'Neuro'-peptides in glia: Focus on NPY and galanin

Ruud Ubink[1,*], Laura Calza[2] and Tomas Hökfelt[1]

[1]Department of Neuroscience, Karolinska Institutet, Berzelius väg 1, SE-171 77 Stockholm, Sweden
[2]Dipartimento di morfofisiologia veterinaria e produzioni animali, University of Bologna, 40064 Ozzano Emilia, Bologna, Italy

The term neuropeptide was advanced by de Wied and collaborators in the early seventies. At that time, they defined neuropeptides as endogenous substances synthesized in nerve cells and involved in nervous system functions. Since then, several studies have revealed that the very same 'neuropeptides' are also expressed in non-neuronal cells. It is therefore generally accepted that the original definition of these peptides was too limited and, consequently, it has recently been revised. Among the non-neuronal cells that synthesize neuropeptides are several glial cell types.

Glia, the largest population of cells in the nervous system, have long been falsely regarded as merely supporting cells for the nearby neurons. However, it is now clear that reciprocal neuron–glia interactions play an important role in information processing in the CNS [1]. Thus, glia take part in signalling mechanisms and are able to release neurotransmitters such as glutamate, and they show Ca^{2+} fluctuations in response to specific stimuli. This review aims to highlight some recent findings on yet another 'neuronal' feature of glial cell types: their expression of neuropeptides [2]. (The early findings on this topic were reviewed in the mid 1990s [3–6]. This article will not deal with the vast literature concerning certain other peptides, such as growth factors and cytokines, that are well known to be present in glia [6,7]; nor will it review the important fact that glia also express neuropeptide receptors [8–11].)

Many neuropeptides are expressed in glia

In the CNS, all three major classes of glia – oligodendrocytes, microglia and, in particular, astrocytes – have been reported to express neuropeptides. These neuropeptides include opioid peptides, angiotensin, neuropeptide Y (NPY), somatostatin, substance P, cholecystokinin (CCK), atrial natriuretic peptide (ANP) and several others, as summarized in Table 1 [12–43]. Many studies are based on tissue cultures, and in some cases only the presence of transcripts or the peptide immunoreactivity has been demonstrated, but some peptides have also been reported to be expressed *in vivo* in the normal brain of rats and even humans. However, there are multiple examples of neuropeptides present in cultured glia that cannot be detected in resting glia in the adult brain. Moreover, nerve injury and other pathological processes influence glial neuropeptide expression [44].

Glial neuropeptide expression is region-specific and regulated

Just as neuropeptides are heterogeneously distributed among neuronal populations and brain regions, their expression in glia is highly regional. Thus, Shinoda and collaborators showed that astrocytes express specific neuropeptide genes depending on the brain region from which they were divided [20]. Similarly, an *in vivo* study on the distribution of angiotensinogen mRNA showed marked regional distribution patterns (e.g. very high levels in astrocytes in the hypoglossal nucleus but no expression in the lateral septum) [14]. Viljin *et al.* [19] found that levels of proenkephalin mRNA in cultured neonatal hypothalamic astrocytes were comparable to levels present in cultured embryonic striatal and hypothalamic neurons. Moreover, levels of transcript were different when comparing several brain regions, and seemed to decrease during development. Similar results were obtained not only for proenkephalin but also for somatostatin mRNA [20]. In fact, several of these neuropeptides are under developmental regulation: for example, expression of both NPY and somatostatin in these cells is turned off shortly after birth [21]. Interestingly, after the postnatal downregulation of glial peptide transcripts, injury in adulthood can cause upregulation again, suggesting replication of an ontogenetic expression pattern [4,20].

Further evidence for the regulated expression of neuropeptides by glia comes from studies of primary cultures of human and rat astrocytes [4,29]. For example, phorbol 12-myristate-13- acetate (PMA) potently increases NPY protein and mRNA levels in astrocytes, suggesting regulation via the protein kinase C (PKC) pathway. Interleukin-1β induces NPY expression in human, but not rat, astrocytes; this induction involves cFos and JunB [30]. Interestingly, basic fibroblast growth factor (bFGF) has a similar effect on NPY expression in both rat and human astrocytes, whereas forskolin increases NPY levels only in human astrocytes [29]. These experimental findings raise the possibility that growth factors might be endogenous regulators of glial peptide expression, a view supported by their temporal, spatial and developmental expression. However, an interaction between

* Current address: Department of Pharmacology, N.V. Organon, Molenstraat 110, 5340 BH Oss, The Netherlands.
Corresponding author: Tomas Hökfelt (tomas.hokfelt@neuro.ki.se).

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Table 1. Neuropeptides expressed in glia

Neuropeptide	Detection level	Glial cell	Cell system	Refs
Angiotensin	mRNA	Astrocytes	*In vivo* and cell culture	[12]
	mRNA and peptide	Astrocytes	*In vivo* and cell culture	[13]
	mRNA	Astrocytes	*In vivo* and cell culture	[14]
Atrial natriuretic peptide (ANP)	Peptide	Astrocytes and Bergmann glia	*In vivo*	[15–17]
Carnosine	Peptide	Astro-oligodendrocytes	*In vivo*	[18]
		Bergmann glia		
		Tanycytes and ependyma		
Enkephalin	mRNA	Astrocytes	Cell culture	[19]
	mRNA	Astrocytes	Cell culture	[20,21]
	mRNA and peptide	Astrocytes	Cell culture	[22]
				[23]
	mRNA	Pituicytes	*In vivo*	[24]
	Peptide	Astrocytes	Slice culture	[25]
	mRNA	Glial precursors	Slice culture	[25]
	Peptide	Oligodendrocyte precursors	Cell culture	[26]
Dynorphin	Peptide	Oligodendrocyte precursors	Cell culture	[26]
Galanin	mRNA and peptide	Microglia?	*In vivo* (colchicine)	[27]
		Oligodendrocyte precursors	*In vivo* (colchicine, CSD[a])	[28]
Neuropeptide Y (NPY)	Peptide	Astrocytes	Cell culture	[29,30]
	mRNA and peptide	Olfactory ensheathing cells	*In vivo*	[31–33]
	mRNA and peptide	Schwann cell precursors	*In vivo*	[34]
Nociceptin (orphanin FQ)	mRNA and peptide	Astrocytes	Cell culture	[35]
Oxytocin and vasopressin	Peptides	Pituicytes	*In vivo*	[36]
			Cell culture	[37]
Somatostatin	mRNA and peptide	Astrocytes	Cell culture	[20,21]
	Peptide	Glia (intermediate lobe)	*In vivo*	[38]
Substance P	Peptide and mRNA	Astrocytes	Cell culture	[39]
	mRNA	Microglia	Cell culture (human cells)	[40]
Vasoactive intestinal polypeptide (VIP)	Peptide	Astrocyte	*In vivo*	[41]
	Peptide	Astrocyte	*In vivo* (human)	[42]
	Peptide	Pituicytes	*In vivo*	[43]

[a]Abbreviation: CSD, cortical spreading depression.

growth factors and glial neuropeptides remains to be demonstrated *in vivo*.

Nociceptin (also known as orphanin FQ) is expressed in astrocytes and is regulated by several injury-induced factors, such as reactive oxygen species, ciliary neurotrophic factor, inflammatory mediators and ceramide [35]. Ceramide is generated in response to inflammatory mediators and oxidative stress. It upregulates nociceptin levels 22-fold after 24 h in culture, and this upregulation is mediated by extracellular-signal-related kinase (ERK) and p38 mitogen-activated protein kinase (MAPK). Activation of ERK involves phosphorylation of PKC and cAMP-response-element-binding protein (CREB), and the nuclear factor κB (NF-κB) pathway also seems to be important [35].

Putative roles and functions of neuropeptides in glia

Opioid peptides, ANP, VIP and substance P

Much focus has been on expression of opioid peptides, especially enkephalin in astrocytes [19,20,22]. This is particularly interesting because endogenous opioid systems have long been known to regulate cell proliferation [45] and dendritic growth and spine formation [46]. These studies provide evidence of a developmental role for glial opioid peptides. In addition, oligodendroglia express opioids (both proenkephalin and prodynorphin peptides) in a developmentally regulated manner; these factors could modulate oligodendrocyte maturation and survival, perhaps involving autocrine or paracrine mechanisms [26]

The peptide ANP has been localized in astroglia in canine cerebral cortex [15] and in human cerebral cortex [16] and cerebellum [17]. Cells of the glia limitans were ANP-positive, as were cytoplasmic and fibrous astrocytes and Bergmann glia. McKenzie *et al.* suggested that ANP could influence local vascular dynamics and/or permeability, mitogenesis and growth of glia and neurons [17]. However, these studies were based only on immunohistochemistry; demonstration of the presence of ANP transcript in the corresponding glia with *in situ* hybridization would be valuable. In view of the well-established vasodilatory and trophic effects of vasoactive intestinal polypeptide (VIP), the close relationship between VIP-positive astrocytes and brain vasculature suggests that this peptide could exert similar roles to ANP [42].

The four isoforms of preprotachykinin-A transcripts, substance P immunostaining and mRNA of the substance P receptor neurokinin-1 have all been detected in human fetal microglia cultures [40]. Thus, substance P, which is not only a neuronal messenger but also a major modulator in the immune system, could be a regulator of glial functions and play a role in CNS host defence mechanisms [40].

NPY in olfactory ensheathing cells and Schwann cells

The finding that NPY (both peptide and transcript) is expressed close to the CNS, overlapping with the zone in which incoming axons are sorted before they enter the CNS (Figures 1 and 2), is suggestive of a functional role related to growth, pathfinding and/or trophism. This high level of NPY expression [31–33] occurs in olfactory ensheathing cells [47,48], which support growth of olfactory sensory axons from the olfactory epithelium to

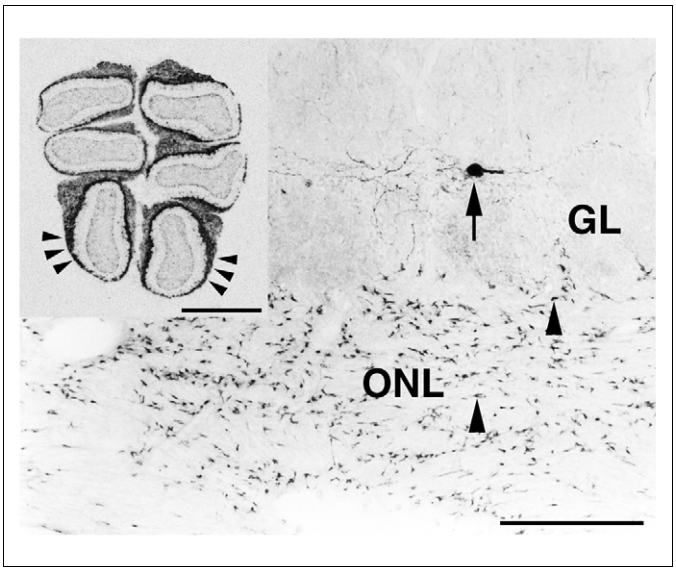

Figure 1. Immunohistochemical analysis (main figure) shows numerous neuropeptide Y (NPY)-positive olfactory ensheathing cells (large arrowheads) in the olfactory nerve layer (ONL), not penetrating into the glomerular layer (GL). Arrow points to an NPY-positive short-axon cell. Inset shows *in situ* hybridization of six sections from the olfactory bulb with very high NPY mRNA levels in the olfactory nerve layer (small arrowheads). Scale bars, 100 μm (main figure) and 3 mm (inset).

the glomerular layer of the olfactory bulb throughout life [49] (Figure 3). It also occurs transiently in Schwann cell precursors [34], which are present at a time during development when many peripheral axons grow towards their target [50] (Figure 3).

It has been suggested by Jessen and Mirsky [51] that survival of sensory neurons in dorsal root ganglia is regulated initially by glia-derived signals as the axons elongate towards their target, and subsequently by

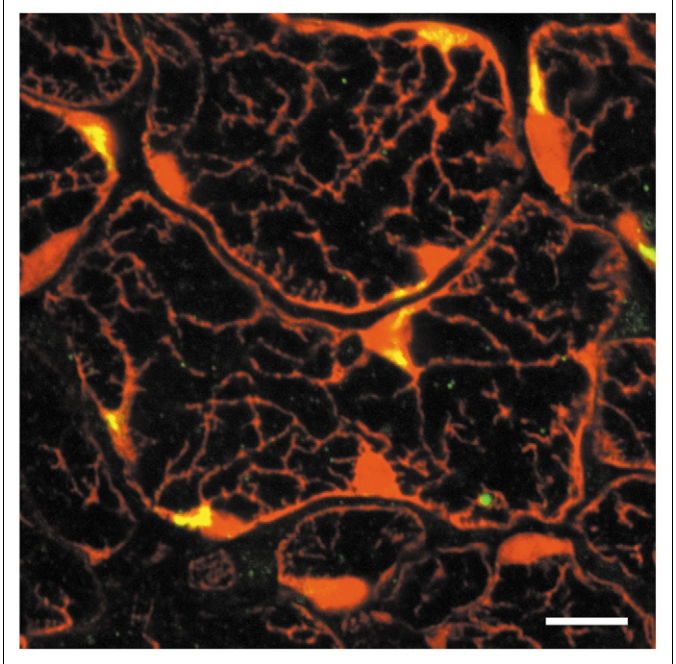

Figure 2. Double-labelling immunofluorescence of olfactory ensheathing cells. Antiserum to S-100 protein labels delicate processes (red), whereas detectable neuropeptide Y immunoreactivity (yellow) is confined to discrete regions of the olfactory ensheathing cells, presumably the Golgi apparatus. Scale bar, 10 μm.

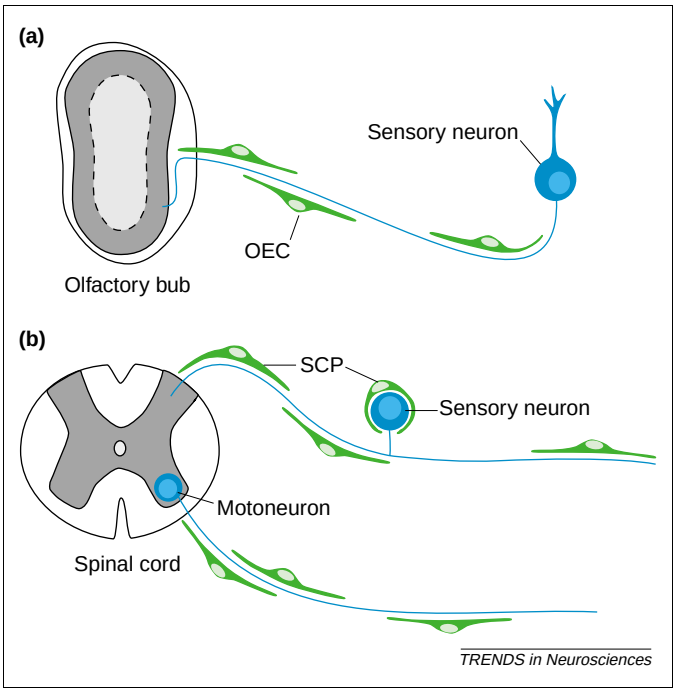

Figure 3. Comparison of olfactory ensheathing cells and Schwann cell precursors. **(a)** Neuropeptide Y (NPY)-positive olfactory ensheathing cells (OEC) around an olfactory sensory neuron projecting to the glomerular layer in the olfactory bulb. **(b)** NPY-positive Schwann cell precursors (SCP) around a sensory dorsal root ganglion neuron and its axons, as well as around the axons of a motoneuron. Whereas olfactory ensheathing cells express NPY during their entire life, Schwann cell precursors do so only for a very restricted period during development. The NPY expression is high close to the spinal cord, perhaps in so-called boundary-cap cells, but the extent to which NPY is present in Schwann cell precursors along the entire axon remains to be further analyzed.

target-derived signals once the axons have reached their target fields. That NPY could be necessary and sufficient in such a role seems unlikely because crossing $NPY^{-/-}$ mice [52] with the P2-IRES-tau-LacZ mice created by Mombaerts *et al.* (in which a single olfactory receptor has been genetically labelled with a tau-LacZ construct, allowing visualization of the entire neuron from the olfactory mucosa to its glomerulus) [53] failed to reveal any defects in olfactory neuron pathfinding (Figure 4).

Recently, however, Hansel *et al.* [54] showed that NPY-deficient mice have decreased proliferation of developing olfactory receptor neurons (Figure 5a,b) and that NPY, via Y1 receptors, PKC and ERK1/2, can induce neuronal precursor proliferation (Figure 5c,d). The source of NPY appears to be developing olfactory receptor neurons, and this source is later switched in the adult rat to a subpopulation of non-neuronal sustentacular cells. On the basis these findings, it seems that throughout life olfactory ensheathing cells that synthesize NPY at a high level have an important role in providing continuous trophic support, revealing a clear role for a peptide produced at least in part in glia.

No study similar to that of Hansel *et al.* [54] has investigated the possible role of NPY in control of proliferation of sympathetic and dorsal root ganglion neurons. Moreover, it has yet to be determined which, if any, receptors are around at the time, when NPY is expressed in Schwann cell precursors.

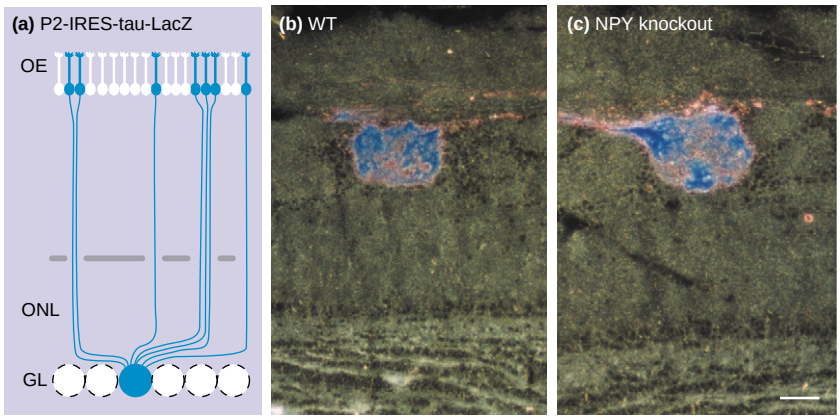

Figure 4. Crossing neuropeptide Y (NPY) knockout mice with the Mombaerts P2-Ires-tau-LacZ mice. **(a)** In the Mombaerts mice, olfactory sensory neurons expressing the P2 odorant receptor are genetically labelled (blue). These neurons project to two glomeruli in the glomerular layer (GL) of each olfactory bulb (one lateral and one medial; for simplicity, only one side is shown here). After crossing the mice, the corresponding glomeruli are labelled both in wild-type (WT) **(b)** and NPY knockout **(c)** animals. Scale bar, 70 μm. Abbreviations: OE, olfactory epithelium; ONL, outer nuclear layer.

White [55] has shown that NPY can indirectly stimulate neurite outgrowth from cultured dorsal root ganglia by releasing neurotrophin 3 from spinal cord slices in culture, but it is not clear whether this occurs *in vivo*. NPY also stimulates the proliferation of smooth muscle cells of blood vessels, acting both via Y1 and Y2 receptors [56]. This raises the interesting possibility that such trophic effects might be utilized in the form of olfactory-ensheathing-cell transplantation, for repair of central pathways including spinal cord lesions [57,58].

Galanin in glia

Galanin protein and preprogalanin mRNA are expressed in a subset of small glia of the adult rat brain after intraventricular administration of colchicine [27], which blocks fast axonal transport and mitosis. The colchicine-induced upregulation of galanin in glia is strongly dependent on thyroid hormone [59], possibly via a thyroid-hormone-responsive element on the galanin promoter [60]. Moreover, oligodendrocyte precursor cells express thyroid hormone receptors, which could be involved in galanin upregulation, and thyroid hormone is a key regulator for oligodendrocyte maturation that also triggers cell cycle exit [61,62]. Recent studies show that galanin and its transcript can be transiently expressed during cortical spreading depression by a population of NG2-positive glia [28]. NG2, a major chondroitin sulfate proteoglycan, could be a marker for oligodendrocyte precursors [63,64], which are small (10–15 μm diameter) cycling cells, abundant in both grey and white matter [63,65]. In addition, the galanin-positive glia seen after colchicine treatment co-localize with NG2 (Figure 6).

Galanin is upregulated in several types of neurons under various experimental conditions, including peripheral nerve injury [66] and treatment with vinblastine [67], a mitosis inhibitor closely related to colchicine. The elevated galanin levels in dorsal root ganglion neurons could promote trophic processes after injury [68], such as neurite outgrowth via the galanin R2 receptor [69]. The detection of galanin and galanin receptors prenatally in dorsal root ganglion neurons [70] and abundantly even in embryonic stem cells [71], supports a developmental role. It is possible that galanin can regulate proliferation, migration and differentiation of neurons and oligodendrocyte precursors via autocrine and/or paracrine mechanisms. In other systems, galanin influences growth processes, including tumour development. However, these effects are equivocal – both growth

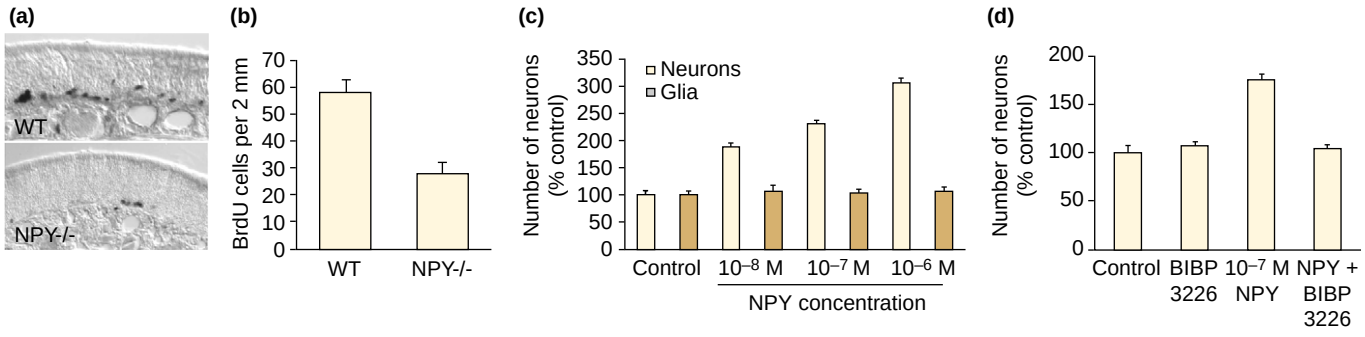

Figure 5. Neuropeptide Y (NPY) has a proliferative effect on olfactory neurons. **(a,b)** Bromodeoxyuridine (BrdU) labelling of adult mouse brain shows decreased postnatal neurogenesis in the precursor cell population of NPY knockout mice compared with that of wild-type (WT) mice. Immunohistochemical results are shown in (a) and quantitative data in (b). **(c)** After 24 h incubation of one-day-old primary olfactory neuronal cultures with NPY, there is an increase in neuron number, as demonstrated by immunohistochemical staining with neuron-specific tubulin. **(d)** After 24 h incubation with NPY there is an increased number of neurons in olfactory mucosal cultures, and this increase is blocked by the NPY Y1-receptor antagonist BIBP3226. Reproduced, with permission, from Ref. [54], © (2002) Macmillan Publishers Ltd (http://www.nature.com).

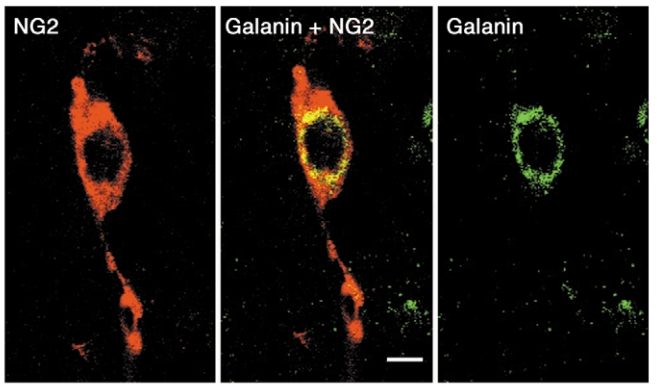

Figure 6. Double labelling of a cell in the corpus callosum of rat after intraventricular colchicine injection, showing galanin expression (green) together with the oligodendrocyte-precursor-specific marker NG2 (red). Scale bar, 5 μm.

stimulating [72] and antiproliferative [73,74] effects have been observed.

Can peptides be released from glia?

In neurons, neuropeptides are typically stored and released from large (electron) dense-core vesicles (LDCVs). Little is known about neuropeptide storage and release from glia. After colchicine treatment, electron microscopy shows the presence of NPY-immunoreactive granules, apparently associated with the Golgi apparatus and possibly representing LDCVs, in olfactory ensheathing cells [31]. Recently, Krzan *et al.* [75] have transfected cortical astrocytes with pro-ANP fused with emerald-green fluorescent protein, resulting in fluorescent puncta presumably representing secretory granules. Using a reporter of cumulative exocytosis, they obtained evidence that ANP is released by regulated Ca^{2+}-dependent exocytosis from astrocytes.

Concluding remarks

The findings summarized here expand the overall view on the function of neuropeptides and our understanding of neuron–glia interactions. Taken together, there is now strong evidence that neuropeptides are synthesized in astrocytes, oligodendrocytes, microglia and Schwann cells. In many cases, glial neuropeptide levels are very low and culturing, lesions or other manipulations cause upregulation and/or induction. The presence of neuropeptide receptors on glia already, and often preferentially, during prenatal life, strongly suggests that glia–glia and glia–neuron interactions are important during development as well as in the adult under normal and pathological conditions. Many of these effects might be autocrine and/or paracrine, but neuronal and glial elements probably also interact through complex cross-talk mechanisms. However, several questions remain to be answered. These include: (i) is the transcript indeed translated into peptide, (ii) is the peptide immunoreactivity specific (which could be answered by demonstrating presence of transcript), (iii) to what extent is the peptide expressed not only under culture conditions but also *in vivo*, and (iv) what are, in individual cases, the targets of neuropeptides released from glia – peptide receptors on glia, on neurons, or on both?

Acknowledgements

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