

## Cellular localization of a chromogranin B-like derived peptides in leeches

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**Michel Salzet & Martine Verger-Bocquet**

Laboratoire de Neuroimmunologie des Annélides, UMR CNRS 8017, SN3, IFR 118,  
Université des Sciences et Technologies de Lille. 59650 Villeneuve d'Ascq, France.

*Correspondence to:* Prof. Michel Salzet  
Laboratoire de Neuroimmunologie des Annélides,  
UMR CNRS 8017, SN3,  
Université des Sciences et Technologies de Lille.  
59650 Villeneuve d'Ascq, FRANCE.  
PHONE: +33 3 2033 7277 FAX: +33 3 2004 1130  
E-MAIL: michel.salzet@univ-lille1.fr

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### **Abstract**

Using immunocytochemistry techniques, we demonstrated specific immunostaining with antibodies rose against fragment (547-560) and (614-626) of bovine chromogranin B (CGB) at the level of brain and the tegument of the leech *Theromyzon tessulatum*. The used of these antibodies on leech sections revealed immunopositive labeling in neurons and glial cells of the central nervous system (CNS), and epidermal glandular cells of the tegument. Colocalization between two antibodies rose against different fragment of bovine CGB have been demonstrated in neurons and glial cells of leech CNS like in vertebrates. Finally, the whole of the data showed for the first time the presence in leeches of CGB like derived peptides.

## 1. Introduction

Chromogranins are acidic proteins which are found throughout neuroendocrine system [1]. They comprise several defined proteins which have been named chromogranin A [2, 3], chromogranin B [4] [5]; secretogranin II [6] [7], 7B2 [8], ProSAAS [9]. These proteins distribution have been undergone in rat brain in great detail [1,6,10–12]. Interestingly, prochromogranin B (CGB) is known to be processed in several peptides *i.e.* GAWK, CCB, BAM-1745, OA21, OA8, LE-20, NL-10, OY-10 presenting several different biological functions [5]. In addition to their roles as helper proteins in the packaging of peptides, they may serve as pro-hormones to generate biologically active peptides such as vasostatin-1 and secretolytin [13, 14]. These molecules derived from CGA and CGB respectively, possess antimicrobial properties [13, 14]. From the C-terminal part of CGB, two peptides have been identified in both rat and bovine endocrine tissue *i.e.* the peptide 11 (PE11) at position 552–562 [15] and secretolytin at position 614–621 [13]. We have recently demonstrated that immune cells also contain CGA and CGB [16–18] like proenkephalin [19]. Moreover, secretolytin is shown to be released from monocytes *in vitro* under interleukin 6 stimulation [20]. In the present study, we demonstrate using the immunocytochemical technique that chromogranin B derived peptides like a leech secretolytin are also present in leeches like we also previously demonstrated for enkelytin, another antimicrobial neuropeptide derived peptide, the proenkephalin [21–23].

## 2. Material and Methods

### 2.1. Animals

Mature specimens of the rhynchobdellid leech *T. tessulatum*, reared under laboratory conditions as described by Malecha [24, 25] were used in this study.

### 2.2. Antiserum

Polyclonal antisera used in immunocytochemistry were kindly provided by Dr M.H. Metz-Boutigue (INSERM U 338, Strasbourg, France). These rabbit antibodies recognize synthetic peptides corresponding to bovine secretolytin (CGB 614–626), and the C-terminal part of bovine chromogranin B (CGB 547–560) [19].

### 2.3. Immunocytochemical procedures

Anterior parts of *T. tessulatum* were fixed overnight at 4°C in Bouin-Hollande fixative (+10% HgCl<sub>2</sub> saturated solution) [26]. They were then embedded in paraffin and serially sectioned at 7 µm. After removal of paraffin with toluene, the sections were successively treated with the anti-CGB diluted 1:200 and with goat anti-rabbit IgG conjugated to horseradish peroxidase as described elsewhere. The specificity of anti-CGB was tested on consecutive sections mounted on different

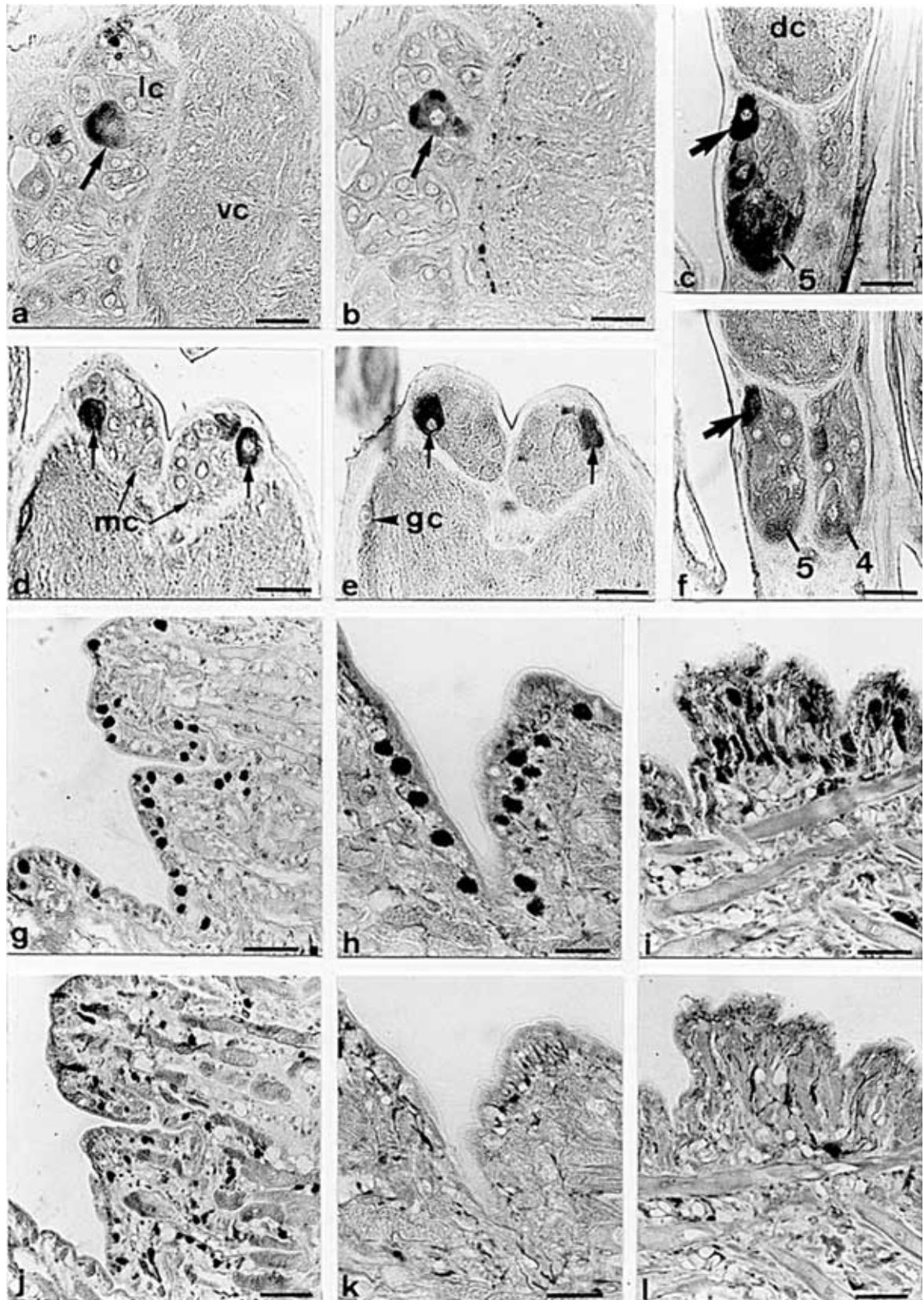
slides by pre-adsorbing the antiserum overnight at 4°C with the homologous antigen [synthetic peptide, Neo-system (Strasbourg, France)] at a concentration of 350 µg/ml of pure antiserum.

## 3. Results and Discussion

### 3.1. Central nervous system localization

Immunocytochemical experiments were performed on leech tissues. Neurons (Fig. 1a, c, d) and glial cells (Fig. 1e) in the compartments of the supra-oesophageal ganglion are stained with both antisera directed against the peptide E11 [15] and the secretolytin [13], allowing to suspect the presence of such molecules in leech nervous system. Immunopositive staining with anti-CGB (547–560) and (614–626) in same neurons have also been detected (Fig. 1a–1f) reflecting that the C-terminal part of CGB is present in leech neurons and that leech CGB processing will occurs in leech like in vertebrates [27]. Moreover, besides data obtained at the level of the brain, similar results were found in epithelial glandular cells of the tegument (Fig. 1g–1j) as we previously obtained for enkelytin, an antimicrobial peptide derived from proenkephalin processing [21]. Furthermore, the specificity of the staining was checked by pre-adsorbing the antibody with its specific synthetic peptide (Fig 1k, 1l).

Taken together, these data are in lines with the ones, we obtained with another endocrine marker, the enkelytin [21]. This peptide is present in brain, nervous and immune systems as well as the tegument like for the ones we detected with the anti-CGB (614–626) which is corresponding to bovine secretolytin [13]. Indeed, enkelytin like secretolytin, with their high antibacterial activities, further associates opioid peptides with immune related activities [28]. We surmise that immune or neural signalling may lead to enhanced proenkephalin and CGB proteolytic processing freeing both opioid peptides and enkelytin or secretolytin-like peptide [21]. In this scenario, the opioid peptides would stimulate immunocyte chemotaxis and phagocytosis as well as the secretion of cytokines. During this process, the simultaneously liberated enkelytin and leech secretolytin would attack bacteria immediately, allowing time for the immunocyte stimulating capabilities of the opioid peptides to manifest themselves [16–18]. This hypothesis is further supported by the presence of specific met-enkephalin receptors on these cells. Thus, it appears that many of the mammalian molecular and cellular survival strategies first appeared in organisms that evolved at least 500 million years ago. They may have evolved first to supplement immune actions, *i.e.*, enkelytin, leech secretolytin by covering the latency period before total or partial immune activation occurs [16–18].



**Figure 1:** Cellular localization of the chromogranin B derived-like peptide in *T. tessulatum*

**a-f:** CGB derived-like peptide immunoreactivity detected in some neurons of lateral (lc) (Fig. a) and median (mc) (Fig.d) of subesophageal ganglion This immunoreactivity is also observed in fibers of dorsal (dc) (Fig. b, c, arrow) commissure. Scale bar = 35  $\mu$ m. Interestingly same cells (neurons and glial cells (gc) are stained by the anti-CGB (547-560) (a,c,d) and (614-626) (b, e, f).

**g-i:** Adjacent sections of epidermal and muscular area of the leech treated with anti-CGB (614-626). The staining is present at the level of the subepidermal glandular structures (g) and in more internal glandular structures (j). Control of specificity by preadsorption of anti-CGB (614-626) by the homologous antigen (secretolytin) is shown: (h, i) positive immunostaining ; (k, l) after preadsorption of the antiserum.

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