membrane, a finding confirmed by microscopy. The cells were then perfused (2 ml/min) with albumin-free buffer solution, and the perfusate collected at timed intervals. The histamine content of the perfusate was determined by alkaline condensation with O-phthalaldehyde followed by acidification and measurement of the fluorescence (excitation 350 nm, emission 440 nm; Shore, Burkhalter & Cohn, 1959) or by bioassay on the superfused guinea-pig ileum. In further experiments, the perfusate was led directly to a mixing coil and to a flow-cell spectrofluorimeter which enabled the chemical histamine assay to be carried out automatically, with the fluorescence intensity being displayed on a chart recorder.

The cells appeared to retain their functional integrity since the low resting histamine output could readily be increased up to 200-fold by chemical or immunological challenge. In 12 experiments, administration of the polymer 48/80 (0.1 ml volume) into the perfusion medium gave a reproducible and dose-dependent release of histamine from the cells. The threshold (0.05-0.1 μg) and maximal (1-2 μg) doses of 48/80 were similar to those observed with incubated mast cells. With initial high doses of 48/80 (0.5-2 μg), subsequent administration in the same experiment gave a very diminished histamine release, presumably due to depletion of the stored mast cell histamine. However, with lower doses of 48/80 (0.05-0.2 μg), several comparable responses could be obtained.

This method, which can readily be adapted to study other mediators such as 5-hydroxytryptamine, and other cell types, should give a better understanding of the kinetics of mediator release from specific inflammatory cells and provide a simple technique for the rapid screening of drugs which alter such release.

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The inhibitory transmission to the internal anal sphincter

A.O. ADEBANJO, N. AMBACHE & J. VERNEY

Medical Research Council, Department of Physiology, Royal College of Surgeons of England, London WC2A 3PN

Inhibition of this sphincter by the sacral nerves is atropine-resistant (Langley & Anderson, 1895a; Garrett & Howard, 1975). Our results show that this sacral inhibitory post-ganglionic transmission resembles that present in the retractor penis (Luduena & Grigas, 1966, 1972; Ambache, Killick & Zar, 1975). It is likely that the other atropine-resistant sacral inhibitory effects described by Langley & Anderson (1895b) are similarly mediated.

Procedure: The anal canal is excised by a wide perineal incision. The anus is slit and a 1-2 cm width of mucosa, adjoining the skin, is dissected off the underlying sphincters. A plane of separation is found between the pink-brown external sphincter (skeletal) and the white smooth muscle band of the internal sphincter. Strips from > 50 rabbit, pig, dog, cat and guinea-pig sphincters were suspended in Krebs-Henseleit (35° C) and stimulated transmurally (1-20) pulses at 1 min intervals; supramaximal voltage; 0.2 ms 10 Hz).

Results: Transmural stimulation elicited repeatable inhibitions unaffected by pentolinium (93 μM) but abolished by tetrodotoxin (0.63 μM). Pure relaxations were obtained without 'rebound' (e.g. Figure 1) in most preparations; only a few had an additional (adrenergic) motor component which disappeared after guanethidine, 10 μM, or phentolamine, 5.3 μM.

That acetylcholine is not the inhibitory parasympathetic transmitter was shown by: (a) its opposite, contractile effect, except once (cat sphincter; cf. monkey, Rayner, 1971) when, in high doses (277 μM), acetylcholine, like nicotine, produced a pentolinium-susceptible inhibition. (b) Persistence of these neurogenic inhibitions in atropine (0.029-29 μM); and (c) failure of physostigmine (7.7 μM) to potentiate the atropine-resistant inhibitions.

The inhibitory transmission is not adrenergic or tryptaminergic, persisting after propranolol

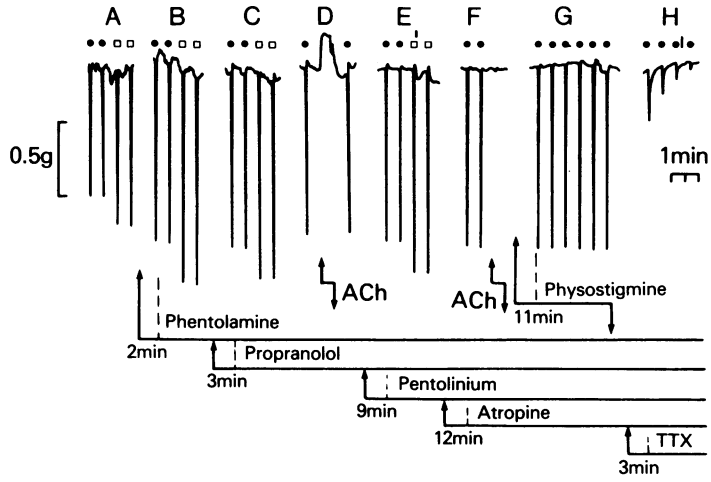

Figure 1 Rabbit sphincter ani internus: neurogenic inhibition is not cholinergic or adrenergic. The inhibitory responses, elicited at 1 min intervals by 10 (•) or 20 (□) pulses, persist (B-G) in phentolamine (5.3 μM), propranolol (6.8 μM), pentolinium (93 μM) and atropine (29 nM) but vanish, at H, in tetrodotoxin (TTX, 0.63 μM). They are not mimicked by 28 μM acetylcholine (ACh, 28 μM), which is motor before atropine (cf. D and F); and are not potentiated by physostigmine (G, 7.7 μM). The preparation did not respond to ATP (18 μM; not shown).

(6.8 μM) or butoxamine (6.6 μM) ± phentolamine (5.3 μM) or phenoxybenzamine (5.8 μM); and after guanethidine (10 μM), bretylium (24 μM) or LSD (125 nM).

Transmission by ATP, histamine or prostaglandins (E and F), was excluded.

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