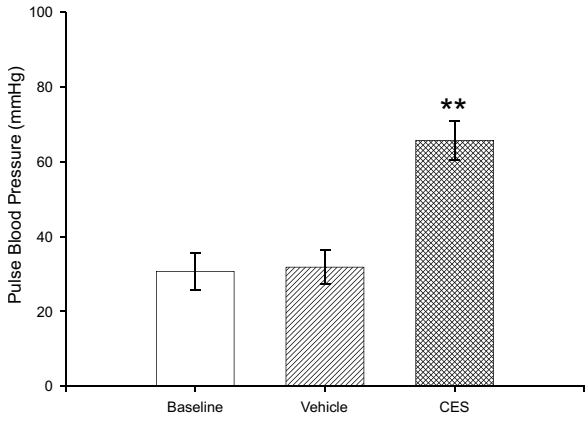

Fig. 2. Effect of eyestalk CES on pulse blood pressure. Bars represent the mean of 10 experiments and vertical lines the standard error of the mean. ** = $p < 0.01$ *vs* Vehicle.

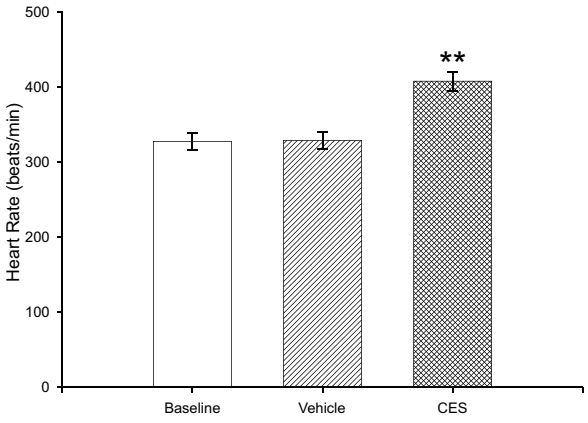

Fig. 3. Effect of the eyestalk CES on heart rate. Bars represent the mean of 10 experiments and vertical lines the standard error of the mean. ** = $p < 0.01$ *vs* Vehicle.

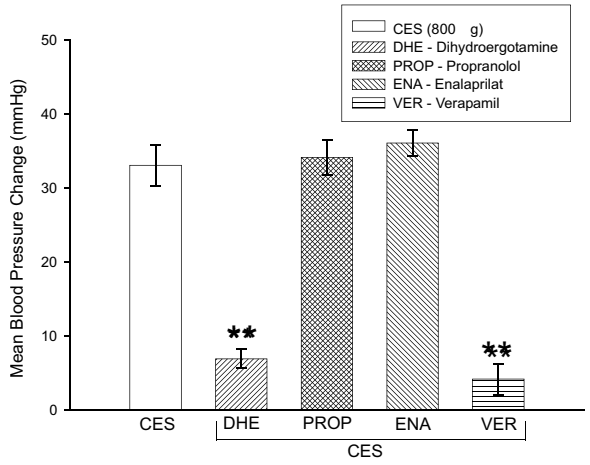

Fig. 4. Mean blood pressure change after 800 μg eyestalk CES. Influence of pretreatment (15 min) with dihydroergotamine, propranolol, enalaprilat and verapamil. Bars represent the mean of 10 experiments with each drug and vertical lines the standard error of the mean. ** = $p < 0.01$ *vs* Vehicle.

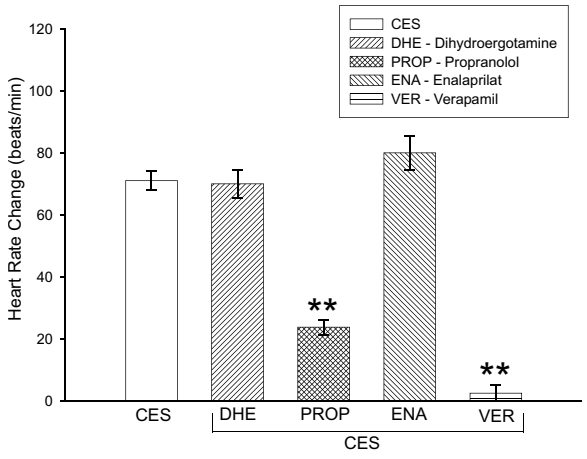

Fig.5. Heart rate change after 800 μg eyestalk CES. Influence of pretreatment (15 min) with dihydroergotamine, propranolol, enalaprilat and verapamil. Bars represent the mean of 10 experiments and vertical lines the standard error of the mean. ** = $p < 0.01$ *vs* Vehicle.

Enalapril pretreatment did not modify the cardiovascular changes induced by CES on blood pressure or heart rate ($p > 0.05$) (Figs. 4 and 5).

Verapamil significantly reduced the cardiovascular responses to CES in both mean blood pressure and heart rate by 28.7 ± 2.10 mmHg and 68.5 ± 2.50 beats/min, respectively, this verapamil blockade was highly significant $F(13) = 38.02$, $p < 0.0001$) (Figs. 4 and 5).

DISCUSSION

Experimental evidence indicates that neuropeptides of the crustacean nervous system, including the eyestalk, have biological activity on many organs from diverse animal species; as it was demonstrated on neural and cardiovascular tissues from invertebrates and vertebrates (1, 10, 11, 29, 36). On the other hand biochemical experiments indicate that some substances isolated from crustaceans nervous system show a structural homology with other cardioexcitatory peptides obtained from mollusks, gastropods and insects; and the presence of both, neural circuits and specific receptors for these peptides have also been found in mammalian cardiovascular tissues (2, 37, 38).

The employed extraction procedure eliminated proteins, low molecular weight substances and lipids: allowing isolation of peptides into the CES. Intravenous administration of CES in all our experimental animals produced a significant increase in systolic and diastolic blood pressure, with a concomitant increase in heart rate, which indicates an important stimulation of the rat cardiovascular system. This effect had an average duration of 20 minutes, which means there is some deactivating mechanism which could be operating at plasma such as peptidases, kidney excretion or liver biotransformation into inactive compounds.

There is no previous experimental report about the effect of cardiovascular excitatory shrimp neural extracted CES on the rat cardiovascular system of the effect of these neuropeptides enzimatic deactivationat cardiovascular activity.ood pres. Previous *in vitro* studies performed in our laboratory showed CES contains peptides having a positive inotropic and chronotropic effects on isolated rat atria and significant vasopressor effects on isolated thoracic aortic rings, which means CES contains substances having a stimulatory action on these organs.

Our results coincide with other researcher's findings that have demonstrated a cardioaccelerating effect on mollusks and snails hearts induced by cardioactive neuropeptides obtained from crustacean and insect nervous systems, and biochemical studies have suggested some degree of structural homology between them (36-38).

Dihydroergotamine produced an important reduction of the hypertensive response to CES, injection but did not change the tachycardia induced by assayed CES. These findings suggest a role of vasopressor α-adrenergic receptors in the hypertensive response induced by CES. However, this experiment does not ruled out some facilitatory effect of CES on noradrenaline release from sympathetic nerve terminals. Dihydroergotamine is not a selective $\acute{\alpha}$ adrenergic blocker, it can antagonize serotonin at $5HT_{1A,}$ $5HT_{2B}$ and $5HT_{2C}$ receptors; so, we can not disregard a serotonin agonist effect of CES. Previous administration of propranolol significantly reduced the tachycardia induced by CES without any modification on blood pressure, suggesting the β adrenergic receptors mediate this tachycardic response. The effects of CES are similar to those produced by catecholamines such as adrenaline but the presence of this catecholamine can be ruled out because it is not found in the eyestalk of *Peneaus vanameii.*

Pretreatment with enalapril did not modify CES-induced cardiovascular changes, suggesting that angiotensin II synthesis does not play a role in the cardiovascular response produced by this neural fraction.

Previous administration of verapamil produced a highly significant decrease of both the hypertensive and tachycardic response to CES, indicating an important role of calcium channels in the cardiovascular response to this substance. These findings have not been previously reported, but are consistent with former results obtained; showing "in vitro" that verapamil significantly reduces the effects of CES on isolated atrial contraction strength and aortic contractile response. There is a report in invertebrates showing that exposure of heart cells of *Lymnaea* snail to these cardioactive peptides changes the opening and closing kinetics of calcium channels (6).

In conclusion, CES extracted from shrimp eyestalk Peneaus *vanameii* contains a cardioaccelerating and pressor substance of peptidic nature, and whose effects were antagonized by verapamil and dihydroergotamine, suggesting an $\acute{\alpha}$-adrenergic, calcium-ediated action; without any involvement of angiotensin II receptors because enalapril did not introduce any change on its actions.

ACKNOWLEDGEMENTS

This work was supported by Grant C-I 2-0430932/00 from Consejo de Investigación de la Universidad de Oriente and from Grant 09-11-5009-2009 from CDCH Universidad Central de Venezuela.

REFERENCES

1. **Kleinholz LH.** Separation and purification of crustacean eyestalk hormones. Am Zool 1966; 6:161-167.

2. **Kleinholz LH.** Crustacean neurosecretory hormones and physiological specificity. Am Zool 1976; 16:151-166.
3. **Dockray GF, Vaillant C, Williams RG.** New vertebrate brain gut peptide related to molluscan neuropeptide and opioid peptide. Nature.1981; 293(22):656-658.
4. **Greenberg MJ, Price DA.** Invertebrate neuropeptides: Native and naturalized. Annu Rev Physiol 1983; 45:271-288.
5. **Arechiga H, Huberman A, Naylor E.** Hormonal modulation of circadian neural activity in *Carcinus maenus*. Proc R Soc Lon B.1974; 187:299-313.
6. **Bucket KJ, Dockray GF, Osborne NN, Benjamin PR.** Pharmacology of the myogenic heart of the pond snail *Lymnaea stagnalis*. J Neurophysiol. 1990; 63:1413- 1424.
7. **Sasaki T, Satake H, Minakata H, Takeda M.** Characterization of crustacean cardioactive peptide as a novel insect midgut factor: isolation, localization and stimulation of alpha- amylase activity and gut contraction. Endocrinology 2005; 145: 5671-5678.
8. **Vehovszky A, Agrícola HJ, Elliot CJ, Ohtami M, Karpati L, Hernacoli L.** Crustacean cardioactive peptides (CCAP) related molluscan peptides (M-CCAPs) are potential extrinsic modulators of the bucal feeding network in the pond snail *Lymnaea stagnalis*. Neurosci Lett 2005; 373:200-205.
9. **Arechiga H, Cabrera-Peralta C, Huberman A.** Functional characterization of the neurodepressing hormone in the crayfish. J Neurobiol 1979; 10(4):409-422.
10. **Morris H, Pánico M, Karplus A, Lloyd PE, Riniker B.** Elucidation by FAB- MS of the structure of a new cardioactive peptide in *Aplysia*. Nature1985; 300(16):643-644.
11. **Wharton J, Gulbenkian S.** Peptides in the mammalian cardiovascular system. Regulatory Peptides (Polak, JM Ed) 1 Ed Birkhauser Verlag-Bassel. Boston pp. 76-95. 1989.
12. **Choung JS, Webster SG.** Expression and release patterns of neuropeptides during embryogenic development and hatching of the green shore crab *Carcinus maena*. Development. 2004; 131:4751-4761.
13. **Clark AC, Del Campo ML, Euver J.** Neuroendocrine control of larval ecdisis behaviour in *Drosophila melanogaster*: complex, regulation by partially redundant neuropeptide. J Neurosci 2004; 24:4283-4292.
14. **Page T, Larimer J.** Neural control of circadian rhythmicity in the crayfish. The locomotor activity rhythm. J Comp Physiol. 1975; 97: 59-89.
15. **Price DA.** Evolution of a molluscan cardioregulatory neuropeptide. Am Zool 1986; 26: 1007-1015.
16. **Trube A, Audehm U, Dircksen H.** Crustacean cardioactive peptide immunorreactive neurons in the ventral nervous system of the crayfish. J.Comp Neurobiol 1994; 34: 80-93.
17. **Weimann J, Skiebe P, Heinzel H, Soto C, Kopell N, Jorge-Rivera J, Marder E.** Modulation of oscillator interactions in the crab stomatogastric ganglion by crustacean cardioactive peptide. J Neurosci 1997; 175(5):1748-1760.
18. **Aguilar C, Peña A, Barreto V.** Efecto de la hormona neurodepresora sobre la actividad de la aurícula aislada de la rata. Saber 1998; 10(2): 44-48.
19. **Huang EY, Li JY,Wang CH, Chen JC.** The cardiovascular effect of PRFFamide and (Tic) Amide a possible agonist and antagonist of neuropeptide FF (NPFF). Peptides 2000; 21:205-210.
20. **Predel R, Herbert Z, Eckert M.** Neuropeptides in perisympathetic organs of *Manduca sexta*: specific composition and changes during development. Peptides 2003; 24:1457-1464.
21. **Walker S, Stell W.** Gonadotropin Releasing hormone (GnRF), molluscan cardioexcitatory peptide (FMRFamide), Enkephalin and related neuropeptides affect goldfish retinal ganglion cell activity. Brain Res. 1986; 384:262-273.
22. **Kivipelto L, Majane EA, Yang HY, Panula P.** Immunohistochemical distribution and partial characterization of FLFQPQR-Famide like peptides in the central nervous system of rats. J Comp Neurol 1989; 286:269-287.

23. **Wirsig-Wiechmann C.** The nervous terminalis in the chick: A FMRFamide immunoreactive and ACHE positive nerve. Brain Res 1990; 523:175-179.
24. **Yukimura T; Unger T; Rascher W; Lang W and Ganten, D.** Central peptidergic stimulation in blood pressure control: role of enkephalin in rats. Clin Sci 1981; 61: 347s-350s.
25. **Geraerts WPM, van Leeuwen JP, Nuyt K, de With ND.** Cardioactive peptides of the CNS of the pulmonate snail *Lymnaea stagnalis*. Experientia. 1981; 37:1168-1170.
26. **Gouarderes C, Kar S, Zajac JM.** Presence of neuropeptide FF receptors on primary afferent fibers of the rat spinal cord. Neuroscience 1996; 74 (1): 21-27.
27. **Allard M, Zajac JM, Simonnet G.** Autorradiographic distribution of receptors FLFQPQRFamide. A morphine modulation peptide, in rat central nervous system. Neuroscience 1992; 49(1):101-116.
28. **Allard M, Rousselot P, Lombard M, Theodosis DT.** Evidence for neuropeptide FF (FLFQPQRFamide) in rat dorsal root ganglion. Peptides.1999; 20:327-333.
29. **Li L, Kelley WP, Billimoria CP, Christie AE, Pulver SR, Sweedler JV, Marder E.** Mass spectrometric investigation of the neuropeptide complement and release in the pericardial organs of the crab *Cancer borealis*. J Neurochem 2003; 87:642-656.
30. **Audsley N, Weaver RJ.** A comparison of the neuropeptide from retrocerebral complex of adult male and female *Manduca sexta* using MALDI-TOF mass spectrometry. Regulat Pept 2004; 116:127-137.
31. **Dulcis D, Levine RB.** Innervations of the heart of the adult fruit fly, *Drosophila melanogaster*. J. Comp Neurol. 2003; 465:560-576.
32. **Wine JJ.** The structure of tonic flexor motor neurons in crayfish abdominal ganglia. J. Comp. Physiol. 1974; 93:315-336.
33. **Huberman A, Arechiga H, De la Rosa J, Aramburo C.** Isolation and purification of neurodepressing hormone from the eyestalk of *Procamburus bouvieri* (Ortmann). Eur J Biochem 1979; 99:203-208.
34. **Wong TM, Greenberg MJ, Tse SY.** Cardiovascular effects of intraventricular injection of FMRFamide, MET-Enkephalin and their common analogues in the rat. J. Comp. Biochem Physiol 1985; 81C:175-179.
35. **Higgins W, Price DA, Greenberg MJ.** FMRFamide increases the adenylate cyclase activity and cyclic AMP level on molluscan heart. Eur J Pharm 1978; 48: 425-430.
36. **Lloyd PE, Kupfermann I, Weiss KR.** Two endogenous neuropeptides (SCP_A and SCP_B) produces a cAMP mediated stimulation of cardiac activity in *Aplysia*. J Comp Physiol A 1985; 156:659-667.
37. **Stangier J, Hilbich C, Keller R.** Occurrence of crustacean cardioactive peptide (CCAP) in the nervous system of an insect *Locusta migratoria*. J Comp Physiol B 1989; 159:5-11.
38. **Keller R.** Crustacean neuropeptides: Structures, function and comparative aspects. Experientia 1992; 48:439-446.