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The variation of noradrenaline output with frequency of nerve stimulation and the effect of morphine on the cat nictitating membrane and on the guinea-pig myenteric plexus

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Morphine has been shown to inhibit contractions of the cat nictitating membrane elicited by nerve stimulation both *in vivo* and *in vitro* (Trendelenburg, 1957; Kosterlitz & Taylor, 1959; Thompson, 1960). This action of morphine is of interest since it is a specific effect (Cairnie, Kosterlitz & Taylor, 1961) and appears to involve an inhibition of noradrenaline (NA) release. We have now examined the effect of morphine on NA release from the cat nictitating membrane.

The responses of the medial smooth muscle of the cat nictitating membrane and the guinea-pig myenteric plexus-longitudinal muscle preparation were recorded *in vitro*. The tissues were bathed in Krebs solution at 37° C and stimulated supramaximally by electrical field stimulation (1 ms rectilinear pulses). The NA outputs were measured by transferring the fluid surrounding the tissues to a cascade system in which the NA was assayed on superfused rabbit arterial preparations (Hughes, 1972).

Morphine caused a dose-dependent inhibition of the contraction of the nictitating membrane to electrical stimulation. This inhibition could be reversed by the addition of naloxone (0·27 μM) to the bath. The effect of morphine was most prominent at low frequencies of stimulation (0·1–2 Hz), thus confirming the observations of Cairnie *et al.* (1961). At 1 Hz the ED_{50} for morphine was 0·5 μM.

After stimulation with trains of 100 pulses, the outputs of NA from the nictitating membrane were not significantly different at 0·2, 1, 5 and 15 Hz, the mean output being $8 \pm 1{\cdot}9$ (mean $\pm$ S.E.) (pg/pulse)/g of tissue ($n=18$). The noradrenaline output was markedly reduced by morphine and again this effect was most prominent at the lower frequencies of stimulation. Phenoxybenzamine (29·3 μM) produced a 23-fold increase in noradrenaline output at each frequency without altering the frequency-output relationship. The fraction of the total tissue content of NA which was released by one pulse after phenoxybenzamine was $4\text{–}8 \times 10^{-5}$. This constancy of output per pulse at varying frequencies contrasts strongly with that found in the rabbit vas deferens and portal vein (Hughes, 1972), in which the output/pulse increases as the frequency is increased. In these tissues of the rabbit, morphine had no depressant effect on NA release.

In the guinea-pig myenteric plexus, morphine (1·33 μM) did not depress evoked NA output although the release of acetylcholine is depressed by this drug (Paton, 1957). In this tissue, the output of NA per pulse at 16 Hz was twice that at 2 Hz. Phenoxybenzamine (29·3 μM) increased the output 7-fold at both frequencies.

The different types of frequency-output relationships found in the various tissues may represent multiple mechanisms for the control of NA release. It may be significant that sensitivity to morphine appears to be linked with only certain types of frequency-output relationships both in the cholinergic (Greenberg, Kosterlitz & Waterfield, 1970) and adrenergic systems.

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The effect of nerve stimulation on the depletion of noradrenaline by reserpine in the heart, vas deferens and anococcygeus muscle of the rat

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Most adrenergically innervated peripheral organs are depleted of their noradrenaline (NA) content by reserpine to a roughly similar degree. An exception is the vas deferens which is relatively resistant (Sjöstrand & Swedin, 1968). Two possible explanations are either that the rate of depletion is dependent on the firing frequency in the nerves and this is low in the vas deferens, or that 'short' adrenergic neurones in the vas deferens are less easily depleted than the long adrenergic neurones elsewhere. We have investigated these by studying the effect of artificial stimulation on the rate of depletion of the nerves to the heart, vas deferens and anococcygeus. The latter has a dense adrenergic innervation uniformly distributed throughout the muscle like the vas deferens, but unlike the vas deferens, the neurones are conventional 'long' ones (Gillespie & McGrath, 1972).

Rats were given various doses of reserpine intraperitoneally, the vas deferens and anococcygeus muscles removed 24 h later, and the NA content measured. A dose of 200 μg/kg caused 80–90% depletion of NA in both muscles. The time course of depletion with such a dose was then examined in all three organs. After 6 h