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# Effect of the cholinesterase--inhibiting substance galanthamine on evoked visual potentials in rats

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**Abstract.** We studied the effect of intravenous injection of the cholinesterase inhibitor galanthamine (GAL) in doses from 0.025 to 5.0 mg/kg on electrically evoked field potentials in rat visual cortex. In all the experiments the amplitude of late components of evoked potentials was significantly reduced, while early components remained unaffected. These findings indicate that cortical cells are inhibited by acetylcholine (ACh). Furthermore, combined application of a muscarinic receptor blocker (atropine) and GAL reliably suppressed the effects of galanthamine. These observations suggest that ACh-induced inhibition may be mediated by activation of GABAergic interneurones that possess muscarinic receptors.

Short communication

**Key words:** ACh, acetylcholine, AChE, acetylcholinesterase, dLGN, dorsal lateral geniculate nucleus, GAL, galanthamine

Recently, clinical interest has been focused on cholinesterase inhibitors as a consequence of the "cholinergic hypothesis" of age-related memory deficits (Bartus et al. 1985). Galanthamine, a reversible selective inhibitor of acetylcholinesterase (AChE) (Thomsen and Kewitz 1990, Bickel et al. 1991, Holl et al. 1992), can offer several advantages compared to other agents and is currently used for symptomatic treatment of cognitive deficits and memory impairment in Alzheimer's disease (Thomsen et al. 1990). However, more recent studies have suggested that the cholinergic system may not be primarily involved in memory processing (Cheal 1981, Collerton 1986, Decker and McGaugh 1991). Clinical pharmacological treatment trials using cholinesterase inhibitors have resulted at best in only partial stabilisation of memory deficits in Alzheimer's disease patients (Summers et al. 1981, Gauthier et al. 1990). Application of acetylcholine (ACh) to central neurones can cause not only rapid and/or slow excitation but also inhibition (for review see Krnjević 1979). Further pharmacological and electrophysiological analysis has indicated that ACh-induced inhibition in the cerebral cortex is due to the relatively rapid excitation of GABAergic interneurones (Randic et al. 1964, Sato et al. 1987a,b, Müller and Singer 1989). Increased dopaminergic, adrenergic, serotonergic, glycinergic, or GABAergic function are all believed to enhance memory and are known to reverse induced amnesia (Flood and Cherkin 1986).

To test whether ACh induced inhibition in the visual cortex we investigated the effects of intravenously applied galanthamine on electrically evoked potentials in the visual cortex of adult rats. The experiments were performed on 23 Wistar male rats, weighing 320-400 g, bred in our laboratory colony. The animals were anaesthetised with urethane (1.2 g/kg i.p.) and fixed in a stereotaxic instrument according to the method of Paxinos and Watson (Paxinos and Watson 1982). To maintain a constant level of anaesthesia, about 10% of the initial dose of urethane was administered intraperitoneally before the animals began to show signs of awakening. The body temperature was thermostatically held between 36°and 38°C.

One bipolar concentric stimulation electrode, model SNE-100, was inserted stereotaxically in the dorsal lateral geniculate nucleus (dLGN: AP= -4.3 mm from bregma, L= 3.5mm, 4.0 mm below the dura). Evoked potentials were recorded from silver ball electrodes implanted epidurally over cortical area 17 (A/P= -9.0 mm from bregma, L= 2-3mm). The indifferent electrodes were placed laterally over nonvisual areas. Electrode positions were adjusted under electrophysiological control to obtain maximum evoked responses. The dLGN was stimulated with a single 50 μs square-wave pulse every 30 s. Stimulus strengths of the dLGN were adjusted to yield maximal evoked potentials (200 -250 μA).

Data acquisition was performed on-line with a laboratory computer and consisted of compilation of mean evoked potentials from 20 repetitions at 30 s intervals. As the response parameter we measured off-line the evoked potential amplitude between the maximal positive and negative deflection before and after administration of the AChE inhibitor GAL and an ACh blocking agent (atropine).

Galanthamine (obtained from Pharmachim - Bulgaria) and atropine (obtained from Sigma) were diluted in 1ml Ringer solution and injected intravenously over a period of about one minute at the doses 0.025, 0.25, 2.5 and 5.0 mg/kg.

After the termination of the experiments, the animals were killed by an overdose of Nembutal, and their brains were perfused by 10% formalin through the carotid arteries. The location of the electrodes was verified by conventional histological techniques using the Nissl staining method. Numerical data are expressed as mean value +/- standard error of the mean (SEM).

In all experiments we observed a dose-dependent decrease in the negative wave amplitude of the cortical evoked potentials following the administration of galanthamine (Fig. 1A). Doses of 0.025, 0.25, 2.5 and 5.0 mg/kg produced 30, 35, 70 and 75% decreases in amplitude, respectively (Fig. 1B). The effects of GAL started just after injection and was especially rapid and strong at doses 2.5 and 5.0 mg/kg (Fig.1A). It was interesting to note that GAL at a dose of 0.025 mg/kg produced a long lasting

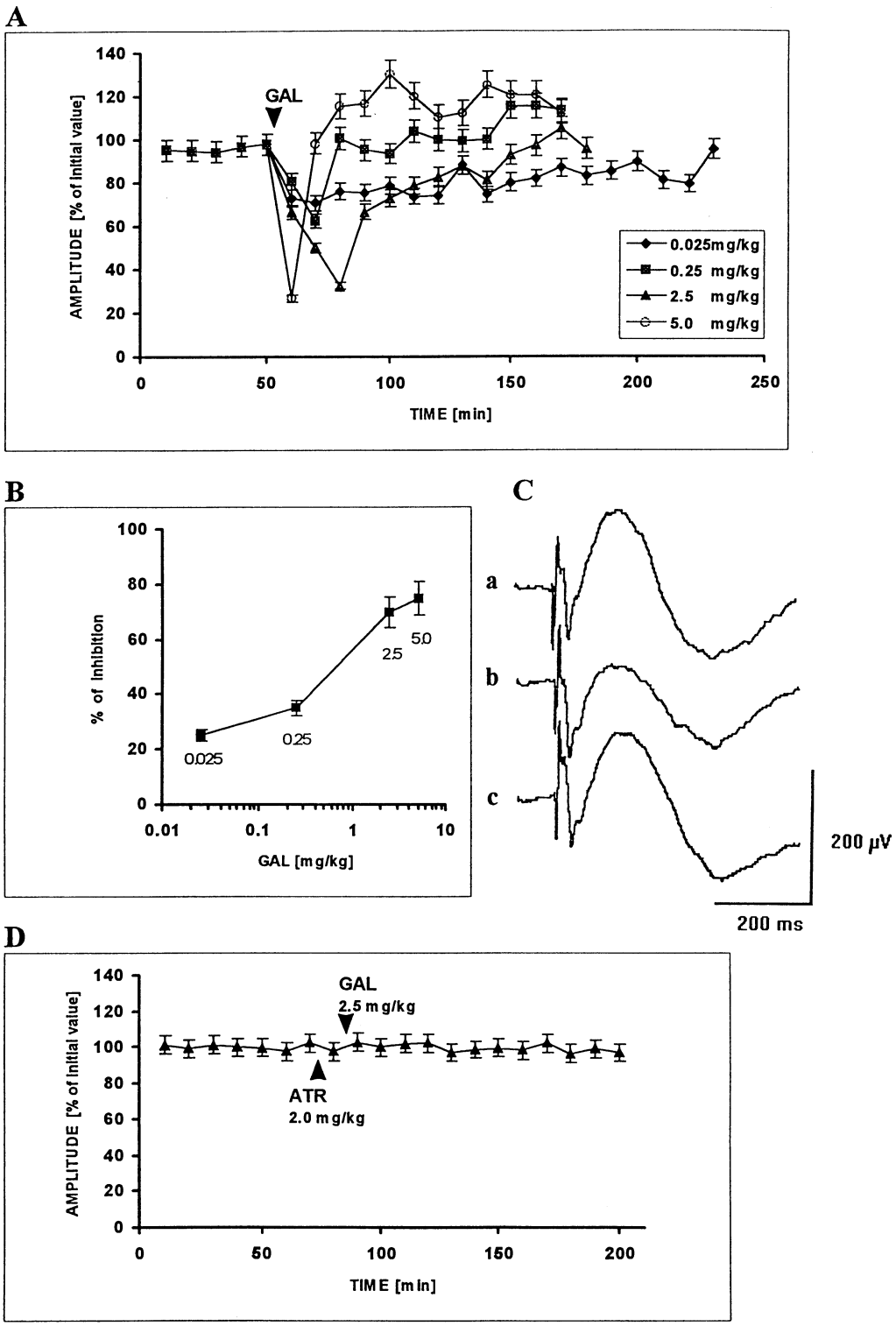


Fig. 1. A, dose-dependent effect of the systemic administration of the cholinesterase inhibitor galanthamine (GAL) on cortical responses evoked by electrical stimulation of the dorsal lateral geniculate nucleus (dLGN); B, dose-response curve for the percentage decrease in the changes of cortical evoked potentials amplitude following GAL injection; C, original mean traces recording of evoked potential in visual cortex before (a) and after (b, c) the GAL administration at a dose of 0.025 mg/kg; D, blockage of the effects of galanthamine injection by pre-treatment of the muscarinic acetylcholine receptor blocker atropine. Arrows indicated galanthamine (GAL) and atropine (ATR) injection. Each points represents mean value +/- SEM (bars).

amplitude inhibition of evoked cortical potentials. However, at a dose of 5.0 mg/kg we have observed first a very rapid and strong suppression and then weak long lasting facilitation of the cortical evoked potentials. In both cases full recovery was observed 2 h after the GAL injection. In a total of 4 experiments we tested the effects of combined administration of acetylcholinesterase inhibitor and the muscarinic receptor blocker atropine. In all experiments the application of atropine reliably suppressed the effects of galanthamine. Pre-treatment with atropine at a dose of 2.0 mg/kg completely blocked the effects of GAL-induced depression of the cortical evoked potentials after i.v. GAL administration at a dose of 2.5 mg/kg (Fig. 1D).

These results indicate that intravenous application of GAL depressed or fully eliminated the late component of evoked potentials. These findings suggest that cortical polysynaptic responses are inhibited by ACh and confirm earlier reports indicating that acetylcholine has both excitatory and inhibitory effects in the cortex (Randic et al.1964, Sato et al.1987a,b, Müller and Singer 1989). This depressant effect was blocked by atropine which suggests that muscarinic ACh receptors are involved in this process. The same effect was observed in a slice of rat prefrontal cortex (Vidal and Changeux 1989) and in single-unit activity of cat visual cortex (Müller and Singer 1989) after ionotophoretic application of acetylcholine and scopolamine or atropine. Müller and Singer (1989) have observed that this depressant effect of ACh was also blocked by bicuculine methiodide, a $GABA_A$-receptor antagonist. *In vitro* studies also confirm that bicuculine abolishes the depressant effect of ACh, which suggests that cholinergic inhibition is mediated by activation of GABAergic interneurones (McCormick 1992). There is anatomical evidence that GABA within the cortex is contained in intrinsic neurones (for review see Parnavelas 1990). Immunohistochemical studies involving use of an antibody against GABA have shows that about 15% of the neurones in rat visual cortex are GABAergic (Lin et al. 1986, Meinecke and Peters 1987). It has been shown that a subpopulation of GABA-containing neurones of the mammalian neocortex also contains AChE (Hallanger et al. 1986). Anatomical evidence indicates that muscarinic receptors are largely located on neuronal elements intrinsic to the cortex, especially to layers I, III-IV and VI (Sahin et al. 1992). Pitler and Alger (1992) concluded that GABAergic inhibitory interneurones possess muscarinic receptors. Activation of these receptors increases the excitability of the interneurones, and synaptically released ACh increases interneuronal activity. All the anatomical and electrophysiological evidence quoted above suggests the existence of cholinergic/GABAergic interaction in the rat visual cortex. Our results also confirm this interaction and may suggest that GAL, uses a non-cholinergic mechanism, similar to those of cholinergic drugs, in memory processing. Therefore, it is interesting to note that in old rats, decreased AChE activity impaired acquisition in the Morris water-maze, a learning and memory test (Biegon et al. 1986). The weak enhancement of amplitude of evoked potentials observed after rapid and strong inhibition at a dose of 5.0 mg/kg may suggest interactions between cholinergic and other, non-GABAergic systems. A significant increase of norepinephrine (NE) and dopamine (DA) levels was observed after systemic administration of cholinesterase inhibitors (Cuadra et al. 1994). There is also evidence that DA affects cholinergic neurones. Activity of cortically projecting neurones in the nucleus basalis is regulated in an excitatory manner by central DA neurones (Day and Fibiger 1992).

Additional electrophysiological studies are needed to elucidate this question.

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Bartus R.T., Dean R.L., Pontecorvo M.J., Flicker C. (1985) The cholinergic hypothesis: a historical overview, current perspective and future directions. In: Memory dysfunction: an integration of animal and human research from pre-clinical and clinical perspective (Eds. D.S. Olton, E. Gamzu and S. Corkin). Acad. of Sci., New York, p. 421-450.

Bickel U., Thomsen T., Fischer J. P., Weber W., Kewtiz H. (1991) Galnathamine: pharmacokinetics, tissue distribution and cholinesterase inhibition in brain of mice. Neuropharmacology 30: 447-454.

Biegon A., Greenberger V., Segal M. (1986) Quantitative histochemistry of brain acetylcholinesterase and learning rate in the aged rat. Neurobiol. Aging 7: 215-217.

Caudra G., Summers K., Giacobini E. (1994) Cholinesterase inhibitor effects on neurotransmitters in rat cortex in vivo. J. Pharmacol. Exp. Ther. 270: 277-284.

Cheal M.L. (1981) Scopolamine disrupts maintenance of attention rather than memory processes. Behav. Neural. Biol. 33: 163-187.

Collerton C. (1986) Cholinergic function and intellectual decline in Alzheimer's disease. Neuroscience 19: 1-28.

Day J., Fibiger H.C. (1992) Dopaminergic regulation of cortical acetylcholine release. Synapse 12: 281-286.

Decker M.W., McGaugh J.L. (1991) The role of interactions between cholinergic system and other neuromodulatory systems in learning and memory. Synapse 7: 151-168.

Flood J. F., Cherkin A. (1986) Scopolamine effects on memory retention in mice: a model of dementia? Behav. Neurol. Biol. 45: 169-184.

Gauthier G., Bouchard R., Lamontagne A., Bailey P., Bergman H., Ratner J. (1990) Tetrahydroaminoacrine-lecithin combination treatment in patients with intermediate-stage Alzheimer's disease. N. Engl. J. Med. 322: 1272-1276.

Hallanger A.E., Wainer B.H., Rye D.B. (1986) Colocalization of gamma-aminobutric acid and acetylcholinesterase in rodent cortical neurons. Neuroscience 19: 763-769.

Holl G., Straschill M., Thomsen T., Fischer J. P., Kewitz, H. (1992) Effect of the cholinesterase inhibiting substance galanthamine on human EEG and visual evoked potentials. Electroencephalogr. Clin. Neurophysiol. 82: 445-452.

Krnjević K. (1979) Chemical nature of synaptic transmission in vertebrates. Physical. Rev. 54: 418-540.

Lin C.-S., Lu S. M., Schmechel D.E. (1986) Glutamic acid decarboxylase and somatostatin immunoreactivities in rat visual cortex. J.Comp.Neurol. 244: 369-383.

McCormick D. A. (1992) Neurotransmitter actions in the thalamus and cerebral cortex and their role in neuromodulation of thalamocortical activity. Prog. Neurobiol. 39: 337-388.

Meinecke D. L., Peters A. (1987) GABA immunoreactive neurons in rat visual cortex. J. Comp. Neurol. 261: 388-404.

Müller C. M., Singer W. (1989) Acetylcholine-induced inhibition in the cat visual cortex is mediated by a GABAergic mechanism. Brain Res. 487: 335-342.

Parnavelas J. G. (1990) Neurotransmitters in the cerebral cortex. In: Progress in Brain Research (Eds. H.B.M. Uylings, C.G. VanEden, J.P.C. DeBruin, M.A. Corner M. A. and M.G.P. Feenstra). Vol. 85. Elsevier, Amsterdam p. 13-29.

Paxinos G., Watson C. (1982) The rat brain in stereotaxic coordinates. Academic Press, Sydney.

Pitler T. A., Alger B. E. (1992) Cholinergic excitation of GABAergic interneurons in the rat hippocampal slice. J. Physiol. (Lond.) 450: 127-142.

Randic M., Siminoff R., Straughan D. W. (1964) Acetylcholine depression of cortical neurons. Exp. Neurol. 9: 236-242.

Sahin M., Bowen W. D., Donoghue J. P. (1992) Location of nicotinic and muscarinic cholinergic andu-opiate receptors in rat cerebral neocortex: evidence from thalamus and cortical lesions. Brain Res. 579: 135-147.

Sato H., Hata Y., Hagihara K., Tsumoto T. (1987a) Effects of cholinergic depletion on neuron activities in the cat visual cortex. J. Neurophysiol. 58: 781-794.

Sato H., Hata Y., Masui H., Tsumoto T. (1987b) A functional role of cholinergic innervation to neurons in the cat visual cortex. J. Neurophysiol. 58: 765-780.

Summers W.K., Viesselman J.O., Marsh G.M., Candelora K. (1981) Use of THA in treatment of Alzheimer-like dementia: pilot study in twelve patients. Biol. Psychiatry 16: 145-153.

Thomsen T., Blickel U., Fischer J. P., Kewitz H. (1990) Galanthamine hydrobromide in a long-term treatment of Alzheimer's disease. Dementia 1: 46-51.

Thomsen T., Kewitz H. (1990) Selective inhibition of human acetylcholinesterase by galanthamine in vitro and in vivo. Life Sci. 64: 1553-1558.

Vidal C., Changeux J.-P. (1989) Pharmacological profile of nicotinic acetylcholine receptors in the rat prefrontal cortex: An electrophysiological study in a slice preparation. Neuroscience 29: 261-270.

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