first detectable at approximately 12 days and reached adult levels at about 56 days. The relationship between ACh content and ChAc activity within each corneal extract was also logarithmic. Compared with ACh and ChAc, AChE activity showed less variation among different families and an entirely different relationship to age. A sharp peak in AChE was observed in the region of 3–10 days, and activity levelled off at adult values at values at about 25 days.

The coincidence of the opening of the immature rabbit's eyelids (approximately 10 days after birth) with the first appearance of ACh and ChAc in the epithelium, together with the long time taken to achieve maturity of the cholinergic system, do not support a neural role for the bulk of ACh in the cornea.

W.S.W. acknowledges receipt of a Van Ameringen Fellowship of the Foundations' Fund for Research in Psychiatry and a Wellcome Trust Travelling Fellowship.

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Some pharmacological properties of RX 67668—a new anticholinesterase

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Cis-2-phenyl-1-(N-pyrrolidinyl) cyclohexane hydrochloride (RX 67668) has been shown to be an anticholinesterase whose chemical structure is unrelated to the chemical structures of established anticholinesterases. In a comparative study with a number of other anticholinesterases only RX 67668 was able to reverse the neuromuscular blockade induced by D-tubocurarine at doses which did not also produce symptoms of muscarinic stimulation.

The inhibitory effect of RX 67668 on acetylcholinesterase (acetylcholine acetylhydrolase, 3.1.1.7) and butyrylcholinesterase (acetylcholine acetyl-hydrolase, 3.1.1.8) was initially measured *in vitro* using the method of Michel (1949). Concentrations of anticholinesterases necessary to produce 50% inhibition of the enzymes were determined. It was found that approximately 5×10^{-6}M RX 67668 was necessary to produce 50% inhibition of both acetylcholinesterase and butyrylcholinesterase.

Among the pharmacological tests used to assess anticholinesterase activity *in vivo* are the rat chromodacryorrhoea test (Burgen, 1949) and mouse miosis test (Schneider, 1970). Using these tests a dose of 1·4 mg/kg i.p. RX 67668 was needed to reduce by half the dose of methacholine necessary to produce red tears, whilst a dose of 7·2 mg/kg s.c. RX 67668 was necessary to reduce the pupil diameter of the mouse to 50% of the control value. Comparable doses for neostigmine are 0·036 mg/kg i.p. and 0·06 mg/kg s.c. respectively.

Anticholinesterases find their principal clinical use in the reversal of muscle relaxation at the termination of surgical procedures. Experiments using either the

rat anterior tibialis preparation of the cat tibialis preparation demonstrated that RX 67668 was effective in reversing tubocurarine-induced muscle blockade at doses in the range 0·3–1·0 mg/kg, i.v. At these doses, unlike animals treated with comparable doses of other anticholinesterases, the RX 67668-treated animals exhibited no signs of muscarinic stimulation such as salivation, lachrymation, urination or defaecation.

This apparent lack of muscarinic activity was investigated by studying the effect of RX 67668 on the flow of saliva induced by electrical stimulation of the chorda tympani nerve in the cat. It was found that, although RX 67668 increased the flow of saliva produced in response to electrical stimulation at doses greater than 0·3 mg/kg, i.v., the drug did not induce a spontaneous flow of saliva at doses up to 20 mg/kg, i.v. In contrast neostigmine produced a spontaneous flow of saliva at doses greater than 0·03 mg/kg, i.v.

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Differential effects of drugs on the acetylcholine output from the myenteric plexus and the responses of the longitudinal muscle of the guinea-pig ileum

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In the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum, morphine (1–2·5 μM), noradrenaline (3 μM) and adrenaline (0·3–0·6 μM) inhibit the contractile responses to electrical field stimulation at low frequencies (0·017–1 Hz) and reduce the output of acetylcholine (ACh) obtained in the presence of eserine (7·7 μM) by 65–85%, whereas the responses and output obtained at 10 Hz are unaffected (Cowie, Kosterlitz & Lydon, 1968; Paton & Zar, 1968; Paton & Vizi, 1969; Kosterlitz, Lydon & Watt, 1970).

Similar depressions of ACh output were obtained with hexamethonium (140 μM) (Greenberg, Kosterlitz & Waterfield, 1970) and $MnCl_2$ (125 μM) but, in contrast to the findings with morphine and the catecholamines, the contractile responses were not inhibited. When the release of ACh was depressed by about 80% by hexamethonium, addition of morphine reduced the ACh output by a further 10%; the contractile response obtained in the absence of eserine was not reduced by hexamethonium but was depressed by hexamethonium plus morphine. Similar results were obtained with $MnCl_2$ (125 μM) and morphine (1 μM).

It is possible that the ACh output observed in the presence of eserine is not identical with the output responsible for the contraction of the longitudinal muscle and that hexamethonium and Mn-ions depress only the output in the presence of eserine. Another possibility is a release of ACh from two different pools of ACh; both pools would be sensitive to the depressant actions of morphine and adrenaline