tetraethylammonium or triethylcholine (Roberts, 1962) producing postsynaptic depression and presynaptically enhancing transmitter release. This explanation appears unlikely in view of the known presynaptic depressant actions of barbiturate drugs (Schoepfle, 1957; Weakley, 1969) and, at the concentrations used in our experiments, the lack of an apparent action on mepps.

The possibility that intrinsic and extrinsic ACh act through different groups of the receptor population cannot be ruled out. The results indicate a need for caution in the interpretation of results obtained when employing extrinsic agonists.

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The effects of hexamethonium, morphine and adrenaline on the output of acetylcholine from the myenteric plexus-longitudinal muscle preparation of the ileum

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When the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum is immersed in Krebs solution containing eserine (7·7 μM) and choline (20 μM) and is stimulated supramaximally by an electrical field, the amount of acetylcholine (ACh) released per pulse decreases with increasing frequency of stimulation (Cowie, Kosterlitz & Watt, 1968; Paton & Zar, 1968). Similar results have now been obtained in the corresponding preparation of the rabbit, except that the absolute values for the amount of ACh released are only one-tenth of that obtained in the guinea-pig. Whereas in the guinea-pig the ACh output due to low frequencies of stimulation (0·016–0·33 Hz) is depressed by morphine (0·25–1 μM), it does not affect the output in the rabbit.

In the guinea-pig, hexamethonium (140 μM), morphine (0·25–1 μM) and adrenaline (0·5 μM) depress ACh output more at low than at high frequencies of stimulation. At a frequency of 0·016 Hz any of the three drugs depresses the output by 55 to 85%. After hexamethonium has been added in a concentration that has a maximum effect, morphine or adrenaline causes a further reduction in output of 50% or more. These observations suggest that the sites of action of these drugs overlap.

Morphine (0·25–1 μM) depresses the ACh output induced by single pulses at a frequency of 0·016 Hz by 55 to 65% but the output from a train of 10 pulses applied at intervals of 20 ms is depressed to a lesser extent. When the number of pulses per train is increased to 100, there is no longer a significant difference between the outputs of ACh obtained with or without morphine.

When the preparation is stimulated in the absence of eserine and the mechanical responses to electrical stimulation are recorded, morphine and adrenaline depress the responses at 0·016 Hz. This depressant effect is much reduced at 1 Hz and absent at 10 Hz. Hexamethonium has scarcely any effect on the mechanical responses at any of the frequencies.

The site of action of hexamethonium is uncertain. In the superior cervical ganglion of the cat hexamethonium has a greater blocking effect at 8 Hz than at 0·5 Hz (Riker & Komalahiranya, 1962). The fact that in the myenteric plexus preparation the output of acetylcholine is decreased more at low than at high frequencies may indicate that, in this preparation, the depressant effect of hexamethonium is not due to its ganglion-blocking action.

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Presynaptic inhibition of acetylcholine release by endogenous and exogenous noradrenaline at high rate of stimulation

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Noradrenaline (NA) has been shown to be a potent inhibitor of the acetylcholine (ACh) release due to nerve stimulation from nerve terminals of longitudinal muscle strip of guinea-pig ileum (Paton & Vizi, 1969; Vizi, 1968; Kosterlitz, Lydon & Watt, 1970) and of guinea-pig colon (Beani, Bianchi & Crema, 1969). Inhibition was obtained at low stimulation frequencies (0·1–2 Hz), but not at higher ones (>5 Hz), and it was suggested that it might be exerted through α-receptors (Paton & Vizi, 1969; Vizi, 1968; Kosterlitz, Lydon & Watt, 1970).

In order to study the effect of noradrenaline on ACh output at a high rate of stimulation, an " intermittent " stimulation method was used. A longitudinal muscle strip of guinea-pig ileum was bathed in 3·5 ml Krebs solution at 37° C containing eserine sulphate (2×10^{-6} g/ml) and was stimulated by field stimulation. At " intermittent " stimulation the trains of 2–10 shocks with intervals of 50–1,000 ms were delivered at a frequency of 0·1 Hz and repeated until enough ACh had been collected for assay on the guinea-pig ileum. The volley output of *n*th shock was calculated as follows:

$$\frac{a}{i}\sum_{K=i} x_k = \frac{a}{i} iV_i,$$ where i=1st . . .*n*th shock, a the total number of shocks delivered, n the number of shocks in one train, V_i the average volley output when $n=i$ and x_k the volley output produced by kth shock. The volley output by the first shock in one train was taken as the volley output by stimulation of 0·1 Hz [(11·7±0·3 ng/g)/volley; mean±S.E.M., n=26], and the volley output by the 2nd, 3rd . . . *n*th shock was calculated. The output per volley fell