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Effects of sympathetic nerve stimulation on electrical activity of Auerbach's plexus and intestinal smooth muscle tone

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It is well known that movement of an intestine is inhibited by sympathetic nerve stimulation. The site of action of catecholamines released by sympathetic nerve stimulation is, however, obscure. Recent histochemical studies indicated that fluorecence of catecholamines was observed mainly around nerve cells in Auerbach's plexus (Norberg, 1964; Jacobowitz, 1965). These observations suggested that the site of action of catecholamines released from the sympathetic nerve endings might be in Auerbach's plexus (Norberg & Sjoqvist, 1966). The present experiments were designed to test effects of sympathetic nerve stimulation on electrical activity of Auerbach's plexus and tone of intestinal smooth muscle.

Male guinea-pigs, 350 to 600 g, were killed by a blow on the head; from a small piece of ileum, longitudinal muscle with perivascular nerve was dissected (Finkleman, 1930) and mounted in a rectangular Lucite chamber filled with 10 ml of Locke Ringer solution at $36 \pm 0{\cdot}5°$ and gassed with 5% CO_2 in oxygen. Both ends of the preparation were sewn with stainless pins to spread the preparation. One end was fixed to the chamber and the other was attached to an isometric force-displacement transducer (Nihon Koden, SS-IT) connected to a recorder. Resting tension was adjusted to 1 g. Electrical activity of Auerbach's plexus was simultaneously recorded by a floating fine glass suction electrode (Tip diameter: 30 to 100 μm) according to Sato, Takayanagi & Takagi (1973). To stimulate the sympathetic nerve, square-wave monophasic pulses of 0·3 to 1 ms duration at 40 Hz were applied to the perivascular nerve for 0·5 to 10 s at supra-maximal voltage through Ag-AgCl electrodes. Those preparations that were relaxed by sympathetic nerve stimulation were used. Locke Ringer solution used had the following composition (mM): NaCl 154, KCl 5·6, $CaCl_2$ 2·2, MgCl 2·1, $NaHCO_3$ 5·9 and glucose 2·8, the pH was 7·8.

Drugs used were guanethidine sulphate (Nippon Ciba Geigy Co. Ltd, Japan), nicotine bitartrate (Nakarai

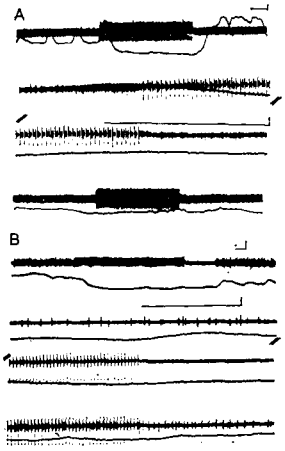

FIG. 1. Effects of sympathetic nerve stimulation on electrical activity of Auerbach's plexus and tension of longitudinal muscle. Upper trace: electrical activity. Lower trace: tension. Vertical calibration: 2 μV and 100 mg. Horizontal calibration: 1 s. Large spikes are artifacts originated in electrical stimulation.
A: Type 1 responses. The top pair: control responses to sympathetic nerve stimulation. The second and third pairs: control responses recorded at a high sweep velocity to observe the spike between stimuli. Note that spikes from Auerbach's plexus were unaffected, notwithstanding that intestinal tone was decreased under and after sympathetic nerve stimulation. The bottom pair: responses in the presence of guanethidine (3×10^{-6} g ml^{-1}).
B: Type 2 responses. The top pair: control responses to sympathetic nerve stimulation. The second and third pairs: control responses recorded at a high sweep velocity. Note that the spike frequency was greatly reduced under and after sympathetic nerve stimulation. The bottom pair: responses recorded at a high sweep velocity in the presence of guanethidine (3×10^{-6} g ml^{-1}).

* Correspondence.

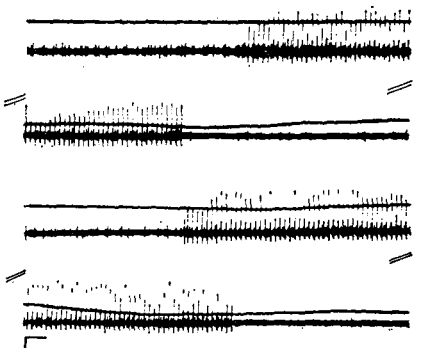

FIG. 2. Effects of sympathetic nerve stimulation on electrical activity of Auerbach's plexus and tension of longitudinal muscle (Type 3 responses). Upper trace: electrical activity. Lower trace: tension. Vertical calibration: 2μV and 100 mg. Horizontal calibration: 0·1 ms. Large spikes are artifacts originated in electrical stimulation. The top and second pairs: control responses to sympathetic nerve stimulation. The third and bottom pairs: responses in the presence of hexamethonium (10^{-5} g ml^{-1}).

Chemicals Co. Ltd, Japan) and hexamethonium bromide (Yamanouchi Seiyaku Co. Ltd, Japan).

We have already classified spikes from Auerbach's plexus in the guinea-pig ileum into two typical patterns: one is termed a single spike unit which is modified by many drugs affecting cholinergic transmission, the other is a burst unit which is hardly affected by drugs (Sato & others, 1973). The single spike unit therefore, is considered to be concerned with cholinergic transmission (Sato & others, 1973; Wood, 1975) while the burst unit was unaffected by sympathetic nerve stimulation, so the single spike unit only was used.

Spontaneous spikes from Auerbach's plexus were recorded from 6 different sites in each preparation. Experiments were carried out on 40 different preparations. Effects of sympathetic nerve stimulation on them were classified into three types.

Type 1: In most sites tested sympathetic nerve stimulation was without any effect on electrical activity of the plexus, while it relaxed the smooth muscle (Fig. 1A). The spontaneous spikes insensitive to sympathetic nerve stimulation were greatly reduced by exogenous noradrenaline as reported by Sato & others (1973). Relaxation of the longitudinal muscle induced by sympathetic nerve stimulation was not observed after administration of guanethidine (3×10^{-6} g ml^{-1}) (Fig. 1A). Type 1 responses were observed from about 70% of all the sites tested. When nicotine (3×10^{-6} g ml^{-1}) was applied, nicotine-induced spikes observed from these sites were also unaffected by sympathetic nerve stimulation. Type 2: Sympathetic nerve stimulation inhibited the electrical activity of Auerbach's plexus and relaxed the longitudinal muscle (Fig. 1B). Both the inhibitory effects were blocked by guanethidine (3×10^{-6} g ml^{-1}). Type 2 responses were obtained from about 20% of all the sites tested. Type 3: Sympathetic nerve stimulation caused long-lasting excitation of neurons in Auerbach's plexus, which was not reduced by hexamethonium (10^{-5} g ml^{-1}) but rather potentiated, while tension of longitudinal muscle was decreased by stimulation (Fig. 2).

The histochemical observations of Norberg (1964) and Jacobowitz (1965) suggested that adrenergic innervation of the intestine seems to lie mostly in the Auerbach's plexus. However, Gershon (1967) tested the relation between acetylcholine output from guinea-pig stomach and sympathetic nerve stimulation, and concluded on the mechanism of sympathetic nervous inhibition of gastrointestinal movement that the effect of sympathetic nerve stimulation is mainly due to direct action of the released catecholamines on the smooth muscle itself. Beani, Bianchi & Crema (1969) also investigated the effects of sympathetic nerve stimulation on contraction of guinea-pig isolated terminal colon and acetylcholine release elicited by pelvic nerve and transmural stimulation and suggested that the sympathetic control of gastrointestinal tone and motility was exerted through two different routes: inhibition of the intramural cholinergic plexus and direct relaxation of smooth muscle cells. In this study we have not obtained the clear relation between the inhibition of intestinal tone by sympathetic nerve stimulation and the decrease of spontaneous activity of Auerbach's plexus by stimulation. The present results, with the reports by Gershon (1967) and Beani & others (1969), suggest that the inhibitory effect of sympathetic nerve stimulation on the intestinal tone is due mainly to the inhibitory action of the released catecholamines on the smooth muscle cells and only to a small extent to inhibition of activity of Auerbach's plexus.

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