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GENERATION AND PHENOTYPIC CHARACTERIZATION OF A GALANIN OVEREXPRESSING MOUSE

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Abstract—In most parts of the peripheral nervous system galanin is expressed at very low levels. To further understand the functional role of galanin, a mouse overexpressing galanin under the platelet-derived growth factor-B was generated, and high levels of galanin expression were observed in several peripheral tissues and spinal cord. Thus, a large proportion of neurons in autonomic and sensory ganglia were galanin-positive, as were most spinal motor neurons. Strong galanin-like immunoreactivity was also seen in nerve terminals in the corresponding target tissues, including skin, blood vessels, sweat and salivary glands, motor end-plates and the gray matter of the spinal cord. In transgenic superior cervical ganglia around half of all neuron profiles expressed galanin mRNA but axotomy did not cause a further increase, even if mRNA levels were increased in individual neurons. In transgenic dorsal root ganglia galanin mRNA was detected in around two thirds of all neuron profiles, including large ones, and after axotomy the percentage of galanin neuron profiles was similar in overexpressing and wild type mice. Axotomy reduced the total number of DRG neurons less in overexpressing than in wild type mice, indicating a modest rescue effect. Aging by itself increased galanin expression in the superior cervical ganglion in wild type and transgenic mice, and in the latter also in preganglionic cholinergic neurons projecting to the superior cervical ganglion. Galanin overexpressing mice showed an attenuated plasma extravasation, an increased pain response in the formalin test, and changes in muscle physiology, but did not differ from wild type mice in sudomotor function. These findings suggest that overexpressed galanin in some tissues of these mice can be released and via a receptor-mediated action influence pathophysiological processes. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: nerve injury, extravasation, sensory ganglia, sympathetic ganglia, pain.

Galanin is in most species a 29 (30 in human) amino acid peptide with an amidated C-terminal and was first isolated from the porcine intestine (Tatemoto et al., 1983). Galanin was subsequently isolated/cloned from bovine (see Rökaeus and Brownstein, 1986), rat (Vrontakis et al., 1987; Kaplan et al., 1988), human (Bersani et al., 1991; Evans and Shine, 1991; Schmidt et al., 1991; McKnight et al., 1992) and several other species showing a high degree of homology, whereby the N-terminal 14 amino acids are conserved in all species so far analyzed, with the exception of tuna fish (see Langel and Bartfai, 1998). Galanin derives from a large precursor molecule with 123 amino acids which also harbors a second peptide termed galanin message-associated peptide (GMAP) (Rökaeus and Brownstein, 1986). Initial studies showed that galanin is widely distributed both in the central and peripheral nervous system (Rökaeus et al., 1984; Ch'ng et al., 1985; Ekblad et al., 1985a; Melander et al., 1985, 1986; Skofitsch and Jacobowitz, 1985a,b, 1986; Furness et al., 1987). Several review articles (Bartfai et al., 1993; Merchenthaler et al., 1993; Crawley, 1995; Leibowitz, 1995; Kalra and Kalra, 1996; Zigmond et al., 1996; Fuxe et al., 1998; Kerr et al., 2000b; Xu et al., 2000; Gundlach, 2002; Vrontakis, 2002; Ubink et al., 2003) and books (Hökfelt et al., 1991, 1998) have reported on manifold functional aspects of galanin.

In the sympathetic nervous system, i.e. in the rat superior cervical ganglion (SCG), galanin-like immunoreactivity (LI) is normally seen in only a few neurons (Strömberg et al., 1987). Following transection of the two efferent carotid nerves galanin is strongly upregulated in the neuronal cell bodies of rat and mouse SCG (Rao et al., 1993; Klimaschewski et al., 1994, 1996; Mohney et al., 1994;

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Abbreviations: CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; EDL, extensor digitorum longi; FITC, fluorescein isothiocyanate; GalOE, galanin overexpressing; GMAP, galanin message-associated peptide; HPLC, high performance liquid chromatography; ir, immunoreactive; LI, like immunoreactivity; $mRNA^+$, mRNA-positive; NOS, nitric oxide synthase; NP, neuron profile; NPY, neuropeptide Y; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PDGF-B, platelet-derived growth factor B; RIA, radioimmunoassay; rOD, relative optical density; SCG, superior cervical ganglion; SSC, standard saline citrate; TSA, tyramide signal amplification; VAChT, vesicular acetylcholine transporter; VIP, vasoactive intestinal polypeptide; WT, wild type.

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Schreiber et al., 1994; Zhang et al., 1994; Shadiack et al., 1995).

In sensory ganglia, such as dorsal root ganglia (DRGs), galanin is normally expressed only at low levels in a few small neurons, both in the rat (Ch'ng et al., 1985; Skofitsch and Jacobowitz, 1985a) and mouse (Corness et al., 1996; Shi et al., 2001). After axotomy there is a marked upregulation of galanin, mainly in small- and medium-sized neurons in both species (Hökfelt et al., 1987; Villar et al., 1989; Corness et al., 1996; Shi et al., 2001).

Previous studies have shown that in adrenal chromaffin cells and adrenal nerve terminals of various species, galanin and/or GMAP are expressed at low to moderate levels (Bauer et al., 1986; Lundberg et al., 1986; Pelto-Huikko, 1989; Rökaeus et al., 1990; Zentel et al., 1991).

Since galanin under normal circumstances is expressed at very low levels in several systems, we and others (Cai et al., 1999; Steiner et al., 2001; Bacon et al., 2002; Holmes et al., 2003; see Crawley et al., 2002) have generated mice overexpressing galanin (GalOE mice), in our case with the platelet-derived growth factor-B (PDGF-B) promoter (Sasahara et al., 1991) linked to a large fragment of the galanin/GMAP gene including the second intron. It is hoped that such mice can provide some insight into the functional role of galanin released from endogenous stores, complementing multiple studies based on administration of exogenous galanin, and perhaps providing clues for new therapeutic strategies.

The aim of the present work was therefore to define possible changes in behavioral phenotype of GalOE mice. In view of the broad distribution of PDGF-B (Sasahara et al., 1991) a similarly wide galanin expression can be expected. Therefore, to interpret possible phenotypic changes, it is necessary to perform a careful mapping of galanin in the GalOE mice. Here we focus on several peripheral tissues of our GalOE mouse, including sympathetic and sensory ganglia, adrenal gland, skin and muscle as well as spinal cord. We compared young adult GalOE mice with young wild type (WT) C57BL/6 mice, and in some cases also aged GalOE and WT mice. These mice were studied after transection of the carotid nerves or the sciatic nerve, and functional studies on extravasation, pain (formalin test), sweat secretion and muscle function were also carried out. Some of these results have previously been reported in abstract form (Holmberg et al., 2000). We have in a parallel paper also described the galanin distribution patterns in the brain of this GalOE mouse (Kuteeva et al., 2004).

EXPERIMENTAL PROCEDURES

Generation of transgenic mice

The 1.3 kb PDGF-B promoter (Collins et al., 1985; Sasahara et al., 1991) was excised from the psisCAT6a plasmid with *Xba*I and *Hind*III (New England Biolabs Inc., Beverly, MA, USA) and cloned into the *Xba*I/ *Hind*III restricted LITMUS 29 vector (Evans et al., 1995). The galanin/GMAP gene construct, including the second endogenous intron of the galanin gene and containing 5′ *Sal*I and 3′ *Hind*III restriction sites, was ligated into the LITMUS/PDGF-B promoter plasmid. The galanin/GMAP fragment was created by polymerase chain reaction (PCR), from both mouse cDNA and genomic DNA. In a second round, the PCR products from the first round were used as overlapping templates, and the 5′- and 3′-terminal primers contained restriction sites for *Sal*I and *Hind*III, respectively. The PDGF-B promoter/galanin gene construct (see Fig. 1a) was excised from the XL-1 blue plasmid backbone using *Xba*I and *Hind*III. The excised PDGF-B promoter/galanin fragment was injected at a concentration of 2–3 ng/μl into pronuclei from fertilized mouse oocytes (Hogan et al., 1986) from a cross between F1(C57Bl/6×CBA) mice. The experimental procedures were approved by the local ethics committee (Stockholms Norra Djurförsöksetiska Nämnd). The experiments conformed to the guidelines of the Society for Neuroscience on the ethical use of animals. The number of animals was minimized, and precautions were taken to minimize animal suffering.

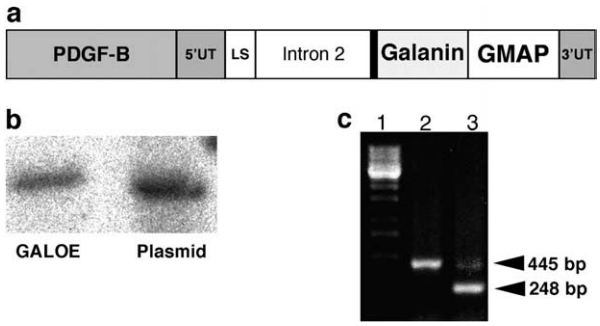

Fig. 1. (a–c) (a) Schematic drawing of the galanin/GMAP construct consisting of the PDGF-B promoter, the 5′ untranslated region, a leader sequence (LS), a second intron from the galanin gene followed by the signaling sequence (black), the galanin/GMAP cDNA, and finally a 3′ untranslated region. (b) Southern blot of GalOE DNA. A galanin plasmid was used as a control. (c) PCR products from genotyping. Lane 1 shows the 1 kb stair, lanes 2 and 3 the 445 bp WT band and the 248 bp GalOE band, respectively. In lane 3 a weak 445 bp band is seen indicating endogenous galanin.

DNA preparation and genotyping

Mouse tail biopsies were lysed in a buffer [pH 8.0; 50 mM Tris, 100 mM EDTA, 100 mM NaCl, 1% (w/v) SDS] with 100 μg/ml Proteinase K (Boehringer Mannheim, Mannheim, Germany) overnight at 55 °C. DNA was extracted in 2-propanol and centrifuged at 13,000 r.p.m. for 10 min at 4 °C, washed in 70% ethanol and dissolved in Tris–EDTA buffer (10 mM, 1 mM; pH 8.0). DNA was amplified using PCR with specific primers for galanin 5′-TGCCTCCCTAGAGTCGACGAGGGATCCTCGTGCGCT-3′ and 5′-AGGCATCCCAAGTCCCAGAGTGGCTGA-3′ and *Taq*DNA polymerase (2.5 U; Sigma, St. Louis, MO, USA) at 95 °C for 45 s, at 54 °C for 1 min and at 72 °C for 1 min for 30 cycles, and finally at 72 °C for 10 min. The PCR product was separated on a 1.4% (w/v) agarose gel containing 5 μl ethidium bromide (10 mg/ml)/100 ml gel, and visualized with UV-light showing a 445 base pair WT band or a 248 base pair GalOE band.

Southern blot

Genomic DNA (10 μg) from GalOE and WT mice was cleaved using a *Bam*HI restriction enzyme (100 U/μl) (NEB) in a 10× *Bam*HI buffer containing 1% bovine serum albumin overnight at 37 °C. As a control, 0.1 ng of plasmid was cleaved with the same enzyme, but for 1 h at 37 °C. As the size of the plasmid construct (ca 3×10^3 bp) represents approximately $1/10^6$ of the size of the mouse genome (ca 3×10^9 bp), 0.1 ng of plasmid represents 10× more copies of the gene than what is contained in 10 μg genomic DNA. DNA was separated on a 0.7% agarose gel containing ethidium bromide, then blotted over to a Hybond-N membrane