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Immunocytochemical distribution of angiotensin-I converting enzyme in the central nervous system of insects and speculations about its possible function*

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Abstract. Insect peptidyl-dipeptidase A [angiotensin I – converting enzyme (ACE)] is a soluble single-domain peptidyl-dipeptidase that has many properties in common with the C-domain of mammalian somatic ACE and with the single-domain mammalian ACE. In agreement with a variety of insects, immunocytochemical studies reveal the presence of an ACE-like protein in *Locusta migratoria*. ACE-like immunoreactivity is present in neurosecretory cells of the pars intercerebralis. These cells have axons projecting into the nervus corporis cardiaci I and into the storage part of the corpus cardiacum, a neuroendocrine organ directly releasing into the aorta. The localisation of ACE in neurosecretory cells is consistent with its proposed role as a processing enzyme that is involved in the generation of active peptide hormones.

INTRODUCTION

The original discovery in insects of an angiotensin-I converting enzyme homologue was rather unexpected (Cornell et al., 1995; Lamango & Isaac, 1994). In mammals this angiotensin converting enzyme (EC 3.4.15.1, called peptidyl peptidase A or simply abbreviated to ACE) represents a Zn^{2+} metalloprotease which plays an important role in blood pressure homeostasis (Corvol et al., 1995). ACE cleaves the carboxyterminal His-Leu dipeptide from angiotensin I, converting it to a potent vasopressor, angiotensin II (Ondetti & Cushman, 1982). In mammals, apart from angiotensin I, only bradykinin, a vasodilatory peptide (Erdös & Skidgel, 1987), and the natural stem cell regulatory peptide, N-acetyl-Ser-Asp-Lys-Pro, are known to be in vivo substrates for the ACE enzyme (Ehlers & Riordan, 1989; Azizi et al., 1996).

In insects, blood pressure regulation is not a main issue and the presence in insects of the typical vertebrate type ACE substrate, angiotensin I, has not yet been detected. Otherwise, the enzymatic activity described for the ACE homologues purified from *Musca domestica*, *Drosophila melanogaster* and *Haematobia exigua irritans*, all dipteran insects, is strikingly similar to the activity profile described for mammalian ACE (Lamango & Isaac, 1994; Cornell et al., 1995; Wijffels et al., 1996).

This seemingly conflicting information makes the search for the physiological significance of ACE homologues in insects even more challenging.

Insect ACE mainly differs at two levels in regard to mammalian ACE. First, only a single domain enzyme is described in contrast to both the double domain somatic ACE and single domain testicular ACE demonstrated in mammals. Second, the presence of a secretory signalling peptide at the amino-terminal end of the pre-enzyme, as deduced from ORF translation of cloned cDNA's, refers to the occurrence of secreted solubilized ACE in the circulation and/or at least in the introduction into the secretory pathway. In mammals both sACE and tACE represent membrane-linked proteases which, to a minor extent, can be proteolytically released into the bloodstream.

PHYSIOLOGICAL SIGNIFICANCE OF ACE IN INSECTS

Recently, ACE was also found in the fly's haemolymph (Isaac & Lamango, 1994). Previous to this finding, the ACE functional hypothesis focused towards a direct role in the regulation of organ muscle contraction as seen in the presence of an in vitro demonstrated ACE cleavage site in insect tachykinins (Isaac et al., 1998a). The latter peptides are known to modulate muscle contraction in insects (Schoofs et al., 1997). The recent observation that neurosecretory cells in the brain and suboesophageal ganglion of *Locusta migratoria,* which contain Lom-MT immunoreactive material, are also reactive for ACE, has altered our thinking about the physiological role of insect ACE. Now, it is thought that the enzyme might function as a real intracellular convertase responsible for the fine trimming of neuropeptides following their liberation from their precursor by an alpha-subtilisin-like prohormone

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convertase (Isaac et al., 1997; 1998b; Schoofs et al., 1998). Indeed, recombinant *Drosophila* ACE cleaves the dipeptides Lys-Arg and Arg-Arg from the C-terminus of FSPRLGKR and FSPRLGRR, respectively, to yield FSPRLG. The generated FSPRLG is relatively resistant to further hydrolysis by ACE, allowing the Gly extended peptide to serve as a substrate for a peptidylglycine α-amidating mono-oxygenase that in turn assures functionality of this peptide (Isaac et al., 1998a).

In the above context, knowledge of the correct distribution of ACE immunoreactivity in the insect neural tissue becomes more important since co-localization data might open the way for identifying additional natural ACE substrates in insects.

DISTRIBUTION OF ACE IN THE INSECT NERVOUS SYSTEM

The distribution of ACE immunoreactivity seems to be very similar in different insect species. In the brain, immunoreactivity is localised in both the neuropile regions and in neurosecretory cells. In the cockroach, *Leucophaea maderae*, the beetle, *Leptinotarsa decemlineata*, the fly, *Neobellieria bullata*, the locust, *Locusta migratoria* and the lepidopterans, *Bombyx mori* and *Mamestra brassicae*, ACE immunoreactivity has been reported as well in neurosecretory cells of the pars intercerebralis (Schoofs et al., 1998). In *Locusta migratoria*, ACE immunoreactivity is apparent in median neurosecretory cell bodies (Isaac et al., 1998a) from which the axons project into the contralateral nervus corporis cardiaci I (NNC I) (Rademakers, 1977; Konings et al., 1988). Accordingly, immunoreactivity was observed in the NCC I which ramifies into the CCs where immunoreactivity was also observed (Fig. 1). In agreement with the results from *Locusta*, ACE immunoreactivity has also been found in the corpora cardiaca of the walking stick insect, *Carausius morosus*, the Colorado potato beetle, *Leptinotarsa decemlineata* and the cockroach, *Leucophaea maderae.*

The observed distribution of ACE in the insect nervous system suggests a possible pleiotropic role for this enzyme. First, the enzyme localised in the neuropile might function as an inactivator of peptidic neurotransmitters following their release into the synaptic cleft (Lamango et al., 1997). The reported wide activity spectrum of the ACE enzyme, which, besides the typical carboxyterminal dipeptidase activity, also functions as an endopeptidase (Erdös, 1990), supports this hypothesis.

Furthermore, the demonstration of ACE inside neurosecretory cells suggests a possible role in the intracellular processing of peptides from their macromolecular precursors. The reported ability of ACE to convert Lom-MT I into a hexamer ready for amidation strongly supports this hypothesis. In addition, ACE was found to be present in pheromone biosynthesis activating neuropeptide (PBAN) cells of the suboesophageal ganglion in several lepidopteran insects (Schoofs et al., 1998). PBAN has the same FXPRLamide carboxyl terminal sequence as Lom-MT. Both peptides belong to the same family. Finally, the observation that ACE is present in the storage part of the

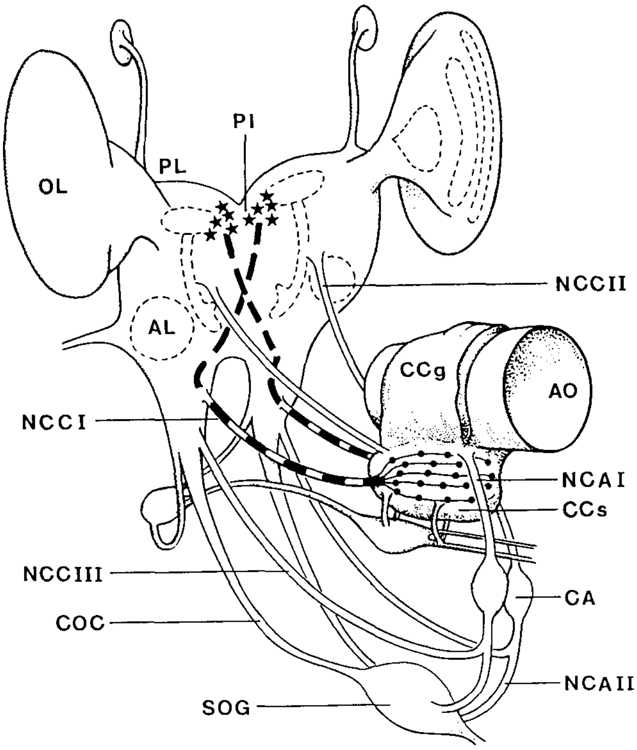

Fig. 1. Diagram of the central nervous system of *Locusta migratoria* (Vullings et al., 1998) showing the immunodistribution of ACE. Immunoreactive cells in the pars intercerebralis (shown by stars) have axons projecting into the NCC-I and ramifying into the storage part of the corpus cardiacum (dashed lines). AL – antennal lobes; AO – aorta; CA – corpus allatum; CCg – glandular part of the corpus cardiacum; CCs – storage part of the corpus cardiacum; COC – circumoesophageal connectives; NCA I – nervus corporis allati I; NCA II – nervus corporis allati II; NCC I – nervus corporis cardiaci I; NCC II – nervus corporis cardiaci II; NCC III – nervus corporis cardiaci III; OL – optic lobes; PI – pars intercerebralis; PL – pars lateralis; SOG – suboesophageal ganglion.

corpora cardiaca suggests that ACE originating in neurosecretory cells might be released into the haemolymph (De Loof et al., 1998). This latter observation is most puzzling. The controlled release of ACE in the hemolymph, as demonstrated in house flies and locusts, points towards an important regulatory role of the ACE enzyme in both the fast proteolytic activation and/or the sudden metabolic clearance of regulatory peptides in the circulation.

DISTRIBUTION OF ANGIOTENSIN-I IN THE CENTRAL NERVOUS SYSTEM OF *LOCUSTA MIGRATORIA*

Angiotensin-I-like immunoreactivity was localised in the central nervous system of the African locust, *Locusta migratoria.* The properties of the antibody were described previously (Laurent et al., 1995, 1998). The immunopositive reaction was completely abolished with bovine angiotensin. Angiotensin-I-like immunoreactivity was observed in cells of the pars intercerebralis of the protocerebrum (Fig. 2). These cells have axons projecting into the nervus corporis cardiaci I and into the storage or neurohaemal part of the corpus cardiacum. Many neuro-

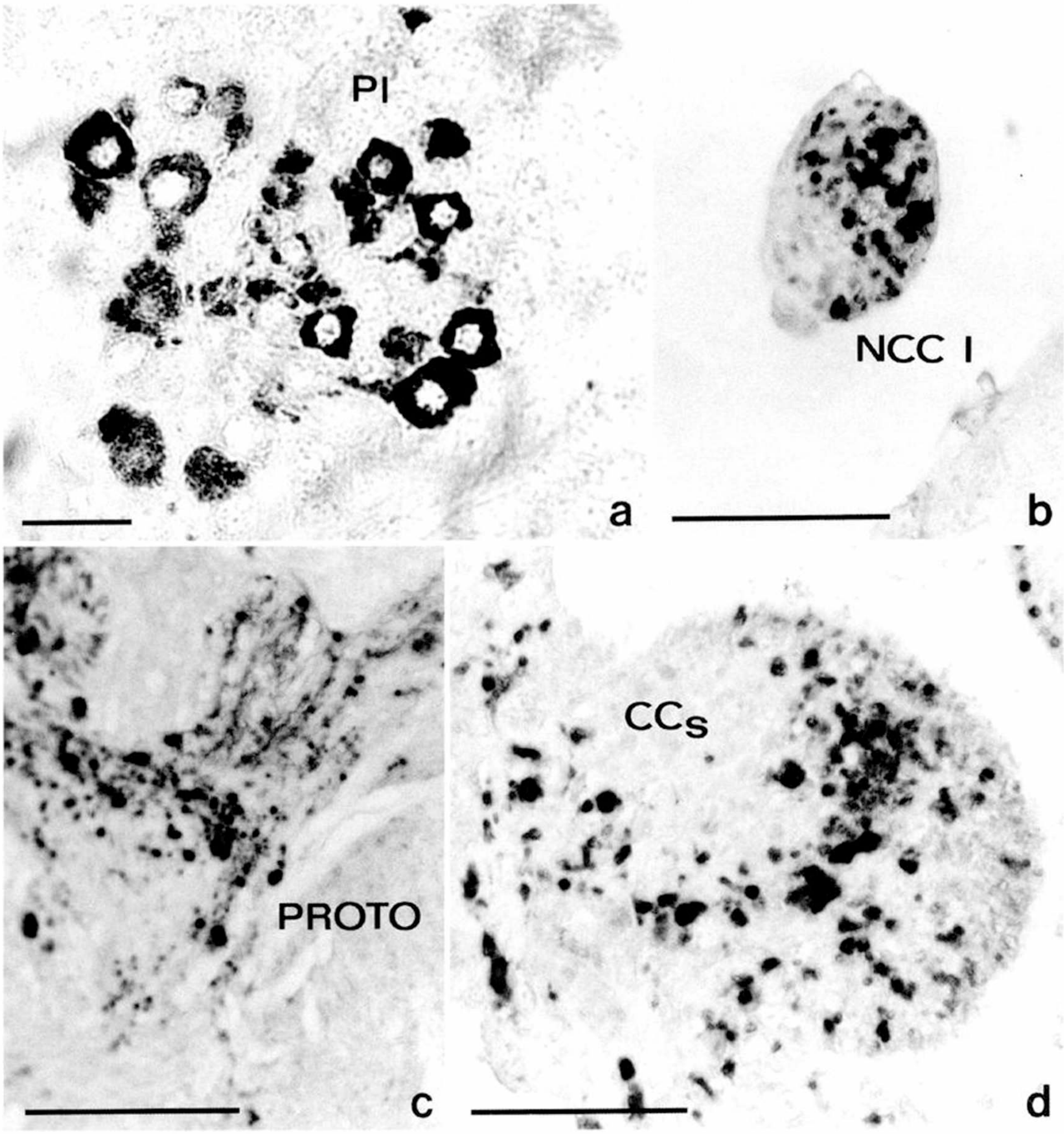

Fig. 2. Immunocytochemical distribution of angiotensin-I in the central nervous system of *Locusta migratoria*. (a) Angiotensin-I-like immunoreactivity in the pars intercerebralis of the protocerebrum. (b) Angiotensin-I-like immunoreactivity in a cross section of the nervus corporis cardiaci I. (c) Angiotentensin-I-like immunoreactivity in axons of neurosecretory cells of the pars intercerebralis. (d) Angiotensin-I-like immunoreactivity in the storage part of the corpus cardiacum. CCs – storage part of the corpus cardicum; NCC I – nervus corporis cardiaci I; PI – pars intercerebralis; PROTO – protocerebrum. Scale bar = 25 µm.

peptides are present in nerves projecting from the brain to the corpus cardiacum, which is one of the most important neuroendocrine organs of insects (Veelaert et al., 1998; Vullings et al., 1998). This indication about the presence, in *Locusta*, of an angiotensin-I-like peptide needs further evidence by either direct purification and/or cloning of the corresponding gene.

DISCUSSION

The discovery of in vitro prohormone-processing activity of insect ACE provides a possible explanation for intracellular co-localization of the enzyme with FXPRLamides, and provides evidence for a new role for ACE in the processing of peptide hormones and transmitters. These data are in line with the ones recently found in leeches and molluscs (Salzet & Stefano, 1998; Stefano & Salzet, 1998; Stefano et al., 1998). In these animals, ACE and a second enzyme, the neutral endopeptidase, are involved in prohormone-processing activities in immunocytes (Salzet et al., 1997, Salzet & Stefano, 1998; Stefano & Salzet, 1998; Stefano et al., 1998). Moreover, at the level of the leech brain, ACE is present in dense-core secretory granules in both neuron perikarya and in nerve fibers in the neurohaemal area (Vandenbulcke et al., 1997). In addition, immunocytochemical data suggest the presence of an angiotensin-I-like peptide in the central nervous system of *Locusta migratoria*. Purification of this angiotensin-like factor is in progress. In the gypsy moth, *Lymantria dispar*, both bovine angiotensin II and bovine ACE induce synthesis of immunodetectable ecdysteroids by pupal testis in vitro but are antagonistic to coincubated testis ecdysiotropin (TE) (Loeb et al., 1998)

This suggests that the angiotensin – angiotensin-converting enzyme-like system might be very well conserved during evolution. However, this does not exclude the possibility that the functions of the angiotensin-like peptide and ACE in insects could be different from those in mammals.

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REFERENCES

Azizi M., Rousseau A., Ezan E., Guyene T.-T., Michelet S., Grognet J.-M., Lenfant M., Corvol P. & Menard J. 1996: Acute angiotensin-converting enzyme inhibition increases the plasma level of the natural stem cell regulator N-acetyl-Seryl-aspartyl-lysyl-proline. *J. Clin. Invest.* **97**: 839–844.

Cornell M.J., Williams T.A., Lamango N.S., Coates D., Corol P., Soubrier F., Hoheisel J., Lehrach H. & Isaac E. 1995: Cloning and expression of an evolutionary conserved single domain angiotensin converting enzyme from Drosophila melanogaster. *J. Biol. Chem.* **270**: 13613–13619.

Corvol P., Williams T.A. & Soubrier F. 1995: Peptidyl dipeptidase A: angiotensin I-converting enzyme. In Barrett A.J. (ed.): *Methods in Enzymology. Vol. 248*. Academic Press, London, pp. 283–305.

De Loof A., Schoofs L., Huybrechts R., Veelaert D., Macours N., Isaac E., Salzet M. & Loeb M. 1998: Search for a renin-angiotensin system (RAS) in insects. In Brunnhofer V. & Soldán T. (eds): *Book of Abstracts, VIth Eur. Congr. Entomol.* Institute of Entomology, České Budějovice, pp. 114–115.

Ehlers M.R.W. & Riordan J.F. 1989: Angiotensin-converting enzyme: new concepts concerning its biological role. *Biochemistry* **28**: 5311–5322.

Erdös E.G. 1990: Angiotensin-converting enzyme: new concepts concerning its biological role. *Hypertension* **16**: 363–370.

Erdös E.G. & Skidgel R.A. 1987: The angiotensin-I converting enzyme. *Lab. Invest.* **57**: 245–358.

Isaac R.E. & Lamango N.S. 1994: Peptidyl dipeptidase activity in the hemolymph of insects. *Biochem. Soc. Trans.* **22**: S292.

Isaac R.E., Lamango N.S., Nachman R.J., Strey A. & Hayes T.K. 1997: Angiotensin-converting enzyme and the metabolism of regulatory peptides in insects. *Ann. N. Y. Acad. Sci.* **814**: 339–341.

Isaac R.E., Schoofs L., Wiliams T.A., Veelaert D., Sajid M., Corvol P. & Coates D. 1998a: A novel peptide-processing activity of insect peptidyl-dipeptidase A (angiotensin I-converting enzyme): the hydrolysis of lysyl-arginine and arginyl-arginine from the C-terminus of an insect prohormone peptide. *Biochem. J.* **330**: 61–65.

Isaac R.E., Schoofs L., Williams T.A., Corvol P., Veelaert D., Sajid M. & Coates D. 1998b: Towards a role for angiotensin-converting enzyme in insects. *Ann. N. Y. Acad. Sci.* **839**: 288–292.

Konings P.N.M., Vullings H.G.B., Siebinga R., Diederen J.H.B. & Jansen W.F. 1988: Serotonin-immunoreactive neurons in the brain of Locusta migratoria innervating the corpus cardiacum. *Cell Tissue Res.* **254**: 147–153.

Lamango N.S. & Isaac R.E. 1994: Identification and properties of a peptidyl dipetidase in the housefly, Musca domestica, that resembles mammalian angiotensin-converting enzyme. *Biochem. J.* **299**: 651–657.

Lamango N.S., Nachman R.J., Hayes T.K., Strey A. & Isaac R.E. 1997: Hydrolysis of insect neuropeptides by an angiotensin-converting enzyme from the housefly, Musca domestica. *Peptides* **18**: 47–52.

Laurent V., Bulet P. & Salzet M. 1995: A comparison of the leech Theromyzon tessulatum angiotensin I-like molecule with forms of vertebrate angiotensinogens: a hormonal system conserved in the course of evolution. *Neurosci. Lett.* **190**: 175–178.

Laurent V., Stefano G.B. & Salzet M. 1998: The leech angiotensin-converting enzyme. *Ann. N. Y. Acad. Sci.* **839**: 500–502.

Loeb M.J., De Loof A., Schoofs L. & Isaac E. 1998: Angiotensin II and angiotensin-converting enzyme as candidate compounds modulating the effects of testis ecdysiotropin in testes of the gypsy moth, Lymantria dispar. *Gen. Comp. Endocrinol.* **112**: 232–239.

Ondetti M.A. & Cushman D.W. 1982: Enzymes of the renin-angiotensin system and their inhibitors. *Annu. Rev. Biochem.* **51**: 283–308.

Rademakers L.H.P.M. 1977: Identification of a secretomotor center in the brain of Locusta migratoria, controlling secretory activity of the adipokinetic hormone producing cells of the corpus cardiacum. *Cell Tissue Res.* **184**: 381–395.

Salzet M. & Stefano G.B. 1998: Evidence for an invertebrate neuroimmune system: Neuropeptide processing in leech-host communication. *Trends Comp. Biochem. Physiol.* **5**: 85–98.

Salzet M., Salzet-Raveillon B., Cocquerelle C., Verger-Bocquet M., Pryor S.C., Rialas C.M., Laurent V. & Stefano G.B. 1997: Leech immunocytes contain pro-opiomelanocortin: nitric oxide mediates hemolymph proopiomelanocortin processing. *J. Immunol.* **159**: 5400–5411.

Schoofs L., Veelaert D., Vanden Broeck J. & De Loof A. 1997: Peptides in the locusts, Locusta migratoria and Schistocerca gregaria. *Peptides* **18**: 145–156.

Schoofs L., Veelaert D., De Loof A., Huybrechts R. & Isaac R.E. 1998: Immunocytochemical distribution of angiotensin I-converting enzyme-like immunoreactivity in the brain and testis of insects. *Brain Res.* **785**: 215–227.

Stefano G.B. & Salzet M. 1999: Invertebrate opioid precursors: Evolutionary conservation and the significance of enzymatic processing. *Int. Rev. Cytol.* **154**: 255–287.

Stefano G.B., Salzet B. & Salzet M. 1999: Mytilus edulis hemolymph contains propiomelanocortin: LPS and morphine stimulate differential processing. *Mol. Brain Res.* (in press).

Vandenbulcke F., Laurent V., Verger-Bocquet M., Stefano G.B. & Salzet M. 1997: Biochemical identification and ganglionic localization of leech angiotensin-converting enzyme. *Mol. Brain Res.* **49**: 229–237.

Veelaert D., Schoofs L. & De Loof A. 1998: Peptidergic control of the corpus cardiacum-corpora allata complex of locusts. *Int. Rev. Cytol.* **182**: 249–302.

Vullings H.G.B., Diederen J.H., Veelaert D. & Van der Horst D.J. 1999: Multifactorial control of the release of hormones from the locust retrocerebral complex. *Microsc. Res. Tech.* **45**(3): 142–153.

Wijffels G., Fitzgerald C., Gough J., Riding G., Elvin C., Kemp D. & Willadsen P. 1996: Cloning and characterization of angiotensin-converting enzyme from the dipteran species, Haematobia irritans exigua, and its expression in the maturating male reproductive system. *Eur. J. Biochem.* **237**: 414–423.

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