are contracted by low concentrations of prostaglandins of the E and F series, the rat colon being more sensitive to F's than to E's (Ferreira & Vane, 1967). Pretreatment of the assay tissues for 2 h (before bathing them in blood) with indomethacin (5 μg/ml) and phenoxybenzamine (2 μg/ml) increased their sensitivity to and specificity for prostaglandins (Gilmore, Vane & Wyllie, 1968). Muscular work was produced by electrical stimulation of the distal end of the cut sciatic nerve at 4 Hz (pulses of 5-7 v., 0.1 ms duration) for 5-10 minutes.

Stimulation of the sciatic nerve was often accompanied by the release into the femoral venous blood of an unidentified substance which relaxed the assay tissues. This relaxation waned and was superseded, usually during the fifth minute of exercise, by contractions of the assay tissues (45/51 trials). The contractions, indicative of PLS release, were sustained for up to 20 min after the exercise was over. Gallamine (3 mg/kg i.v.) prevented both muscle contraction and PLS output which could then be obtained by direct stimulation of the muscles. PLS release was also demonstrated in 6 experiments in which the vascularly isolated gracilis muscle was stimulated either via the gracilis nerve, or directly after gallamine.

The relative contractions of the assay tissues suggested that the PLS was mainly of the E series; the concentration released (assayed as prostaglandin E_2) ranged from 0.5-4.5 ng/ml (mean ± s.e. = 2.18 ± 0.24 ng/ml). When the large increase in blood flow which accompanied the muscle work is taken into account, it is evident that the output of PLS per min was substantial.

Indomethacin (2 mg/kg i.v.), an inhibitor of prostaglandin biosynthesis (Vane, 1971) was given in 17 dogs. Muscular exercise was no longer accompanied by PLS output, re-enforcing the conclusion that the contractor substance detected was a prostaglandin. The initial relaxation was still present. Thus PLS is released as a consequence of muscle contraction.

Prostaglandins of the E-series are potent vasodilators in the hind limb, so that this release may contribute to the functional hyperaemia. The fact that the release outlasts the exercise suggests that PLS contributes more to post-exercise hyperaemia than to that which occurs during exercise.

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Modulation of frequency-dependent noradrenaline release by calcium, angiotensin and morphine

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Adrenergic transmitter release appears to be regulated by a number of mechanisms at the site of release. These mechanisms may vary at different neuroeffector sites giving rise to different patterns of transmitter release in various tissues. Thus in the rabbit portal vein and vas deferens, when the external calcium ion concentration was 2.54 mM, the output/pulse of noradrenaline increased with the frequency of nerve stimulation whereas in the cat nictitating membrane and mouse vas deferens, the output/pulse was constant over the frequency range 0.2-15 Hz. These differences were not due to variations in transmitter uptake or metabolism (Hughes, 1972; Henderson & Hughes, unpublished observations). Further clarification of the mechanisms underlying the frequency-output relationship may be achieved by studying agents which modify the relationship.

All the experiments were carried out on isolated tissues bathed in Krebs solution at 36 ±1° C. Noradrenaline output was determined by measuring the overflow into the bathing fluid (Hughes, 1972).

When the external calcium concentration was increased from 2.54 to 5.08 mM, the output of noradrenaline from the rabbit vas deferens was preferentially increased at low frequencies of

stimulation (0.5-5 Hz) and the frequency-output slope reduced to near zero. The reverse occurred when the calcium concentration was reduced to 1.27 mM, there was a proportionately greater reduction of output at the lower frequencies of stimulation and the frequency-output slope increased threefold. In the mouse vas deferens raising the calcium concentration from 2.54 to 5.08 mM preferentially increased output at low frequencies, thus causing the output/pulse of noradrenaline to decrease with frequency. Conversely decreasing the calcium concentration to 1.27 mM reduced the output at low frequencies thus causing the output of noradrenaline to increase with frequency. Therefore, modification of the external calcium concentration altered the frequency-output relationship at various sites such that the noradrenaline output/pulse increased, decreased or remained constant with stimulus frequency.

In the rabbit portal vein and vas deferens, tissues in which the output/pulse increased as the frequency was increased (calcium concentration 2.54 mM), angiotensin (0.2-1 μM) increased the noradrenaline output at low frequencies (0.5-5 Hz), with little or no effect at higher frequencies. Thus in the presence of angiotensin the output/pulse remained constant between 0.5 and 16 Hz. In the mouse vas deferens, angiotensin (1 μM) did not alter the output/pulse at low or high frequencies. Morphine (1 μM) depressed the output at low frequencies in the mouse vas deferens so that in the presence of morphine the output/pulse increased with stimulus frequency. In the rabbit portal vein and vas deferens, morphine had no effect.

The possibility therefore arises that differences in the patterns of noradrenaline output may reflect different mechanisms involved in calcium utilization by the release process. Modulation of these mechanisms by drugs can lead to frequency-dependent effects on noradrenaline output.

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Acetylcholine synthesis from [^{14}C]-choline in isolated segments of guinea-pig ileum

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The study of transmitter mechanisms in adrenergic nerves has been greatly facilitated by the use of radio-labelled noradrenaline and its precursors. Few comparable studies have been carried out with radio-labelled acetylcholine or choline. Uptake and release have been investigated with brain synaptosomes (Clouet & Williams, 1974), phrenic nerve-diaphragm preparations (Chang & Lee, 1970; Potter, 1970), autonomic ganglia (Friesen, Kemp & Woodbury, 1965), and heart (Wallach, Goldberg & Shideman, 1967; Buterbaugh & Spratt, 1968). Only one study has been carried out with intestine (Mattila & Idänpään-Heikkilä, 1968).

Longitudinal muscle–Auerbach's plexus preparations were dissected from guinea-pig terminal ileum, as described by Paton & Aboo Zar (1968), and mounted in an organ bath (8 ml) in Krebs-Henseleit solution containing physostigmine (0.05 μg/ml). The strips were incubated for 75 to 300 min in [^{14}C]-choline at a radioactive concentration of 0.125 μCi/ml corresponding to choline concentrations of 4.2 to 7.0 μM; these concentrations of choline did not affect the tone of the muscle. The incorporation of the ^{14}C-label into acetylcholine in the tissue was determined as described by Potter & Murphy (1967).

Strips which were stimulated at 0.1 Hz during the period of incubation showed increased incorporation of radioactivity attributable to acetylcholine amounting to 171% of that in unstimulated strips ($P < 0.02$, $n = 6$) for a 75 min period of incubation. However, there was a significant decrease to 66% and 70% of control ($P < 0.001$ for each) for periods of incubation of 150 min and 225 min, respectively. These findings suggest that the rate of incorporation of [^{14}C]-choline into acetylcholine is increased by field stimulation of cholinergic neurones, but if the incubation time is prolonged the increased turnover of label caused by stimulation leads to loss of choline by metabolic degradation or by incorporation into other molecules such as phospholipids.