Inhibition of ^{3}H-noradrenaline release from sympathetic nerves of guinea-pig atria by a presynaptic α-adrenoceptor mechanism

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Brown & Gillespie (1957) were the first to show that phenoxybenzamine caused an increase in noradrenaline (NA) efflux in response to sympathetic nerve stimulation. The effect has been attributed to blockade of the loss of released NA either at its post-junctional site of action (α-adrenoceptors) or by neuronal or extraneuronal uptake.

The mechanism of action of phenoxybenzamine has been explored in experiments with guinea-pig isolated atria in which adrenergic transmitter stores had been labelled by incubation with ^{3}H-NA. The intrinsic sympathetic nerves were stimulated with electrical field pulses at 5 Hz for 30 s periods. Efflux of radioactive substances into the fluid bathing the atria was measured during resting periods and during periods when the nerves were stimulated.

Phenoxybenzamine (1×10^{-7} to 1×10^{-5}M) caused a concentration-dependent increase of tritium in response to sympathetic nerve stimulation and also increased the positive inotropic response. With 1×10^{-5}M, the increase in tritium efflux was more than 6-fold.

Since β-adrenoceptors predominate in the atria, and in rabbit heart with which Starke & Schümann (1972) observed similar effects, phenoxybenzamine cannot be increasing efflux by blocking the post-junctional site of action of NA. Furthermore, propranolol did not increase stimulation-induced efflux.

Cocaine (1×10^{-7} to 3×10^{-4}M) and desipramine (3×10^{-8} to 1×10^{-4}M), which inhibit neuronal uptake, caused only slight increases (<2-fold) in tritium efflux. Normetanephrine (5×10^{-6} and 1×10^{-5}M), which inhibits extraneuronal uptake, had no effect on tritium efflux. Consequently, it is unlikely that blockade of NA uptake is responsible for the greatly increased efflux caused by phenoxybenzamine.

Kirpekar & Puig (1971) postulated the presence of α-adrenoceptors at terminal adrenergic axons through which transmitter release was inhibited, and suggested that blockade of these receptors by phenoxybenzamine removed the inhibition. In atria, tritium efflux in response to sympathetic nerve stimulation was increased more than 2-fold by three other α-adrenoceptor antagonists (each in a concentration of 1×10^{-5}M): phentolamine, azapetine and dihydroergotamine.

Further evidence that phenoxybenzamine was acting on α-adrenoceptors to enhance NA release was obtained from experiments in which the receptors were protected from blockade by the presence of the agonistic drugs methoxamine or oxymetazoline. These drugs markedly reduced or abolished the effect of phenoxybenzamine (1×10^{-6} to 1×10^{-5}M). With stimulation at a frequency of 5 Hz, the α-adrenoceptor agonists did not significantly decrease tritium efflux, hence the inhibition may be maximal with endogenously released noradrenaline at this frequency of stimulation.

However, exogenous NA (1×10^{-6}M) decreased the effects of stimulation on tritium release and force of contraction.

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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.