J. Physiol. (1986), **372**, *pp.* 457–483
With 1 *plate and* 13 *text-figures*
Printed in Great Britain

CHARACTERISTICS OF PHASIC AND TONIC SYMPATHETIC GANGLION CELLS OF THE GUINEA-PIG

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(*Received 19 December 1984*)

SUMMARY

1. Intracellular recording techniques have been used to determine the electrophysiological properties of sympathetic neurones in ganglia of the caudal lumbar sympathetic chain (l.s.c.) and in the distal lobes of inferior mesenteric ganglia (i.m.g.) isolated from guinea-pigs.

2. Passage of suprathreshold depolarizing current initiated transient bursts of action potentials in 97% of l.s.c. neurones, but only 13% of i.m.g. cells ('phasic' neurones). Most i.m.g. neurones fired continuously during prolonged depolarizing pulses ('tonic' neurones).

3. Passive membrane properties varied; mean cell input resistance was similar between groups, but phasic neurones had smaller major input time constants on average than had tonic cells.

4. Current–voltage relations determined under both current clamp and voltage clamp were linear around resting membrane potential ($\sim$60 mV), where membrane conductance was lowest. Instantaneous and time-dependent rectification varied in the different neurone types.

5. The current underlying the after-hyperpolarization following the action potential was significantly larger on average in tonic i.m.g. cells than in phasic neurones, although its time course (τ = 100 ms) was similar.

6. Phasic neurones fired tonically when depolarized after adding the muscarinic agonist, bethanechol (10^{-5} M to 10^{-4} M), to the bathing solution. Bethanechol blocked a proportion of the maintained outward current (presumably the M-current, I_M, Adams, Brown & Constanti, 1982) in phasic neurones; this current was small or absent in tonic neurones.

7. Transient outward currents resembling the A-current (I_A, Connor & Stevens, 1971*a*) were evoked in tonic but not in phasic neurones by depolarization from resting membrane potential. I_A could only be demonstrated in phasic neurones after a period of conditioning hyperpolarization.

8. After a step depolarization to ~ -50 mV, I_A reached peak amplitude at about 7 ms and then decayed with a time constant of about 25 ms in both neurone types.

9. Activation characteristics of I_A were similar for phasic and tonic neurones, but inactivation curves, although having the same shape, were shifted to more depolarized voltages in tonic neurones. That is, I_A was largely inactivated at resting membrane potential in phasic, but not tonic neurones.

10. It is concluded that the discharge patterns of the two populations of sympathetic neurones result from differences in the voltage-dependent potassium channels present in their membranes. The anatomical occurrence of the different cell types suggests that phasic neurones are vasoconstrictor and tonic neurones are involved with visceral motility.

INTRODUCTION

In intracellular studies of neurones in a number of different autonomic ganglia of guinea-pigs (Weems & Szurszewski, 1978; Kreulen & Szurszewski, 1979) and cats (Griffith, Gallagher & Shinnick-Gallagher, 1980; Decktor & Weems, 1981, 1983; Julé & Szurszewski, 1983), two distinct patterns of repetitive discharge have been described. A prolonged suprathreshold depolarizing current pulse passed through the recording micro-electrode leads either to a brief burst of action potentials at the beginning of the depolarization or to maintained repetitive firing at a slower regular rate throughout the duration of the current pulse. Neurones with these different firing characteristics have been designated 'phasic' and 'tonic' neurones, respectively. The observed proportions of neurones of each type have varied between the different ganglia studied. It seems possible that the different discharge patterns are characteristic of neurones with different functions.

Quantitative neuroanatomical tracing studies of lumbar sympathetic pathways in cats using horseradish peroxidase (McLachlan & Jänig, 1983; Baron, Jänig & McLachlan, 1985*b*) have enabled us to identify two populations of sympathetic ganglion cells that project to functionally distinct target organs. The neurones in the caudal lumbar sympathetic chain distal to the last lumbar splanchnic nerve (l.s.n.) send their axons in the spinal nerves to the hind limb, and the vast majority of these innervate blood vessels in skeletal muscle and skin (McLachlan & Jänig, 1983). In contrast, most post-ganglionic axons in the hypogastric nerve arise from cell bodies in the distal lobes of the inferior mesenteric ganglion (i.m.g.) (Baron *et al.* 1985*b*). Functionally, hypogastric axons are primarily involved with regulation of motility and secretion in the bladder, rectum and internal reproductive organs (Langley & Anderson, 1895*a–c*). Most of the vascular innervation of the pelvic organs is provided by ganglion cells lying along the hypogastric nerves or in the pelvic plexuses (Langley & Anderson, 1895*d*; Sjöstrand, 1965). These data provide the basis for the classification of sympathetic ganglion cells in these locations into vascular and visceral populations.

We have now undertaken a systematic study of the sympathetic neurones from these two anatomical sources in guinea-pigs. Their destinations in hind limb and pelvic organs have been confirmed, and their electrophysiological properties examined. Most remarkably, the responses to maintained depolarizing current in virtually all lumbar chain neurones are 'phasic', while the majority of distal i.m.g. cells have 'tonic' firing patterns. The occurrence of voltage-dependent membrane channels, particularly three different potassium conductances, have been found to differ in the different neurone types, and provide explanations for the phasic and tonic discharge patterns. Preliminary brief reports of these observations have been presented (Cassell, Clark & McLachlan, 1984; McLachlan, Clark & Cassell, 1984).

METHODS

Guinea-pigs (180–250 g, either sex) were anaesthetized with urethane (1–1·5 g/kg I.P.) and perfused through the descending thoracic aorta with physiological saline of the following ionic composition (mM): Na^+, 151; K^+, 4·7; Mg^{2+}, 1·2; Ca^{2+}, 2; Cl^-, 144·4; H_2PO_4, 1·3; HCO_3, 16·3; glucose, 7·8; pH 7·2–7·4, gassed with 95% O_2, 5% CO_2, at room temperature. Either the caudal lumbar sympathetic chain (l.s.c.) from the level of L4 white ramus to below L6 sympathetic ganglion (segmental numbering according to Baron, Jänig & McLachlan, 1985*a*) or the i.m.g. were dissected from the animal under flowing oxygenated saline. These ganglia with their associated nerve trunks were pinned out on the transparent silicone-rubber-coated base of a plastic recording chamber of volume about 0·8 ml. The chamber was transferred to the stage of an inverted compound microscope (magnification up to ×350) and perfused with saline of the above composition warmed to 35 °C at a flow rate of about 5 ml/min.

Intracellular recordings were made from ganglion cells using micro-electrodes pulled from 1 mm fibre-filled glass capillary tubing and filled with 0·5 M-KCl; satisfactory impalements were obtained only with electrodes having resistances in the range 80–150 MΩ. Data in this paper are from cells in which stable impalements were maintained for a minimum of 15 min before recordings were taken. Passive electrical properties were determined at 10–15 min intervals throughout an impalement; data have been included only if values remained stable for two or more of these determinations.

Electrophysiological properties of neurones were determined using single electrode discontinuous current clamp (s.e.c.c.) for voltage recording, and single electrode voltage clamp (s.e.v.c.) (Axon Instruments, Inc.). In s.e.c.c. mode, constant current is passed briefly and then voltage is sampled (cf. s.e.v.c). If cycling frequencies are high relative to the membrane time constant, but low relative to the discharge of micro-electrode capacitance, passive properties can be determined during passage of small hyperpolarizing current pulses (0·01–0·1 nA, 150–400 ms duration) without the serious distortion introduced by passing current through high-resistance micro-electrodes via a bridge circuit (see McLachlan, 1974). Care was taken to adjust the cycling frequency of the current-passing mode so that the effects of electrode capacitance were minimized; this usually meant that the cycling rate was between 1·0 and 2·0 kHz during the recording of electrotonic potentials. Measurements were made of cell input resistance (R_{in}) from the steady-state level of the voltage response, and the major cell input time constant (τ_{in}) determined from the single exponential fitted to the onset of this response between 20 and 70% of its final amplitude (see McLachlan, 1974). R_{in} was also determined from the slope of the current–voltage relation between resting membrane potential (r.m.p.) and 20 mV more negative. The cycling rate was usually increased to between 2 and 3·5 kHz in s.e.v.c. mode in order to improve stability; this was possible provided the depth of fluid covering the micro-electrode tip was kept low. During large voltage commands, the clamp gain was reduced somewhat below optimum to prevent oscillation, but the clamped potential was always flat to within 5% of the command value in a few milliseconds, except where specified in the Results. Action potentials initiated by brief (30–50 ms) depolarizing currents were recorded in bridge mode when the amplitude was measured, but other recordings of potential were normally made in s.e.c.c.

Voltage and current records (filtered at 3 kHz) were either digitized and analysed as the experiment progressed, or recorded on FM tape (Hewlett–Packard 3968A) and digitized subsequently. In a few early experiments, data were photographed directly from the oscilloscope on recording film. Generally, the sampling rate for digitization was 1 kHz or greater. For very slow responses, sampling was occasionally reduced to 300 or 500 Hz. Analysis was performed on a DEC 11/23 microcomputer, programs being written in assembler and FORTRAN; data were stored in files on floppy disk. Averages were made of 10–50 sweeps when appropriate depending on the signal-to-noise ratio. Steady-state values were calculated over the final 10 ms of the voltage–current pulse, while 'instantaneous' values were determined between 10 and 20 ms after the beginning of the pulse, averaging the signals over the subsequent 5 ms; the period was chosen so as to avoid the current transient which varied in duration in different ganglion cells.

Properties of the A-current (I_A) were examined in both phasic l.s.c. or tonic i.m.g. neurones, but properties of I_A in neurones of the less common type in a particular ganglion (e.g. phasic neurones in the i.m.g.) or of the 'low resistance' group (see Results) were not studied in detail. In

order to manipulate the experimental values used to describe the inactivation and activation characteristics of I_A (see Results), logistic curves of the form:

$$P_1 [1+\exp (P_2(V+P_3))]^{-1} \quad (1)$$

were fitted to the experimental data points shown in Fig. 13. The three parameters, P_1 to P_3, that describe the relation between peak amplitude of I_A and membrane potential, V, were used to test for significant differences between data from different neurone types. This logistic curve is identical in form to the Boltzmann equation, used by others to describe similar relations for ion channels (Hodgkin & Huxley, 1952; Belluzzi, Sacchi & Wanke, 1985).

I_A was computed for different voltage steps according to the equation:

$$I_A = g_A(V-V_A) = \bar{g}_A A^4(V,t) B(V,t) (V-V_A) \quad (2)$$

(see Connor & Stevens, 1971*b*), where g_A = A-conductance, V = membrane potential, V_A = reversal potential for I_A (−90 mV), $\bar{g}_A$ = maximum value of A-conductance, t = time, and A and B are described by the equations:

$$\tau_A \mathrm{d}A(V,t)/\mathrm{d}t + A(V,t) = A(V, \infty),$$

$$\tau_B \mathrm{d}B(V,t)/\mathrm{d}t + B(V,t) = B(V, \infty),$$

given the initial conditions $A(V,0) = A(V_h, \infty)$, $B(V,0) = B(V_h, \infty)$ and $V = V_h$ for $t < 0$, and $V = V_c$ for $t > 0$, where V_h = holding potential from which membrane is stepped, and V_c = potential to which membrane is stepped. The index of A derived by Connor & Stevens (1971*b*) was used; it was difficult to resolve the rising phase of recorded I_As (see Results) to test whether another value would have been more appropriate. Values of τ_A and τ_B of 3 and 25 ms, respectively, were found to permit typical recorded I_A time courses to be simulated (see Fig. 12). Values of $\bar{g}_A$ were arbitrarily selected so that currents of the amplitudes measured experimentally (Fig. 13) could be reproduced; the selected values were about twice the average largest values determined experimentally. Measurements of peak I_A at membrane potentials less negative than −50 mV were sometimes contaminated with inward current, even in the presence of tetrodotoxin (TTX). To allow for this, the slope of the derived activation curve for I_A was arbitrarily increased by 20%.

Data are presented as mean ± S.E. of mean except where indicated. Statistical significance between populations of data was tested using Student's t test.

Histology

Sympathetic ganglion cells were labelled retrogradely with horseradish peroxidase (HRP) applied at an operation under pentobarbitone anaesthesia (35 mg/kg) 2 days before the animals were killed. Perfusion and histological techniques have been described elsewhere (McLachlan & Jänig, 1983; McLachlan, Oldfield & Sittiracha, 1985). HRP was applied as a 40% solution (w/v) in H_2O to the freshly cut axons of a muscle nerve in the hind limb, or to one hypogastric nerve (see Pl. 1).

RESULTS

Destination of axons of neurones in the two locations studied

The anatomy of the lumbar sympathetic outflow to the inferior mesenteric ganglion (i.m.g.) and to the hind limb is illustrated in Pl. 1. The arrangement of the white and grey rami and of the lumbar splanchnic nerves (l.s.n.) varied between animals but the pattern of the nerve pathways was consistent and resembled that described for the cat and rabbit (see Langley & Anderson, 1896; Baron *et al.* 1985*a*). One (sometimes two) l.s.n. run from the sympathetic trunk opposite each white ramus to the i.m.g. at spinal levels L2, L3 and L4. The most caudal splanchnic nerve arises from the trunk between L3 and L4 or at the rostral end of the ganglion to which projects the most caudal white ramus (L4). This ganglion will be referred to as L4 ganglion (see Baron *et al.* 1985*a*). The more caudal ganglia of the sympathetic chain have only grey rami which, like those of more rostral lumbar ganglia, run caudally for some distance over the vertebrae to joint the spinal nerve of the next segment.

When HRP was applied to the nerves to gastrocnemius and soleus muscles, about 500 ganglion cells were labelled mainly in the central part of ganglia L5 and L6 of the lumbar sympathetic chain (see Pl. 1), with smaller numbers decreasing in the rostral direction as far as L2 (see also McLachlan & Jänig, 1983; McLachlan *et al.* 1985). As this represents about 10 % of the cells in L5 and L6 ganglia, a substantial proportion of neurones in the paravertebral ganglia at this level is likely to be involved with vasoconstriction in hind-limb muscles. Most of the remainder are probably associated with the vasculature in hind-limb skin (McLachlan *et al.* 1985). It has recently been shown that very few neurones in equivalent ganglia project their axons via the sacral chain to the pelvic viscera in the cat (see Kuo, Hisamitsu & de Groat, 1984).

The i.m.g. in the guinea-pig usually consists of one proximal and one distal lobe lying rostral and caudal to the inferior mesenteric artery (see Kreulen & Szurszewski, 1979). The colonic nerves arise primarily from the proximal lobe with a variable (occasionally substantial) projection from the distal lobe. Two thick hypogastric nerves project from the distal lobe to the pelvic plexuses. When HRP was applied to one hypogastric nerve, the majority of labelled sympathetic ganglion cells (about 70 %) lay in the distal lobe of the i.m.g. (see Pl. 1) with most of the remainder in the proximal i.m.g. and a few scattered through paravertebral ganglia L1–L3. Many labelled cells in the distal lobe were clustered close to the point of exit of the hypogastric nerve. Electrophysiological studies were restricted to this region of the distal lobe.

Classification of neurones on the basis of firing pattern

Intracellular recordings were made from sympathetic neurones lying in the caudal lumbar chain ganglia (L5 and L6) and in the distal lobe of the i.m.g. When depolarizing current pulses of between 0·05 and 0·5 nA amplitude and 150–350 ms duration were passed through the recording micro-electrode, action potentials were initiated in the majority of cells at a threshold depolarization of between 15 and 25 mV. At just sub-threshold depolarizations, local responses were first observed about 20 ms after the onset of the current pulse in neurones of the l.s.c. (Fig. 1*Aa*); in most i.m.g. cells, the first sign of a local response occurred much later – about 100 ms after the onset of the depolarization (Fig. 1*Ba*). Action potentials initiated at threshold occurred at the same latencies as the local responses (Fig. 1*b*). Increases in the strength of the current pulse increased the number and frequency of action potentials occurring during the pulse in cells from both locations (Fig. 1*c* and *d*). However, the pattern of firing was quite different. Almost all (109/113) neurones in the l.s.c. discharged only at the beginning of the depolarizing current pulse and then firing ceased. In contrast, the majority (105/121) of neurones impaled in the i.m.g. fired rhythmically and continuously throughout the current pulse. Cessation of firing after an initial burst in l.s.c. neurones and maintenance of lower frequency discharge in i.m.g. neurones were observed even if depolarizing current was passed continuously for several seconds. These two firing patterns have been described in other autonomic neurones and have been designated 'phasic' and 'tonic' respectively (Griffith *et al.* 1980; Decktor & Weems, 1981, 1983). The remainder of each population discharged in the alternative mode, i.e. four tonic neurones were identified in the l.s.c. and sixteen phasic neurones in the i.m.g.

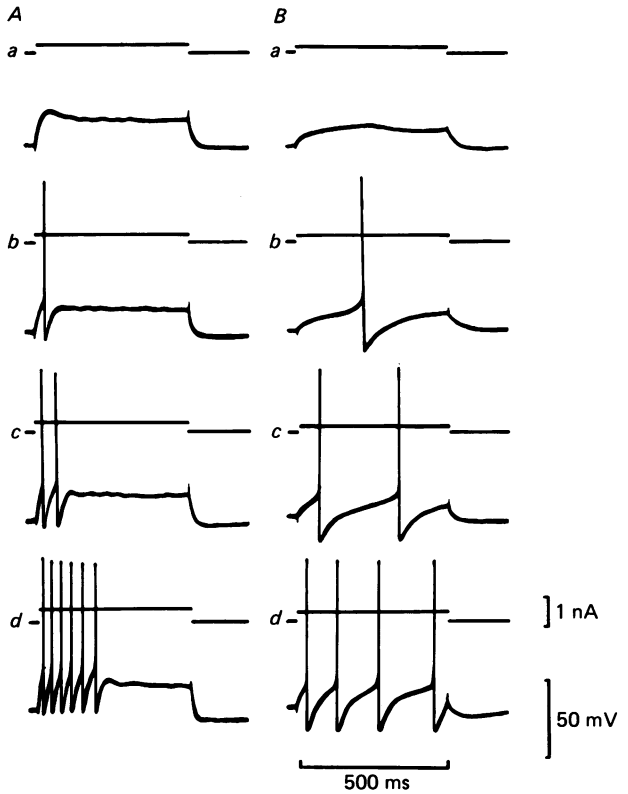

Fig. 1. Responses of different guinea-pig sympathetic neurones to depolarizing current passed through the recording micro-electrode. *A*, cell in lumbar sympathetic ganglion L5; *B*, cell in the distal lobe of the i.m.g. Magnitude of current increased progressively from *a* (just subthreshold) to *d*. The neurone in *A* discharges phasically at the onset of the current pulse, while the neurone in *B* fires tonically throughout the passage of current.

Passive electrical properties of l.s.c. and i.m.g. neurones

After impalement of a neurone, r.m.p. and cell input resistance (R_{in}) gradually increased and then stabilized after some 15–30 min. During this period, it was possible to characterize the neurones according to their firing pattern, and to examine their synaptic input. Once stable, r.m.p. in different cells lay in the range −48 to −78 mV, that of phasic neurones in the l.s.c. being on average about 3 mV less negative than that of tonic i.m.g. cells ($P < 0{\cdot}01$; see Table 1). Measurements were taken only after this initial period; subsequently, r.m.p. usually remained within ±5 mV until the electrode was withdrawn or the impalement lost.

When current pulses of small amplitude (0·01–0·1 nA) were passed through the recording micro-electrode, voltage responses were recorded in neurones of both types which reached a new steady level after between 100 and 300 ms (see Fig. 4). The voltage transient at the onset of a small hyperpolarization (< 15 mV) was found to follow a single exponential time course for at least the latter 80% of its amplitude. The time constant of this exponential, or major input time constant, τ_{in}, of the cell, reflects the membrane time constant (Rall, 1960).

TABLE 1. Passive membrane properties of phasic and tonic sympathetic neurones

	L.s.c. phasic ($n = 43$)	I.m.g. phasic ($n = 5$)	I.m.g. tonic ($n = 40$)
R.m.p. (mV)	57·7 ± 1·0	56·0 ± 2·4	61·4 ± 0·9
Cell input resistance, R_{in} (MΩ)	175 ± 9	165 ± 19	193 ± 16
Cell input time constant, τ_{in} (ms)	27·2 ± 1·6	27·3 ± 2·9	41·6 ± 2·2
Apparent cell capacitance (pF)	164 ± 11	159 ± 15	246 ± 13
Threshold current (nA)	0·24 ± 0·04	0·22 ± 0·05	0·50 ± 0·08
Threshold voltage (mV)	24·0 ± 0·9	26·0 ± 2·9	27·1 ± 1·2

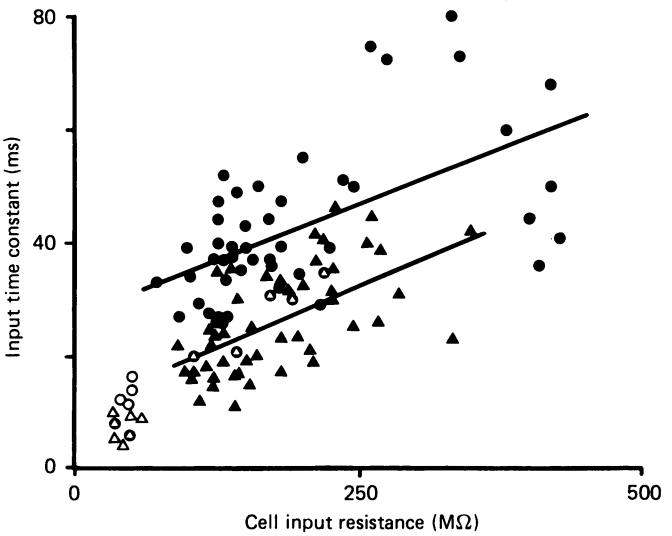

Fig. 2. Graph relating cell input resistance and major cell input time constant for sympathetic neurones of l.s.c. (triangles) and i.m.g. (circles). Cells in the i.m.g. that fired phasically in response to depolarizing current shown as circles with inset triangles. The subgroup of neurones having very low input resistances are indicated by the open symbols. Regression lines fitted to data for tonic neurones (filled circles, upper line) and phasic l.s.c. cells (filled triangles, lower line) have $r = 0·57$ and 0·60 respectively. The intercepts of these lines are significantly different ($P < 0·01$), but their slopes are not.

R_{in} determined from small hyperpolarizations ranged from 34 to 425 MΩ in different cells, and τ_{in} from 4·2 to 83 ms. When τ_{in} was plotted against R_{in} for neurones in the different ganglia (Fig. 2), it was evident that the data for phasic neurones in the l.s.c. only partly overlapped those for the tonic i.m.g. cells. The distributions of R_{in}, τ_{in} and apparent cell capacitance ($= \tau_{in}/R_{in}$) determined in l.s.c. and i.m.g. neurones are shown in Fig. 3. Although there is considerable overlap in these histograms, several differences are apparent. While mean values of R_{in} were similar between cell groups, mean values of τ_{in} and apparent cell capacitance were both significantly lower ($P < 0·001$) in l.s.c. neurones than in tonic i.m.g. cells (see Table 1). Phasic neurones identified in the i.m.g. had properties that fell amongst those recorded from the l.s.c. (see Table 1).

A small proportion of neurones (16 % in l.s.c., 13 % in i.m.g.) had particularly low

values of R_{in}, and appeared to represent a separate cell type (open symbols in Fig. 2; shaded columns in Fig. 3). Data from these cells are not included in Table 1. Neurones with similar properties were called Type II cells by Crowcroft & Szurszewski (1971) in an earlier study of the guinea-pig i.m.g. We do not think these cells were

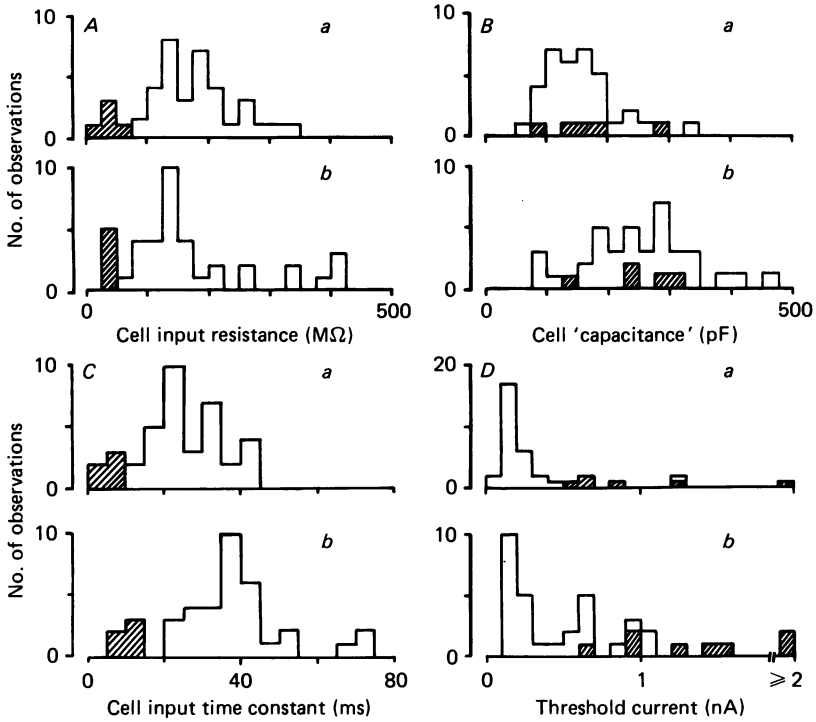

Fig. 3. Membrane properties of neurones of the l.s.c. (*a*) and the i.m.g. (*b*). Frequency distributions of cell input resistance (*A*), apparent cell capacitance ($= \tau_{in}/R_{in}$) (*B*), cell input time constant (*C*) and threshold current for initiation of an action potential (*D*). Hatched areas show data obtained in neurones in the group having very low input resistance.

damaged by the impalement as they had brief (1–2 ms) action potentials of large amplitude (120–130 mV) and particularly high r.m.p.s (mean $70{\cdot}1 \pm 1{\cdot}5$ mV, $n = 12$) which remained stable for periods as long as 4 h. These neurones had very high thresholds for initiation of action potentials (usually 35–40 mV positive of r.m.p.). The majority appeared to be phasic as only three out of six in the i.m.g. could be classified as tonic. However, it is not obvious that this classification should be applied because their high threshold made it difficult to fire more than one action potential. The major characteristic of this group of neurones appeared to be a very high resting conductance to K^+.

Current–voltage relations of phasic and tonic neurones

The relation between current and voltage was studied in both s.e.c.c. and s.e.v.c. modes. In s.e.c.c., depolarizations greater than about 20 mV were accompanied by

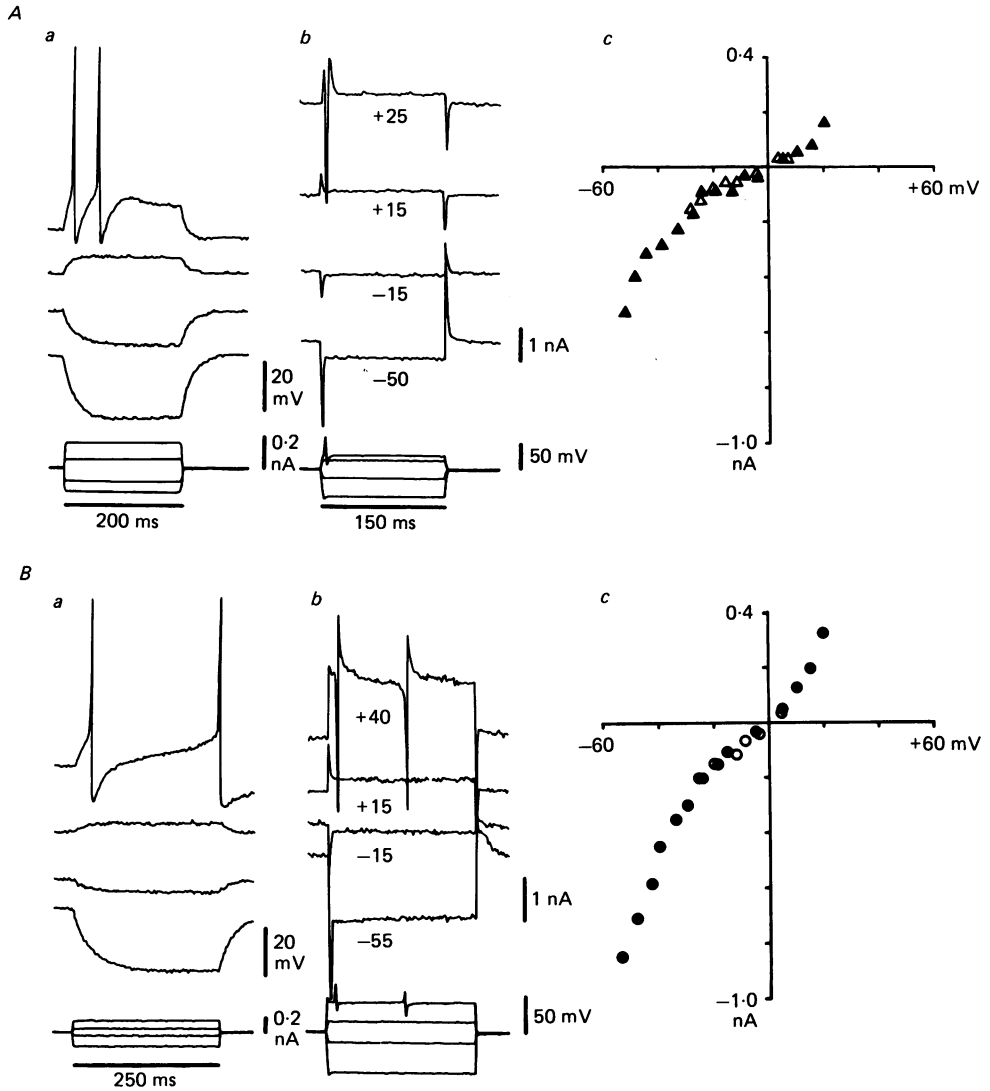

Fig. 4. Current–voltage relations of a sympathetic neurone in a ganglion of the l.s.c. (*A*) and of another in the i.m.g. (*B*). Examples of records of voltage (above) and current (at the bottom) in current clamp shown in *a*, and of current (above) and voltage (at the bottom) in voltage clamp in *b*. Numbers in *b* indicate the voltage command steps from resting membrane potential (−55 mV in *A*; −60 mV in *B*). *c*, plot of steady-state current–voltage relations determined in voltage clamp (filled symbols) and current clamp (open symbols) for sub-threshold ranges of membrane potential.

action potential discharge of the phasic or tonic pattern (see above). Plots of current amplitude against steady-state voltage response were linear around r.m.p., but showed rectification, usually in both depolarizing and hyperpolarizing directions. When the voltage was stepped (in s.e.v.c.) from r.m.p. to different depolarized and hyperpolarized levels, the same current–voltage relation was determined (see Fig. 4).

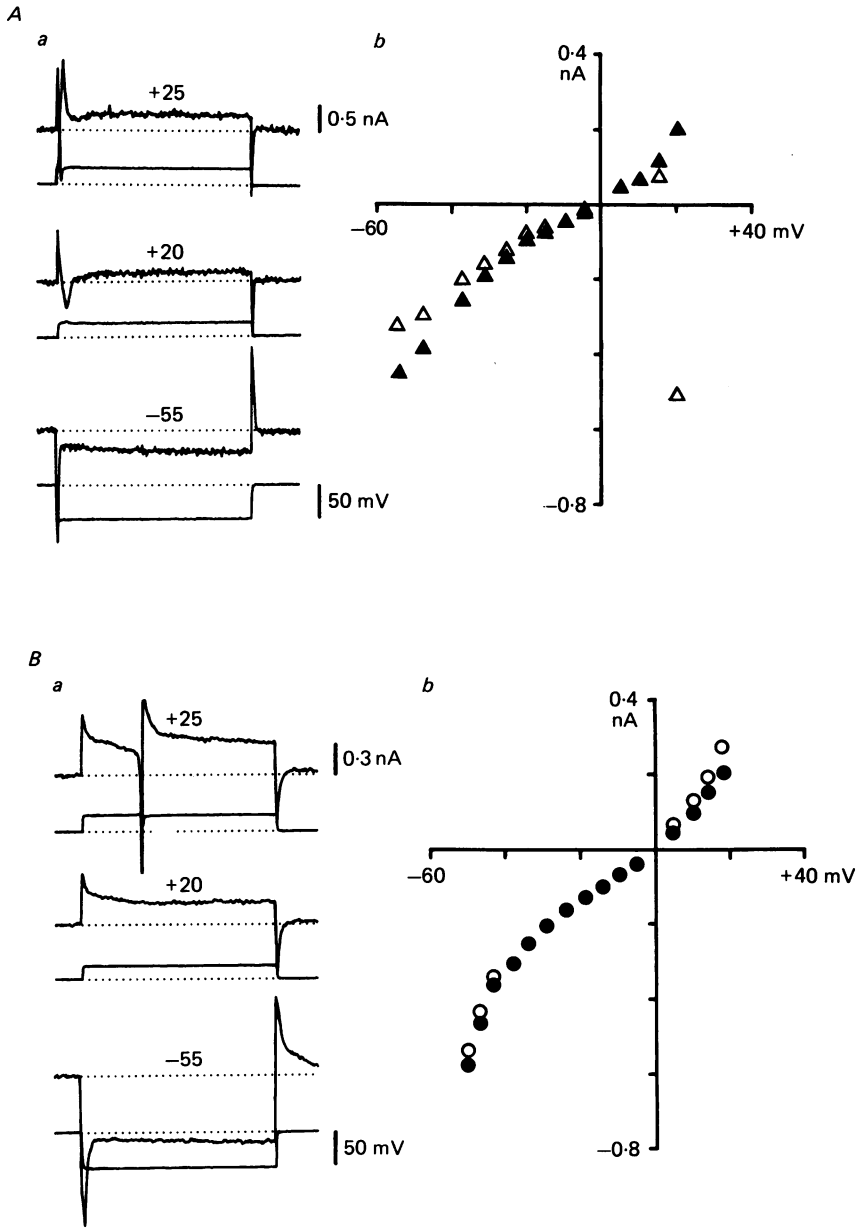

Fig. 5. Instantaneous and steady-state current–voltage relations of a phasic neurone in the l.s.c. (*A*) and of a tonic neurone in the i.m.g. (*B*). *a*, sample depolarizing and hyperpolarizing current (above) and voltage (below) records in voltage clamp at resting membrane potential (−60 mV in *A*; −58 mV in *B*) during 250 ms voltage command steps as indicated. *b*, plot of instantaneous (open symbols) and steady-state (filled symbols) current–voltage relations determined in the subthreshold range. Instantaneous currents measured about 10 ms (*A*) and 20 ms (*B*) after the beginning of the voltage step to avoid onset current transients. Both of these neurones show inward rectification during large hyperpolarizing voltage steps. Just subthreshold responses to depolarizing commands show early inward current in *A*, but outward current in *B*.

Large brief 'action currents' were recorded at threshold voltage steps (> 15 mV), either at the onset of a depolarizing step in phasic neurones (Figs. 4*A* and 5*A*), or after a delay of between 50 and 100 ms in tonic neurones (Fig. 5*B*). Larger voltage steps initiated more than one action current. During these currents, voltage control was poor, but the command potential (±3%) was usually restored within 10 ms of the peak current.

In all phasic neurones, at the beginning of depolarizing voltage steps that were just subthreshold for the initiation of the action current, a transient inward current lasting 20–50 ms was observed (Fig. 5*Aa*). In contrast, at the onset of depolarizing steps preceding the action current in tonic neurones, the outward current was initially greater than the leak current, indicating a transient phase of increased membrane conductance (Fig. 5*Ba*). In all tonic neurones, a transient outward current of similar time course was observed at the break of a hyperpolarizing voltage command step of > 25 mV (Figs. 4*Bb* and 5*Ba*). A rather smaller amplitude transient outward current could also be observed at the break of large hyperpolarizations in 38% of phasic neurones. These currents lasted for some 50–70 ms, and had characteristics (see below) similar to those of the transient potassium current called the A current (I_A) (Connor & Stevens, 1971*a*; Neher, 1971).

Most of the neurones exhibited instantaneous (< 20 ms) anomalous rectification with hyperpolarizations > about 30 mV (Figs. 4 and 5). This was more pronounced in tonic than in phasic neurones, only 68% of the latter showing it clearly. Time-dependent current relaxations during hyperpolarizing voltage commands were not observed with steps < 30 mV from r.m.p. However, in 48% of phasic neurones, and 25% of tonic neurones, slow inward current relaxations over about 200 ms were observed during large hyperpolarizing steps (Figs. 4*A*, 5*A* and *B*). These resembled currents described in cat motoneurones (Barrett, Barrett & Crill, 1980) and guinea-pig hippocampal neurones (Halliwell & Adams, 1982) that have been called I_Q. In another 25% of tonic neurones, but no phasic neurones, small outward current relaxations occurred in the same voltage range (see e.g. Fig. 4*B*).

Action potentials in phasic and tonic neurones

The current required to bring most ganglion cells to threshold was < 0·5 nA, and the threshold voltage was between 15 and 25 mV positive of r.m.p. However, in some neurones (about 40% of i.m.g. and 13% of l.s.c. cells, in addition to those in the low R_{in} group), currents of up to 1·5 nA were required (Fig. 3). The threshold voltage in these neurones was usually over 30 mV positive of r.m.p. The depolarizing current necessary to bring the cells to threshold was on average higher ($P < 0·01$) in i.m.g. than in l.s.c. neurones (see Table 1). This was apparently due to the activation of I_A in i.m.g. cells (see below).

Brief depolarizing pulses (30–50 ms duration) from r.m.p. were used to initiate action potentials (see Fig. 6). These had amplitudes of between 75 and 120 mV, and durations at half-amplitude of 1–2 ms. An after-hyperpolarization followed each action potential; this lasted from between 100 and 600 ms in different cells, being more prominent in tonic i.m.g. neurones.The after-hyperpolarization was on average slightly smaller ($P < 0·05$) in phasic l.s.c. neurones (mean ± S.E. of mean, 9·6 ± 0·9 mV, $n = 25$) than in tonic i.m.g. cells (12·3 ± 0·8 mV, $n = 23$), while its duration was

shorter in some of the cells (mean 300 ± 18 ms (l.s.c.); 334 ± 12 ms (i.m.g.)). The current underlying the after-hyperpolarization was recorded in voltage clamp at resting potential; by applying similar brief depolarizations that were just suprathreshold for an action current, the mean amplitude of the tail current in phasic l.s.c. neurones (0·10 ± 0·01 nA, $n = 22$) was found to be significantly smaller ($P < 0·01$) than

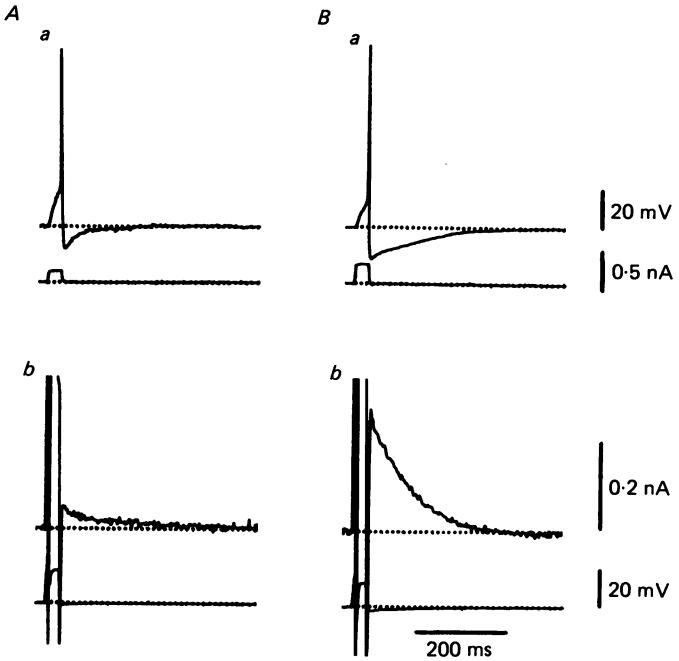

Fig. 6. Action potentials and after-hyperpolarizations of a phasic sympathetic neurone of the l.s.c. (*A*) and a tonic neurone in the i.m.g. (*B*) initiated by brief (30 ms) suprathreshold stimuli. *a*, voltage (above) and current (below) records in current clamp and *b*, current (above) and voltage (below) records in voltage clamp at resting membrane potential (−55 mV in *A*; −57 mV in *B*). Time scale applies throughout.

that in tonic neurones (0·17 ± 0·02 nA, $n = 23$). However, a few phasic neurones had large amplitude currents, and some tonic neurones small ones, despite similar clamp potentials. Phasic neurones with large post-spike currents fired only once over a very wide range of depolarizations. The time constant of the major exponential decay of the currents was slightly longer ($P < 0·01$) in l.s.c. neurones (132 ± 9 ms) than in tonic i.m.g. cells (104 ± 5 ms). As the former value is likely to be affected by the difficulty in fitting exponentials to small, slow and noisy signals, the more prominent after-hyperpolarization in tonic i.m.g. cells primarily results from the larger amplitude of the underlying current. Phasic i.m.g. cells usually exhibited small after-hyperpolarizations.

In some neurones in each location, an initial faster outward current (τ between 7 and 15 ms) was also recorded (see also Freschi, 1983); in s.e.c.c., a biphasic after-hyperpolarization was observed in these cells. Both the slow tail current and the brief early current were abolished at −60 mV when the K^+ concentration in the

bathing solution was raised to 20 mM (four neurones); the brief current, unlike the slow current, was abolished in solutions containing 10 mM-tetraethylammonium chloride, and not in solutions containing either no Ca^{2+}, or added Mn^{2+} (2 mM). Thus, the initial faster current presumably represents the delayed rectifier, while the slower current is a consequence of the calcium-activated K^+ conductance that follows calcium influx during the action potential in mammalian sympathetic neurones (McAfee & Yarowsky, 1980).

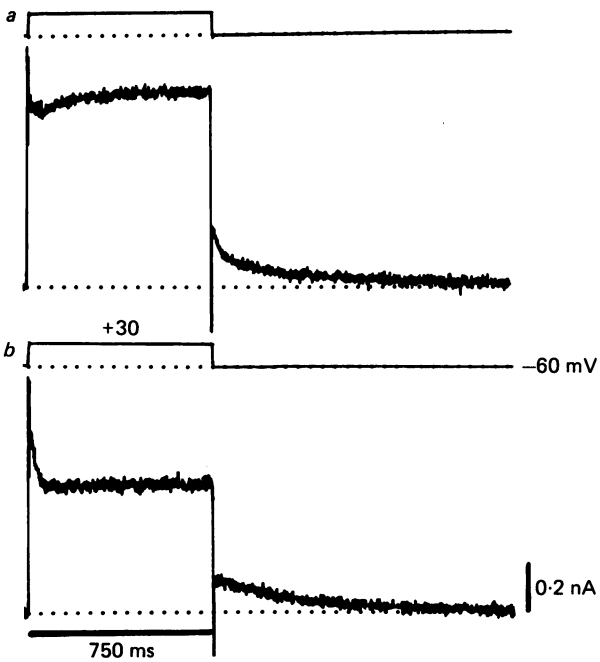

Fig. 7. Currents recorded in a phasic l.s.c. neurone during and after +30 mV voltage clamp command steps from −60 mV, *a*, in solution containing TTX (0·5 μM) and *b*, in the same solution after adding bethanechol (5×10^{-5} M). The early fast component ($\tau \approx 30$ ms, in *a*) of the residual outward current following repolarization was abolished in the presence of bethanechol; the slow component ($\tau \approx 350$ ms) was unaffected.

Effect of a muscarinic agonist on action potential firing patterns

The phasic nature of the discharge pattern displayed by l.s.c. neurones could be explained if an increase in membrane conductance develops slowly and is sustained during maintained depolarization. Such a current, the M-current, I_M, has been reported in rat superior cervical ganglion cells (Constanti & Brown, 1981) as well as in bull-frog sympathetic neurones, in which its properties have been extensively described (Adams, Brown & Constanti, 1982). Under the conditions of the present experiments (i.e. temperature, membrane potential, etc.), this current would be expected to have a relatively fast time course of activation/inactivation.

When the neurones were clamped near resting membrane potential (−60 mV), and stepped for 500–1000 ms to 30–40 mV more positive, action currents were usually initiated at the onset of the depolarization. These were abolished by the addition

of TTX (0·3–0·6 μM) to the bathing solution in some experiments, but an early inward current (duration <20 ms) usually remained (just visible on the upstroke of the current record in Fig. 7). Following an initial decline in outward current if an action current had been initiated (i.e. after some 200–400 ms), a steady level of outward current was usually maintained until the end of the depolarization (see Figs. 7 and 8). This sequence of events was observed in both l.s.c. and i.m.g. neurones,

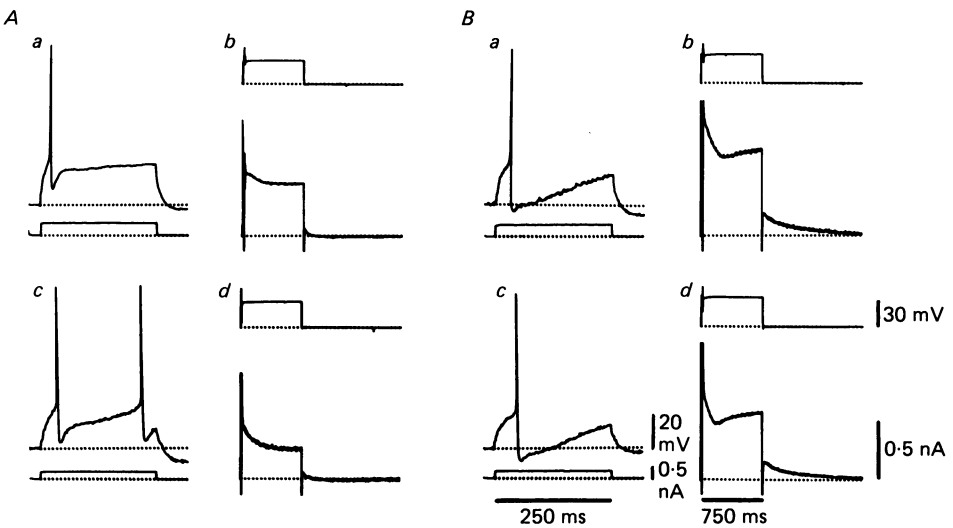

Fig. 8. Responses of a phasic neurone in the l.s.c. (*A*) and a tonic neurone in the i.m.g. (*B*) in control solution (*a* and *b*) and in the presence of bethanechol (5×10^{-5} M) (*c* and *d*). Voltage (above) and current (below) records of (*a*, *c*) action potentials evoked by depolarizing current pulses (0·5 nA in *a*; 0·3 nA in *c*) at -58 mV (*A*) and -62 mV (*B*); (*b* and *d*) averages of ten responses to voltage command steps to -28 mV (*A*) and -27 mV (*B*).

with some variation in the number of initial action currents, and the development in other cells of a slow outward current (Fig. 8*B*) during the later stage of the pulse. In a few tonic neurones, action currents occurred throughout the voltage step.

When the membrane potential was returned to near -60 mV, there was a variable period during which outward current persisted. In some (mostly phasic) neurones, only an early brief phase of outward current was observed (Fig. 8*A*), but in many others the outward current decayed more slowly with a major time constant of several hundred milliseconds following an initial fast component (Fig. 7). After subtraction of the major component, the amplitude of the early phase of the repolarization was usually $< 0·1$ nA. In phasic neurones, this component had a time constant between 25 and 45 ms, as would be expected if it represented the closing of M-channels under these conditions (Constanti & Brown, 1981; Adams *et al.* 1982). Furthermore, the addition of the muscarinic agonist, bethanechol (10^{-5} to 10^{-4} M) reduced or abolished the fast component without affecting the slow component (Fig. 7; also reported for hippocampal neurones, Brown & Griffith, 1983). In tonic cells, an early

component with a longer time constant (between 45 and 75 ms) was identified, but its amplitude was largely unaffected by the addition of bethanechol.

The addition of bethanechol to the bathing solution transformed the phasic discharge of action potentials in l.s.c. neurones during depolarizing current into a repetitive discharge typical of tonic neurones (Fig. 8*A*; see also Brown & Constanti, 1980; Brown, Adams & Constanti, 1982). Furthermore, at a concentration of 5×10^{-5} M, the level of maintained outward current during a voltage-clamp step to -30 mV was markedly reduced (by $0{\cdot}15 \pm 0{\cdot}04$ nA after allowing for changes in

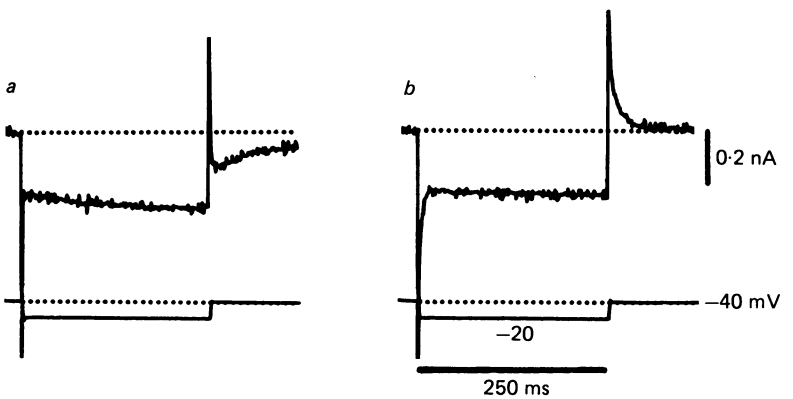

Fig. 9. Current relaxations elicited during hyperpolarizing steps at depolarized membrane potentials. Current (above) and voltage (below) records in l.s.c. (*a*) and tonic i.m.g. (*b*) neurones during voltage-clamp steps from -40 to -60 mV. Inward current relaxation is prominent in l.s.c. but not i.m.g. neurones.

leakage current, $n = 6$, see Fig. 8*Ab* and *d*), in addition to the reduction of the fast component of the recovery current. In five tonic neurones, bethanechol produced a much smaller reduction ($P < 0{\cdot}02$) in the maintained level of current during the same voltage command step ($0{\cdot}03 \pm 0{\cdot}01$ nA, see Fig. 8*B*), while three other tonic neurones fired repetitively at high frequencies when depolarized to this extent in the presence of bethanechol.

In phasic neurones, rectification of the current–voltage relation at depolarized potentials was reduced but never abolished; little effect could be detected in tonic neurones. In both neurone types, the addition of bethanechol increased R_{in}, inhibited the calcium-activated potassium conductance (see also North & Tokimasa, 1983) and I_A (E. M. McLachlan & J. F. Cassell, unpublished observations), and lowered action potential threshold. Details of these effects will be reported elsewhere.

The presence of current relaxations that might reflect the closing of M-channels could also be demonstrated in phasic neurones by holding the membrane potential at -40 mV and stepping it to -60 mV (Fig. 9). This procedure produced small relaxations in some tonic cells, but on average the relaxation was less than 25% of the amplitude of that observed in phasic neurones. I_A was always present at the end of such hyperpolarizations in tonic but not always in phasic neurones (Fig. 9). I_M therefore seems to be relatively small or absent in tonic i.m.g. neurones.

Transient outward current in phasic and tonic neurones

The transient outward current that was initiated by depolarization was much more prominent in tonic than in phasic neurones, and appeared to delay the onset of the action potential in i.m.g. cells (see earlier). The properties of this current (I_A) in rat sympathetic ganglion cells have recently been described in detail (Galvan & Sedlmeir, 1984; Belluzzi *et al.* 1985), but differ in some aspects from those of phasic and tonic

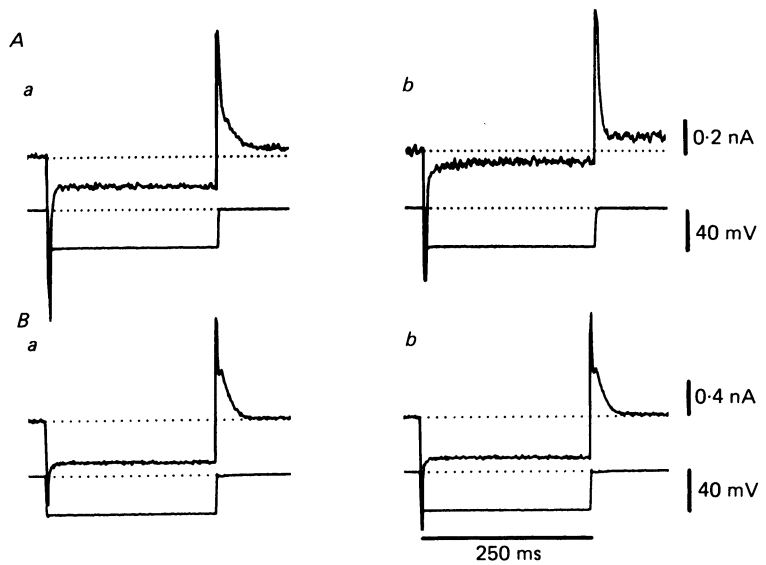

Fig. 10. Effects of Ba^{2+} and Mn^{2+} on transient outward currents (I_A) elicited at the break of hyperpolarizing steps in tonic neurones. Current (above) and voltage (below) records during voltage-clamp steps from −50 to −90 mV in two i.m.g. cells, *a*, in normal bathing solution and *b*, after replacing Ca^{2+} (2 mM) with Ba^{2+} (in *A*) and Mn^{2+} (in *B*).

neurones in the guinea-pig. As mentioned above, I_A was always observed at the break of a hyperpolarizing step of > 25 mV from r.m.p. in tonic neurones, but could be detected in less than half of the phasic neurones under these conditions. These observations suggest that I_A in tonic neurones differs from that in phasic neurones in either magnitude or voltage sensitivity, or both. Several properties of I_A were therefore studied, initially to confirm that the same type of voltage-dependent potassium channel was present in both neurones. A few experiments were performed in the presence of TTX (0·3–0·6 μM) and TEA (10 mM); this extended the range over which the currents could be examined, but at these concentrations of TTX the fast inward current was not completely blocked. The results were qualitatively the same whether or not these substances were present. In the experiments in which the effects of ions and drugs were tested, I_A was often initiated by stepping to a potential positive of r.m.p. in order to increase its amplitude (see below).

When the K^+ concentration in the bathing solution was increased from 4·7 to 20 mM, the reversal potential for I_A was shifted close to r.m.p. In both phasic ($n = 2$)

and tonic ($n = 4$) neurones clamped at r.m.p., both I_A at the end of a large hyperpolarizing step from r.m.p., and the inward current relaxation during the hyperpolarization (I_Q, Halliwell & Adams, 1982) when present, were abolished or reversed in the higher K^+ concentration. In addition, cell input resistance was

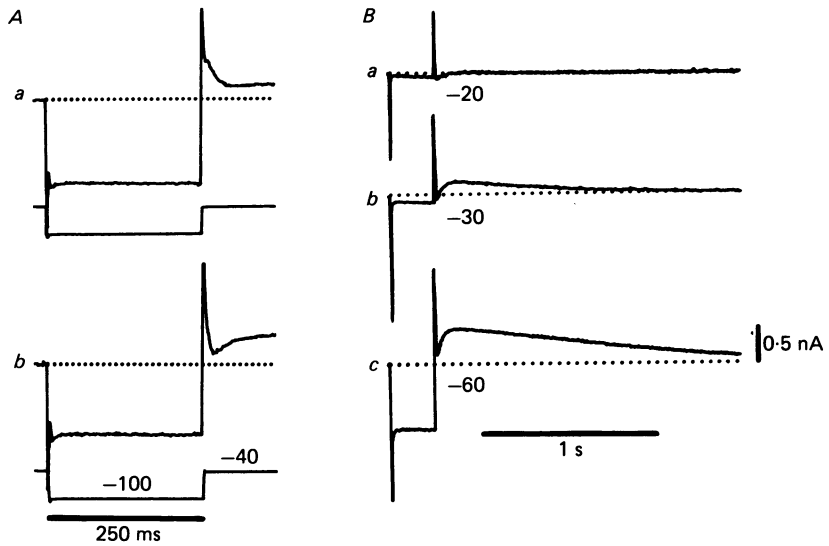

Fig. 11. Prolonged slow outward current activated at the break of hyperpolarization in a tonic neurone held at −40 mV. *A*, currents (above) in response to −60 mV voltage command steps (records below) in *a* control solution, and *b* solution containing 3 mM-4-AP. *B*, currents recorded in the presence of 4-AP (3 mM) during and after 250 ms hyperpolarizations to *a* −60, *b* −70, and *c* −100 mV (size of voltage command steps indicated on records).

decreased, and the holding current increased, in a K^+ concentration of 20 mM in all cells tested.

Removal of Ca^{2+} from the bathing solution and its replacement with Ba^{2+} (2 mM) abolished I_A in both phasic ($n = 3$) and tonic ($n = 5$) neurones (Fig. 10*A*). However, replacement of Ca^{2+} with Mn^{2+} had little effect on I_A in four tonic neurones and one phasic neurone (Fig. 10*B*, cf. Galvan & Sedlmeir, 1984), so that it is unlikely that I_A is necessarily dependent on Ca^{2+} movement. Anomalous rectification, calcium-activated potassium conductances and I_Q were all abolished by both Mn^{2+} and Ba^{2+}, and resting conductance was decreased. The addition of 4-aminopyridine (4-AP) (1 mM) reduced I_A in one phasic and two tonic neurones, and a higher concentration (3 mM) virtually abolished it in another tonic neurone (see Fig. 11).

Slower and more prolonged transient outward currents were elicited at the break of a period of hyperpolarization (after I_A) in seven out of seventeen tonic and none out of eleven phasic neurones if the holding potential was 10–20 mV positive of r.m.p. (Fig. 11). These currents increased with both the amplitude and duration of the hyperpolarization, from thresholds of about −25 mV and 200 ms respectively. In different cells, the prolonged transient currents were not blocked by substitution of

Ba^{2+} for Ca^{2+} ions (Fig. 10*A*), or by the addition of 4-AP (Fig. 11), both of which abolished or reduced I_A. The long currents were also not blocked if Ca^{2+} were replaced with Co^{2+}, which did not abolish I_A. Their onset could be observed when I_A was blocked (Fig. 11); the slow currents rose to a peak in 80–120 ms and then declined exponentially with a time constant between 300 and 900 ms in different cells. The properties of these currents were not studied further in the present experiments.

Conductance change during I_A

The magnitude of the conductance change in tonic neurones during I_A was determined from the size of I_A at its peak (about 10 ms after the voltage step, see below) in two ways, either (a) relative to the leak resistance at the end of a depolarizing voltage step, or (b) using the relation $g_A = I_A/(V_c - V_A)$ for currents at the break of a hyperpolarizing step. For large currents elicited by steps of ± 30 mV around r.m.p., cell conductance was on average doubled at the peak of I_A (a: $+92 \pm 22\%$, $n = 5$; b: $+94 \pm 12\%$, $n = 7$), i.e. g_A was about 5 nS. This would be consistent with observed increases in clamp error during large I_A. Similarly, the largest I_As recorded in the experiments illustrated in Fig. 13 corresponded to $g_A = 10$ nS, with average values of about 5 nS. These values are likely to represent the conductance with only a proportion of all A-channels open (i.e. $< g_A$). In phasic neurones (see Fig. 13), peak g_A was about half that in tonic neurones.

The transient in the current record at the make and break of a voltage command step obscured the rising phase of I_A, but its time course could be revealed by adding current transients of opposite polarity. In thirteen tonic and eight phasic neurones, the current transients were removed by adding together duplicate records shifted in time so that the onset and break of a voltage step were aligned (see Fig. 12*Aa*). The procedure had the technical limitation that, after passage of large or prolonged currents through some micro-electrodes, the current transient at the break of the voltage step was larger than at the onset. This artifact tended to lead to underestimation of the rise time of I_A.

In four other tonic and two other phasic neurones, the current transients were removed by adding together the currents flowing during depolarizing and hyperpolarizing voltage steps (250 ms duration). Voltages were matched so as to identify I_A when the potential was stepped to a particular level, usually -40 or -45 mV. This procedure eliminated not only the current transient (see Fig. 12*B*) but also distortions due to slower current relaxations such as the M-current in phasic neurones.

The rise time of I_A determined in tonic neurones varied from 4 to 13 ms (mean $7{\cdot}2 \pm 0{\cdot}6$ ms, $n = 15$) and in phasic neurones from 4 to 7 ms (mean $5{\cdot}1 \pm 0{\cdot}4$ ms, $n = 8$); rise time appeared to increase slightly with larger depolarizing steps. The decay of I_A in tonic neurones was exponential with a time constant (τ_B) of $22{\cdot}1 \pm 1{\cdot}6$ ms ($n = 17$); in any one neurone, τ_B was little affected by the holding potential or the potential at which I_A was initiated, in the potential range -120 to -40 mV. In phasic neurones, time constants of exponentials fitted to the decay phase of I_A between 60 and 20% of its peak amplitude ranged from 5·5 to 25·5 ms (mean $11{\cdot}7 \pm 2{\cdot}0$ ms, $n = 10$). However, only in the cases having the larger time constants were the decays well described by single exponentials. In most others, there was evidence of inward current at all potentials at which I_A could be detected. It has therefore not been

possible to detect any major difference in time course of I_A between the two types of neurone in these experiments. Recorded I_As elicited near r.m.p. could be well fitted by eqn. (2) using $\tau_A = 3$ ms and $\tau_B = 25$ ms (Fig. 12*Ab*), and these 'typical' values were used for computer simulations (see Cassell & McLachlan, 1986).

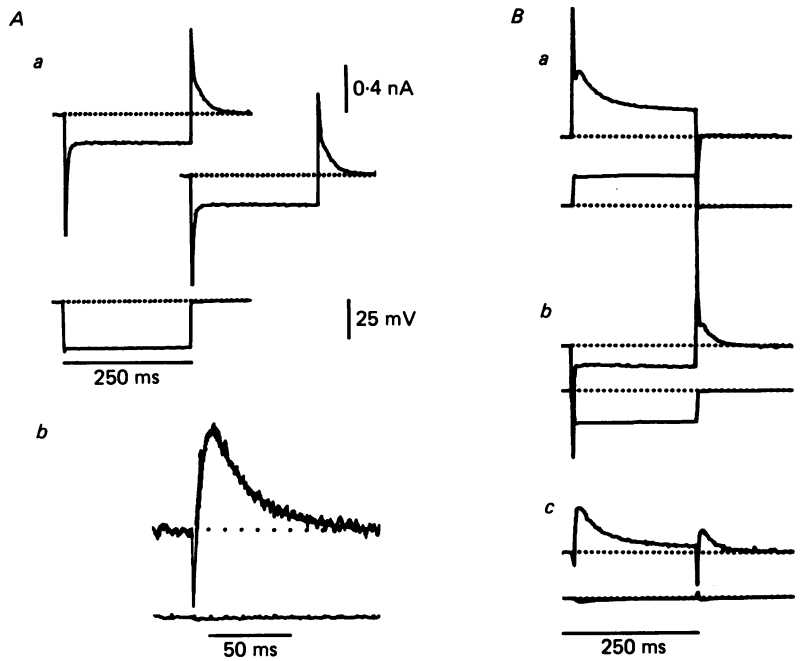

Fig. 12. Time course of I_A in tonic neurones determined after removal of current transients. *A*, addition of duplicate records shifted in time. *a*, duplicates of averaged current records in response to −30 mV step from a holding potential of −60 mV (r.m.p.), with corresponding voltage record (below); *b*, result of addition of records shown in *a* (enlarged). The time course of I_A is well fitted by eqn. (2) with $\tau_A = 3$ ms, $\tau_B = 25$ ms. *B*, addition of responses to depolarizing and hyperpolarizing voltage steps. Averaged current (above) and voltage (below) records from another neurone held at −70 mV and stepped +25 mV (*a*), and held at −45 mV and stepped −25 mV (*b*). The averaged records have been summed in (*c*). Current and voltage calibrations apply to *Aa* and *Ba* and *b*; gains in *Ab* × 4 and in *Bc* × 2.

It is evident in Fig. 12*Ba* that the depolarizing step from −70 to −45 mV elicited a larger and more prolonged I_A and also more steady outward current (after I_A) in this tonic neurone than the same voltage step in Fig. 12*Bb*. Because the test pulses (250 ms duration) in these experiments were presented at intervals of > 2 s, it seemed likely that the differences resulted from a longer period of conditioning hyperpolarization. Although part of the prolongation might be explained by the initiation of the slow current mentioned above, I_A would also be expected to vary with the duration of conditioning hyperpolarization (Galvan & Sedlmeir, 1984). This was tested using command pulses of constant amplitude presented at intervals of > 2·5 s.

At a holding potential of −60 mV in tonic neurones, the amplitude of I_A was reduced if the conditioning hyperpolarization lasted <200 ms, and increased a further 10–40 % for more prolonged hyperpolarizations (up to 600 ms). τ_B increased progressively with the duration of preceding hyperpolarization by some 30–50 % to a plateau level for hyperpolarizations > about 400 ms. Both I_A amplitude and τ_B remained relatively constant with longer hyperpolarizations (up to 2 s studied).

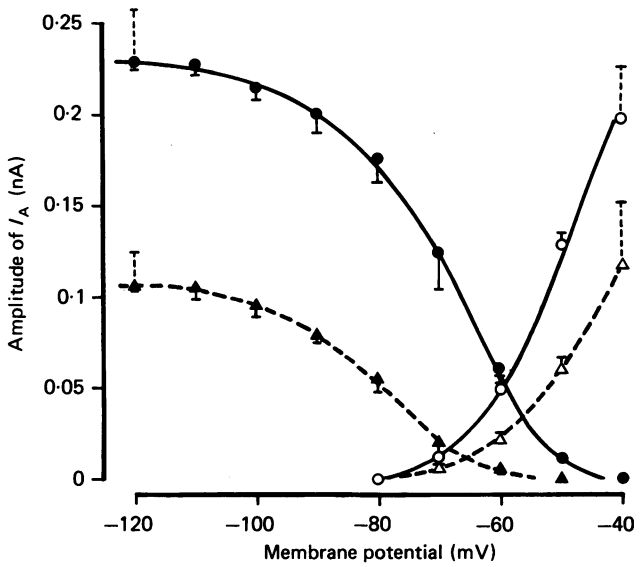

Fig. 13. Measurements of peak I_A in phasic neurones (triangles) and in tonic neurones (circles) in experiments designed to determine characteristics of inactivation (filled symbols) and activation (open symbols) (see text). Continuous vertical bars indicate S.E. of mean for normalized data, dashed bars S.E. of mean for currents to which data were normalized. Further details in text.

Activation/inactivation characteristics of I_A in phasic and tonic neurones

Curves describing activation and inactivation of I_A were constructed using an experimental protocol similar to that of Connor & Stevens (1971*b*). Neurones were clamped at −80 mV and depolarized to different levels to define activation, and held at −40 mV and hyperpolarized to different levels to define inactivation (I_A elicited at the break of the step). This protocol assumes that I_A resulting from these depolarizing steps provides an accurate measure of activation of A channels before a significant increase in the number of inactivated channels can occur. Experimental data are shown in Fig. 13 for six phasic and six tonic neurones. To construct these curves, the data points were first normalized to the plateau for the inactivation curve, and to the value of −40 mV for the activation curve. Logistic curves (eqn. (1) Methods) were fitted to the pooled normalized data points and subsequently scaled to the mean observed currents to show the differences between phasic and tonic neurones. Standard errors for the normalized points are indicated by continuous vertical bars, and for the measured currents by dashed bars (Fig. 13).

The forms of the curves (Fig. 13) for phasic and tonic neurones were virtually identical, there being no significant differences between the values for the parameters of the logistic curves describing their shape (i.e. P_1, P_2). The curves used to define activation could be overlaid, but that describing inactivation for tonic neurones was shifted to the right of the curve for phasic neurones, the values for P_3 being significantly different ($P < 0{\cdot}02$); potentials at 50 % of the plateau level were -84 mV for phasic neurones, and -71 mV for tonic neurones. In addition, the maximum inactivation current for tonic neurones was more than twice that for phasic neurones ($P < 0{\cdot}01$). The mean value for activation current at -40 mV was also larger in tonic than phasic neurones although the data were not significantly different.

The activation curve (A (V, ∞)) can be derived from these experimental data by dividing the measured currents by the driving force ($V_c - V_A$), and taking the fourth root of these conductances (see Methods). Because the driving force is constant during the experimental protocol to determine inactivation, B (V, ∞) is conventionally treated in normalized form (see Connor & Stevens, 1971*b*). The derived inactivation and activation curves are not accurate descriptions of the processes underlying I_A because of two factors. First, the experimental design assumes that τ_B, the time constant of the decay phase of I_A, is infinitely large relative to τ_A, the time constant of the rising phase. For molluscan neurone somata (Connor & Stevens, 1971*b*), with values of about 400 and 20 ms respectively, the derived curves proved reasonable, as they could be used to predict the behaviour of the neurone. However, for sympathetic neurones, with time constants of about 25 and 3 ms, the error in estimating A and B would be expected to be greater. Secondly, and more importantly, many of the voltage steps used experimentally fell in the region of overlap between the curves, especially for tonic neurones. Thus, for example, steps designed to demonstrate activation reached voltages at which significant numbers of channels were not inactivated. Consequently, the derived values for A and B could not be used to reproduce Fig. 13 using eqn. (2). In practice, the best reconstruction of the experimental curves was achieved by empirically displacing the relation describing both A and B to more hyperpolarized potentials using parallel shifts of 5 mV and 3 mV respectively (see also Connor *et al.* 1977). Shifts of smaller or larger magnitude produced large discrepancies in the region of overlap (i.e. -60 to -70 mV). Half-inactivation points thus became -87 mV for phasic and -74 mV for tonic neurones. A ratio of 0·56 of values for $\bar{g}_A$ (see eqn. (2)) was necessary to reproduce the sizes of the observed currents in phasic relative to tonic neurones. These adjusted relations have been used to test the effect of I_A on synaptic potentials (see Cassell & McLachlan, 1986).

DISCUSSION

The present study describes the characteristics of two groups of mammalian sympathetic neurones that can be distinguished on the basis of their pattern of discharge in response to depolarizing current. Virtually all neurones in the caudal l.s.c. showed a brief burst of action potentials at the onset of depolarization, while most of the neurones in the distal lobe of the i.m.g. fired rhythmically and continuously when depolarized. A small proportion of i.m.g. cells discharged phasically; the properties of these neurones resembled those of l.s.c. cells, rather than those of the rest of the i.m.g. neurones. Functionally, most of the paravertebral neurones

probably have vasoconstrictor functions in the hind limb; the prevertebral population is mainly involved with motility and secretion in the pelvic organs (see Introduction).

Phasic and tonic firing patterns have been described previously in several ganglia of the guinea-pig and cat. In cat pelvic ganglia, 93% of neurones were identified as tonic (Griffith *et al.* 1980). The proportion of tonic neurones reported in studies of the coeliac and superior mesenteric ganglia varies between about 60% (Kreulen & Szurszewski, 1979) and 35% (Decktor & Weems, 1983). In studies of the i.m.g., either only tonic firing patterns (Crowcroft & Szurszewski, 1971) or about 40% tonic/60% phasic neurones (Weems & Szurszewski, 1978; Kreulen & Szurszewski, 1979; Julé & Szurszewski, 1983) have been described, but it is not clear from these reports how many of the neurones may have been projecting in the colonic nerves. In the present study, which was confined to the region from which neurones project in the hypogastric nerves, 87% were tonic and about 12% phasic. In contrast, 72% of neurones in cat renal ganglia discharged phasically (Decktor & Weems, 1981), as did 96% of the l.s.c. neurones described here. When the functions served by the components of the outflow from these various ganglia are considered, it appears that these distinctive discharge patterns may provide a basis for the broad classification of sympathetic neurones as either vasoconstrictor or visceral.

Several differences in the membrane properties of these two neurone groups were identified. In general, tonic neurones had larger membrane time constant (τ_{in}) than had phasic neurones (cf. Decktor & Weems, 1983), although the mean value for R_{in} was not significantly higher. Determination of the membrane electrical properties of these neurones is complicated by the presence of unknown numbers of dendrites. It is not known whether the geometry of l.s.c. and i.m.g. neurones differs, although the average electrical length of a cable equivalent to the dendritic tree of neurones of both types seems to be about 0·5 λ (J. F. Cassell & E. M. McLachlan, unpublished observations, see also McLachlan, 1974). As R_{in} is determined by both geometry and membrane resistivity (R_m), the derived value for apparent cell capacitance does not simply reflect cell surface area. On the assumptions that specific membrane capacitance is constant and 1 μF/cm^2, and that τ_{in} reflects the membrane time constant (but see Lux, 1967; Jack & Redman, 1971), R_m of these neurones (25 KΩ cm^2, phasic; 40 KΩ cm^2, tonic) is even higher than that, e.g. measured recently in hippocampal neurones *in vitro* (Brown, Fricke & Perkel, 1981). However, the possibility exists that membrane ion channels are not uniformly distributed (G. D. S. Hirst, E. M. McLachlan & S. J. Redman, unpublished observation), so that it is difficult at present to identify the basis for the differences in passive electrical properties.

Action potentials in both phasic and tonic sympathetic neurones arose in many cases at similar voltage thresholds about 20 mV positive of r.m.p. However, whereas the action potential occurred at the onset of depolarization in phasic neurones, and a small inward current could always be detected just below threshold, the action potential in tonic neurones was initiated only after a delay, and often without detectable inward current. The delay was apparently caused by the transient outward current, I_A, which was not observed in phasic neurones except after a period of conditioning hyperpolarization. In phasic neurones, as in rat s.c.g. cells (Galvan & Sedlmeir, 1984; Freschi, 1983) and a number of other neurones (e.g. Connor & Stevens, 1971*a*; Adams *et al.* 1982) I_A was largely inactivated at r.m.p. In contrast,

I_A was readily observed after small depolarizations from r.m.p. in tonic neurones of the i.m.g.

The initiation of the action potential in tonic neurones was also delayed under conditions in which the soma was voltage clamped, suggesting that the site at which the active response was generated lay some distance from the soma. From the apparent electrical length of the processes of the neurones (see above), the voltage would be expected to be only a few millivolts lower than the somatic voltage command at the end of the processes. However, the processes would be much less depolarized than the (clamped) soma during I_A, so that a distant site would achieve threshold only after I_A had ceased. In some other neurones, particularly of the tonic type, the somatic voltage that was just threshold for the action potential was some 30–45 mV positive of r.m.p.; this is also consistent with a distant locus for the initiation of the action potential.

Action potentials in both types of neurone appear to possess a calcium component, which is most obviously expressed as a prolonged potassium current ($\tau = 100$ ms) (McAfee & Yarowsky, 1980). This current was on average larger in tonic than in phasic neurones, suggesting that calcium influx may be greater. This might reflect a greater number of calcium channels. The large after-hyperpolarization produced by this conductance might contribute to the slow rates of repetitive firing in these cells (see Connor & Stevens, 1971*b*). The other feature likely to be responsible for the rhythmic firing of tonic neurones is their prominent I_A; this transient current has been shown to account for slow repetitive discharge characteristics even in the absence of a prolonged potassium current (see Connor, 1978). A large calcium-activated potassium conductance was present in a few phasic neurones, in which discharge was usually limited to a single action potential. However, as this current was small in most phasic neurones, and generally larger in tonic neurones, it cannot be responsible for determining phasic discharge behaviour in sympathetic neurones (cf. Madison & Nicoll, 1984).

While the high frequency of the initial discharge in phasic neurones (see Fig. 1) can be explained by the absence of I_A, the termination of firing seems to follow because of I_M (Adams *et al.* 1982). The magnitude of I_M was significantly greater in phasic than in tonic neurones. This current was identified by the addition of a muscarinic agonist, bethanechol, which converted phasic discharges into repetitive firing (see also Brown & Constanti, 1980). In some tonic neurones, I_M could not be detected at all under the conditions of our experiments. It was evident, however, that significant rectification occurs in both neurone types that is not due to I_M.

There were many similarities in the properties of I_A in phasic and tonic neurones. As has been described for other neurones (Connor & Stevens, 1971*a*; Neher, 1971; Adams *et al.* 1982; Galvan & Sedlmeir, 1984; Belluzzi *et al.* 1985), the membrane channels for I_A appear to be selective for K^+; movement of K^+ is blocked by Ba^{2+} and by drugs such as 4-AP. The current is observed when the membrane is subjected to depolarizing voltage steps, particularly after a conditioning period of hyperpolarization. The time course of I_A can be described by a function (eqn. (2)) similar to the Hodgkin–Huxley description of the transient inward sodium current. The inactivation time constant, τ_B, is short relative to the activation time constant as in other vertebrate sympathetic neurones (see also Adams *et al.* 1982; Galvan &

Sedlmeir, 1984; Belluzzi *et al.* 1985; cf. molluscan neurones, Connor & Stevens, 1971*b*). While I_A appears to be responsible for delaying the initiation of action potentials in tonic neurones of the guinea-pig i.m.g., it seems unlikely that there could be any effect on action potential repolarization (Belluzzi *et al.* 1985) given the time course of activation of I_A in these cells.

Phasic and tonic neurones differ in that peak conductance of A-channels is significantly larger in neurones of the tonic type. This might reflect different numbers of channels. Recent measurements of I_A in rat superior cervical ganglion cells using a two-micro-electrode voltage clamp (Belluzzi *et al.* 1985) yielded peak conductances of about 500 nS at 37 °C (cf. about 100 nS at 25 °C (Galvan & Sedlmeir, 1984) and about 30 nS at 37 °C (E. M. McLachlan, personal observations) using a single micro-electrode clamp). In experiments on guinea-pig ganglion cells at 35 °C, peak conductances recorded with a single micro-electrode were only about 10 nS in i.m.g. cells (see above), and 2 nS in myenteric neurones (Hirst, Johnson & van Helden, 1985). As there is no evidence that the quality of voltage-clamp control was significantly impaired in those experiments in which only one electrode had been inserted, there must either be a substantial species difference in the numbers of A-channels present, or else some experimental factor such as efflux of potassium or influx of Ca^{2+} following the double impalement may have affected A-channel conductance.

The voltage sensitivity of A-channels also differs between phasic and tonic neurones in the guinea-pig, not with regard to their activation, but rather in that they are inactivated at more depolarized membrane potentials in tonic neurones. Half-inactivation occurs at -87 mV in phasic neurones, more than 10 mV more hyperpolarized than in tonic neurones (-74 mV). The time constant for removal of inactivation in both neurone types was about 100 ms, consistent with some other observations (Adams *et al.* 1982; Galvan & Sedlmeir, 1984; but see Belluzzi *et al.* 1985). Although all other characteristics of the A-channels were found to be the same in the two types of neurones, it appears that voltage-sensitivity is not necessarily constant for this membrane channel.

The consequence of greater peak conductance and less inactivation is that substantial I_A exists in tonic neurones in the region of membrane potential around r.m.p. This means that A-channels probably contribute to resting membrane conductance, despite their time-dependent inactivation (see Connor, 1978). This idea would be consistent with the slightly more negative r.m.p.s (-3 mV on average) recorded in tonic neurones (see Table 1). Furthermore, it seems that this current plays an important dynamic role in membrane conductance in the range of membrane potentials in which rhythmic low-frequency discharges are observed. In most tonic neurones, the effect of the calcium-activated potassium conductance after the action potential would tend to hold the neurones hyperpolarized, increasing I_A reactivation, and potentiating its effects. It is therefore likely that the firing pattern of these neurones follows primarily from the properties of this transient potassium conductance.

In conclusion, the data presented here suggest that sympathetic paravertebral neurones associated with the vasculature of the hind limb, and prevertebral neurones involved with the diverse non-vascular functions of the pelvic organs, can be differentiated on the basis of their discharge pattern during passage of constant

depolarizing current. These appear to result from differences in the voltage-dependent potassium channels present in the membrane of each neurone type: (a) l.s.c. neurones discharge phasically because of the potassium current, I_M, which is small or absent in distal i.m.g. cells, and (b) i.m.g. neurones fire tonically at lower frequencies because of the combined effects of the transient potassium current, I_A, which is initiated by depolarization from resting potential in these cells, and the lack of significant I_M. The calcium-activated potassium conductance following the action potential may contribute to slowing firing frequency.

The passive electrical properties of these neurones also differ, and this may reflect differences in neuronal geometry as well as differences in the populations of membrane ion channels open at resting potential. While the population of phasic neurones in the l.s.c. appears to be relatively homogeneous, the cells of the distal i.m.g. have more varied characteristics. This is not surprising if neuronal properties are specialized according to the function of individual sympathetic pathways.

The National Heart Foundation of Australia provided support for Dr Clark. We are very grateful to Drs S. J. Redman and C. Bell for their helpful comments, and Karen Walls for technical assistance.

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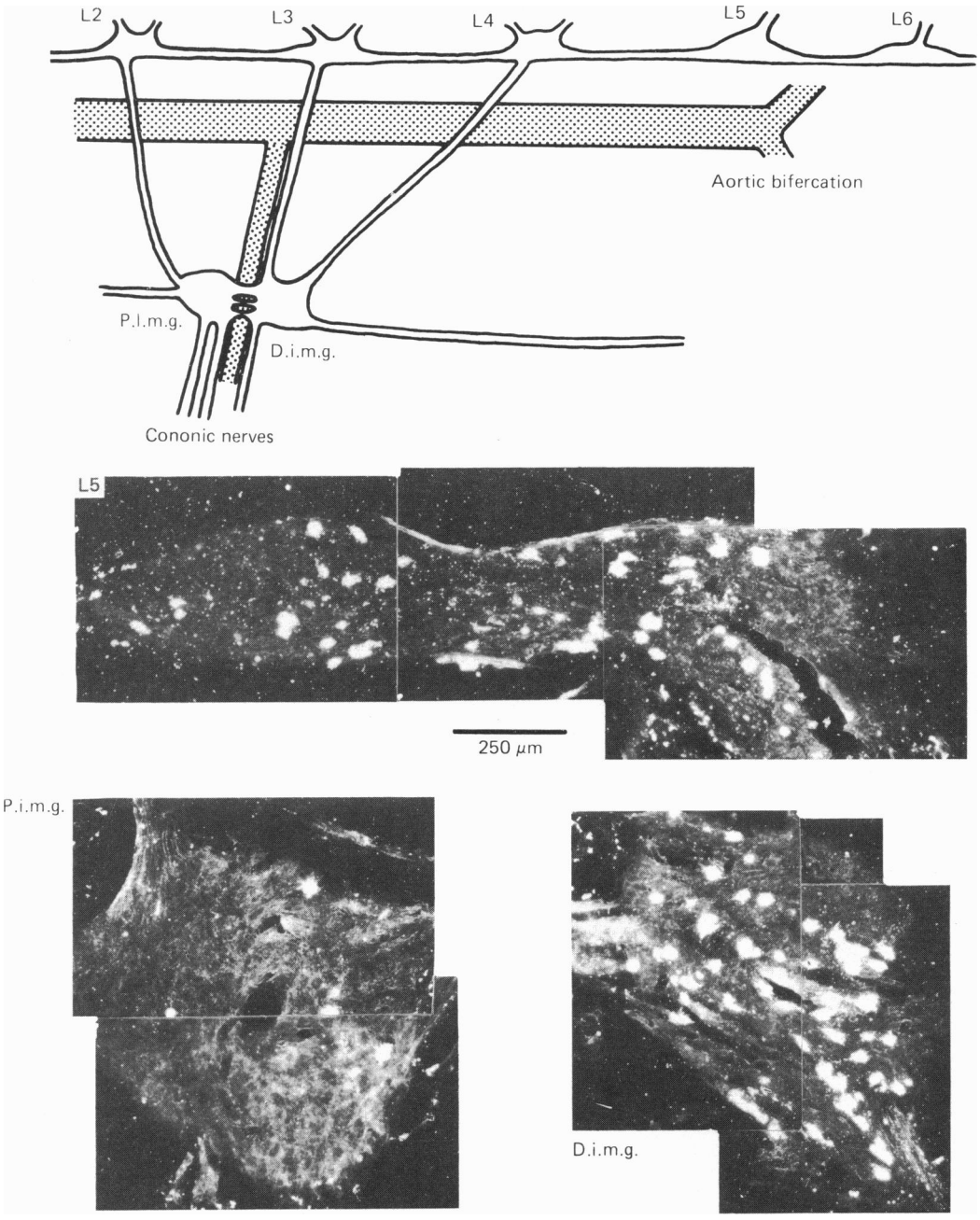
L2
L3
L4
L5
L6
Aortic bifercation
P.l.m.g.
D.i.m.g.
Cononic nerves
L5
250 μm
P.i.m.g.
D.i.m.g.

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EXPLANATION OF PLATE

Anatomy of the lumbar sympathetic chain and the inferior mesenteric ganglion (i.m.g.) in the guinea-pig. Diagram of the arrangement of nerves (above) with dark-field photomicrographs of sections (30 μm) cut from lumbar sympathetic ganglion L5, and from the proximal (p.i.m.g.) and distal (d.i.m.g.) lobes of the i.m.g. (below). Some neurone cell bodies in each section are densely filled with granules of HRP reaction product and appear white. The filled neurones in L5 ganglion were labelled retrogradely after injection of HRP into the ipsilateral gastrocnemius muscle in one animal, and those in the i.m.g. were labelled after applying HRP to the cut axons of one hypogastric nerve in another animal. From experiments of this kind, it can be concluded that large numbers of sympathetic neurones in the lumbar sympathetic chain at this level project to the vasculature of the muscles of the hind limb, and that most neurones in the distal lobe of the i.m.g. project in the ipsilateral hypogastric nerve. Calibration applies to micrographs only.