ISJ 12: 173-175, 2015 ISSN 1824-307X

LETTER TO EDITOR

On the existence of possible pituitary adenylate cyclase-activating polypeptide adenylate type 1 receptor in earthworms

L Molnár[1], E Pollák[1], I Somogyi[1], P Engelmann[2]

[1]Department of Comparative Anatomy and Developmental Biology, Faculty of Sciences, University of Pécs, Ifjúság u. 6, H-7624 Pécs, Hungary
[2]Department of Immunology and Biotechnology, Clinical Center, University of Pécs, Szigeti u. 12, H-7643 Pécs, Hungary

Accepted May 29, 2015

To the Editor

Neuropeptides, expressed by both invertebrate and vertebrate nervous systems (Grimmelikhuijzen and Hauser, 2012) play critical roles in both synaptic and non-synaptic signalling mechanisms regulating the functions of nervous, endocrine and immune systems (Nässel and Larhammar, 2013). In contrast to vertebrates the invertebrate nervous system contains high number of neurosecretory cells that produce and elaborate several neurohormones (most of them are peptides) that act on peripheral tissues *e.g.,* body wall muscles, alimentary canal, excretory and genital organs (Taghert and Nitabach, 2012). However, some of the neuropeptides are neuromodulators in the central nervous system mediating the firing mechanism and synaptic transmission of neurons (van den Pol, 2012). While broad range of neuropeptides is known from some invertebrates like planarians, roundworms, insects or molluscs (De Haes *et al*., 2014), but less is known from the annelids, especially from earthworms.

Most of the neuropeptides, like proctolin, FMRFamide-like peptides, neuropeptide Y (Lengvari *et al.*, 2001), substance P and ACTH-like peptide (Aros *et al*., 1980), believed to be regulator molecules in earthworms had only been identified by immunocytochemical stainings. There are only a few neuropeptides, *e.g.,* CAPA-peptides (Herbert *et al*., 2009) and pituitary adenylate cyclase activating polypeptide (PACAP)-like peptides (Somogyvári-Vígh *et al*., 2000) which expression, pattern and function have investigated by various experimental protocols in details.

PACAP is a highly conserved member of the VIP/secretin/glucagon peptide family found in neuronal elements of both the CNS and several peripheral tissues of vertebrates. It acts as a pleiotropic neuropeptide via three heptahelical G-protein-linked receptors, one PACAP-specific (PAC1) receptor and two receptors that are shared with VIP (VPAC1 and VPAC2). PACAP has been implicated in a variety of central nervous functions, including hypophysiotropic function, learning and memory formation, psychomotor function, and the response to nerve injury. Furthermore, it is a potent antiapoptotic and neurotrophic substance (Vaudry *et al*., 2009).

By means of immunohistochemistry PACAP-like activity was identified in the central and peripheral nervous system of some earthworm species, namely *Eisenia fetida*, *Lumbricus terrestris* and *L. polyphemus* (Reglödi *et al*., 2000). Two distinct forms of PACAP-like molecules (consist from 27 or 38 amino acids) were found in the CNS of *L. polyphemus* of which the smaller molecule proved to be the predominant form (Somogyvári-Vígh *et al*., 2000). Applying whole mount preparations to immunohistochemical observations Molnár and colleagues (2006) showed that each PACAP-like molecules occurred in distinct neuron populations of both *L. terrestris* and *E. fetida* and concluded that these molecules might mediate distinct physiological processes in earthworms. The biological activity of the purified HPLC fractions of both PACAP27 and PACAP38-like peptides isolated from the earthworm CNS was investigated in *in vitro* experimental conditions. Both PACAP-like molecule fractions from earthworms have activated adenylate cyclase and possessed sequence similarities to mammalian PACAP isoforms (Somogyvári-Vígh *et al*., 2000).

Occurrence, distribution and cellular transport of the specific PAC1-receptor in neural structures of the ventral nerve cord ganglia in *E. fetida* was investigated by light and electron microscopic immunocytochemistry. The experimental results revealed that PAC1-receptor-like immunoreactivity localized both on intracellular membranes (endoplasmic reticulum cisternae) and special domains of plasma membrane (synaptic membrane) of neurons as well. It suggests a compound transport and presentation process of the receptor molecule on the cell surface which supposes its role in synaptic transmission (Molnár *et al*., 2008).

Corresponding author:
Peter Engelmann
Department of Immunology and Biotechnology
Clinical Center, University of Pécs
Pécs, H-7643, Szigeti u. 12, Hungary
E-mail: engelmann.peter@pte.hu

A possible role of PACAP-like peptides in mediation of both embryogenesis (Boros *et al*., 2008) and posterior regeneration of *E. fetida* (Várhalmi *et al*., 2008) was suggested based on the quantitative analysis of the expression of PACAP-like peptides applying one of the most sensitive immunoserological methods, the radioimmunoassay. During both the embryonic development and segment regeneration the overexpression of PACAP-like proteins was detected and a characteristic antero-posterior gradient found in regenerating ventral nerve cord ganglia suggesting that they had a strong influence on cell cycle and tissue differentiation in earthworms (Boros *et al*., 2008, Várhalmi *et al*., 2008). The expression of PAC1-receptor was investigated by immunocytochemical and immunoserological methods (Western blot and Far Western blot) during the embryonic development of *E. fetida* and showed its existence in germinal layers and developing ventral nerve cord ganglia (Boros *et al*., 2010).

According to the PACAP and PAC1/VPAC receptor localization studies in vertebrates it was clear that their expression is not restricted only to neuroendocrine and separate vegetative organs (Vaudry *et al*., 2009). We observed PAC1-like immunoreactivity in coelomocytes by means of immunocytochemistry and Western blot. Among coelomocytes the amoebocyte subgroup expressed more abundantly this receptor, but whether this immunocyte is only a responder and/or a producer of the neuropeptide was not clear. To answer this, first immunoserological experiments (RIA) were performed from coelomocytes of regenerating earthworms and proved the PACAP-like immunoreactivity in this immune cell compartment as well (Somogyi *et al*., 2009). These immunological cross-reaction based results should be further supported by molecular biological approaches to isolate and characterize the mRNA transcripts for PACAP and PAC1 receptor from earthworms.

Recent molecular evidence from other phyla (Pirger *et al*., 2010; Lugo *et al*., 2013) and our findings strengthen the notion for evolutionary conservation of this neuropeptide family across animal kingdom.

Acknowledgements

We acknowledge the financial support of Medical Faculty Research Foundation, University of Pécs (PTE-ÁOK-KA 34039/10-06 and 2013/09), the János Bolyai Research Foundation of the Hungarian Academy of Sciences, the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 ‘National Excellence Program’. The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

References

Aros B, Wenger T, Vigh B, Vigh-Teichmann I. Immunohistochemical localization of substance P and ACTH-like activity in the central nervous system of the earthworm *Lumbricus terrestris* L. Acta Histochem. 66: 262-268, 1980.

Boros A, Reglödi D, Herbert Z, Kiszler G, Nemeth J, Lubics A, *et al*. Changes in the expression of PACAP-like compounds during the embryonic development of the earthworm *Eisenia fetida*. J. Mol. Neurosci. 36:157-65, 2008.

Boros A, Somogyi I, Engelmann P, Lubics A, Reglödi D, Pollák E, *et al*. Pituitary adenylate cyclase-activating polypeptide type 1 (PAC1) receptor is expressed during embryonic development of the earthworm. Cell Tissue Res. 339: 649-653, 2010.

De Haes W, Van Sinay E, Detienne G, Temmerman L, Schoofs L, Boonen K. Functional neuropeptidomics in invertebrates. Biochim. Biophys. Acta 1854: 812-826; 2015.

Grimmelikhuijzen CJ, Hauser F. Mini-review: the evolution of neuropeptide signaling. Regul. Pept. 177: S6-S9, 2012.

Herbert Z, Zougman A, Pollák E, Boros A, Kapan N, Molnár L. Identification of novel neuropeptides in the ventral nerve cord ganglia and their targets in an annelid worm, *Eisenia fetida*. J. Comp. Neurol. 514: 415-432, 2009.

Krajniak KG. Invertebrate FMRFamide related peptides. Protein Pept. Lett. 20: 647-670, 2013.

Lengvari I, Csoknya M, Lubics A, Szelier M, Hámori, J. Proctolin immunoreactive elements in the nervous system of earthworm (*Lumbricus terrestris*). Acta Biol. Hung. 45: 337-345, 1993.

Lugo JM, Carpio Y, Morales R, Rodríguez-Ramos T, Ramos L, Estrada MP. First report of the pituitary adenylate cyclase activating polypeptide (PACAP) in crustaceans: conservation of its functions as growth promoting factor and immunomodulator in the white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol. 35: 1788-1796, 2013.

Molnár L, Pollák E, Boros A, Reglödi D, Tamás A, Lengvári I, *et al*. Comparative Anatomy of PACAP Immunoreactive Structures in the Ventral Nerve Cord Ganglia of Lumbricid Oligochaetes. Ann. NY Acad. Sci. 1070: 427-430, 2006.

Molnár L, Pollák E, Boros A, Shioda S, Nakajo S, Tamás A, *et al*. PAC1 receptor localization in a model nervous system: light and electron microscopic immunocytochemistry on the earthworm ventral nerve cord ganglia. Regul. Pept. 145: 96-104, 2008.

Nässel DR, Larhammar D. Neuropeptides and peptide hormones. In: Galizia CG, Liedo PM (eds), Neurosciences - From Molecule to Behavior: a university textbook, Springer-Verlag, Berlin Heidelberg, pp 213-237, 2013.

Pirger Z, Lubics A, Reglodi D, Laszlo Z, Mark L, Kiss T. Mass spectrometric analysis of activity-dependent changes of neuropeptide profile in the snail, *Helix pomatia*. Neuropeptides 44: 475-483, 2010.

Reglödi D, Lengvari I, Szelier M, Vigh S, Arimura A. Distribution of PACAP-like immunoreactivity in the nervous system of oligochaeta. Peptides 21: 183-188, 2000.

Somogyi I, Boros A, Engelmann P, Varhalmi E, Nemeth J, Lubics A, *et al*. Pituitary adenylate cyclase-activating polypeptide-like compounds could modulate the activity of coelomocytes in the earthworm. Ann. NY Acad. Sci. 1163: 521-523, 2009.

Taghert PH, Nitabach MN. Peptide neuromodulation in invertebrate model systems. Neuron 76: 82-97, 2012.

van den Pol AN. Neuropeptide transmission in brain circuits. Neuron 76: 98-115, 2012.

Varhalmi E, Somogyi I, Kiszler G, Nemeth J, Reglodi D, Lubics A, *et al.* Expression of PACAP-like compounds during the caudal regeneration of the earthworm *Eisenia fetida*. J. Mol. Neurosci. 36: 166-174, 2008.

Vaudry D, Falluel-Morel A, Bourgault S, Basille M, Burel D, Wurtz O, *et al*. Pituitary adenylate cyclase-activating polypeptide and its receptors: 20 years after the discovery. Pharmacol. Rev. 61: 283-357, 2009.