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CHOLINESTERASE INHIBITION BY GALANTHAMINE AND LYCORAMINE

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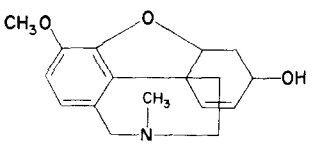
Abstract—Galanthamine, a recently isolated alkaloid, has been found to inhibit the acetylcholinesterase of muscle and the cholinesterase of plasma and brain. The inhibition of the muscle enzyme by galanthamine is greater than that produced by pyridostigmin, a compound of known usefulness, but less than that of neostigmin. The muscle cholinesterase inhibition produced by lycoramine was found to be about equal to that of pyridostigmin. These alkaloids are thus of interest because of the possibility of their therapeutic usefulness.

GALANTHAMINE and lycoramine are alkaloids which have recently been isolated from plant bulbs of the Amaryllidaceae family.[1–4] Galanthamine is an amine believed to have the chemical structure:



A slightly different structure has been assigned to galanthamine by Proskurnina and Yakovleva.[5] The spectral analysis of samples from different sources appears to be identical. Galanthamine is referred to as lycoremine by Uyeo and Kobayashi.[2] Lycoramine is the dihydro-derivative of galanthamine and contains no double bond in the hydroxyl-substituted ring. These alkaloids structurally resemble morphine, which is known to influence cholinesterase activity, since it can antagonize the inhibition of plasma cholinesterase by prostigmine and inhibit the esterases of plasma and red blood cells.[6, 7] Thus, since galanthamine has been reported to inhibit cholinesterase, to be of benefit in the treatment of myasthenia gravis,[7] and to increase the sensitivity of muscle to acetylcholine,[8] we have determined the cholinesterase activity of a muscle homogenate in the presence of each compound, and compared the inhibition to that produced by pyridostigmin and neostigmin, compounds with known activity and usefulness. Fig. 1 shows that galanthamine hydrochloride inhibits muscle cholinesterase more than pyridostigmin bromide, but less than neostigmin bromide. Lycoramine hydrobromide produced about the same amount of inhibition as pyridostigmin. Galanthamine hydrochloride was also found to inhibit the cholinesterase of dog plasma (Fig. 1) and the cholinesterase of cat brain; the brain enzyme was inhibited 50 per cent at a 2×10^{-5} M concentration.

We used a muscle homogenate prepared from the entire hind limb musculature of a dog and all the curves plotted from the activity of muscle enzymes were obtained with

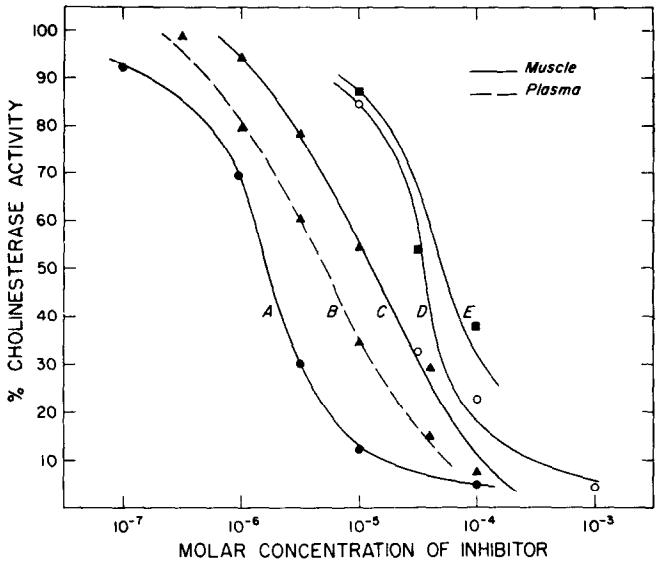


FIG. 1. Inhibition of muscle and plasma cholinesterase.
A. Neostigmin Br. B. Galanthamine HCl (plasma).
C. Galanthamine HCl. D. Pyridostigmin Br. E. Lycoramine HBr.

the same batch of homogenate. The muscles were homogenized in and washed three times with saline and then diluted 1 : 5 with a bicarbonate buffer with a final molar concentration of $NaHCO_3$ 0·025, NaCl 0·15, $MgCl_2$ 0·04, and equilibrated with 95% N_2 and 5% CO_2. The activity of 0·92 g (wet weight) of muscle was measured at 10 min intervals for 30 min in the Warburg apparatus using a 0·01 M acetylcholine chloride substrate. Twelve flasks were used to determine each point on the curves depicting muscle activity, including corrections for pressure, heat-inactivated tissue, and spontaneous hydrolysis of the substrate. The inhibitor and the substrate were tipped into the homogenate simultaneously from different side arms and incubated with shaking for 10 min prior to beginning measurement of enzyme activity. Homogenates prepared and tested in this way hydrolyse acetyl-β-methylcholine, show only slight activity in splitting butyrylcholine or benzoylcholine and are inhibited by an excess of an acetylcholine substrate. The dog muscle enzyme which these alkaloids inhibit can thus be considered chiefly acetylcholinesterases.

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