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**Direct Determination of Acetylcholine Release by Radioimmunoassay
and Presence of Presynaptic M₁ Muscarinic
Receptors in Guinea Pig Ileum**

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Radioimmunoassay for acetylcholine (ACh) with a sensitivity of 10 pg/tube was applied to the direct determination of ACh output from the nerve endings in longitudinal muscle strips of guinea pig ileum. The strips were preincubated with an irreversible cholinesterase inhibitor and superfused with Krebs' solution under various experimental conditions. Pirenzepine (0.1—10 μ M) and atropine (10—100 nM) produced an increase in electrically evoked ACh output through the inhibition of presynaptic muscarinic receptors. Contractile response to endogenous ACh released by electrical stimulation was enhanced by pirenzepine and atropine at lower concentrations, whereas the highest concentrations of pirenzepine (10 μ M) and atropine (100 nM) caused a reduction in the enhanced contractile response and a significantly diminished response, respectively. These results demonstrate that the concentrations of pirenzepine and atropine, effective in inhibiting presynaptic muscarinic receptors, differ from those inhibiting postsynaptic muscarinic receptors and suggest the possibility that presynaptic M₁ muscarinic receptors regulating ACh output may be present in the guinea pig ileum.