Lignocaine (1–100 μg/ml), cocaine (10–100 μg/ml) anoxia (5% CO_2 in N_2) or cooling (22–32° C) either had no effect on the TTX-resistant contractions or simultaneously depressed responses to acetylcholine or KCl. Hyoscine (2–10 μg/ml) or morphine (10–100 μg/ml) did not affect the contractions selectively, and methysergide (1–20 μg/ml), mepyramine (1 μg/ml) and polyphloretin phosphate (10–1,000 μg/ml) in doses which antagonized responses to 5-hydroxytryptamine, histamine and prostaglandin E_2 respectively were mainly without effect.

We conclude that the TTX-resistant contractions were probably due to direct electrical excitation of the smooth muscle cells. Paton (1955) found that atropine completely blocked contractions of guinea-pig ileum to single pulses of short duration and concluded that the response was mediated entirely by cholinergic nerves. The situation is clearly not so with repetitive stimulation. Furthermore, TTX seems likely to be more suitable than atropine for analysing nerve mediated responses since TTX would be expected to block all nerves whereas the response after atropine would include a component from inhibitory nerves (Ambache, 1955).

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Evidence for a non-adrenergic inhibitory nervous pathway in guinea-pig trachea

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Electrical stimulation of the isolated intact guinea-pig trachea causes a biphasic response, initially excitatory and then inhibitory. The excitatory response was abolished by atropine (10 ng/ml) (Farmer & Coleman, 1970).

In the present study, the nature of the inhibitory response has been examined. Propranolol (10–100 ng/ml, n=4) and guanethidine (100–300 ng/ml, n=4) reduced but never completely abolished the inhibitory response. The optimum stimulation period for the inhibitory response increased in the presence of these drugs from 7 s to 12 s. A small inhibitory response was also obtained in tracheas in which sympathetic nerve function was abolished by pretreatment with syrosingopine (5 mg/kg I.P. 16–20 h before the experiment, n=54) or with 6-hydroxydopamine (2×25 mg/kg I.V. on day 1, 2×50 mg/kg I.V. on day 7, experiments performed on days 8–10, n=2). In these preparations the inhibitory response was not modified by propranolol (1 μg/ml) or guanethidine (5 μg/ml).

These results show that the inhibitory response to electrical stimulation consists of two distinct responses, an adrenergic inhibitory response and a non-adrenergic inhibitory response (NAIR). Further investigation of the NAIR carried out in tracheas from syrosingopine pretreated guinea-pigs and in the presence of atropine (100 ng/ml) to eliminate adrenergic and cholinergic effects respectively. The NAIR was abolished by lignocaine (100–300 μg/ml, n=5) and by tetrodotoxin (10–100 ng/ml, n=5), suggesting that it is of nervous origin.

An attempt was made to characterize the transmitter mediating the NAIR. It was concluded that the transmitter was unlikely to be (1) histamine—NAIR not blocked by mepyramine (0·1–1·0 μg/ml, n=5) or burimamide (1–10 μg/ml, n=5), (2) 5-hydroxytryptamine—NAIR not blocked by methysergide (10 μg/ml, n=4), (3) a prostaglandin—NAIR not blocked by the prostaglandin synthesis inhibitor indomethacin (1–3 μg/ml, n=5) or (4) cyclic 3′5′-AMP—NAIR was neither potentiated by the phosphodiesterase

inhibitor theophylline (1·0–30 μg/ml, n=4) nor blocked by the phosphodiesterase activator imidazole (0·1–1·0 mg/ml, n=4).

Burnstock has reported the presence of a non-adrenergic inhibitory nervous pathway in gastro-intestinal muscle (Burnstock, 1972). They suggested that the neurotransmitter mediating the inhibitory response is probably ATP. In the present study the adenosine uptake blocking drug dipyridamole (1–3 μg/ml) potentiated the NAIR in 9 out of 11 experiments and unmasked the inhibitory response to ATP (10 μg/ml) and adenosine (10 μg/ml) added to the bathing medium.

It is concluded that electrical stimulation of the guinea-pig trachea, in addition to activating cholinergic and adrenergic nervous pathways, may activate a separate and distinct inhibitory nervous pathway. The results of initial experiments suggest some similarity to the non-adrenergic inhibitory nervous pathways in gastro-intestinal muscle described by Burnstock (1972).

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Further studies on the interaction between acetylcholine antagonists and anticholinesterases on cat soleus muscle

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It was shown previously by Brimblecombe & Everett (1969a, 1970a) that certain acetylcholine antagonists, notably N-ethyl-2-pyrrolidylmethyl-cyclopentylphenylglycollate (PMCG), caused augmentation of the twitch, indirectly or directly elicited, in both slow-twitch (soleus) and fast-twitch (flexor digitorum longus) muscles of the cat hind limb. It was also shown (Brimblecombe & Everett, 1968b, 1970b) that the same compounds prevented or reversed sarin-induced twitch augmentation in these muscles. The experiments described here were designed to explore the latter effects in more detail.

The method used for recording contractions of the soleus was essentially that described by Buller & Lewis (1965a, b) and all drugs were injected intra-arterially via the sural artery. The acetylcholine antagonists used were the glycollates PMCG and N-methyl-4-piperidylphenylcyclohexyl glycollate (PPCG), the latter both as the racemate and as its R and S enantiomers, and the carboxylate 4′-N-methylpiperidyl-1-phenylcyclopentane carboxylate (G3063).

Sarin (5 μg) was administered to both legs. In one leg 2 mg of an acetylcholine antagonist was given 5 min previously. All the compounds at this dose level which produced little or no twitch augmentation protected the muscle from sarin-induced twitch augmentation. When the augmented maximal twitch on the unproected side had reached a steady state, doses of the acetylcholine antagonist, beginning at 0·1 mg and increasing by a factor of 2, were given. A dose-dependent reduction in maximal twitch tension occurred and dosing was continued until the control tension was reached.

Experiments were also carried out with neostigmine. The acetylcholine antagonists protected the muscle from the effects of 10 μg neostigmine in a dose-dependent manner. Complete protection was given by 2 mg while 1 and 0·5 mg gave only partial protection. The maximal twitch augmentation produced by neostigmine could also be reversed by the acetylcholine antagonists.

The two enantiomers of PPCG, which differ by a factor of not less than 20 in their potency as acetylcholine antagonists, are approximately equiactive in causing augmentation of maximal twitch and in preventing or reversing anticholinesterase-induced twitch augmentation showing that these effects are unrelated to the acetylcholine antagonist activities of the compounds.