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SYMPATHETIC CONTROL OF LOWER OESOPHAGEAL SPHINCTER MOTILITY IN THE CAT

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SUMMARY

1. The action of adrenaline, noradrenaline and efferent sympathetic fibres on the smooth muscle of the lower oesophageal sphincter (l.o.s.) was studied *in vivo* on the anaesthetized cat and *in vitro* with the sucrose gap method.

2. Adrenaline and noradrenaline produce a marked depolarization of the circular muscle of the l.o.s. This effect is suppressed by dihydroergotamine or phentolamine, and greatly reduced by atropine; it remains unaltered by hexamethonium.

3. Sympathetic fibres are excitatory for the l.o.s. They come from the stellate ganglion or run along the splanchnic nerve: the fibres arising from the stellate ganglion (mainly by the cardiac branch of the ganglion) join the vagus nerve at the thoracic level; the fibres running along the splanchnic nerve pass through the coeliac ganglion without synapsing; their cellular bodies lie probably in the ganglia of the sympathetic chain.

4. Repetitive stimulation (20–40 Hz) of these fibres induce, with a latency of 5–8 sec, a sustained or rhythmic contraction of the l.o.s. This response is suppressed by dihydroergotamine, and greatly reduced by atropine, while hexamethonium has no effect.

5. Stimulation of sympathetic fibres induces a facilitation of the vagal excitatory responses and an inhibition of the vagal inhibitory responses of the l.o.s.

6. Our data show that the sympathetic response of the l.o.s. results from the stimulation of adrenergic receptors which are located not only on muscular fibres but also, and chiefly, on intrinsic neurones. Thus, the sympathetic control of the l.o.s. is mainly exerted through cholinergic myenteric neurones which could be excited either directly or indirectly by inhibition of inhibitory intrinsic neurones.

INTRODUCTION

In the cat, the lower oesophageal sphincter (l.o.s.) consists of smooth muscle and has a double innervation: intrinsic (intramural neurones) and extrinsic (vagal and sympathetic supply). In a previous publication (Gonella, Niel & Roman, 1977), we

have studied the vagal component of the extrinsic innervation. The present paper deals with the sympathetic control.

From a classical point of view, the sympathetic post-ganglionic fibres end directly on the smooth muscle fibres. In fact, by means of histofluorescence techniques, many authors have shown that adrenergic endings are also mainly located on the Auerbach's neurones (Norberg, 1964, 1967; Jacobowitz, 1965; Costa & Gabella, 1968, 1971; Baumgarten & Lange, 1969; Furness & Costa, 1974). These data about the sympathetic innervation of the gastrointestinal tract also apply to the oesophagus and l.o.s. Many results about the role of the sympathetic nerves are controversial (see Ingelfinger, 1958). Some of the authors (Zeller & Burget, 1937; Hwang, Essex & Mann, 1947; Ingelfinger, 1958) have only attributed a negligible role to the sympathetic innervation of the l.o.s. Carlson (1922) claimed that sympathetic nerves could exert both motor and inhibitory effects on the area. But, according to Jourdan, Hutet, Sagols & Faucon, (1955) the sympathetic supply of the l.o.s. would represent only the motor component of the extrinsic innervation whereas the vagal fibres would have an inhibitory action. They based their opinion on the fact that after resection of the stellate ganglion in the dog, the permanent spasm of the l.o.s. induced by the section of the two cervical vagi was suppressed. The motor action of the sympathetic tract has been confirmed by Baisset & Montastruc (1956) who demonstrated, in the dog too, that the stimulation of the stellate ganglion induced a contraction of the l.o.s.

We have re-examined the sympathetic control of the l.o.s. using electromyographic techniques. The experiments consisted of stimulating the sympathetic nerves and ganglia and recording simultaneously both electromyogram (e.m.g.) of l.o.s. smooth muscle and intraluminal pressure modifications. Experiments were mainly performed *in vivo* on acute cats. A few animals underwent acute experiments two weeks after a previous thoracic section of the splanchnic nerve. The sucrose gap method was also used to test the responses of circular muscle strips from l.o.s. to adrenaline, noradrenaline and various other drugs.

METHODS

In vivo experiments

Acute experiments. Adult cats of either sex were used. Anaesthesia (sodium thiopentone) surgical procedures, stimulating and recording techniques have been described in a previous paper (Gonella *et al.* 1977). The approach of the left stellate ganglion was carried out by resection of the three first ribs. A thoracic window made on the left side by resection of the 8th, 9th and 10th ribs led to the l.o.s. and left splanchnic nerve. A further dissection of the subdiaphragmatic muscles allowed us to isolate the left splanchnic nerve from the sympathetic chain to the coeliac ganglion. The approach to the right nerves was carried out by passing in the right thoracic or abdominal cavities from the left dissected areas.

Semichronic experiments. These animals first underwent a unilateral section of the splanchnic nerve during an aseptic surgical operation. Anaesthesia was performed by I.P. injection of a sodium pentobarbitone (Nembutal) solution (30 mg/kg). The left splanchnic nerve was dissected at the thoracic level as indicated for acute experiments; it was cut just above the diaphragm. The surgical wound was then closed again and the pleural vacuum restored. After the delay required for nerve fibre degeneration (13 or 14 days), the animals underwent an acute experiment identical to that previously described.

Sucrose gap experiments. The surgical phase was identical to that described for acute experiments. Helical strips of circular muscle from distal thoracic oesophagus and l.o.s. (0·8 mm width

and 3·5 cm length) were dissected and placed horizontally in a sucrose gap apparatus identical to that used by Bülbring & Tomita (1969). The e.m.g. was recorded by two silver–silver chloride electrodes connected to the DC amplifier of a cathode ray (Tektronix 502) oscilloscope by means of a cathode follower (Clottes, 1969). Mechanical activity of the strips was recorded using a diode transducer transforming mechanical tension into potential difference (Talbot, Lilienthal, Beser & Reynolds 1951).

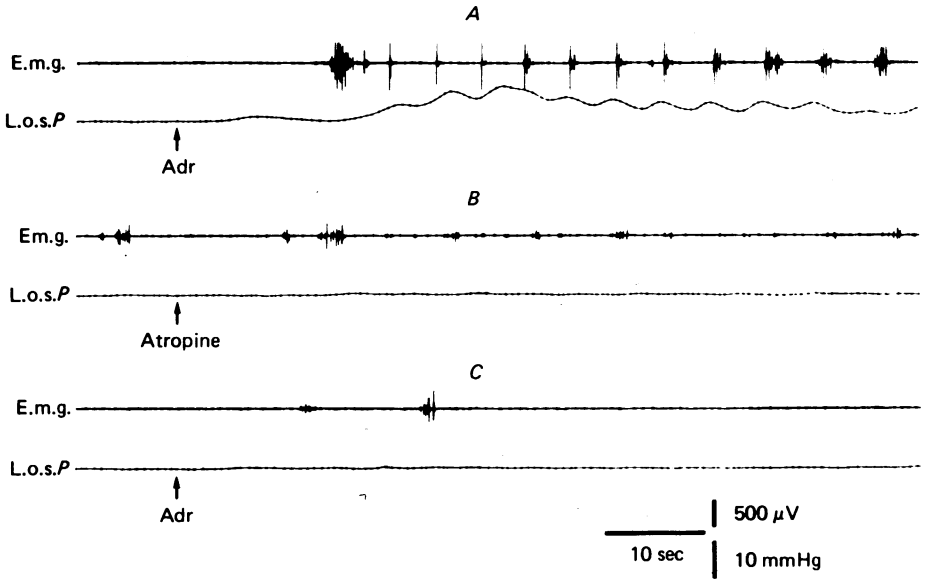

Fig. 1. Action of atropine on the electrical and mechanical responses of l.o.s. to an intravenous injection of adrenaline (*in vivo* experiment). *A*, control; *B*, injection of atropine; *C*, adrenaline under atropine. The arrows indicate the time of adrenaline (Adr, 4 μg/kg) or atropine (0·05 mg/kg) injection. E.m.g.: bipolar recording (RC, 0·3 sec); L.o.s. *P*: intraluminal pressure.

RESULTS

1. *Effects of noradrenaline or adrenaline in vivo*

The I.V. injection of noradrenaline or adrenaline (2–3 μg/kg) elicited a tachycardia, a blood pressure rise and, after 5–8 sec latency, a powerful contraction of the l.o.s. which was either sustained or rhythmic and lasted about 10–20 sec. The motor effect of noradrenaline or adrenaline was located to the l.o.s. primarily. However a slight response could be recorded 1 or 2 cm above the sphincter area.

The l.o.s. response to small doses of noradrenaline or adrenaline (2–3 μg/kg) was suppressed (Fig. 1) by atropine (0·05–0·1 mg/kg). But for higher doses (5–6 μg/kg) noradrenaline and adrenaline were still able to produce a l.o.s. response which was antagonized by dihydroergotamine (1 mg/kg).

2. *Action of noradrenaline and adrenaline on circular muscle strips from the l.o.s. and oesophageal body*

On the muscle of oesophageal body noradrenaline and adrenaline (10^{-5}–10^{-7} g/ml.) induced either a hyperpolarization or a depolarization triggering spikes and a contraction of the strip (Fig. 2).

On the sphincter muscle, noradrenaline and adrenaline (10^{-5}–10^{-7} g/ml.) always elicited a depolarization of about 5–10 mV generally without generating spikes but producing all the same a contraction of the strip (Fig. 2). This response was antagonized by α-blockers (dihydroergotamine or phentolamine; 10^{-6} g/ml.; Fig. 3) and often replaced by a slight hyperpolarization which was suppressed by a β-blocker such as propranolol (10^{-5} g/ml.). The noradrenaline or adrenaline depolarizing effect was markedly reduced by atropine (10^{-6} g/ml.) (Fig. 3), but not affected by hexamethonium (10^{-6} g/ml.).

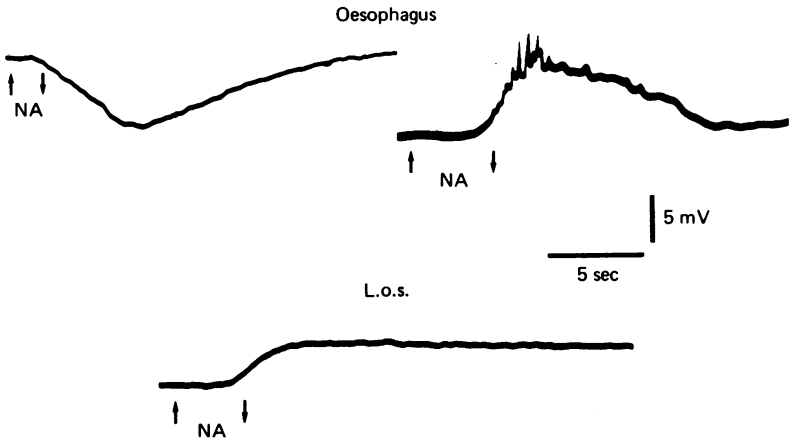

Fig. 2. Action of noradrenaline on circular muscle of l.o.s. and oesophagus. E.m.g. recording with the sucrose gap technique. On oesophagus strips (dissected 4 cm above the l.o.s.), noradrenaline (10^{-6} g/ml.) induced a hyperpolarization: upper left tracing (3 times out of 5); however a depolarization with superimposed spikes could be observed: upper right tracings (2 times out of 5). On strips from the l.o.s., the same concentration of noradrenaline always caused a depolarization without spikes (sixteen animals).

3. *Effects of splanchnic nerve stimulation in vivo*

A : stimulation of the thoracic splanchnic nerve. The splanchnic nerve was stimulated in the thorax between the 7th and 8th rib; it was either ligated or even severed above the stimulating electrodes. Stimulation by a train of stimuli (30 Hz; 0·5 msec; 10 V during 8 or 10 sec) induced first a blood pressure rise and after a long latency (5–8 sec) a motor response of l.o.s. This response assumed two main forms:

(*a*) a single and gradual rise of the endoluminal pressure followed by a very slow return to the basal level;

(*b*) a rhythmic contractile activity, i.e. several pressure waves superimposed on a rise in base-line pressure.

Spike potentials were recorded during both responses. In case of a rhythmic response (type *b* above), each pressure peak was generally (but not always) associated with a burst of spikes, however the rise in base-line pressure did not show any corresponding electrical activity. Besides during the type *a* response, spike potentials occurred as a transient burst only just before or during the ascending phase of the pressure change.

We tried to record a possible slow variation of muscle resting polarization by using monopolar records, long time constant, or even DC amplification. These experiments were not conclusive, mainly because of the displacements of the pressure electrode and the eventual interfering artifacts of movement.

The effects of splanchnic nerve stimulation were confined to l.o.s. and did not occur on the oesophageal body. In addition they were not affected by adrenalectomy.

B: study of the splanchnic path supplying the l.o.s. In order to study the course of the splanchnic fibres supplying the l.o.s., the stimulating electrodes were moved,

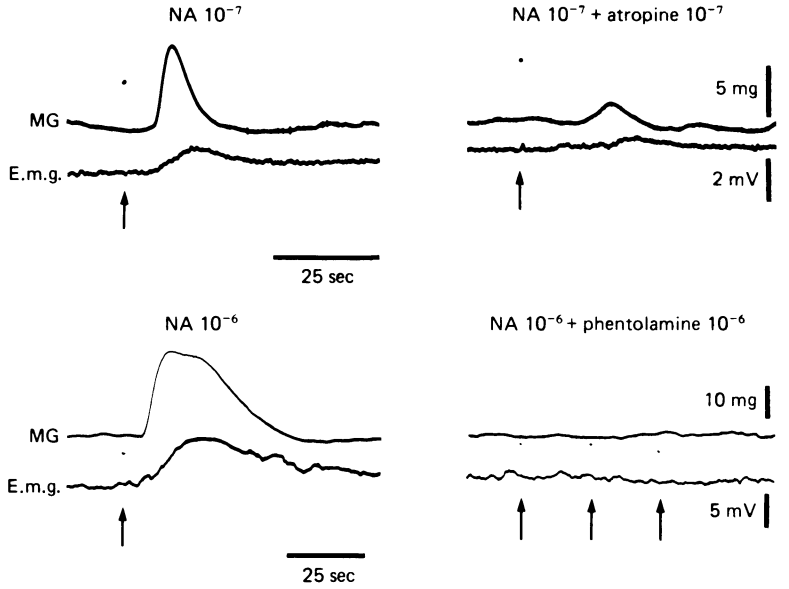

Fig. 3. Action of atropine and phentolamine on the electrical and mechanical responses of two different muscle strips from l.o.s. (sucrose gap technique). Upper trace: mechanical responses (MG); lower trace: electrical responses (e.m.g.). The arrows indicate the time of noradrenaline (NA) application (0·1 ml.).

more and more caudally along the splanchnic nerve. For each successive site, the nerve was cut above the stimulating electrodes. A contraction of the l.o.s. occurred after stimulation at the following sites: the supra-diaphragmatic part of the splanchnic nerve before and after severing the small nerve bundles leaving or reaching the main trunk; the subdiaphragmatic part of the splanchnic nerve and the coeliac ganglion (at those levels the contractions of the l.o.s. were induced by fairly slight stimulations: trains of stimuli at 30 Hz; 0·5 msec; 3 V; 3 sec duration) the most dorsal nervous branches leaving the coeliac ganglion.

C: do the splanchnic fibres innervating the l.o.s. make any synapse within the coeliac ganglion? To answer this question two kinds of experiments have been performed.

(*a*) Direct application of drugs to the coeliac ganglion. In five acute experiments, solutions of atropine or hexamethonium have been applied on the coeliac ganglion or even injected into it. No significant or reproducible difference was noted between the effects of pre- and post-coeliac stimulations.

(*b*) Chronic section of splanchnic nerve above the coeliac ganglion. In six cats

under aseptic conditions the left sympathetic ganglion chain and splanchnic nerve were cut just above the diaphragm. A fortnight later (period required for Wallerian degeneration of nerve fibres) these cats underwent the stimulation of the left coeliac ganglion during an acute experiment. This stimulation failed to elicit any clear response of the l.o.s. in five cats. In the 6th one, a response was recorded the amplitude of which was smaller than that observed in intact cats.

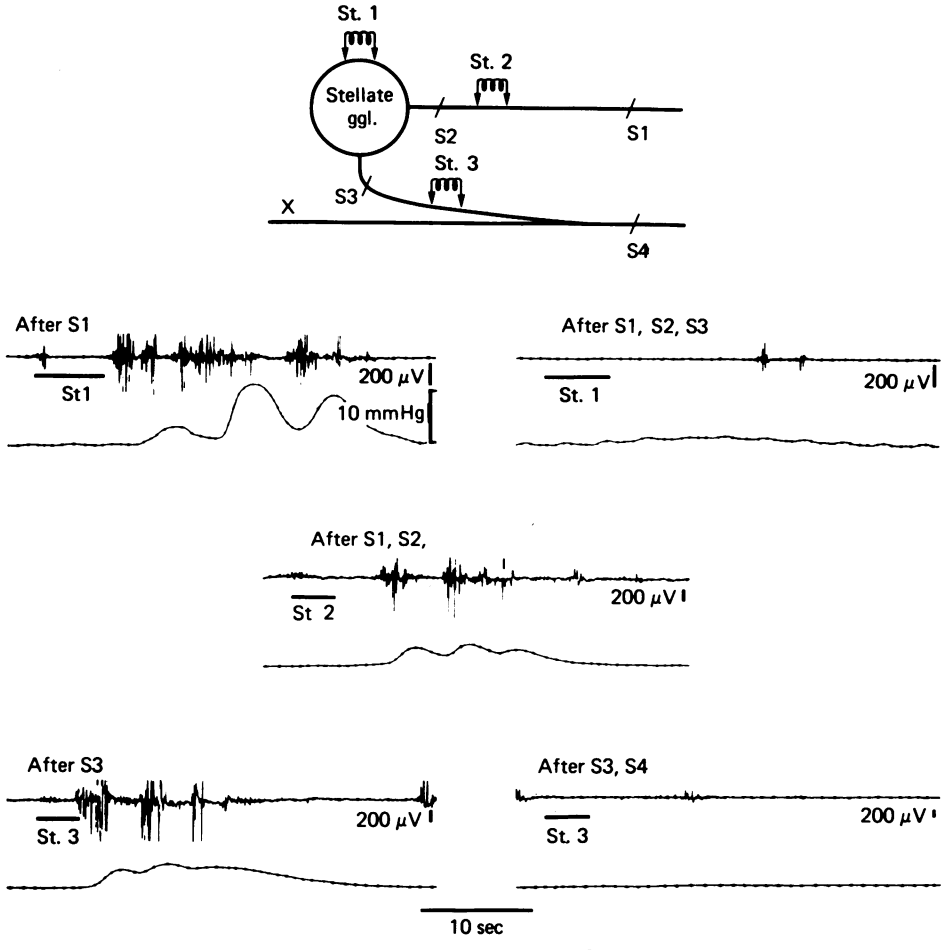

Fig. 4. *In vivo* stimulation of the sympathetic efferent fibres supplying the l.o.s. Upper trace: e.m.g. bipolar recording (RC = 0·06 sec); lower trace: intraluminal pressure. X: vagus nerve. S1, S2, S3, S4: levels of successive sections of the sympathetic nerve pathways to the l.o.s. St_1, St_2, St_3: stimulation of the stellate ganglion, the sympathetic ganglion chain, and the cardiac branch of the stellate ganglion respectively. St_1 induces a motor activity of the l.o.s. even after bilateral section of the splanchnic nerves and the sympathetic ganglion chains just above the diaphragm (S1). This response disappears after section of both the cardiac branch (S3) and the sympathetic ganglion chain just below the stellate ganglion (S2). Stimulations performed below the level of the different sections (St_2 and St_3) still produce responses which disappear after section of the dorsal and ventral trunks of the vagus nerve (S4). Stimulation parameters: 30 Hz; 0·5 msec; 12 V.

4. *Effects obtained by stimulating other sympathetic nerves*

Stimulation of the stellate ganglion by a train of stimuli (30 Hz; 0·5 msec; 10–12 V; 3 or 4 sec duration) produced after 5–8 sec latency a contraction of the l.o.s. which lasted 15 or 20 sec and looked very much like those obtained by stimulating the splanchnic nerve (see §3*A*).

These responses remained intact after the following sections were carried out, one after another, on the same animal: bilateral cervical vagotomy; excision of the two coeliac ganglia or bilateral section of splanchnic nerves and sympathetic ganglion chains just above the diaphragm (Fig. 4: St_1 after S1); interruption of connexions between the two stellate ganglia (left and right) just above the stimulated ganglion, and section of the sympathetic cervical trunk.

These responses disappeared after severing *both* the cardiac branch of the stellate ganglion *and* the sympathetic ganglion chain just below the stellate ganglion (Fig. 4: St_1 after S1, S2, S3).

After these last sections a motor response of the l.o.s. reoccurred when stimulating the sympathetic ganglion chain or the cardiac branch of the stellate ganglion below the level of sections (Fig. 4: St_2 after S1 and S2; St_3 after S3). The later stimulation seemed to be more effective than the former. These responses were abolished by severing both dorsal and ventral trunks of the vagus nerve, a few centimetres above the l.o.s. (Fig. 4: St_3 after S3 and S4).

5. *Action of some pharmacological agents on the responses elicited by stimulation of the sympathetic nerves*

A: dihydroergotamine. The I.V. injection (1 mg/kg) of dihydroergotamine (α-blocker) caused a blood pressure decrease from 120 to 60 mmHg within 15 sec. Then, a recovery occurred and the blood pressure returned roughly to its initial value. Nevertheless stimulations of the splanchnic nerve or the stellate ganglion usually were no longer effective (Fig. 5) although a very slight response of the l.o.s. sometimes remained.

B: atropine. Atropine injected I.V. at a dose of 0·1 mg/kg suppressed, or at least greatly reduced, the motor effects of sympathetic nerve stimulation (Fig. 6).

C: dihydroergotamine and atropine together. Atropine or ergotamine, when injected apart, were sometimes unable to block completely the response of the l.o.s. triggered by stimulation of the sympathetic nerves. However when the two drugs were injected together, the sympathetic response of the l.o.s. was totally abolished.

D: hexamethonium. In a previous publication (Gonella *et al.* 1977) we provided evidence that the vagus nerve carried excitatory fibres to the l.o.s. Their stimulation elicits a depolarization of the smooth muscle fibres recorded as e.j.p.s which may give rise to spike potentials inducing a contraction of the sphincter. These excitatory fibres are cholinergic and activate intramural cholinergic neurones, so their effect is abolished by hexamethonium.

We have tested the action of hexamethonium on the excitatory effects induced on the l.o.s. by the vagus and sympathetic nerves; the disappearance of the vagal responses providing evidence of the action of hexamethonium.

A test response of the l.o.s. was first recorded after stimulating at the following

sites: cervical vagus, stellate ganglion, thoracic vagus just above the diaphragm (dorsal or ventral trunk), splanchnic nerve just above the diaphragm, splanchnic nerve above the coeliac ganglion, coeliac ganglion, dorsal post-coeliac root. Hexamethonium was then injected I.V. (30 mg/kg); it elicited an important but transient

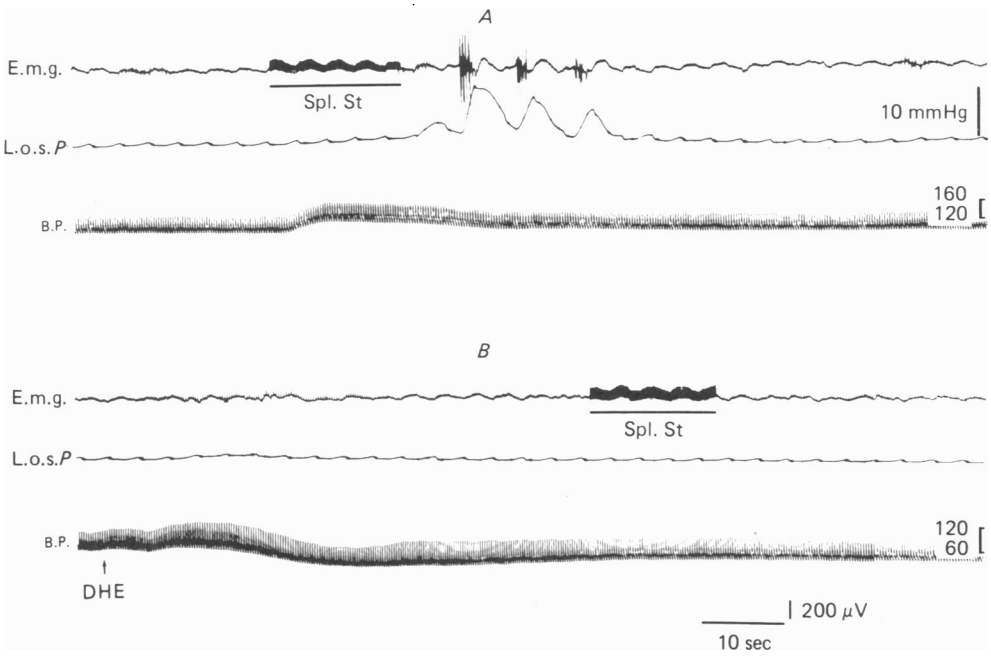

Fig. 5. Effect of dihydroergotamine on l.o.s. response evoked by splanchnic nerve stimulation. E.m.g.: bipolar recording (RC: 0·06 sec); L.o.s. *P*: intraluminal pressure; B.P.: arterial blood pressure. Spl. St: splanchnic nerve stimulation (30 Hz; 0·5 msec; 16V during 13 sec). *A* and *B* recordings were made on the same animal. *A*, control; *B*, after dihydroergotamine (DHE) injection (1 mg/kg) at the arrow. Notice in *B* the decrease of blood pressure and the suppression of the sympathetic response of the l.o.s.

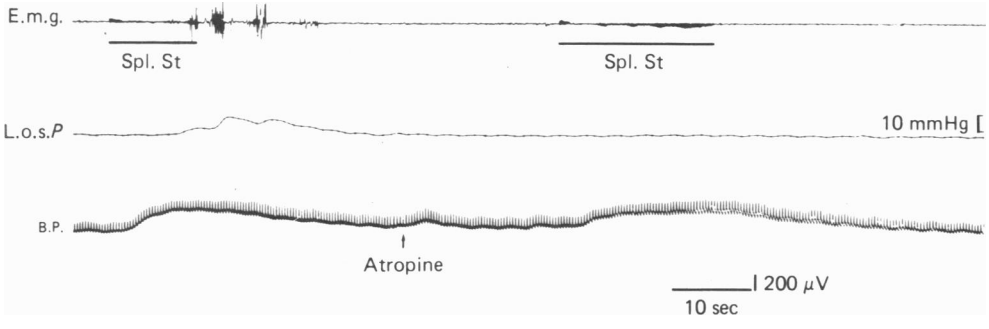

Fig. 6. Effect of atropine on l.o.s. response evoked by thoracic splanchnic nerve stimulation. E.m.g.: bipolar recording (RC = 0·06 sec); L.o.s. *P*: endoluminal pressure; B.P.: arterial blood pressure; Spl. St: splanchnic nerve stimulation (30 Hz; 0·5 msec; 16V). Notice that, after atropine (0·1 mg/kg), the rise in blood pressure is still present while the response of the l.o.s. is abolished.

fall of the arterial pressure and an intense e.m.g. activity of the lower oesophagus (l.o.s. included) lasting 10–20 min.

After the end of this hypermotility, the previous stimulations were performed again. They were ineffective on blood pressure. As for the l.o.s., stimulation of the cervical vagus had no more effect, but those of the thoracic vagus, the stellate ganglion, the splanchnic nerve and the coeliac ganglion were still effective and even more effective. The effect of the pre- and post-coeliac stimulation remained unchanged: the l.o.s. response elicited by the precoeliac stimulation did not become smaller than the response elicited by the post-coeliac stimulation.

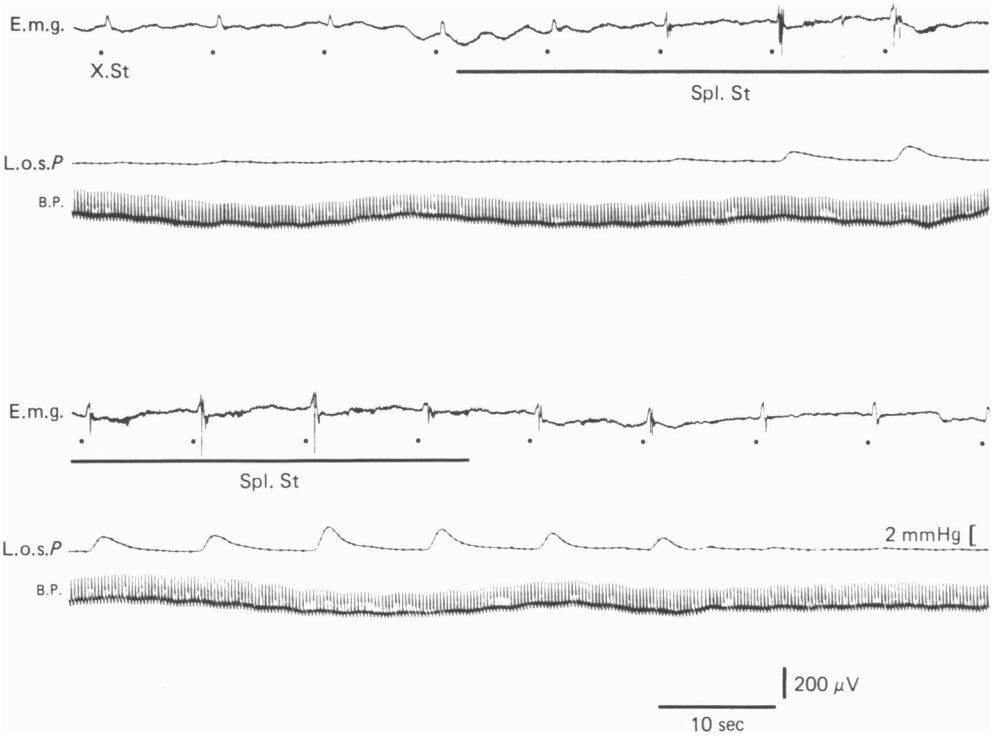

Fig. 7. Effects of a subthreshold stimulation of the splanchnic nerve on the e.j.p.s evoked by vagus nerve stimulation. E.m.g.: monopolar recording (RC = 5 sec); L.o.s. *P*: intraluminal pressure; B.P.: arterial blood pressure; X. St: vagal stimulation, single shock (0·5 msec; 5 V at 9 sec intervals); Spl. St: splanchnic nerve stimulation (2 Hz; 0·5 msec; 6 V during 80 sec). Tracings without interruption. Vagal stimulations evoke e.j.p.s which do not give rise to spikes, there is no intraluminal pressure variation. During splanchnic nerve stimulation, there is a gradual decrease in e.j.p.s amplitude, presumably due to a sustained depolarization of smooth muscle, allowing e.j.p.s to generate spikes, thereby inducing l.o.s. contractions. This facilitation persists as long as 20 sec after the end of splanchnic nerve stimulation.

6. *Effects of simultaneous stimulation of sympathetic and parasympathetic efferent fibres*

A: effects of a long lasting subliminal splanchnic nerve stimulation on the excitatory responses elicited on the l.o.s. by vagal stimulation. The cervical vagus stimulations

were adjusted to elicit only e.j.p.s. The thoracic splanchnic nerve stimulation with a train of stimuli at 2 Hz, 0·5 msec, 6 V was subliminal and did not produce any l.o.s. response. But when associated with vagal stimulation, it resulted in the following phenomena: the e.j.p.s decreased in amplitude but they became able to trigger spike potentials thereby producing a rise of the endoluminal pressure (Fig. 7). This facilitation of the vagal responses took place gradually and then lasted several seconds after the end of splanchnic stimulation.

B: effects of a splanchnic nerve stimulation on the i.j.p.s elicited on the l.o.s. by a vagal stimulation under atropine. In a previous publication (Gonella *et al.* 1977) it has been shown that the vagal supply to the l.o.s. included not only excitatory fibres but also inhibitory ones, the stimulation of which elicited i.j.p.s corresponding to a hyperpolarization of the smooth muscle fibres. These inhibitory responses were generally observed after atropine treatment which suppressed the excitatory responses.

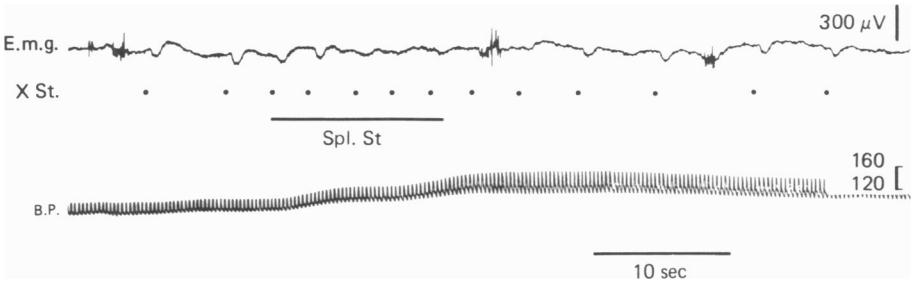

Fig. 8. Effects of splanchnic nerve stimulation on i.j.p.s evoked by vagal stimulation under atropine (0·1 mg/kg). E.m.g.: monopolar recording (RC = 1·2 sec); B.P.: arterial blood pressure; X. St: vagal stimulation (2 shocks at 100 Hz; 0·5 msec; 15 V); Spl. St: splanchnic nerve stimulation (30 Hz; 0·5 msec; 6 V). Vagal stimulations evoke i.j.p.s. Splanchnic nerve stimulation suppresses i.p.j.s. This inhibition lasts several seconds after the end of splanchnic nerve stimulation. Bursts of spikes correspond to a spontaneous electrical activity of the longitudinal muscle.

When a supraliminal stimulation (30 or 40 Hz; 0·5 msec; 6–10 V) was applied under atropine to the splanchnic nerve, a complete disappearance of the vagal i.j.p.s was observed after a delay of about 4–5 sec (Fig. 8). This effect lasted several seconds after the end of the sympathetic stimulation. An identical phenomenon occurred after I.V. injection of adrenaline or noradrenaline (3–5 μg/kg). The blockade of i.j.p.s was suppressed by a previous injection of dihydroergotamine (1 mg/kg).

DISCUSSION

The motor effect of adrenaline and noradrenaline on the l.o.s. has been previously described by different authors on the cat, the dog, the opossum, the monkey and man (Ingelfinger, 1958; Clark & Vane, 1961; Botha, 1962; Christensen, 1971).

Our recordings with the sucrose gap technique show that this action is achieved through a depolarization of the muscle cells. This depolarization is induced by α-adrenergic receptors since it is antagonized by dihydroergotamine or phentolamine. The hyperpolarization sometimes recorded after injection of α-blockers is under the

control of β-adrenergic receptors since this effect is suppressed by propranolol. Anyhow, this inhibitory action is slight and cannot be correlated with any observable phenomenon during acute experiments.

Similar results were obtained by Ellis, Kauntze & Trounce (1960) with *mechanical* records from circular strips of human oesophagus and by Christensen & Dons (1968) on circular strips from cat l.o.s. In addition, we have noticed that the motor effects of adrenaline or noradrenaline decreased in the presence of atropine, a result already reported by Christensen & Daniel (1966) while they studied the e.m.g. responses to adrenaline of longitudinal strips from the cat lower oesophagus. Like Christensen & Daniel, we conclude that the motor action of adrenaline or noradrenaline on l.o.s. is mainly achieved through a cholinergic mechanism. The weak response still observed under atropine is very likely due to a direct effect of noradrenaline on the muscle.

The hyperpolarization induced on the oesophageal body by noradrenaline or adrenaline depends on α-adrenergic receptors since it disappears under ergotamine. This fits well with the inhibitory action of noradrenaline on the non-sphincteric areas of the digestive tract. The cases of depolarization observed in our experiments express very likely individual variations of the oesophageal body reactivity to noradrenaline. This variability has been previously described by Ellis *et al.* (1960) on strips of human oesophageal body.

Concerning the sympathetic innervation of the l.o.s., our experiments have shown that two main pathways do exist: one following the splanchnic nerve, the other one issuing from the stellate ganglion. The effects triggered by splanchnic nerve stimulation do not result from a humoral release of adrenaline by adrenals, since they persist after adrenalectomy. They indicate the existence of a sympathetic subdiaphragmatic recurrent loop innervating the l.o.s. This is a confirmation of earlier results reported by Carlson (1922) and by Clark & Vane (1961). According to Mitchell (1953) sympathetic fibres arise from the coeliac ganglion, and reach the l.o.s. running along the gastric arteries. Yet we have not been able to demonstrate any synaptic relay within the coeliac ganglion (see results 3*C* and 5*D*). So, it seems likely that fibres within the splanchnic nerves are already postganglionic sympathetic fibres probably having their cell bodies in the ganglia of the sympathetic chain.

The effects obtained by stimulating the stellate ganglion are in agreement with results reported by Jourdan *et al.* (1955) and by Baïsset & Montastruc (1956), showing the existence, in the dog, of excitatory fibres arising from the stellate ganglion and following the thoracic vagus to reach the l.o.s. In addition one of us (J. Gonella, unpublished results) has brought histological evidence of such an innervation by observing retrograde chromatolysis pictures in the stellate ganglion after a chronic section of the thoracic vagus in the cat. These chromatolysis pictures were located in the medio-ventral area of the ganglion, the region where Hamberger, Norberg & Sjoquist (1963) had located, by histofluorescence technique, only adrenergic neurones.

The responses of l.o.s. following stimulation of the sympathetic supply are antagonized by dihydroergotamine and also by atropine. These results are clearly consistent with those obtained *in vitro* (sucrose gap technique) by testing directly the action of noradrenaline on circular strips from the l.o.s. Then, it is likely that stimulation of

sympathetic nerves calls into play adrenergic fibres. These results are in keeping with histological data of Baumgarten & Lange (1969) who found fluorescent noradrenergic fibres supplying the intramural ganglia and the circular smooth muscle fibres of the cat l.o.s. Hence it seems unlikely that the long latency of the sympathetic responses could be only due to an overflow of transmitter from adrenergic fibres supplying blood vessels and ganglia. We suggest that a possible explanation of this long latency may be found in the action of sympathetic transmitter on intrinsic neurones. On account of the following data: (1) the facilitation of vagal excitatory responses by a moderate sympathetic stimulation; (2) the action of atropine on the sympathetic responses; (3) the existence of an adrenergic innervation of the myenteric plexus (Baumgarten & Lange, 1969), we reach the conclusion that the adrenergic fibres might excite the intrinsic cholinergic neurones of l.o.s., thereby inducing a release of acetylcholine as already postulated by Christensen & Daniel (1966). Another striking effect of sympathetic stimulation is the inhibition of the vagal inhibitory responses, i.e. the cancellation of i.j.p.s. This inhibition seems to be exerted on the inhibitory myenteric neurones since noradrenaline causes the muscle to depolarize and the i.j.p.s should be increased in amplitude if the myenteric inhibitory circuits were not affected by the sympathetic stimulation. So, it is likely that the adrenergic fibres exert a dual action on the myenteric neurones: a facilitation for excitatory neurones and an inhibition for inhibitory ones. It is possible that in fact this dual effect corresponds to a disturbance of the balance which normally exists within the myenteric plexus between excitatory and inhibitory influences. The existence of interconnexions between excitatory and inhibitory intramural pathways was postulated by Furness (1969) in the colon and by Hirst (1975) in the small intestine of guinea-pig. In previous experiments on the cat lower oesophagus *in vitro* (Gonella *et al.* 1977) we obtained a more direct proof of such connexions, for the vagal excitatory responses of l.o.s. were suppressed by a distension of the thoracic oesophagus: the intramural inhibitory neurones excited by distension elicited in the aboral direction not only a muscular hyperpolarization, as stated by Diamant (1973), but also an inhibition of intramural cholinergic excitatory neurones of the same region. Thus, if the excitatory intramural cells are inhibited when the inhibitory neurones are activated, then when the inhibitory neurones are inhibited by stimulating the sympathetic nerves, there should be a facilitation of excitatory neurones. Or conversely, if the sympathetic fibres facilitate the excitatory intramural neurones, the inhibitory ones should be inhibited. Further experiments are necessary in order to elucidate the exact mechanism of action of sympathetic fibres. Nevertheless, it seems clear that the sympathetic motor control of the l.o.s. is achieved chiefly through an action at the level of myenteric neurones and, to a lesser degree, through a direct action on muscle cells. In both cases α-adrenergic receptors are involved. The action on myenteric neurones might result from an inhibition of inhibitory nerve cells or from a direct facilitation of excitatory ones, or even from both mechanisms operating simultaneously, the final result being an increased release of acetylcholine. Evidence for such an increased release of acetylcholine under the action of noradrenaline has been recently obtained by ourselves on muscular strips from l.o.s. previously loaded with radioactive choline. These last results will be published soon.

Finally, either in the l.o.s. or in the non-sphincteric areas of the gastro-intestinal tract, the sympathetic control appears to be exerted in the same way, i.e. mainly by an indirect action on myenteric ganglia and accessorily by a direct action on muscle cells. Yet, in the two cases the resulting effect is exactly opposite: in the l.o.s. there is an excitation due to a depolarization of the muscle and to a facilitation of the excitatory intramural neurones (possible consequence of the inhibition of inhibitory cells), while for the non-sphincteric areas there is an inhibition due to a muscle hyperpolarization and to an inhibition of intrinsic cholinergic neurones (see Jule & Gonella, 1972; Furness & Costa, 1974).

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