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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REFERENCES

COWIE, A. L., KOSTERLITZ, H. W. & WATT, A. J. (1968). Mode of action of morphine-like drugs on autonomic neuro-effectors. *Nature, Lond.*, **220**, 1040–1042.

PATON, W. D. M. & ZAR, M. A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle fibres. *J. Physiol., Lond.*, **194**, 13–33.

RIKER, W. K. & KOMALAHIRANYA, A. (1962). Observations on the frequency dependence of sympathetic ganglion blockade. *J. Pharmac. exp. Ther.*, **137**, 267–274.

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

from the first stimulation to a final constant output per volley that depends, as the rate of decline, on the frequency of stimulation applied. The higher the frequency the faster is the decline in volley output. There is a second power relationship between the rate of decline of ACh output per volley and the stimulus interval (stimulus interval $= \frac{1}{\text{frequency}}$).

Noradrenaline (0·25–1·0 μg/ml) reduced the ACh volley output even at high frequency of stimulation (3–20 Hz) and short volleys (2–10 shocks in one train). Noradrenaline reduced the ACh volley output to the level of that produced by continuous stimulation of 10 Hz [(1·4–1·9 ng/g)/volley]. The ACh outputs by the first shocks was as much reduced by noradrenaline at high frequency stimulation as the output was higher than (1·4–1·9 ng/g)/volley. This action of noradrenaline was antagonized by phentolamine (2 μg/ml for 20 min).

Guanethidine (10 μg/ml) reduced the resting ACh output [(43·5±3·6 ng/g)/min] by 54%. The volley outputs due to low (0·1 Hz) and high frequency stimulation (intermittent stimulation, 10 Hz, 5 shocks in one train) were reduced by 65% and 46%, respectively. However, with continuous stimulation at 10 Hz, guanethidine failed to reduce the ACh output. The inhibitory action of guanethidine is probably due to the noradrenaline released by it. This explanation is supported by the fact that guanethidine was ineffective in strips depleted of noradrenaline by 6-hydroxydopamine pretreatment of the guinea-pig (2×70 mg/kg intravenously 24 and 6 h). In such strips the ACh output was higher at resting condition (75%) and at 0·1 Hz stimulation (80%). The output induced by 10 Hz [(945±56 ng/g)/min] was unaffected.

The fact that noradrenaline added or released is also capable of reducing ACh output when the firing is of high rate but short in duration suggests that noradrenaline plays a general modulator role in controlling the output of ACh presynaptically.

REFERENCES

BEANI, L., BIANCHI, C. & CREMA, A. (1969). The effect of catecholamines and sympathetic stimulation on the release of acetylcholine from the guinea-pig colon. *Br. J. Pharmac.*, **36**, 1–17.

KOSTERLITZ, H. W., LYDON, R. J. & WATT, A. J. (1970). The effects of adrenaline, noradrenaline and isoprenaline on inhibitory α and β-adrenoceptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmac.*, **39**, 398–414.

PATON, W. D. M. & VIZI, E. S. (1969). The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strip. *Br. J. Pharmac.*, **35**, 10–28.

VIZI, E. S. (1968). The inhibitory action of noradrenaline and adrenaline on release of acetylcholine from guinea-pig ileum longitudinal strips. *Arch. exp. Path. Pharmak.*, **259**, 199–200.

The detection and assay of noradrenaline released from isolated tissues during intramural nerve stimulation

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The perfused spleen has been used almost exclusively to study the relationship between noradrenaline output and the frequency of sympathetic nerve stimulation (Brown & Gillespie, 1957; Blakeley, Brown & Ferry, 1963; Haefely, Hurlimann & Thoenen, 1965; Kirpekar & Misu, 1967). The aim of this study was to measure the output of the adrenergic transmitter in other isolated organs and to examine the relationship between output and frequency of nerve stimulation.