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POST-NATAL DEVELOPMENT OF GANGLIA IN THE LOWER LUMBAR SYMPATHETIC CHAIN OF THE RAT

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SUMMARY

1. The initial stages in the development of functional synapses have been examined in ganglia of the lower lumbar sympathetic chain of the rat using intracellular recording techniques.

2. In animals of age up to 7 days post-natal, many impaled cells were inexcitable and possessed no synaptic input. The proportion of excitable cells impaled increased with the age of the animal. Two types of action potential could be identified.

3. Initially the synaptic input consisted of one or a few subthreshold synaptic potentials. The number of preganglionic inputs to each cell increased over the first 1–2 weeks after birth.

4. The quantal content of each input was initially very low. At least some inputs showed an increase in quantal content during development; eventually one or occasionally two inputs became suprathreshold.

5. Voltage-clamp studies indicated that the time course of excitatory synaptic currents did not change during development.

6. The amplitudes of miniature excitatory synaptic currents in animals aged less than 10 days were some three to five times the size of those recorded from mature animals.

INTRODUCTION

Mammalian sympathetic ganglion cells usually receive a number of excitatory synaptic inputs (Blackman & Purves, 1969; Bennett & McLachlan, 1972; Skok, 1973), but little is known of the way in which this adult pattern of innervation develops. The development of nervous connexions to mammalian skeletal muscle is well documented. Initially one and then several axons make contact with the muscle fibres, and channels sensitive to transmitter become restricted to the region of contact (Redfern, 1970; Bennett & Pettigrew, 1974; Dennis, Ziskind-Conhaim & Harris, 1981). Subsequently, one presynaptic terminal grows larger, and releases progressively more quanta of transmitter (Bennett & Pettigrew, 1974), while the other synapses are withdrawn to leave the mature pattern of a single suprathreshold input per muscle fibre. During development of the synapse in mammalian muscle, the average lifetime of transmitter–receptor activated channels decreases to its mature value (Sakmann & Brenner, 1978; Fischbach & Schuetze, 1980).

The mammalian autonomic ganglia in which developmental changes have been studied lie rostrally in the animal and functional synaptic contacts are already present at birth. The subsequent stages of synapse elimination have been well described (Lichtman, 1977; Lichtman & Purves, 1980; Johnson & Purves, 1981). In order to examine the events which occur during the initial stages of synapse formation, recordings have been made from the lower lumbar sympathetic chain (L_4 and L_5 ganglia) taken from rats of post-natal age 1–21 days. This age period was hoped to encompass the time during which neurones differentiate and synaptic contacts are initially made in the lumbar ganglia since neuronal development proceeds rostro-caudally (Jacobson, 1970). Our data indicate that many synaptic contacts in the lower lumbar region of the sympathetic chain became functional in the first few days after birth in the rat. The number of presynaptic axons innervating a ganglion cell progressively increases. Initially, the number of quanta released from each axon is few, but later one or occasionally two of the axons matures so that when stimulated they release sufficient quanta to invariably initiate an action potential in the ganglion cell. However, coincident with the increase in quantal content, a decrease in the size of the response to each quantum was detected. The change in quantal size is discussed in relation to the small number of transmitter–receptor channels activated by individual quanta of transmitter at mature ganglionic synapses (Rang, 1981).

METHODS

Lumbar sympathetic chain ganglia (usually the left) were dissected from male rats (P.V.G. strain) aged between 1 and 21 days post-natal. The animals were killed by decapitation and bled, and the body was pinned in a dissecting chamber through which flowed physiological saline gassed with 95% O_2/5% CO_2 at room temperature (22–28 °C). The lower lumbar sympathetic chain (L_3–L_5 and L_6 if present) was isolated by clearing away connective tissue and cutting the connecting rami and visceral projections. The sympathetic chain was then pinned out in a recording chamber (volume 1 ml) with a base which consisted of a microscope cover-slip coated with clear silicone rubber (Sylgard 184); the preparations were viewed using an inverted compound microscope (magnification ×40 ×400) (for further details see Hirst, Holman & Spence, 1974). Physiological saline of composition (mM): NaCl, 120; KCl, 5; $CaCl_2$, 2·5; $MgCl_2$, 2·0; $NaHCO_3$, 25; NaH_2PO_4, 1; glucose, 22, pH 7·2–7·4, gassed with 95% O_2/5% CO_2 and warmed to 37 °C, was pumped through the recording chamber at 6 bath volumes per minute. Preparations taken from animals younger than 7 days deteriorated if recording periods were longer than 2–3 h (see also Lichtman & Purves, 1980). In a few experiments on rats aged 2, 3 or 4 days, the superior cervical ganglion (s.c.g.) was isolated for study as described above. Some observations on similar experiments in ganglia from adult rats are also reported.

Intracellular recordings were made from ganglion cells using micro-electrodes pulled from 1·5 mm diameter fibre-filled glass capillaries using a Brown–Flaming puller. Electrodes with which satisfactory impalements were obtained had resistances in the range 80–140 MΩ when filled with 0·5 M-KCl. All data reported in this paper are from impalements in which the resting membrane potential settled after a few minutes to a value between −50 and −70 mV. This usually remained stable until the micro-electrode was withdrawn. The passive electrical properties of neurones were determined using a single electrode current clamp (s.e.c.c.), by passing hyperpolarizing current (duration 50–80 ms) through the recording electrode. The current intensity was adjusted so that the amplitude of the resulting electrotonic potential did not exceed 20 mV.

The time course and intensities of post-synaptic excitatory currents were determined using a single electrode voltage clamp (s.e.v.c.) (Axon Instruments). Provided care was taken to ensure that the micro-electrode tip capacitance was kept low (< 1 pF), cycling rates of between 2 and 4 kHz were readily obtained with the pre-amplifier in either s.e.c.c. or s.e.v.c. mode. Tip capacitance was reduced by ensuring that the level of fluid over the preparation did not exceed 50 μm, and by coating

the electrodes with silicone fluid to prevent 'creep' of tissue fluid up the micro-electrode. During intracellular recording, after switching from s.e.c.c. to s.e.v.c. mode, the clamp gain was optimized without oscillation before measuring the synaptic currents. Within a few milliseconds the 'clamped' potential record was flat (see Fig. 7). The maximum residual voltage accepted was 15% of the peak unclamped voltage signal. The error voltage invariably occurred during the rising phase of the excitatory synaptic potential and this will lead to an over-estimation of the excitatory synaptic current rise time. All records of membrane potential and synaptic current, unless stated otherwise, were filtered using a 1·5 kHz flat delay filter (Rockland 432).

Since the input to ganglion cells lying in L_4 and L_5 sympathetic chain ganglia arises from L_2 and above and descends along the sympathetic chain, all preganglionic axons were stimulated via a pair of electrodes placed across the chain approximately 1 mm cranial to the ganglion studied. In each experiment, recordings were made first from ganglion cells in L_5, the stimulating electrodes being placed between L_4 and L_5. Subsequently, recordings were made from L_4, the stimulating electrodes being placed between L_4 and L_5.

The nature of the synaptic input was investigated by applying brief (< 1 ms) stimuli of variable strength, and examining the number and configuration of the evoked excitatory synaptic potentials (e.s.p.s) or currents (e.s.c.s). If necessary, the cells were hyperpolarized by 10–30 mV to eliminate action potentials during this procedure. The abrupt appearance at a given stimulus strength of a large suprathreshold response (> 1 nA peak e.s.c. amplitude) was taken to indicate a single 'strong' fibre input (see Holman & Hirst, 1977). The action potentials initiated by these inputs were not readily blocked by hyperpolarization of the cell. Because of the proximity of the stimulating electrode, it is possible that this procedure underestimates the number of projections since axons with similar thresholds may not be distinguishable (see Lichtman & Purves, 1980). For this reason the absolute number of synaptic inputs, particularly in cells which appeared to have 'strong' inputs, could not be determined.

In some experiments, synaptic transmission was prevented by reducing the Ca^{2+} concentration to 0·25 mM with a concurrent increase in Mg^{2+} concentration to 20 mM, no compensation was made for the increased tonicity. In a few experiments action potentials were abolished by superfusing the preparation with solution containing tetrodotoxin (10^{-7}–10^{-6} g/ml; Sigma).

Neurone size

S.c.g. and ganglia L_4 and L_5 from each of two litter-mates aged 3, 6, 14 and 20 days were fixed in 4% paraformaldehyde–0·5% glutaraldehyde in 0·1 M-phosphate buffer, post-fixed in 1% osmium tetroxide, dehydrated and embedded in Araldite. Semi-thin sections (1–2 μm) were stained with Toluidine Blue. Cross-sectional areas of profiles containing a nucleolus were measured at a magnification of 800× using a digitizing tablet and associated computer.

RESULTS

Anatomical observations

The lowest lumbar sympathetic ganglia (L_4–L_5) on the first post-natal day were very small (< 200 μm long) whereas ganglia at L_3 and the s.c.g. were nearly as long as those taken from animals aged 7 days. Over the first week, the volumes of L_4 and L_5 ganglia increased by a factor of about ten (cf. rat s.c.g., 2-fold; Hendry & Campbell, 1976). Branches from these ganglia to the viscera contain few post-ganglionic axons (Hancock & Peveto, 1979), so that the majority of cells impaled in L_4 and L_5 were probably vasomotor neurones projecting to skin and muscle of the still developing hind limb and tail in L_5 and L_6 spinal nerves (see McLachlan & Janig, 1983).

Cell diameter histograms from 3 day rat s.c.g. were bimodal, being skewed to smaller values (mode 7 μm) with a second larger population with mode about 14 μm (mean diameter $11{\cdot}1 \pm 3{\cdot}1$ μm, S.E. of mean, $n = 79$). By 6 days the smaller cells had largely disappeared (mean $14{\cdot}1 \pm 3{\cdot}4$ μm, $n = 75$). Mean cell diameter in lumbar

ganglia up to 7 days of age was less than in s.c.g. (3 days: $9{\cdot}8 \pm 2{\cdot}9$ μm, $n = 54$; 6 days: $10{\cdot}8 \pm 1{\cdot}6$ μm, $n = 76$). However, by 14 days, the rostrocaudal differential had disappeared and all cell diameter histograms were unimodal, showing a progressive increase to means of $17{\cdot}6 \pm 3{\cdot}8$ μm, $n = 48$ (s.c.g.) and $17{\cdot}5 \pm 3{\cdot}1$ μm, $n = 69$ (lumbar), at 21 days of age (see also Hendry & Campbell, 1976). These observations suggest that at birth the caudally located sympathetic ganglia are less developed than the ganglia at the rostral end of the chain.

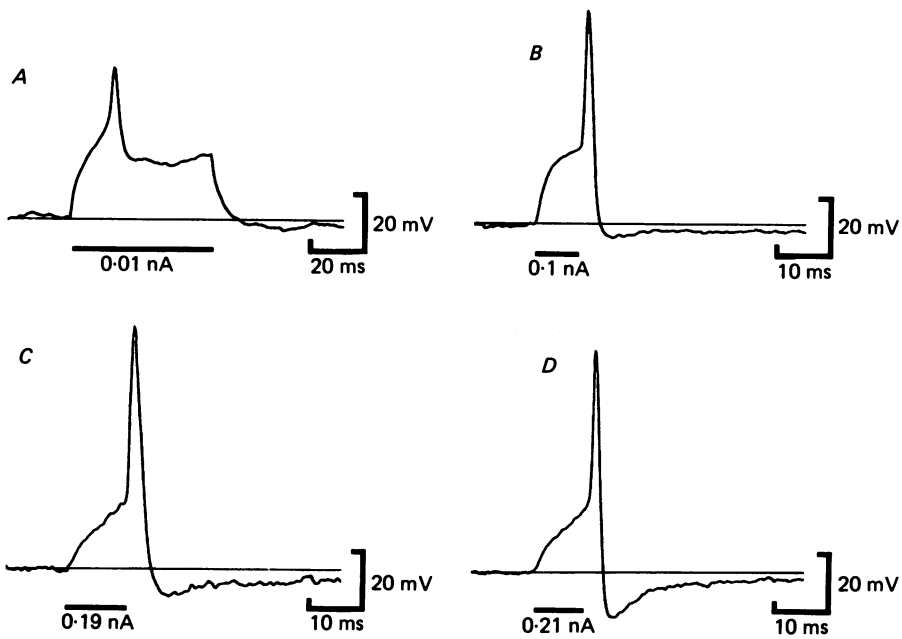

Fig. 1. Action potentials recorded from cells of rat lumbar sympathetic ganglia during post-natal development. Responses to direct depolarizing current pulses in non-innervated cell of the 'primitive' type from an animal aged 4 days (*A*), and innervated cells from animals aged 3 days (*B*), 7 days (*C*) and 21 days (*D*).

Changes in active electrical properties of cells in lumbar sympathetic ganglia during post-natal development

In a proportion of cells impaled in all ganglia studied, no active responses could be evoked by passing depolarizing current (up to 1 nA) through the recording electrode. These cells had input resistances in the range 100–300 MΩ at <7 days, decreasing to <50 MΩ by 21 days, and short time constants (3–5 ms). Inexcitable cells from animals over about 10 days of age usually depolarized slowly without synaptic potentials in response to repetitive stimulation of preganglionic inputs. Such cells are thought to be glial cells (Blackman & Purves, 1969). The proportion of impaled lumbar chain cells which were inexcitable declined rapidly over the first post-natal week.

Action potentials were generated in all other impaled cells in response to depolarizing current (0·1–0·4 nA for 10 ms, or 0·02–0·07 nA for 50 ms) (see Fig. 1). Throughout the period under study, the type of action potential most commonly recorded had a rapid

rise time ($\cong$ 1 ms) and duration at half-maximum amplitude (half-width) of about 1·8 ms (1–4 days: mean 1·8 $\pm$ 0·2 ms, S.E. of mean, $n = 18$; 14 days: 1·8 $\pm$ 0·1 ms, $n = 12$; 21 days: 1·7 $\pm$ 0·1 ms, $n = 13$) (see Fig. 1*B* and *D*). These action potentials had amplitudes greater than 60 mV, which were rapidly reduced by adding tetrodotoxin (10^{-6} g/ml) to the bathing solution. Each neurone with this type of action potential could be shown to receive at least one, and usually several, excitatory synaptic inputs by stimulating the sympathetic chain.

In a small proportion (12 from 101) of excitable cells from animals aged <6 days, the action potentials initiated by direct stimulation had long half-widths (3–8 ms) and small amplitudes (maximum detected 52 mV) (see Fig. 1*A*). These action potentials had high thresholds and were often graded in amplitude by varying the intensity of the depolarizing current. We have no reason to think that the small amplitude and slow time course of these action potentials resulted from damage on impalement as their input resistances were extremely high, ranging from 208 to 850 MΩ (mean 427 $\pm$ 50 MΩ, $n = 12$; see Fig. 2*B*). Unfortunately, impalements of these cells rarely lasted for longer than 5 min and the ionic mechanisms underlying these action potentials have not yet been investigated. As these action potentials resemble those recorded from differentiating spinal neurones (Baccaglini & Spitzer, 1977), the cells from which such records were obtained will be referred to as 'primitive' cells. It was not possible to demonstrate the presence of synaptic input to these cells.

Another developmental change in the time course of action potentials was noted in a proportion (47 %) of innervated cells of rats aged 5–7 days (Fig. 1*C*), and in occasional cells at 14 and 21 days. The action potentials of neurones at 5–7 days had normal amplitudes but longer half-widths (mean 2·56 $\pm$ 0·27 ms, S.E. of mean, $n = 18$) than did those of the innervated cells of younger or older animals. A frequency histogram of action potential half-widths for the 5–7 day age group was bimodally distributed; there was no apparent correlation between half-width and amplitude.

Changes in passive electrical properties of lumbar sympathetic neurones during post-natal development

As has been mentioned, 'primitive' cells had high input resistances (Fig. 2*B*); electrotonic potentials in them could be described by single exponentials with time constants in the range 4·8–14·3 ms (mean 9·5 $\pm$ 0·8 ms, S.E. of mean, $n = 9$).

The input resistances of neurones which received synaptic inputs were much lower than those of 'primitive' cells. The mean input resistance fell from about 150 MΩ to about 100 MΩ after the first week (see Fig. 3). Meanwhile, however, the cell time constant increased from about 5 to 8 ms (see Figs. 2 and 3). Many electrotonic potentials showed an initial rapid decline before decaying with an exponential time course (Fig. 2*B*). The initial rapid decline probably reflects current spread into the dendritic tree. As the initial faster decay did not last for longer than 10 % of the final cell time constant, the cell dendrites must be electrically short, i.e. less than 0·5 space constants (Jack & Redman, 1971; see also McLachlan, 1974). The proportion of cells whose electrotonic potentials gave evidence of dendritic current spread increased during the developmental period examined. This might suggest that cell processes develop over this time. Also plotted in Fig. 3 is the mean input capacitance of neurones at different developmental stages. It is not known if the specific membrane

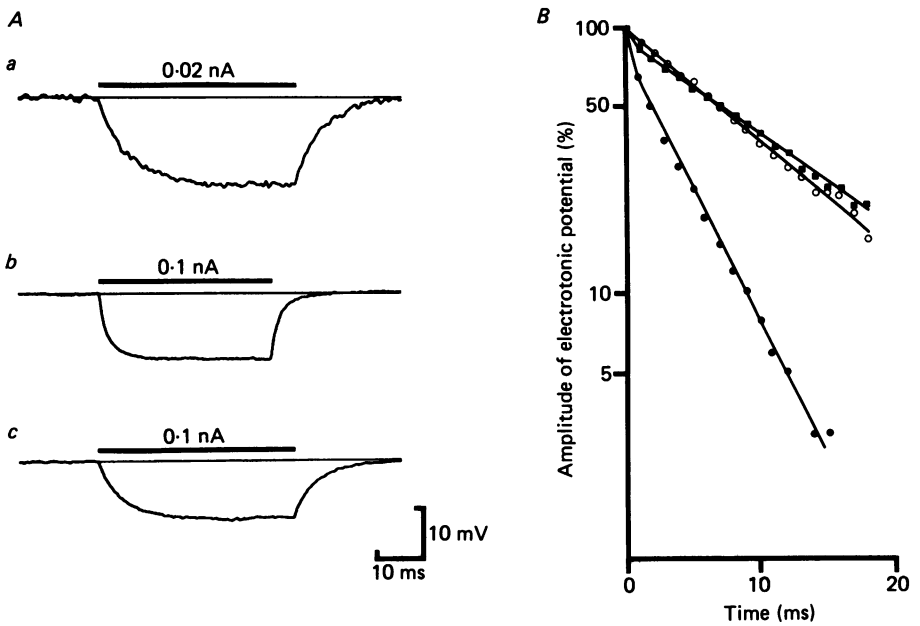

Fig. 2. Passive electrical properties of lumbar sympathetic ganglion cells during development. *A*, responses to direct hyperpolarizing current in a 'primitive' cell from a lumbar ganglion from an animal aged 4 days (*a*), and from innervated cells from animals aged 3 days (*b*) and 21 days (*c*). *B*, semilogarithmic plot of time course of onset of voltage responses in *A* of 'primitive' cell (○), 3 day cell (●) and 21 day cell (■).

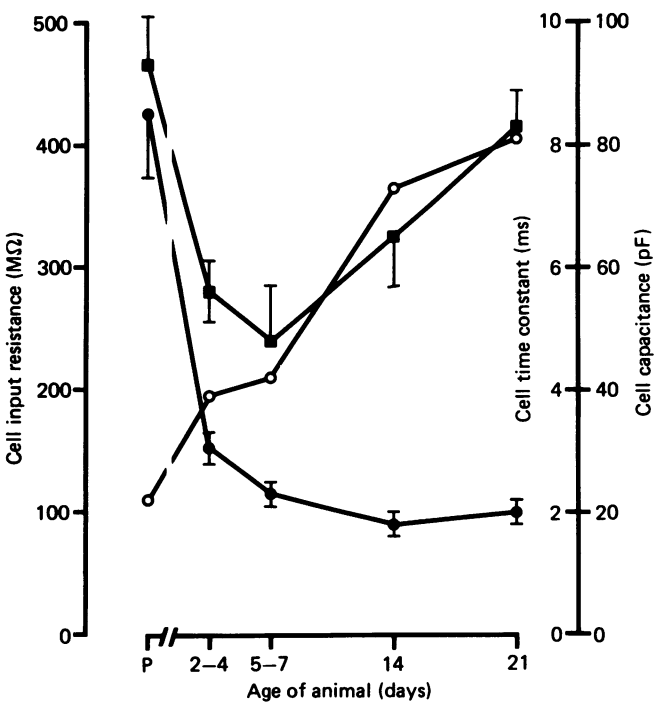

Fig. 3. Changes in passive electrical properties of sympathetic ganglion cells during post-natal development. Mean values (±S.E. of mean) of cell input resistance (●) and cell time constant (■) at different stages, together with average cell capacitance (○) derived from these data. Data from primitive cells is plotted separately (P).

capacitance of these neurones changes during development. If this were not the case, the cell capacitance would be a measure of cell surface area, suggesting that during this period the neurones we impaled nearly doubled in diameter; this is in accord with the histological observations. Furthermore, if the specific membrane capacitance does not change, it would appear that during maturation, the specific membrane resistance increases.

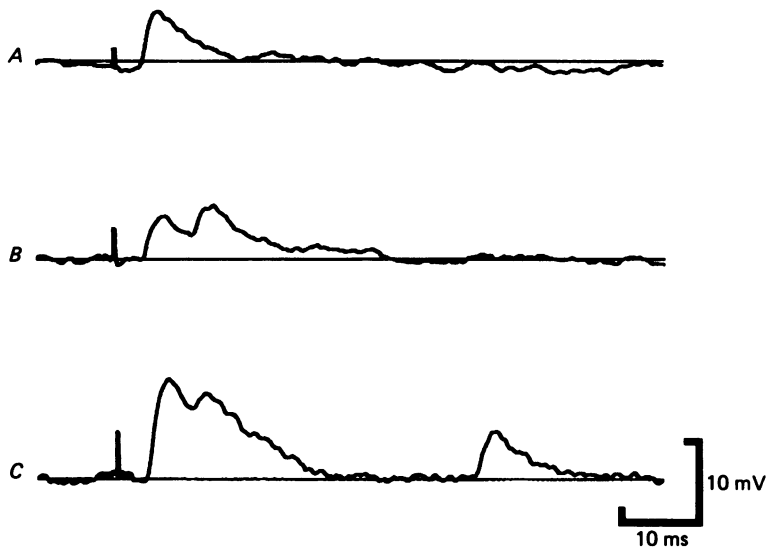

Fig. 4. Excitatory synaptic potentials (e.s.p.s) recorded from a lumbar sympathetic ganglion cell of animal aged 2 days. Stimuli of increasing voltage (*A–C*) applied to preganglionic axons proximal to L_4 ganglion. A spontaneous e.s.p. can be seen to right of *C*.

Development of synaptic connexions in lumbar sympathetic ganglia

Preganglionic stimuli failed to initiate detectable synaptic potentials in 'primitive' cells. In contrast, preganglionic stimuli evoked e.s.p.s in all cells with brief all-or-none action potentials. With animals in the youngest age group (1–4 days) e.s.p.s had amplitudes of some 5–10 mV, and each ganglion cell received only a few (one to four) inputs (see Fig. 4). A proportion of these innervated cells had input resistance of over 250 MΩ but, unlike 'primitive' cells, their action potentials were brief. It is possible that these cells represent a transitional stage between the two cell types. The amplitude of the individual e.s.p.s was similar to that of the miniature excitatory synaptic potentials (m.e.s.p.s) in the same cell (Fig. 4*C*), suggesting that the e.s.p.s had very low quantal contents. Despite this, few failures of release were detected during the first few stimuli.

In the majority of neurones from animals older than 3 days, the combined excitatory synaptic input evoked by synchronous supramaximal stimulation was suprathreshold for the initiation of a post-ganglionic action potential. However, at post-natal stages up to 7 and even 14 days, many of the several subthreshold preganglionic inputs failed intermittently during repetitive stimulation at frequencies of 0·5 Hz or even less. At this stage of synapse formation, it was apparent that the

cells in L_4 ganglia usually received more or larger synaptic inputs than did cells in L_5 ganglia, and that presynaptic failure occurred less readily.

As well as having several subthreshold inputs, a small proportion (8 %) of neurones in the youngest group could be shown to possess a 'strong' synaptic input (see Methods). That is, when such an input was stimulated, it invariably initiated a post-ganglionic action potential which was followed by an after-depolarization

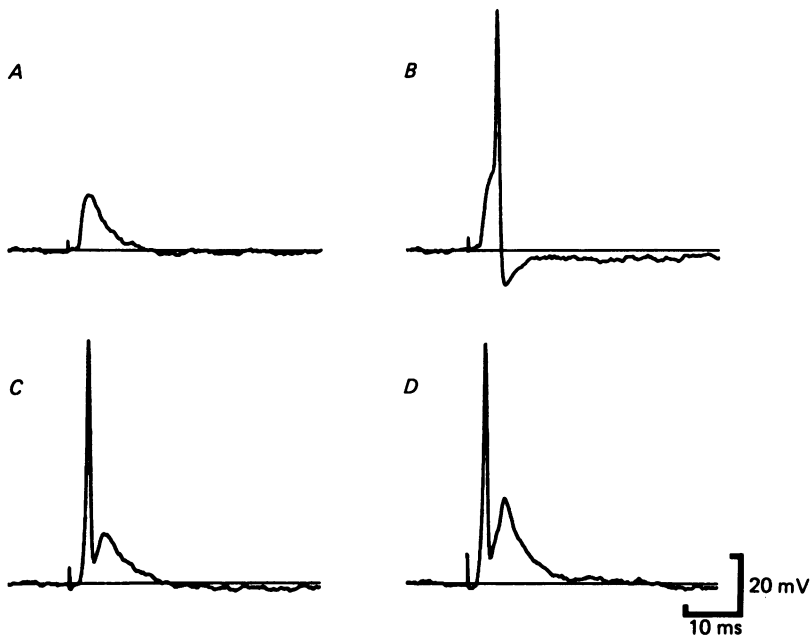

Fig. 5. Synaptic input to a lumbar sympathetic ganglion cell in an animal aged 21 days. Stimuli of increasing voltage (*A–D*) to descending sympathetic chain. A suprathreshold response (*C*) as well as several subthreshold e.s.p.s are evoked in this cell.

(Holman & Hirst, 1977). 'Strong' inputs never failed during brief trains of repetitive stimuli at frequencies up to 25 Hz. The proportion of neurones which received 'strong' inputs progressively increased, being 16 % at 5–7 days, 36 % at 14 days, and 84 % at 21 days. A proportion (20 %) of cells from animals aged 21 days received two 'strong' inputs, while all ganglion cells at each developmental stage could be shown in addition to receive several subthreshold inputs (Fig. 5). This pattern of innervation was the same as that found in adult rat lower lumbar ganglia. In a few neurones in preparations from animals aged 14 days, in excess of eight discrete excitatory inputs could be clearly distinguished. This was rarely seen at the other stages examined.

In a few experiments, recordings were made from 2–4 day-old rat s.c.g. cells. These cells had input resistances (mean 164 ± 24 MΩ, $n = 10$) and time constants ($5{\cdot}3 \pm 0{\cdot}7$ ms) similar to those of cells in the lower lumbar chain at the same age. However, no 'primitive' cells, and very few inexcitable cells were impaled. Unlike ganglion cells in L_4 and L_5 at this age, many of the innervated cells received in excess of eight excitatory inputs, and about 20 % had 'strong' synaptic inputs. These observations suggest that the synaptic connexions to s.c.g. cells are at a more advanced stage of development in the early post-natal period.

Time course of e.s.c.s recorded from sympathetic ganglion cells of animals of different ages

If the stimulus strength to the lumbar sympathetic trunk was adjusted so as to excite only a single axon, the resultant e.s.p. was almost invariably of insufficient amplitude to initiate an action potential. The underlying e.s.c. was determined under conditions of voltage clamp (see Fig. 6*A* and *B*). The e.s.c. was maximal after about

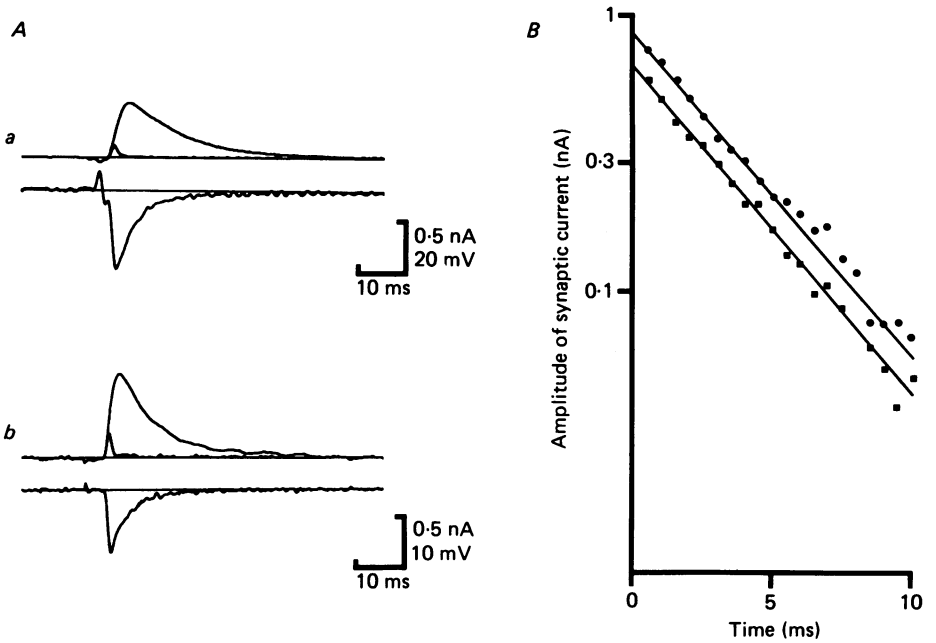

Fig. 6. Excitatory synaptic currents (e.s.c.s) recorded from lumbar sympathetic ganglion cells at different developmental stages. *A*, averaged synaptic responses of potential (upper trace) and current (lower trace) from cells of animals aged 3 days (*a*) and 21 days (*b*). Superimposed traces recorded in current clamp and voltage clamp modes. *B*, semi-logarithmic plot of time course of decay of e.s.c.s in 3 day cell (●) and 21 day cell (■).

1 ms, and it decayed with a time course that could be described by a single exponential (Fig. 6*C*). The time constant of this exponential was remarkably constant between cells and did not change during the developmental period examined. When the cells were 'clamped' at between −55 and −65 mV, the time constants of decay of e.s.c.s in cells from animals at different ages were 4·2±0·2 ms (mean±S.E. of mean, $n = 13$, 1–4 days); 3·9±0·2 ms ($n = 14$, 5–7 days); 3·6±0·3 ms ($n = 20$, 14 days) and 4·0±0·3 ms ($n = 9$, 21 days). These values did not differ from those obtained in s.c.g. cells from animals 2–4 days old (4·2±0·1 ms, $n = 9$). Taken together these data suggest that the kinetics of channel closure at these mammalian cholinergic synapses do not alter during development.

Several other incidental observations were made on the synaptic currents which are consistent with those reported for e.s.c.s in other mammalian ganglion cells (Rang, 1981; Derkach, Selyanko & Skok, 1983). In one experiment, the temperature was reduced from 37 to 27 °C; the time constant of decay of the e.s.c. changed from

4·2 to 7·2 ms. E.s.c.s of both immature and mature ganglion cells displayed only slight voltage sensitivity with membrane hyperpolarization. The time constant of e.s.c. decay increased by up to 25% when the membrane potential was changed from rest to a value of 30 mV more hyperpolarized. In some cells, however, there was virtually no change in time course. Plots of e.s.c. amplitude *versus* membrane potential in the range −40 to −100 mV were linear, with extrapolated reversal potentials of between −15 and +10 mV. This was again so for both immature and mature synapses. The time course of e.s.c.s of sympathetic ganglion cells was influenced by the extracellular calcium concentration; doubling the external calcium increased the time constant of decay of the e.s.c. by about 25%.

In some experiments, it was possible to voltage clamp the post-synaptic responses initiated by 'strong' fibre stimulation. Most of these synaptic currents, which had peak amplitudes up to 3 nA, also had a single exponential time course of decay with a time constant of about 4 ms, but in a few other cells they were slightly prolonged. Since in these cases the stimuli invariably excited several axons, the possibility that the prolongation of time course reflected dispersed conduction velocities of the preganglionic axons could not be excluded.

Changes in spontaneous e.s.p.s and e.s.c.s

Spontaneous m.e.s.p.s were not always detected in lumbar sympathetic ganglion cells which could be shown to receive an excitatory synaptic input. This could have arisen either because they had a small amplitude or because their frequency was extremely low, often as low or lower than 0·03 Hz (see also Blackman & Purves, 1969; McLachlan, 1975). M.e.s.p.s were never detected in 'primitive' or inexcitable cells.

In recordings from ganglia of younger animals (<7 days) m.e.s.p.s often had very large amplitudes (see Figs. 2 and 7); on occasions m.e.s.p.s initiated action potentials. Preganglionic stimuli sometimes initiated bursts of m.e.s.p.s (at frequencies up to 20 Hz) which continued to occur for many minutes (see for example Figs. 7 and 8*A*). Most of the quantitative data described in this paper comes from such cells. These bursts of m.e.s.p.s were not abolished by rapidly perfusing the ganglia with solutions containing either tetrodotoxin (1×10^{-6} g/ml) or a reduced extracellular calcium concentration (0·25 mM).

The time course of all m.e.s.p.s was indistinguishable from that of e.s.p.s evoked in the same ganglion cell; if transmitter release was depressed in low Ca^{2+}/high Mg^{2+} solutions so that the majority of stimuli failed to evoke a response, the remaining occasional e.s.p.s were always similar in amplitude and time course to the m.e.s.p.s.

It was possible to increase m.e.s.p. frequency in cells in older preparations by repetitive preganglionic stimulation (frequencies 5–20 Hz); this was particularly obvious in cells which received a 'strong' synaptic input. However, at this later developmental stage, most m.e.s.p.s were only about 1 mV in amplitude and many were barely distinguishable from recording noise. Larger amplitude m.e.s.p.s occurred very infrequently (see McLachlan, 1975).

The possibility was considered that the large amplitudes of m.e.s.p.s in younger preparations simply reflected the passive properties of the ganglion cells (Fig. 3). Cells with higher input resistances and lower capacitances (at 1–7 days) would be expected to present greater impedances than those with lower resistances and higher capaci-

tances (at 14–21 days). Calculations of the membrane potential changes produced by brief synaptic currents (time to peak $\simeq 1$ ms followed by an exponential decay of time constant 4 ms with a peak amplitude of 0·1 nA) in two cells, one with an input resistance of 140 MΩ and capacitance of 40 pF, and the other with an input resistance of 100 MΩ and capacitance of 80 pF, indicated that these would differ by a factor of less than two. The peak membrane potential changes were 4·8 mV and 2·5 mV respectively.

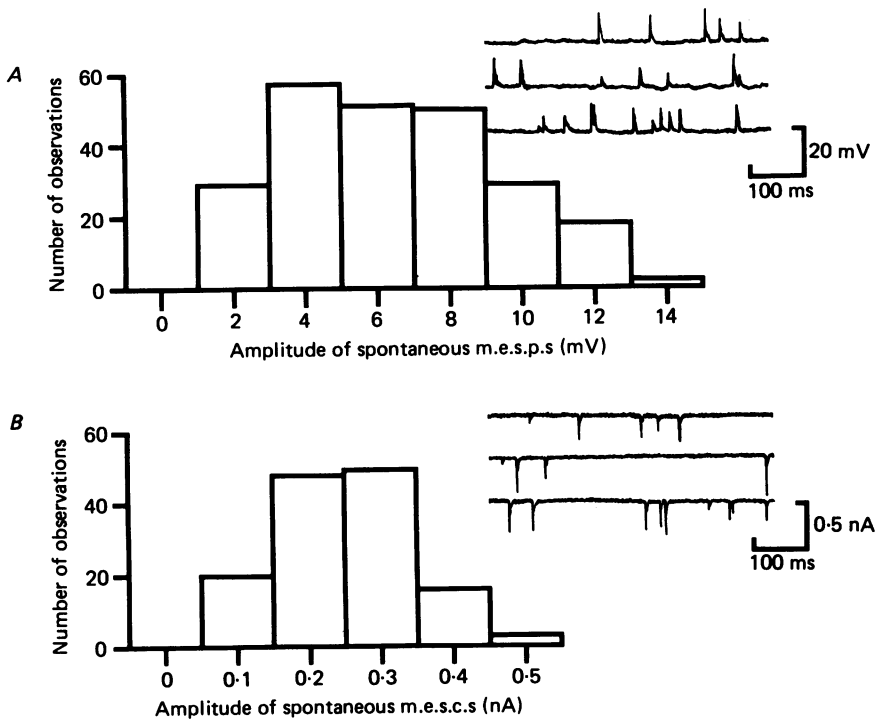

Fig. 7. Amplitude frequency distributions of spontaneous m.e.s.p.s (*A*) and m.e.s.c.s (*B*) recorded in a lumbar sympathetic ganglion cell of an animal aged 6 days. Insets show examples of records from which measurements were made.

When ganglion cells were voltage clamped, the amplitudes of spontaneous miniature excitatory synaptic currents (m.e.s.c.s) could be determined (Fig. 7*B*). M.e.s.c.s underlying the large m.e.s.p.s in the immature ganglion cells had amplitudes in the range 0·1 to 0·6 nA, and their distribution was unimodal. The time courses of m.e.s.c.s of all sizes were the same and no inflexions on the rising phases of 'large' m.e.s.c.s could be detected. The population of m.e.s.c.s recorded in ganglion cells from young animals (<7 days) were generally larger than those recorded from older animals (21 days) (Fig. 8).

The proportion of cells with large quantal responses fell during the developmental period under study. This has been quantitated by taking an arbitrary minimum amplitude of 5 mV (to exclude the effect of the passive membrane properties of immature neurones) or 0·2 nA. On this basis 37 % of all cells (nineteen from fifty-two)

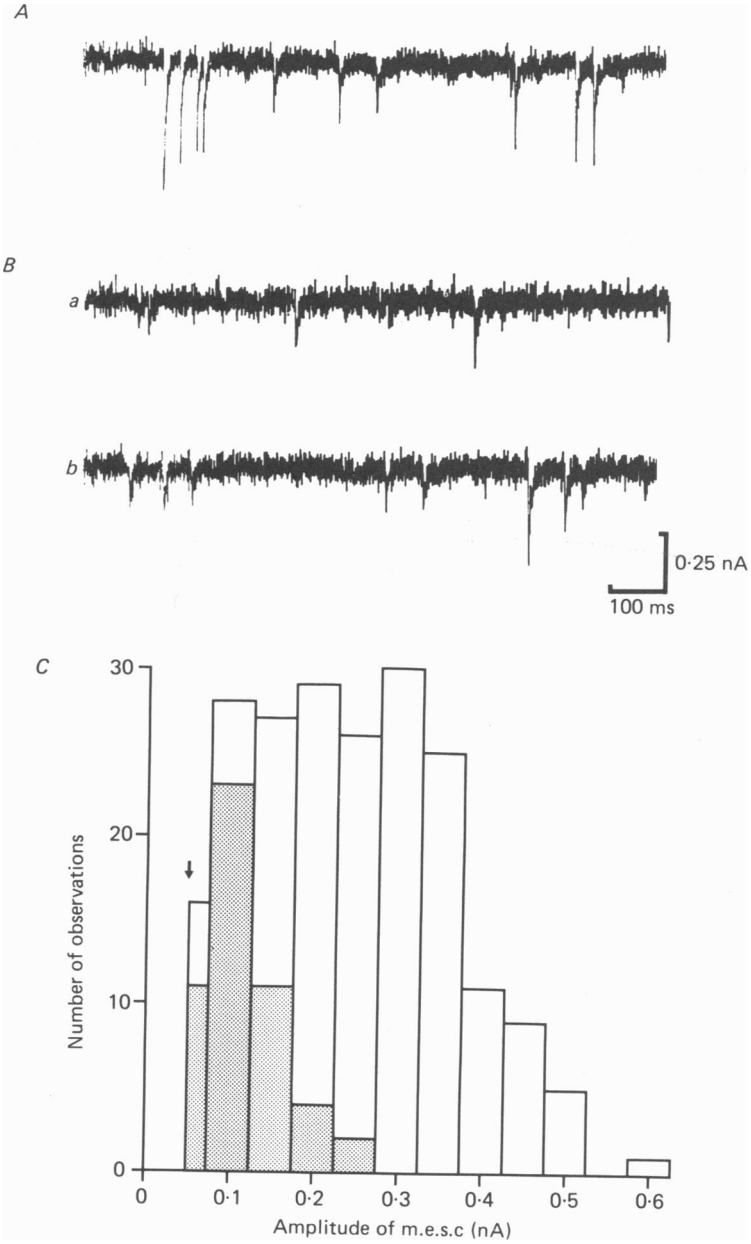

Fig. 8. Spontaneous m.e.s.c.s recorded from lumbar sympathetic ganglion cells from animals aged 4 days (*A*) and 21 days (*B*). Membrane potential clamped at −70 mV in *A* and *B a*, and −100 mV in *B b*. *C* shows amplitude frequency distribution of m.e.s.c.s recorded at −70 mV membrane potential; data from the same cells as illustrated in *A* and *B*. Open columns, 4 day animal; stippled columns, 21 day animal. Arrow indicates level of recording noise.

from animals aged 2–4 days had large spontaneous events. The proportion of cells fell from 39% (twenty-two from fifty-six, 5–7 days) and 15% (three from nineteen, 14 days) to 8% (two from thirty, 21 days of age). These observations suggest that, during post-natal development, as the quantal content of the synaptic inputs becomes larger, the amplitude of each quantal response decreases.

DISCUSSION

Recordings from the lower lumbar sympathetic chain ganglia of young rats (up to 1 week of age) indicated the presence of three groups of cells. One of these was characterized by having moderate input resistances ($\cong$200 MΩ), and lacking the ability to generate action potentials in response to membrane depolarization. The nature of these cells is unknown. Their input resistances were higher than those of glial cells present in older animals; this could be related to differences in cell size. Unlike adult glial cells which depolarize during repetitive neuronal activity due to extracellular K^+ accumulation (Kuffler, Nicholls & Orkand, 1966), the membrane potential of these cells did not change during repetitive preganglionic fibre stimulation. While this might imply that these cells are not glial, it could simply reflect the paucity of synaptic connexions at this stage. Alternatively these inexcitable cells could be preneuronal cells which are unable to generate action potentials. Such cells are found in *Xenopus* embryonic spinal cord, where they are electrically coupled to neighbouring cells and lack voltage-dependent membrane channels (Spitzer, 1982). However, such embryonic cells show voltage-dependent uncoupling from neighbouring cells; this is reflected in a sudden increase in input resistance during point injection of depolarizing current. No such voltage-dependent uncoupling was detected during current injection into inexcitable sympathetic chain cells.

The second group was characterized by having high input resistances and by generating action potentials of slow time course and variable amplitude. These cells had no demonstrable synaptic input, and were only detected in tissues from animals aged less than 6 days post-natal. The slow time course and variability of amplitude of the action potentials is reminiscent of the calcium action potentials present in neurones of *Xenopus* embryos; subsequently the nature of the membrane channels changes, so that tetrodotoxin-sensitive action potentials are generated (Baccaglini & Spitzer, 1977). We have as yet not attempted to characterize the ionic mechanism underlying the action potentials of these immature sympathetic neurones.

The third type of cell generated brief action potentials with amplitudes in excess of 60 mV which were largely abolished by tetrodotoxin. This type of cell was present throughout the developmental period studied and these action potentials were identical to those generated by most adult autonomic ganglion cells. At about 7 days of age, the action potential in some of these cells became slightly prolonged; we have no explanation for this observation. Although brief action potentials appeared at the same time as the first functional synaptic inputs, a causal relationship between these events cannot be concluded.

During the first 3 weeks of post-natal development, the input resistance of the ganglion cells fell. This presumably reflects the increase in mean cell diameter. However, superimposed on the fall in input resistance was an increase in cell time

constant. The simplest explanation for this observation would be that the specific membrane resistance of the cells was increasing. Developmental changes of specific membrane resistance in a variety of tissues have been documented (Harvey, 1980).

Each of the neurones which generated a brief action potential could be shown to receive at least one excitatory synaptic input. In the first post-natal week, the proportion of cells having synaptic inputs increased, as did the number of inputs received by each cell. This contrasts with more rostrally located autonomic ganglia in which the cells have multiple innervation at birth (Lichtman, 1977; Lichtman & Purves, 1980; Johnson & Purves, 1981), and is consistent with the rostrocaudal progression of development in the nervous system. This is also reflected in cell sizes; at 1–3 days post-natal rat submandibular ganglion cells and rabbit ciliary ganglion cells average 18 and 24 μm diameter respectively compared with less than 10 μm for the cells of rat lower lumbar ganglia.

During the first 3 weeks of post-natal development the nature of synaptic connexions changed. At the initial stages of synapse formation, when it was possible to evoke only a few subthreshold e.s.p.s, the amplitudes of m.e.s.p.s were similar. This implies that, early in development, as at other synapses (Bennett & Pettigrew, 1974; Dennis *et al.* 1981), the quantal content is low. Many of the inputs were depressed by repetitive stimulation, the terminal failing to release except when stimulated at long intervals (see also Dennis *et al.* 1981). This might be related to the low numbers of synaptic vesicles present at ganglionic synapses soon after contacts are formed (Litchman, 1977; Hruschak, Friedrich & Giacobini, 1982; E. M. McLachlan, personal observations). Later, release became better maintained and the quantal content of one or sometimes two contacts increased until when stimulated it released sufficient transmitter to initiate a post-synaptic action potential. Unfortunately, the presence of this 'strong' input in rat sympathetic ganglion cells prevented assessment of the total numbers of inputs received. As a consequence it has not been possible to demonstrate the elimination of excess synaptic inputs over the post-natal period. These changes in ganglionic transmission occur over the time during which sympathetic vasomotor axons establish their contacts with the mesenteric vasculature (Hill, Hirst & van Helden, 1983). Fluorescence histochemical studies suggest that the innervation of the femoral vessels develops over a similar time course (G. D. S. Hirst and E. M. McLachlan, unpublished observations).

The time constant of decay of the e.s.c.s recorded from rat lumbar sympathetic ganglion cells is the same as that reported for rabbit s.c.g. cells (Derkach *et al.* 1983). During the development of synaptic contacts at the mammalian skeletal neuromuscular junction, the average lifetime of post-synaptic receptor channel decreases (Sakmann & Brenner, 1978; Fischbach & Schuetze, 1980) but this is not the case for receptors on chick muscle (Schuetze, 1980; Harvey & van Helden, 1981). Our observations on the time course of synaptic currents recorded from developing mammalian ganglion cells suggests that a developmental change in channel kinetics does not occur at these synapses. Together with the difference in the rate of channel closure indicated by the time courses of the e.s.c.s (<1 ms at rat neuromuscular junction *versus* 4 ms in sympathetic ganglia) this emphasizes the difference between these cholinergic receptors. This has previously been evident from their different sensitivities to nicotinic receptor blocking drugs (see e.g. Landmesser, 1972).

During the development of synapses in ganglia, the size of m.e.s.p.s and the underlying m.e.s.c.s tended to fall. The sizes of synaptic vesicles in immature and adult preganglionic terminals are similar (Lichtman, 1977; E. M. McLachlan, personal observations), so that it seems more likely that developmental changes occur post-synaptically. At maturity, the number of channels activated by a quantum of transmitter at mammalian ganglion cells is about 100 (Rang, 1981; Derkach *et al.* 1983). This implies that each synapse is saturated after the release of a quantum of transmitter (Rang, 1981). Since the current underlying each quantal response progressively decreases during development, either the mean channel conductance decreases or the number of channels under a synaptic contact becomes less. We favour the latter hypothesis since this would account for the fall in variability in quantal size during development (see Fig. 8). Individual synaptic contacts should therefore fail to show any variability in the response to the release of a quantum. This has been shown to be the case for the Ia excitatory synapses on α-motoneurones (Edwards, Redman & Walmsley, 1976; Jack, Redman & Wong, 1981*a*; Hirst, Redman & Wong, 1981) and there again the best explanation would seem to be one of receptor saturation (Jack, Redman & Wong, 1981*b*). However, there is considerable variation in the amplitudes of quantal responses arising from a single preganglionic axon (McLachlan, 1975; Fig. 8). If receptor saturation does occur, the variation must reflect the variation in the number of channels between individual subsynaptic receptor patches at the multiple sites of contact that are formed by individual preganglionic axons and autonomic ganglion cells, along with a component of noise contamination.

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