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Some mechanisms which may modulate noradrenaline release in guinea-pig isolated ileum

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Guinea-pig isolated ileum pre-incubated with [^{3}H]noradrenaline, released ^{3}H-label during coaxial stimulation at 3·2–12·8 Hz. This evoked ^{3}H-overflow seems due to nerve stimulation, and may be frequency-dependent. The ^{3}H-overflow evoked by coaxial stimulation was increased with 2·6 μM phentolamine, reduced at threshold stimulation by 0·56 μM prostaglandin E_1, but the tendency for 2·8 μM indomethacin to increase the evoked ^{3}H-overflow was not statistically significant. The results indicate that the release of noradrenaline from guinea-pig ileum may be modulated by activation of presynaptic α-adrenoceptors, whereas prostaglandins act mainly at a postsynaptic site to modulate the sympathetic response.

Noradrenaline may inhibit its own release from sympathetic neurons in various tissues, by activating presynaptic α-adrenoceptors (Farnebo & Hamberger 1970; Häggendal 1970; Langer 1970). In contrast, stimulation of presynaptic β-adrenoceptors may increase noradrenaline release (Adler-Graschinsky & Langer 1975; Stjärne & Brundin 1975). Some prostaglandins (PGs) can also reduce noradrenaline release (Hedqvist 1969), although probably by acting at a site other than presynaptic α-adrenoceptors (Stjärne 1973).

Modulation of noradrenaline release has not been fully investigated in the gut. In the guinea-pig, inhibitory presynaptic α-adrenoceptors are thought to occur in the sympathetic neurons of the ileum (Drew 1977), and PGE_1 reduces the overflow of ^{3}H-label by perivascular nerve stimulation in the taenia caecum pre-incubated with [^{3}H]noradrenaline, whereas indomethacin causes an increase (Ishii et al 1977). We have used guinea-pig isolated ileum incubated with [^{3}H]noradrenaline, to investigate the effects of adrenoceptor antagonists, PGE_1 and indomethacin on the stimulated release of ^{3}H-label.

METHODS

Male albino or coloured guinea-pigs approximately 400 g were stunned and bled. Segments of distal ileum 4 to 5 cm long were removed at least 8 cm from the caecum. These were incubated for 90 min in 1 ml modified Krebs solution (mM: NaCl 118·6; $CaCl_2$ 2·7; KCl 4·7; KH_2PO_4 1·2; $MgSO_4$ 0·1; $NaHCO_3$ 25·0 and dextrose 10·4), containing 25 μl 1-[7-^{3}H]noradrenaline (^{3}H-NA) (9·8 Ci mmol^{-1}) and 0·57 mM ascorbic acid, to protect the noradrenaline from oxidation. The solution was maintained at 37 °C and bubbled with 5% CO_2 in O_2. After incubation, the ileum was suspended under a 1 g load in a 10 ml isolated organ bath for coaxial stimulation (Paton 1955). The tissue was then washed for 30 min by continuous overflow of 2 litres Krebs solution at 37 °C, containing 0·57 mM ascorbic acid (used for all subsequent experiments) and bubbled with 5% CO_2 in O_2.

Coaxial stimulation of the ileum was provided by a Grass S88 stimulator. Voltage was just maximal (8–20 V) at a pulse width of 0·5 ms and frequencies from 0·1 to 12·8 Hz. Longitudinal muscle responses were recorded using an isometric transducer (Grass Instrument Co. Ltd. FT03C) and pen recorder. In each experiment, 4 min rest preceded 4 min stimulation. The bath fluid was collected after each period, the tissue washed once and the bath refilled with fresh Krebs solution. From each collection 0·4 ml was added to 10 ml scintillant and counted for radioactivity in an Intertechnique SL30 liquid scintillation spectrometer; ^{3}H-overflow from the ileum was expressed as μCi min^{-1} g^{-1} wet weight. To minimize the effect of the ^{3}H-label depletion, frequency response curves for ^{3}H-overflow were constructed with equal numbers of experiments beginning at the low or at the high end of the frequency scale. The results are therefore expressed as the evoked ^{3}H-overflow, calculated as a percentage of the resting overflow.

Noradrenaline (NA) extraction and assay. The method used followed that of Welch & Welch (1969). Sections of ileum were homogenized in a 150 W ultrasonic disintegrator (MSE) and fluorescence was

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recorded spectrometrically. NA was assayed by comparison with standard NA concentrations which were extracted and prepared at the same time as the ileum samples, thus providing an internal standard for correction of recovery losses.

Uptake of ^{3}H-label was determined from aliquots of the organic and aqueous layers obtained during extraction, by comparison with similar extractions of standard ^{3}H-NA.

Drugs used. PGE_1 (Upjohn Ltd. and Miles Laboratories Ltd.), indomethacin (Merck, Sharp and Dohme), phentolamine mesylate (CIBA), propranolol hydrochloride (ICI), histamine acid phosphate (BDH), tetrodotoxin (Sankyo), (−)-noradrenaline bitartrate and (−)-[7-^{3}H]noradrenaline (Radiochemical Centre, Amersham).

Indomethacin 2·8 μM was freshly prepared by dissolving 2 mg in 4 ml ethanol and adding to 2 litres Krebs solution. The scintillant was PCS (Amersham-Searle): toluene (1 : 1 v/v). For extraction and assay of noradrenaline, solutions were prepared according to Welch & Welch (1969).

Results are expressed as medians and semi-quartile ranges and analysed by the Wilcoxon matched-pairs signed rank test, the Mann-Whitney U-test, or the Spearman rank correlation. Where P values are not given, 'significant' means $P \leqslant 0{\cdot}05$.

RESULTS

Endogenous NA, and uptake of ^{3}H-label

The NA content of the ileum was 2·2 (0·9 to 3·2) μg g^{-1} (n = 9), and this was not significantly changed by incubation with 25 μl ^{3}H-NA (2·0 (range: 0·7 to 4·6) μg g^{-1}, n = 4).

During incubation with ^{3}H-NA, 18·6 (15·1 to 35·2) μCi ^{3}H-label g^{-1} was taken up by the ileum (n = 5). Phentolamine 2·6 or 13·2 μM, or propranolol 0·4 μM, did not significantly affect the uptake of ^{3}H-label during incubation ($P > 0{\cdot}4$ for each, n = 6, 5 and 4 respectively).

^{3}H-overflow evoked by coaxial stimulation

Coaxial stimulation at frequencies above 1·6 Hz evoked ^{3}H-overflow from the ileum (Fig. 1). Tetrodotoxin 0·7 μM prevented both the twitch response and the evoked ^{3}H-overflow, whereas histamine 0·3–6·5 μM, in the presence of 0·7 μM tetrodotoxin, contracted the ileum but did not greatly stimulate ^{3}H-release (Fig. 1).

The evoked ^{3}H-overflow per pulse during coaxial stimulation declined from approximately 7 nCi g^{-1} at 0·1 Hz to approximately 1 nCi g^{-1} at 0·8–12·8 Hz (Fig. 2).

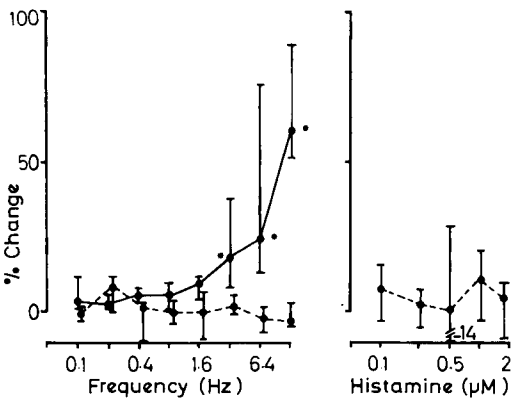

FIG. 1. Coaxial stimulation of guinea-pig ileum (1·6 to 12·8 Hz) significantly increased ^{3}H-overflow at each frequency, after incubation with ^{3}H-NA (●—●; $P < 0{\cdot}05$; n = 10). Evoked ^{3}H-overflow was expressed as the percentage of the ^{3}H-overflow at rest (medians and semiquartile ranges). Tetrodotoxin 0·7 μM significantly reduced this evoked ^{3}H-overflow at 3·2–12·8 Hz (●- - -●; n = 6; $*P < 0{\cdot}03$). Histamine, in the presence of 0·7 μM tetrodotoxin did not greatly affect ^{3}H-overflow (medians and ranges; n = 4).

Effect of adrenoceptor antagonists on coaxially stimulated ^{3}H-overflow

Phentolamine 2·6 μM significantly increased the regression coefficient for ^{3}H-overflow evoked at 1·6–12·8 Hz, but comparing each frequency separately the ^{3}H-overflow was significantly increased

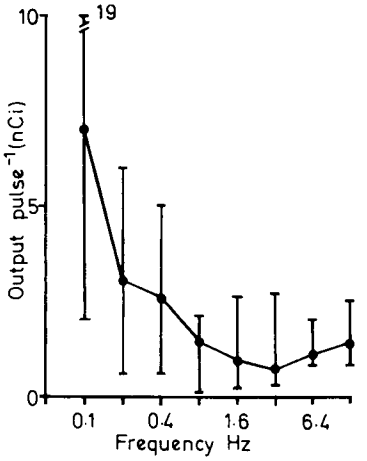

FIG. 2. Coaxially-stimulated output of ^{3}H-label per pulse decreased from approximately 7 nCi g^{-1} at 0·1 Hz, to approximately 1 nCi g^{-1} at 0·8–12·8 Hz. (Spearman rank correlation: $P < 0{\cdot}01$; n = 10 for each frequency). Points represent the median and the bars represent the semiquartile ranges, calculated by subtracting the resting from the stimulated overflow and dividing by the number of pulses.

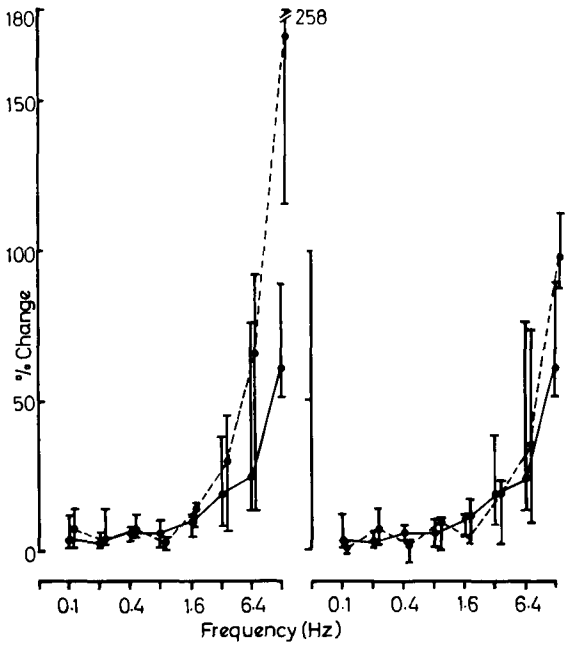

FIG. 3. Phentolamine 2·6 μM added 30–40 min before each experiment (left-hand graph, ●- - -●; n = 10) significantly increased the evoked ^{3}H-overflow only at 12·8 Hz ($P = 0{\cdot}003$), when compared with controls (●—●; n = 10). Overall, the regression coefficient for 1·6–12·8 Hz was significantly increased ($P < 0{\cdot}001$). Propranolol 0·4 μM added 30–40 min before each experiment (right-hand graph, ●- - -●; n = 10), had no significant effect (regression coefficient: $0{\cdot}1 > P > 0{\cdot}05$). Points represent the median percentage of the ^{3}H-overflow at rest, the bars semiquartile ranges.

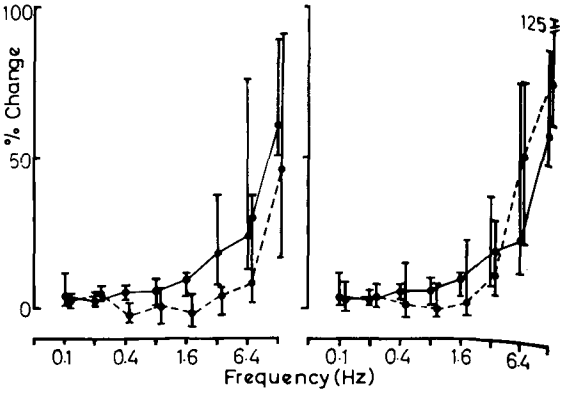

FIG. 4. PGE_1 0·56 μM added 30–40 min before each experiment (left-hand graph, ●- - -●; n = 10) significantly reduced the evoked ^{3}H-overflow at 3·2 Hz ($P = 0{\cdot}03$), when compared with controls (●—●; n = 10). Overall, the regression coefficient for 1·6–12·8 Hz was not significantly reduced ($P > 0{\cdot}1$). Indomethacin 2·8 μM added 30–40 min before each experiment (right-hand graph, ●- - -●; n = 10), had no significant effect (regression coefficient: $P > 0{\cdot}1$). Points represent the median percentage of the ^{3}H-overflow at rest, the bars semiquartile ranges.

only at 12·8 Hz (Fig. 3). The overall tendency for propranolol to reduce the regression coefficient was not statistically significant (Fig. 3).

Effect of PGE_1 or indomethacin on coaxially stimulated ^{3}H-overflow

PGE_1 14 nM or 0·56 μM did not significantly affect the regression coefficient for ^{3}H-overflow evoked at 1·6–12·8 Hz; comparing each frequency separately, these concentrations of PGE_1 significantly reduced the ^{3}H-overflow only at 3·2 Hz (Fig. 4 for 0·56 μM PGE_1). Indomethacin 2·8 μM had no significant effect on the evoked ^{3}H-overflow (Fig. 4).

DISCUSSION

The NA content of the distal ileum was similar to that found by Bennett & Houghton (1976); at least part of this store could be labelled by incubation with ^{3}H-NA. During coaxial stimulation, ^{3}H-label was released from the ileum and this overflow was blocked by tetrodotoxin. In similar experiments where the perivascular nerve was stimulated, the evoked ^{3}H-overflow strongly correlated with the sympathetic response (Sanger 1977), suggesting that the ^{3}H-label was released mainly as a consequence of noradrenergic stimulation. However, the possibility that other neurons also take up and release ^{3}H-label cannot be excluded.

The 'output per pulse' for ^{3}H-overflow evoked by coaxial stimulation may not be exact since the spontaneous NA overflow during and between each pulse is not known; Cowie et al (1978) suggest that in the presence of eserine, spontaneous acetylcholine overflow from the guinea-pig ileum is suppressed during stimulation. At frequencies of stimulation which significantly increased the overflow of ^{3}H-label (3·2–12·8 Hz), the ^{3}H 'output per pulse' seems independent of frequency. At lower frequencies, the 'output per pulse' apparently declined with increasing frequency, as in human taenia coli pre-incubated with ^{3}H-NA (Houghton & Bennett 1978). Higher rates of stimulation may stimulate more noradrenergic neurons than at low frequencies, or perhaps different types of noradrenergic neurons are present.

Henderson et al (1972, 1975), using a myenteric plexus-longitudinal muscle preparation from guinea-pig ileum, found that the overflow of intact noradrenaline per pulse was frequency-dependent. However, metabolized NA was not measured and

metabolism may depend on stimulation frequency (Langer 1970).

Phentolamine, but not propranolol, in concentrations which did not affect the uptake of ^{3}H-NA by the ileum, increased the evoked ^{3}H-overflow, particularly during high-frequency stimulation. Phentolamine might therefore inhibit an ileal presynaptic α-adrenoceptor negative feedback mechanism (Drew 1977). The existence of presynaptic β-adrenoceptors strongly modulating NA release seems unlikely from our experiments.

PGE_1 significantly reduced the evoked ^{3}H-overflow only at 3·2 Hz, increasing the threshold of stimulation at which sympathetic neurons released ^{3}H-label. Indomethacin 2·8 μM had no effect on the evoked ^{3}H-overflow, suggesting that prostanoids synthesized either spontaneously, in response to electrical stimulation or to NA (Botting & Salzmann 1974; Botting 1977) are not important modulators of NA release from the ileum. In contrast, prostanoid synthesis seems to be an important modulator of NA release from guinea-pig isolated taenia caecum (Ishii et al 1977).

Kadlec et al (1978) reported that 30 nM PGE_2 reduced the ^{3}H-overflow evoked at 5 Hz, but 50 μM indomethacin caused an increase. However, this concentration of indomethacin may inhibit phosphodiesterase (Flower 1974), so that the increased concentration of cyclic AMP may facilitate evoked NA overflow (Langer et al 1975).

PGE_1 or PGE_2 usually antagonized, whereas indomethacin strongly facilitated the inhibition of muscle contraction induced in guinea-pig ileum by NA or perivascular nerve stimulation (Gintzler & Musacchio 1974; Sanger & Watt 1976, 1978; Ehrenpreis et al 1978). In this tissue, antagonism by prostanoids would therefore seem to be mainly postsynaptic, while activation of presynaptic α-adrenoceptors may reduce the release of NA.

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