of low doses of bethanidine, would be compensated for by the concomitant increase in sensitivity of the nictitating membrane to its action.

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Extrinsic and intrinsic acetylcholine and barbiturate effects on frog skeletal muscle

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Del Castillo & Katz (1957) demonstrated that tubocurarine simultaneously reduced the depolarization response of frog skeletal muscle to both neurogenically released (intrinsic) and electrophoretically applied (extrinsic) acetylcholine (ACh). Quilliam (1955) reported that barbiturate drugs decreased the ACh contracture of the frog isolated ileo-fibularis muscle without a concomitant block of neuromuscular transmission. He also noted (unpublished observations) that the depolarizing response to ACh added to the fluid bathing the frog isolated toe muscle was profoundly decreased by low concentrations of barbiturate compounds which left the muscle action potential undiminished. Thesleff (1956) suggested that pentobarbitone might be more effective in reducing the response to extrinsic than to intrinsic acetylcholine. Since these findings seem to imply some difference in the action of intrinsic and extrinsic ACh, we have investigated this phenomenon further on the frog isolated motor nerve-sartorius muscle preparation.

Transmission was blocked with 6–10 mM Mg for the observation of end-plate potentials. An intracellular microelectrode recorded the end-plate potentials (epps) and miniature end-plate potentials (mepps), and also the electrophoretic potentials produced by pulses of current through an acetylcholine-filled micropipette placed on the end-plate region. These potentials were evoked concurrently, photographed, and also monitored on a chart-recorder.

Concentrations of amylobarbitone (2 to 8×10^{-5} g/ml) which were without neuromuscular blocking action produced a large depression of the electrophoretic potentials, while there was little or no effect on the mepps or epps. Essentially similar results were obtained using thiopentone (2 to 8×10^{-5} g/ml).

Two possibilities exist. First, the observed differences were due to different rates of ACh release, neurally and electrophoretically. The effect of amylobarbitone on electrophoretic potentials of different time course but of comparable amplitude was examined and found to be the same. Second, barbiturate drugs might be acting like

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.