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.

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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.

The bioassay technique depends on transferring the Krebs solution bathing the donor tissue to a cascade system where the active material is assayed on a series of superfused vascular tissues. This technique allows the detection of as little as 50 pg of noradrenaline added to the 4 ml donor bath. The rabbit portal vein (Hughes & Vane, 1967) and the rabbit vas deferens were used as donor tissues and the intramural nerves excited by transmural stimulation (1 ms, supramaximal voltage at 1–16 Hz for 120, 240 and 480 pulses).

The release of noradrenaline-like material was seen at all frequencies, and was abolished in the presence of bretylium (10^{-6}–10^{-5} g/ml) or tetrodotoxin (5×10^{-7} g/ml). The contractions of the assay vessels to noradrenaline and to the released material were also abolished by phentolamine (10^{-9}–10^{-8} g/ml). Further proof that the active material was noradrenaline was obtained by combining the relatively large outputs from the vas, concentrating the samples over alumina, and estimating the noradrenaline fluorimetrically.

The most obvious feature of the outputs from the vein and vas deferens was the much greater output per pulse seen when the frequency was increased from 1–4 Hz to 8 Hz and above. Thus at 2 Hz the mean output from the vein was $11{\cdot}9\pm0{\cdot}86$ (pg/pulse)/g (mean$\pm$S.E.M.); this increased to $30{\cdot}7\pm3{\cdot}97$ (pg/pulse)/g at 8 Hz (ten experiments each). This difference was significant ($P<0{\cdot}01$), and was not altered by treating the tissues with cocaine. In the vein and the vas deferens, cocaine (5 μg/ml) increased the noradrenaline output by 200–300% at both low (1–4 Hz) and high (8–16 Hz) frequencies of stimulation.

These experiments indicate that the neuronal uptake mechanism for noradrenaline is as active at high as at low frequencies of stimulation. The differences in output per pulse contrast with results obtained in the spleen. Metabolism of the noradrenaline may contribute to this difference in measured outputs (Langer, 1970) and this and other possible mechanisms are being investigated.

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Motor transmission in the vas deferens: the inhibitory action of noradrenaline

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It is generally held that the post-ganglionic motor transmitter in the guinea-pig vas deferens is noradrenaline. The following findings do not support this view: