CO-EXISTENCE OF NADPH-DIAPHORASE, FIBROBLAST GROWTH FACTOR-2 AND FIBROBLAST GROWTH FACTOR RECEPTOR IN SPINAL AUTONOMIC SYSTEM SUGGESTS TARGET-SPECIFIC ACTIONS

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Abstract—In the rat spinal cord, we found substantial co-existence of fibroblast growth factor-2, fibroblast growth factor receptor (type-I or \( \text{fGF} \)) immunoreactivity and reduced nicotinamide adenine dinucleotide phosphate (NADPH)-diaphorase activity (a histochemical marker for neuronal nitric oxide synthase) in preganglionic autonomic cell groups of intermediate layers VI, VII and X. Anti-fibroblast growth factor and anti-nitric oxide synthase binding sites were confined to the cytoplasm of reactive neurons as judged by immunogold electron microscopy. Within the major autonomic nucleus, i.e. intermediolateral column, three different populations were identified: (i) fibroblast growth factor and fibroblast growth factor receptor, (ii) fibroblast growth factor/NADPH-diaphorase and (iii) NADPH-diaphorase-only stained cell groups. Sympathoadrenal neurons were prelabelled with fluorescent tracer Fast Blue and co stained for fibroblast growth factor-like protein and NADPH-diaphorase, suggesting heterologous diversification of neuronal phenotypes and functional organization in the spinal autonomic system.

Our findings suggest intriguing roles for nitric oxide and fibroblast growth factor-2 cytokine in the preganglionic sympathetic spinal cord system: The "short-term" diffusible messenger nitric oxide may act as "tonic" and/or "phasic" signal within rostrocaudally oriented function-specific preganglionic units necessary for integrated target control. The "long-term" messenger fibroblast growth factor-2 may be involved in, for example, cytokine-dependent regulation of neuronal NADPH-diaphorase/nitric oxide synthase. Furthermore, co-existence of NADPH-diaphorase, fibroblast growth factor-2 and receptor in sympathoadrenal neurons suggest mutual target-specific regulatory functions, e.g. hormone release and blood perfusion or maintenance of phenotype and plasticity responsiveness of adrenal medullary tissue.

Key words: central nervous system, autonomic motor neurons, sympathoadrenal system, nitric oxide synthase, adrenal gland, retrograde labelling.

The autonomic nervous system is considered the major efferent part of the peripheral nervous system that regulates the function of all visceral target organs in the body, with the exception of skeletal muscle. Of the three components, the sympathetic, parasympathetic and enteric systems, the somata of sympathetic preganglionic neurons reside in the intermediate zone between the dorsal and ventral gray of the thoracolumbar spinal cord, where they are organized in a ladder-like fashion of rostrocaudally and mediolaterally segmented cell groups referred to as autonomic spinal cord nuclei. These nuclei are composed of heterogeneous subgroups reflecting precise functional organization for the sympathetic outflow to peripheral targets. In all parts of the nervous system, neuronal subgroups may be identified according to their neurochemical or biophysical properties, which provide valuable markers for studying neuronal phenotype and functional diversification. One way to further characterize neuronal phenotypes is to find correlations between the known patterns of, for example, neuropeptides or neurotransmitters and novel messenger molecules.

Since the discovery of nitric oxide synthase (NOS), which generates the free radical molecule nitric oxide (NO) in the CNS, unique functions have been proposed for this gaseous molecule, e.g. neural signalling, learning and memory or cytotoxic and/or injury-induced neuronal dysfunctions. Besides expression in the CNS, striking patterns of NOS and its associated NADPH-diaphorase activity have been found in peripheral ganglia, extra- or intramural neurons or fibre plexuses, suggesting multiple functions of NO as, for example, a non-cholinergic, non-adrenergic transmitter for peripheral autonomic subsystems. In spinal autonomic nuclei, NOS and NADPH-diaphorase co-localize within choline acetyltransferase-positive sympathetic...
and parasympathetic cell groups, thus documenting substantial co-existence of the NO system with the classical neurotransmitter acetylcholine in central cholinergic systems.

Recently, novel functions have also been documented for the fibroblast growth factors (FGFs), primarily known as mitogenic and angiogenic mesenchymal growth factors, as important regulatory messengers in nervous system development, maintenance and regeneration. The two prototypic family members, acidic and basic FGF (designated FGF-1 and -2), were originally purified from pituitary and adrenal glands. At present, the FGF family comprises nine different, yet structurally related, molecules (FGFs 1–9) serving as pleiotropic and multifunctional cytokines involved in mesoderm and neuroectoderm-derived cell proliferation and differentiation. The biological actions of FGF's are mediated by ligand binding to cell surface receptors (FGF-Rs). Besides low-affinity FGF-Rs, members of cell surface heparan sulphate proteoglycans, four different genes of high-affinity FGF-Rs (designated FGF-R1–4) are presently known. Meanwhile, widespread expression of FGF, FGF-R and FGF mRNAs has been shown in central and peripheral neurons of various mature brain areas, while their distribution patterns and possible functions within defined neural systems have been investigated only recently. Accordingly, much needs to be learned about the co-existence of FGF with other neurochemical properties or messenger molecules, first yet important steps towards unravelling the significance of regulatory molecules in specific loci of the CNS.

As the presence of FGF-2 is not well-documented in the spinal autonomic neurons and because of the intriguing expression of FGF and NOS in the CNS, we have used NADPH-diaphorase histochemistry and NOS, FGF-2 and FGF-R immunohistochemical detection for documentation of their cellular distribution and possible co-existence in spinal autonomic subnuclei and in sympathoadrenal neurons identified by retrograde tracing in vivo.

**EXPERIMENTAL PROCEDURES**

**Materials and antibodies**

Chloral hydrate, Fast Blue (FB) and Triton X-100 were purchased from Sigma Chem. Co., Munich, Germany. Heparin (25,000 units) was obtained from Hoechst, Frankfurt, Germany. Reduced β-NADPH, glycine, nitroblue tetrazoliumchloride and Tris were bought from Riedel, Hamburg, Germany. Monoclonal anti-bovine basic FGF type II (IgG1k), polyclonal anti-FGF-R1 (fg), raised against an external region peptide of the chicken FGF-R which is cross-reactive with fg receptor in rat tissue, and FGF-R antigen were purchased from Upstate Biotechnology, Lake Placid, NY, U.S.A. Recombinant bovine or human basic FGF-2 protein was from Progen, Heidelberg, Germany. Preimmune animal sera, biotinylated anti-mouse immunoglobulin G and anti rabbit immunoglobulin G peroxidase kits were from Vector Laboratories (Burlingame, CA, U.S.A.). Affinity purified polyclonal anti-NOS raised in rabbits against the synthetic peptide from the C-terminal of the cloned rat cerebellar NOS was supplied by Euro-Diagnostica B.V., Apeldoorn, The Netherlands. The chromogen 3,3'-diaminobenzidine was supplied as tablets by Kementec, Copenhagen, Denmark. One nanometre (1 nm) colloidal gold-coupled goat-anti rabbit immunoglobulin G and 5 nm gold-labelled goat anti-mouse immunoglobulin G were from Amersham, Braunschweig, Germany. All other products including buffer ingredients were of analytical grade and were purchased from Merck, Darmstadt, Germany. Electron microscopic material was from Plano, Marburg, Germany. Frozen sections were cut on a Cryotome (Reichert–Jung), thick sections on a Vibratome (Oxford Instruments, U.K.) and ultrathin sections on an Ultracut (Reichert–Jung, Heidelberg, Germany). Photographic documentation was made on 400 ASA black/white (Ilford, U.K.) or colour films (Fujichrome, Tokyo, Japan).

**Animals and tissue preparation**

For light microscopy, female 90-day-old Wistar rats (n = 10) were anaesthetized with chloral hydrate (350 mg/kg body weight, i.p.), transcardially perfused with isotonic NaCl/heparin followed by freshly prepared 4% (w/v) paraformaldehyde in 100 mM sodium phosphate-buffered saline, pH 7.4. After dissection, the spinal cords were postfixed overnight in 10% buffered formaldehyde. For retrograde labelling in vivo, a separate animal group (n = 5) received unilateral injections of the fluorescent tracer FB (2% w/v aqueous solution) into the left adrenal medulla. Five days later, FB-injected animals were perfusion-fixed and spinal cords with prelabelled intermediolateral (IML) cell column treated as given above. Spinal cords were cryopreserved with 15% (w/v) sucrose overnight and cryosectioned (25 μm) in the horizontal plane. For electron microscopic immunolabelling, five anaesthetized animals (n = 5) were transcardially perfused with 3% (w/v) paraformaldehyde/0.25% (v/v) glutaraldehyde in 60 mM sodium phosphate buffer, pH 7.3. The spinal cords were dissected and postfixed for 30 min, horizontal 15 μm-thick sections were cut (Vibratome) and processed for immunogold electron microscopy.

**Histochemistry and immunohistochemistry**

From the spinal cords, a pool of serially cut frozen sections was collected in separate 24 mm multwells filled with ice-cold phosphate-buffered saline. For better orientation, every sixth section of each cord was processed for NADPH-diaphorase reaction according to an improved histochemical protocol which allows for reliable and optimal staining results. Parallel frozen sections of intermediate spinal cord areas were mounted on protein-coated slides, air-dried and preincubated with blocking buffer (100 mM Tris–HCl, pH 7.6/2% preimmune serum) followed by 18 h incubation at 4°C with (i) monoclonal anti-basic FGF-2, diluted 1:250 in blocking buffer, (ii) polyclonal anti-FGF-R1, diluted 1:300 in blocking buffer. After several rinses with buffer, visualization of primary antibody was performed by secondary antibody detection (biotinylated horse anti-mouse or goat anti-rabbit immunoglobulin G peroxidase) and subsequent incubation in fresh 0.1% (v/v) diaminobenzidine/0.01% (v/v) H2O2 in Tris–HCl, pH 7.6, for 10 min in the dark. Controls were carried out with omission of primary antibody or incubation with antibody (1:100) preadsorbed with antigen (1 μg basic FGF or 2.5 μg chicken FGF-R protein, for 6 h on a shaker).

**Immunogold electron microscopy**

Vibratome thick sections (100 μm) were transferred to incubation buffer: 0.5% (w/v) sodium borohydrate/0.1% (w/v) glycine in 60 mM sodium phosphate, pH 7.3, for 15 min, then in 0.5% (w/v) bovine serum albumin (BSA)/0.1% (w/v) gelatine for 30 min. Finally, preincubated sections were incubated with anti-NOS and anti-FGF-2
antibodies, diluted 1:250 in BSA/gelatine, overnight at 4°C. After washing (BSA/gelatine, 2 h on a shaker), thick sections were incubated with goat anti-rabbit immunoglobulin G coupled to 1 nm colloidal gold and goat anti-mouse immunoglobulin G coupled to 5 nm gold (1:50 in BSA/gelatine) for 6 h at 37°C (on a shaker), washed extensively in buffer (20 min on a shaker) and fixed in 2.5% (v/v) buffered glutaraldehyde (10 min). To improve detectability, the size of the gold particles was increased by silver enhancement.17 Thick sections were then dehydrated in graded ethanol series and flat-embedded in Araldite plastic. Selected tissue areas with silver-enhanced, immunoreactive cell groups visible in 1-μm plastic sections (Fig. 4a, b) were ultrathin-sectioned (60-100 nm), double-stained and photographed by a Zeiss EM-10 electron microscope.

RESULTS

Fibroblast growth factor-2 and receptor immunolocalization

Horizontal frozen sections were stained for NADPH-diaphorase to show the rostrocaudal topography of the main autonomic cell groups of the lower thoracic rat spinal cord and for appropriate orientation in immunohistochemically stained serial sections (Fig. 1). At the lower thoracic spinal cord segments Th7 to lumbar segments L1, FGF-2 immunoreactivity was detected in the neuronal cell groups known as the IML, intercalated and central autonomic nuclei of the intermediate spinal cord gray in Rexed's laminae VI, VII and Xc (Fig. 2a-c). In addition to perikaryal staining, small oval-shaped and densely stained structures reminiscent of astrocyte nuclei were also found to be FGF-2 positive (cf. Fig. 2a, b). Capillaries or nerve fibres were not stained. In parallel sections stained for FGF-R, marked similarities were found to FGF-2 staining patterns with respect to topographical distribution, neuronal localization and cytoplasmic expression of FGF-R immunoreactivity (Fig. 2d-f), suggesting coexistence of both markers which, however, remains to be shown by double-labelling experiments. In addition to neuronal localization, the FGF-R antibody also demarcated capillary walls in spinal cord tissue (not shown). Immunostaining was completely negative when antigen-preadsorbed antibody was used or when primary antibody was omitted from the immunohistochemical protocol (data not shown).

Co-localization of NADPH-diaphorase and fibroblast growth factor 2

NADPH-diaphorase histochemistry was applied to spinal cord sections prior to FGF-2 immunohistochemistry (Fig. 3). Combined NADPH-diaphorase/FGF-2 labelling revealed the presence of three apparently differently stained cell groups of the IML column: (i) NADPH-diaphorase-only stained neurons, (ii) neurons double-stained with NADPH-diaphorase/FGF-2, and (iii) neurons with FGF-2 immunoreactivity (Fig. 3a, b).

Ultrastructural localization of fibroblast growth factor-2 and nitric oxide synthase

Thick sections of spinal cords were processed for the silver-enhanced immunogold method. In 1-μm plastic sections, anti-FGF-2 or anti-NOS binding sites are seen as small gold particles over neuronal somata (Fig. 4a, c). Ultrasections of these areas reveal the subcellular distribution of gold-labelled anti-FGF and anti-NOS binding sites which are mainly localized in the cytosol (Fig. 4b, d).

Retrograde labelling of sympathoadrenal neurons

From the variously stained subgroups of the IML cell column, sympathoadrenal projection neurons were retrogradely labelled in vivo by FB and the spinal cord sections immunostained for FGF-2-like protein. Although FGF immunoreactivity is clearly present, it is not as strong as that detected routinely in these neurons (cf. Fig. 3), possibly due to weaker antibody reaction, if not antigen masking, elicited by the amount of fluorescent dye. Results from these experiments show that the retrogradely labelled and fluorescent cell bodies belong to the FGF-2-positive cell group in the IML column (Fig. 5).
DISCUSSION

In this report, FGF-2 and FGF-R immunostaining and immunoelectron microscopic detection have been documented in autonomic nuclei of the rat spinal cord. Although FGF and FGF-R expression has been investigated in central and peripheral neuronal regions, including spinal cord (see next paragraph), their presence in the spinal autonomic nuclei, to the best of our knowledge, has not yet been documented. Furthermore, we provide evidence for substantial co-existence of NADPH-diaphorase enzyme activity, a marker for neuronal NOS expression, and FGF-2 immunoreactivity within neurons. In addition, in vivo prelabelled sympathoadrenal neurons have been co-stained for NADPH-diaphorase and FGF-2-like protein. Based on their distinct immunostaining patterns and co-distribution within target-specific neuronal subgroups, our findings suggest intriguing roles for the two messengers in the autonomic spinal cord system and in adrenomedullary innervation in particular.

Fibroblast growth factor and fibroblast growth factor receptor in the spinal cord and its autonomic nuclei

In the normal rat spinal cord, FGF-1 and -2 immunolocalization and FGF-R mRNA have been
found in somatic ventral motor neurons and dorsal sensory neurons \( ^{21,38,69} \) and fibres of the dorsal columns (fasciculus cuneatus and gracilis) and in regional astrocytes. \(^{29,40} \) From this and previous reports by others, we conclude that FGF-2 and FGF-R are also present in autonomic neurons of the intermediate spinal cord gray. While FGF-R1 \((f_{lg})\) is predominantly expressed by neurons, FGF-R2 and 3 are mainly found in glial cells. \(^{38,72} \) In our study, FGF-R immunolocalization was confined to neuronal cells and, in turn, may reflect the presence of FGF-R1 \((f_{lg})\)-like protein.

In selected areas of nerve tissue, disparate patterns of FGF and FGF-R have been reported in various cell types, e.g. neurons, glia or endothelium, \(^{62} \) nerve fascicles \(^{40} \) or extracellular matrix. \(^{5} \) FGF-2-immunoreactivity has been variably found in either cytosol or within the nucleus. \(^{93} \) Findings which might reflect (i) the existence of FGF-2 isoforms \((18,000-23,000\) or \(43,000\) mol. wt isoforms), or (ii) the variable subcellular distribution of “nuclear” and/or “cytosolic” FGF within perikarya. \(^{25} \) In our study, well-characterized monoclonal FGF-2 and polyclonal FGF-R antibody species \(^{28,31} \) adequate blocking experiments (with antigen-preadsorbed antibody) and optimal tissue fixation procedures have been used for reliable FGF immunohistochemistry. \(^{33} \)

In the spinal cord, FGF-1 immunoreactivity and mRNA expression co-localize with motor and sensory neurons, suggesting sequestration of FGF-1 protein in FGF-synthetizing neurons. \(^{20} \) Likewise, spinal cord motoneurons and interneurons reveal strong FGF-2 immunoreactivity; \(^{38} \) accordingly, they may accumulate considerable levels of FGF protein. Members of the FGF family are candidates for autocrine and/or intracrine (i.e. intracellularly acting) pathways in the nervous system, as regulators for cell proliferation and transmitter synthesis, direct gene transcription \(^{13} \) or neuronal survival. \(^{44} \) In our study, anti-FGF-2 binding sites were found in the cytosol of preganglionic somata by immunogold electron microscopy; thus, intracrine regulatory functions of FGF-2 similar to those proposed for other neuron classes \(^{42} \) may be working in preganglionic neurons as

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Fig. 3. Co-localization of NADPH-diaphorase activity (blue) and FGF-2 immunoreactivity (light brown) in the spinal IML nucleus. (a) Overview at horizontal plane, double-stained segmented IML cell groups. (b) Single and double staining identifies several subpopulations as seen at higher magnification: FGF-2-only (arrow A, light brown), NADPH-diaphorase/FGF-2 (arrow B, brown) and NADPH-diaphorase-only stained groups (arrow C, blue). Scale bar = 50 \(\mu\)m.
Fig. 4. Pairs of light and electron micrographs of preganglionic IML neurons immunogold-labelled for anti-NOS and anti-FGF-2 binding sites. In plastic sections inspected by light microscopy (left panel), immunogold particles are seen over “black-sprinkled” IML perikarya, thus visualizing NOS (a) or FGF-2 (c) like expression. Methylene Blue/Azur-II counterstain. By electron microscopy (right panel), anti-NOS (b) and anti-FGF-2 binding sites (d) are visualized by immunogold particles distributed in the cytoplasm. Silver-enhanced immunogold (normally round-shaped and size-matched particles) is detectable as irregular-shaped electron-dense particles. N, nucleus; rER, rough endoplasmatic reticulum. Scale bars = 10 μm (a, c); 0.5 μm (b, d).

well. On the other hand, FGF-R mRNA is expressed by spinal cord neurons, which may contain FGF proteins, supporting the notion of autocrine and/or paracrine pathways of FGF in the nervous system. However, FGF-1 and -2 lack conventional signal sequences; thus, availability of these cytokines from “FGF secretory cells” by classical secretory mechanisms has been questioned. Notably, cytokines which are devoid of signal sequences may be released via exocytosis mechanisms. With the exception of the primary release of FGF-2 from dead or damaged cells (i.e. “lesion factor”), the mechanisms of release, as well as the sources and targets of FGF, are still obscure in nerve tissue. The existence of such auto/paracrine functions of FGF under physiological conditions awaits experimental evidence. Neverthe-
which raises the possibility of retrogradely acting prelabelled in vivo by the retrograde fluorescent tracer FB as detectable in spinal cord sections by UV illumination. (b) Mediate spinal cord layers. These nuclei are organized in heterogeneous target-specific cell groups,3*5' defined by topographical, neurochemical or functional parameters, that account for the dynamic responses and specificity of the autonomic actions in the body.36 In the spinal cord, there is substantial co-existence of NADPH diaphorase/NOS staining with preganglionic neurons that contain the classical transmitter acetylcholine (i.e. about 50% in the IML cell group). In the present investigation, a subpopulation of FGF-2-immunoreactive IML neurons co-stained for NADPH-diaphorase activity, supporting the notion that the novel messenger molecule NO is synthesized within FGF-2-containing neurons. Similar to its partial co-expression with cholinergic neurons in the spinal cord, NADPH-diaphorase is confined to subgroups of FGF-2-positive autonomic neurons, suggesting functional rather than phenotypical linkages between NO formation and subclasses of neurons.

Both messengers have so far been shown to exert quite different mechanisms of actions in the nervous system; thus, their co-existence in neurons cannot be easily explained. Three classical NO-mediated functions have been characterized: (i) endothelium relaxation, (ii) neurotransmission and (iii) cell-mediated immune responses.7 In the nervous system, NO has been designated as a "neurotransmitter-like" agent, as it does not leave the cell via the vesicle-related neurosecretory pathway, the well-known route for classical stimulus-coupled transmitter release.45 The gaseous molecule NO readily diffuses from its sources (NO-producer cell) to reach its putative target cells,11 such as nearby somata of other neurons or glia, afferent or efferent synaptic partners (interneurons or peripheral ganglia) or non-neuronal tissue (smooth muscle). Some of these putative NO target cells have been shown to contain soluble guanylyl cyclase,46,47 the presently accepted "NO receptor" of the NO/cyclic GMP system.7 Release of NO from cells which contain the L-arginine-dependent NO pathway may either be constitutive or stimuli-controlled. According, NO represents a "short-term" intercellular signal molecule which acts in a time scale of seconds.11 In conclusion, the NO system in spinal autonomic areas may represent a "tonic" and/or "phasic" modulatory mechanism within anatomically and functionally heterogeneous preganghonic groups or integrated efferent control of dynamic peripheral target functions in vegetative homeostasis. Integrated action(s) of NO within spinal thoracolumbar preganglionic units is (are), for example, highlighted by the rapid sequential, i.e. reflexory target organ activation, of adrenomedullary catecholamine hormone secretion, cardiac rate and force or vasomotory control in response to stress conditions.

By contrast, FGF cytokines are known to act as "long-term" trophic messengers in the range of hours or days in development,7 or within weeks, months or even throughout lifetime in postnatal or adult nervous system maintenance and regeneration.64 NOS expression in non-neuronal cells, i.e. macrophages and glial cells, is regulated by the cytokines interleukin-1 or interferone gamma in vitro12,25 and, therefore, direct or indirect regulation of NOS by FGF-2 cannot be ruled out. In fact, FGF-2 administration in vivo prevented loss of NADPH-diaphorase activity in lesioned preganglionic neurons,76 supporting the idea that messenger co-existence may not be acciden-

![Fig. 5. Presence of FGF-2-immunoreactivity in sympatoadrenal neurons of the IML column. (a) IML neurons were prelabelled in vivo by the retrograde fluorescent tracer FB as detectable in spinal cord sections by UV illumination. (b) Retrogradely labelled IML neurons were immunostained for anti-FGF-2 as seen by light microscopy.](image)
Nitric oxide synthase and fibroblast growth factor-2: co-existence in the sympathoadrenal system: implications for mutual target-specific actions?

Based on the documented co-distribution of NADPH-diaphorase activity, NOS and FGF-2-like proteins, a link between anatomical localization and physiological or pharmacological effects of NO or FGF thus appears justified.

In the adrenal medulla, NOS immunoreactivity has been found in intrinsic ganglion cells, bundles and dense varicose fibre networks embedding the chromaffin cell and in perivasculat fibrils. Supporting the notion that splanchic control of NO-generating sympathoadrenal neurons may be two-fold for chromaffin cell function, i.e. catecholamine secretion, and/or for adrenal blood perfusion rates via vasodilatory actions of NO to promote access of hormones to systemic circulation. Besides acetylcholine, corelease of non-cholinergic transmitter(s) by splanchic terminals has been proposed for catecholamine secretion in isolated perfused adrenals. In similar perfusion models, splanchic stimulation resulted in NO-mediated stimulation of soluble guanylyl cyclase and cyclic GMP levels in adrenomedullary tissue and chromaffin cells. Likewise, adrenal vascular tone was affected by the NO precursor l-arginine. However, adrenomedullary vasodilation is blocked by the NO inhibitor nitroarginine methyl ester without affecting hormone secretion; thus, separate but parallel neural regulation of NO versus acetylcholine may be operating in vivo. Notably, FGF-induced synthesis of endothelium-derived relaxing factor, which is identical to NO, strongly suggests mutual actions of both messengers in, for example, adrenal vascular tone control. There is also evidence for NOS immunoreactivity in chromaffin cells, which also immunostain for FGF-like proteins and FGF gene expression. However, their putative roles in chromaffin cell function have to be further substantiated by, for example, co-localization studies. Finally, FGF-2 and possibly also other neurotrophic cytokines, such as glutamatergic and NOS-containing sympathetic ganglionic neurons, may have unique, if not mutual, role(s) for autonomic efferent control and for endocrine target organ functions, which may be assessed further by co-localization studies, and physiological and pharmacological investigations of defined central and peripheral nervous systems and the targets they innervate.

CONCLUSION

Besides splanchnic autonomic control of peripheral targets by the classical neurotransmitter acetylcholine, the free radical NO and FGF cytokine(s) may have unique, if not mutual, role(s) for autonomic efferent control and for endocrine target organ functions, which may be assessed further by co-localization studies, and physiological and pharmacological investigations of defined central and peripheral nervous systems and the targets they innervate.

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