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EFFECTS OF GALANIN SUBCHRONIC TREATMENT ON MEMORY AND MUSCARINIC RECEPTORS

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Abstract—The cholinergic pathways, which originate in the basal forebrain and are responsible for the control of different cognitive processes including learning and memory, are also regulated by some neuropeptides. One of these neuropeptides, galanin (GAL), is involved in both neurotrophic and neuroprotective actions. The present study has evaluated in rats the effects on cognition induced by a subchronic treatment with GAL by analyzing the passive avoidance response, and the modulation of muscarinic cholinergic receptor densities and activities. [^{3}H]-N-methyl-scopolamine, [^{3}H]-oxotremorine, and [^{3}H]-pirenzepine were used to quantify the density of muscarinic receptors (MRs) and the stimulation of the binding of guanosine 5′-(γ-[^{35}S]thio)triphosphate by the muscarinic agonist, carbachol, to determine their functionality. Some cognitive deficits that were induced by the administration of artificial cerebrospinal fluid (aCSF) (i.c.v. aCSF 2 μl/min, once a day for 6 days) were not observed in the animals also treated with GAL (i.c.v. 1.5 mmol in aCSF, 2 μl/min, once a day for 6 days). GAL modulates the changes in M_1 and M_2 MR densities observed in the rats treated with aCSF, and also increased their activity mediated by $G_{i/o}$ proteins in specific areas of the dorsal and ventral hippocampus. The subchronic administration of the vehicle was also accompanied by an increased number of positive fibers and cells for GAL around the cortical tract of the cannula used, but that was not the case in GAL-treated rats. In addition, the increase of GAL receptor density in the ventral hippocampus and entorhinal cortex in the aCSF group was avoided when GAL was administered. The number of acetylcholinesterase (AChE)-positive neurons was decreased in the nucleus basalis of Meynert of both GAL- and aCSF-treated animals. In summary, GAL improves memory-related abilities probably through the modulation of MR density and/or efficacy in hippocampal areas.

Key words: galanin, memory, muscarinic receptors, [^{35}S]GTPγS, autoradiography, immunohistochemistry.

INTRODUCTION

Galanin (GAL) is a neuropeptide composed of 29 aminoacids in the rat, which is widely distributed in peripheral organs and in the Central Nervous System (CNS). GAL involvement in neuroprotective and neurotrophic actions has been reported in different models of brain damage in addition to its classical roles in other neuronal functions such as feeding, nociception, learning and memory (Lang et al., 2007). GAL immunoreactivity has been reported in cortical cholinergic innervation from the septum-basal forebrain complex (Melander et al., 1985), and also in monoaminergic and GABAergic neurons (Melander et al., 1986). Regarding the cholinergic system, GAL and GAL receptors have been found located in areas involved in cognitive processes (Hökfelt et al., 1987; Melander et al., 1988). The effects of exogenous GAL on the central cholinergic system differ according to the area studied. *In vitro* studies demonstrate that GAL inhibits both basal and K^+-stimulated acetylcholine (ACh) release in the ventral hippocampus and cortical slices (Fisone et al., 1987; Wang et al., 1999). GAL increases ACh release in the hippocampus when it is infused in the medial septum or in the nucleus of the diagonal band of Broca in freely moving rats (Elvander et al., 2004), probably by activation of cholinergic neurons (Jhamandas et al., 2002). Moreover, the perfusion of GAL by microdialysis increases striatal ACh levels in anesthetized animals (Antoniou et al., 1997). The effects of GAL on ACh release in awake rats seem to depend on the site of administration (Ögren and Pramanik, 1991; Amoroso et al., 1992; Antoniou et al., 1997) and also on the receptor type present in each area (Laplante et al., 2004).

The effects of GAL on memory also depend on the dose and the brain area studied (Sundstrom et al., 1988; Malin et al., 1992; Robinson and Crawley, 1993; Ukai et al., 1995; Ögren et al., 1996, 1999; Schött et al., 2000; Kinney et al., 2002, 2003; Elvander et al., 2004). GAL at low doses enhances ACh release and improves cognition (Ögren et al., 1996; Elvander et al., 2004),

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Abbreviations: aCSF, artificial cerebrospinal fluid; ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; ANOVA, analysis of variance; βA, beta-amyloid; CNS, Central Nervous System; EGTA, ethylene glycol tetraacetic acid; GAL, galanin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; [^{3}H]-NMS, [^{3}H]-N-methyl-scopolamine; i.c.v., intracerebroventricular; Iso-OMPA, tetraisopropyl pyrophosphoramide; MR, muscarinic receptor; nbM, nucleus basalis of Meynert; PBS, phosphate-buffered saline; [^{35}S]GTPγS, guanosine 5′-(γ-[^{35}S]thio)triphosphate; STL, step-through latency.

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whereas high–medium doses had no effect or induced cognitive deficits (Ukai et al., 1995; Ögren et al., 1996). Intriguingly, both GAL over-expression and GAL gene silencing in genetically modified mice produce cognitive deficits and a reduction of cholinergic markers in the basal forebrain (O'Meara et al., 2000; Steiner et al., 2001). On the other hand, GAL is up-regulated in cholinergic forebrain neurons after hippocampal and/or cortical lesion (Cortés et al., 1990). GAL also reduces the activation of intracellular second messengers mediated by muscarinic receptors (MRs) (Palazzi et al., 1991).

As has been stated above, the role of GAL in the ventral hippocampus has been widely studied. Indeed, the majority of cholinergic projections containing the neuropeptide have been found in the septo-hippocampal projection which innervates the ventral hippocampus and, to a lesser degree, the dorsal hippocampus. This is consistent with the greater number of high-affinity sites for [^{125}I]-GAL in the ventral hippocampus in comparison with the dorsal hippocampus (Fisone et al., 1987; Melander et al., 1988).

An enhancement of galaninergic innervations within the basal forebrain is also present in certain neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (AD) (Chan-Palay, 1988; Beal et al., 1990; Mufson et al., 1993). GAL receptor density is increased in the hippocampus, amygdala, entorhinal cortex and nucleus basalis of Meynert (nbM) of AD patients, although its regulation seems to vary during the different stages of the disease (Rodríguez-Puertas et al., 1997a; Mufson et al., 2000; Pérez et al., 2002). Additionally, GAL immunoreactivity has also been reported in amyloid plaques and in their associated neurites (Diez et al., 2000; Mufson et al., 2005). Nevertheless, it is still unclear whether GAL hyperinnervation in AD patients contributes to cognitive deficits or, on the contrary, has a neurotrophic and neuroprotective effect on cholinergic neurons.

In the present work we study the interaction between the galaninergic and cholinergic systems by evaluating the effects of a subchronic treatment with a low dose of GAL on learning and memory of rats, and on different neurochemical parameters of the cholinergic system (by measuring MR density and activity and acetylcholinesterase (AChE) activity) in the basal forebrain. In addition, the effects of the treatment on the galaninergic system were analyzed (GAL receptor density and endogenous GAL immunoreactivity).

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley rats (250–275 g) were divided into four groups. The first group (untreated) was comprised of unmanipulated rats. A transcranial cannula was implanted in each animal from the other groups following the same stereotaxic coordinates. One of these groups was not treated (sham), but the animals were handled daily in a similar way to the other operated rats. A third group of rats was treated with artificial cerebrospinal fluid (aCSF), the vehicle in which the neuropeptide GAL was dissolved for the fourth group of treatment. Animal care and all experiments were carried out according to guidelines approved by the Ethics Committee of the University of the Basque Country (UPV/EHU) following the European Communities Council Directive (86/609/EEC) of 24 November 1986 and the recommendations from the Directive (207/526/EEC).

Materials

[^{3}H]-N-methyl-scopolamine ([^{3}H]-NMS) (70–87 Ci/mmol), [^{3}H]-pirenzepine (86 Ci/mmol), [^{3}H]-oxotremorine (75.8 Ci/mmol), [^{125}I]-GAL (2200 Ci/mmol) and guanosine 5′-(γ-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) (1250 Ci/mmol) were purchased from Perkin Elmer (Waltham, MA, USA). Rat GAL was obtained from Bachem (Weil am Rhein, Germany), carbachol, atropine, GTPγS, sodium citrate, tetraisopropyl pyrophosphoramide (Iso-OMPA) and the Tris–maleate buffer were purchased from Sigma (St Louis, MO, USA); cupric sulfate and potassium ferricyanide from Merck and acetylthiocholine iodide from Fluka Analytical. The following primary antibody was used: rabbit polyclonal antiserum for GAL (Rabbit anti-GAL, developed by Dr. Elvar Theodorsson, Linköping, Sweden), followed by rhodamine-conjugated goat anti-rabbit immunoglobulin (Jackson Immunoresearch, Baltimore, PA, USA). All other chemicals were obtained from standard sources and were of the highest purity commercially available.

Surgery and treatments

Surgery was performed under chloral hydrate anesthesia (400 mg/kg; i.p.) to compare the results with a previous study (Barreda-Gómez et al., 2005). The cannulas were implanted following the stereotaxic coordinates in the left lateral ventricle: −1 mm anteroposterior and −1.5 mm laterodorsal to the Bregma and 3.5 mm ventral from the top of the skull. The guide cannulas were fixed to the skull by dental cement and the animals were allowed to recover for a six-day period prior to the daily infusion of GAL (1.5 nmol), which was dissolved in aCSF (148 mM NaCl, 2.7 mM KCl, 0.85 $MgCl_2{\cdot}6H_2O$, 1.2 mM $CaCl_2{\cdot}2H_2O$; pH 7.4 adjusted with 1 mM K_2HPO_4), or aCSF (2 μl/1 min) for a further six-day period. The sham group of rats was similarly treated, but did not receive aCSF. After surgery the rats received one dose (2.25 mg/kg; i.m.) of oxytetracycline. The injection was administered by using a microinjection pump (CMA/102, Sweden) connected to a Hamilton syringe. During the infusion the rats were slightly restrained by hand and, once finished, the injection cannula was left inside the guide cannula for 60 s in order to prevent backflow. Finally a dummy cannula was inserted into the guide. After the rats had completed the passive avoidance test, there was a 24-h GAL wash-out period and then they were anesthetized, decapitated and their brains removed and either frozen at −80 °C (for the experiments of autoradiography; $n = 7$) or fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS 0.1 M; pH 7.4) containing 0.3% picric acid (for the