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Effects of Alpha 1- and Alpha 2-Adrenoreceptor Stimulation on Galanin mRNA Expression in Primary Cultured Superior Cervical Ganglion Neurons

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Abstract

Galanin (Gal) is a 29-amino-acid neuropeptide which is expressed in superior cervical ganglion (SCG) neurons and plays a trophic role in the adult animal and acts as an inhibitory modulator of cholinergic and noradrenergic neurotransmission. Whether activation or inhibition of alpha-adrenoreceptors influences Gal mRNA expression in SCG neurons remains unknown. Here, we have evaluated the possible regulation of Gal mRNA expression with acute (4 h) and chronic (4 days) stimulation of alpha 1- and alpha 2-adrenoreceptor agonists or antagonists in primary cultured SCG neurons. The results showed that the amount of Gal mRNA expression in cultured SCG neurons increased significantly after chronic stimulation with alpha 2-adrenoreceptor antagonist yohimbine compared with control SCG neurons at the same time point, whereas the amount of Gal mRNA expression decreased significantly after chronic stimulation with alpha 2-adrenoreceptor agonist clonidine as compared with that in control group. All these effects were not dose-dependent on the administration of alpha 2-adrenoreceptor agonist clonidine or alpha 2-adrenoreceptor antagonist yohimbine. Alpha 1-adrenoreceptor agonist phenylephrine or antagonist prazosin chronic stimulation did not have effects on Gal mRNA expression. Acute exposure of these agents did not have effects on Gal mRNA expression. The present study showed that Gal may be regulated by activation or inhibition of alpha 2-adrenoreceptors, but not alpha 1-adrenoreceptors in sympathetic neurons.

Key Words: Adrenoreceptor, Galanin, Superior cervical ganglion, Sympathetic neuron

INTRODUCTION

Galanin (Gal) is a 29-amino-acid neuropeptide which is distributed widely in the central and peripheral nervous systems including superior cervical ganglion (SCG) neurons (Norberg *et al.*, 2004; Holmberg *et al.*, 2005). Gal-like immunoreactivity is normally seen in only a few neurons of SCG. Gal overexpressed mice exhibited a strong Gal-like immunoreactivity in most SCG neuron profiles. The overexpression of the peptide in SCG neurons was paralleled by increased mRNA levels (Brumovsky *et al.*, 2006). Gal is induced in autonomic neurons after peripheral nerve lesion. Following transection of the two efferent carotid nerves Gal is strongly upregulated in the neuronal cell bodies of rat and mouse SCG (Holmberg *et al.*, 2005). Gal plays a trophic role in the adult animal and acts as an inhibitory modulator of cholinergic and noradrenergic neurotransmission (Miller *et al.*, 1999; Van Hoomissen *et al.*, 2004; Landry *et al.*, 2005; Hobson *et al.*, 2006). Gal is co-localized with norepinephrine (NE) in several regions of the brain, such as locus coeruleus, bed nucleus of the stria terminalis, amygdala, cortex and hippocampus (Hokfelt *et al.*, 1998; Kozicz, 2001; Xu *et al.*, 2001; Holmes *et al.*, 2002; Barrera *et al.*, 2006; Ogren *et al.*, 2006; Simpson *et al.*, 2006). Gal hyperpolarizes noradrenergic locus coeruleus neurons at high concentrations (10^{-6}-10^{-7} mol/L) and enhances NE-induced hyperpolarization at low concentrations (10^{-9} mol/L) (Hokfelt *et al.*, 1998). It has been shown that Gal expression was regulated by NE in the magnocellular neurons of supraoptic nucleus in an ex vivo acute model of rat hypothalamic slices (Melnikova *et al.*, 2006) and in cultured dorsal root ganglion (DRG) neurons

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(Yang *et al*., 2009). Many factors, such as leukemia inhibitory factor (LIF), nerve growth factor (NGF), non-neuronal cells, and potassium, have been demonstrated to be involved in the upregulation of Gal in cultured SCG neurons (Klimaschewski, 1997; Girard *et al*., 2002; Liu *et al*., 2010). The use of dissociated sympathetic neurons in vitro has provided a considerable amount of information concerning adrenoreceptors which regulate sympathetic transmitter release (Boehm and Huck, 1997). Whether activation or inhibition of alpha-adrenoreceptors influences Gal mRNA expression in SCG neurons remains unknown. In the present study, we have evaluated the possible regulation of Gal mRNA expression by selective alpha 1- and alpha 2-adrenoreceptor agonists or antagonists in primary cultured SCG neurons.

MATERIALS AND METHODS

SCG cell culture preparations

Bilateral SCGs were dissected out from newborn Wistar rats. The animals were obtained from the Experimental Animal Center of Shandong University of China. Prior to culture, SCGs were digested with 0.25% trypsin (Sigma) in D-Hanks solution at 37°C for 10 min, and then centrifuged and triturated in growth media supplemented with 2.5% fetal bovine serum (Gibco). The dissociated SCG neurons were cultured in flasks (Costar, Corning, NY, USA) for detecting Gal mRNA by RT-PCR. The flasks were pre-coated with poly-L-lysine prior to plating SCG neurons. SCG neurons were plated at a density of 5×10^5 cells/ml in flasks. All cultures were maintained in the DMEM/F12, supplemented with 5% fetal bovine serum, 2% B-27 supplement (Gibco), L-glutamine (0.1 mg/ml, Sigma), penicillin (100 U/ml), and streptomycin (100 μg/ml). SCG cells were cultured in culture media at 37°C with 5% CO_2 for 24 h and then maintained in culture media containing cytarabine (ara-C) (5 μg/ml) for another 24 h to inhibit growth of non-neuronal cells.

Exposure of alpha-adrenoreceptor ligands on SCG neurons

For acute stimulation, SCG cell cultures were prepared as described above and allowed to grow processes for 6 days with media change every 2 days, followed by the addition of alpha 1-adrenoreceptor agonist phenylephrine (10^{-5} mol/L), alpha 1-adrenoreceptor antagonist prazosin (10^{-6} mol/L), alpha 2-adrenoreceptor agonist clonidine (10^{-5} mol/L), and alpha 2-adrenoreceptor antagonist yohimbine (10^{-5} mol/L), respectively, for 4 h.

For chronic stimulation, SCG cell cultures were prepared as described above and allowed to grow processes for 2 days, followed by the addition of alpha 1-adrenoreceptor agonist phenylephrine (10^{-5} mol/L), alpha 1-adrenoreceptor antagonist prazosin (10^{-6} mol/L), alpha 2-adrenoreceptor agonist clonidine (10^{-5} mol/L), and alpha 2-adrenoreceptor antagonist yohimbine (10^{-5} mol/L), respectively. Cultures were then incubated for additional 4 days with media change every 2 days. The changed culture media would contain each agonist or antagonist with the exact concentration.

For various concentrations stimulation, the ligands that have effects on mRNA expression of Gal were used at different concentrations (10^{-6} mol/L, 10^{-5} mol/L, 10^{-4} mol/L, respectively).

RNA extraction and RT-PCR

The mRNA levels of Gal in SCG neurons at 4 h or 4 days after administration of alpha-adrenoreceptor agonists or antagonists were analyzed by RT-PCR, with β-actin mRNA as an internal control. Total SCG cell RNA of each flask was isolated by TRIzol (Gibco). cDNA synthesis was performed with M-MLV reverse transcriptase. The gene-specific primers were synthesized by use of the published cDNA sequences for Gal and β-actin. The synthetic oligonucleotide primer sequences for Gal and β-actin were as follows:

Gal 5'-ATG CCA ACA AAG GAG AAG AG-3' (upper primer) and 5'-AGG TGC AAG AAA CTG AGA AA-3' (lower primer).

β-actin 5'-ATC ATG TTT GAG ACC TTC AAC-3' (upper primer) and 5'-CAT CTC TTG CTC GAA GTC CA-3' (lower primer).

The predicted size of the amplified Gal and β-actin DNA products were 224 bp and 317 bp, respectively.

PCR amplification was performed for 35 cycles. The cycle profile included denaturation for 45 s at 94°C, annealing for 60 s at 53°C, and extension for 45 s at 72°C. PCR was performed within the range that demonstrates a linear correlation between the amount of cDNA and the yield of PCR products.

The amplified products were analyzed by standard agarose gel electrophoresis and stained with ethidium bromide, visualized by a UV transilluminator and photographed. The photographs were scanned and the electrophoresis gel images were analyzed quantitatively by using an Image J analysis software. The levels of Gal mRNA were expressed as the ratio of the gene to β-actin.

Statistical analysis

Data are expressed as mean ± SD. Statistical analysis was evaluated with SPSS software by one-way ANOVA followed by the Student-Newman-Keuls test for significance to compare the differences among various groups. Significance was accepted at $p<0.05$.

RESULTS

Effects of acute exposure alpha-adrenoreceptor ligands on Gal mRNA expression

To evaluate the possible regulation of Gal mRNA expression by acute exposure of selective alpha 1- and alpha 2-adrenoreceptor agonist or antagonist in primary cultured SCG neurons, the levels of Gal mRNA were estimated by RT-PCR after 4 h stimulation with these agonists or antagonists. The ratio of Gal mRNA/β-actin mRNA in acute alpha 1-adrenoreceptor agonist phenylephrine (10^{-5} mol/L) stimulated SCG neurons is 0.6991 ± 0.0669. The ratio of Gal mRNA/β-actin mRNA in acute alpha 1-adrenoreceptor antagonist prazosin (10^{-6} mol/L) stimulated SCG neurons is 0.7209 ± 0.0561. The ratio of Gal mRNA/β-actin mRNA in acute alpha 2-adrenoreceptor agonist clonidine (10^{-5} mol/L) stimulated SCG neurons is 0.7920 ± 0.0847. The ratio of Gal mRNA/β-actin mRNA in acute alpha 2-adrenoreceptor antagonist yohimbine (10^{-5} mol/L) stimulated SCG neurons is 0.7600 ± 0.0607. The ratio of Gal mRNA/β-actin mRNA in acute control group is 0.7698 ± 0.0570. The levels of Gal mRNA expression were not significantly changed after acute simulation with these agents (Fig. 1).

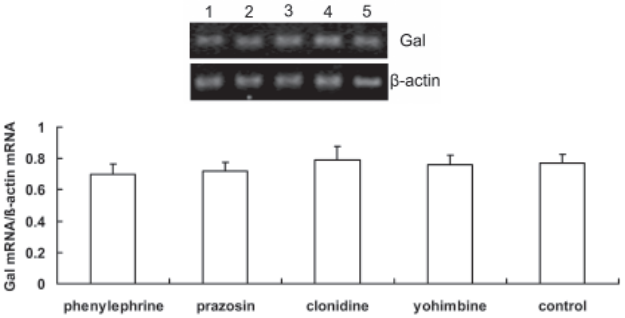

Fig. 1. Effects of acute exposure of alpha 1- and alpha 2-adrenoreceptor agonist or antagonist on Gal mRNA expression in SCG neurons were investigated by RT-PCR. Lane 1: alpha 1-adrenoreceptor agonist phenylephrine treatment. Lane 2: alpha 1-adrenoreceptor antagonist prazosin treatment. Lane 3: alpha 2-adrenoreceptor agonist clonidine treatment. Lane 4: alpha 2-adrenoreceptor antagonist yohimbine. Lane 5: control. The ratio of Gal mRNA/β-actin mRNA in acute alpha 1-adrenoreceptor agonist phenylephrine, alpha 1-adrenoreceptor antagonist prazosin, alpha 2-adrenoreceptor agonist clonidine, alpha 2-adrenoreceptor antagonist yohimbine stimulated SCG neurons is 0.6991 ± 0.0669, 0.7209 ± 0.0561, 0.7920 ± 0.0847, and 0.7600 ± 0.0607, respectively. The ratio of Gal mRNA/β-actin mRNA in acute control group is 0.7698 ± 0.0570. Bar graphs with error bars represent mean ± SD (n=5).

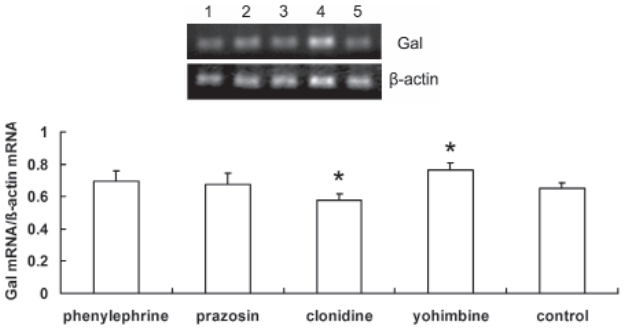

Fig. 2. Effects of chronic exposure of alpha 1- and alpha 2-adrenoreceptor agonist or antagonist on Gal mRNA expression in SCG neurons were investigated by RT-PCR. Lane 1: alpha 1-adrenoreceptor agonist phenylephrine treatment. Lane 2: alpha 1-adrenoreceptor antagonist prazosin treatment. Lane 3: alpha 2-adrenoreceptor agonist clonidine treatment. Lane 4: alpha 2-adrenoreceptor antagonist yohimbine. Lane 5: control. The ratio of Gal mRNA/β-actin mRNA in chronic alpha 1-adrenoreceptor agonist phenylephrine, alpha 1-adrenoreceptor antagonist prazosin, alpha 2-adrenoreceptor agonist clonidine, alpha 2-adrenoreceptor antagonist yohimbine incubated SCG neurons is 0.6927 ± 0.0647, 0.6729 ± 0.0726, 0.5764 ± 0.0377, and 0.7655 ± 0.0417, respectively. The ratio of Gal mRNA/β-actin mRNA in chonic control group is 0.6484 ± 0.0374. Bar graphs with error bars represent mean ± SD (n=5). *p<0.05 vs. control.

Effects of chronic exposure alpha-adrenoreceptor ligands on Gal mRNA expression

To evaluate the possible regulation of Gal mRNA expression by chronic exposure of selective alpha 1- and alpha 2-adrenoreceptor agonists or antagonists in primary cultured SCG neurons, the levels of Gal mRNA were estimated by RT-PCR after 4 days stimulation with these agonists or antagonists. The ratio of Gal mRNA/β-actin mRNA in chronic alpha 1-adrenoreceptor agonist phenylephrine (10^{-5} mol/L) incubated SCG neurons is 0.6927 ± 0.0647. The ratio of Gal mRNA/β-actin mRNA in chronic alpha 1-adrenoreceptor antagonist prazosin (10^{-6} mol/L) incubated SCG neurons is 0.6729 ± 0.0726. The ratio of Gal mRNA/β-actin mRNA in chronic alpha 2-adrenoreceptor agonist clonidine (10^{-5} mol/L) incubated SCG neurons is 0.5764 ± 0.0377. The ratio of Gal mRNA/β-actin mRNA in chronic alpha 2-adrenoreceptor antagonist yohimbine (10^{-5} mol/L) incubated SCG neurons is 0.7655 ± 0.0417. The ratio of Gal mRNA/β-actin mRNA in chronic control group is 0.6484 ± 0.0374. The levels of Gal mRNA expression in cultured SCG neurons increased significantly after chronic stimulation with alpha 2-adrenoreceptor antagonist yohimbine compared with control SCG neurons at the same time point (p<0.05), whereas the levels of Gal mRNA expression decreased significantly after chronic stimulation with alpha 2-adrenoreceptor agonist clonidine as compared with that in control group (p<0.05). Alpha 1-adrenoreceptor agonist phenylephrine or antagonist prazosin chronic stimulation did not have effects on Gal mRNA expression (Fig. 2).

Effects of chronic exposure of different concentrations of alpha 2-adrenoreceptor ligands on Gal mRNA expression

To evaluate the possible regulation of Gal mRNA expression by chronic exposure of various concentrations of alpha 2-adrenoreceptor agonist or antagonist in primary cultured SCG neurons, the levels of Gal mRNA were estimated by RT-PCR after 4 days stimulation with different concentrations (10^{-6} mol/L, 10^{-5} mol/L, 10^{-4} mol/L, respectively) of clonidine or yohimbine. The ratio of Gal mRNA/β-actin mRNA in chronic alpha 2-adrenoreceptor agonist clonidine (10^{-6} mol/L, 10^{-5} mol/L, 10^{-4} mol/L) incubated SCG neurons is 0.5977 ± 0.0621, 0.5352 ± 0.0619, 0.5147 ± 0.0476, respectively. The levels of Gal mRNA expression decreased significantly after higher concentration (10^{-5} mol/L, 10^{-4} mol/L) stimulation with clonidine as compared with that in control group (0.6493 ± 0.0396) (Fig. 3). The ratio of Gal mRNA/β-actin mRNA in chronic alpha 2-adrenoreceptor antagonist yohimbine (10^{-6} mol/L, 10^{-5} mol/L, 10^{-4} mol/L) incubated SCG neurons is 0.6518 ± 0.0441, 0.7074 ± 0.0615, 0.7174 ± 0.0404, respectively. The levels of Gal mRNA expression increased significantly after higher concentration (10^{-5} mol/L, 10^{-4} mol/L) stimulation with yohimbine as compared with that in control group (0.6221 ± 0.0379) (p<0.05) (Fig. 4).

DISCUSSION

Alpha-adrenoreceptor subtypes alpha 1- and alpha 2-adrenoreceptors are expressed in cultured sympathetic neurons (Shivachar and Eikenburg, 1999; Trendelenburg *et al.*, 2003; Brum *et al.*, 2006). Activation or inhibition of distinct adrenoreceptors may result in different effects (Chen *et al.*, 2006). The dual pro- and anti-inflammatory role of the sympathetic nervous system in inflammatory conditions may relate to an intricate shift from beta-to-alpha adrenergic signaling (Straub and Harle, 2005). In the present study, the different effects of selective alpha 1- and alpha 2-adrenoreceptor ligands on Gal mRNA expression in primary cultured SCG neurons were observed. The results showed that the amount of Gal mRNA expression in cultured SCG neurons increased significantly after chronic stimulation with alpha 2-adrenoreceptor antagonist yohimbine (10^{-5} mol/L) compared with control SCG neurons

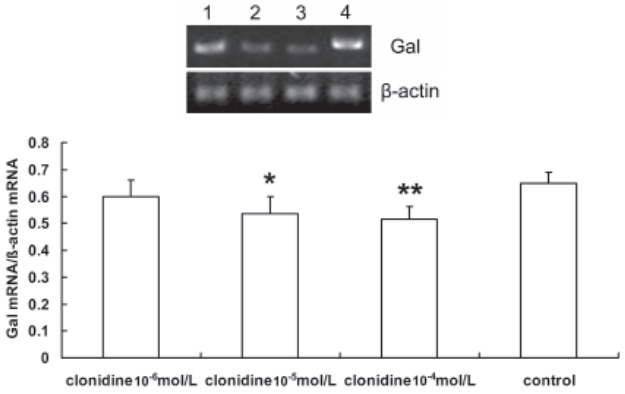

Fig. 3. Effects of chronic exposure of different concentrations of alpha 2-adrenoreceptor agonist clonidine on Gal mRNA expression in SCG neurons were investigated by RT-PCR. Lane 1: clonidine 10^{-6} mol/L. Lane 2: clonidine 10^{-5} mol/L. Lane 3: clonidine 10^{-4} mol/L. Lane 4: control. The ratio of Gal mRNA/β-actin mRNA in chronic clonidine 10^{-6} mol/L, 10^{-5} mol/L, and 10^{-4} mol/L incubated SCG neurons is 0.5977 ± 0.0621, 0.5352 ± 0.0619, and 0.5147 ± 0.0476, respectively. The ratio of Gal mRNA/β-actin mRNA in control group is 0.6493 ± 0.0396. Bar graphs with error bars represent mean ± SD (n=5). **p*<0.05 vs. control, ***p*<0.01 vs. control.

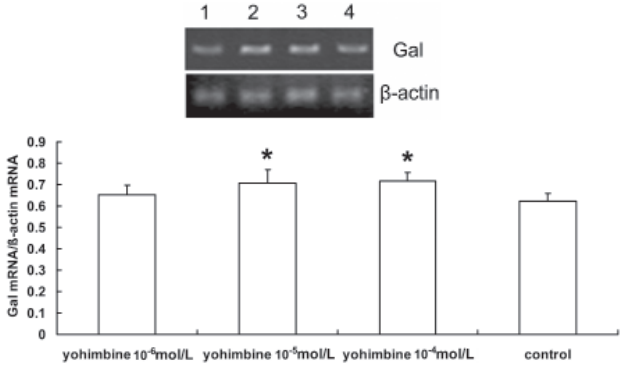

Fig. 4. Effects of chronic exposure of different concentrations of alpha 2-adrenoreceptor antagonist yohimbine on Gal mRNA expression in SCG neurons were investigated by RT-PCR. Lane 1: yohimbine 10^{-6} mol/L. Lane 2: yohimbine 10^{-5} mol/L. Lane 3: yohimbine 10^{-4} mol/L. Lane 4: control. The ratio of Gal mRNA/β-actin mRNA in chronic yohimbine 10^{-6} mol/L, 10^{-5} mol/L, and 10^{-4} mol/L incubated SCG neurons is 0.6518 ± 0.0441, 0.7074 ± 0.0615, and 0.7174 ± 0.0404, respectively. The ratio of Gal mRNA/β-actin mRNA in control group is 0.6221 ± 0.0379. Bar graphs with error bars represent mean±SD (n=5). **p*<0.05 vs. control.

at the same time point, whereas the amount of Gal mRNA expression decreased significantly after chronic stimulation with alpha 2-adrenoreceptor agonist clonidine (10^{-5} mol/L) as compared with that in control group. The effects alpha 2-adrenoreceptor ligands on Gal mRNA expression were not dose-dependent after chronic stimulation. Alpha 1-adrenoreceptor agonist phenylephrine (10^{-5} mol/L) or antagonist prazosin (10^{-6} mol/L) chronic stimulation did not have effects on Gal mRNA expression. Acute stimulation of these agents did not have effects on Gal mRNA expression.

The neuropeptide Gal is involved in neuronal differentiation and neurite outgrowth during development and is markedly up-regulated in response to peripheral nerve lesion. Depolarization of primary cultured sympathetic neurons of the rat SCG elevated Gal mRNA levels which appeared to correlate more closely with Gal peptide production (Girard *et al.*, 2002). The neurotrophic actions of Gal are demonstrated by the up-regulation of Gal expression both in DRG neurons and in SCG neurons after nerve injury (Holmberg *et al.*, 2005; Holmes *et al.*, 2005). And also, exogenous Gal significantly increased the mean axonal length and the number of branch points (Suarez *et al.*, 2006) and capsaicin-evoked neuropeptide release (Yang *et al.*, 2008). In the present study, upregulation or downregulation of Gal mRNA expression stimulated by distinct adrenoreceptors in cultured SCG neurons implicated that Gal may also involved in modulation of responses in peripheral nerve fibers. The evidence has accumulated that Gal is involved in nociception and expressed in both DRG neurons and SCG neurons (Holmberg *et al.*, 2005; Liu and Li, 2008). Sympathetically-maintained pain is thought to be the result of an injury induced coupling between the sympathetic postganglionic neurons and primary afferent nociceptors (Gold *et al.*, 1997). Different subtypes of adrenoreceptors have different actions. Activation or inhibition of distinct adrenoreceptors influences tone of sympathetic nervous system or neurotransmitter synthesis or release (Chen *et al.*, 2006). In the present study, the regulation of Gal mRNA expression in SCG neurons by activation of alpha 2-adrenoreceptors may be involved in the mechanism of sympathetic-sensory coupling even the mechanism is not fully understood.

These adrenoreceptors couple to and activate only certain G protein types, thus leading to specific intracellular signals (Wettschureck and Offermanns, 2005). Activation of the inhibitory $G\alpha_i$ protein by alpha 2-adrenoreceptors leads to the inhibition of adenylyl cyclase, thus resulting in decreased cellular cAMP levels (Hein, 2006). Alpha 2-adrenoceptors inhibited either electrically or K^+-evoked NE release from sympathetic neurons (Boehm and Huck, 1997). In the present study, the levels of Gal mRNA expression in cultured SCG neurons decreased significantly after chronic stimulation with alpha 2-adrenoreceptor agonist clonidine, whereas Gal mRNA expression was promoted by chronic stimulation with alpha 2-adrenoreceptor antagonist yohimbine. Both facilitatory and inhibitory effects on Gal mRNA expression in SCG neurons by administration of alpha 2-adrenoreceptor ligands implicated that Gal mRNA expression was closely related to the activity of alpha 2-adrenoreceptors. Alpha 1-adrenoreceptors are coupled to G_q/11-mediated pathways, which increase intracellular inositol-trisphosphate (IP_3) and Ca^{2+} concentrations (Hein, 2006). And also, the alpha 1-adrenoreceptor agonist phenylephrine was found to elicit a concentration-dependent increase in NE release from rat sympathetic neurons (Boehm and Huck, 1997). However, in the present study chronic stimulation with alpha 1-adrenoreceptor agonist phenylephrine or alpha 1-adrenoreceptor antagonist prazosin did not have effects on Gal mRNA expression which implicated that Gal mRNA expression was not sensitive to the activity of alpha 1-adrenoreceptors. Acute exposure of these agents did not have effects on Gal mRNA expression suggested that alpha 2-adrenoreceptor agonists and antagonists are gentle stimulators on Gal mRNA expression. Different effects on Gal mRNA expression in cultured SCG neurons by activation of different alpha-adrenoreceptor subtypes at different time point implicated that functional effects of alpha-adrenoreceptors

vary with different experimental conditions.

In conclusion, the upregulation of Gal mRNA expression in SCG neurons by selectively inhibition of alpha 2-adrenoreceptor and the dowregulation of Gal mRNA expression by selectively activation of alpha 2-adrenoreceptor may related to neurotrophic actions and may be involved in the mechanism of sympathetic-sensory coupling in sympathetically-maintained pain. The continued investigation of these receptors in primary cell culture will provide further valuable insights into the mechanisms that regulate neuropeptide expression.

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REFERENCES

Barrera, G., Hernandez, A., Poulin, J. F., Laforest, S., Drolet, G. and Morilak, D. A. (2006) Galanin-mediated anxiolytic effect in rat central amygdala is not a result of corelease from noradrenergic terminals. *Synapse* **59**, 27-40.

Boehm, S. and Huck, S. (1997) Receptors controlling transmitter release from sympathetic neurons in vitro. *Prog. Neurobiol.* **51**, 225-242.

Brum, P. C., Hurt, C. M., Shcherbakova, O. G., Kobilka, B. and Angelotti, T. (2006) Differential targeting and function of alpha2A and alpha2C adrenergic receptor subtypes in cultured sympathetic neurons. *Neuropharmacology* **51**, 397-413.

Brumovsky, P., Hygge-Blakeman, K., Villar, M. J., Watanabe, M., Wiesenfeld-Hallin, Z. and Hokfelt, T. (2006) Phenotyping of sensory and sympathetic ganglion neurons of a galanin-overexpressing mouse--possible implications for pain processing. *J. Chem. Neuroanat.* **31**, 243-262.

Chen, Q., Li, D. P. and Pan, H. L. (2006) Presynaptic alpha1 adrenergic receptors differentially regulate synaptic glutamate and GABA release to hypothalamic presympathetic neurons. *J. Pharmacol. Exp. Ther.* **316**, 733-742.

Girard, B. M., May, V., Bora, S. H., Fina, F. and Braas, K. M. (2002) Regulation of neurotrophic peptide expression in sympathetic neurons: quantitative analysis using radioimmunoassay and real-time quantitative polymerase chain reaction. *Regul. Pept.* **109**, 89-101.

Gold, M. S., Dastmalchi, S. and Levine, J. D. (1997) Alpha 2-adrenergic receptor subtypes in rat dorsal root and superior cervical ganglion neurons. *Pain* **69**, 179-190.

Hein, L. (2006) Adrenoceptors and signal transduction in neurons. *Cell Tissue Res.* **326**, 541-551.

Hobson, S. A., Holmes, F. E., Kerr, N. C., Pope, R. J. and Wynick, D. (2006) Mice deficient for galanin receptor 2 have decreased neurite outgrowth from adult sensory neurons and impaired pain-like behaviour. *J. Neurochem.* **99**, 1000-1010.

Hokfelt, T., Xu, Z. Q., Shi, T. J., Holmberg, K. and Zhang, X. (1998) Galanin in ascending systems. Focus on coexistence with 5-hydroxytryptamine and noradrenaline. *Ann. N. Y. Acad. Sci.* **863**, 252-263.

Holmberg, K., Kuteeva, E., Brumovsky, P., Kahl, U., Karlström, H., Lucas, G. A., Rodriguez, J., Westerblad, H., Hilke, S., Theodorsson E., Berge, O. G., Lendahl, U., Bartfai, T. and Hökfelt, T. (2005) Generation and phenotypic characterization of a galanin overexpressing mouse. *Neuroscience* **133**, 59-77.

Holmes, A., Yang, R. J. and Crawley, J. N. (2002) Evaluation of an anxiety-related phenotype in galanin overexpressing transgenic mice. *J. Mol. Neurosci.* **18**, 151-165.

Holmes, F. E., Mahoney, S. A. and Wynick, D. (2005) Use of genetically engineered transgenic mice to investigate the role of galanin in the peripheral nervous system after injury. *Neuropeptides* **39**: 191-199.

Klimaschewski, L. (1997) Regulation of galanin in rat sympathetic neurons in vitro. *Neurosci. Lett.* **234**, 87-90.

Kozicz, T. (2001) Axon terminals containing tyrosine hydroxylase- and dopamine-beta-hydroxylase immunoreactivity form synapses with galanin immunoreactive neurons in the lateral division of the bed nucleus of the stria terminalis in the rat. *Brain Res.* **914**, 23-33.

Landry, M., Liu, H. X., Shi, T. J., Brumovsky, P., Nagy, F. and Hokfelt, T. (2005) Galaninergic mechanisms at the spinal level: focus on histochemical phenotyping. *Neuropeptides* **39**, 223-231.

Liu, H., Liu, Z., Xu, X., Yang, X., Wang, H. and Li, Z. (2010) Nerve growth factor regulates galanin and neuropeptide Y expression in primary cultured superior cervical ganglion neurons. *Pharmazie* **65**, 219-223.

Liu, Z. and Li, Z. (2008) Regulation of galanin and galanin receptor 2 expression by capsaicin in primary cultured dorsal root ganglion neurons. *In Vitro Cell. Dev. Biol. Anim.* **44**, 379-384.

Melnikova, V. I., Raison, D., Hardin-Pouzet, H., Ugrumov, M. V., Calas, A. and Grange-Messent, V. (2006) Noradrenergic regulation of galanin expression in the supraoptic nucleus in the rat hypothalamus. An ex vivo study. *J. Neurosci. Res.* **83**, 857-863.

Miller, M. A., Kolb, P. E., Leverenz, J. B., Peskind, E. R and Raskind, M. A. (1999) Preservation of noradrenergic neurons in the locus ceruleus that coexpress galanin mRNA in Alzheimer's disease. *J. Neurochem.* **73**, 2028-2036.

Norberg, A., Griffiths, W. J., Hjelmqvist, L., Jornvall, H. and Rokaeus, A. (2004) Identification of variant forms of the neuroendocrine peptide galanin. *Rapid. Commun. Mass. Spectrom.* **18**, 1583-1591.

Ogren, S. O., Kuteeva, E., Hokfelt, T. and Kehr, J. (2006) Galanin receptor antagonists: a potential novel pharmacological treatment for mood disorders. *CNS Drugs* **20**, 633-654.

Shivachar, A. C. and Eikenburg, D. C. (1999) Differential effects of epinephrine and norepinephrine on cAMP response and g(i3)alpha protein expression in cultured sympathetic neurons. *J. Pharmacol. Exp. Ther.* **291**, 258-264.

Simpson, K. L., Waterhouse, B. D. and Lin, R. C. (2006) Characterization of neurochemically specific projections from the locus coeruleus with respect to somatosensory-related barrels. *Anat. Rec. A. Discov. Mol. Cell Evol. Biol.* **288**, 166-173.

Straub, R. H. and Harle, P. (2005) Sympathetic neurotransmitters in joint inflammation. *Rheum. Dis. Clin. North Am.* **31**, 43-59.

Suarez, V., Guntinas-Lichius, O., Streppel, M., Ingorokva, S., Grosheva, M., Neiss, W. F., Angelov, D. N. and Klimaschewski L. (2006) The axotomy-induced neuropeptides galanin and pituitary adenylate cyclase-activating peptide promote axonal sprouting of primary afferent and cranial motor neurones. *Eur. J. Neurosci.* **24**, 1555-1564.

Trendelenburg, A. U., Philipp, M., Meyer, A., Klebroff, W., Hein, L. and Starke, K. (2003) All three alpha2-adrenoceptor types serve as autoreceptors in postganglionic sympathetic neurons. *Naunyn. Schmiedebergs Arch. Pharmacol.* **368**, 504-512.

Van Hoomissen, J. D, Holmes, P. V., Zellner, A. S., Poudevigne, A. and Dishman, R. K. (2004) Effects of beta-adrenoreceptor blockade during chronic exercise on contextual fear conditioning and mRNA for galanin and brain-derived neurotrophic factor. *Behav. Neurosci.* **118**, 1378-1390.

Wettschureck, N. and Offermanns, S. (2005) Mammalian G proteins and their cell type specific functions. *Physiol. Rev.* **85**, 1159-1204.

Xu, Z. Q., Tong, Y. G., Hokfelt, T. (2001) Galanin enhances noradrenaline-induced outward current on locus coeruleus noradrenergic neurons. *Neuroreport* **12**, 1779-1782.

Yang, X., Liu, Z. and Li, Z. (2009) Effects of norepinephrine on galanin expression in dorsal root ganglion neurons in vitro. *Curr. Ther. Res. Clin. E.* **70**, 19-28.

Yang, X., Liu, Z., Wang, L., Liu, H., Wang, H. and Li, Z. Z. (2008) Exogenous galanin regulates capsaicin-evoked substance P release from primary cultured dorsal root ganglion neurons. *Neuro. Endocrinol. Lett.* **29**, 911-916.