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CHARACTERISTICS OF SYNAPTIC INPUT TO THREE CLASSES OF SYMPATHETIC NEURONE IN THE COELIAC GANGLION OF THE GUINEA-PIG

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SUMMARY

1. Intracellular recordings from sympathetic neurones in the isolated coeliac ganglion of guinea-pigs have been used to define the synaptic input to three subtypes of neurone, classified on the basis of their discharge during maintained depolarizing current as phasic neurones, neurones with prolonged after-hyperpolarizations (LAH), and tonic neurones.

2. The three classes of neurone were distributed characteristically in different parts of the ganglion.

3. Passive membrane properties differed between the three neurone types. Mean input resistance was highest in phasic neurones and was inversely related to the size of the prolonged calcium-activated potassium conductance in LAH neurones. Mean input time constant was highest in tonic neurones, because of significantly higher cell capacitance.

4. Phasic and LAH neurones usually received one suprathreshold ('strong') as well as several subthreshold excitatory synaptic potentials (ESPs) from the *ipsilateral* splanchnic nerve. In general, the amplitude and number of splanchnic inputs were greater, and the occurrence of two strong inputs more common, in phasic than in LAH neurones. The input to tonic neurones was small and usually subthreshold, even with supramaximal splanchnic stimulation. In a few (mostly tonic) neurones lying close to the midline, small ESPs were evoked by *contralateral* splanchnic stimulation.

5. Antidromic action potentials were evoked in more than half of all neurones by high voltage coeliac nerve stimulation. In addition, multiple small subthreshold ESPs were recorded in virtually all tonic neurones (99%) on coeliac nerve stimulation. In contrast, coeliac stimulation rarely evoked a few very small ESPs in LAH neurones (9%), but no synaptic response in phasic neurones.

6. In about half of the tonic neurones tested (but no phasic or LAH neurones), small ESPs were evoked by stimulation of the intermesenteric nerve.

7. Slow depolarization elicited by repetitive activation of splanchnic and coeliac nerve trunks, at voltages supramaximal for the fast cholinergic responses, were

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recorded from about half of both phasic and tonic neurones, but only one of twenty-four LAH neurones. These responses commonly faded during subsequent trials, so that it was difficult to characterize them.

8. The data indicate that the three broad groups of coeliac neurone, classified on the basis of their voltage- and calcium-dependent potassium conductances, receive different patterns of synaptic input. The differences may be related to the three major functions of vasoconstriction, motility and mucosal secretion in the small intestine.

INTRODUCTION

Mammalian sympathetic neurones (like many central neurones) can be differentiated on the basis of their discharge during maintained depolarization (see Weems & Szurszewski, 1978; Decktor & Weems, 1983; Cassell, Clark & McLachlan, 1986). Two firing patterns are observed, namely, short phasic bursts early after the onset of depolarization or tonic firing at lower frequency that persists for as long as depolarization is maintained. These patterns result from the presence of different voltage- and calcium-dependent potassium conductances activated at potentials less negative than resting membrane potential (RMP) and as a consequence of the initiation of action potentials (Cassell *et al.* 1986; Cassell & McLachlan, 1987). In some central neurones, phasic firing has been thought to result primarily from activation of K^+ channels by Ca^{2+} influx during the action potential (Madison & Nicoll, 1984), but such a conductance change occurs in lumbar sympathetic neurones of both phasic and tonic types (Cassell *et al.* 1986). In lumbar neurones, phasic discharge appears to result because of activation of M-channels (K^+ conductances that open slowly after depolarization; Brown, Adams & Constanti, 1982), whereas tonic firing occurs in neurones without many M-channels and is dependent on transient K^+ conductances (A-channels) which, in contrast to these channels in other neurones, are not mainly inactivated at potentials positive of RMP (Cassell *et al.* 1986).

In the coeliac ganglion of guinea-pig and rabbit (Cassell & McLachlan, 1987), some neurones discharge phasically because they also possess another Ca^{2+}-activated K^+ conductance which has slow kinetics; this conductance resembles those described in myenteric neurones (Hirst, Johnson & van Helden, 1985), nodose neurones (Fowler, Greene & Weinreich, 1985), preganglionic neurones (Yarom, Sugimori & Llinás, 1985; Yoshimura, Polosa & Nishi, 1986) and some other sympathetic neurones (Christian & Weinreich, 1988). These neurones (called LAH) have a prolonged after-hyperpolarization after the action potential that lasts several seconds; they normally discharge only once over a wide range of depolarization amplitudes (Cassell & McLachlan, 1987).

The present experiments have examined the distribution of phasic, LAH and tonic sympathetic neurones in the coeliac ganglion of the guinea-pig and both the origin and type of synaptic input. We have already reported that multiple excitatory synaptic potentials (ESPs) are recorded in tonic but not phasic coeliac neurones upon supramaximal stimulation of the coeliac nerves (Meckler & McLachlan, 1988). Here we have determined the relative effectiveness of the cholinergic synaptic input from

various sources in initiating action potentials in the three different classes of neurone. Further, we have found that slow potential changes evoked by trains of nerve stimuli at voltages supramaximal for the cholinergic responses appear to be restricted to only some neurone types. An attempt is made to correlate the data with what is known of the distribution of the three major kinds of neurochemically identified ganglion cells (Macrae, Furness & Costa, 1986), whose terminations in the wall of the small intestine (Costa & Furness, 1984) imply distinct functional roles.

METHODS

Guinea-pigs (160–260 g, either sex) were anaesthetized with urethane (1–1·5 g kg^{-1} I.P.) and perfused through the descending thoracic aorta with oxygenated physiological saline as described previously (Cassell *et al.* 1986). A preparation consisting of both splanchnic nerves, both left and right halves of the coeliac ganglion and the attached coeliac nerve trunks was dissected out and pinned in a plastic recording chamber on a silicone rubber base. In most experiments, only the left half was pinned securely for impalement. In some experiments, the superior mesenteric ganglion (SMG) and the intermesenteric nerve (IMN) remained connected to the preparation. The experimental chamber was perfused with physiological saline (containing 2 mM-Ca^{2+}, see Cassell *et al.* 1986), gassed with 95% O_2–5% CO_2, and warmed to 35 °C, at a flow rate of about 5 ml min^{-1}.

Intracellular recordings were made as described previously (Cassell *et al.* 1986; Cassell & McLachlan, 1987), although in many cases impalements were not held for more than a few minutes, the aim being to sample as many cells as possible. Recording conventions were the same as in our earlier reports, with most recordings made at RMP in current clamp, or with the neurone clamped at RMP (zero holding current) using a single electrode voltage clamp; RMP and holding current are indicated by dotted baselines in the figures. Responses were studied during and after voltage command steps from RMP without adding tetrodotoxin or other drugs.

The most critical criterion for cell classification proved to be the decay of the outward tail current recorded with the membrane clamped at RMP immediately after a brief depolarization had elicited an uncontrolled 'action current' (see Cassell *et al.* 1986). Tail currents were fitted by a single exponential or by the sum of two exponentials using equations described previously (Cassell & McLachlan, 1987). The amplitude of the faster tail current, present in all neurones, was measured at its peak as soon as the membrane potential returned to the holding potential after the 'action current'. The time constant of its single-exponential decay was determined directly in phasic and tonic neurones, and in LAH neurones after fitting and substracting the second slower tail current as described in Cassell & McLachlan (1987). Peak amplitude of the slower tail current occurs some 750 ms after the action current. Peak amplitudes of the underlying conductance changes (g_{K1} and g_{K2}) were calculated from $g_K = I/(V_H - E_K)$.

Synaptic responses were evoked via close-fitting suction electrodes applied to the splanchnic, coeliac and intermesenteric nerves, using 0·5–1 ms pulse widths and voltages up to 90 V. In most experiments, the suction electrodes were located about 10 mm from the ganglion along the greater splanchnic nerves and 8 mm away along the coeliac nerves.

Data have been expressed as mean ± standard error of the mean (S.E.M.) throughout and significant differences determined using Student's *t* test (Zar, 1984).

RESULTS

General observations

The present data were obtained from 318 neurones impaled in different regions throughout most of the left coeliac ganglion and the more medial parts of the right coeliac ganglion in twenty-nine preparations.

The cells were classified as phasic, LAH or tonic by their discharge characteristics during maintained depolarizing current (Fig. 1), as described previously (Cassell *et al.*

1986; Cassell & McLachlan, 1987). Their location in the coeliac ganglion was noted and details of their synaptic input were obtained. Only 175 of the coeliac neurones sampled were held for sufficiently long also to determine their membrane properties (see Cassell *et al.* 1986). The classification of neurones was not always easy, especially if the impalements were held for only a few minutes. It was confirmed during the

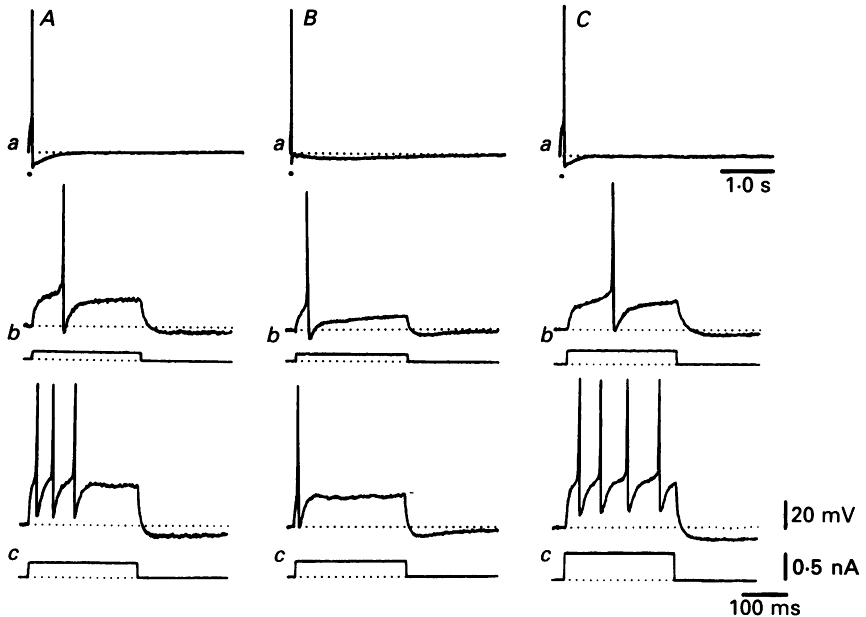

Fig. 1. Responses of three types of guinea-pig coeliac neurone to depolarizing current passed through the recording microelectrode. *A*, phasic neurone; *B*, LAH neurone; *C*, tonic neurone. *a*, action potentials elicited by 50 ms steps of 0·4, 0·45 and 0·5 nA respectively (at times indicated by dots) showing relative durations of after-hyperpolarization in each cell type. *b* and *c*, voltage responses (upper traces) to longer steps of (*b*) just-threshold and (*c*) approximately twice-threshold current (lower traces). Time calibration in *Ca* applies throughout *a*; that in *Cc* applies in *b* and *c*; vertical calibrations apply throughout.

course of these experiments that tonic neurones can always be classified on the basis of their synaptic input from the coeliac nerves (see Meckler & McLachlan, 1988), although not all of these cells discharged tonically when initially impaled. On this basis, the remaining 143 neurones were readily subdivided into tonic and non-tonic neurones and their location noted.

Neurones were always allocated to one of the three classes after careful consideration of the available data, although a small number of neurones had intermediate properties. This approach may have obscured real distinctions, but it seems premature at this stage to compound the classification. Of the proportions of 14 % phasic, 49 % LAH and 37 % tonic neurones in the population studied in detail, tonic neurones may be underrepresented. This would have resulted because of two approaches that developed during the experiments: (i) once we were confident that we could classify tonic neurones by their pattern of synaptic input (see below), we did not always wait to record their membrane properties as we were more concerned to

obtain secure impalements that would enable us to distinguish between phasic and LAH neurones under voltage clamp (see Methods), and (ii) we tended to avoid the medial part of the ganglion where tonic neurones were most frequently encountered, because we were trying to sample as many phasic neurones as possible. Further, some regions of the ganglion were not sampled; for example, the surface of the renal pole in most preparations was disrupted by cut preganglionic fibre tracts that had projected to the adrenal gland (see Fig. 2).

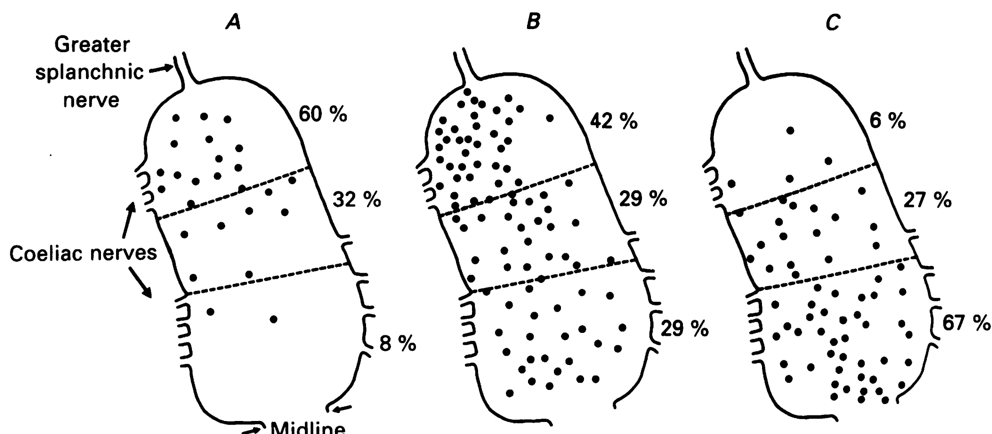

Fig. 2. Schematic diagram of the distribution of the three classes of neurone in the left coeliac ganglion. *A*, phasic ($n = 25$); *B*, LAH ($n = 87$); *C*, tonic ($n = 63$). Percentages are indicated for each type in the dorsal, middle and ventral lobes (separated by dashed lines). Truncated nerves to the right are connectives to the superior mesenteric ganglion.

Location of neurones of different classes

Each side of the coeliac ganglion consists of two or three lobes, with bridging tissue of variable shape; the location of every impaled cell was marked on a schematic map of one side partitioned into three 'lobes'. Phasic neurones were most frequently located in the dorsal lobe (near the point of entry of the major splanchnic nerve, Fig. 2*A*), with few present towards the midline. Tonic neurones were located medially with the majority in the ventral lobe (Fig. 2*C*). The more common LAH neurones were distributed throughout the ganglion, but the majority we sampled were present in the dorsal lobe (Fig. 2*B*). The proportions of neurones identified as phasic: LAH: tonic were 25:68:7% of those impaled in the dorsal lobe, 15:53:32% of those impaled in the middle lobe, and 4:38:58% of those impaled in the ventral lobe.

Ca^{2+}-activated g_Ks

Neurones of all types showed evidence of g_{K1}, the calcium-activated potassium conductance following the action potential which decays exponentially with a time constant of about 130 ms (Cassell *et al.* 1986). There was a general relationship between the amplitude of g_{K1} and cell capacitance (*C*) across all neurones (Table 1), suggesting a correlation between the number of these K^+ channels and cell membrane surface area.

Because the slow conductance change was often too small to produce a detectable after-hyperpolarization, LAH neurones could sometimes only be distinguished from phasic neurones after averaging at least ten tail currents after initiation of an 'action current' under voltage clamp (see Methods). The smallest detectable slow tail current had 0·02 nA peak amplitude. Slow tail currents were fitted by the sum of two

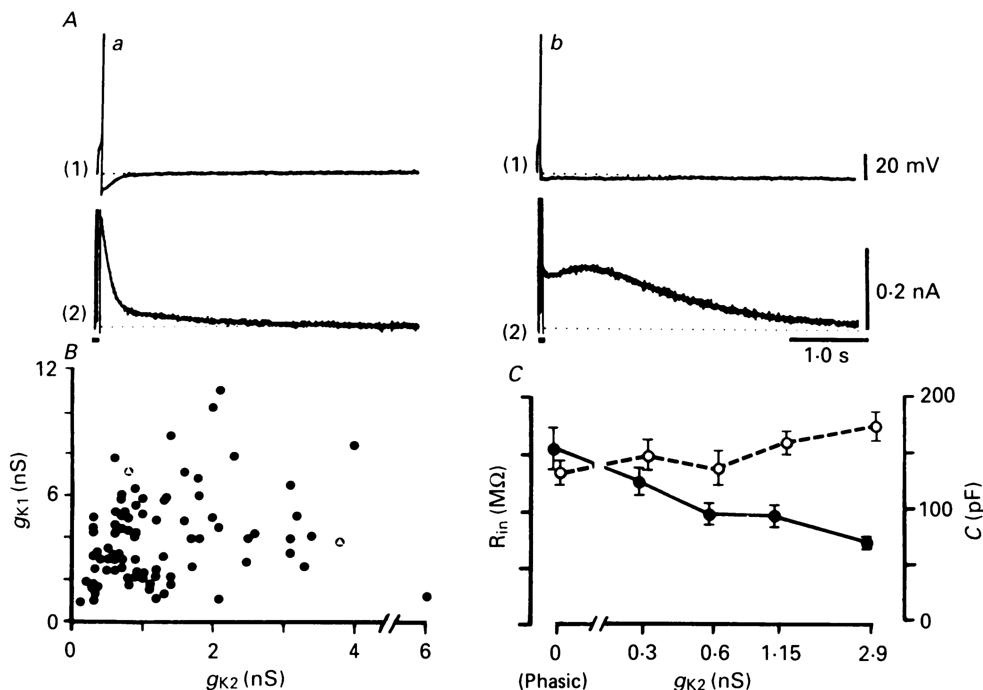

Fig. 3. Calcium-activated K^+ conductances in LAH neurones. *A*, records in two neurones (*a*, *b*) with very different ratios of g_{K1} to g_{K2}: (1) action potentials initiated by just-threshold current steps (50 ms, 0·5 and 0·4 nA respectively), and (2) tail currents evoked after action currents under voltage clamp of the soma (by +27 and +33 mV steps from −60 mV respectively). Bars below mark the time of the depolarizing steps. *B*, relation between amplitudes of g_{K1} and g_{K2} in LAH neurones; symbols containing open triangles indicate those illustrated in *A*. *C*, plots of mean cell input resistance (R_{in}, ●, continuous line) and mean apparent cell capacitance (*C*, ○, dashed line) against g_{K2} amplitude for phasic neurones ($g_{K2} = 0$) and four subgroups of LAH neurones (see text). Vertical bars indicate ±S.E.M.; S.E. of mean g_{K2} for each subgroup are obscured by the symbols.

exponentials with an onset delay of 60 ms (see Cassell & McLachlan, 1987) (τ_{on} = 294·8 ± 7·5 ms and τ_{off} = 1·40 ± 0·05 s, n = 74). Peak amplitude of g_{K2} varied over a considerable range between cells (0·1–6·3 nS), with consequently variable effects in the size of the after-hyperpolarization; mean g_{K2} was 1·28 ± 0·11 nS (n = 87). The ratio of the amplitude of g_{K2} to that of g_{K1} ranged from 0·06 to 4·8 (see Fig. 3*A*), there being no significant correlation between the two values (r = 0·22, Fig. 3*B*). The properties of the LAH population were examined after subdividing them arbitrarily into four groups with $g_{K2} \leqslant 0·4$ nS, $0·4 < g_{K2} \leqslant 0·8$ nS, $0·8 < g_{K2} < 2$ nS and $g_{K2} \geqslant 2$ nS, respectively (see Fig. 3*C* and Table 2).

TABLE 1. Passive membrane properties of coeliac neurones

	Phasic $n = 25$	LAH $n = 87$	Tonic $n = 63$
RMP (mV)	−59·8 ± 1·2	−56·0 ± 0·4	−63·1 ± 1·2
R_{in} (MΩ)	156·9 ± 19·5	99·8 ± 4·6	95·9 ± 5·9
τ_{in} (ms)	18·0 ± 1·8	14·8 ± 0·7	25·8 ± 1·3
C (pF)	135·0 ± 11·0	156·9 ± 5·7	299·7 ± 16·0
Threshold current (nA)	0·3 ± 0·03	0·36 ± 0·02	0·59 ± 0·08
g_{K1} amplitude (nS)	2·8 ± 0·8	3·9 ± 0·2	5·2 ± 0·6
g_{K1} decay time constant (ms)	143·1 ± 14·0	130·0 ± 3·0	138·5 ± 6·8

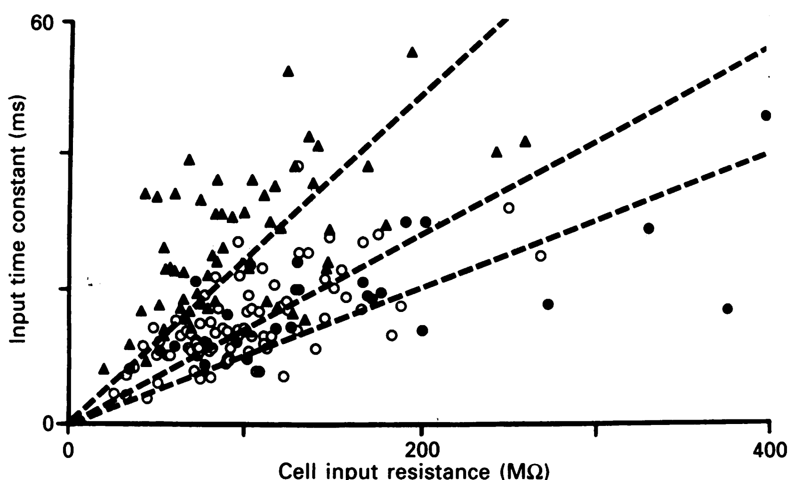

Fig. 4. Relationship between cell input resistance and cell input time constant for phasic (●), LAH (○) and tonic (▲) coeliac neurones. Regression lines (dashed) fitted through the origin have significantly different slopes (0·10, 0·14 and 0·24, respectively).

Passive membrane properties

In contrast to the findings of earlier studies of the coeliac ganglion (Kreulen & Szurszewski, 1979; Decktor & Weems, 1983), the properties of the three classes of coeliac neurone differed in several ways (Table 1). The relationships between R_{in} and τ_{in} for the three neurone populations (Fig. 4) have regression lines calculated through the origin with significantly different slopes ($P < 0·05$; Zar, 1984). When linear regressions were calculated from the data points alone, r values were 0·7, 0·66 and 0·57 for phasic, LAH and tonic neurones, but only the intercepts of the lines derived from the LAH and tonic populations differed significantly from each other ($P < 0·001$).

Phasic neurones. The input resistance (R_{in}) was on average higher ($P < 0·001$) in phasic neurones than in either LAH or tonic neurones while C was lower than in tonic

neurones ($P < 0.001$). The resultant τ_{in} was on average slightly higher than in LAH neurones, but significantly lower than in tonic neurones ($P < 0.01$). Twelve of fourteen phasic neurones (86%) had current–voltage ($I–V$) relations that were linear in the subthreshold range extending down to E_K; the remaining cells showed evidence of anomalous rectification.

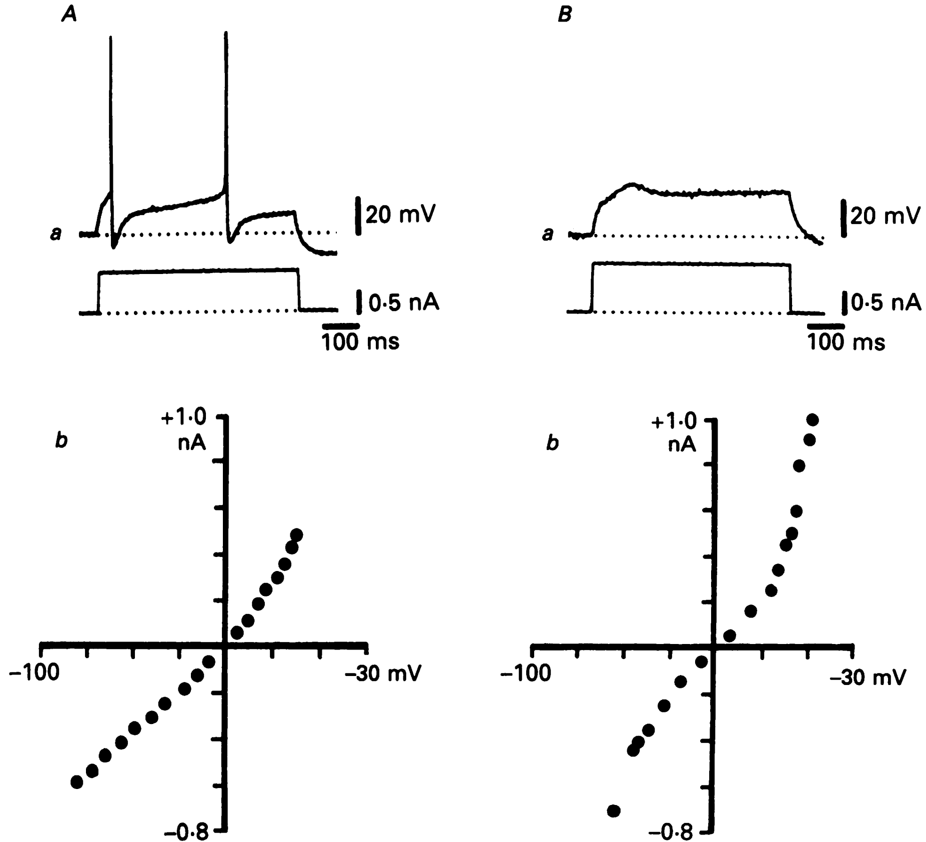

Fig. 5. Responses of (*A*) 'normal' and (*B*) 'high threshold' tonic neurones. *a*, suprathreshold depolarizing current (lower traces) that initiated two action potentials in the normal neurone (*A*, upper trace) produced only a small local response in the high threshold neurone (*B*, upper trace). *b*, steady-state current–voltage relations in the subthreshold range of potentials in the same two neurones.

LAH neurones. RMP did not vary significantly between the subgroups of LAH neurones, although on average it was significantly lower than that of both phasic and tonic neurones ($P < 0.001$). In a comparison that includes the phasic neurones (Fig. 3*C*), it can be seen that, with increasing g_{K2}, mean R_{in} decreased and C increased slightly, so that there was an overall decrease in mean τ_{in}. The majority of LAH neurones (49/57) had linear $I–V$ curves (see Cassell & McLachlan, 1987), with some evidence of anomalous rectification in the remaining cases.

Tonic neurones. On average, R_{in} in tonic neurones was similar to that of LAH cells,

but they had much larger values of C. As in the inferior mesenteric ganglion (IMG, Cassell *et al.* 1986), the amplitude of the depolarizing current necessary to bring tonic neurones to threshold was significantly higher than for phasic or LAH neurones ($P < 0.05$), reflecting the different voltage sensitivity of inactivation of I_A in tonic neurones (Cassell *et al.* 1986). This was confirmed directly in experiments on two tonic and two LAH coeliac neurones, the latter having I_A with characteristics similar to those in lumbar phasic neurones (Cassell *et al.* 1986). More than half of the tonic neurones (19/34) had I–V relations showing anomalous rectification (cf. IMG neurones, Cassell *et al.* 1986), those in the other cells being linear (see Fig. 5). Time-dependent current relaxations during hyperpolarizing voltage commands were rare in all types of coeliac neurone.

Nearly half (43%) of the tonic neurones were classified during the experiments as 'high threshold', i.e. it was difficult or even impossible to depolarize the cell sufficiently via the recording electrode to initiate an action potential (see Fig. 5). These neurones were located throughout the ganglion in the same regions as other tonic neurones. Their passive electrical characteristics were largely indistinguishable from those of other tonic neurones, except for a significantly higher RMP (-67.5 ± 1.7 mV, $n = 24$, cf. -59.4 ± 1.4 mV, $n = 27$, $P < 0.001$). This was, however, not the sole reason for the higher threshold as many of these cells could not be excited by depolarizing current steps even from more positive holding potentials (e.g. -55 mV). The only other feature was a generally larger cell capacitance (331 ± 29 pF, $n = 27$, cf. 276 ± 17, $n = 36$, $P < 0.2$), suggesting that the 'high threshold' neurones had more extensive surface areas. In addition, the splanchnic inputs to this group of neurones were overall less likely to initiate action potentials (Table 2). These observations would be consistent with a distant site of action potential initiation, possibly along the axon, but at least distant from both the soma and the splanchnic synaptic contacts.

Synaptic input

Synaptic potentials were evoked by stimulation of axons in the splanchnic, coeliac and intermesenteric nerves, and also occurred spontaneously. In some cells, short bursts of subthreshold ESPs of relatively constant amplitude occurred at apparently random intervals, independent of experimental intervention. More rarely, these gave rise to action potentials. These events, presumably the result of spontaneous discharge within the ganglion (see Cassell & McLachlan, 1986), were observed in 19% of phasic, 15% of LAH and 22% of tonic neurones and bore no relationship to the position of the cell in the ganglion.

As described below, the synaptic input received by each neurone type differed characteristically. Within any neurone class, there was no correlation between type of input and cell location. Further, there were no differences between the neurone classes in the stimulation voltages applied to the splanchnic nerve that produced threshold responses.

Synaptic input to phasic neurones

Splanchnic nerves. Stimulation of preganglionic fibres in the splanchnic nerve evoked ESPs and action potentials in all phasic neurones in the ipsilateral coeliac

ganglion. As described previously for phasic lumbar neurones (Cassell & McLachlan, 1986), the response at threshold voltage (3–15V) was a subthreshold ESP (Fig. 6*Aa*) in the majority of cases (75%). These responses occurred at a latency of 5–20 ms (mean $13{\cdot}0 \pm 1{\cdot}8$ ms, $n = 6$) implying conduction velocities (CVs) of 0·5–2·0 m s^{-1}. Increasing stimulus voltage recruited additional ESPs which usually initiated an action potential by summation (Fig. 6*Ab*). However at some particular small adjustment in stimulus strength (e.g. between *b* and *c* in Fig. 6*A*), the response in 89% of neurones changed abruptly in configuration and became an all-or-none

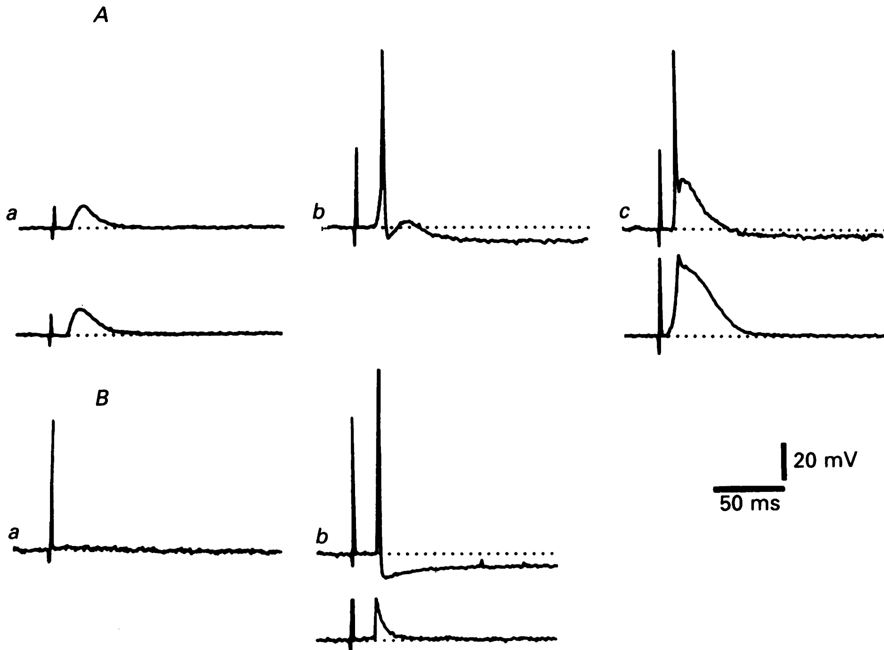

Fig. 6. Responses of a phasic neurone to nerve stimulation. *A*, graded stimulation of splanchnic nerves from just threshold in *a* to maximal in *c*. A small increase in voltage between *b* and *c* recruits a 'strong' fibre with a marked change in configuration of the response. Lower traces in *a* and *c* during 25 mV hyperpolarization of the soma. *B*, coeliac nerve stimulation produces no response in *a* but a further small increase in voltage evokes an antidromic response in *b*. Lower trace shows the axon spike when the soma is hyperpolarized by 15 mV. Note stimulus artifact truncated in lower traces.

action potential characterized by an extremely rapid rising phase and an after-depolarization. This indicated excitation of a single 'strong' (Str) preganglionic fibre (Holman & Hirst, 1977), which often increased the frequency of spontaneous ESPs. In 25% of phasic neurones, the threshold response was a Str input. There was no difference in CV between Str fibres and those evoking subthreshold ESPs (subs). In a few cells (10%), supramaximal splanchnic stimulation resulted only in several subs, with no Str input.

When action potentials were blocked by hyperpolarizing the neurones by some 25–40 mV (by passing current via the recording microelectrode), neurones with Str

inputs were found to have underlying synaptic responses with a fast peak and a decay phase which was not simply exponential (see Hirst & McLachlan, 1986).

The relative strength of the suprathreshold inputs could be subclassified into: (i) 'small Str' fibres which evoked action potentials with after-depolarizations only 5–10 mV in amplitude at RMP and peak responses < 20 mV in amplitude when the soma was hyperpolarized to −85 mV; (ii) conventional 'Str' fibres which evoked action potentials with after-depolarizations 10–20 mV in amplitude and responses 20–35 mV in amplitude with RMP set at −85 mV; (iii) 'big Str' fibres which evoked action potentials with after-depolarizations > 20 mV in amplitude and which could only be blocked by hyperpolarizing the soma by > 30 mV. When the action potentials were blocked, the peak amplitude of the synaptic response was usually > 40 mV.

TABLE 2. Synaptic input to different classes of coeliac neurone

	Phasic	LAH				Tonic	
Subtype...		1	2	3	4	1	2
Splanchnic							
No response	0	0	0	0	0	4	2
Subs only	3	2	1	2	1	13	15
Subs–AP	0	2	1	3	2	10	2
Small Str	1	5	1	7	2	3	4
Str	11	9	10	13	6	4	0
Big Str	6	2	6	5	4	0	1
2 Str	7	0	2	2	0	1	0
Coeliac							
No response	16	10	6	19	5	1	0
Anti only	11	6	12	14	7	0	0
Few subs	0	0	0	1	0	1	1
Few + anti	0	2	1	1	2	2	1
Many subs	0	0	0	1	0	15	8
Many + anti	0	0	0	0	0	16	18
$n \geqslant$	28	20	21	32	15	35	24

Numbers indicate the number of neurones with responses consisting of subthreshold ESPs (sub), Strong inputs (Str), or antidromic (anti) action potentials (APs) to supramaximal stimulation of ipsilateral splanchnic nerve and coeliac nerves. LAH neurones subdivided into subtypes 1–4 with progressively increasing amplitude of g_{K2}. Tonic neurones subdivided into normal (1) and high-threshold (2). For details of synaptic inputs and subgroups see text.

About 25% of phasic neurones received two Str inputs (2 Str); although these rarely caused firing of two action potentials, two distinct peaky components could be distinguished during graded stimulation when the membrane was hyperpolarized (see Hirst & McLachlan, 1986).

Stimulation of the contralateral splanchnic nerve failed to evoke any response in three phasic neurones tested (all in the dorsal lobe).

The distribution of different types of fast cholinergic input to phasic and other classes of neurone are shown in Table 2.

Coeliac and intermesenteric nerves. Stimulation of the coeliac nerves, usually with much higher voltages than were applied to the splanchnic nerves, evoked antidromic action potentials (Fig. 6*Bb*) in 41% of phasic neurones and no response in the

remaining cells. Latencies for the antidromic responses ranged from 10 to 22 ms (mean $15{\cdot}8 \pm 1{\cdot}6$ ms, $n = 8$) implying CVs of 0·36–0·8 m s^{-1}. Antidromic responses could be distinguished from ESPs by the time course of the fast-rising electrotonic axon spike observed when the soma was hyperpolarized (Fig. 6*Bb*, see Julé, Krier & Szurszewski, 1983). Although a proportion of coeliac neurones do not project their axons in the coeliac nerves, and additionally some axons may have been damaged in dissection, antidromic responses in these fine unmyelinated fibres *in vitro* are often difficult to elicit (see Julé *et al.* 1983), so that the number of failures may be unrealistically high.

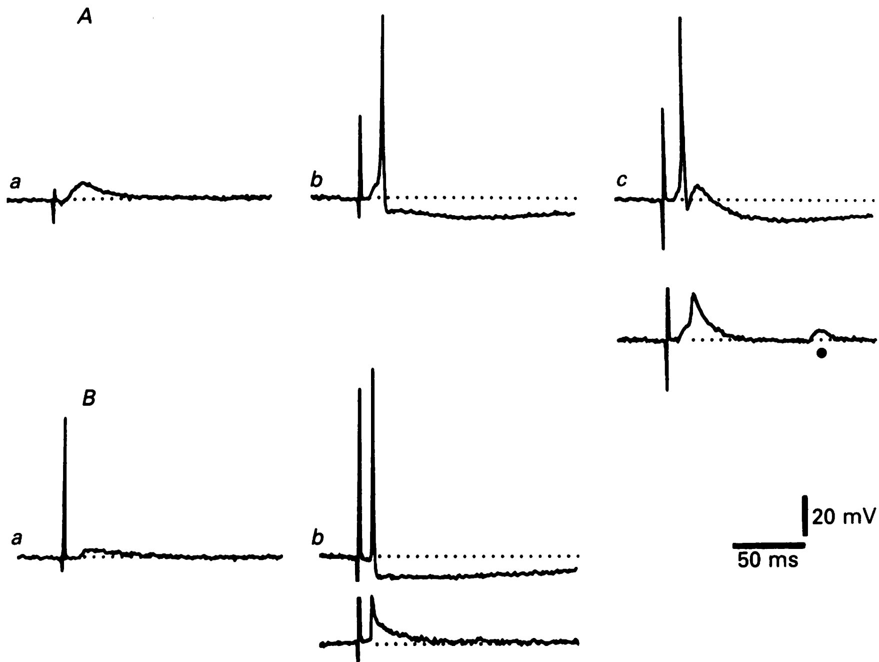

Fig. 7. Responses of an LAH neurone to nerve stimulation. *A*, ESPs evoked by graded stimulation of splanchnic nerve (*a–c*) sum to initiate an action potential. Hyperpolarization of the soma by 20 mV (lower trace in *c*) reveals that the maximal response consists of summed synaptic potentials with a fast peak, classified as a 'small strong' input; spontaneous ESP marked by dot. *B*, coeliac nerve stimulation evokes a small subthreshold ESP (*a*) at a voltage just subthreshold for the antidromic response (*b*). Lower trace shows both axon spike and small ESP during membrane hyperpolarization.

Stimulation of the IMN just rostral to the IMG failed to evoke any synaptic response in three phasic cells tested.

Synaptic input to LAH neurones

Splanchnic nerves. Synaptic responses were evoked in all LAH neurones by graded stimulation of the splanchnic nerves; these largely resembled those in phasic neurones (Fig. 7*A*), with the majority (84%) having Str inputs. Latencies again ranged from 5 to 20 ms and CVs did not differ significantly for Str and weak inputs. However, the amplitude of the underlying synaptic potentials (revealed during membrane hyperpolarization) was usually smaller than in phasic neurones

(Fig. 7*Ac*). Using the same grading system (see above) to describe the inputs, the splanchnic inputs to LAH neurones were found to be generally weaker than those to phasic neurones (see Table 2). For example, only 5% of LAH neurones received two Str inputs. There was no relationship between the 'strength' of the synaptic input and g_{K2} amplitude.

In four of thirty-eight cells tested, contralateral splanchnic stimulation produced one or two small ESPs of variable amplitude.

Coeliac and intermesenteric nerves. Stimulation of the coeliac nerves evoked antidromic action potentials (Fig. 7*Bb*) in 52% of LAH neurones; again, much higher voltages were required than to evoke synaptic responses from the splanchnic nerves. Latencies of antidromic responses ranged from 6 to 35 ms (mean 14·2 ± 1·3 ms, $n = 32$), implying CVs of 0·23–1·3 m s^{-1}. In contrast to phasic neurones, however, one or a few ESPs of a few mV amplitude were recorded in 7·5% of 134 LAH neurones (Fig. 7*Ba*). The threshold for these ESPs was in some cases lower, and in others higher, than for the antidromic action potential.

Stimulation of the IMN just rostral to the IMG evoked a small subthreshold ESP in one of eighteen LAH cells tested.

Synaptic input to tonic neurones

Splanchnic nerves. Synaptic responses were evoked in 90% of tonic neurones by graded stimulation of the splanchnic nerves; cells in which no responses were recorded were all located in the ventral lobe. The majority (67%) of tonic neurones received only subthreshold ESPs, even when stimulated with stimuli up to 15 × threshold (Fig. 8*A*); in about one-third of these, the summed ESPs initiated action potentials when stimulated synchronously. Strong inputs were evoked in 23% of tonic neurones but half of these were classified as 'small Str' by the criteria outlined above (see Table 2). Latencies of threshold responses ranged from 3 to 20 ms (mean 8·8 ± 0·6 ms, $n = 34$), implying CVs of 0·5–3·3 m s^{-1}, and were significantly shorter than in the other neurone types ($P < 0.001$); there was no detectable difference in latency between Str and weak inputs.

In seven of twenty-six cells tested, contralateral splanchnic stimulation produced one or two small ESPs of variable amplitude (cf. Decktor & Weems, 1983); none of these cells was located in the dorsal lobe.

Coeliac and intermesenteric nerves. Stimulation of the coeliac nerves evoked antidromic action potentials (Fig. 8*Bc*) in 59% of tonic neurones; as for phasic and LAH neurones, much higher voltages were required than to evoke synaptic responses from the splanchnic nerves. Latencies of antidromic responses ranged from 5 to 25 ms (mean 11·9 ± 1·0, $n = 22$), implying CVs of 0·32–1·6 m s^{-1}.

In marked contrast to phasic and LAH neurones, all tonic neurones exhibited multiple small amplitude ESPs on stimulation of the coeliac nerves (Fig. 8*B*). In any one cell, these were recruited over a wide range of stimulus voltages (usually 10–50 V), with notably little correlation between threshold voltage and latency. The synaptic response was evoked more readily than the antidromic action potential (ratio of threshold voltages antidromic : synaptic = 2·4 ± 0·05, $n = 31$). Latencies of threshold synaptic responses ranged from 5 to 60 ms (mean for threshold ESPs, 19·0 ± 1·1 ms, $n = 48$) implying CVs of 0·1–1·6 m s^{-1}.

Thus, most commonly, during graded increases in stimulus strength, ESPs were

initially evoked at long latencies (Fig. 8*Ba*), then action potentials were initiated by summation of many ESPs (Fig. 8*Bb*) and finally an antidromic action potential was evoked at a shorter latency by high voltage stimuli (Fig. 8*Bc*). In some cases, two action potentials were elicited by supramaximal stimulation from the summed synaptic response as well as the antidromic potential.

ESPs evoked by coeliac nerve stimulation resembled those evoked by splanchnic stimulation in the same cell, both in time course and their reversible blockade by

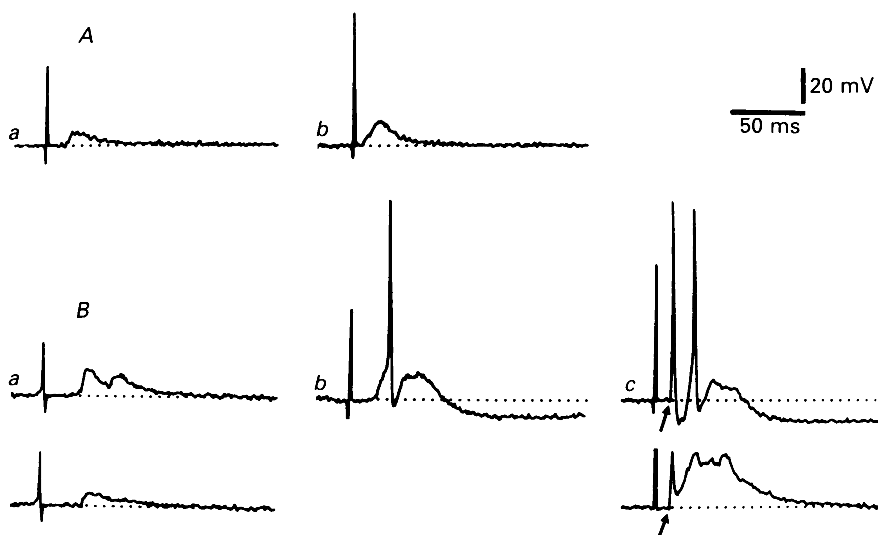

Fig. 8. Responses of a tonic neurone to nerve stimulation. *A*, graded splanchnic stimuli evoke only subthreshold ESPs (*a* and *b*). *B*, graded coeliac stimuli (*a–c*) evoke many subthreshold ESPs which sum to initiate an action potential. At high voltages (*c*), an antidromic response (marked by arrows) is evoked at a shorter latency than the synaptic responses. Membrane hyperpolarization by 15 mV in lower traces in *a* and *c*.

d-tubocurarine (10^{-5}–10^{-4} M) or hexamethonium (10^{-4} M) ($n = 7$). They differed however in having greater variability of amplitude between stimuli and in their failure to facilitate when stimulated repetitively. Splanchnic ESPs, like those evoked in most sympathetic ganglion cells by stimulation of preganglionic axons, usually increase in amplitude over the first few stimuli in a train. If stimulation frequency is high (> 5 Hz, see McLachlan, 1975), subsequent responses become smaller until a steady state is reached. In contrast, during a train of coeliac stimuli, individual ESPs tended to fail intermittently, and the response to supramaximal stimulation decreased progressively in amplitude.

The number of coeliac ESPs evoked in a given cell was difficult to determine. With progressive increase in stimulus voltage, the impression was of virtually continuous gradation in amplitude. When the membrane was clamped at RMP and excitatory synaptic currents were recorded, at least fifteen could be distinguished in many cells. However, the variability of amplitude of responses at a given latency made it impossible to count these accurately. The impression was that as many as forty axons may impinge on a single neurone. In preparations in which only part of the

coeliac nerves was stimulated, the number of synaptic responses in a given cell was reduced, suggesting that the input arises in diverse parts of the periphery.

Stimulation of the IMN just rostral to the IMG evoked synaptic responses in three of eight tonic cells tested. In each case, the responses were small ESPs and the neurones were located in the ventral lobe close to the connectives to the SMG.

Figure 9*A* summarizes the different patterns of splanchnic and coeliac inputs to the three classes of coeliac neurone.

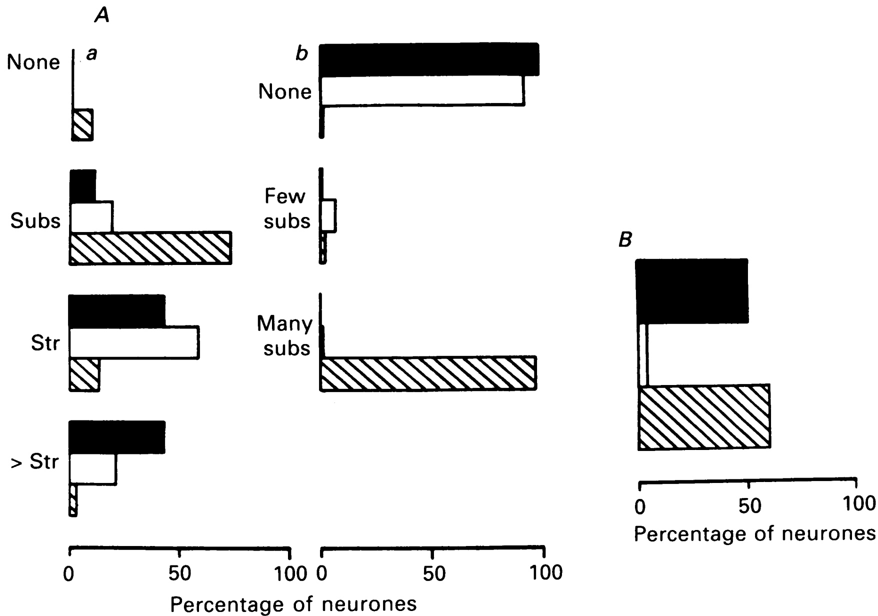

Fig. 9. Patterns of synaptic input in the three different classes of neurone: phasic neurones (filled columns), LAH neurones (open columns) and tonic neurones (hatched columns). *A*, proportions of each neurone type showing different grades of synaptic response to supramaximal (*a*) splanchnic and (*b*) coeliac stimuli. 'Subs' are subthreshold ESPs, 'Str' includes 'small Str' and normal strong inputs, '> Str' includes 'big Str' and '2 Str' inputs (see text). *B*, proportions of each neurone type showing slow responses to repetitive activation of incoming axons.

Slow responses to repetitive nerve stimuli

In addition to conventional cholinergic ESPs, slow synaptic responses have been reported to occur in neurones of the coeliac ganglion (Dun & Ma, 1984) following repetitive activation of incoming axons. Trains of stimuli at 15–30 Hz, 200 ms to 2 s duration, applied to splanchnic and coeliac nerves evoked trains of action potentials followed by slow depolarizations in six of eleven phasic, one of twenty-four LAH and sixteen of twenty-seven tonic neurones (see Fig. 9*B*). In tonic neurones, responses were usually evoked by independent stimulation of both nerve trunks, and they summed. No trains were evoked by coeliac stimulation in phasic neurones. Neurones exhibiting slow responses were located throughout the ganglion except in the dorsal lobe near the splanchnic nerve. No slow hyperpolarizations were detected.

Most responses were elicited only if stimulus voltage was 2–12 × that necessary to

recruit all fast ESPs (see also Dun & Ma, 1984) and had a very variable time course, commonly peaking at about 1 s after the train and decaying over periods ranging up to minutes. Peak amplitude ranged from 1 to nearly 20 mV and was generally graded with stimulus voltage and with the number of stimuli in each train (Fig. 10*A*). However, in the majority of neurones, maximal responses were < 6 mV in amplitude, and they proved extremely difficult to study because of marked fading with successive trials (Fig. 10*B*). Intertest intervals of at least 5 min were often necessary before another slow depolarization could be evoked, but even then usually all that could be established from the records was whether or not a response had occurred.

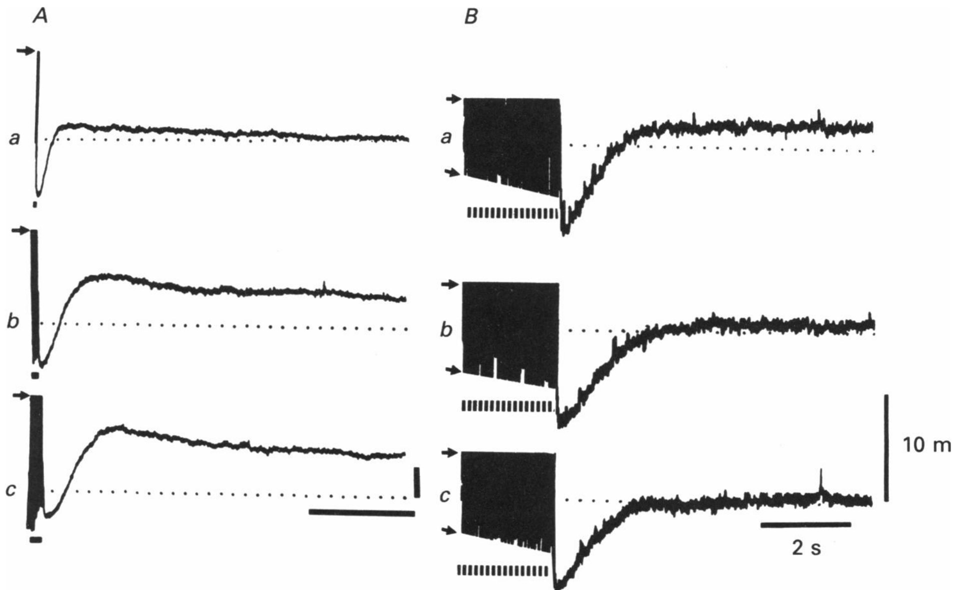

Fig. 10. Slow synaptic responses. *A*, membrane depolarization in a phasic neurone following (*a*) one, (*b*) four and (*c*) seven splanchnic stimuli (at times marked by bars) at 40 V, 20 Hz. These graded responses were the largest and most readily evoked slow responses observed in any neurone. *B*, membrane depolarization in a tonic neurone following 2 s trains of coeliac stimuli (dashed bars) at 60 V, 15 Hz progressively fades when tested at > 6 min intervals (*a*–*c*). Action potentials evoked during trains of stimuli have been truncated (arrows). Calibration bars have values shown in *B*.

The trains of action potentials, but not the slow responses, were blocked reversibly by *d*-tubocurarine (10^{-4} M, $n = 4$) and neither was affected by atropine (10^{-7} M, $n = 2$) (cf. Dun & Ma, 1984).

Attempts to determine the underlying conductance changes by recording current during a response evoked when the membrane potential was clamped at RMP were largely unsuccessful because of small and noisy signals and the inability to average several responses. When small hyperpolarizing voltage steps (10 mV, 100–200 ms long) were applied during a response in voltage clamp, or current steps applied in current clamp, evidence of conductance decreases of up to 30% were observed in each of three cells.

We examined whether the repetitive high-voltage stimuli used to evoke slow responses modified either passive membrane properties or Ca^{2+}-activated g_Ks. If a

substance was released by these stimuli, the previous history of synaptic activity might have been responsible for the variations in these parameters between neurones. Table 3 shows the data for the three neurone types obtained in (a) 'train' experiments in which we tested for slow synaptic responses whenever we obtained a satisfactory impalement, and (b) 'no-train' experiments in which we used only occasional single stimuli at voltages too low to evoke a slow response. The only significant differences were the values for τ_{in} and g_{K1} amplitude in phasic neurones. It is not clear what these changes imply, and further experiments would be required to test them, particularly in view of the small sample size. Furthermore, because almost all LAH neurones are discharged by supramaximal splanchnic stimuli, the absence of differences in g_{K2} amplitudes between 'train' and 'no-train' experiments also implies that repetitive activation of this conductance did not change its amplitude in the long term.

TABLE 3. Effect of repetitive high voltage stimuli on membrane properties

	R_{in} (MΩ)	τ_{in} (ms)	n	g_{K1} (nS)	g_{K1} (ms)	g_{K2} (nS)	g_{K2} (s)	n
Phasic								
(a)	178±26	21±3*	13	3·6±1·0*	140±15	—	—	10
(b)	134±29	14±1	12	0·8±0·2	154±37	—	—	3
LAH								
(a)	109±13	15±2	18	3·4±0·4	128±6	1·3±0·2	1·4±0·1	18
(b)	97±5	15±1	68	4·0±0·3	130±3	1·3±0·1	1·4±0·1	68
Tonic								
(a)	93±12	26±2	22	6·8±1·4	128±10	—	—	7
(b)	97±7	25±2	41	4·6±0·7	142±8	—	—	19

* Significant difference between values in (a) and (b), $P < 0.05$.

(a) refers to experiments in which trains of stimuli > 40 V, 30 Hz of 0·2 or 2 s duration were applied to the incoming nerve trunks; (b) refers to experiments in which only low-voltage single stimuli were presented.

DISCUSSION

In these experiments, three classes of coeliac neurones have been identified, confirming our earlier observations (Cassell & McLachlan, 1987) and providing details of the membrane properties of each neurone type. In addition, it has been shown that the synaptic input of both preganglionic and peripheral origin differs characteristically for each of the three cell types, providing further evidence for the suggestion that the different classes of neurone are associated with distinct functional sympathetic pathways (see Cassell & McLachlan, 1987).

Many of the properties of the coeliac neurone populations overlapped, but several differences were significant. RMP was on average lowest in LAH neurones, and highest in tonic neurones, whereas mean τ_{in} and C were lower in phasic and LAH than in tonic neurones. Mean R_{in} was inversely related to the amplitude of g_{K2} in phasic and LAH neurones (Fig. 3*D*). As LAH neurones with the smallest g_{K2} had many features similar to those of the phasic neurones which lacked this conductance,

phasic and LAH neurones might seem to represent a continuum. However, the synaptic input to these classes was clearly different, with all subgroups of LAH neurones having weaker splanchnic inputs than phasic neurones, as well as occasional peripheral inputs and no detectable slow reponses (Table 2).

The membrane characteristics of each electrophysiological class of neurone are similar but not identical to those determined previously in other sympathetic ganglia. In general coeliac neurones had lower R_{in} and τ_{in} than the neurones in the lumbar sympathetic chain (LSC) and IMG (see Cassell *et al.* 1986), with τ_{in} in coeliac phasic neurones significantly lower ($P < 0{\cdot}02$) than in lumbar phasic neurones. In both populations τ_{in} and C were significantly lower in phasic than in tonic neurones (see Cassell *et al.* 1986), but the differences were smaller in the coeliac than in the lumbar, mainly because of significantly lower R_{in} in coeliac tonic neurones than in the IMG ($P < 0{\cdot}001$). Current–voltage relations in most coeliac neurones were linear in the subthreshold range of potentials to beyond -90 mV, in contrast with lumbar neurones which most frequently exhibited anomalous rectification (Cassell *et al.* 1986). Some of these differences may result from differences in cell size. The significance of cell properties in determining cell behaviour will be clarified when ongoing activity and the factors which influence it *in vivo* are better understood.

The three classes of neurone generally receive different patterns of synaptic input from the central nervous system (via the splanchnic nerve) and from the periphery (via the coeliac nerves): (i) *phasic neurones* receive very powerful preganglionic connections, that guarantee a relay function for the majority of these neurones in at least one pathway; they have no peripheral input; (ii) most *LAH neurones* also have suprathreshold central inputs, but these are smaller and less powerful than those in phasic neurones; a few LAH neurones receive small inputs from the periphery; (iii) *tonic neurones* normally integrate a number of subthreshold ESPs for excitation, and the vast majority of these inputs arise in the periphery; only a small proportion of tonic neurones are driven by a preganglionic connection.

The patterns of synaptic input were surprisingly consistent bearing in mind the not infrequent damage to the axons during dissection. For example, the absence of splanchnic input to 10% of tonic neurones might reflect such damage. Although the other types of neurone always received splanchnic input, the input to tonic neurones frequently consisted of only two or three axons, so that this might have been more readily lost. Alternatively, it is possible that the spinal input to tonic neurones arises from more caudal spinal segments than the axons in the greater splanchnic nerve.

It should be noted that the role of the prolonged g_K in modulating action potential frequency in LAH neurones may be relatively minor. Even the largest conductance increases produced by either g_{K1} or g_{K2} have little or no effect on the ability of Str preganglionic inputs to initiate a postsynaptic discharge, although some 'small Str' inputs might conceivably be blocked. Subthreshold ESPs are not reduced in amplitude, only abbreviated in time course, by the combined effects of a decreased τ_{in} and an increased driving force (see Edwards, Hirst & Silinsky, 1976). However, summation of ESPs to reach threshold would clearly be affected by the level of hyperpolarization, and this would be important in integration of subthreshold inputs.

Slow synaptic responses were evoked by high-voltage repetitive stimulation in

about half of the phasic and the tonic neurones tested, but in only one of twenty-four LAH neurones (Fig. 10). Such responses have been described by others, particularly in the IMG where they can be evoked by colonic (Kreulen & Peters 1986), venous (Keef & Kreulen, 1986) or ureteric (Amann, Dray & Hankins, 1988) distension. We found slow responses small and difficult to elicit in coeliac neurones, although readily evoked and larger in five of six IMG neurones we tested (unpublished observations). As in a previous report (Dun & Ma, 1984), slow responses in coeliac neurones were not blocked by cholinergic blockers and appeared to involve a reduction in conductance, presumably to K^+ ions. The importance of these small variable responses in coeliac neurones seems questionable because of the unphysiological voltages required to stimulate the axons from which they are derived. However, it has apparently proved relatively easy to evoke such responses in the IMG by natural stimuli (Keef & Kreulen, 1986; Kreulen & Peters, 1986; Amann *et al.* 1988). Clearly an increase in cell resistance during the slow potential would potentiate the effectiveness of sub-threshold ESPs in initiating action potentials (see Dun & Ma, 1984). The current interest in neuropeptides as potential mediators of the slow response should however not obscure the need to determine the physiological mechanisms by which it is elicited *in vitro* as well as *in vivo*.

The distribution of neurone types throughout the coeliac ganglion (Fig. 2) can be compared with the distribution of neurochemical types. Immunohistochemical double-labelling techniques (Macrae *et al.* 1986) showed that the great majority of coeliac neurones are noradrenergic; of these, 50% are immunoreactive to neuropeptide Y (NPY) and 25% to somatostatin (SOM), while the remaining 25% are not stained using any known antibody. In our experiments, phasic neurones were most commonly impaled in the dorsal lobe where the majority of neurones are NPY-positive. This correlation seems to parallel that already reported for lumbar paravertebral ganglia (McLachlan & Llewellyn-Smith, 1986), with the inference that phasic neurones are vasoconstrictor. Tonic neurones were rarely present in the dorsal lobe, being most common in the ventral lobes, where they were impaled slightly more often than LAH neurones. Tonic neurones predominate in the IMG where the majority of neurones are SOM-positive (Hökfelt, Elfvin, Elde, Schultzberg, Goldstein & Luft, 1977). Somatostatin-positive neurones and those without a known peptide are found in about equal proportions in the medial lobes of the coeliac ganglion (Macrae *et al.* 1986). However, about half of the cells we characterized in the ganglion were LAH neurones; these were the majority of those sampled in the dorsal lobe.

While it is possible that there is no correlation between neurochemistry and membrane characteristics, the concept of a link is hard to reject as the same number of subgroups can be classified on each basis. Furthermore, the patterns of synaptic connectivity described here support a functional distinction. On the other hand, it is quite likely that the population we sample electrophysiologically is biased by soma dimensions. For example, phasic neurones have a lower mean cell capacitance (C, see Table 1), and subjectively it was generally harder to obtain 'good' impalements from them, so that they may be underrepresented quantitatively in our sample. Because of these quantitative difficulties, it remains uncertain whether all vasoconstrictor neurones in the coeliac ganglion are phasic, and whether the function that is reflexly

modified by peripheral afferent axons is motility or secretion. The relation between peptide content and electrophysiological behaviour will require further experimentation to clarify.

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REFERENCES

AMANN, R., DRAY, A. & HANKINS, M. W. (1988). Stimulation of afferent fibres in the guinea-pig ureter evokes potentials in inferior mesenteric ganglion neurones. *Journal of Physiology* **402**, 543–554.

BROWN, D. A., ADAMS, P. R. & CONSTANTI, A. (1982). Voltage-sensitive K-currents in sympathetic neurons and their modulation by neurotransmitters. *Journal of the Autonomic Nervous System* **6**, 23–35.

CASSELL, J. F., CLARK, A. L. & McLACHLAN, E. M. (1986). Characteristics of phasic and tonic sympathetic ganglion cells of the guinea-pig. *Journal of Physiology* **372**, 457–483.

CASSELL, J. F. & McLACHLAN, E. M. (1986). The effect of a transient outward current (I_A) on synaptic potentials in sympathetic ganglion cells of the guinea-pig. *Journal of Physiology* **374**, 273–288.

CASSELL, J. F. & McLACHLAN, E. M. (1987). Two calcium-activated potassium conductances in a subpopulation of coeliac neurones of guinea-pig and rabbit. *Journal of Physiology* **394**, 331–349.

CHRISTIAN, E. P. & WEINREICH, D. (1988). Long-duration spike afterhyperpolarizations in neurons from the guinea pig superior cervical ganglion. *Neuroscience Letters* **84**, 191–196.

COSTA, M. & FURNESS, J. B. (1984). Somatostatin is present in a subpopulation of noradrenergic nerve fibres supplying the intestine. *Neuroscience* **13**, 911–919.

DECKTOR, D. L. & WEEMS, W. A. (1983). An intracellular characterization of neurones and neural connexions within the left coeliac ganglion of cats. *Journal of Physiology* **341**, 197–211.

DUN, N. J. & MA, R. C. (1984). Slow non-cholinergic excitatory potentials in neurones of the guinea-pig coeliac ganglia. *Journal of Physiology* **351**, 47–60.

EDWARDS, F. R., HIRST, G. D. S. & SILINSKY, E. M. (1976). Interaction between inhibitory and excitatory synaptic potentials at a peripheral neurone. *Journal of Physiology* **259**, 647–663.

FOWLER, J. C., GREEN, R. & WEINREICH, D. (1985). Two calcium-sensitive spike after-hyperpolarizations in visceral sensory neurones of the rabbit. *Journal of Physiology* **365**, 59–75.

HIRST, G. D. S., JOHNSON, S. M. & VAN HELDEN, D. F. (1985). The slow calcium-dependent potassium current in a myenteric neurone of the guinea-pig ileum. *Journal of Physiology* **361**, 315–337.

HIRST, G. D. S. & McLACHLAN, E. M. (1986). Development of dendritic calcium currents in ganglion cells of the rat lower lumbar sympathetic chain. *Journal of Physiology* **377**, 349–368.

HÖKFELT, T., ELFVIN, L. G., ELDE, R. P., SCHULTZBERG, M., GOLDSTEIN, M. & LUFT, R. (1977). Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. *Proceedings of the National Academy of Sciences of the USA* **74**, 3587–3591.

HOLMAN, M. E. & HIRST, G. D. S. (1977). Junctional transmission in smooth muscle and the autonomic nervous system. In *Handbook of Physiology, The Nervous System*, vol. 1, *Cellular Biology of Neurons*, part 1, ed. KANDEL, E. R., pp. 417–461. Bethesda, MD, USA: American Physiological Society.

JULÉ, Y., KRIER, J. & SZURSZEWSKI, J. H. (1983). Patterns of innervation of neurones in the inferior mesenteric ganglion of the cat. *Journal of Physiology* **344**, 293–304.

KEEF, K. D. & KREULEN, D. L. (1986). Venous mechanoreceptor input to neurones in the inferior mesenteric ganglion of the guinea-pig. *Journal of Physiology* **377**, 49–59.

KREULEN, D. L. & PETERS, S. (1986). Non-cholinergic transmission in a sympathetic ganglion of the guinea-pig elicited by colon distension. *Journal of Physiology* **374**, 315–334.

KREULEN, D. L. & SZURSZEWSKI, J. H. (1979). Nerve pathways in celiac plexus of the guinea pig. *American Journal of Physiology* **237**, 90–97.

MACRAE, I. M., FURNESS, J. B. & COSTA, M. (1986). Distribution of subgroups of noradrenaline neurons in the coeliac ganglion of the guinea-pig. *Cell and Tissue Research* **244**, 173–180.

McLACHLAN, E. M. (1975). An analysis of the release of acetylcholine from preganglionic nerve terminals. *Journal of Physiology* **245**, 447–466.

McLACHLAN, E. M. & LLEWELLYN-SMITH, I. J. (1986). The immunohistochemical distribution of Neuropeptide Y in lumbar pre- and paravertebral sympathetic ganglia of the guinea pig. *Journal of the Autonomic Nervous System* **17**, 313–324.

MADISON, D. V. & NICOLL, R. A. (1984). Control of the repetitive discharge of rat CA1 pyramidal neurones *in vitro*. *Journal of Physiology* **354**, 319–331.

MECKLER, R. L. & McLACHLAN, E. M. (1988). Axons of peripheral origin preferentially synapse with tonic neurones in the guinea pig coeliac ganglion. *Neuroscience Letters* **86**, 189–194.

WEEMS, W. A. & SZURSZEWSKI, J. H. (1978). An intracellular analysis of some intrinsic factors controlling neural output from inferior mesenteric ganglion of guinea pigs. *Journal of Neurophysiology* **41**, 305–321.

YAROM, Y., SUGIMORI, M. & LLINÁS, R. (1985). Ionic currents and firing patterns of mammalian vagal motoneurons *in vitro*. *Neuroscience* **16**, 719–737.

YOSHIMURA, M., POLOSA, C. & NISHI, S. (1986). Electrophysiological properties of sympathetic preganglionic neurons in the cat spinal cord *in vitro*. *Pflügers Archiv* **406**, 91–98.

ZAR, J. H. (1984). *Biostatistical Analysis*. Englewood Cliffs, NJ, USA: Prentice-Hall Inc.